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Analysis of paternal lineages in selected Caucasian, Central Asian and Greek horse populations with Y chromosomal markers

Diplomarbeit

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vorgelegt von
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1. Introduction

1.1. Development of horse breeding

Humans adapted plants and animals like they could use their qualities best. This process is called domestication (Zeder et al. 2006). The domestication of horses shaped trade, the spread of cultures and transportation. Due to horses' speed and endurance men were able to cover long distances, which had not been possible before (Outram et al. 2009). First evidence for domestic horses was found in the steppe of Kazakhstan. This indication can be dated back to around 5500 years ago in the culture of Botai. In the first step, domesticated horses were distributed to Eastern Europe and later into the southwestern parts of Asia (Guimaraes et al. 2020). From the early days onwards humans aimed to achieve certain characteristics in horses. Thus, breeding goals were set and the breeding mares and stallions were chosen accordingly (Librado et al. 2017).

Within this thesis autochthonous horse breeds from Central and Western Asia, the Caucasus Region and Greece will be analysed with regard to their ancestry and demography. These parts of the world were the centre of culture in the early ages (Willekes 2013). In Western and Central Asia the harsh environmental conditions forced people to live a nomadic lifestyle from the seventh or eighth century Before Common Era (BCE) onwards. Therefore, horses with a great endurance, a high tolerance to climate extremes and a strong will to survive were bred. Until the 20th century this lifestyle was common (Willekes 2013). In antiquity, horses were not kept for the daily work in the field in the Mediterranean area since it was more expensive to keep horses there compared to Western and Central Asia, due to the climatic and environmental conditions. In Ancient Greece heavy field work was carried out by donkeys, oxen or mules. Horses were used for purposes like sport, spectacle or warfare. Hence, only wealthy people could afford those (Willekes 2013). The horses on Greek islands and on the mainland were surefooted and adapted to the (semi-)mountainous surroundings. Those animals were not bred for carrying heavy armours. When Greek armies got more involved in wars with tribes from Asia Minor, the main objective became breeding horses best suited for the Greek war efforts. Thus, the horses needed to become heavier and their average withers hight rose as well (Willekes 2013).

In general, from the early days on different populations of horses were crossbred and it is hard to re-establish the history of horse breeding from today's point of view (Librado et al. 2016). Nowadays, molecular genetic methods are used to investigate the open questions left on the historic development and genetic composition of modern-day horse breeds. Apart from

autosomal polymorphic markers and the strictly maternally inherited mitochondrial DNA (mtDNA), the enigmatic male sex chromosome, the Y chromosome, is an interesting and very useful marker (Librado et al. 2016).

1.2. Characteristics of the breeds included in this thesis

1.2.1. Armenian Horses

Armenia is a country with a great history of horse breeding. The Armenian horses were highly valued in ancient times. Petroglyphes dating back to 3000 BCE show men in wagons, chariots and plows pulled by horses. Additionally, the oldest wagons in the world dating back to 2000 BCE were found in Armenia (Davis 2007). There is no literature available about the history and ancestry of the horses living in Armenia today.

1.2.2. The Tushuri – a Georgian horse breed

The Tushuri Horse (see Fig. 1) is bred in the mountains of East Georgia. It is one of the eldest horse populations in the Caucasus region. It descends from Georgian breeds which were bred between the first and third century of Common Era (CE). The Tushuri was later influenced by Eastern breeds like the Turkoman, the Arabian and the Persian Horse. These horses have a great ability of endurance, are sure footed and able to adapt easily to extreme temperature changes. These are all skills needed in the mountainous environment of their origin (Rousseau 2017). The predominant colours are grey, chestnut, black and sorrel. Their average withers height is around 1.34 m. In 1990 around 1500 Tushuris were living in Georgia (DAD-IS 2020).

Fig. 1: Tushuri Horse.

https://www.picuki.com/media/1951690208420426734 (access 28.10.2020)

1.2.3. Greek ponies and horses

In course of this thesis eight native Greek breeds were analysed. Each breed's origin lies in different parts of Greece (see Fig. 2 and Tab. 1).

Fig. 2: Origin of Greek breeds analysed on a physical map of Greece.

The region of origin of each breed is marked with an arrow. The colour of the arrow varies depending on the breed. Further explanation concerning those colours is given in Tab. 1.

https://de.m.wikipedia.org/wiki/Datei:Greece_map_CIA_1996.jpg (access 30.10.2020)

Tab. 1: Explanation Fig. 2.

Colour of the Arrow	**Breed**
blue	Andravida Horse
white	Creta Pony
grey	Lesvos Pony
violet	Peneia Pony
yellow	Pindos Pony
brown	Rodos Small Horse
red	Skyros Pony
green	Thessalian Pony

1.2.3.1. Andravida Horse

The Andravidas breed is also known as Eleia or Eilia. This breed originated from the Andravidas regions which are part of the plain of Eleia in the west of the Peloponnesus. This breed was developed at the beginning of the last century by crossing Anglo-Norman stallions with mares native to the regions mentioned above (Hendricks 2007). Around 1920, the descendants of these horses were crossbred with Nonius stallions. The Nonius breed itself originated when an Anglo-Norman stallion was crossbred with Spanish-Neopolitan mares in the early 18th century (Hendricks 2007). In 2011 a herd book for the Andravidas was established (DAD-IS 2020). The colours which occur most frequently within this breed are chestnut, bay, brown and red roan. Horses of grey colour are rare. Their average withers height is 1.5-1.63m. They are mainly used as draft and riding horses and for farm work (Hendricks 2007). According to the Domestic Animal Diversity Information System (DAD-IS) (2020) of the Food and Agriculture Organisation (FAO) ten horses were left in 2004.

Fig. 3 shows a picture of an Andravida Horse.

Fig. 3: Andravida Horse.

https://www.horsebreedspictures.com/wp-content/uploads/2016/09/Andravida-Horse-Stallion.jpg (access 28.10.2020)

1.2.3.2. Creta Pony

The Creta Pony is also called Messara Horse. This breed has its origin around 1500 CE on the island of Crete when horses were imported by Turkish invaders. It was originally bred in the Messaras mountains in the region of Heraklion. Today, this breed can be found all over the island (Kugler 2010). One of the characteristics of this breed is their ability to pace (Hendricks 2007). This skill is possibly a result of the crossbreeding of local horses with Arabian-like horses during the time of Turkish occupation (Kugler 2010). The Creta Pony also shows similarity with horses which were illustrated in the Minoan time. Due to the climate and environmental conditions some ancient characteristics have been maintained despite human interventions in breeding (Amaltheia 2013a). In 1994 a herdbook was established and the number of horses is on slight rise due to conservation efforts. Nowadays, they are mainly used as riding horses (Kugler 2010). Their average height is 1.32 to 1.42 m and the predominant colours are grey and brown. The DAD-IS of the FAO lists this breed as critical maintained (DAD-IS 2020).

Fig. 4 depicts a Creta Pony.

Fig. 4: Creta Pony.

https://en.wikipedia.org/wiki/Messara_horse (access 01.11.2020)

1.2.3.3. Lesvos Pony

The Lesvos Pony (see Fig. 5) is called Midili by local people. In the 19th century, a small horse resembling the Rodos Pony was reported to live on the island. This horse vanished during the two World Wars (Kugler 2010). The Aegean small bay horse which migrated to the Balkan Peninsula from either Central Anatolia or Asia Minor could be a common ancestor of the horses on Rhodes, Skyros and Lesvos (Kugler 2010). Due to the crossbreeding with Thoroughbreds, these horses are now of a larger size and no standard exterior can be defined. The breed is not officially recognised and a breeding association has not been implemented. Furthermore, no research has been conducted concerning the breed's origin. At the moment, around 300 ponies live on the island and the breed is on the verge of extinction (Kugler 2010).

Fig. 5: Lesvos Pony.

http://www.arca-net.info/admin/breedatlas/BA_suche.asp (access 30.10.2020)

1.2.3.4. Peneia Pony

The Peneia or Pinia Pony evolved in the region of Eleia (western Peloponnesus) with its typical semi-mountainous scenery. Peneia Ponies are heavier than other indigenous breeds in Greece. The predominant colours are grey, brown, roan and occasionally bay. Their withers height varies greatly from 1.24 to 1.42 m. They are mostly used as pack animals, for riding and farm work (see Fig. 6). Moreover, they are often trained in a gait called “aravani” which is a kind of a pace, in order to make the ride easier. They are also crossed with Thoroughbreds and the hybrids are very tough and useful horses (Hendricks 2007). The DAD-IS of the FAO lists the Peneia Pony as an endangered maintained breed. The size of the population is on a fall. In 2017 around 140 animals belonged to this breed, in 2019 only 70 horses were left (DAD-IS 2020).

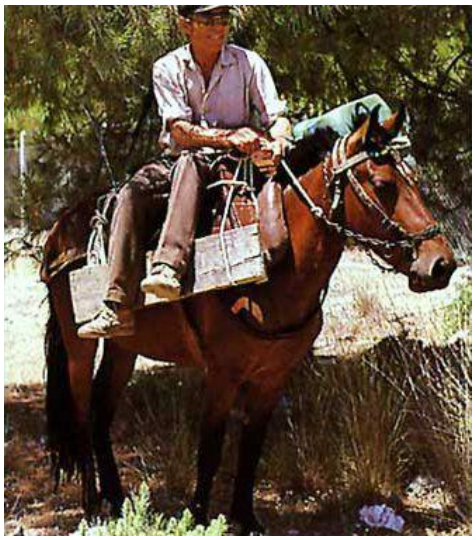


Fig. 6 Peneia Pony.

https://www.horsebreedspictures.com/wp-content/uploads/2016/09/Peneia-Pony-Horse.jpg (access 30.10.2020)

1.2.3.5. Pindos Pony

The Pindos Pony is also known as Samotraki Pindos (see Fig. 7). This breed can be found in the Pindus mountains in Epirus and Thessaly. The origin of this breed is uncertain, but its exterior implies a strong influence of oriental breeds. The average height is 1.14 to 1.23 m. The ponies are predominantly bay, brown or occasionally grey but never skewbald or piebald. Pindos ponies have a good stamina and they are used as pack animals, for riding and light draft (Hendricks 2007). The FAO lists the Pindos Pony as endangered-maintained. In 2019 the population of this breed was around 5200 horses (DAD-IS 2020).

Fig. 7: Pindos Pony.

https://alchetron.com/Pindos-Pony (access 30.10.2020)

1.2.3.6. Rodos Small Horse

In 1920 and 1930 Italian agronomists described this breed. Until the 20th century there was a similar breed on the island of Lesvos, but it disappeared. The common ancestor of the horses on the islands of Rhodes, Skyros and Lesvos could be a Aegean small bay horse that immigrated from Asia Minor or Central Anatolia (Amaltheia 2013b). The Rodos Small Horse plays an important role in the cultural heritage of Rhodes. But, for a long period of time the Rodos Small Horse was not within human care. The horses roamed the island freely and no efforts were made to preserve this breed. Hence, the numbers declined massively. The last horses survived in the area of the Arachangelos mountains on the island of Rhodes. In 2001, the Faethon Foundation was founded to preserve this breed. A genealogical studbook was created (Amaltheia 2013b). In 2007 merely eight mares and three stallions were left. The number of horses is not increasing but aging horses can be replaced by young ones. Their average height is 0.80 to 1.15 m (Kugler 2010).

Fig. 8 shows a Rodos Small Horse.

Fig. 8: Rodos Small Horse.

https://www.rhodesguide.com/magazine/article/Rodos-Pony-Rhodes-Small-Horses/ (access 31.10.2020)

1.2.3.7. Skyros Pony

Multiple breeds which developed between the 30° and the 40° latitude show a strong resemblance to miniature horses. These breeds include the Carpathian, the Sardinian Pony, the Skyros Pony, the Caspian, the Turkish Mytilene and the Chinese Guoxia (Hendricks 2007). The exact origin of Skyros Pony is not known but it is considered that the common ancestor of the Rodos Small Horse, the Lesvos Pony and the Skyros Pony is the Aegean small bay horse. The island of Skyros is situated in the Aegean Sea and this breed developed in the southeastern parts of the island. The landscape is stony and mountainous and they live there as a semi-wild population (Laliotis and Avdi 2017). Around 100 ponies are still on the island. Their average height is 0.93 to 1.05 m. The predominant colour is bay but grey, brown and seldomly dun can also occur (Hendricks 2007). This breed is well studied and three types of horses are defined. Type A is the purest, Type B has faced an influence of crossbreeding and Type C is larger because of excessive breeding with other breeds (Kugler 2010).

Fig. 9 depicts a Skyros Pony.

Fig. 9: Skyros Pony.

https://www.greece-is.com/giving-skyros-endangered-ponies-a-future/ (access 31.10.2020)

1.2.3.8. Thessalian Pony

The Thessalian Pony is also known as Thessaly Horse. For a long period of time, this breed was thought to be extinct, but a small number of animals survived. This breed originated in northern Greece. BCE, these ponies were used in the cavalry. For instance, Alexander the Great's horse 'Bucephalus' was presumably a Thessalian pony. Alexander's father, Philipp, acquired many horses from the regions around Feragana for military use (Hendricks 2007). Therefore, the Thessalian descends from the Turkoman horse. Prior to the World Wars the breed was relatively small (1.34 m on average). After the war, the demand for larger horses was on the rise. Thus, the Greek authorities decided to cross Thessalian ponies with Anglo-Arabs, Arabs and Lipizzaners. The predominant colours are grey, chestnut, brown and bay (Hendricks 2007). Within the DAD-IS of the FAO this breed is listed as endangered-maintained. The latest data of the population shows a rise in Thessalian Ponies. In 2009 only 40 animals were left. Within ten years the number increased by thirty times (DAD-IS 2020).

Fig. 10 shows a Thessalian Pony.

Fig. 10: Thessalian Pony.

https://www.pinterest.com/pin/545357836107801089/ (access 31.10.2020)

1.2.4. The history of horse breeding in the Iranian and Turkmenistan border area

The ancient, original Turkmenian is regarded to be extinct, although the name is still used for horses bred in Turkmenistan and Persian Turkmenistan. Since 1000 BCE this ancient breed was known to be an excellent racehorse. Turkmenian horses were very popular in ancient times (Hendricks 2007). For example, they were used to improve indigenous Greek horse breeds which thereby gained a lighter appearance. After having been bred with horses from the Feragana valley, the endurance of these Greek breeds also improved (Hendricks 2007). The Roman Empire was conducive to spread Turkmenian horses all around known world. Modern descendants of these horses are the Akhal-Teke, Turkoman and the Iomud (Hendricks 2007). The influence of Turkmenian horses on European and Northern African breeds was enormous. The old Caspian and Turkmenian horses may have been the first hotblooded horses and also the ancestors of the Barb and Arab horse (Hendricks 2007). When the English Thoroughbred studbook was established, many Turkmenians were imported to the United Kingdom to improve the Thoroughbred. The pedigree of one of the three founder stallions of the English Thoroughbred, Darley Arabian, was also heavily influenced by the Turkmenian horse. Also, it is believed that Byerley Turk, another founder stallion of the Thoroughbred, was a Turkmenian (Hendricks 2007).

1.2.5. Iranian horse breeds

The three most important horse breeds in Iran are the Caspian Miniature Horse, the Kurd Horse and the Turkoman (Hosseini et al. 2016). Their regions of origin are shown in Fig. 11.

Fig. 11: Regions of origin of Iranian horse breeds on a physical map of Iran.

The different regions are marked with arrows in diverse colours. The black arrows show the region of origin of the Caspian Miniature Horse. The red arrows show the region where the origin of the Kurd Horse is. The arrow in an orange colour shows the region where the Turkoman has its origin.

https://www.freeworldmaps.net/asia/iran/map.html (access 02.11.2020)

1.2.5.1. Caspian Miniature Horse

The Caspian Miniature Horse was thought to be extinct for over one thousand years. In 1965, it was rediscovered by Louise Firouz around the Caspian Sea. In the same year, a study was initiated to determine the number of horses, the nature and history of this breed. Their height was 1.04-1.24 m. Despite good environmental conditions the horses became smaller with an average height around 0.91 m (Hendricks 2007). Further breeding should lead to reversion to the original size. The Caspian is characterized by its ability to jump and has very elegant paces (Hendricks 2007). The Caspian Miniature Horse could be one of the last descendants of the wild living Oriental horses and one of the forefathers of modern-days hotblooded horses. It is thus of great importance to determine whether this breed has been continuously bred or not (Hendricks 2007). Nowadays, the Caspian Miniature Horse is bred all around the world.

Especially in the United Kingdom, the United States of America, Australia and New Zealand. There is no danger of extinction for this breed, but they remain rare (Hendricks 2007). In its country of origin, the Iran, the Caspian Miniature Horse is close to extinction. Therefore, projects to conserve this breed have been started (Amoli et al. 2017).

Fig. 12 depicts a Caspian Miniature Horse.

Fig. 12: Caspian Miniature Horse.

https://www.pinterest.com/pin/658370039247327705/ (access 03.11.2020)

1.2.5.2. Kurd Horse

The Kurd Horse (see Fig. 13) is mainly found in the province of Kurdistan in the north-western part of Iran. The origin of this breed can be retraced to 600 BCE (Fotovati 2000). The latest published number of horses in Iran was around 2700 in 2004. The breed is grouped in three families: Afshari, Jaaf and Sajabi. They are adapted to the hilly regions and cumbersome roads of Kurdistan. This breed is mostly used for horse polo (chogan) and dressage (Amjadi et al. 2017).

Fig. 13: Kurd Horse

https://betterkurdistan.wordpress.com/2013/03/31/kurdish-horse/ (access 27.10.2020)

1.2.5.3. Turkoman

This breed is also known as Turkmenian, Turk, Turkmene and Turkmen. As mentioned before, the ancient pure-bred horse is thought to be extinct today but the horses raised in Persian Turkmenistan are still called Turkoman (Hendricks 2007). The Turkoman's origin lies in the open steppe of the Iran. These horses belong to the Oriental breeds. They were raised by the tribe of Turkomans in big herds of mares and stallions which used to graze on pastures throughout the year. Only animals with an approved record were allowed for breeding (Hendricks 2007). They must show their ability for long distance travelling and racing. Beginning in the 1960s Thoroughbreds were brought to the Iran for racing and Turkomans were crossbred with those to improve speed. But those attempts were not successful. Today, Turkoman horses' pasture around the Atrek River (Hendricks 2007). The predominant colours are chestnut, black, grey, brown and bay. There average height is 1.52 to 1.63 m. They are used as riding horses and their appearance reflects their speed and endurance (see Fig. 14) (Hendricks 2007).

Fig. 14: Turkoman

https://ifpnews.com/the-turkoman-horse (access 03.11.2020)

1.2.6. Turkmenistan's horse breeds

1.2.6.1. Akhal-Teke

Akhal-Tekes originated around the eight century CE from the ancient Turkmenian horses which were considered to be the best horses in Central Asia. The Akhal-Teke's origin lies in the Kopet Mountains promontory and in the oases of Akhal and Tejen southeast of the Kara Kum desert. These regions are on the border between Iran and Turkmenistan (see Fig. 15) (Hendricks 2007). Akhal means pure and Teke is the name of the tribe that bred those horses for centuries. The Tekes were nomadic people and it was important for the horses to cover long distances without fatigue. To be deemed breedable, the animals had to prove that they were the best in racing and would impart their qualities to their offspring (Hendricks 2007).

Fig. 15: Physical map of Turkmenistan.

The Akhal and Tejen oases are mapped in green. They are the origin of the breed Akhal-Teke.

http://www.akhaltekesaustralia.cm.au/origins.html (access 31.10.2020)

Breeds in Iran, Afghanistan, Iraq and Turkey often descendent directly from the Akhal-Teke and its ancient forefathers. Thanks to famous stallions like Penteli, Muar and Absent the Akhal-Teke is often present in modern warmblood pedigrees. The look of Akhal-Tekes is unique. The colour of this breed is often a conspicuous golden dun (see Fig. 16). The horses also frequently shimmer metallic. Other colours are black, bay and grey. Akhal-Tekes often have white markings on legs and the face (Hendricks 2007).

When Turkmenistan joined Russia, this breed was not needed anymore. As the feuds between the tribes had been settled, the raising of the horses was considered to be too expensive. Consequently, the Akhal-Tekes were sold to other countries like India, Afghanistan, Turkey or Iran. They were replaced with cheaper working horses. In 1988, the Akhal-Tekes were near extinction. Feral herds were founded in Turkmenistan and one year later the number of horses started increasing again (Hendricks 2007).

Another problem was that purebred Akhal-Tekes were bred with Thoroughbreds to make them faster. The quality of the descendants could not reach the high standards. In 1973, only three pure stallions and eighteen purebred mares were left in Russia because of the extensive

crossbreeding. A few breeders realised the seriousness of the situation and started breeding only with purebred animals. Nowadays, it is important to sustain a healthy gene pool (Hendricks 2007).

Fig. 16: Akhal-Teke

https://en.wikipedia.org/wiki/Akhal-Teke#/media/File:Dagat-Geli.jpg (access 01.11.2020)

1.3. The Y chromosome and its inheritance

1.3.1. Characteristics of the Y chromosome and its use for studying paternal lines

Y chromosomes developed approximately 180 million years ago when it attained a locus determining for the male sex. The size of the Y chromosome in horses is approximately 40-50 million basepairs (bp). Hence, it is one of the smallest chromosomes of the genome. Two thirds thereof are heterochromatic, only the distal third of the segment is euchromatic (Janečka et al. 2018). The euchromatic sequence contains approximately 15 megabases (Mb) (Raudsepp et al. 2004). It is not possible for most parts of the equine Y chromosome to recombine with the X chromosome during meiosis (Janečka et al. 2018). The pseudoautosomal region (PAR) is the only part of the Y chromosome that recombines with the X chromosome (Paria et al. 2011). The equine PAR connects directly to the euchromatic region (Janečka et al. 2018). Nevertheless, most of the Y chromosome is haploid, male specific (MSY) and is not involved in the process of recombination (Janečka et al. 2018). According to Jobling and Tyler-Smith (1995) the MSY region of the Y chromosome is also called non-recombining region (NRY). Consequently, the NRY is directly passed on to male offspring of a stallion. Therefore, the whole NRY of an individual descends from a single male ancestor from whom it was passed down the following generations. Similarly, the mitochondrial DNA (mtDNA) is passed on from the mother to the descendants (Jobling and Tyler-Smith 1995). Fig. 17 shows

the inheritance of mtDNA and autosomal, Y chromosomal sequences. As no recombination takes place, the only way to generate new variation on the NRY in a population are spontaneous mutations that arise in the germline (Jobling et al. 1996). Those mutations can be insertions and deletions (INDELs), single nucleotide polymorphisms (SNPs) and copy number variants (CNVs) (Doan et al. 2012). The mutations, that accumulate over the years, serve as Y chromosomal markers and due to the lack of recombination the allelic states at multiple markers can be straightforwardly combined into haplotypes (HTs). Y chromosomal HTs can then be used to trace paternal lineages and reveal genetic history in horses as well as humans and other mammals.

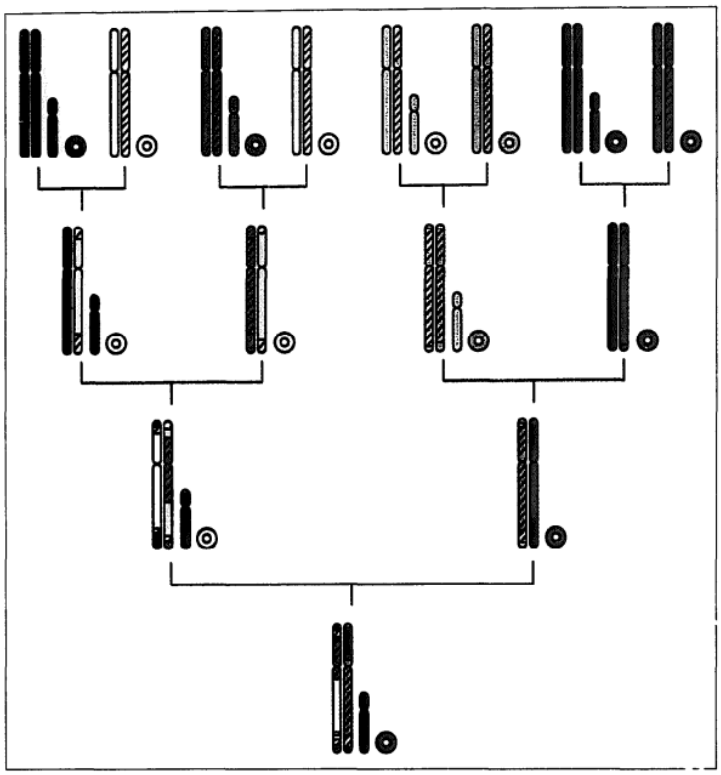


Fig. 17: Inheritance of mtDNA and autosomal, Y chromsomal sequences (resumed from Jobling MA and Tyler-Smith C 1995).

Y chromosomes (small chromosome) and mtDNA (circle) sequences do not recombine in each generation in contrast to autosomal sequences (large chromosomes).

1.3.2. The NRY in horses

A lack of diversity is observed when comparing the NRY of horses (Lindgren et al. 2004). This is probably the consequence of a small effective population size of males, which means that a small number of males could effectively reproduce. Several aspects of the reduction in effective population size in horses were studied. Due to their mating habits in the wild, only a few males are able to pass their genetic information on to the offspring. Feral horses generally live in groups which are socially stable (Lindgren et al. 2004, Keiper 1986, Warmuth et al. 2012). Normally, these groups include one stallion, a few mares and the offspring up to an age of two years. Within this social group the stallion fathers all the descendants (Keiper 1986). Furthermore, it is assumed that several bottlenecks occurred in the early stages of domestication (Lindgren et al. 2004, Warmuth et al. 2012). Another aspect which has to be considered is that during the process of domestication few male horses had genetic influence on the domestic horse (Lindgren et al. 2004). Since the establishment of breeds, around 400 years ago, male horses are mostly only admitted for breeding when they fulfil defined criteria. Also, breeders have a big influence on which sires they use for their mares to get the best offspring.

1.4. Standard of knowledge of genetic characterisation of paternal lineages with Y chromosomal markers

In order to trace back the population's genetic development of paternal lines, polymorphic Y chromosomal markers located on the NYR have already been established for other species such as humans (Underhill and Kivisild 2007).

Due to the low sequence diversity on the horse Y chromosome, early studies have not been able to find suitable markers for differentiating and tracing stallion lines (Wallner et al. 2003, Wallner et al. 2004, Lindgren et al. 2004, Wallner et al. 2013).

