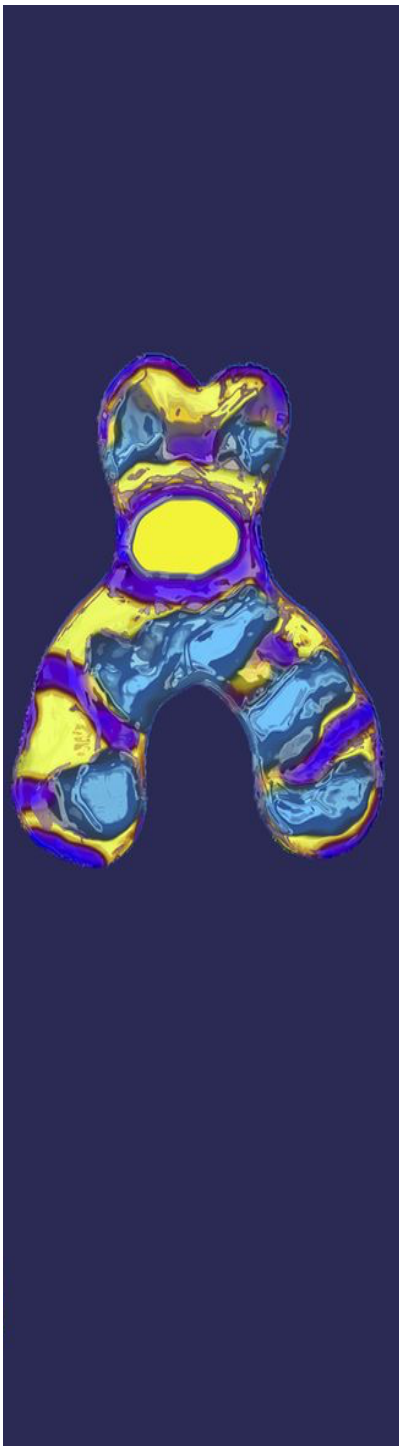

Northern European horse breeds: a close look at their ancestry from the male perspective

vetmeduni vienna

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1.0 Introduction

The ancestor of the horse (Equus ferus) migrated across the Bering strait land bridge 2.6 million years ago originally colonising eastern Eurasia before expanding across the continent around 160 thousand years ago (Hendricks, 2007; Lindsay, Opdyke, & Johnson, 1980; Warmuth et al., 2012). The modern day horse (Equus ferus callabus) was domesticated around 5.5 thousand years ago coinciding with the rapid development of human civilisation, enabling rapid communication, transforming trade routes and spreading language and faith across the continent to northern Europe (Frankopan, 2015; Kelekna, 2009; Warmuth et al., 2013). The site or sites of domestication are unknown however, presumed sites include: The Pontic Caspian Steppe and the northern steppe of Kazakhstan due to the high prevalence of Eneolithic remains (Anthony & Ringe, 2015; Zeder, Bradley, Smith, & Emshwiller, 2006). The earliest domestic horse sample was excavated in Hungary which is close to Ukraine and in line with the theory described by Warmuth et al., (2012) that domestication occurred in western Eurasia. This marks the first era in which horse movement was influenced by humans.

Domestic horse herds were composed of a number of mares and comparatively few stallions. The use of only a few stallions at domestication, probably because they were difficult to catch and handle, caused a severe loss of diversity resulting in the Y chromosome being very similar in all modern horses. The Y chromosome is only present in males and is preserved as it is passed from father to son. Lack of changes in the Y chromosome as it is passed from father to son enables us to identify the sire line and use it to trace the movement of horses (Jobling & Tyler-Smith, 2003).

Previous studies using this method have already discovered that there was a second latter movement of horse starting with Neapolitan Spanish horses during the 16th century and continuing into the 19th century with the movement of Arabian and Turkoman horses responsible for the foundation of the English Thoroughbred. Organised breeding strategies further reduced the diversity in the Y chromosome; as a result, the majority of modern horse breeds descend paternally from only a few oriental stallions which originate from a group of horses that had the same Y chromosome around 700 years ago (Wallner et al., 2017). This is known as a crown group and is shown by the shaded region of figure 1

below. The oriental stallions can be divided in to lineages of Arabian and Turkoman descent, shown in figure 2 below. Most domestic horses therefore only represent a fraction of the male lineages which would have been present in the ancestor Equus ferus which roamed the earth 5.5 thousands years ago (Wallner et al., 2017).

Having said this, some Northern European native breeds, such as the: Shetland Pony, Icelandic horse, Norwegian Fjord and Swedish Coldblood horse, escaped this second influential wave and form private Y chromosome groups outside of the crown separating from the common ancestor at different time periods, see figure 2 below. For example, the Shetland pony diverges from the common ancestor around 1,000-1,300 years ago (Felkel et al., 2018). Northern European breeds may therefore be pivotal for the discovery of ancient migratory pathways and studying these breeds could provide a fundamental link between the modern horse and the Equus ancestor.

Our questions therefore are:

- How do native breeds to the UK, Irish and other Northern European horses fit into this picture?
- Have these horses been influenced and if so by which breeds?

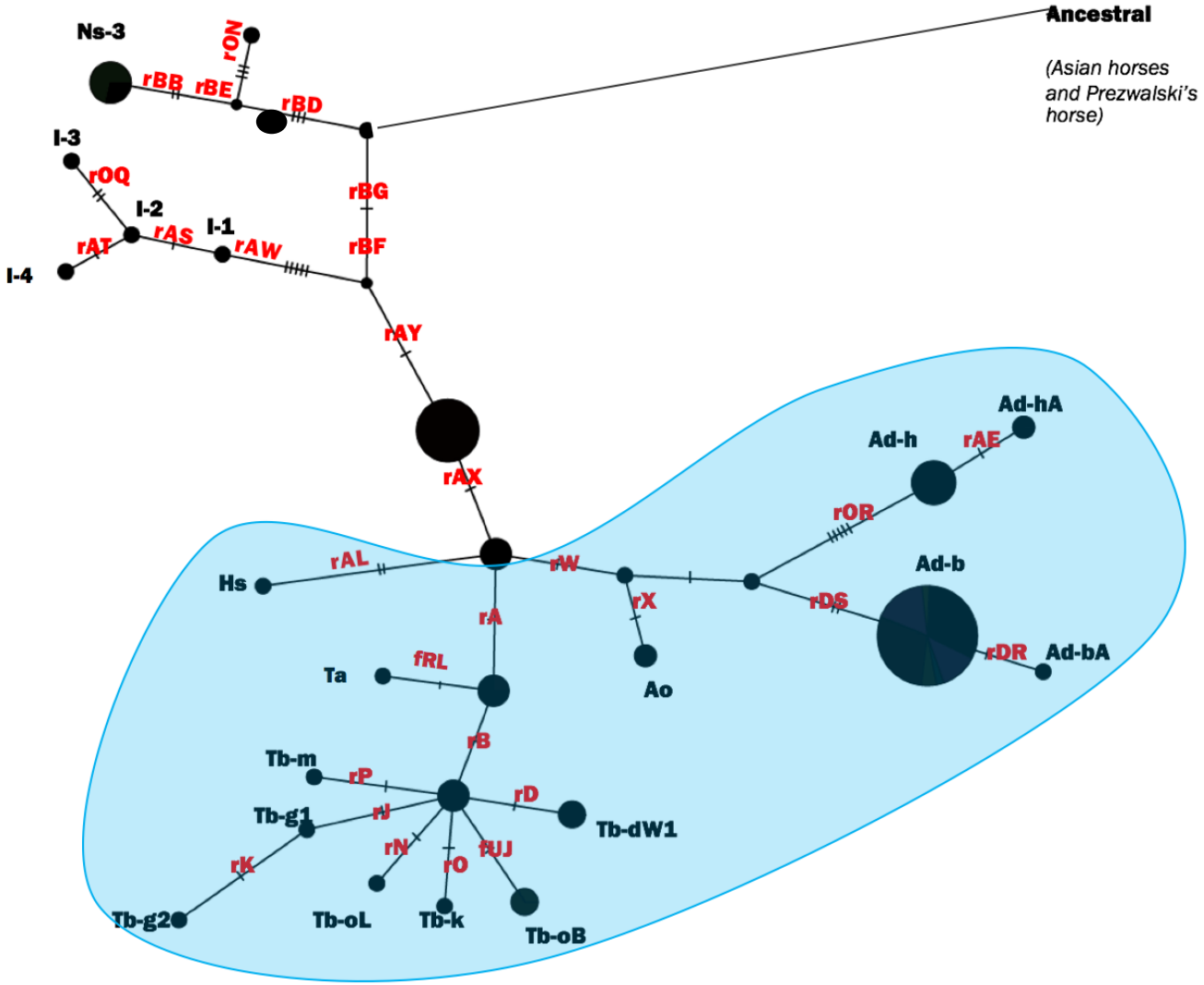

Figure 1: Y chromosome tree showing the crown group (shaded blue) containing breeds affected by the Spanish and Oriental movement adapted from Wallner et al., (2017):

Nf = Norwegian Fjord Ns-3 = Shetland I 1-4 = Icelandic Hs= horse of Spanish origin (Lippizan, Lusitano, Haflinger) Ta= Modern Arabs Tb = Turkoman Ad-h = Coldblooded European horses Ad-hA = Coldbooded German Draught Ad-b = Friesian Ad-bA= Connemara Ao = Arab