In 2013, Wallner et al. succeeded for the first time in describing a few polymorphic markers on the NRY. They used Next Generation Sequencing (NGS) technologies to screen for such markers. Around 180 kilobases (kb) of the Y chromosome were screened in several breeds like Welsh-A and -B, Standardbred, several Warmblood and Thoroughbred and one Przewalski Horse. It was possible for them to determine six different HTs within domesticated horses and two HTs for the Przewalski Horse. It was discovered domestic horses' NRYs are genetically different from the Przewalski horse. Furthermore, it was also possible to determine

the great influence of the English Thoroughbred on modern-day breeds (see Fig. 18) (Wallner et al. 2013).

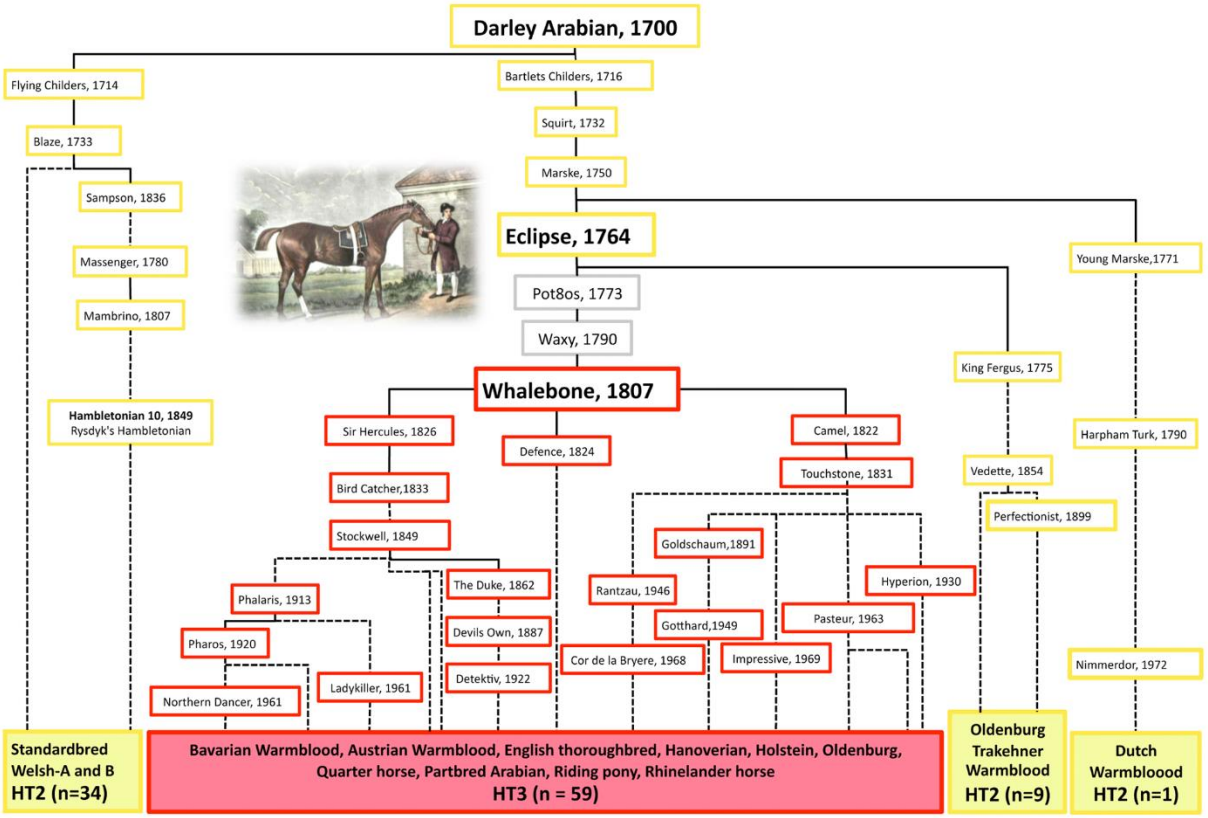


Fig. 18: Influence of the English Thoroughbred on modern-day's breeds (image from Wallner et al. 2013).

In 2017, Wallner et al. published the first genealogy of the Y chromosome. 1.46 Mb of the MSY were scanned in 52 horses of 21 different breeds. They determined three INDELs and 49 single nucleotide variants. In conjunction with four previously published MSY variants (Wallner et al. 2013) it was possible to determine 24 HTs (Wallner et al. 2017). The extremely low diversity of sequences on the Y chromosome was further confirmed by these findings (Wallner et al. 2017). In accordance with these results it was possible to establish a phylogenetic tree with the donkey and the Prezwalski horse as two outgroups. Two private Northern European HTs cause an incisive split in the MSY lineage and form two independent branches: I (Icelandic Horse) and N (Norwegian Fjord Horse and Shetlandpony). It was possible to group all other HTs within one haplogroup (HG) (Crown Group) which developed around 700 years ago. The predominance of the Crown HG was attributed to Oriental horses that were imported to Europe (Wallner et al. 2017). Inside the Crown Group four HGs branched off: A, L, S and T. HG A

includes the breeds Trakehner, Arabian, Connemara Pony and South German Draft Horse. Within HG A the HTs Ao (Arabian, Trakehner), Ad-h (South German Draft Horse) and Ad-b (Connemara Pony) could be determined. Iberian influenced horses belong to HG L and Sorraia horses to HG S. Two thirds of the samples tested were grouped inside HG T. HT Tb was widely spread by the English Thoroughbred. Moreover, HT Tb-d is carried by descendants of Darley Arabian. Offspring of Whalbone spread HT Tb-dW1 which developed out of Tb-d (Wallner et al. 2017). The dominance of the Crown Group in breeds nowadays can be attributed to the use of Oriental horses as a refiner for European breeds. These Oriental lineages were mainly HT Tb and HT Ao. The origin of HT Tb is the Turkoman and HT Ao is conveyed from "Original Arabians" (Wallner et al. 2017). Fig. 19 shows the phylogenetic tree established by Wallner et al. (2017).

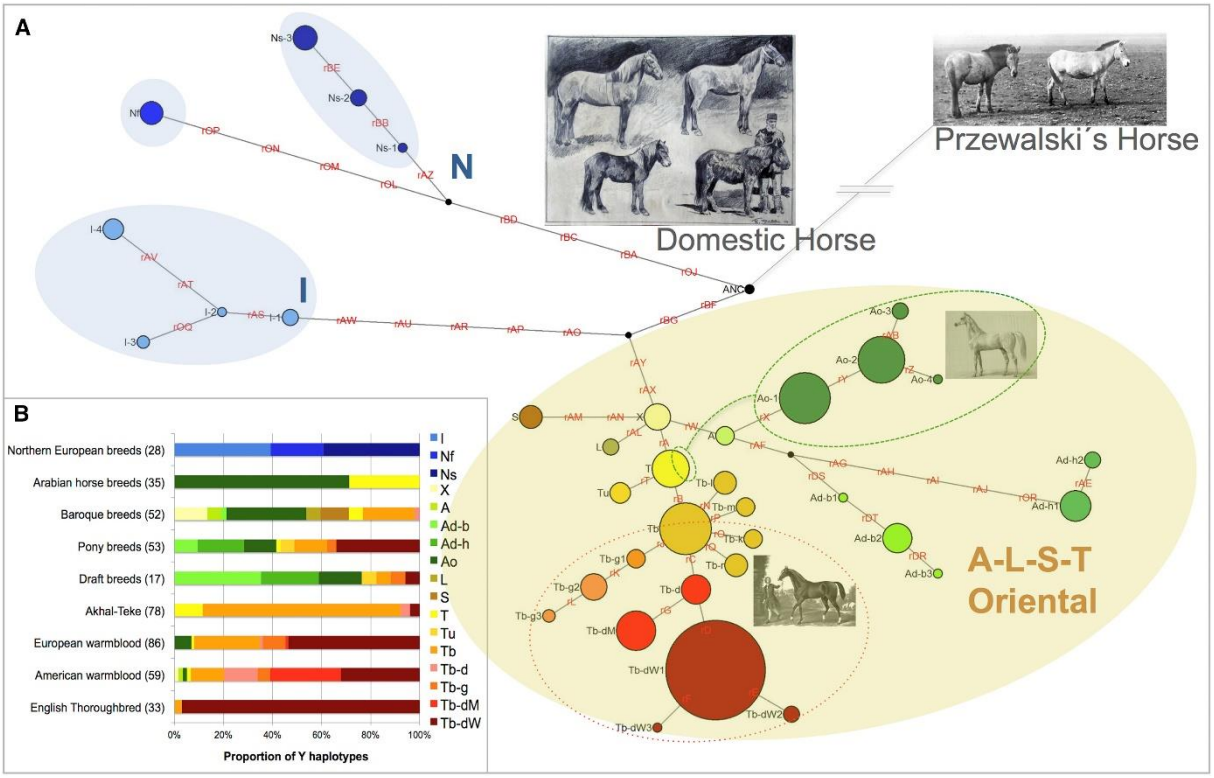


Fig. 19: Phylogenetic tree (figure from Wallner et al. 2017).

A: Network of HTs of 52 domestic horses analysed by Wallner et al. 2017. The different HTs are mapped as circles. The diameter of the circles represents the number of samples per HT. On the lines between the HTs one can see the HT determining markers in red. The Crown Group HTs (A-L-S-T) are highlighted in yellow, red and green. The green dotted line frames

the HTs which are influenced by Arabian Horses. The HTs effected by Thoroughbreds are marked by a red dotted line. Northern European breeds (N, I) are outside the Crown Group.

B: The relative frequency of different HTs by breed and group of breeds is given. In parentheses the number of samples per group of breeds is shown.

Felkel et al. (2018) extended the dataset of the previous MSY studies and tested American, Asian and European horses. The Asian animals broaden the MSY phylogeny of horses because they showed a broader variety of HTs than American or European breeds. Although most of the Asian samples were again part of the Crown, with three new HTs (C, Ta, Ao-m) inside the Crown (Felkel et al. 2018), some Asian horses classified outside the Crown and represented the HTs J, M, O, Y which had not been described before. HT J was found in the Korean Jeju Pony and HT M could be detected in the Mongolian Horse. HT O is a HT which branched off from all other HGs a long time before domestication (Felkel et al. 2018). It was detected in horses from Mongolia, Vietnam and Myanmar (Felkel et al. 2018, Chilla 2019). Other than a private HT Y, the Yakutian horse also carried HT O and a HT from the Crown Group (Ad-h2). Fig. 20 depicts the phylogenetic tree established by Felkel et al. (2018).

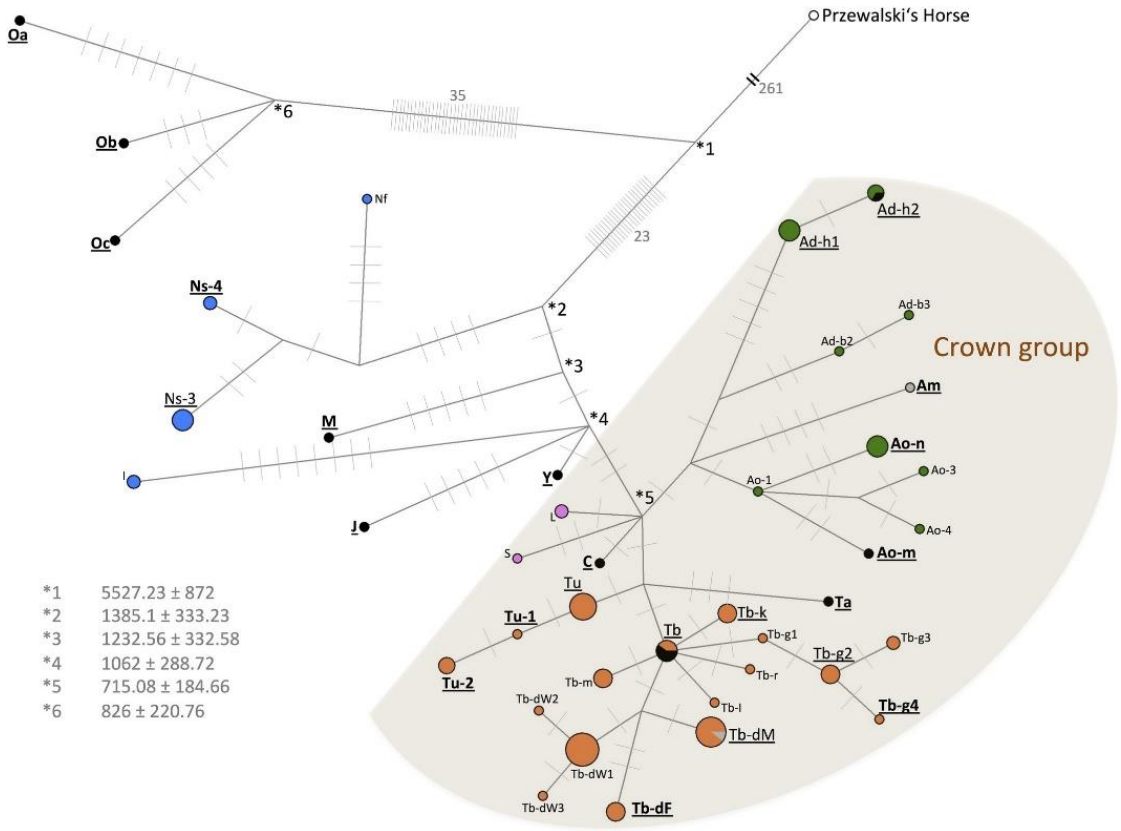


Fig. 20: Phylogenetic tree (image from Felkel et al. 2018).

HT network established after the analysis of 104 male domestic horses and 1 Przewalski horse (Felkel et al. 2018, Wallner et al. 2017). The Crown Group (ALST) defined in Wallner et al. (2017) is highlighted in grey. The bold HTs were defined in Felkel et al. (2018) and the underlined HTs could be found within in the analysis of the dataset (Felkel et al. 2018). Mutations between the HTs are shown in hatch marks along the branches. The numbers next to the branches show the number of mutations.

The dating of the nodes was calculated with an assumption of a generation time of seven years. The nodes are marked with numbers and asterisks. Also, the standard deviation was calculated.

American samples are mapped in grey, Asian specimens in black and Northern European in blue. HG A is depicted in green, the orange colour marks HG T and pink is specific for HGs S and L.

In a recent study, published by Felkel et al. (2019), 5.8 Mb of the MSY were screened. It was achieved to divide the Crown Group in three HGs A, H and T. Within HG H the previously

described HGs L and S (Wallner et al. 2017) were united. Additionally, HG C which was detected by Felkel et al. (2018) was grouped inside HG H (Felkel et al. 2019). The most recent common ancestor of the Crown Group was dated to around 500 CE. According to this work, HT O branched off from the remaining lineages around 13000 years ago (Felkel et al. 2019).

1.5. The aim of this project

The diversity of MSY HTs in American and European horses was diminished because of a strong male-biased selection in breeding. For this thesis Y chromosomal HTs spectra in horses from Armenia, Georgia, Turkmenistan, Iran, the Greek mainland (Peloponnese, Thessaly, Elis) and some Greek islands (Creta, Rhodes, Lesvos, Skyros) will be screened by genotyping key variants published in Felkel et al. (2019). 153 samples, provided by Dr. Nikos Kostaras, Prof. Gus Cothran and Dr. Rytis Juras will be analysed and the MSY pattern of these remote horse populations will be compared to the one in transboundary breeds. The autochthonous breeds in the Caucasus Region, in Central and Western Asia and on the Greek mainland and islands will not be as heavily affected by those breeding strategies. We therefore expect that Greek, Central and Western Asian horse populations have a broader HT spectrum than intensively selected breeds. The aim of this study is to shed light on the origin and breeding history of these remote horse populations.

This thesis was authorised by the Ethical Board of the University of Veterinary Medicine of Vienna. The identity of the horses analysed will not be revealed.

2. Material and Methods

2.1. Material

In Tab. 2, Tab. 3, Tab. 4 and Tab. 5 the used reagents, devices, materials, programs and software are shown.

2.1.1. Reagents

Tab. 2: Reagents used

Chemical	**Producer**
2x KASP® Master Mix	LGC Group, Great Britain
KASP® Assay Mix	LGC Group, Great Britain
PCR-Primer	Sigma-Aldrich, USA
10x buffer	Sigma-Aldrich, USA
Deoxynucleotide (dNTP) Mix	Fermentas, Latvia
Magnesium Chloride (MgCl2)	Solisbiodyne, Estonia
DNA polymerase I Thermus aquaticus (Taq, Biotaq-Polymerase)	Agrobiogen, Germany
Agarose powder	VWR International, USA
Atlas ClearSight DNA Stain	Bioatlas, Estonia
Loading buffer: 30 % Glycerin BromPhenolBlau	 Roth, Austria Sigma-Aldrich, USA
TRIS-Borat-EDTA-buffer (TBE-buffer)	Roth, Austria
TRIS-EDTA (TE)	Roth, Austria
Kb ladder	Fermentas, Latvia
DNA isolated from blood and hair cells	Various sources
QX Alignment Marker 15bp/1kb	QIAgen, Germany
QX Alignment Marker 15bp/3kb	QIAgen, Germany
QX DNA Size Marker 25–500bp (50 ul) v2.0	QIAgen, Germany
QX DNA Size Marker FX174/HaeIII (50 ul)	QIAgen, Germany
ΦX174 DNA-Hae III Digest	New England Biolabs® Inc., USA
DNA Dilution buffer	QIAgen, Germany

2.1.2. Devices

Tab. 3: Devices used

Device	Producer
Vortexer (MS 2 Minishaker®)	IKA®, Germany
Plate spinner (Labnet MPS 1000 Mini plate spinner ®)	Sigma-Aldrich, Austria
Centrifuge	Eppendorf, Austria
Electronic pipette	Eppendorf, Austria
Pipettes	Gilson®, USA
CFX Real-Time System C1000 Touch®	Bio-Rad, USA
SimpliAMP™ Thermocycler	Applied Biosystems™, USA
Electrophoresis chamber	Peqlab, USA
Molecular Imager® Gel Doc™ XR+	Bio-Rad, USA
QIAxcel® Advanced System	QIAgen, Germany

2.1.3. Used Materials

Tab. 4: Materials used

Material	Producer
Eppendorf Tubes	Eppendorf, Austria
96 well plate white	Bio-Rad, USA
Eppendorf Fast PCR Tube Strips	Eppendorf, Austria
Microseal® B Adhesive Optical Sealer	Biozym, Germany
Pipette Filter Tips 20, 200, 1000 µl	Greiner Bio One, Austria
Pipette Filter Tips 10, 20 µl, yellow	Sarstedt, Germany

2.1.4. Programs and Software

Tab. 5: Programs and Software used

Software	Producer
Excel®	Microsoft Office®, USA
Bio-Rad CFX Manager 3.1®	Bio-Rad, USA

Doc XR + Gel Documentation System	Bio-Rad, USA
ABI 3130xl Genetic Analyzer	ThermoFisher Scientific, USA
QIAxcel® ScreenGel Software	QIAgen, Germany

2.1.5. Used samples

36 hair samples from male individuals used in this thesis were provided by Dr. Nikos Kostaras from Amaltheia, Greece. A technician of the Institute of Animal Breeding and Genetics of the University of Veterinary Medicine Vienna isolated the DNA from hair samples using Nexttec™ DNA Isolation Kit. Genomic DNA was provided from 117 male horses by Prof. Gus Cothran and Dr. Rytis Juras from Texas A&M University as part of a scientific collaboration. Within this thesis, provided DNA material in a concentration of 5-10 ng/µl was analysed. Samples Y_PR_19_058 and Y_PR_065 contained <5 ng/µl. In total, DNA samples of 153 male horses from 14 different breeds were used. The sampling was arbitrary. The breeds analysed are indigenous and for most of them no pedigree information is available. Therefore, it cannot be guaranteed these animals are not related to each other. The sample set consists of ten Akhal-Tekes, 15 Andravida Horses, ten Armenian Horses, ten Caspian Miniature Horses, ten Creta Ponies, ten Kurd Horses, ten Lesvos Ponies, 13 Peneia Ponies, eight Pindos Ponies, nine Rodos Small Horses, 25 Skyros Ponies, three Thessalian Ponies, ten Turkoman and ten Tushuris (samples are listed in Tab. 7 and Fig. 23). The samples were collected in Armenia, Georgia, Greece, Iran and Turkmenistan (see Tab. 6 and Fig. 21 and 22). Tab. 8 and Fig. 24 show the distribution of the breeds within the countries.

Tab. 6: Origin of samples used

Origin of samples	**Number of samples**
Armenia	2
Greece	93
Georgia	18
Iran	30
Turkmenistan	10
In total	***153***

Fig. 21: Regions of origins.

The samples' countries of origin are marked blue and shown on the map.

https://map1.maploco.com/visited-countries/mine.php?c1=m9bt6kiao0-cjfmwie61e-hc6sffljie-b685yij7sw-4ckvwko7lw (access 19.07.2020)

Tab. 7: Horse breeds and number of samples per breed.

Breed	**Number of samples**
Akhal-Teke	10
Andravida Horse	15
Armenian Horse	10
Caspian Miniature Horse	10
Creta Pony	10
Kurd Horse	10
Lesvos Pony	10
Peneia Pony	13
Pindos Pony	8
Rodos Small Horse	9
Skyros Pony	25
Thessalian Pony	3
Turkoman	10
Tushuri	10
In total	***153***

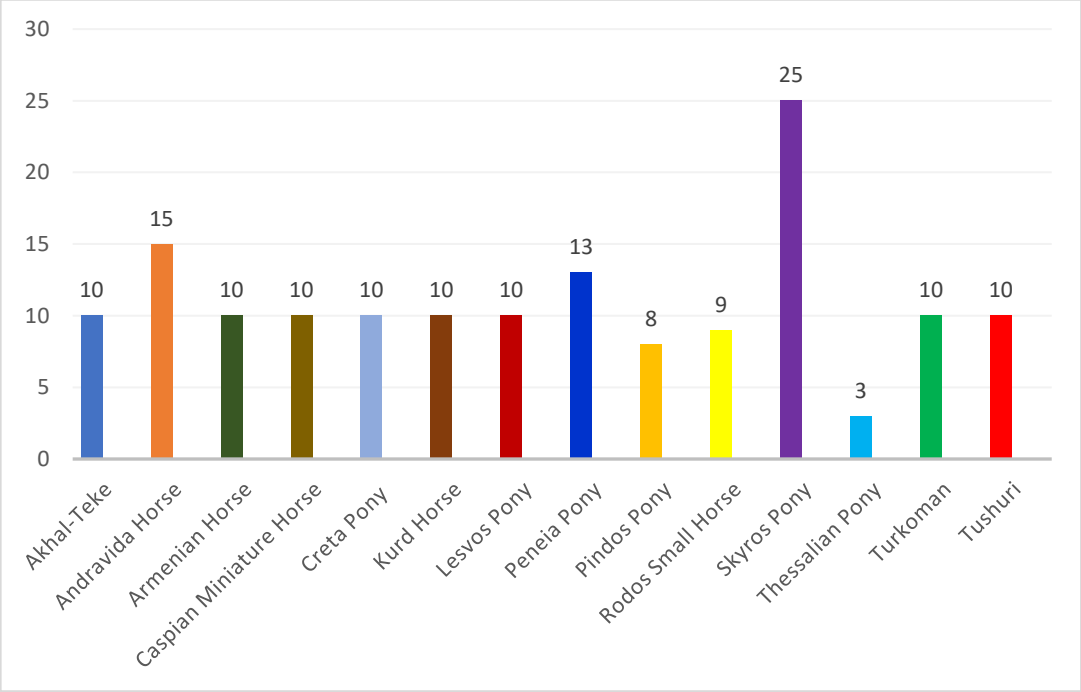


Fig. 22: Distribution of the breeds.

In this Fig. the number of DNA samples per breed is shown as a bar chart. The breeds are shown in different colours.

Tab. 8: Number of samples per breed and per country

Country	**Breed**	**Number**
Armenia	Armenian Horse	2
Georgia	Armenian Horse	8
	Tushuri	10
Greece	Andravida Horse	15
	Creta Pony	10
	Lesvos Pony	10
	Peneia Pony	13
	Rodos Small Horse	9
	Pindos Pony	8
	Skyros Pony	25
	Thessalian Pony	3
Iran	Kurd Horse	10

	Turkoman	10
	Caspian Miniature Horse	10
Turkmenistan	Akhal-Teke	10
In total		***153***

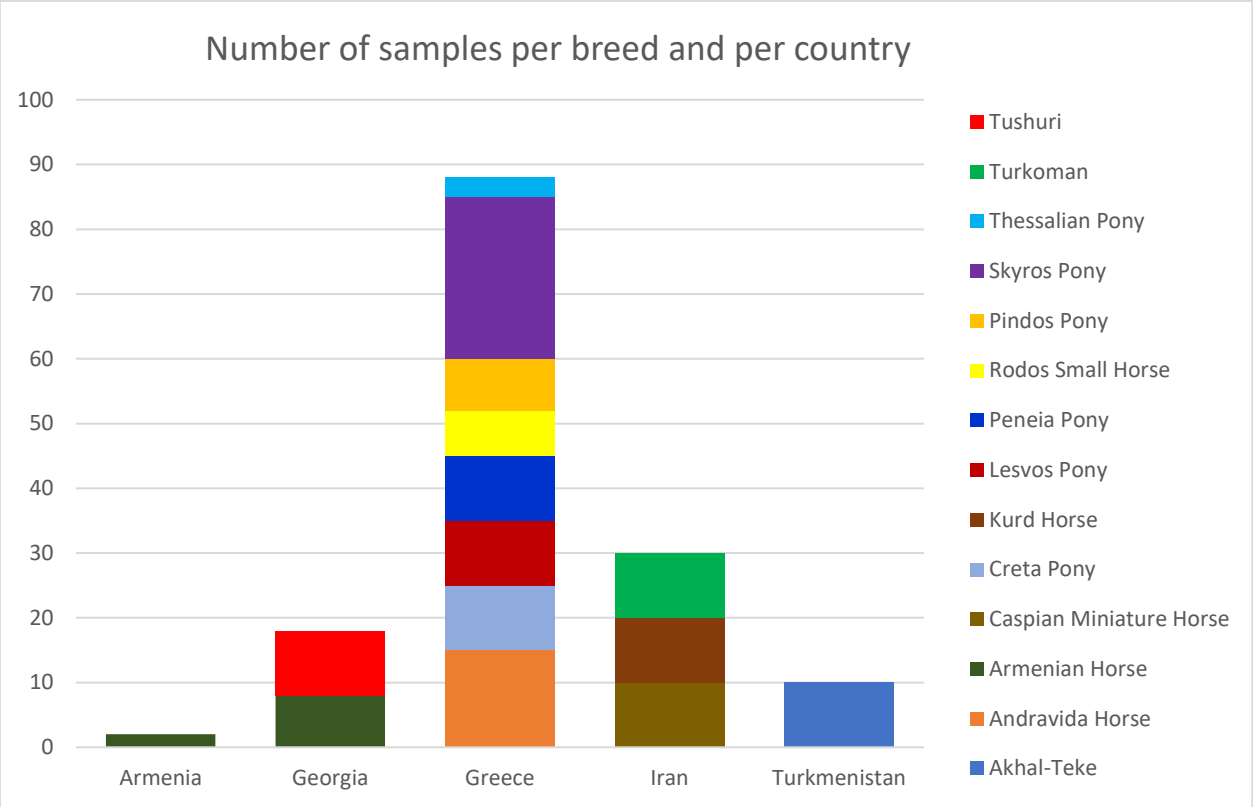


Fig. 23: Number of samples per breed and per country.