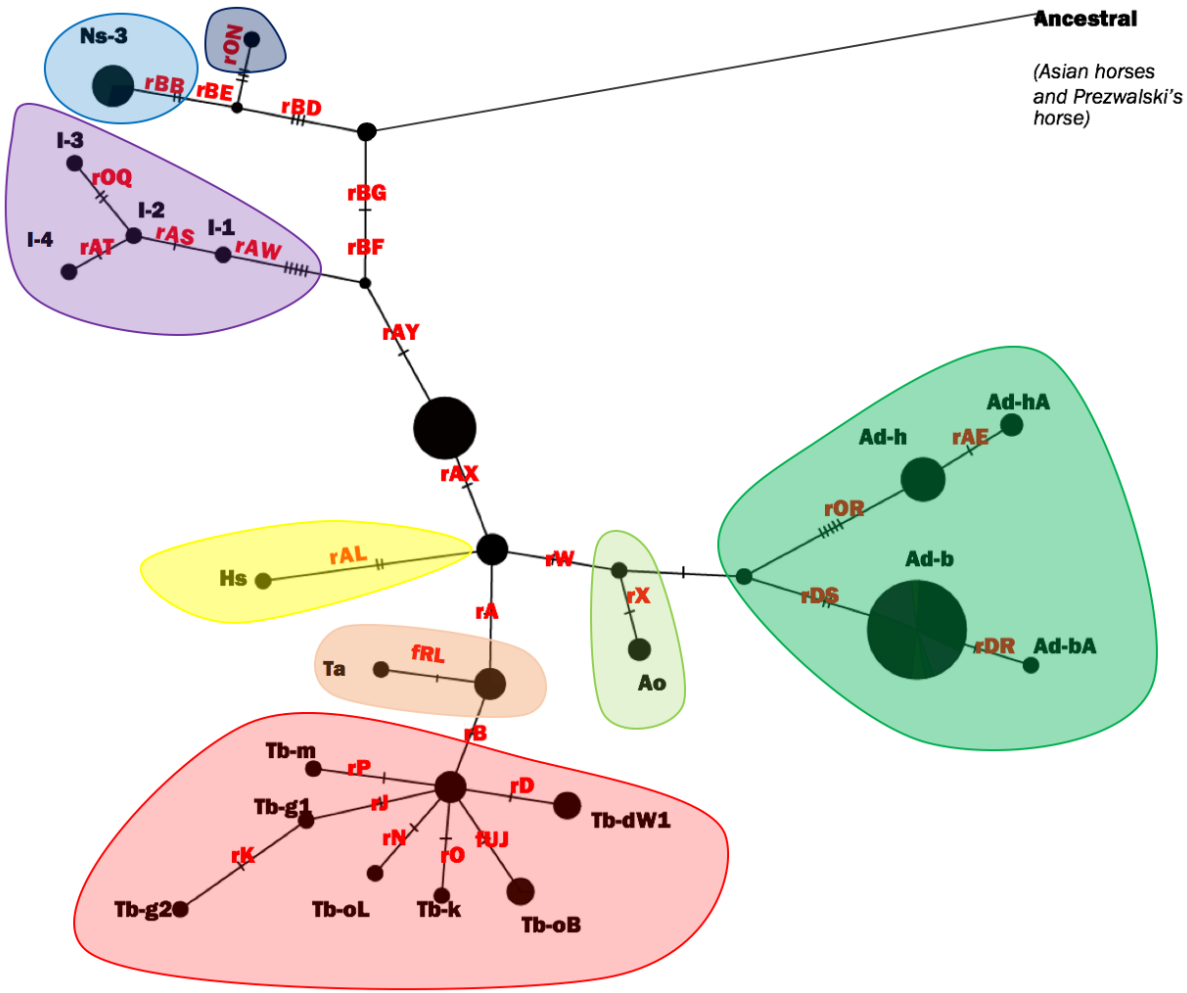

Figure 2: Y Chromosome network displaying the origin of male lineage groups adapted from Wallner et al., (2017)

Spanish horses (Hs- yellow)**; Turkoman** horses: Thoroughbred/ Thoroughbred type horses (Tb - red), Modern Arabs (Ta, orange); **Arabs:** Pure Arabians (Ao – light green), European coldblooded horses and European ponies (Ad- dark green)

Nf = Norwegian Fjord Ns-3 = Shetland I 1-4 = Icelandic Hs= horse of Spanish origin (Lipizzaner, Lusitano, Haflinger) Ta= Modern Arabs Tb = Turkoman Ad-h = Coldblooded European horses Ad-hA = Coldblooded German Draught Ad-b = Friesian Ad-bA= Connemara Ao = Arab

2.0 How the Y chromosome diagram works

When you decode the genetic sequence of the Y chromosome you get a string of A's, T's C's and G's which looks like this:

ACTGCGTACGATTCGAGCTAGCTACGAAGCTAGCATCGATCA

When comparing the Y chromosomes of two individuals the two chromosomes have the same sequence over most of its length as above but occasionally there are some changes for example, the G in the code below has been substituted for an A in the modern horse:

Ancestral variant

Ancestral horse:

CTGCGTACGATTCGAGCTA G CTACGAAGCTAGCATCGATCA

Modern horse:

CTGCGTACGATTCGAGCTA A CTACGAAGCTAGCATCGATCA

Derived variant

These changes can be breed or region specific and we can put horses into 'groups' based on their Y chromosome sequence differences. The red letters in the figures above represent the changes (mutation) in the Y chromosomal code that differ to that of the ancient horses causing a derived variant.

For example, all Shetland ponies (Ns- 3 group) will have the derived variant for rBD, rBE and rBB but will have the ancestral variant for all other variants including rON which is specific to the Norwegian fjords. The derived variant for rBB is 'T' whereas the ancestral variant is 'C'. Only Shetland ponies will have the 'C' variant, shown in figure 3 below. Similarly, Spanish horses (Hs group in figure 1 and 2 above) will have the mutant state for rBG, rBF, rAY, rAX and rAL but will have the ancestral state for all other variants.

Y Chromosome sequence
Shetland pony

AAAAA[C||T]AACAGTTTAG
A

rBB_C Ancestral (All other horses)

rBB_T Derived (Shetland ponies)

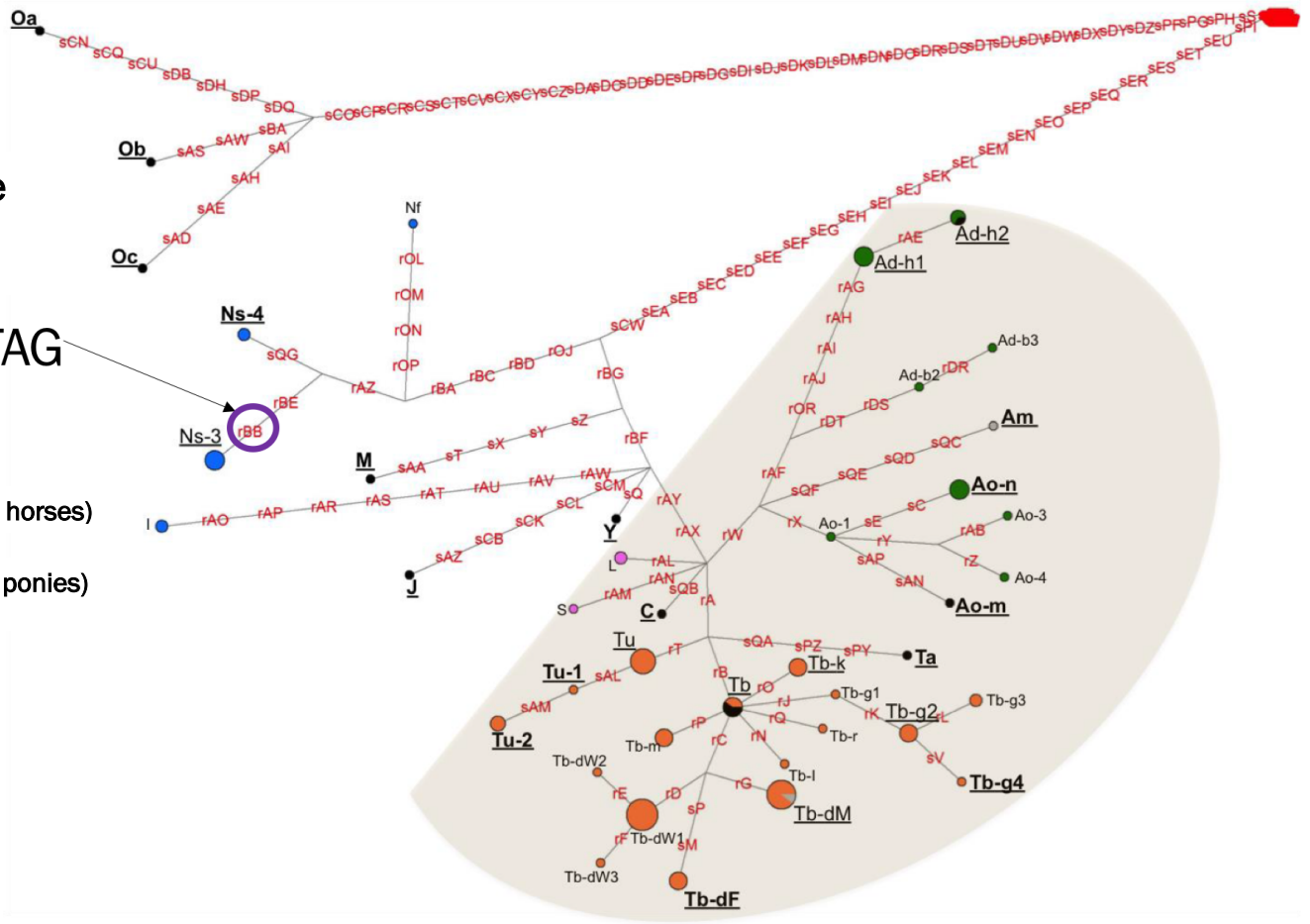

Figure 3: Y chromosome tree showing ancestral and derived states for the variant rBB specific to the Shetland pony adapted from Wallner et al., (2017)

Oa/Ob/Oc = Yakut Siberian Nf = Norwegian Fjord Ns-4 = Shetland Ns-3 = Shetland M= Mongolian I = Icelandic J = Jeju island Y= Yakut Siberian L = Lippizan S = Lusitano C = Haflinger Tu= Modern Arabs Tb = Turkoman Ta = Arab Ad-h1 = Coldblooded European horses Ad-h2 = Coldblooded German Draught Ad-b = Native UK and Irish ponies Am = South American Ao = Arab

Variants described in the above figures make up the backbone of the horse Y chromosome tree. These variants were identified though sequencing the Y chromosome (reading the code) of many individuals. Once the Y chromosome variants have been identified it is possible to build a Y chromosome tree, putting the variants in order depending on their presence or absence within a horse or breed (as described above in section 2). This is a very expensive technique however, once a variant has been identified we can look for it in other horses or horse breeds using KASP technology, see 5.0 Methods. This way we can analyse many, many horses and identify horses which have unusual groupings and are therefore good candidates for whole Y chromosome sequencing as they may possess new variants.

This data was published in: (Felkel et al., 2018; Felkel et al., 2019; Wallner et al., 2017). A more complex network showing all the published variant and groups can be seen in Appendix 3 and can be found in the supplementary file of Felkel et al., (2019).

To date, the Y chromosomal grouping for the following breeds remains unknown: Clydesdale, Cleveland Bay, Exmoor, Eriskay, New Forest, Spotted pony, Irish Draught, Suffolk Punch, Percheron and Dale.

****** Important note when reading results ******

The Y chromosome is inherited directly from father to son and does not change except for the mutations described above. Because of this, most recent cross breeding is the one that is reflected in the Y chromosome. For example, the grandsire maybe a Belgium draught but if the sire is a Barb, it is the Barb Y chromosome that gets passed on to the son and the Belgium draught Y chromosome is lost. Therefore, only the most recent paternal history is reflected in the Y chromosome.

****** Important note when reading results ***

3.0 Our native breeds

Many of our native breeds are rare with many having only a few or one remaining founding sire line. It is important to complete research such as this to gain a deeper understanding of their history. Hair samples were kindly donated by a number of owners across the UK. The number of samples collected per breed and their population status is shown below in table 1.