In this Fig. the number of samples per breed and per country is shown as a bar chart. Breeds are shown in different colours.

2.2. Methods

2.2.1. Quantitative Real-Time PCR

Quantitative Real-Time PCR (qPCR) has changed molecular biology and has delivered new possibilities to analyse samples (Gadkar and Filion 2014). The process of quantitative Real-Time PCR occurs in a closed system, as target DNA is amplified and the generated product can be measured throughout the amplification process by the emitted fluorescence signal of the dyes (Singh and Roy-Chowdhuri 2016). The fluorescence signal is proportional to the DNA

template available in the beginning (Kubista et al. 2006). Since their inception, qPCR technologies have improved significantly. Even though the basic principle has remained unchanged, modern, state-of-the-art qPCR systems are more affordable, faster and more sensitive than their predecessors (Gadkar and Filion 2014). Moreover, the results can be visualised immediately ("real-time") on a computer (Singh and Roy-Chowdhuri 2016). Within this thesis, competitive allele-specific PCR (KASP®) Technology is used which can be analysed with any qPCR instrument (LGC Group 2014).

2.2.2. KASP® Technology

KASP® technology is a genotyping technology which uses a competitive allele-specific PCR, concerted with a reporting system based on fluorescence to discover INDELs or SNPs (He et al. 2014). It can be analysed with any Real-Time PCR instrument (LGC Group 2014). For KASP® analysis, the 2x KASP® Master Mix and the KASP® Assay mix are needed (LGC Group 2014).

The Assay mix is specific for the variant to test and contains two different allele-specific forward primers which are competitive and one universal reverse primer (LGC Group 2013). Each forward primer has an inimitable tail sequence and these tail sequences play an important role in the process of signal generation because they match with a fluorescence resonant energy transfer (FRET) cassette (LGC Group 2014). One FRET cassette is labelled with hexachloro-fluorescein (HEX), the other with 6-Carboxyfluorescein (FAM) (LGC Group 2014).

The 2x KASP® Master Mix includes the FRET cassette, the Taq polymerase, $MgCl_2$, the buffer solution and nucleotides, which are needed for building DNA strands (LGC Group 2013). To perform KASP® analysis, the Assay mix and the 2x KASP® Master Mix are mixed with the DNA sample. Then a thermocycle protocol is started (LGC Group 2013). Initially, the Taq polymerase is activated at a temperature of 94 °C for 15 minutes. Subsequently, the DNA template is denaturated and at a temperature of 61 °C to 55 °C the allele-specific forward primer and the reverse primer bind to the corresponding allele (LGC Group 2013). Upon template elongation, the tail is added to the newly synthesised DNA strands. This process is repeated during the following PCR cycles and each time a sequence complement is generated (LGC Group 2013). Thus, the labelled dye is no longer quenched and a signal is emitted. The competitive binding of the allele-specific forward primers makes it possible to differentiate between two alleles. Samples homozygous at the locus tested, emit one fluorescent signal. If

the sample is heterozygous at the locus tested, both dyes will emit fluorescent signals and the induced signal is mixed (LGC Group 2013).

Fig. 25 shows the principle of KASP® technology.

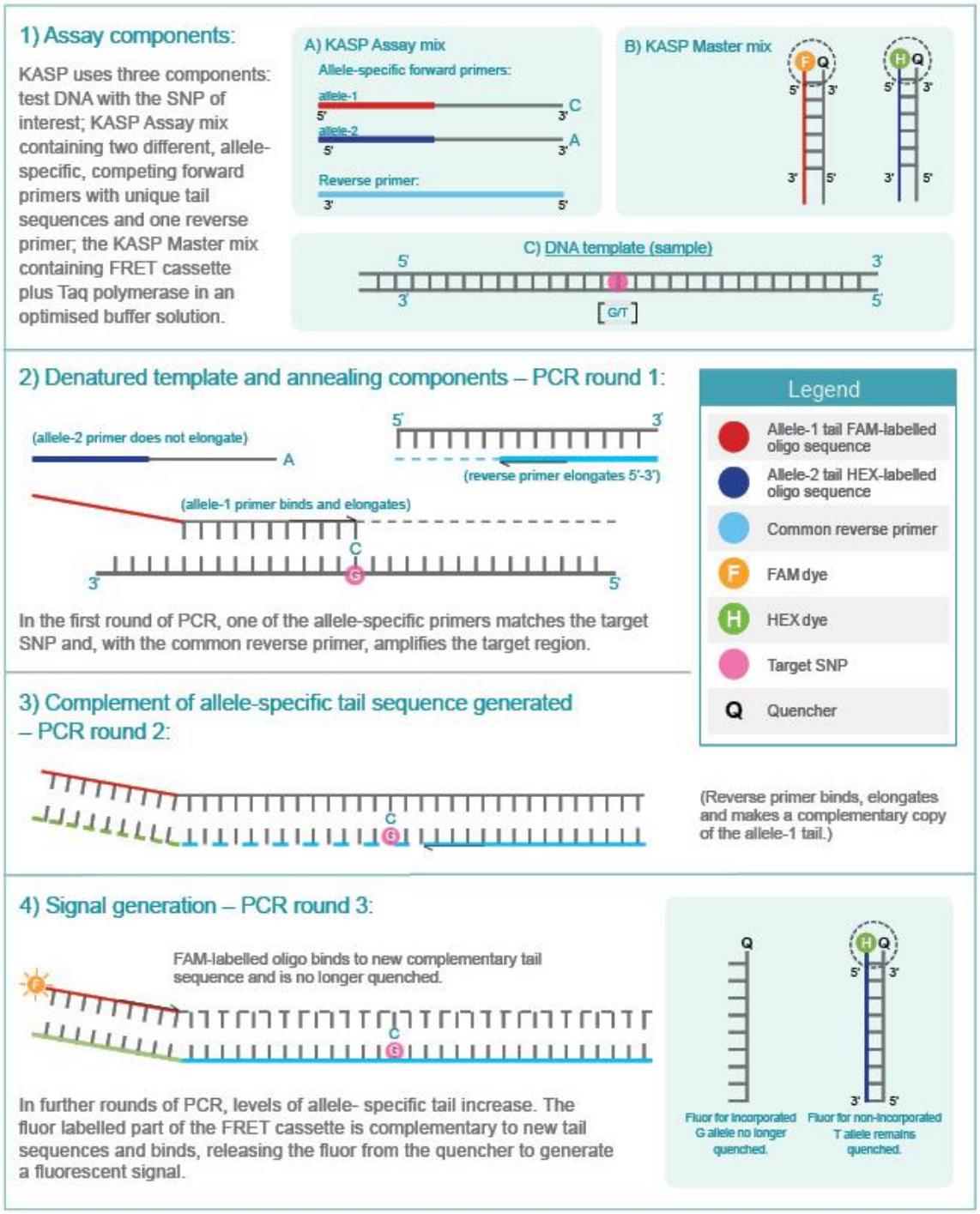


Fig. 24: Scheme of the mechanism of KASP® technology.

This Fig. explains schematically the mechanism of KASP® technology. It was taken over from LGC Group.

https://biosearch-cdn.azureedge.net/assetsv6/how-does-kasp-work.jpg (access 19.07.2020)

2.2.3. Steps necessary before starting a KASP® analysis run

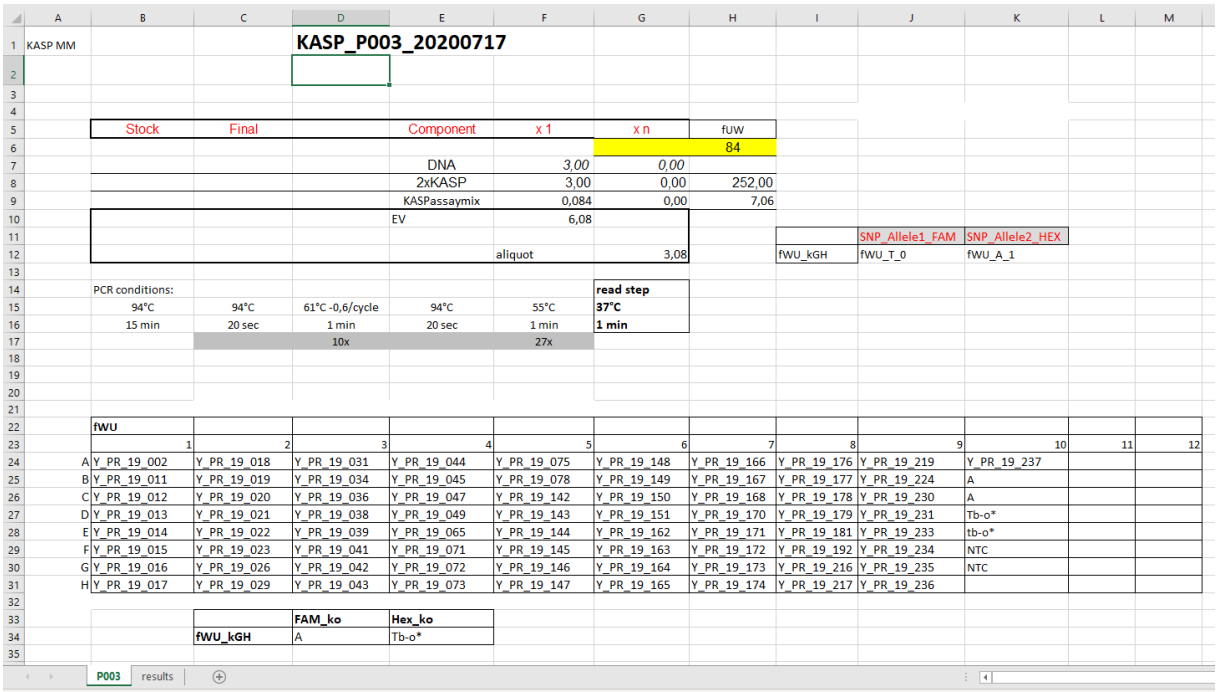
Before starting a KASP® analysis run, an EXCEL® file (established and provided by Mag. med. vet. Doris Rigler) was completed by choosing the specimens and positive controls (an example is given in Fig. 26). Also, the volumes of 2x KASP® and KASP® Assay mix for preparing the Master Mix were calculated with this sheet. For each run four positive controls, namely genomic DNA from male horses with known allelic status (two for each dye - FAM and HEX), and two non-template controls (negative controls or NTC) were analysed together with the samples of unknown allelic status. This procedure was performed for 44 different assays.

2.2.4. Performing MSY genotyping

The samples chosen before were sorted on a rack. In the PCR Master Mix laboratory, the 2x KASP® and KASP® Assay mix were pipetted into a 1.5 ml Eppendorf tube, vortexed and centrifuged. Following, 3 µl of the assay specific Master Mix were pipetted into the wells of a 96 well plate needed for the qPCR. In another laboratory 3 µl DNA were added to each Master Mix well. Tab. 9 shows the components in each well.

Tab. 9: KASP® components in each well

Components	**Amount (µl)**
DNA	3.00
2x KASP®	3.00
KASP® Assay mix	0.084
Final volume	***6.08***



KASP MM

KASP_P003_20200717

Stock	Final	Component	x 1	x n	fUW
					84
		DNA	*3,00*	*0,00*	
		2xKASP	3,00	0,00	252,00
		KASPassaymix	0,084	0,00	7,06
		EV	6,08		
			aliquot	3,08	

	SNP_Allele1_FAM	SNP_Allele2_HEX
fWU_kGH	fWU_T_0	fWU_A_1

PCR conditions:					**read step**
94°C	94°C	61°C -0,6/cycle	94°C	55°C	**37°C**
15 min	20 sec	1 min	20 sec	1 min	**1 min**
		10x		27x	

fWU

	1	2	3	4	5	6	7	8	9	10	11	12
A	Y_PR_19_002	Y_PR_19_018	Y_PR_19_031	Y_PR_19_044	Y_PR_19_075	Y_PR_19_148	Y_PR_19_166	Y_PR_19_176	Y_PR_19_219	Y_PR_19_237		
B	Y_PR_19_011	Y_PR_19_019	Y_PR_19_034	Y_PR_19_045	Y_PR_19_078	Y_PR_19_149	Y_PR_19_167	Y_PR_19_177	Y_PR_19_224	A		
C	Y_PR_19_012	Y_PR_19_020	Y_PR_19_036	Y_PR_19_047	Y_PR_19_142	Y_PR_19_150	Y_PR_19_168	Y_PR_19_178	Y_PR_19_230	A		
D	Y_PR_19_013	Y_PR_19_021	Y_PR_19_038	Y_PR_19_049	Y_PR_19_143	Y_PR_19_151	Y_PR_19_170	Y_PR_19_179	Y_PR_19_231	Tb-o*		
E	Y_PR_19_014	Y_PR_19_022	Y_PR_19_039	Y_PR_19_065	Y_PR_19_144	Y_PR_19_162	Y_PR_19_171	Y_PR_19_181	Y_PR_19_233	tb-o*		
F	Y_PR_19_015	Y_PR_19_023	Y_PR_19_041	Y_PR_19_071	Y_PR_19_145	Y_PR_19_163	Y_PR_19_172	Y_PR_19_192	Y_PR_19_234	NTC		
G	Y_PR_19_016	Y_PR_19_026	Y_PR_19_042	Y_PR_19_072	Y_PR_19_146	Y_PR_19_164	Y_PR_19_173	Y_PR_19_216	Y_PR_19_235	NTC		
H	Y_PR_19_017	Y_PR_19_029	Y_PR_19_043	Y_PR_19_073	Y_PR_19_147	Y_PR_19_165	Y_PR_19_174	Y_PR_19_217	Y_PR_19_236			

	FAM_ko	Hex_ko
fWU_kGH	A	Tb-o*

P003 results

Fig. 25: Master Mix sheet used to calculate the components of the Master Mix (example for assay fUW).

Information about the pipetting scheme including the controls on a 96 well plate, volumes used for each well and the volume needed in total are given in the upper panel. The unit used is µl. The table in the middle shows the temperature cycling for the KASP® analysis. The control samples for the assay fWU_kGH are depicted at bottom of the Fig.

Furthermore, an adhesive optical sealer was used to seal the plate. In the next step, the plate was spun for a few seconds in a plate spinner. Then the CFX Real-Time System C1000 Touch® (BioRad) was started to process the qPCR. The thermocycling protocol adopted by Mag. med. vet. Doris Rigler to obtain higher fluorescence signals, that allow a more reproducible discrimination of the two alleles is given in Tab. 10. This profile does not comply with the standard protocol recommended by LGC Group in terms of amplification steps.

Tab. 10: KASP®_plus_3 protocol used by the Institute of Animal Breeding and Genetics (University of Veterinary Medicine Vienna).

During the second step the temperature is decreased by 0.6 °C per cycle to achieve a target temperature of 55 °C for the last ten cycles. (modified table adopted from LGC Group

https://biosearch-cdn.azureedge.net/assetsv6/KASP-thermal-cycling-conditions-all-protocols.pdf (accessed 22.07.2020)

Step	**Protocol Stage**	**Temperature**	**Duration**	**Number of cycles for each stage**
1	Activation	94 °C	15 min	1
2	Denaturation	94 °C	20 seconds	10
	Annealing/Elongation	61 °C – 55 °C	60 seconds (decrease 0,6 °C per cycle to achieve 55 °C)	
3	Denaturation	94 °C	20 seconds	30
	Annealing/Elongation	55 °C	60 seconds	
4	Original Stage (Read Stage)	30 °C	60 seconds	1

2.2.5. Data Generation

After completion of the PCR, the fluorescence signal emitted by DNA samples is measured in each well and the values are displayed in the cluster plot using the program Bio-Rad CFX Manager 3.1®. The horizontal X-axis in the output shows the value of fluorescence from the FAM dye and the vertical Y-axis depicts the signal from the HEX dye (LGC Group 2019). If the sample tested is homozygous for the X signal oligonucleotide, only the FAM signal will be significantly increased compared to the no-template controls (Allele 1 in Fig. 27). The same applies for the homozygous HEX samples (LGC Group 2019). If a sample is heterozygous, both signals are observed. The heterozygous data points are mapped in the middle of the plot (LGC Group 2019). Also, it is important for the NTCs to be pictured closely together at the

onset of the plot in order to ensure that no signal is emitted from them (LGC Group 2019). The absolute value of the fluorescence signal which is generated during this process is included in a table (see at Tab. 12). If the KASP® results did not allow a clear grouping of the samples, as depictured in Fig. 27, a recycle step was done to determine if the samples are part of the FAM or HEX group. Every recycle strengthens the ability of the dyes to emit more fluorescent colour and to therefore get clearer signals which can be detected. For the recycle, the protocol explained in Tab. 11 was used.

Tab. 11: KASP® recycle protocol recommended by the producer LGC Group

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

In the following, the results are depicted as a cluster plot and are also listed in a table. The table was exported to an EXCEL®-sheet. Exemplarily, Fig. 27 and Tab. 12 show a cluster plot and a table of the results of one KASP® Real-Time PCR run.

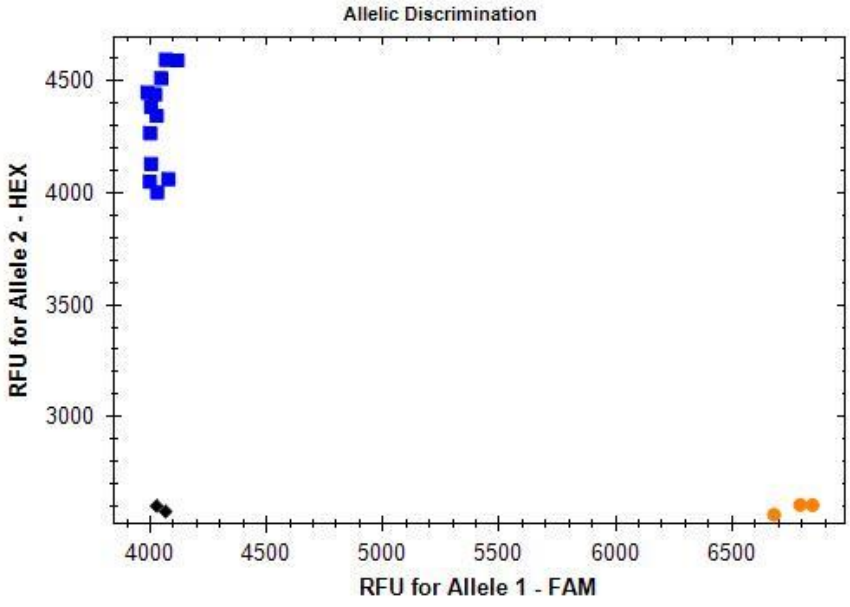


Fig. 26: Cluster plot qDR assay.

qDR is the name of the sequence of a used Y chromosomal marker. The samples of the assay are pictured as data points. Samples which were tested homozygous for ’Allele 1’ are shown

as orange dots (FAM). Samples homozygous for ’Allele 2’ are shown as blue squares (HEX). The NTCs are mapped in black cuboids.

Tab. 12: Results of the qDR assay as a table.

Relative Fluorescence Unit (RFU) 1 and RFU2 are the absolute values of the fluorescent signal and those numbers are assigned to FAM (RFU1) and HEX (RFU2).

The column “call” shows which allele is predicted by the program. ’Allele 1’ means that the FAM signal was explicitly emitted. ‘Allele 2’ represents a clear HEX signal. ‘No Call’ means that the samples could not be assigned to either allele. Ao-aD and T are the positive controls, NTC is the non-template control. In column 6 the allele of the sample is shown (see below).

Well	Sample	Call	RFU1	RFU2	qDR
F02	Ao-aD	Allele 2	3998,60	4048,09	qDR_T_1
G02	Ao-aD	Allele 2	4067,87	4590,91	qDR_T_1
D02	T	Allele 1	6680,63	2564,28	qDR_G_0
E02	T	Allele 1	6792,82	2608,21	qDR_G_0
A03	NTC	No Call	4066,22	2578,85	No Call
H02	NTC	No Call	4027,45	2603,56	No Call
A01	Y_PR_19_004	Allele 2	4030,03	4000,13	qDR_T_1
B01	Y_PR_19_006	Allele 2	4003,12	4380,33	qDR_T_1
C01	Y_PR_19_030	Allele 2	4078,01	4059,13	qDR_T_1
D01	Y_PR_19_032	Allele 2	3989,07	4445,08	qDR_T_1
E01	Y_PR_19_033	Allele 2	4027,88	4341,97	qDR_T_1
F01	Y_PR_19_035	Allele 2	3999,93	4264,17	qDR_T_1
G01	Y_PR_19_070	Allele 2	4117,14	4588,56	qDR_T_1
H01	Y_PR_19_175	Allele 2	4021,24	4436,62	qDR_T_1
A02	Y_PR_19_218	Allele 2	4003,84	4127,32	qDR_T_1
B02	Y_PR_19_229	Allele 2	4047,61	4508,04	qDR_T_1
C02	Y_PR_19_238	Allele 1	6845,82	2607,26	qDR_G_0
		KASP_P011_20200723			
		SNP_Allele1_FAM	SNP_Allele2_HEX		
		qDR_G_0	qDR_T_1		

2.2.6. Selection of the assays

There are certain alleles at several markers which determine the MSY HGs (see in general Fig. 28). When testing the samples, the results are '_1' or '_0'. '_1' indicates that the sample is carrying the derived allele and is therefore part of the HG it was tested for. In contrast '_0' means that the sample has the ancestral allele and is therefore not part of this group. For example, if a sample is tested as rAX_C_1, it carries the allele 'C' at this locus and the sample is accordingly part of the Crown HG.

Only little was known about the descent of the breeds analysed in this thesis. Therefore, all samples were first tested for the marker rAX to determine whether they are part of the Crown or not. Samples with the results rAX_C_1 were further tested for markers that determine the Crown HGs. The markers tested first were rB, rA, fVZ, rC, rW and fYR. rA, fVZ, rC and rB belong to the HG T (see at Fig. 28 and Tab. 13). The marker rW determines HG A and fYR determines HG H (Felkel et al. 2019). Because of the history of the breeds studied in this thesis the marker rB (determining HG Tb) was tested first. Samples with the result rB_C_0 were then tested for rA and fVZ to determine if they are part of HG T or not (Wallner et al. 2017). Samples which were rA_T_0 and fVZ_G_0 were then tested for rW.

If the samples were rW_A_1, they belong to HG A (Wallner et al. 2017, Felkel et al. 2019). The only sample which was rW_G_0 was tested for fYR to determine if it belongs to HG H.

The samples with a typing outcome for the derived allele on marker rB (rB_G_1) were tested with the markers fWU, fAAC, rC, fWM, rG, rD, fUJ, fQI, rJ, fWY, qGH, rN and sP. The samples with the derived allele for rW were further tested with rAF, sQF, qCU, rX, sE, rDT, rOR, fZC, qDR, sAN, fUS, fST, rY and qEW. Those samples which showed the ancestral allele at the Crown determining marker rAX (rAX_T_0) are located outside the Crown and were further tested for rBG, sES, sCO, fAR and fFQ. See Fig. 28 for a graphical overview.

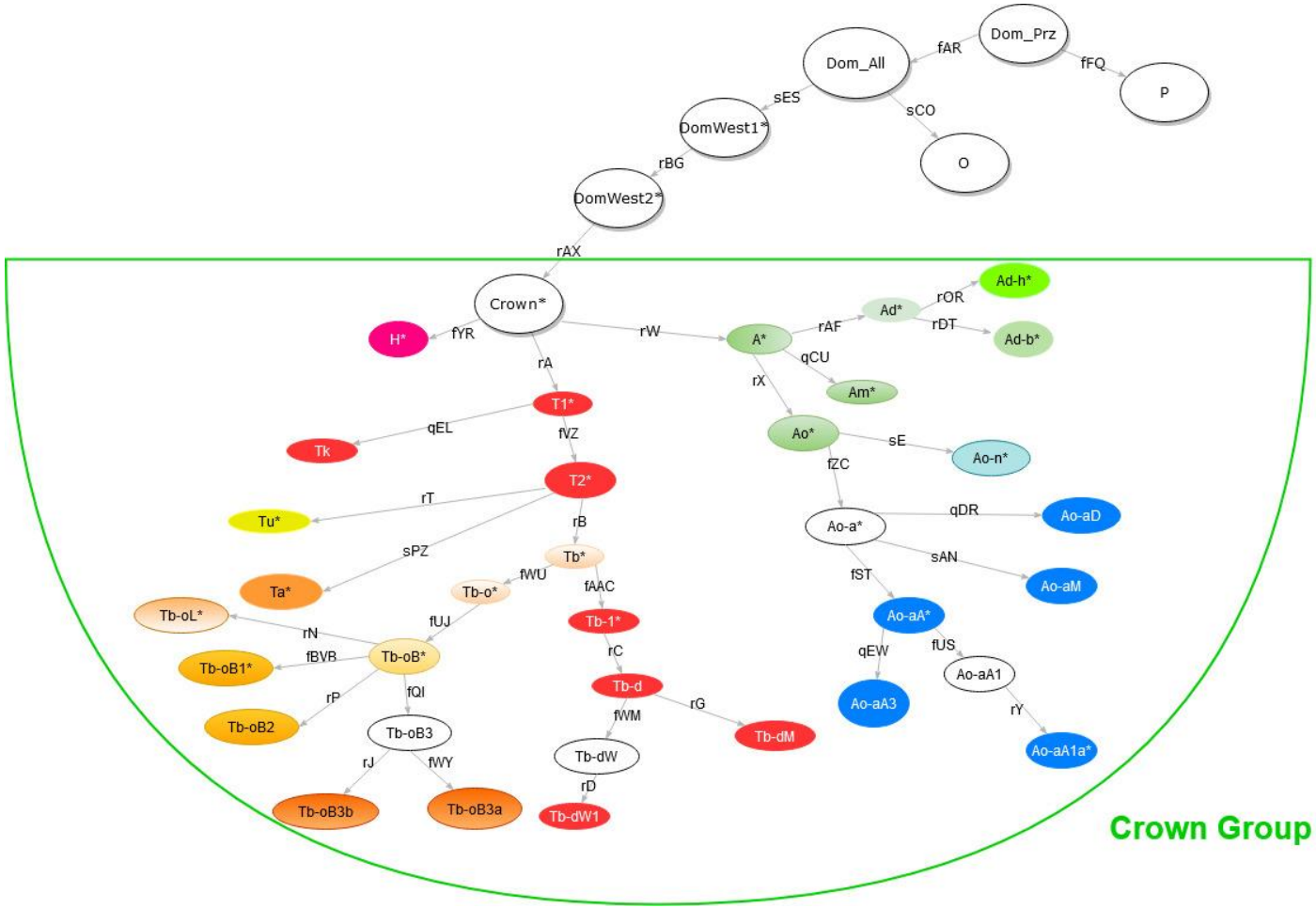


Fig. 27: Simplified phylogenetic genealogical tree based on the markers used (modified after Felkel et al. 2019).