Table 1: Number of samples per breed and their UK population status

Breed	Population Status (UK)	Number of Samples
Horse Breeds		
Cleveland Bay	<300[1]	3
Irish Draught	2000[2] (worldwide)	3
Clydesdale	500-900[1]	10
Shire	900-1500[1]	5
Suffolk Punch	<300[1]	2
Ardennes	Common[4] (worldwide)	1
Percheron	300[3]	7
Pony Breeds		
Dartmoor	300-500[1]	7
Exmoor	300-500[1]	4
Eriskay	<300[1]	16
Fell	500-900[1]	14
Dale	<300[1]	1
Shetland	Common[4] (worldwide)	5
New Forrest	1500-3000[1]	1
Spotted pony	Rare[4] (worldwide)	2
Total		**81**

[1] (RBST, n.d.-b) [2](International Museum of the Horse, n.d.)[3] (HeavyHorses, 2017)

[4] (Hendricks, 2007)

4.0 Breed background

In order to contextualise the data generated it is important to have an understanding of horse history; mainly: the import and export of stallions, breeding fashions, breeding laws and agricultural/economical changes which impacted native horse breeds. A brief description of the breeds male history is described below.

4.1 Horse breeds

4.1.1 Cleveland Bay

The Cleveland Bay originated in Cleveland (north east Yorkshire) and was historically used as a pack horse, excavating from mines and also as the mount of the 'chapman' (sales man). In the late 17th century the breed was heavily influenced by Spanish and Barb horses which were imported to the north east of the UK through the port of Whitby. The breed is well known for its use as a royal carriage horse during the golden era of carriage driving in the 18th century starting with the reign of George II (1727). As the roads improved a demand for a faster coach horse led to a cross between the Cleveland Bay and a Thoroughbred to create a new breed, the Yorkshire coach horse which is now extinct (Cleveland Bay Horse Society of North America, n.d.).

Sadly, the development of the railways and the improvement of the roads led to the decline of the Cleveland bay which became critically endangered by 1880 (Cleveland Bay Horse Society, n.d.). The breed continued to be used for agriculture as it was good over rough ground with heavy loads however, its adaptation to agriculture is coupled with an affinity for pulling artillery which led to further decline during the world wars (Edwards, 2016). In 1962 only four stallions remained; the colt Mulgrave Supreme (1961) was bought by Queen Elizabeth II and made publicly available for stud. His line is prevalent within the Cleveland Bay and the continuation of the breed is primarily attributed to him. Despite this, there are fewer than 300 breeding Cleveland Bays in the UK and their breed status remains critical (Edwards, 2016; RBST, n.d.-b).

4.1.2 Irish Draught

The Irish Draught has had a fundamental role in: agriculture, sport and welfare for thousands of years. The Irish Draught is thought to have been influenced by the heavy Flemish (now known as the Belgium Draught) and norther French horses that were brought with the Anglo-Norman invasion of 1172. In the 16th century trade with Spain and the resultant admixture of native draught horses with lighter horses native to the Iberian peninsula is thought to be the cause of the breeds light build (Edwards, 1992; International Museum of the Horse, n.d.). The Irishman's love of hunting shaped the breed to perform both agriculturally and under the saddle producing a careful and brave individual. After the famine (1847) Clydesdale and Shire horses were introduced with little success as the draught developed poor quarter and limb conformation and stamina. This practice was stopped and Thoroughbred was introduced to correct these traits and improve shoulder conformation (idhba, n.d.). Inevitably, during the world wars numbers declined dramatically (Edwards, 1992).

Today, the Irish Draught makes an excellent hunter or sport horse, when bred with a Thoroughbred, and has produced many world class eventing and show jumping individuals. The most significant lines are those of the King of Diamonds and Clover Hill stallions who were both world ranking show jumping sires. The success of the sport horse has slowly lead to the decline of the pure bred Irish Draught which is thought to be endangered with only 2000 individuals left worldwide (International Museum of the Horse, n.d.).

4.1.3 Clydesdale

The Clydesdale was founded in the Valley of Clyde in Scotland and has taken many different forms until its recent establishment 150 years ago. It is thought to have been influenced by many pony breeds including the now extinct Galloway pony.

Warfare has undoubtedly shaped the heavy breeds. In the 15th century horse imports increased exponentially as 'improvement' of the native breeds for warfare was sort. James III of Scotland (1460-1488) is thought to have brought the founding stock for the "Great horses' of England which were in turn the founding stock for the Shire. James IV of

Scotland (1488-1513) introduced lighter horses from Spain and France to introduce speed in hunters and race horses as well as heavy horses from Poland. James V of Scotland (1528-1542) brought horses from Denmark and passed a law as part of the 'Black Acts' to try to regain the church. The breed of horse act of 1535 stated that any person who had an enclosed deer park must have at least two mares of above 13hh high in order for them to be bred with horses no shorter than 14hh high in order to raise the size of the native horses. Should the time arise their offspring would be used to sustain war. Colts were also castrated if they did not meet the height requirement. Henry VIII (1509-1547) is also thought to have introduced the Spanish Jennet horse and also the North African Barbs (Beck, 1992).

The breed was solidified in the 18th century when the 6th Duke of Hamilton and John Praterson of Lochlyoch imported Flemish horses to increase their size. It is thought that Shire mares were introduced to the Clydesdale. The most significant Clydesdale sire line the Prince of Wales and Darnley stallions although, it is thought there is one founding sire known as Glancer (Edwards, 1992).

4.1.4 Shire

The Shire horse is a descendant of the Great English war horse of the 16th century and was founded in the Fens. It wasn't until the end of the 16th century that the modern shire was established. As with the Clydesdales, the Flemish horses have thought to have impacted the Shire as the Dutch contractors who drained the fens brought their own Flemish horses. Friesian is thought to have been introduced to the breed for refinement, improving movement. The breeds foundation sire is the Packington Blind Horse (Edwards, 1992, 2016).

4.1.5 Suffolk Punch

The Suffolk Punch is the oldest of Britain's Heavy horses and was established at the beginning of the 16th century. It is thought that the Suffolk Punch was influenced by the Danish Jutlander during the middle ages. During the 19th century trade between Denmark and the UK lead to further import of the Danish Jutlander. In the 18th century however, the Danish Jutlander had been subjected to improvement and bred with the Danish

Frederiksborg which has a Spanish ancestry. During the 19th century, the Danish Frederiksborg was also crossbred with Arabs. Every Suffolk Punch horse can be traced back to Thomas Crisp's horse of Ufford (1768) (Edwards, 1992, 2016).

4.1.6 Ardennes

The Ardennes, also known as the Ardenner or Ardennais, is one of the oldest heavy draught horses originating from the Ardennais mountains of Belgium and France. These horses have been bred on the plateau for approximately 2000 years. The breed is energetic, durable and has great endurance. It is thought that admixture with Arabs occurred in the 18th century followed by the taller Belgium Draught, which introduced Flemish lines to the British horse breeds, to increase size and strength (Hendricks, 2007).

4.1.7 Percheron

The Percheron is a French draught originating from the Le Perche district of Normandy, France. It is unknown when the Percheron was first imported to England; it is possible that ancestral horses were brought to England by William the Conqueror (1066). During the 19th century thousands of the lighter draughts were imported into England to drive the omnibuses (British Percheron Horse Society, n.d.).

4.2 Pony breeds

4.2.1 Exmoor

It is widely accepted that the Exmoor is one of Britain's oldest pony breed which is supported by images of Exmoor type horses depicted on the Beaux Tapestry (1066). The Exmoor pony was founded on the stunning but challenging national park to the west of Somerset. Until the early 1800's, Exmoor was a national forest however, in 1818 part of it was deforested to produce marshy moorland and enclosed. This significant change in landscape is thought to solidify the Exmoor breed and resulted in the origin of two herds; one belonging to Sir Thomas Achland and one to Mr John Knight. Just before the deforestation there is speculation that the stallion Katerfelto, probably of Spanish origin, had influenced the Exmoor. Mr Knight tried to 'improve' the breed with Barb Horse and his son continued his legacy breeding Exmoor ponies with Welsh Cobs; neither were a great

success. In contrast, Sir Achland kept the natural characteristics of the Exmoor and today the herd is known as the Anchor herd due to his distinctive Anchor brand mark. The majority of Exmoors today are descendants of the Anchor herd (Edwards, 1992). After the second world war only 50 Exmoor ponies remained as they were taken to feed the city. Although numbers have increased they still remain endangered (exmoorponysociety, n.d.; RBST, n.d.-b)

4.2.2 Dartmoor

The rough and harsh micro-environment of Dartmoor, similar to that of Exmoor, would suggest that the Dartmoor would be isolated from external admixture. The nearby ports of Plymouth and Exeter however, make this unlikely. Henry I (1100-1135) is thought to have introduced eastern blood and as late as the end of the 19th century Dartmoor ponies were bred with: Welsh Cobs (Dinarth Spark), Hackneys, Arabs (the Leat), Barbs and potentially small Thoroughbreds. During the industrial revolution the pony almost disappeared as Shetlands were bred with them in order to produce a 'pit pony' which was small enough to fit down the tin mines. In an effort to save the breed the Polo and Riding Pony Society began to introduce stallions to a section of the Dartmoor pony stud book, improving the quality of the pony.

4.2.3 Eriskay pony

Throughout Scottish history, Western Isles ponies have been an essential part of the community, agriculture and the economy. The sure footed, hardy ponies were well adapted to the course vegetation on the hills thus they were cheap to keep and were the best pack animals for transporting peat and seaweed. For these reasons, the Island ponies were common up until their decline which began when the treaty of 1707 was signed, marking the unity of England, Wales and Scotland and the start of Great Britain. This union triggered many changes agricultural and economical changes; the exportation of kelp deprived the highlanders of fertiliser and deforestation for charcoal lead to the loss of: fertile top soil, game, timer and shelter for dwellings and the native animals. Decline of the native pony is linked to the loss of ancient highland society.

Although decline started in the mid 1700's rapid decline did not occur until the 19th century, the native ponies were exported off the Western Isles and crossbred with heavier horses such as the: Clydesdale, Highland 'Garrons', Norwegian Fjords and Arabs to produce create powerful agricultural animals. These crossbred could not be transported to the Western Islands due to poor docking facilities and in any case the course vegetation was not suitable or plentiful enough to support high energy input horses and cattle. This isolation between the Western Isles of Scotland and the mainland means these native ponies are probably one of the most ancient and pure breeds in the United Kingdom. John walker (18th century) stated:

" The horses in the Highlands, and especially the Islands, are the only aboriginal and unmixed breeds now extant in Britain. The first race of horses reared in England, and the south of Scotland, is now unknown, and is probably extinguished by the various breeds introduced by other countries in the course of many ages"

John Walker in Beck (1992)

The exportation of western Isle ponies had a devastating effect on the population; it was only at the beginning of the revival (1968) when there were only a few ponies remaining on the island of Eriskay that the Western Isle type pony became known as the 'Eriskay pony'. In the 1960's and 1970's it was thought that there were no Eriskay stallions left so six western Isles highland stallions were introduced in order to save the breed from extinction. These stallions were: Rhum Darroch, Callum of Creagach, Prince (formerly Nicoldene), Balelone, Fingal and Pharic of Hunthall. These stallions were not extensively used due to the discovery of a pure bred Eriskay colt named Eric. Eric had three sons; Prince of Caolas, Gille-Eodhnan and Balachan; which became prolific within the breed maintaining the purity of the Eriskay (Beck, 1992; McGilivray, 2019).