HTs are given in coloured ellipses, markers tested (in black letters) and relevant HTs are pictured.

Tab. 13: Determined haplotypes with their Y chromosome specific marker.

The HTs are based on the alleles at different INDELs or SNPs (markers). The two alleles are given for each marker tested and the ancestral allele of a marker is displayed with '_0'. The allele which determines the respective HG is '_1' and in addition bold. The information about the markers fXX and rXX was published in Felkel et al. 2019. The data about flanking regions of the markers qXX is given in the appendix. The dye which is used for KASP® technology to detect the different alleles is given in columns three and four. The marker fBVB is a tetranucleotide microsatellite and the alleles were determined via fragment length analysis (as described in subsection 2.3.8.).

Haplogroup	**Marker**	**Allele 1 – FAM**	**Allele 2 – HEX**
A	rW	rW_G_0	**rW_A_1**
Ad*	rAF	rAF_G _0	**rAF_A_1**
Ad-b*	rDT	rDT_T_0	**rDT_C_1**
Ad-h*	rOR	rOR_G_0	**rOR_A_1**
Am*	qCU	qCU_T_0	**qCU_A_1**
Ao	rX	rX_G _0	**rX_T_1**
Ao-a	fZC	fZC_T_0	**fZC_C_1**
Ao-aA*	fST	fST_T_0	**fST_A_1**
Ao-aA1a*	rY	rY_ACC_0	**rY_AC_1**
Ao-aA3	qEW	qEW_T_0	**qEW_C_1**
Ao-aD	qDR	qDR_G_0	**qDR_T_1**
Dom_All	fAR_sFA	**fAR_A_1**	fAR_T_0
DomWest1*	sES	**sES_A_1**	sES_C_0
T1*	rA	**rA_A_1**	rA_T_0
T2*	fVZ	fVZ_G_0	**fVZ_C_1**
Ta*	sPZ	sPZ_A_0	**sPZ_T_1**
Tb*	rB	rB_C_0	**rB_G_1**
Tb-1*	fAAC	fAAC_A_0	**fAAC_T_1**
Tb-d*	rC	rC_T_0	**rC_C_1**
Tb-dM	rG	rG_A_0	**rG_G_1**
Tb-dW1	rD	rD_TT_0	**rD_DEL_1**
Tb-o*	fWU_kGH	fWU_T_0	**fWU_A_1**

Tb-oB*	fUJ	fUJ_T_0	**fUJ_C_1**
Tb-oB1*	+ fBVB	+ fBVB_204_0	+ **fBVB_208_1**
Tb-oB3a	fWY	fWY_G_0	**fWY_A_1**
Tb-oL	rN	rN_G _0	**rN_A_1**
Tu*	rT	rT_C_0	**rT_T_1**
Crown- non A, H, T	rAX	**rAX_C_1** **rA_T_0** **rW_G_0** **fYR_T_0**	rAX_T_0

2.2.6.1. Constructing haplotypes

HTs are determined by Y chromosomal markers (Wallner et al. 2013). By catenating the KASP® genotyping results of the markers, HTs can be reconstructed. If samples gave no results for a marker after testing it several times, the allele at the most terminal marker giving an unambiguous result was used to determine the HT (see Tab. 14).

Tab. 14: Rebuilding haplotypes

HTs with the corresponding Y chromosomal markers

Haplogroup A	Marker												
A	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_T_0	rAF_G_0	sQF_G_0	rX_G_0
Ad*	rAX_C_1	rB_C_0			rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_T_0	rAF_A_1		
Ad-b*	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_C_1			
Ad-h*	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_A_1				
Am*	rAX_C_1	rB_C_0			rW_A_1	fzC_T_0	sE_C_0	qCU_A_1					
Ao*	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_T_0	rAF_G_0	sQF_G_0	rX_T_1
Ao-a*	rAX_C_1	rB_C_0			rW_A_1	fZC_C_1	rY_ACC_0	fST_T_0				qDR_G_0	sAN_G_0
Ao-aA*	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_ACC_0	fST_A_1	fUS_T_0	qEW_T_0			
Ao-aA1a*	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_AC_1						

Ao-aA3	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_ACC_0	fST_A_1	fUS_T_0	qEW_C_1			
Ao-aD	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_ACC_0	fST_T_0			rDT_T_0	qDR_T_1	
Non-Crown Group	Marker												
Dom_All	rAX_T_0	rBG_CTT_0				sES_C_0	sCO_A_0	fAR_A_1	fFQ_C_0				
DomWest1*	rAX_T_0	rBG_CTT_0	rB_C_0	rA_T_0	fVZ_G_0	sES_A_1							
Haplogroup T	Marker												
T1*	rAX_C_1	rB_C_0	rW_G_0	fYR_T_0	rA_A_1	fVZ_G_0			qEL_A_0				
T2*	rAX_C_1	rB_C_0	rW_G_0	fYR_T_0	rA_A_1	fVZ_C_1	sPZ_A_0	rT_C_0					
Ta*	rAX_C_1	rB_C_0	rW_G_0	fYR_T_0	rA_A_1	fVZ_C_1	sPZ_T_1						
Tb*	rAX_C_1	rB_G_1				fWU_T_0	rD_TT_0	fAAC_A_0					
Tb-1*	rAX_C_1	rB_G_1		rC_T_0		fWU_T_0	rD_TT_0	fAAC_T_1					
Tb-dM	rAX_C_1	rB_G_1		rC_C_1		fWU_T_0	rD_TT_0	fAAC_T_1	fWM_T_0	qGH_G_0	sP_A_0	rG_G_1	

Tb-dW1	rAX_C_1	rB_G_1	rA_A_1		rW_G_0	fWU_T_0	rD_DEL_1						
Tb-o*	rAX_C_1	rB_G_1	rA_A_1	rC_T_0	fQI_G_0	fWU_A_1		fAAC_A_0		fUJ_T_0	rN_G_0		
Tb-oB*	rAX_C_1	rB_G_1		rC_T_0		fWU_A_1	fWY_G_0		rJ_G_0	fUJ_C_1		fQI_G_0	rP_T_0
Tb-oB3a	rAX_C_1	rB_G_1		rC_T_0		fWU_A_1	fWY_A_1						
Tb-oL	rAX_C_1	rB_G_1				fWU_A_1	fWY_G_0		rJ_G_0	fUJ_T_0	rN_A_1	fQI_G_0	
Tb-oB1*	rAX_C_1	rB_G_1		rC_T_0		fWU_A_1	fWY_G_0		rJ_G_0	fUJ_C_1		fQI_G_0	fBVB_208_1
Tu*	rAX_C_1	rB_C_0	rW_G_0	sPZ_A_0	rA_A_1	fVZ_C_1	rT_T_1						
Haplogroup	Marker												
Crown-Non A, T, H	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_G_0	fYR_T_0							

2.2.7. Genotyping the microsatellite fBVB

One of the markers, ‘fBVB’ (former nomenclature ‘qCO’), used in this study was the MSY tetranucleotide microsatellite (Felkel et al 2019).

2.2.7.1. Definition of microsatellites

Microsatellites are DNA sequences which consist of short repetitions of up to five bps (Bennett 2000). Microsatellites are also known as short tandem repeats (STRs) or simple sequence repeats (SRSs). They are repeated tandemly (Gadgil et al. 2017). Microsatellites usually occur in non-coding regions of the genome (Lo et al. 2019).

2.2.7.2. Determination of Alleles

fBVB is a MSY tetranucleotide microsatellite with a difference of four nucleotides between two alleles (Felkel et al. 2019). The length difference of four bps between the alleles cannot be distinguished with KASP® Technology or Agarose Gel Electrophoresis. Therefore, to genotype fBVB, a detailed fragment length analysis has to be performed.

2.2.8. Methods used to determine fBVB fragment length

2.2.8.1. Polymerase Chain Reaction – PCR

PCR is a frequently used method to amplify DNA sequences (Garibyan and Avashia 2014). This method needs a polymerase enzyme which is thermostable. In general, Taq Polymerase is used (Ramesh et al. 1992). One forward and one reverse primer are used to amplify the template DNA. Additionally, a DNA polymerase specific buffer, dNTPs, $MgCl_2$ and sterile water are required (Lorenz 2012).

2.2.8.2. Agarose Gel Electrophoresis

Gel electrophoresis is a method used to separate the fragments of DNA based on their molecular weight. This means that larger fragments migrate slower. Additionally, the fragment size can also be determined by standardising the gel. Therefore, a fragment of known size is included on the gel and compared to the fragment whose size is in question (Davis et al. 1986).

The amplification products of fBVB (see below) were checked with agarose gel electrophoresis before doing an ABI 3130xl Genetic Analyzer and QIAxcel® Advanced System analysis.

2.2.8.3. ABI 3130xl Genetic Analyzer

ABI 3130xl Genetic Analyzer is an electrophoresis instrument to measure the exact length of fluorescently labelled PCR products (ThermoFisher 2012).

2.2.8.4. PCR amplification of the fBVB microsatellite

Firstly, samples which were fUJ_C_1 were sorted on a rack in accordance with the scheme outlined in the EXCEL® sheet in Tab. 15 and Fig. 29 (established and provided by Mag. med. vet. Doris Rigler). The components of the Master Mix were also calculated within this file. Those components include the fluorescence (FAM) labelled forward Primer, the reverse Primer (Primer sequence is given in Fig. 29), 10x Buffer, dNTP Mix, $MgCl_2$, Taq polymerase and water. Similarly, to the qPCR procedure four positive controls and two NTCs were needed.

Tab. 15: Calculation of the Master Mix for each well.

Stock (concentration units)	Final (concentration units)		Component	x 1 (µl)	x n (µl)
					45
			DNA	2.00	90.00
20	0.40	µM	Primer_forward	0.40	18.00
20	0.40	µM	Primer_reverse	0.40	18.00
10	1.00	X	10x buffer	2.00	90.00
2	0.20	mM	dNTP Mix	2.00	90.00
15	1.50	mM	$MgCl_2$	2.00	90.00
1		u	Taq	0.10	4.50
			H_2O	11.10	499.50
End volume (µl)				**20.00**	**900**

The nomenclature for the fBVB alleles was fBVB_204_0 for ‘Allele 1’ and fBVB_208_1 for ‘Allele 2’. The positive controls for ‘Allele 1’ were four samples out of HG A and the positive controls for ‘Allele 2’ were Y_01_001, Y_01_003, Y_01_004 and Y_01_005. Also two NTC’s were analysed within this run.

fBVB-Typisierung					20200812
Stock	Final		Component	x 1	x n
					45
			DNA	2,00	90,00
20	0,40	muM	Primer_fwd	0,40	18,00
20	0,40	muM	Primer_rev	0,40	18,00
10	1,00	x	10x Puffer	2,00	90,00
2	0,20	mM	dNTP Mix	2,00	90,00
15	1,50	mM	MgCl 2	2,00	90,00
1		u	Taq	0,10	4,50
			H2O	11,10	499,50
			EV	20,00	
				aliquot	18,00

PCR conditions:

95°C	95°C	58°C	72°C	72°C
05:00	00:30	00:40	00:40	5 min
		37 x		

Pipettierschema

	1	2	3	4	5	6	7	8
	Y_PR_19_274	Y_PR_19_396	Y_PR_19_274	Y_PR_19_396	A (Y_PR_19_028)	NTC		
	Y_PR_19_383	Y_PR_19_397	Y_PR_19_383	Y_PR_19_397	A (Y_PR_19_059)	NTC		
	Y_PR_19_384	Y_PR_19_398	Y_PR_19_384	Y_PR_19_398	A (Y_PR_19_066)			
	Y_PR_19_385	Y_PR_19_399	Y_PR_19_385	Y_PR_19_399	A (Y_PR_19_077)			
	Y_PR_19_386	Y_PR_19_400	Y_PR_19_386	Y_PR_19_400	Y_PR_01_3			
	Y_PR_19_393	Y_PR_19_402	Y_PR_19_393	Y_PR_19_402	Y_PR_01_4			
	Y_PR_19_394	Y_PR_19_454	Y_PR_19_394	Y_PR_19_454	Y_PR_01_6			
	Y_PR_19_395	Y_PR_19_494	Y_PR_19_395	Y_PR_19_494	Y_PR_01_8			

Fragmentlän flox

qCOfwd_FAM	qCOrev_b	210 bp

Primer:

qCOfwd	ACAACCTAAGTGTCTGTGAATGA
qCOrev_b	CCCAATAATATTCCACTGCGTGT

Fig. 28: EXCEL® sheet to calculate the components of the fBVB PCR amplification (example).

Information about the scheme of pipetting including the used controls on a 96 well plate. Volumes used for each well and the volume needed in total are depicted. The unit used is µl. The concentration units of stock and final solution are specified in column 3. The table in the middle shows the temperature profile for the PCR. The undermost table describes the scheme of pipetting of the Master Mix for the typing of fBVB.

2.2.8.5. Executed procedure

The components of the Master Mix were pipetted into a 1.5 ml Eppendorf tube, then the Taq polymerase was centrifugated for a few seconds before adding it to the tube. In the following

step the Eppendorf tube was vortexed and centrifugated. Afterwards, 18 µl Master Mix were pipetted into each tube needed of an eight tubes strip. When finished, 2 µl genomic DNA were added. Afterwards the lid was carefully placed upon it.

2.2.8.6. Process of PCR

After finishing the preparations, the samples were centrifugated again and the PCR was started. In Fig. 29 the protocol used by the Institute of Animal Breeding and Genetics of the University of Veterinary Medicine Vienna is described.

2.2.8.7. Agarose Gel preparation

In order to produce the 1.5 % agarose gel used for this test, 1.5 g agarose was diluted with 100 ml 0.5 x TBE buffer and bringing to boil. Then, the fluid gel was cooled down to 60 °C and 2 µl Atlas ClearSight DNA Stain per 100 ml gel were added and carefully mixed. When needed, the gel is taken out of the warming cupboard and filled in a tray. In order to produce the wells required, ridges were put into the trays.

2.2.8.8. Procedure of gel electrophoresis

After cooling at room temperature for at least 20 minutes, the gel was solidified. It was put into an electrophoresis chamber. By removing the ridges, the wells required for samples were received. Accordingly, 0.5 x TBE buffer was filled into the chamber until a thin layer of fluid covered the gel. Afterwards, 5 µl of the fBVB PCR product mixed with loading dye were pipetted into every slot. In the last slot of each row a kb ladder was pipetted. A kb ladder (DNA ladder) is a mixture of DNA strands with defined length, which was used to calibrate the length of the PCR products analysed. Next, an electric field was generated through voltage application for 45 minutes. After 45 minutes had passed, the electric field was removed and the DNA in the gel was visualised with Doc XR + system.

2.2.8.9. Procedure of ABI 3130xl Genetic Analyzer

The PCR products (32 samples) were sent to the company Agrobiogen to determine their fragment length on an ABI 3130xl Genetic Analyzer.

2.2.9. Sideproject: QIAxcel® Advanced System to determine fragment length of fBVB alleles

2.2.9.1. QIAxcel® Advanced System

QIAxcel® Advanced System is a capillary electrophoresis system used to measure DNA fragment sizes in the genome. This instrument contains many microoptical collectors and diodes which emit light. Due to capillary force the PCR products ascend inside the capillary

tubes in the QIAxcel® Advanced gel cartridges. The DNA fragments migrate through the gel matrix inside the capillaries and depending on their length, fragments pass a detection point (principle given in Fig. 30) (QIAgen 2013). A photomultiplier tube records the signal which is emitted and transfers it to the QIAxcel® ScreenGel Software. The interpretation of data is performed with this software (QIAgen 2013). There are different cartridges with varying abilities to separate the fragments into the requested length categories. In comparison to slab-gel methods, the QIAxcel® Advanced instrument provides a better precision and more straightforward interpretation of data. Additionally, this system only needs 0.1 µl DNA sample per run (QIAgen 2013). According to the manufacturer's instructions, a size difference of 4 bp between short PCR products (length about 200 bps) can be determined with this system when using QIAxcel DNA High Resolution Kit. So, in general this system could be applied for distinguishing the two alleles of the microsatellite fBVB, and in the course of this project it was tested whether it definitely applies. This analysis was performed with the help of Dr. rer. nat. Sabine Hammer at the Institute of Immunology at the University of Veterinary Medicine Vienna.

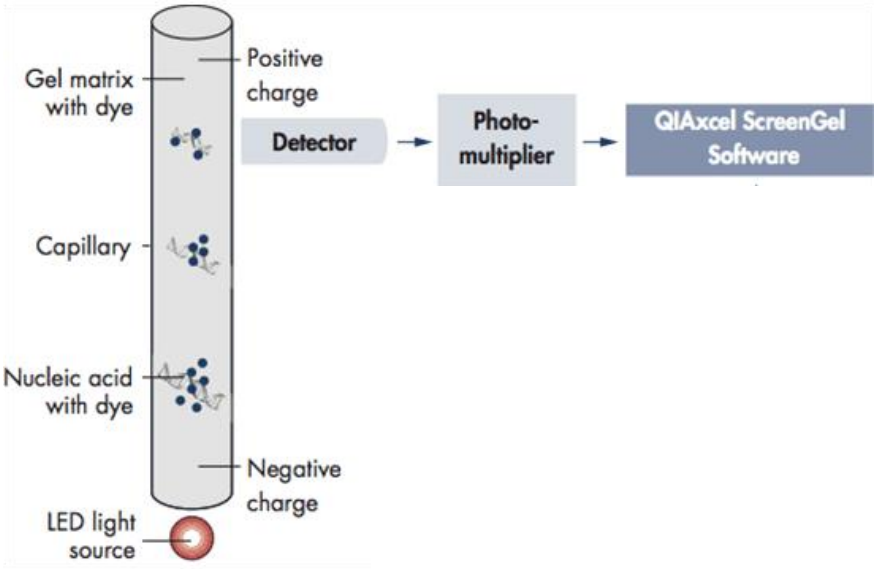


Fig. 29: Modified scheme of QIAxcel® Advanced.

An electric field is connected to the capillary which is filled with gel. The field separates the molecules of nucleic acid by size. The molecules are detected when they wander to the positively loaded side. Subsequently, the generated signals pass through a photomultiplier.

The QIAxcel® ScreenGel Software transmutes the signal into a gel image and an electropherogram (QIAgen 2013).

https://www.gene-quantification.de/qiaxcel-pure-excellence.pdf (access 16.08.2020)

Even when the sample contains merely a small number of nucleic acids, stable results can still be achieved due to the high sensitivity of detection.

2.2.9.2. Preparations for QIAxcel®

fBVB was amplified with PCR, as described above, with the only difference that the forward primer was not fluorescently labelled. The same individuals were amplified as for the ABI 3130xl Genetic Analyser analysis. Aliquots of the PCR products were checked on an Agarose Gel. The analysis was performed at the Institute of Immunology at the University of Veterinary Medicine Vienna. In an EXCEL® sheet, the numbers of PCR samples for QIAxcel® analysis were aligned in rows of twelve. A kb ladder with 15-1000 bp with 100 bp steps each was chosen for the first two analysis runs. A third run was executed with another kb ladder with 15-3000 bp.

2.2.9.3. Procedure of QIAxcel®

First, it was necessary to load the gel cartridge. Afterwards, the buffer tray was filled and loaded. After placing the samples in the capillary electrophoresis system, the procedure was started (QIAgen 2013). Three runs were carried out. During the first run DNA was absorbed for 20 sec. In the following run the time was adapted to 10 seconds. 219 bp had been chosen as a cut-off point for fBVB_204. Any values above 221 bp were defined as fBVB_208_1. For the third run the absorption time was 20 seconds and the cut-off point chosen was 226 bp. All the values above 227 bp were specified as fBVB_208_1.

3. Results

In European and American horse populations the variety of Y chromosomal HTs is reduced due to a strongly male-dominated selection in breeding. In course of this thesis, it was hypothesized that autochthonous horse populations in Western and Central Asia, the Caucasus Region and Greece are exempted from these breeding strategies and therefore display a greater Y chromosomal variability. Hence, Y chromosomal HTs were determined in horses from Armenia, Georgia, Greece, Iran and Turkmenistan by genotyping key variants. Dr. Nikos Kostaras, Dr. Rytis Juras and Prof. Gus Cothran provided 153 samples. The spectrum of HTs of these local horse populations were analysed and compared to patterns of HTs in breeds studied beforehand.

Firstly, the samples were genotyped with KASP® Technology and ABI 3130xl Genetic Analyzer. Afterwards, their HTs were determined. Finally, all the results of the different tests participated within this thesis were summarised and saved in an EXCEL® file. The data in its entirety is given in the appendix.

3.1. Distribution of MSY haplotypes

3.1.1. Results of testing for the Crown haplogroup with Marker rAX

In this thesis, 153 samples were analysed. In the first step all of them were tested for rAX, a marker that determines whether a sample clusters into the Crown haplogroup or not. Samples which carry the allele rAX_C_1 are part of the Crown Group. In Tab. 16, Tab. 17, Fig. 31 and Fig. 32 the distribution of horses inside and outside the Crown in absolute numbers per country and per breed are shown. Out of 153 samples only 27 did not belong to the Crown Group, those specimens carry the ancestral T allele at variant rAX (rAX_T_0). All those samples had been collected on the islands of Rhodes and Skyros in Greece. All samples gathered in Armenia, Georgia, Iran and Turkmenistan belonged to the Crown Group.

Tab. 16: Distribution of Crown and Non-Crown HTs per country of origin.

Country	Crown Group	Non-Crown
Armenia	2	0
Georgia	18	0
Greece	66	27
Iran	30	0
Turkmenistan	10	0
In total	***126***	***27***

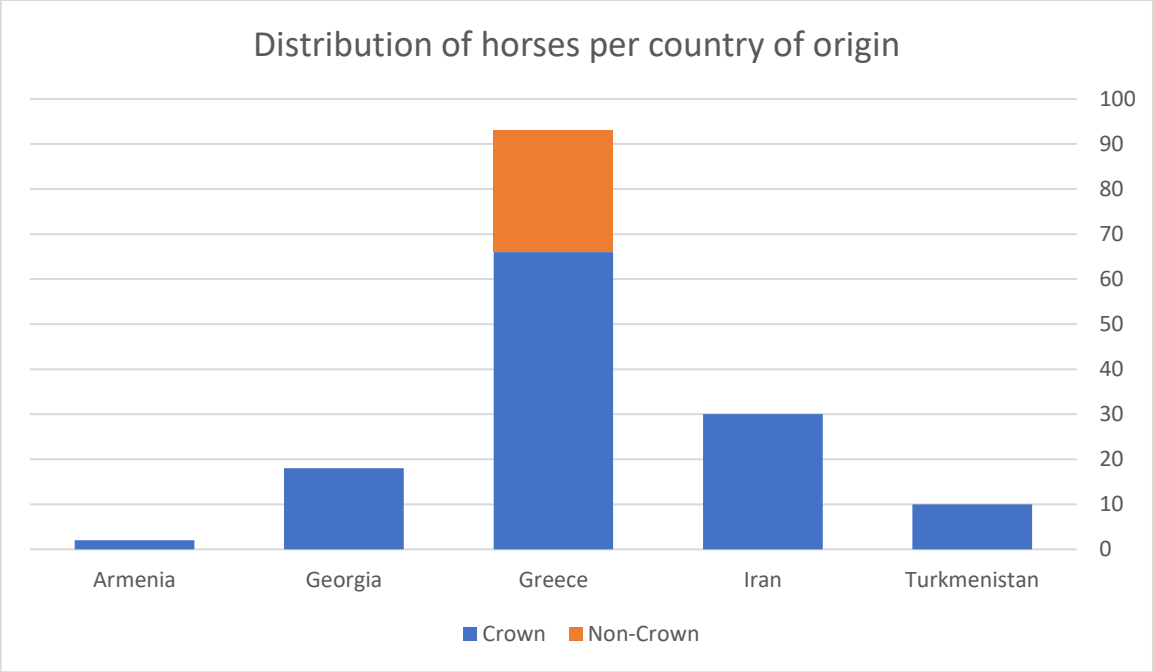


Fig. 30: Distribution of Crown and Non-Crown Group HTs per country of origin.

The distribution of the horses in the Crown or Non-Crown Group per country of origin is shown as a vertical bar chart. The samples which had rAX_C_1 as a result and therefore are part of

the Crown Group are shown in blue. The samples which had rAX_T_0 as a result and therefore are not part of the Crown Group are shown in orange.

On the horizontal axis, the number of horses in absolute numbers is depicted. On the axis of ordinates, the countries are mentioned.

Tab. 17: Distribution of Crown and Non-Crown Group HTs per breed.