4.2.4 Fell and the Dale

Today the Fell and the Dale are geographically distinct occupying the east and the west of the Pennine range respectively however, both are thought be originally derive from the indigenous British pony. Both breeds notably resemble the Friesian horses thought to have been introduced to the native pony stock during the Roman era. During the 18th

century it was thought to have been influenced by the Galloway pony which was used as the mount for the border raiders and is native to the south west of Scotland.

The separating characteristics of the Dale and the Fell are largely due to function and height. These ponies have exceptional strength for their size and were used as pack horses for mountain artillery and to haul loads in the coal mines. As trotting became popular in the late 1700's the Dale was crossed with horses originating from Norfolk or Yorkshire to improve the action of the horse without compromising their agricultural abilities. Today, a number of Dales can be traced back to the Norfolk Cob stallion Shales (1755) descended from the Darley Arabian Thoroughbred. During the 19th century there was admixture with Welsh Cob and Clydesdale (to fulfil the army's demand for 'gunners') although all that visibly remains of the Clydesdale influence are the colours of bay, brown and grey. As with many of the heavy breeds the second world war threatened the existence of the heavy pony resulting in the reorganisation of the Dales pony society in 1964 aiming for preservation rather than improvement (Dales Pony Society, n.d.; Edwards, 1992; RBST, n.d.-a).

The Fell pony was also a pack pony carrying: fleeces, woollen product and a variety of foods. It is also a good trotter and was used for agriculture either under the harness or the saddle, herding flocks and hunting wolves. The Fell is a lighter pony than the Dale, stands up to 14hh high and is known to produce a good small hunter or riding horse. The Galloway stallion Lingcropper (1745) is thought to be the influential Fell stallion line (Edwards, 1992; RBST, n.d.-c; The Fell Pony Society, n.d.).

4.2.5 Shetland

The Shetland pony is small (average 40 inches) hardy pony living off the poor and limited vegetation on the Shetland Isle, Scotland, which has a four mile diameter and is known for particularly harsh winters (Encyclopedia Britannica, n.d.; shetland pony studbook society, n.d.). It's limited height is the result of a genetic mutation in the HMGA2 gene which affects height variability in horses and a number of other species (Frischknecht et al., 2015). This explains why ponies bred in milder conditions did not increase in height (shetland pony studbook society, n.d.). Excavation of pony bones date back to the bronze age making the Shetland one of, if not the oldest, native pony breed in the United Kingdom. The breed has

escaped admixture, which was common on the mainland, due to an inability to dock boats preventing the importation of large horses. Anyhow, poor vegetation and harsh winters made it difficult to sustain breeds of higher input (shetland pony studbook society, n.d.).

The Shetland pony was a pack pony, carting peat and seaweed, and a riding pony despite its size. In the 1850's they were imported to England and bred with native ponies such as the Dartmoor to work in the mines (Encyclopedia Britannica, n.d.).

4.2.6 New Forest

The New Forest ponies have adapted to the heaths and woodland of the New Forest to create a surefooted, hardy, docile and strong pony which today makes a good all-rounder. In 1765 a Thoroughbred called Marske was introduced to improve the appearance and height of the pony. Improvement continued into the late 19th century when Queen Victoria introduced Arab and Barb and Lord Cecil introduced other native pony breeds (Edwards, 2016; Hendricks, 2007).

4.2.7 Spotted Pony

The exact origin of the spotted pony is unknown; it is possible that the pony is native to the British Isles as their leopard–spotted colour would have acted as camouflage in the heaths. However, it is also possible that the pony was imported with the crusaders. Laws implemented in the Tudor era to increase the size of native ponies combined with the fashion of imported Spanish horses lead to the decline of the Spotted Pony. The Spotted Pony gained fashion again in the 19th century as a driving pony however, British Spotted Pony numbers remain depleted as many were exported. Today, there are several performance awards in completions to show the versatility of the pony (Hendricks, 2007).

5.0 Methods

DNA isolation from hair root samples was performed using the nexttec™ Kit (nexttec™, n.d.). Manufactures guide and protocol can be found here:

https://www.nexttec.de/images/stories/pdf/protocol/2019/tissue/10N-Tissue_3.0_nexttec-cleanColumns.pdf

5.1 KASP technology

KASP stands for Kompetitive Allele Specific PCR. PCR copies a section of DNA so there is enough to read a result. To choose a section of DNA you want to amplify you have to use specific markers known as primers. This system allows the identification of the ancestral and derived state of the variant of interest. For example, the rBB variant will have two markers/primers one for the ancestral C allele and one for the mutant/ derived T allele specific to the Shetland.

The markers specific to the variants are combined with the KASP mastermix and the DNA. The KASP mastermix contains fluorescent markers which are complementary to the ancestral (rBB 'C') or mutant state (rBB 'T') and emit light. The fluorescent markers emit a wavelength of light which is read by the machine, figure 4. A more detailed explanation of this technique can be found in Appendix 2. The manufacturers explanation and video tutorial can be found at: https://www.biosearchtech.com/support/education/kasp-genotyping-reagents/how-does-kasp-work