Breed	Crown Group	Non-Crown Group
Akhal-Teke	10	0
Andravida Horse	15	0
Armenian Horse	10	0
Caspian Miniature Horse	10	0
Creta Pony	10	0
Kurd Horse	10	0
Lesvos Pony	10	0
Peneia Pony	13	0
Rodos Small Horse	0	9
Pindos Pony	8	0
Skyros Pony	7	18
Thessalian Pony	3	0
Turkoman	10	0
Tushuri	10	0
In total	***126***	***27***

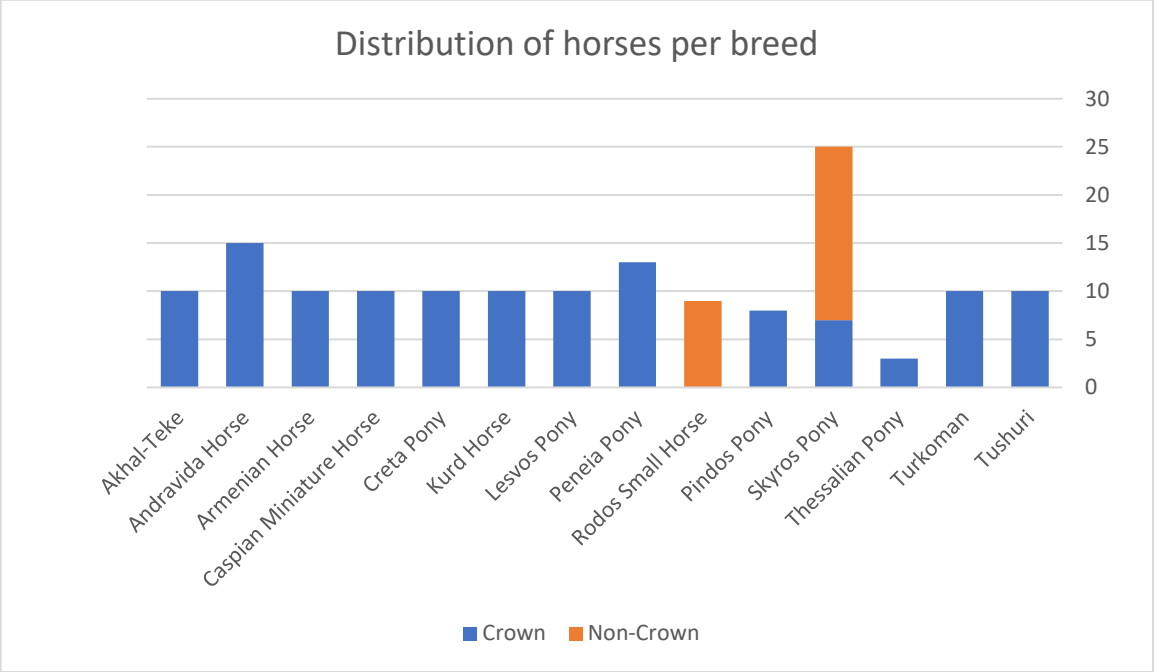


Fig. 31: Distribution of horses Crown and Non-Crown Group haplotypes per breed.

The distribution of the horses in the Crown or Non-Crown Group per breed is shown as a vertical bar chart. The samples which had rAX_C_1 as a result and therefore are part of the Crown Group are shown in blue. The samples which had rAX_T_0 as a result and therefore are not part of the Crown Group are shown in orange.

On the horizontal axis, the number of horses in absolute numbers is depicted. On the axis of ordinates, the countries are shown.

3.2. Distribution of haplotypes outside the Crown Group

Out of 153 samples 27 were not part of the Crown Group. Those specimens were collected on Rhodes and Skyros. It was decided to test the samples which were rAX_0 for marker rBG first and then test those which were rBG_0 for sES (see Fig. 18 and Tab. 14). Out of the Non-Crown Group samples, 26 clustered at the basal node of DomWest1*. Only one sample did not group with HG DomWest1* and was thus further tested with the markers sCO, fAR and fFQ. At the end it was classified as Dom_All (see Fig. 18 and Tab. 14). All nine samples from the island of Rhodes were part of the HG DomWest1*. The remaining 17 DomWest1* samples were collected on Skyros as well as the single individual that grouped onto the basal node Dom_All. Tab. 18 and Fig. 33 show the distribution of the samples outside the Crown Group.

Tab. 18: Distribution of haplotypes outside the Crown Group.

Island	**Haplotype**	**Number of samples**
Rhodes	DomWest1*	9
Skyros	Dom_All	1
	DomWest1*	17
In total		***27***

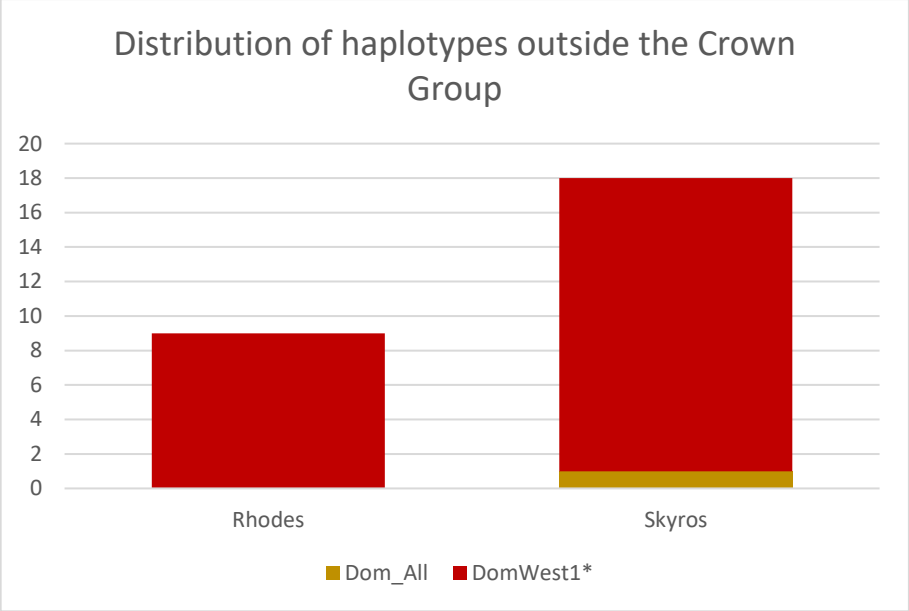


Fig. 32: Distribution of Non-Crown lineages.

The samples which were sES_A_1 are mapped in red. The sample which was sES_C_0 but fAR_A_1 is depicted in a dark yellow.

On the horizontal axis, the number of horses in total numbers is depicted. On the axis of ordinates, each bar is labelled with the name of the island where those sample were collected.

As these two HTs were only found on those two islands, no other countries are depicted in the Fig. above.

3.3. Distribution of Crown haplotypes

The Crown Group is the best investigated group and most modern-day horses are part of it (Felkel et al. 2019). With a percentage of 82% (126 out of 153 samples) the Crown Group

represents the majority of samples investigated in this study. Crown specimens were further tested for markers rW, rA and fYR. rW is determining for HG A*, rA for HG T* and fYR for HG H*. The two samples from Armenia belonged to different HGs. One was part of HG A*, the other one of HG T*. In Georgia, ten out of 14 samples belonged to HG T* and four (29 %) were part of HG A*. A total number of 43 specimens (65 %) collected in Greece grouped inside HG T*. The remaining 35% (23 samples) belonged to HG A*. 70 % of Iranian specimens (21 out of 30 samples) belonged to HG T*. Eight samples (27 %) grouped inside HG A* and one sample (3 %) could neither be attributed to HG T*, A* or H* but was identified as rAX_C_1 (Crown but Non A*, T* or H*). All samples from Turkmenistan grouped in HG T*. In Fig. 34, the distribution of Crown HTs per country is shown.

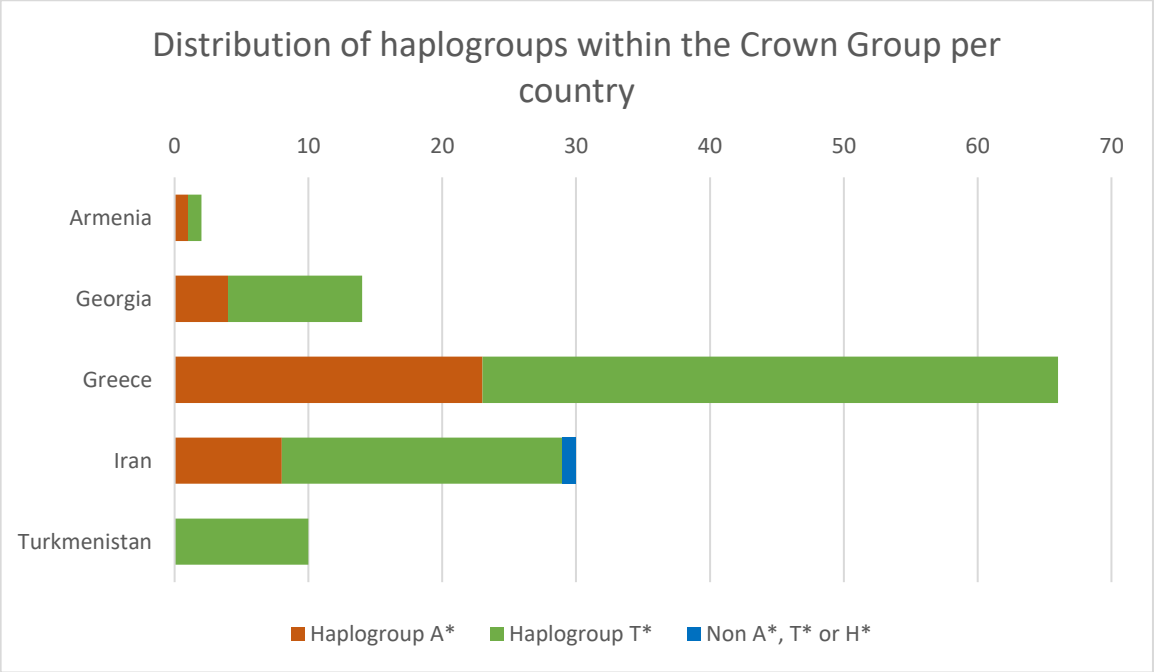


Fig. 33: Distribution of Crown haplogroups by country.

Haplogroup A* is shown in brown, Haplogroup T* in green and the sample which does not belong to one of the three tested HGs is pictured in blue.

The horizontal axis shows the number of samples in absolute numbers. On the axis of ordinates, the countries are mapped.

The Greek samples which were inside the Crown were collected on the mainland and the islands of Crete, Lesvos and Skyros. On Crete, nine horses were part of HG T* and one horse grouped within HG A*. Seven samples (70 %) from Lesvos belonged to HG T*, those samples

represent the majority. The other three (30 %) specimens grouped inside HG A*. Most of the specimens from the island of Skyros were outside the Crown. Of those which group inside the Crown six belong to HG A* and one sample belonged to HG T*. Tab. 19 shows the distribution of the samples per country.

Tab. 19: List of analysed samples per country.

Within this table the distribution of samples within the Non-Crown Group and the haplogroups inside the Crown are listed.

Country	Number of samples	Non-Crown Group	HG A*	HG T*	HG H*	Non A*, T* or H*
Armenia	2	0	1	1	0	0
Georgia	18	0	4	14	0	0
Greece/Crete	10	0	1	9	0	0
Greece/Lesvos	10	0	3	7	0	0
Greece/Pindos	11	0	7	4	0	0
Greece/Peloponnesus	28	0	6	22	0	0
Greece/Rhodes	9	9	0	0	0	0
Greece/Skyros	25	18	6	1	0	0
Iran	30	0	8	21	0	1
Turkmenistan	10	0	0	10	0	0
In total	***153***	***27***	***36***	***89***	***0***	***1***

Further testing was done to do a finer incorporation of the Crown samples into HTs (see at Fig. 35 and Tab. 20). Within HG A*, ten different HTs were determined and within HG T*, 13 different HTs were identified.

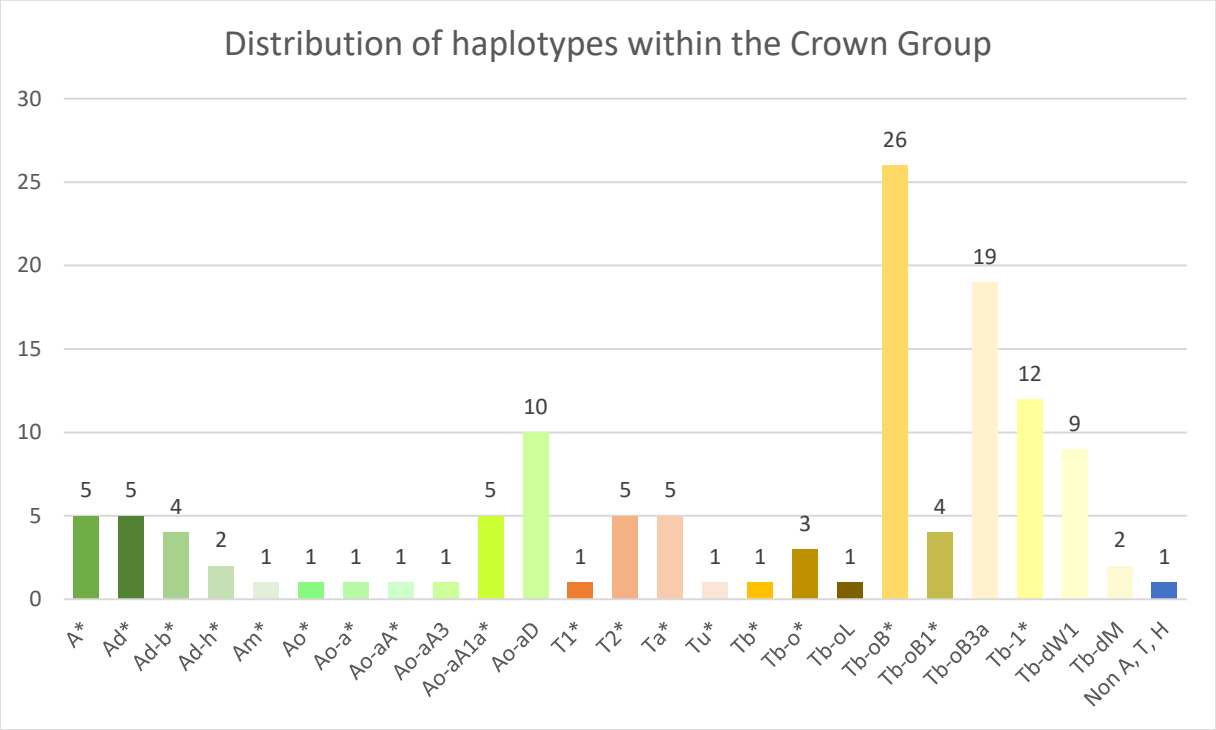


Fig. 34: Distribution of detected Crown haplotypes in the dataset.

The distribution of the different Crown HTs within the entity is shown. In order to provide a better overview every HT is shown in a different colour. HTs which belong to HG A* are shown in green. Those HTs which are grouped within HG T* are shown in orange and yellow. The sample which is in the Crown Group but does not belong to either HG A*, T* or H* is mapped in blue.

The horizontal axis shows 25 different HTs. On the axis of ordinates, the total number of samples per HT is listed.

Tab. 20: Distribution of haplotypes within the Crown Group.

The identified HTs and the total number of samples per HTs are listed.

Haplotype	Total number of samples
A*	5
Ad*	5
Ad-b*	4
Ad-h*	2

Am*	1
Ao*	1
Ao-a*	1
Ao-aA*	1
Ao-aA3	1
Ao-aA1a*	5
Ao-aD	10
T1*	1
T2*	5
Ta*	5
Tb*	1
Tb-o*	3
Tb-oL	1
Tb-oB*	26
Tb-oB1*	4
Tb-oB3a	19
Tb-1*	12
Tb-dW1	9
Tb-dM	2
Tu*	1
Non A, T, H	1
In total	***126***

3.4. Distribution of all haplotypes within the countries and the breeds

Additionally, Tab. 21, Tab. 22, Fig. 36 and Fig. 37 show the distribution of HTs within the breeds and in the different countries.

Tab. 21: Distribution of haplotypes per country.

The different HTs and the absolute number of specimens per HT and per country are listed in the following table.

Country	Haplotype	Absolut number of samples
Armenia	Ao*	1
	Tb-1*	1
Georgia	Ao-a*	1
	Ao-aA1a*	1
	Ao-aD	2
	T1*	1
	T2*	1
	Tb*	1
	Tb-1*	4
	Tb-oB*	3
	Tb-oB1*	2
	Tb-oL*	1
Greece	A*	5
	Ad*	5
	Ad-b*	4
	Ad-h*	2
	Am*	1
	Ao-aA1a*	1
	Ao-aD	5
	Dom_All	1
	DomWest1*	26
	T2*	2
	Ta*	2
	Tb-1*	4
	Tb-dM*	2
	Tb-dW1*	8
	Tb-o*	3
	Tb-oB*	21

	Tu*	1
Iran	Ao-aA*	1
	Ao-aA1a*	3
	Ao-aA3*	1
	Ao-aD	3
	T2*	2
	Ta*	3
	Tb-1*	3
	Tb-oB*	2
	Tb-oB1*	1
	Tb-oB3a	10
	Non A, T, H	1
Turkmenistan	Tb-oB1*	1
	Tb-oB3a	9
In total		***153***

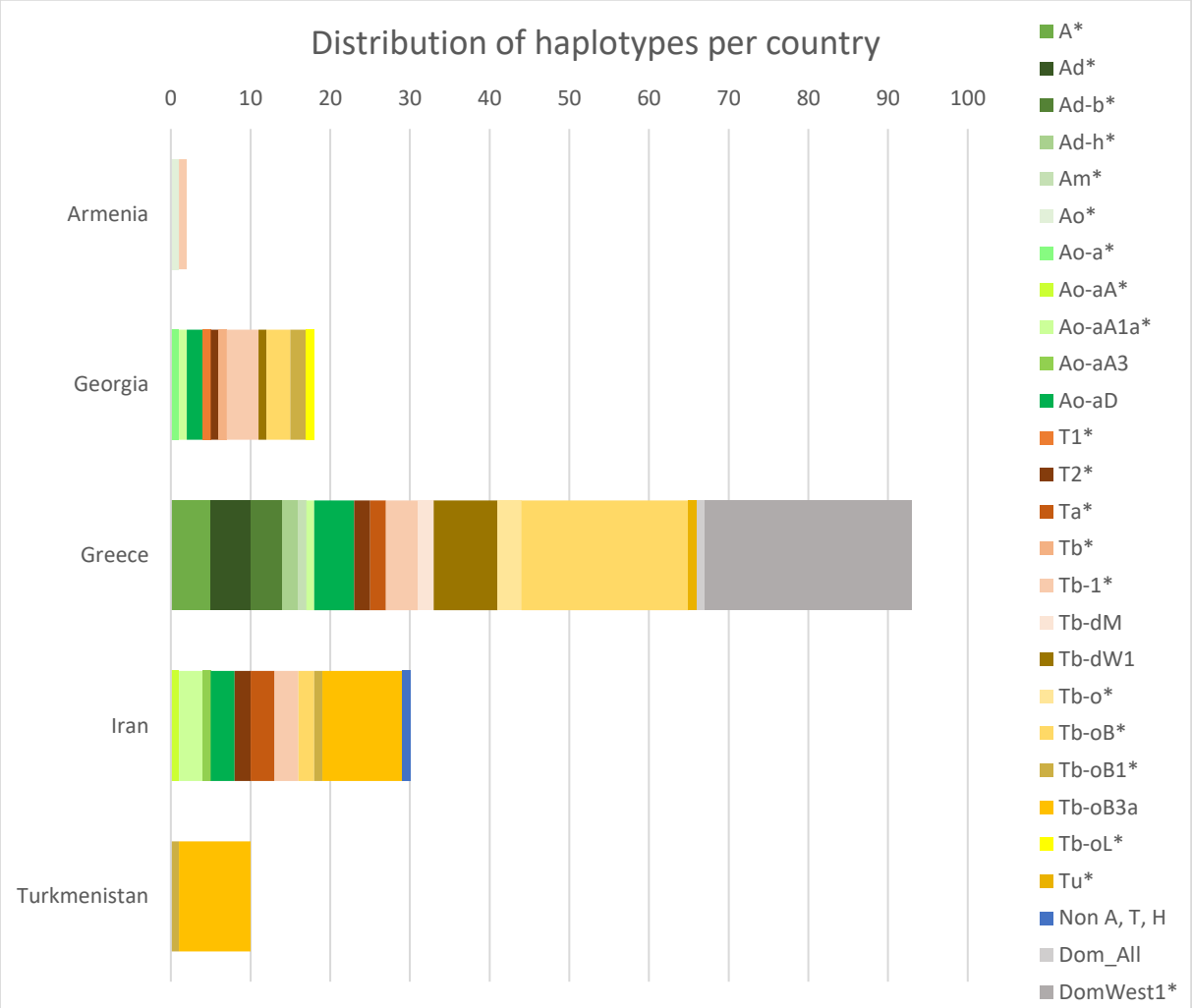


Fig. 35: Distribution of haplotypes per country.

The distribution of HTs within the countries where the samples were taken is shown. Each HT is shown in a different colour to provide a better overview. HTs which belong to HG A* are shown in green. Those HTs which are grouped within HG T* are shown in orange and yellow. The sample which is inside the Crown Group but does not belong to one of the HGs A*, T* or H* is mapped in blue. The specimens which are part of the Non-Crown Group are shown in grey.

On the horizontal axis, the absolute number of specimens per HT is shown. On the axis of ordinates, the countries where the samples have been collected are depicted.

Tab. 22: Distribution of haplotypes within the tested breeds.

Breed	Haplotypes	Absolute number of samples
Akhal-Teke	Tb-oB1*	1
	Tb-oB3a	9
Andravida Horse	Ad-b*	2
	Ad-h*	1
	Ao-aD	2
	Tb-1*	3
	Tb-dM	1
	Tb-dW1	5
	Tu*	1
Armenian Horse	Ao*	1
	Ao-aA1a*	1
	Ao-aD	1
	T2*	1
	Tb-dW1	1
	Tb-1*	2
	Tb-oB*	2
	Tb-oB1*	1
Caspian Miniature Horse	Ao-aA*	1
	Ao-aA3	1
	Ao-aD	1
	T2*	2
	Tb-1*	3
	Tb-oB*	2
Creta Pony	Am*	1
	Tb-oB*	9
Kurd Horse	Ao-aA1a*	3
	Ao-aD	2
	Ta*	3
	Tb-oB3a	1
	Non A, T, H	1

Lesvos Pony	Ao-aD	3
	T2*	1
	Tb-dW1	1
	Tb-oB*	5
Peneia Pony	A*	1
	Ta*	2
	Tb-dW1	1
	Tb-o*	3
	Tb-oB*	6
Pindos Pony	A*	4
	Ad-b*	2
	Ad-h*	1
	Tb-1*	1
Rodos Small Horse	DomWest1*	9
Skyros Pony	Ad*	5
	Ao-aA1a*	1
	Dom_All	1
	DomWest1*	17
	Tb-dW1	1
Thessalian Pony	T2*	1
	Tb-dM	1
	Tb-oB*	1
Turkoman	Tb-oB1*	1
	Tb-oB3a	9
Tushuri	Ao-a*	1
	Ao-aD	1
	T1*	1
	Tb*	1
	Tb-1*	3
	Tb-oB*	1
	Tb-oB1*	1
	Tb-oL*	1
In total		***153***

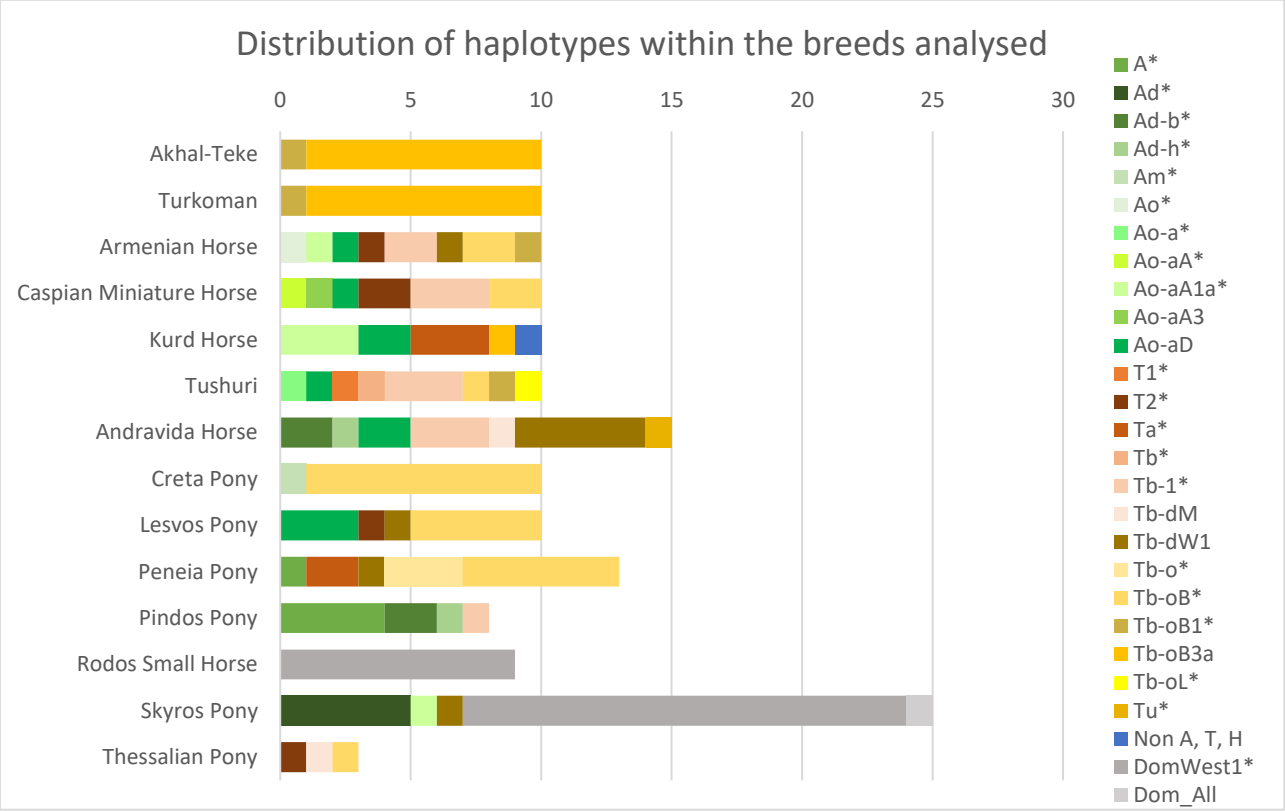


Fig. 36: Distribution of haplotypes within the analysed breeds in the Crown and Non-Crown Group.

The spreading of the HTs within the breeds analysed is shown in this diagram. The samples which belong to HG A* are coloured in green. Those samples which group within HG T* are depicted in yellow and orange colours. The sample which is part of the Crown but does not belong to HT A*, T* or H* is shown in blue. The specimens which group outside the Crown Group are shown in grey colours.

On the horizontal axis, the absolute number of horses is mapped. On the axis of ordinates, the breeds analysed are shown.

3.5. Results of the side project: genotyping fBVB with QIAxcel® Advanced System

First Agarose Gel Electrophoresis was performed to ensure that the fBVB PCR worked for all the samples which were to be tested with ABI 3130xl Genetic Analyzer and QIAxcel® Advanced System. All 32 samples showed the specific amplicon. Fig. 38 shows those results. The PCR product with the expected length of about 200 bp is shown as a black band in Lane 1-19 and Lane 21-44. The kb ladder is pictured on the right side of the image (Lane 20 and Lane 40). The concentrations of nucleic acids per sample are comparable.

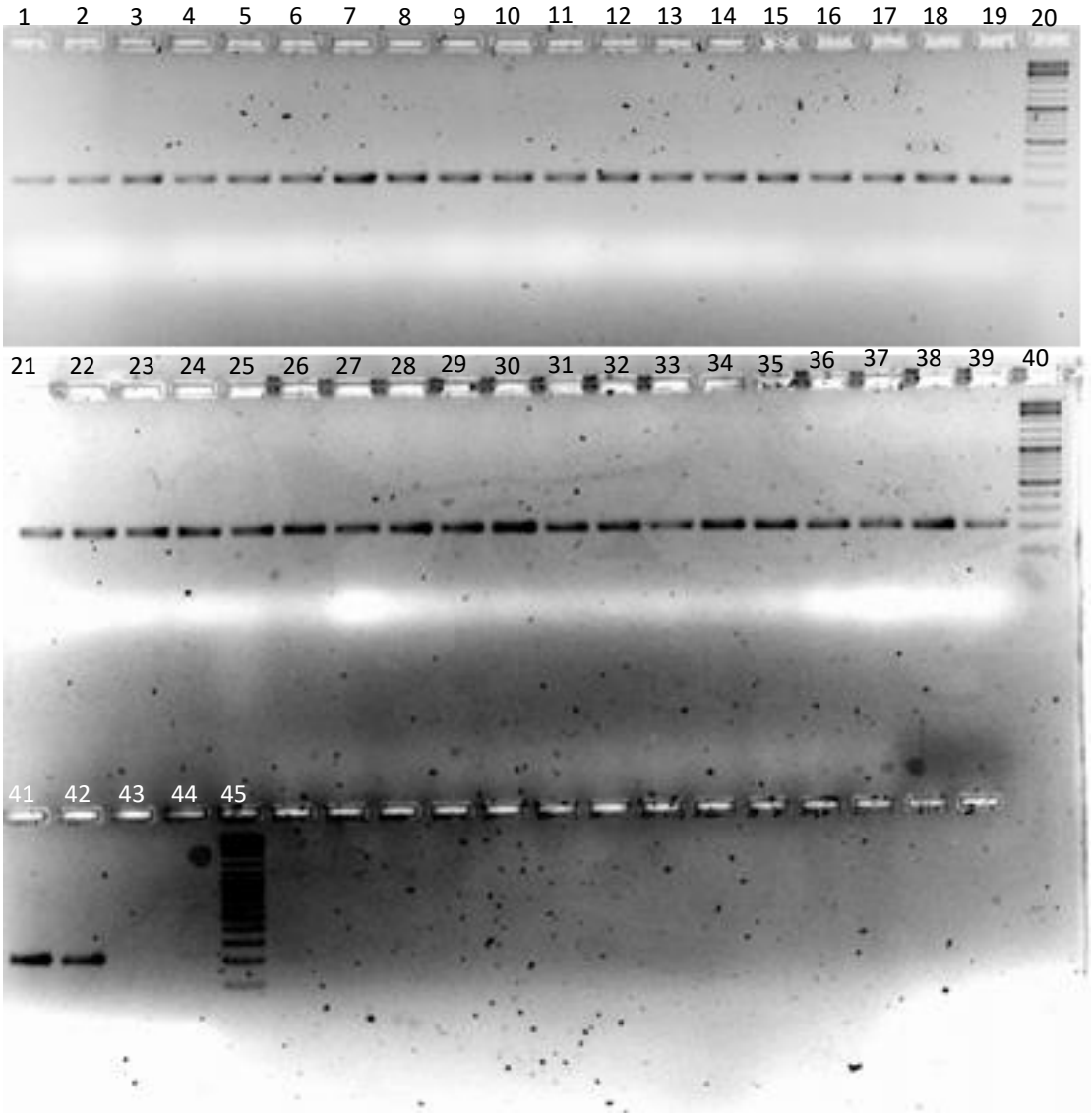


Fig. 37: Results of fBVB PCR product Agarose Gel Electrophoresis visualised with Doc XR + Gel Documentation System.

The genomic DNA is shown in black bands. The kb ladder is pictured on the right side of the image. The concentrations of nucleic acids per sample are similar. There are no bands underneath the last two wells because those are the wells of the NTC.

The lanes are labelled with black or white numbers.

Table 23: Explanation Fig. 37.

In the left column, the number of the lane is shown. In the right column, the name of the sample is written.
Samples Y_PR_19_275, Y_PR_300, Y_PR_347 and Y_PR_358 are not part of the specimens analysed in this thesis.

Lane	Sample
1	Y_PR_19_014
2	Y_PR_19_021
3	Y_PR_19_022
4	Y_PR_19_029
5	Y_PR_19_031
6	Y_PR_19_034
7	Y_PR_19_036
8	Y_PR_19_038
9	Y_PR_19_039
10	Y_PR_19_041
11	Y_PR_19_043
12	Y_PR_19_044
13	Y_PR_19_045
14	Y_PR_19_046
15	Y_PR_19_049
16	Y_PR_19_071
17	Y_PR_19_075
18	Y_PR_19_146
19	Y_PR_19_162
20	Kb ladder
21	Y_PR_19_162
22	Y_PR_19_163
23	Y_PR_19_164
24	Y_PR_19_165
25	Y_PR_19_166
26	Y_PR_19_167
27	Y_PR_19_168
28	Y_PR_19_170

29	Y_PR_19_171
30	Y_PR_19_181
31	Y_PR_19_224
32	Y_PR_19_231
33	Y_PR_19_233
34	Y_PR_19_235
35	Y_PR_19_275
36	Y_PR_19_300
37	Y_PR_19_347
38	Y_PR_19_358
39	H
40	Kb ladder
41	Y_01_001
42	Y_01_005
43	NTC
44	NTC
45	Kb ladder

3.6. Fragment length analysis with the QIAxcel® Advanced System

In this thesis it was tested, whether the QIAxcel® Advanced System can be used for screening the fragment length difference of four nucleotides between two alleles of the tetranucleotide micosatellite fBVB. 32 samples were analysed. The first run was not evaluable. In the second analysis run, the fBVB PCR amplicons showed a length between 216 and 223 bp with this device and 219 bp was chosen as an upper cut-off point for fBVB_204_0. For the second analysis run it was also decided that any values above 221 bp were defined as fBVB_208_1. A third run was executed, using another size marker and different results were obtained. The length of the fBVB microsatellite in this run was determined between 224 and 231 bp. In the third run, any values above 227 bp were attributed to fBVB_208_1. The upper cut-off point was 226 bp. For some samples, the results differed significantly between the runs and accordingly the allele classification (see below).

Fig. 39 and Fig. 40 show examples of different results for the same sample.

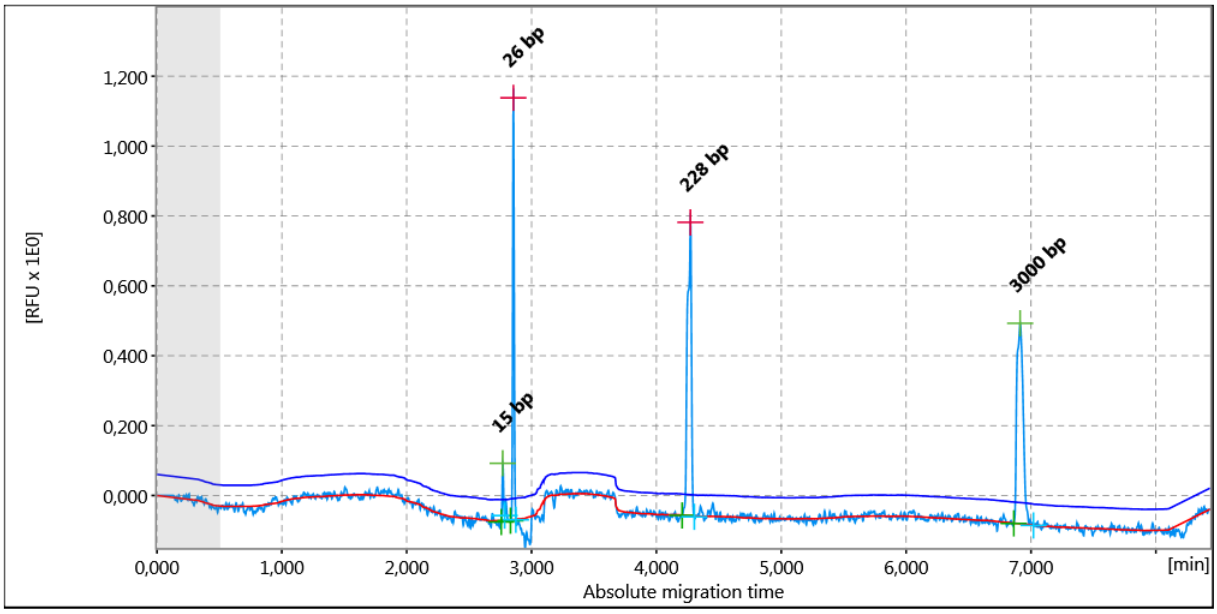


Fig. 38: QIAxcel® analysis of sample Y_PR_19_046 in the third run.

The blue line indicates a threshold value. When a signal passes this value, a peak is detected (QIAgen 2015).

The first peak with a value of 15 bp is the alignment marker and it is used to calibrate the variations of migration time. The second and fourth peak of 26 bp and 3000 bp are the peaks of the size marker. The third peak with a value of 228 bp can be associated with the sample in question.

The horizontal axis shows the absolute migration time of analyte in minutes. The axis of ordinates depicts the intensity in RFU.

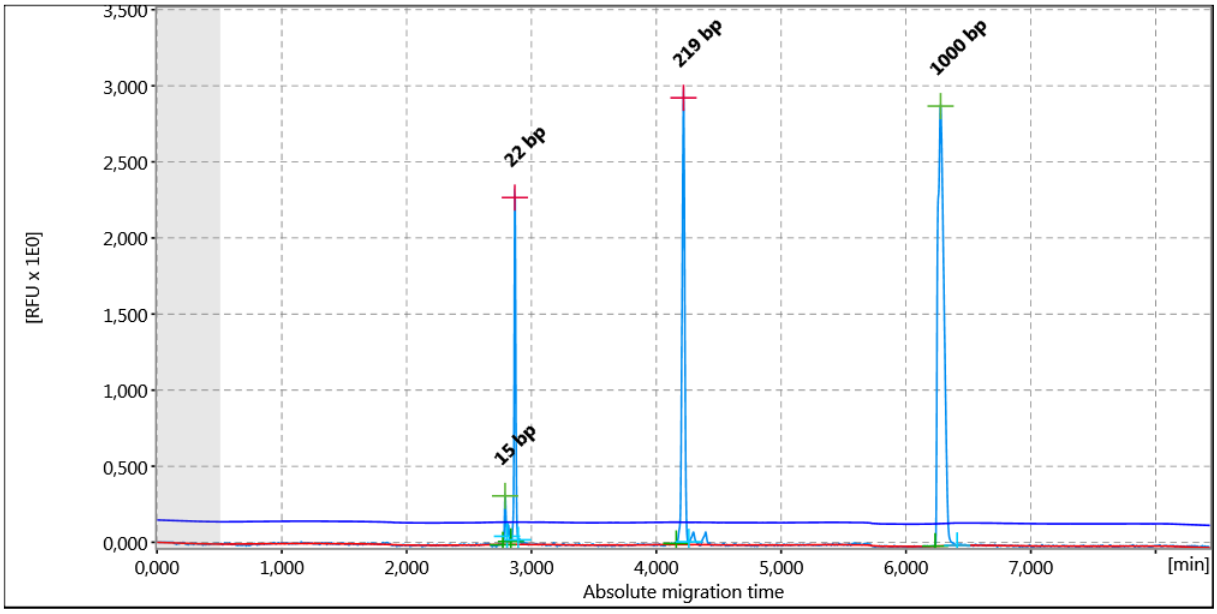


Fig. 39: QIAxcel® analysis of sample Y_PR_19_046. It was determined as fBVB_204_0 in the second run.

The threshold value is indicated by a blue line. Is this value exceeded by a signal a peak is detected (QIAgen 2015).

The alignment marker which is used to calibrate the variations of migration time is depicted with a value of 15 bp. The peaks of 22 bp and 1000 bp can be associated to the size marker. The value of 219 bp (third peak) is connected with the analyte.

The horizontal axis shows the absolute migration time of analyte in minutes. The axis of ordinates depicts the intensity in RFU.

3.7. Comparison of QIAxcel® Advanced System with ABI 3130xl Genetic Analyzer

When using the established method with ABI 3130xl Genetic Analyzer, assumed as the 'Gold standard', four samples were detected as fBVB_208_1. The QIAxcel® Advanced System showed inconsistent results in the three runs performed and its results did not fully match with those of the ABI 3130xl Genetic Analyzer. With QIAxcel® Advanced System five samples were detected as fBVB_208_1 and 21 samples were fBVB_204_0. Six samples were undecided because the different runs showed different results or the value coincided with the cut-off point in both runs (see Tab. 23).

One sample showed unclear results when using ABI 3130xl Genetic Analyzer, but consistent results in the two evaluable QIAxcel® Advanced System runs (run 2: 218 bp, run 3: 226 bp).

Therefore, it was decided to assign sample Y_PR_19_029 as carrying the ancestral allele. In Tab. 23 and Fig. 41 the results of those two methods were compared and the HT was identified. Because of the ambiguous results we got from the QIAxcel® Advanced System for several samples, we conclude that the method is not suitable for reliable determination of fBVB alleles 204 and 208.

Tab. 24: Results of the ABI 3130xl Genetic Analyzer in comparison to QIAxcel® Advanced System to test for HT Tb-oB1* are shown.

Samples which are bold showed different results between QIAxcel® Advanced System and ABI 3130xl Genetic Analyzer. One sample could not be determined within the analysis with ABI 3130xl Genetic Analyzer. It is noted in cursive letters.

The samples which were detected as fBVB_208_1 with QIAxcel® Advanced System are highlighted in yellow. The samples that were assigned to the allele fBVB_204_0 during the analysis with QIAxcel® Advanced System are marked in blue. The samples that could not be clearly classified as one allele despite several analyses with QIAxcel® Advanced System are depicted in green.

For a better understanding the determined allele with QIAxcel® Advanced System is written next to the value of the results.

The unit of the results of QIAxcel® Advanced System and ABI 3130xl Genetic Analyzer is bp.

Sample	QIAxcel® Run 2	QIAXcel® Run 3	ABI 3130xl Genetic Analyzer	Defined allele
Y_PR_19_014	223/208	231/208	208	fBVB_208_1
Y_PR_19_021	222/208	bad	208	fBVB_208_1
Y_PR_19_022	219/204	226/204	204	fBVB_204_0
Y_PR_19_029	*218/204*	*226/204*	*Unclear*	*fBVB_204_0*
Y_PR_19_031	216/204	226/204	204	fBVB_204_0
Y_PR_19_034	219/204	225/204	204	fBVB_204_0
Y_PR_19_036	218/204	225/204	204	fBVB_204_0
Y_PR_19_038	219/204	225/204	204	fBVB_204_0
Y_PR_19_039	218/204	224/204	204	fBVB_204_0

Y_PR_19_041	219/204	226/204	204	fBVB_204_0
Y_PR_19_043	unclear	227	204	fBVB_204_0
Y_PR_19_044	**223/208**	**228/208**	**204**	**fBVB_204_0**
Y_PR_19_045	220	227	204	fBVB_204_0
Y_PR_19_046	**219/204**	**228/208**	**204**	**fBVB_204_0**
Y_PR_19_049	218/204	226/204	204	fBVB_204_0
Y_PR_19_071	217/204	226/204	204	fBVB_204_0
Y_PR_19_075	216/204	225/204	204	fBVB_204_0
Y_PR_19_146	223/208	228/208	208	fBVB_208_1
Y_PR_19_162	218/204	224/204	204	fBVB_204_0
Y_PR_19_163	219/204	225/204	204	fBVB_204_0
Y_PR_19_164	218/204	226/204	204	fBVB_204_0
Y_PR_19_165	218/204	227	204	fBVB_204_0
Y_PR_19_166	218/204	227	204	fBVB_204_0
Y_PR_19_167	**221/208**	**227**	**204**	**fBVB_204_0**
Y_PR_19_168	218/204	226/204	204	fBVB_204_0
Y_PR_19_170	217/204	226/204	204	fBVB_204_0
Y_PR_19_171	218/204	225/204	204	fBVB_204_0
Y_PR_19_181	217/204	225/204	204	fBVB_204_0
Y_PR_19_224	215/204	224/204	204	fBVB_204_0
Y_PR_19_231	218/204	224/204	204	fBVB_204_0
Y_PR_19_233	217/204	224/204	204	fBVB_204_0
Y_PR_19_235	224/208	229/208	208	fBVB_208_1

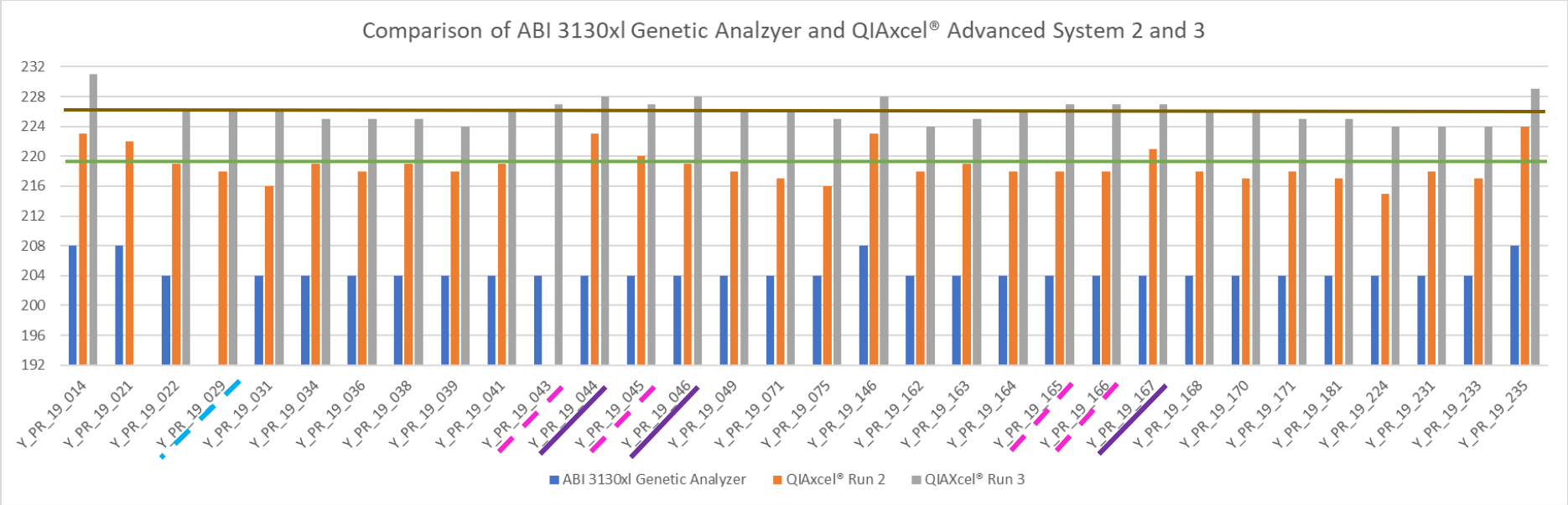


Fig. 40: Comparison of ABI 3130xl Genetic Analyzer and QIAxcel® Advanced System.

One run was carried out with ABI 3130xl Genetic Analyzer. The results are shown in blue.

Three runs were performed with QIAxcel® Advanced System. The fragment lengths determined in the second are depicted in orange. Those of the third run are mapped in grey.

The cut-off point for the second run is shown in green. The one for the third run is depicted in brown.

The samples which were allocated to the wrong HT by QIAxcel® Advanced System are underlined (violet).

The samples that were unclear during analysis with QIAxcel® Advanced System are marked with a dotted line (pink).

One sample was not assigned to one HT during the analysis with ABI 3130xl Genetic Analyzer. It is underlined with a blue dotted line.

The horizontal axis shows the number of each sample. The axis of ordinates shows the length (bp) of each fragment measured.

4. Discussion

The aim of this study was to identify the variety of MSY HTs in a collection of native breeds from Greece, Asia Minor and the Caucasus region. Within this thesis 153 samples from Armenia, Georgia, Greece, Iran and Turkmenistan were analysed. Some of the results put previously believed assumptions into perspective. For the first time MSY HTs outside the Crown HG were found in Europe and a broad diversity of HTs inside the Crown was detected.

4.1. Determining Non-Crown HTs in Europe for the first time

Wallner et al. (2017) and Felkel et al. (2018) assumed that the bulk of modern-day breeds in Europe group within the Crown HG and only a few Northern European breeds cluster outside the Crown (Non-Crown Group). Astonishingly, in the course of this thesis all nine samples gathered on the island of Rhodes and 18 out of 25 (72 %) samples from the island of Skyros grouped outside the Crown. It was thus concluded that these breeds were able to mostly withdraw from the impact of recent breeding with Thoroughbreds or Arabians. Therefore, those horses retained the genetic variation of an original horse population. Determining the origin of these breeds from using written records has proven to be difficult, but the Rodos Small Horse and the Skyros Pony are believed to share a common ancestor: the Aegean small bay horse. This predecessor immigrated from Central Anatolia or Asia Minor (Amaltheia News 2013b). Kugler (2010) mentions that the Lesvos Pony also descends from this breed, but the typical small pony living on Lesvos was thought to be extinct since World War I. But in 2018, the (re)discovery of a small horse breed on Lesvos which is resembling the Aegean type was published (SAVE 2018). However, this breed needs to be further analysed before making conclusions about their relationship with other Greek breeds, especially the Skyros Pony and the Rodos Small Horse. Although, most of the ponies living on the island of Lesvos nowadays are offspring of local horses which mated with breeds like Thoroughbreds (Kugler 2010).

Y chromosomal results of this study support the thesis that the Skyros Pony and the Rodos Small Horse share no common ancestor with the analysed samples of the Lesvos Pony. Because the former two clustered outside the Crown Group whereas the Lesvos Pony was in the Crown or even a Thoroughbred HT (Tb-dW1) was determined (see Table 22, Figure 37).

To get a better understanding of the geographic situation of the three islands Figure 42 shows the location of the islands of Skyros, Rhodes and Lesvos on a map of Greece.

Figure 41: Physical map of Greece.

The islands of Skyros and Rhodes are marked with a blue circle. Around the island of Lesvos a red circle can be found.

https://de.m.wikipedia.org/wiki/Datei:Greece_map_CIA_1996.jpg (access 30.10.2020)

Phenotypically, the Skyros Pony differs from other native Greek breeds (Bömcke et al. 2011). Bömcke et al. (2011) also used microsatellites and pedigree information to compare the Skyros Pony to other Greek breeds (Pindos Pony, Peneia Pony, Creta Pony) and showed that horses from the island of Skyros are clearly distinguishable from any other breed studied (Bömcke et al. 2011). These observations were confirmed within this thesis. The animals living on Skyros and also animals from Rhodes showed both outside the Crown Group HTs DomWest1* and Dom_All. It is worth to notice that those samples remain seated on internal branching points of the MSY phylogeny (for clarification see Fig. 28) and these basal nodes indicate the last common ancestor of a HG. The branching point Dom_All was dated to 13000 and DomWest1* to 2600 years ago (Felkel et al. 2019). This means that the most recent common ancestor

(MRCA) of the specimens which were determined as HT DomWest1* and all other studied horses in this thesis lived 2 600 years ago. The single sample gathered on the island of Skyros showed HT Dom_All. As the branching point Dom_All was dated to 13000 years ago it can be assumed that the MRCA of the HT detected in this horse and the others lived 13000 years ago. This is the first proof of such distantly related MSY HTs being present in an European horse population. Apostolidis et al. (2000) analysed the relationship between five Greek horse breeds (Andravida Horse, Peneia Pony, Creta Pony, Skyros Pony, Thessalian Pony) and investigated the difference among those breeds on a mtDNA level. Apostolidis et al. (2001) used the random amplified polymorphic DNA (RAPD) method to find a way to differentiate between the five Greek breeds mentioned before. Based on mtDNA Apostolidis et al. (2000) showed that none of the breeds found in modern-day Greece have an isolated origin, but that the breeds are heavily mixed through crossbreeding. Additionally, no population specific markers were found when testing samples with RAPD (Apostolidis et al. 2001). Both studies stated that the difference of the exterior of the Skyros Pony can be explained by natural selection and micro-evolutionary processes (Apostolidis et al. 2000, Apostolidis et al. 2001).

The Rodos Small Horse is not as well studied as the Skyros Pony, but this thesis suggests a shared paternal ancestry of the Skyros Pony and the Rodos Small Horse. This assumption is based on the fact that most of the samples analysed carry the same HT DomWest1*. There is also a possibility that both those breeds carry independently evolved HTs both branching off at DomWest1*. A terrific finding was that one of the specimens of Skyros clusters to the basal node of Dom_All.

Finally, the results of this thesis display that the Skyros Pony and the Rodos Small Horse maintained early paternal lineages and they may share a MRCA of the retained autochthonous HTs. This distinct HT pattern in the Skyros Pony and the Rodos Small Horse compared to other breeds analysed within this thesis displays less influence of recent crossing with horse breeds like Thoroughbreds or Arabians.

In the next step, the samples from Skyros and Rhodes need to be further analysed and sequenced to ascertain their private variants and resolve their MSY HTs.

4.2. Diversity inside the Crown Group

Felkel et al. (2019) managed to determine three HGs (A*, H*, T*) inside the Crown Group. As expected, 126 of 153 samples or 83 % of all 153 samples in this thesis grouped inside those defined HGs. However, it was expected to find a large variety of Crown HTs when analysing

those samples. It was hypothesised that the regionally restricted habitats of most breeds analysed in this study contribute to a higher variety of HTs in comparison to transboundary breeds which are common today. Accordingly, 18 out of 126 samples or 14 % of the specimens inside the Crown were located at internal branching points (also called nodes) after genotyping. These nodes include HTs like A*, Ad*, Am*, Ao*, T1* and T2* (see Section 3.3.). In detail, two out of ten (20 %) samples from the Armenian Horse, two out of ten (20 %) from the Caspian Miniature Horse, one out of ten (10 %) specimens from the Creta Pony, one out of ten (10 %) of the Lesvos Pony, one out of 13 samples (8 %) from the Peneia Pony, four out of eight (50 %) specimens from the Pindos Pony, five out of 25 (20 %) samples from the Skyros Pony, one out of three (30 %) samples from the Thessalian Pony and one out of ten (10 %) samples from the Tushuri fulfilled the criteria for grouping on internal nodes. Furthermore, one sample of an Iranian Kurd Horse was grouped within the Crown but it was not possible to cluster it into one of the three known HGs A*, H* and T*. The specimen in question should be sequenced in the future. All those samples illustrate that the horse populations analysed herein are likely to display a greater HT diversity within the Crown Group than has been observed so far. In contrast, most modern-day breeds, especially sport horses, carry the HT Tb-dW1 as a signature of a single thoroughbred sire line (Wallner et al. 2017, Felkel et al. 2019).

In conclusion, the data of the present study shows that the HT diversity inside the Crown exceeds the expectations of Felkel et al. (2019).

4.3. Recent influence of breeding on autochthonous horse populations in Armenia, Georgia, Greece, Iran and Turkmenistan

Due to an excessive use of a small number of stallions, their HTs spread all over the world in the last centuries (Wallner et al. 2013, Felkel et al. 2019). For example, Felkel et al. (2019) managed to determine the HTs inside HG Tb* which are specific for the three founder stallions of the English Thoroughbred. Furthermore, it was possible for Felkel et al. (2019) to determine that HT Tb-dW1 which is present in most of modern-day breeds can be traced back to a stallion named Whalebone born in 1807. Also, HTs specific for Arabian Horses have been recently described (Michaelis 2019). The recent influences on local breeds can be determined with the help of Y chromosomal markers. The results showed that in different regions some breeds have been more heavily influenced by recent refinement than others. Tb* HTs including the signatures of Thoroughbreds are predominant in autochthonous breeds in Armenia, Georgia, Greece, Iran and Turkmenistan.

The regional distribution of HTs is illustrated in Fig. 43, Fig. 44 and Fig. 45. The local characteristics and interesting findings are explained below.

4.3.1. Current situation in Greece

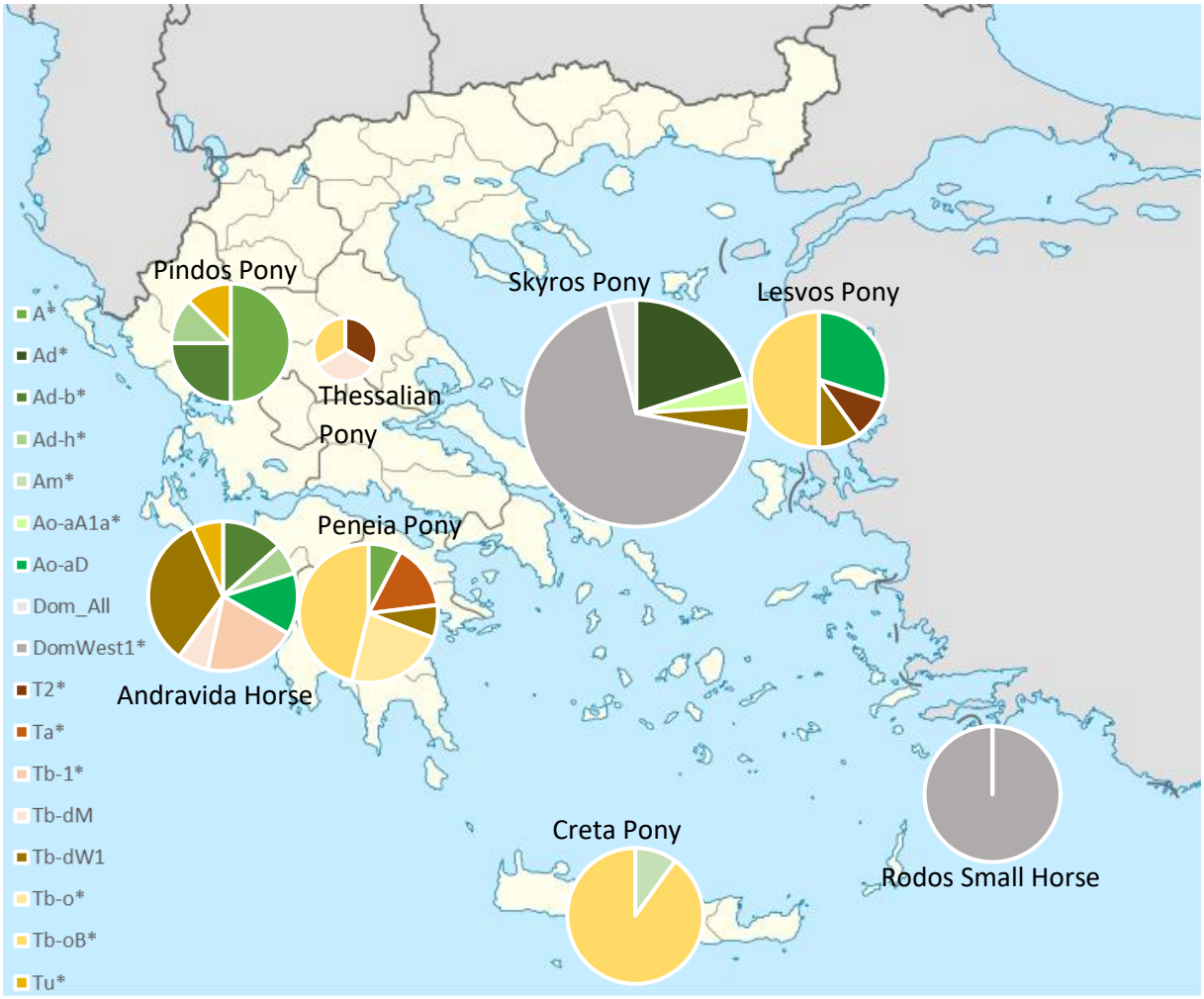


Figure 42: Distribution of the HTs in Greece.

The distribution of the HTs in different regions of Greece is shown. The size of the circles is proportional to the number of samples per breed. For better understanding every HT is depicted in a different colour. HTs inside HG A* are shown in green. HTs which belong to HG

T* are mapped in orange and yellow. The HTs outside the Crown Group are shown in grey colours.

The name of the breeds is written next to the diagrams in black.

https://de.wikipedia.org/wiki/Datei:Greece_location_map.svg (access 09.11.2020)

Exemplarily, the samples of the Andravida Horse showed a wide range of diverse HTs, but an enormous influence of the English Thoroughbred can be asserted. Six out of 15 horses analysed of this breed can be retraced to Darley Arabian because of their HTs Tb-dM and Tb-dW1. Additionally, many of the autochthonous Greek horse breeds from Skyros, Lesvos, the Pindos and Eleia regions showed signatures that are clearly associated with the thoroughbred sire lines after Darley Arabian. It is known that those breeds have been crossbred with Thoroughbreds to fulfil the need for larger riding horses after World War I and II (Hendricks 2007). A fact that is now proven with genetic markers.

Additionally, the Andravida Horse reflects the influence of Norman horses. It is described that Andravidas mares were bred with Nonius stallions to improve the breed (Hendricks 2007). HTs Ad-b* and Ad-h* were detected when analysing samples of the Andravida Horse. In another diploma thesis HTs Ad-h* and Ad-b* were detected as signatures for British and Central European coldbloods (Mühlberger 2019). The impact of Norman horses on this breed is now verified on a genetic basis. Furthermore, three out of eight samples from the Pindos Pony showed the HTs Ad-b* and Ad-h*. The breeding history of this breed is unknown (Hendricks 2007). Following the analysis of Y chromosomal markers an influence of European coldbloods or ponies can be suggested.

In this thesis, it was also possible to determine a pronounced influence of the Arabian Horse on breeds in Greece. Michaelis (2019) managed to assert HTs typical for the Arabian breed. Those HTs were: Ao*, Ao-aA*, Ta* and Ta-a*. These HTs which are specific for the Arabian Horse were determined in samples from the Greek mainland and Greek islands. Some samples from the island of Lesvos and the Andravidas region were allocated to HT Ao-aD* and a few specimens from the island of Skyros were part of HT Ao-aA1a. Both HTs were recently assigned to Arabian Horses by Viktoria Remer (personal communication). The results of this thesis encourage the assumption that the Arabian Horse also has a significant impact on Greek horse breeds.

4.3.2. The influence of breeding on Iranian breeds and their effect on horses worldwide

Local breeds in Iran showed an even more pronounced influence of Arabian Horses. This can be explained with the geographical location close to the Arabian Peninsula. Both the Caspian Miniature Horse and the Kurd Horse reflect this impact as this thesis determined the HTs Ao-aA*, Ao-aA1a, Ao-aA3*, Ao-aD and Ta* which are all specific for the Arabian Horse (see Figure 43). In a study by Sadeghi et al. (2019) SNPs were used to assess matrilineal strains in Persian Arabians. They were able to assert a separate origin of the Persian Arabian from the Caspian Miniature Horse and the Turkoman (Sadeghi et al. 2019). In correspondence to the results of the present study, they observed an interference between Arabian Horses and the Kurdish Horse.

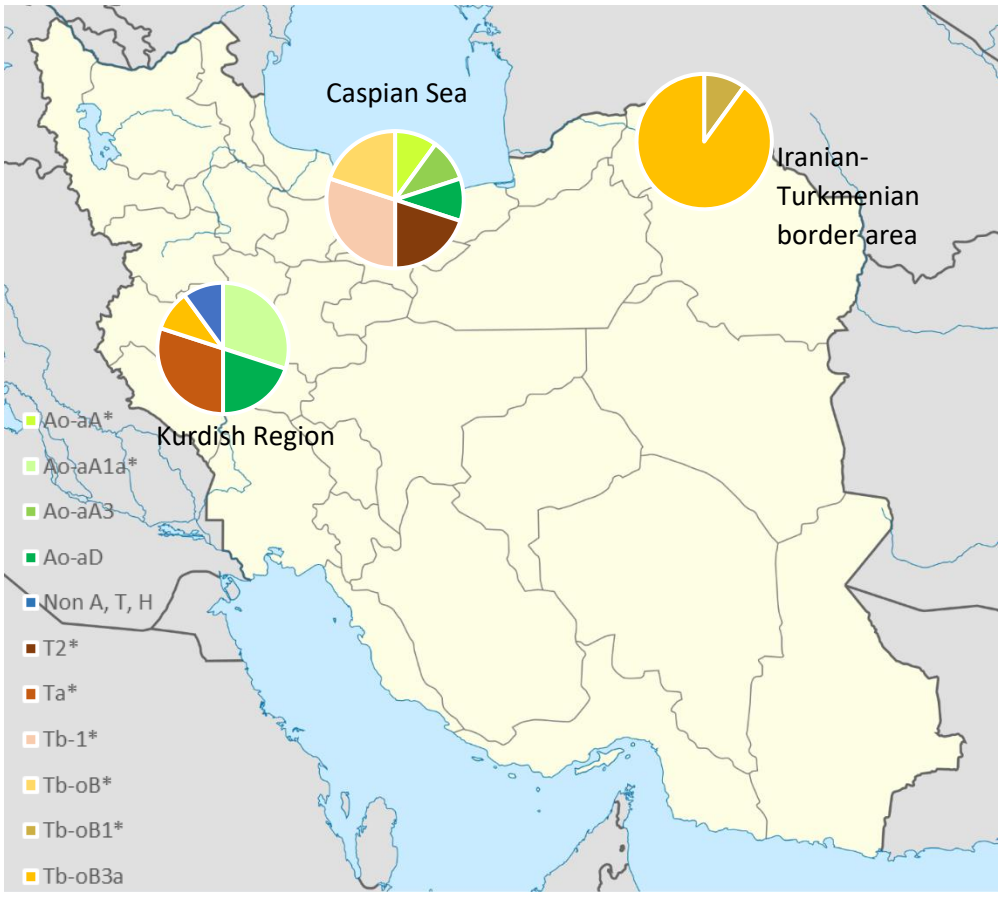


Figure 43: Distribution of the HTs within Iranian breeds.

The distribution of the HTs in different regions of Iran is shown. The circle size is proportional to the number of samples per breed. To get a better overview the HTs detected in Iranian samples are mapped in different colours. HTs inside HG A* are depicted in green. HTs which

belong to HG T* are shown in orange and yellow. The sample which is inside the Crown Group but does not belong to any of the known HGs is depicted in blue.

Important geographic landmarks are written next to the diagrams in black.

https://de.wikipedia.org/wiki/Datei:Iran_location_map.svg (access 10.11.2020)

The forefathers of the English Thoroughbred, the ancient Turkoman and the Arabian Horse, lived in West and Central Asia (Hendricks 2007). One of the founder stallions of the English Thoroughbred is Byerly Turk which carried HT Tb-oB1* (Felkel et al. 2019). It is assumed that he was an ancient Turkoman horse (Hendricks 2007). Thoroughbreds are used as refiners for horse breeds all around the world, due to this excessive use of them the HG Tb* is widely distributed (Hendricks 2007, Felkel et al. 2019). All three analysed Iranian breeds (ten samples per breed) showed at least one sample grouping inside HG Tb*. All ten samples (HT Tb-oB1*, Tb-oB3a) from the Turkoman horse were allocated within HG Tb*. The same results were obtained when analysing the specimen of the Akhal-Teke. Also, this breed has its origin in the Iranian-Turkmenian border area and originated out of the ancient Turkmenian horses around the eight century (Hendricks 2007). It was not possible to determine recent influence (HTs Tb-dW1 or Tb-dM) of crossbreeding Turkomans and Akhal-Tekes with Thoroughbreds. At the moment no further information is available. Also, it is not requested to find the influence of Thoroughbreds on a genetic level within the Arabian and Akhal-Teke horse population.

Additionally, in both the Caspian Miniature Horse and the Kurd Horse the influence of the Tb* clade was evident, but not as strong as in Greek (Lesvos Pony, Creta Pony, Peneia Pony, Andravida Horse) or Caucasian (Armenian Horse, Tushuri) breeds.

4.3.3. The characteristics of the Caucasian horse population

Armenian and Georgian horse breeds also showed pronounced signatures of Thoroughbred and Arabian influence. It was possible to determine the HTs Ao-a*, Ao-aA1a, Ao-aD, Tb-dW1, Tb-oB1* and Tb-oL* (see Figure 45).

The existence of HT Tb-oB1* within this population is a sign of breeding Caucasian breeds with Turkomans or one of the descendants of Byerly Turk. This could be explained by the geographical adjacency to Iran and Turkmenistan and the major influence of the English Thoroughbred. Further investigation is needed to answer this question.

Another interesting finding was the detection of Tb-oL* in one Georgian sample. This HT was initially described in a Lipican horse by Felkel et al. (2019). The Lipizzaner itself was formed by crossbreeding local Karst Horse mares with Spanish stallions. Later, Andalusians, Arabs, Barbs and Neopolitan horses were introduced (Dovc et al. 2006). Further investigation on how HT Tb oL* was (re)introduced to the Caucasus Region needs to be done. Alternatively, it is to be tested in future studies whether HT Tb-oL* has its origin in the Caucasus and was then introduced into the Lipizzaner's.

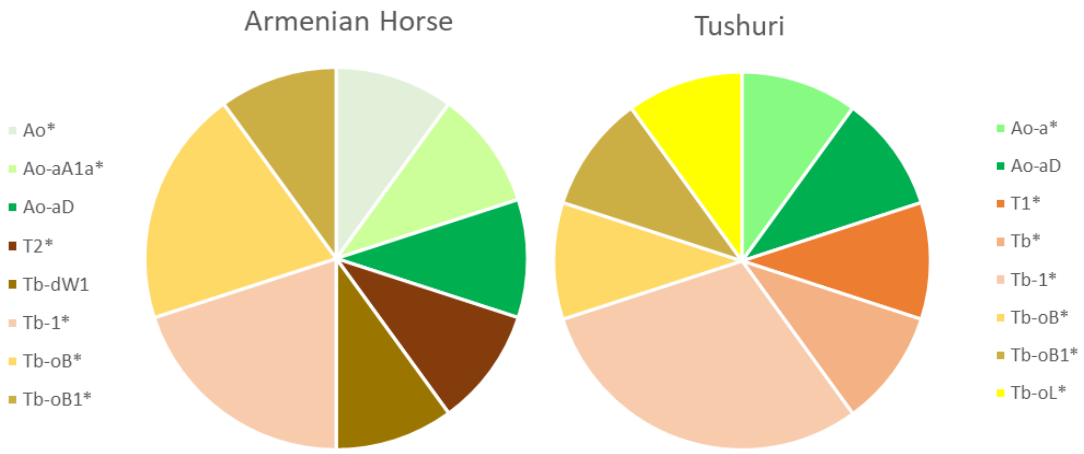


Figure 44: Distribution of HTs within autochthonous breeds in the Caucasus region.

The distribution of the HTs of the samples taken in different regions in Georgia and Armenia is shown. The diagram on the left side is dedicated to the Armenian Horse. On the right side the variety of HTs inside the Tushuri is given. For better visualisation every HT is mapped in a different colour. HTs inside HG A* are shown in green. HTs which belong to HG T* are depicted in orange and yellow.

In conclusion, the effect of reduction of diversity of HTs is variable in the different breeds analysed. The results of this thesis outline that autochthonous breeds in Armenia and Georgia were not able to fully withstand recent breeding influence. The Caucasian breeds show a clustering on internal branching points. Therefore, a unique variability was maintained inside the Crown. Additionally, the region around the Black Sea and the Caspian Sea is known as the region of origin of the Tb* clade. Those findings are confirmed in this thesis. Accessorily, an important finding is the determination of HT Tb-oL* in a breed other than the Lipizzaner.

Future research should include the investigation of other native breeds to gain a more complete understanding of the remaining variability of HTs.

4.4. The importance of autochthonous horse breeds

Both Han et al. (2019) and Chilla (2019) showed in their studies that MSY HT diversity is larger in Asia than in horses on the European or American continent. A large part of the samples in the study by Chilla (2019) was grouped on basal nodes outside of the Crown Group. This is an indicator that breeds in Asia retained genetic variation of a more diverse ancient horse population (Felkel et al. 2018, Han et al. 2019, Chilla 2019).

Nevertheless, HTs which can be found in modern-day breeds were also detected in Asian autochthonous horses. These results show the importance of preserving autochthonous breeds and to prevent excessive breeding with horses which carry modern HTs (Han et al. 2019, Chilla 2019). In this thesis, the samples located on the basal nodes and those which are part of the Non-Crown Group are evidence that autochthonous breeds in Europe account to a greater variety of Y chromosomal HTs than expected. Nonetheless, the enormous influence of English Thoroughbreds and Arabian Horses can be retraced. In every breed analysed except the Rodos Small Horse HTs indicative for Thoroughbreds and Arabians can be found. If these breeds' current genetic status is not preserved, their variety will also diminish and only the predominant HTs will remain.

Apart from the genes, the environment plays an important role in the development of breeds. Horse breeds developed physical characteristics and abilities to survive in their region of origin. Those qualities helped to adapt best to the environment they are living in (Rousseau 2017, Hendricks 2007, Kugler 2010, Cardinali et al. 2016). Horse breeds like the Carpathian, the Caspian, the Chinese Guoxia, the Sardinian Pony, the Skyros Pony and the Turkish Mytilene have two things in common. They resemble more a miniature horse than a pony and the region of their origin lies between the 30° and 40° latitude (Hendricks 2007). For example, Cardinali et al. (2016) analysed ten different Italian horse breeds and asserted the correlation between the surroundings and the exterior of the horses. Both, Italy and Greece, are countries where different horse breeds developed due to the various geographic habitats and cultures. Heavy horses are found in the northern part of the country, whereas on islands and in bay areas smaller horses or ponies developed due to the harsh conditions (Cardinali et al. 2016). Translating those findings to the breeds analysed within this thesis, one can see similar adaptation to the environmental conditions. For example, the largest Greek breed, the Andravida Horse, is found in the northern parts of the Peloponnesus (Hendricks 2007, Kugler 2010). The breeds living on an island or in a bay area (Caspian Miniature Horse, Creta Pony,

Lesvos Pony, Rodos Small Horse, Skyros Pony) have a lower withers height compared to horses living in the mountains or in the steppe (Hendricks 2007, Kugler 2010, Rousseau 2017).

Also, local authorities realised the importance of autochthonous horses for agriculture, culture and tourism. Therefore, breeding programs were initiated and studbooks were established. For example, in 1994 a studbook for the Creta Pony was established and in 2001 the Rodos Small Horse was preserved from extinction when the Faethon foundation was founded (Hendricks 2007, Kugler 2010, Amaltheia 2013b).

The tremendous variety of different Y chromosomal HTs in local breeds is an asset which must be retained and both governments and international organisations should undertake actions to achieve this goal.

4.5. Conclusion

Due to the massive influence of a few breeds on modern-day horse breeds it is more important than ever to preserve this treasure of diversity inside autochthonous breeds. The results of this thesis indicate the importance of additional investigation of remote horse populations. Nevertheless, one must consider that only Y chromosomal markers were used and those markers cannot fully depict the situation on the genome level. Further research should be done to gain a better understanding of the genetic diversity of those breeds.

5. Abstract

Very little is known about the evolutionary history and the influence of other horse breeds on autochthonous breeds in Armenia, Georgia, Greece, Iran and Turkmenistan. In this study the paternal history of horses from those countries was investigated. The male specific part of the Y chromosome (MSY) does not recombine and accordingly the father's MSY region is directly passed on to the male descendants. The MSY is therefore ideal to genetically study the breeding influence of males .

Within this thesis, MSY haplotypes from 153 samples of male horses from the Caucasus region, Western Asia and Greece were determined. The samples were gathered from horses belonging to autochthonous breeds like the Akhal Teke, the Andravida Horse, the Armenian Horse, the Caspian Miniature Horse, the Creta Pony, the Kurd Horse, the Lesvos Pony, the Peneia Pony, the Pindos Pony, the Rodos Small Horse, the Skyros Pony, the Thessalian Pony, the Turkoman and the Tushuri.

45 different Y chromosomal markers were analysed to determine the specific haplotype of the samples, using fluorescent competitive allele specific polymerase chain reaction (KASP® PCR) or QIAxcel® Advanced System and ABI 3130xl Genetic Analyzer. 126 of the 153 DNA samples were assigned to a haplotype within the Crown haplogroup, the predominant haplogroup in modern horses. Within the Crown Group, 36 samples were allocated to the haplogroup A, 89 to haplogroup T and one sample could not be grouped inside any of the previously known haplotypes already described Crown haplogroups. 27 samples from Greek islands did not cluster into the Crown haplogroup. This was the first detection of haplotpyes outside the Crown in Southern Europe.

The results show on the one hand, the influence of recent breeding with Thoroughbreds and Arabians in the autochthonous breeds investigated. On the other hand, the results show that MSY haplotype diversity in autochthonous horse breeds in the European Asian border region is still higher than in modern day breeds.

6. Zusammenfassung

Über die Evolutionsgeschichte und den Einfluss anderer Pferderassen auf autochthone Rassen in Armenien, Georgien, Griechenland, Iran und Turkmenistan ist sehr wenig bekannt. Im Rahmen dieser Arbeit wurde die Geschichte dieser autochtonen Pferderassen mithilfe paternaler Marker untersucht. Der spezifisch männliche Teil des Y-Chromosoms (MSY) rekombiniert nicht und folglich wird die MSY-Region des Vaters direkt an die männlichen Nachkommen weitergegeben. Die MSY ist daher ideal, um den Zuchteinfluss von Hengsten genetisch zu untersuchen.

Im Rahmen dieser Arbeit wurden MSY-Haplotypen von 153 Proben männlicher Pferde aus der Kaukasusregion, Westasien und Griechenland bestimmt. Die Proben stammen von Pferden, die zu autochthonen Rassen wie dem Achal-Tekkiner, dem Andravida Pferd, dem Armenischen Pferd, dem Kaspischen Kleinpferd, dem Kretanischen Pferd, dem Kurdischen Halbblut, dem Mindili Pferd, dem Peneia-Pony, dem Pindos-Pony, dem Rhodos Miniaturpferd, dem Skyros-Pony, dem Thessalischen Pony, dem Turkmenen und dem Tushuri gehören. 45 verschiedene Y-chromosomale Marker wurden analysiert, um den spezifischen Haplotyp der Proben zu bestimmen. Dazu wurde die fluoreszierende kompetitive allelspezifische Polymerase-Kettenreaktion (KASP® PCR), QIAxcel® Advanced System oder ABI 3130xl Genetic Analyzer verwendet. 126 der 153 DNA-Proben konnten einem Haplotyp innerhalb der Kron-Haplogruppe, der vorherrschenden Haplogruppe bei modernen Pferderassen, zugeordnet werden. Innerhalb der Krongruppe gehörten 36 Proben der Haplogruppe A und 89 der Haplogruppe T an. Eine Probe konnte keinem der bisher beschriebenen Haplotypen der Krongruppe zugeordnet werden. 27 Proben von den griechischen Inseln ließen sich nicht in die Kron-Haplogruppe einordnen. Dies war der erste Nachweis von Haplotpyen außerhalb der Krongruppe in Südeuropa.

Die Ergebnisse zeigen einerseits den Einfluss der rezenten Zucht mit Vollblütern und Arabern auf die untersuchten autochthonen Rassen. Andererseits zeigen die Resultate, dass die MSY-Haplotypen-Diversität in autochthonen Pferderassen im europäisch-asiatischen Grenzgebiet immer noch höher ist als bei modernen Sport- und Freizeitpferden.

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Special thanks to collaborators Dr. Nikos Kostaras, President of Amaltheia Association, and Prof. Gus Cothran and Dr. Rytis Juras from Texas A&M University for providing the samples that were analysed as part of this work.

I would also like to thank Dr. rer. nat. Sabine Hammer from the Institute of Immunology at the University of Veterinary Medicine Vienna for making this thesis‘ side project possible.

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9. List of Abbrevations

bp	basepair
BCE	Before Common Era
CE	Common Era
CNV	copy number variant
DAD-IS	Domestic Animal Diversity Information System
dNTP	Deoxynucleotide
FAM	6-Carboxy-Fluorescein
FAO	Food and Agriculture Organisation
FRET	fluorescence resonant energy transfer
HEX	Hexachloro-Fluorescein
HG	haplogroup
HT	haplotype
INDELs	insertion and deletion
KASP	competitive allele-specific PCR
kb	kilobase
mb	megabase
$MgCl_2$	Magnesium Chloride
MRCA	Most Recent Common Ancestor
mtDNA	mitochondrial DNA
MSY	male specific Y chromosomal region
NRY	non-recombining Y chromosome
NTC	Non-template control

RFU	Relative Fluorescence Unit
PAR	pseudoautosomal region
qPCR	quantitative Real-Time PCR
RAPD	randomly amplified polymorphic DNA
RFLP	restriction fragment length polymorphisms
SAVE	Safeguard for Agricultural Varieties in Europe
SNP	single nucleotide polymorphism
SRS	simple sequence repeats
STR	short tandem repeats
Taq	DNA polymerase I Thermus aquaticus
TBE	TRIS-Borat-EDTA-buffer
TE	TRIS-EDTA

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12. Table of Figures

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14. Appendix

14.1. Sequences of the used primers

qCOfwd ACAACCTAAGTGTCTGTGAATGA

qCOrev_b CCCAATAATATTCCACTGCGTGT

14.2. Sequences of the used Y chromosomal markers

qCU

AGTGCTGACAGAAATATAGTTGCCAACCAAGAAAACTATACCCAGCAAAACTGTTATTCA
AAAATGAAGTAGAAATTAAGACATTCTCAGACAAACAGAAGCTGGGAGAACTCATTACCA
CTAGAGCTGCTCTACAACAAATGTTAAAG[T/A]GGTGGCTTAAGTCAAACGAAAGAGTGC
CAGACAGCAGACAACAACATGCATATAAAGCTCTCTGGTAAAGGTCAATGCATAAAGAC
AAATATTGATTTCTGCAGTATTGTAACGTAGGGACATATATCACTTTTAATTCTGTATAAA
ATTT

qDR

GAGAATGTGAAAGAATAATTAATAAAACATGCTCAGGGATCAAAAAAGTAGGGCAATGAT
ATTCAATGTTAGAGTTTTATCAATGTTGAAGAATCATCCAACCATGTGTATGATACTCATA
TTTTGATCCAGATAATGGGAGAAATCAT[G/T]AGTGCCAATAGGGCTCAACTCATAATATG
TGTTTGAAAATTTTCCCATGCTATATTTTCAAAAGACTTATCTAAAAGAAGTGCTATTTTGT
GCCTTTGTTTATGAAGATATCTTCTCAGAGGGGGGAAAACCAAACTGGAGTAAAACAAAT

qEW

GATACATCTTCTTGTAGAAAATGACTAGTCATGCTTCCCTGGCGCATACTTTTGCAAACT
ATTTGCCCTATTGGCCTCTCAGGGCTCTTTGCAAACTTTTTGCTCTGTGATTTTTCTGTAT
AGTTCACAACAATGTAAGAGAAATGTTC[T/C]ATATATTTAAAAACAATTTCAAATACTTTT
TATTTCGTCCTTGAAGTGAAGTACTTCAGCAGTAATGCAACTCAACTCTGAGTGTCTAGA
TCAGTGCTTTTCAAAATTTAGCTATTTTAAAAGCAGCTTGTTTAAATACAGCTTCTTGAGC

qEL

CTCAAAGGCTATTTCTGAAATTCATATAGAAAAGTAAAAATAGCGTTATCTAAAACTGCAG

ATTTTCTATACATACAATTGCAACGCGTTATATGATCTTCTCTACCTTTGTCTTTTTCTATT
TTAAATATTTGTCTTCTTAGATTATC[A/G]CTGTAGAACGGTGTCATTGTTTTAGTATATTC
ACAATATTTCACTGAGTGGGTGAACCATAATTTATTTAGTGTTGTCTGTTGGTGAGCATT
CAAATTATTTTAAGGTTTTAATCATGTAAATAGTGCTTCCCATTATTATACTGTGTGAT

14.3. Distribution of haplotypes within the samples

Table 25: Samples tested with markers rAX, rB, rA, fVZ, rW, fZC, sE, qCU, rOR, rDT, rAF, sQF and rX are shown in the table below. The mutation leading to the haplotype is marked yellow.

Sample	Country	rAX	rB	rA	fVZ	rW	fZC	sE	qCU	rOR	rDT	rAF	sQF	rX
Y_PR_19_050	Greece	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_T_0	rAF_G_0	sQF_G_0	rX_G_0
Y_PR_19_060	Greece	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_T_0	rAF_G_0	sQF_G_0	rX_G_0
Y_PR_19_062	Greece	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_T_0	rAF_G_0	sQF_G_0	rX_G_0
Y_PR_19_065	Greece	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_T_0	rAF_G_0	sQF_G_0	rX_G_0
Y_PR_19_068	Greece	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_T_0	rAF_G_0	sQF_G_0	rX_G_0

Table 26: Samples tested with markers rAX, rB, rW, fZC, sE, qCU, rOR, rDT and rAF are shown in the table below.

The mutation leading to the haplotype is marked yellow.

Sample	Country	rAX	rB	rW	fZC	sE	qCU	rOR	rDT	rAF
Y_PR_19_196	Greece	rAX_C_1	rB_C_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_T_0	rAF_A_1
Y_PR_19_200	Greece	rAX_C_1	rB_C_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_T_0	rAF_A_1
Y_PR_19_201	Greece	rAX_C_1	rB_C_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_T_0	rAF_A_1
Y_PR_19_210	Greece	rAX_C_1	rB_C_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_T_0	rAF_A_1
Y_PR_19_212	Greece	rAX_C_1	rB_C_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_T_0	rAF_A_1

Table 27: Samples tested with markers rAX, rB, rA, fVZ, rW, fZC, sE, qCU, rOR and rDT are shown in the table below.

The mutation leading to the haplotype is marked yellow.

Sample	Country	rAX	rB	rA	fVZ	rW	fZC	sE	qCU	rOR	rDT
Y_PR_19_059	Greece	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_C_1
Y_PR_19_063	Greece	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_C_1

Table 28: Samples tested with markers rAX, rB, rW, fZC, sE, qCU, rOR and rDT are shown in the table below.

The mutation leading to the haplotype is marked yellow.

Sample	Country	rAX	rB	rW	fZC	sE	qCU	rOR	rDT
Y_PR_19_221	Greece	rAX_C_1	rB_C_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_C_1
Y_PR_19_222	Greece	rAX_C_1	rB_C_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_C_1

Table 29: The sample tested with the markers rAX, rB, rA, fVZ, rW, fZC, sE, qCU and rOR is shown in the table below.

The mutation leading to the haplotype is marked yellow.

Sample	Country	rAX	rB	rW	fVZ	rW	fZC	sE	qCU	rOR
Y_PR_19_066	Greece	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_A_1

Table 30: The sample tested with the markers rAX, rB, rW, fZC, sE, qCU and rOR is shown in the table below.

The mutation leading to the haplotype is marked yellow.

Sample	Country	rAX	rB	rW	fZC	sE	qCU	rOR
Y_PR_19_223	Greece	rAX_C_1	rB_C_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_A_1

Table 31: The sample tested with rAX, rB, rW, fZC, sE and qCU is shown in the table below.

The mutation leading to the haplotype is marked yellow.

Sample	Country	rAX	rB	rW	fZC	sE	qCU
Y_PR_19_169	Greece	rAX_C_1	rB_C_0	rW_A_1	fZC_T_0	sE_C_0	qCU_A_1

Table 32: The sample tested with markers rAX, rB, rA, fVZ, rW, fZC, sE, qCU, rOR, rDT, rAF, sQF and rX is shown in the table below.

The mutation leading to the haplotype is marked yellow.

Sample	Country	rAX	rB	rA	fVZ	rW	fZC	sE	qCU	rOR	rDT	rAF	sQF	rX
Y_PR_19_025	Armenia	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_T_0	sE_C_0	qCU_T_0	rOR_G_0	rDT_T_0	rAF_G_0	sQF_G_0	rX_T_1

Table 33: The sample tested with markers rAX, rB, rW, fZC, rY and fST is shown in the table below.

The mutation leading to the haplotype is marked yellow.

Sample	Country	rAX	rB	rW	fZC	rY	fST	QDR	sAN
Y_PR_19_238	Georgia	rAX_C_1	rB_C_0	rW_A_1	fZC_C_1	rY_ACC_0	fST_T_0	qDR_G_0	sAN_G_0

Table 34: The sample tested with markers rAX, rB, rA, fVZ, rW, fZC, rY, fST, fUS and qEW is shown in the table below.

Mutations leading to the haplotype are shown in yellow.

Sample	Country	rAX	rB	rA	fVZ	rW	fZC	rY	fST	fUS	qEW
Y_PR_19_077	Iran	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_ACC_0	fST_A_1	fUS_T_0	qEW_T_0

Table 35: The samples tested with the markers rAX, rB, rA, fVZ, rW, fZC and rY are shown in the table below.

Mutations leading to the haplotype are shown in yellow.

Sample	Country	rAX	rB	rA	fVZ	rW	fZC	rY
Y_PR_19_005	Iran	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_AC_1
Y_PR_19_007	Iran	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_AC_1
Y_PR_19_009	Iran	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_AC_1
Y_PR_19_028	Georgia	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_AC_1

Table 36: The sample tested with the markers rAX, rB, rW, fZC and rY are shown in the table below.

Mutations leading to the haplotype are shown in yellow.

Sample	Counry	rAX	rB	rW	fZC	rY
Y_PR_19_209	Greece	rAX_C_1	rB_C_0	rW_A_1	fZC_C_1	rY_AC_1

Table 37: The sample tested with the markers rAX, rB, rA, fVZ, rW, fZC, rY, fST, fUS and qEW is shown in the table below.

Mutations leading to the haplotype are shown in yellow.

Sample	Country	rAX	rB	rA	fVZ	rW	fZC	rY	fST	fUS	qEW
Y_PR_19_069	Iran	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_ACC_0	fST_A_1	fUS_T_0	qEW_C_1

Table 38: The samples tested with the markers rAX, rB, rA, fVZ, rW, fZC, rY, fST and qDR are shown in the table below.

Mutations leading to the haplotype are marked in yellow.

Sample	Country	rAX	rB	rA	fVZ	rW	fZC	rY	fST	qDR
Y_PR_19_004	Iran	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_ACC_0	fST_T_0	qDR_T_1
Y_PR_19_006	Iran	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_ACC_0	fST_T_0	qDR_T_1
Y_PR_19_032	Greece	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_ACC_0	fST_T_0	qDR_T_1
Y_PR_19_035	Greece	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_ACC_0	fST_T_0	qDR_T_1
Y_PR_19_070	Iran	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_ACC_0	fST_T_0	qDR_T_1

Table 39: The sample tested with the markers rAX, rB, rA, fVZ, rW, fZC,rY, fST, rDT and qDR is shown in the table below.

Mutations leading to the haplotype are marked in yellow.

Sample	Country	rAX	rB	rA	fVZ	rW	fZC	rY	fST	rDT	qDR
Y_PR_19_030	Georgia	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_A_1	fZC_C_1	rY_ACC_0	fST_T_0	rDT_T_0	qDR_T_1

Table 40: The samples tested with the markers rAX, rB, rW, fZC ,rY, fST and qDR are shown in the table below.

Mutations leading to the haplotype are marked in yellow.

Sample	Country	rAX	rB	rW	fZC	rY	fST	qDR
Y_PR_19_033	Greece	rAX_C_1	rB_C_0	rW_A_1	fZC_C_1	rY_ACC_0	fST_T_0	qDR_T_1
Y_PR_19_218	Greece	rAX_C_1	rB_C_0	rW_A_1	fZC_C_1	rY_ACC_0	fST_T_0	qDR_T_1
Y_PR_19_229	Georgia	rAX_C_1	rB_C_0	rW_A_1	fZC_C_1	rY_ACC_0	fST_T_0	qDR_T_1

Table 41: The sample tested with the markers rAX, rW, fZC ,rY, fST and qDR is shown in the table below.

Mutations leading to the haplotype are marked in yellow.

Sample	Country	rAX	rW	fZC	rY	fST	qDR
Y_PR_19_175	Greece	rAX_C_1	rW_A_1	fZC_C_1	rY_ACC_0	fST_T_0	qDR_T_1

Table 42: The sample tested with the markers rAX, rBG, sES, sCO and fAR is shown in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rBG	sES	sCO	fAR
Y_PR_19_195	Greece	rAX_T_0	rBG_CTT_0	sES_C_0	sCO_A_0	fAR_A_1

Table 43: The samples tested with the markers rAX, rBG, rB, rA, fVZ and sES are shown in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rBG	rB	rA	fVZ	sES
Y_PR_19_051	Greece	rAX_T_0	rBG_CTT_0	rB_C_0	rA_T_0	fVZ_G_0	sES_A_1
Y_PR_19_052	Greece	rAX_T_0	rBG_CTT_0	rB_C_0	rA_T_0	fVZ_G_0	sES_A_1
Y_PR_19_053	Greece	rAX_T_0	rBG_CTT_0	rB_C_0	rA_T_0	fVZ_G_0	sES_A_1
Y_PR_19_054	Greece	rAX_T_0	rBG_CTT_0	rB_C_0	rA_T_0	fVZ_G_0	sES_A_1
Y_PR_19_056	Greece	rAX_T_0	rBG_CTT_0	rB_C_0	rA_T_0	fVZ_G_0	sES_A_1
Y_PR_19_057	Greece	rAX_T_0	rBG_CTT_0	rB_C_0	rA_T_0	fVZ_G_0	sES_A_1
Y_PR_19_058	Greece	rAX_T_0	rBG_CTT_0	rB_C_0	rA_T_0	fVZ_G_0	sES_A_1

Table 44: The samples tested with the markers rAX, rBG and sES are shown in the the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rBG	sES

Y_PR_19_182	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_183	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_189	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_190	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_191	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_193	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_197	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_198	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_199	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_202	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_203	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_204	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_205	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_206	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_207	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_208	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_211	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_213	Greece	rAX_T_0	rBG_CTT_0	sES_A_1
Y_PR_19_214	Greece	rAX_T_0	rBG_CTT_0	sES_A_1

Table 45: The sample tested with the markers rAX, rB, rW, fYR, rA, fVZ and qEL is shown in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rB	rW	fYR	rA	fVZ	qEL
Y_PR_19_232	Georgia	rAX_C_1	rB_C_0	rW_G_0	fYR_T_0	rA_A_1	fVZ_G_0	qEL_A_0

Table 46: The samples tested with the markers rAX, rB, rA, fVZ, sPZ and rT are shown in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rB	rA	fVZ	sPZ	rT
Y_PR_19_024	Georgia	rAX_C_1	rB_C_0	rA_A_1	fVZ_C_1	sPZ_A_0	rT_C_0
Y_PR_19_037	Greece	rAX_C_1	rB_C_0	rA_A_1	fVZ_C_1	sPZ_A_0	rT_C_0
Y_PR_19_074	Iran	rAX_C_1	rB_C_0	rA_A_1	fVZ_C_1	sPZ_A_0	rT_C_0
Y_PR_19_076	Iran	rAX_C_1	rB_C_0	rA_A_1	fVZ_C_1	sPZ_A_0	rT_C_0

Table 47: The sample tested with the markers rAX, rB, rW, fYR, rA, fVZ, sPZ and rT is shown in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rB	rW	fYR	rA	fVZ	sPZ	rT
Y_PR_19_180	Greece	rAX_C_1	rB_C_0	rW_G_0	fYR_T_0	rA_A_1	fVZ_C_1	sPZ_A_0	rT_C_0

Table 48: The samples tested with the markers rAX, rB, rA, fVZ and sPZ are shown in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rB	rA	fVZ	sPZ
Y_PR_19_001	Iran	rAX_C_1	rB_C_0	rA_A_1	fVZ_C_1	sPZ_T_1
Y_PR_19_008	Iran	rAX_C_1	rB_C_0	rA_A_1	fVZ_C_1	sPZ_T_1
Y_PR_19_010	Iran	rAX_C_1	rB_C_0	rA_A_1	fVZ_C_1	sPZ_T_1

Table 49: The sample tested with the markers rAX, rB, rW, fYR, rA, fVZ and sPZ are shown in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rB	rW	fYR	rA	fVZ	sPZ
Y_PR_19_225	Greece	rAX_C_1	rB_C_0	rW_G_0	fYR_T_0	rA_A_1	fVZ_C_1	sPZ_T_1

Table 50: The sample tested with the markers rAX, rB, fYR, rA, fVZ and sPZ is shown in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rB	fYR	rA	fVZ	sPZ
Y_PR_19_226	Greece	rAX_C_1	rB_C_0	fYR_T_0	rA_A_1	fVZ_C_1	sPZ_T_1

Table 51: The sample tested with the markers rAX, rB, fWU, rD and fAAC is shown in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rB	fWU	rD	fAAC
Y_PR_19_230	Georgia	rAX_C_1	rB_G_1	fWU_T_0	rD_TT_0	fAAC_A_0

Table 52: The samples tested with the markers rAX, rB, rC, fWU, rD and fAAC are shown in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rB	rC	fWU	rD	fAAC
Y_PR_19_023	Georgia	rAX_C_1	rB_G_1	rC_T_0	fWU_T_0	rD_TT_0	fAAC_T_1
Y_PR_19_026	Armenia	rAX_C_1	rB_G_1	rC_T_0	fWU_T_0	rD_TT_0	fAAC_T_1
Y_PR_19_072	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_T_0	rD_TT_0	fAAC_T_1
Y_PR_19_073	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_T_0	rD_TT_0	fAAC_T_1
Y_PR_19_078	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_T_0	rD_TT_0	fAAC_T_1
Y_PR_19_216	Greece	rAX_C_1	rB_G_1	rC_T_0	fWU_T_0	rD_TT_0	fAAC_T_1
Y_PR_19_234	Georgia	rAX_C_1	rB_G_1	rC_T_0	fWU_T_0	rD_TT_0	fAAC_T_1
Y_PR_19_236	Georgia	rAX_C_1	rB_G_1	rC_T_0	fWU_T_0	rD_TT_0	fAAC_T_1
Y_PR_19_237	Georgia	rAX_C_1	rB_G_1	rC_T_0	fWU_T_0	rD_TT_0	fAAC_T_1

Table 53: The samples tested with the markers rAX, rB, rC, rW, rD and fAAC are shown in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rB	rC	rW	rD	fAAC
Y_PR_19_065	Greece	rAX_C_1	rB_G_1	rC_T_0	rW_G_0	rD_TT_0	fAAC_T_1
Y_PR_19_176	Greece	rAX_C_1	rB_G_1	rC_T_0	rW_G_0	rD_TT_0	fAAC_T_1
Y_PR_19_178	Greece	rAX_C_1	rB_G_1	rC_T_0	rW_G_0	rD_TT_0	fAAC_T_1

Table 54: The sample tested with the markers rAX, rB, rC, fWU, rD, fAAC, fWM and rG is shown in the table below.

Mutations leading to the haplotype are marked in yellow.

Sample	Country	rAX	rB	rC	fWU	rD	fAAC	fWM	rG
Y_PR_19_179	Greece	rAX_C_1	rB_G_1	rC_T_0	fWU_T_0	rD_TT_0	fAAC_T_1	fWM_T_0	rG_G_1

Table 55: The sample tested with the markers rAX, rB, rC, fWU, rD, fAAC, fWM, qGH, sP and rG is shown in the table below.

Mutations leading to the haplotype are marked in yellow.

Sample	Country	rAX	rB	rC	fWU	rD	fAAC	fWM	qGH	sP	rG
Y_PR_19_217	Greece	rAX_C_1	rB_G_1	rC_C_1	fWU_T_0	rD_TT_0	fAAC_T_1	fWM_T_0	qGH_G_0	sP_A_0	rG_G_1

Table 56: The samples tested with the markers rAX, rB, rC and rD are shown in the table below.

Mutations leading to the haplotype are marked in yellow.

Sample	Country	rAX	rB	rC	rD
Y_PR_19_027	Georgia	rAX_C_1	rB_G_1	rC_C_1	rD_DEL_1
Y_PR_19_040	Greece	rAX_C_1	rB_G_1	rC_C_1	rD_DEL_1
Y_PR_19_048	Greece	rAX_C_1	rB_G_1	rC_C_1	rD_DEL_1

Table 57: The samples tested with the markers rAX, rB, rA, rW, fWU and rD are shown in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rB	rA	rW	fWU	rD
Y_PR_19_172	Greece	rAX_C_1	rB_G_1	rA_A_1	rW_G_0	fWU_T_0	rD_DEL_1
Y_PR_19_173	Greece	rAX_C_1	rB_G_1	rA_A_1	rW_G_0	fWU_T_0	rD_DEL_1
Y_PR_19_174	Greece	rAX_C_1	rB_G_1	rA_A_1	rW_G_0	fWU_T_0	rD_DEL_1
Y_PR_19_177	Greece	rAX_C_1	rB_G_1	rA_A_1	rW_G_0	fWU_T_0	rD_DEL_1

Table 58: The samples tested with the markers rAX, rB, fWU and rD are shown in the table below.

Mutations leading to the haplotype are marked in yellow.

Sample	Country	rAX	rB	fWU	rD
Y_PR_19_192	Greece	rAX_C_1	rB_G_1	fWU_T_0	rD_DEL_1
Y_PR_19_219	Greece	rAX_C_1	rB_G_1	fWU_T_0	rD_DEL_1

Table 59: The samples tested with the markers rAX, rB, rC, fWU, fWY, rJ, fUJ and rN are shown in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rB	rC	fWU	fWY	rJ	fUJ	rN
Y_PR_19_042	Greece	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_T_0	rN_G_0
Y_PR_19_047	Greece	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_T_0	rN_G_0

Table 60: The sample tested with the markers rAX, rB, rA, rC, fQI, fWU, fAAC, fUJ and rN are shown in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rB	rA	rC	fQI	fWU	fAAC	fUJ	rN
Y_PR_19_046	Greece	rAX_C_1	rB_G_1	rA_A_1	rC_T_0	fQI_G_0	fWU_A_1	fAAC_A_0	fUJ_T_0	rN_G_0

Table 61: The samples tested with the markers rAX, rB, rC, fWU, fWY, rJ, fUJ, fQI and rP are shown in the table below.

Mutations leading to the haplotype are marked in yellow.

Sample	Country	rAX	rB	rC	fWU	fWY	rJ	fUJ	fQI	rP
Y_PR_19_022	Georgia	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_029	Georgia	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_031	Greece	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_034	Greece	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_036	Greece	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_038	Greece	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_039	Greece	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_041	Greece	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_043	Greece	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_045	Greece	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_049	Greece	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_071	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_075	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0

Table 62: The samples tested with the markers rAX, rB, fWU, fWY, rJ, fUJ, fQI and rP are shown in the table below.

Mutations leading to the haplotype are marked in yellow.

Sample	Country	rAX	rB	fWU	fWY	rJ	fUJ	fQI	rP
Y_PR_19_162	Greece	rAX_C_1	rB_G_1	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0

Y_PR_19_163	Greece	rAX_C_1	rB_G_1	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_164	Greece	rAX_C_1	rB_G_1	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_165	Greece	rAX_C_1	rB_G_1	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_168	Greece	rAX_C_1	rB_G_1	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_170	Greece	rAX_C_1	rB_G_1	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_171	Greece	rAX_C_1	rB_G_1	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_181	Greece	rAX_C_1	rB_G_1	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_224	Greece	rAX_C_1	rB_G_1	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0
Y_PR_19_233	Greece	rAX_C_1	rB_G_1	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	rP_T_0

Table 63: The sample tested with the markers rAX, rB, fWU, fWY, fUJ, fQI and rP is shown in the table below.

Mutations leading to the haplotype are marked in yellow.

Sample	Country	rAX	rB	fWU	fWY	fUJ	fQI	rP
Y_PR_19_166	Greece	rAX_C_1	rB_G_1	fWU_A_1	fWY_G_0	fUJ_C_1	fQI_G_0	rP_T_0

Table 64: The samples tested with the markers rAX, rB, rC, fWU and fWY are shown in the table below.

Mutations leading to the haplotype are marked in yellow.

Sample	Country	rAX	rB	rC	fWU	fWY

Y_PR_19_002	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_A_1
Y_PR_19_011	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_A_1
Y_PR_19_012	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_A_1
Y_PR_19_013	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_A_1
Y_PR_19_015	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_A_1
Y_PR_19_016	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_A_1
Y_PR_19_017	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_A_1
Y_PR_19_018	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_A_1
Y_PR_19_019	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_A_1
Y_PR_19_020	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_A_1

Table 65: The samples tested with the markers rAX, rB, fWU and fWY are mentioned in the table below.

The mutation leading to the haplotype is marked in yellow.

Sample	Country	rAX	rB	fWU	fWY
Y_PR_19_142	Turkmenistan	rAX_C_1	rB_G_1	fWU_A_1	fWY_A_1
Y_PR_19_143	Turkmenistan	rAX_C_1	rB_G_1	fWU_A_1	fWY_A_1
Y_PR_19_144	Turkmenistan	rAX_C_1	rB_G_1	fWU_A_1	fWY_A_1
Y_PR_19_145	Turkmenistan	rAX_C_1	rB_G_1	fWU_A_1	fWY_A_1
Y_PR_19_147	Turkmenistan	rAX_C_1	rB_G_1	fWU_A_1	fWY_A_1

Y_PR_19_148	Turkmenistan	rAX_C_1	rB_G_1	fWU_A_1	fWY_A_1
Y_PR_19_149	Turkmenistan	rAX_C_1	rB_G_1	fWU_A_1	fWY_A_1
Y_PR_19_150	Turkmenistan	rAX_C_1	rB_G_1	fWU_A_1	fWY_A_1
Y_PR_19_151	Turkmenistan	rAX_C_1	rB_G_1	fWU_A_1	fWY_A_1

Table 66: The sample tested with the markers rAX, rB, fWU, fWY, rJ, fUJ, rN and fQI is shown in the table below.

The Mutations leading to the haplotype are marked in yellow.

Sample	Country	rAX	rB	fWU	fWY	rJ	fUJ	rN	fQI
Y_PR_19_231	Georgia	rAX_C_1	rB_G_1	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_heterozygote	rN_A_1	fQI_G_0

Table 67: The samples tested with the markers rAX, rB, rC, fWU, fWY, rJ, fUJ, fQI and fBVB are shown in the table below.

The mutations leading to the haplotype are marked in yellow.

Sample	Country	rAX	rB	rC	fWU	fWY	rJ	fUJ	fQI	fBVB
Y_PR_19_014	Iran	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	fBVB_208_1
Y_PR_19_021	Georgia	rAX_C_1	rB_G_1	rC_T_0	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	fBVB_208_1

Table 68: The samples tested with the markers rAX, rB, fWU, fWY, rJ, fUJ, fQI and fBVB are shown in the table below.

The mutations leading to the haplotype are marked in yellow.

Sample	Country	rAX	rB	fWU	fWY	rJ	fUJ	fQI	fBVB
Y_PR_19_146	Turkmenistan	rAX_C_1	rB_G_1	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	fBVB_208_1
Y_PR_19_235	Turkmenistan	rAX_C_1	rB_G_1	fWU_A_1	fWY_G_0	rJ_G_0	fUJ_C_1	fQI_G_0	fBVB_208_1

Table 69: The sample tested with the markers rAX, rB, rW, sPZ, rA, fVZ and rT is mentioned in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rB	rW	sPZ	rA	fVZ	rT
Y_PR_19_220	Greece	rAX_C_1	rB_C_0	rW_G_0	sPZ_A_0	rA_A_1	fVZ_C_1	rT_T_1

Table 70: The sample tested with the markers rAX, rB, rA, fVZ, rW and fYR is mentioned in the table below.

The mutation leading to the haplotype is shown in yellow.

Sample	Country	rAX	rB	rA	fVZ	rW	fYR
Y_PR_19_003	Iran	rAX_C_1	rB_C_0	rA_T_0	fVZ_G_0	rW_G_0	fYR_T_0