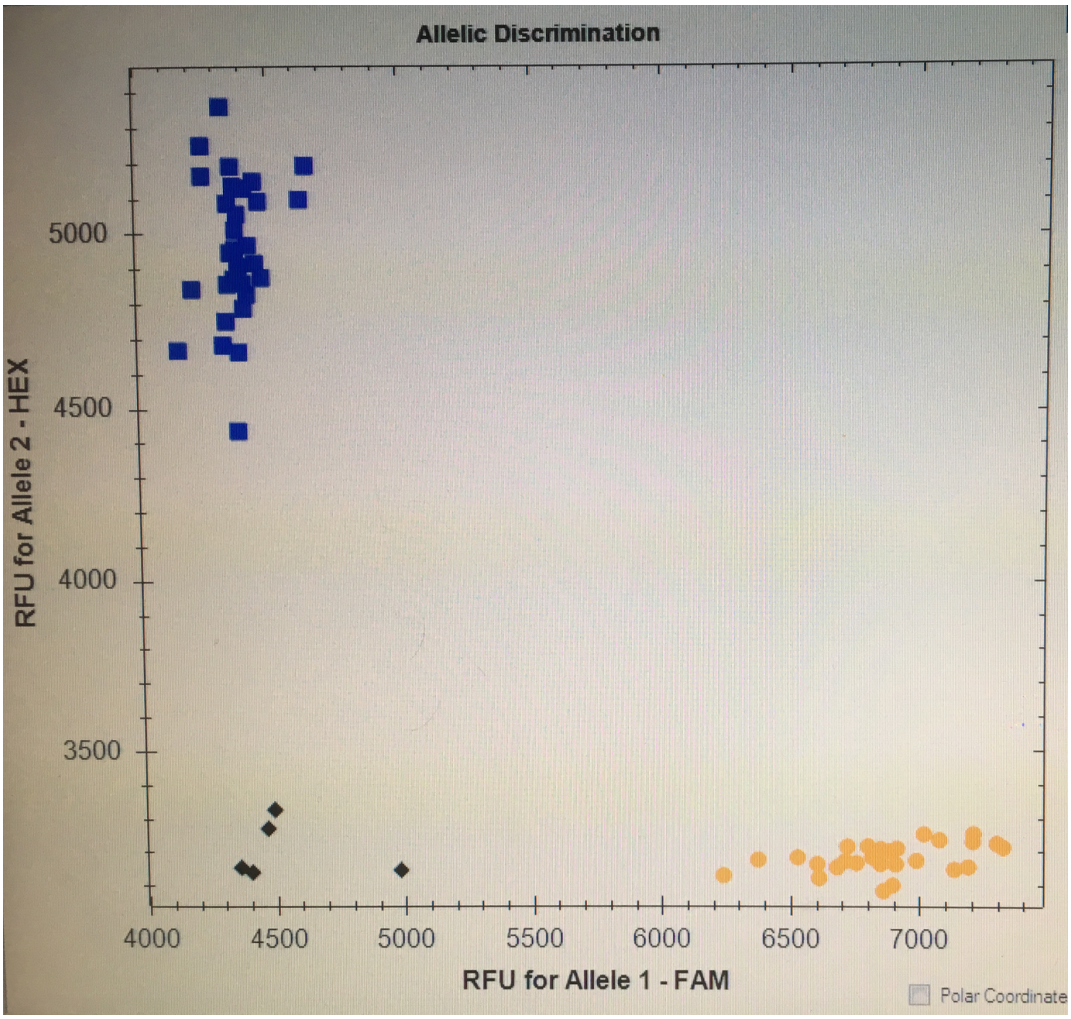

Figure 4: Samples grouped via fluorescent marker wavelength

Blue squares = samples carrying the derived variants; Orange circles = samples carrying the ancestral variant; Black diamonds = negative controls

6.0 Results

The Y chromosome tree for samples used in this project can be seen in figure 4 below. In this data set we have been able to identify Y chromosome groups for the following horses which have not previously been investigated: Clydesdale, Cleveland Bay, Exmoor, Eriskay, New Forest, Spotted pony, Irish Draught, Suffolk Punch, Percheron and Dale.

1) The majority of UK horse and ponies cluster in the Y chromosome group Ad-b. These breeds are the: Clydesdale, Dale, Dartmoor, Fell, Irish Draught, New Forest, Shire and Suffolk Punch. This means that these breeds have either the same or a very similar Y chromosome sequence. To identify differences in the Y chromosome between the breeds the whole Y chromosome needs to be sequenced to find new variants which distinguish these breeds but are not currently published. Here we identify a Y chromosome group specific to British horses regardless of their size, shape and breed.
2) Shetland ponies cluster outside the crown group (Ns) and samples from ponies born in the UK cluster in the same group as those born in mainland Europe suggesting that the male lineages do not differ between the two geographical locations.
3) The Irish Draught and the spotted pony have male lineages that can be attributed to the influence of the Darley Arabian sire line (Tb-dW1). The Darley Arabian line is the most influential of the three founding Thoroughbred sires. As it is known that the Thoroughbred was introduced to both breeds for improvement this is not so surprising. However, with this technology we have been able to identify which founding Thoroughbred sire was used which is beyond the pedigree data currently publicly available online for some Irish Draught horses.
4) The Cleveland bay clusters in the Tb-oB group which may be of Turkoman origin however, further research is required to confirm this.
5) The Eriskay, from descendants of the purebred Eriskay pony Eric, forms a unique cluster between the markers rAY and the rAX whilst the Exmoor and a single Fell line cluster at the base of the Crown group, see figure 4 below. These lineages, along with the Shetland pony and Icelandic horses, have escaped the influence of the Spanish, Arabian and Turkoman horses that were imported to Britain from the 16th century. These breeds therefore represent the most ancient known male

lineages in the UK along with the Shetland pony. These lines may be of interest for conservation.

6) The majority of Fell lines cluster at the Ad-b locus with many of the other British horse breeds however, some rare lines were also identified. One Fell clustered between variants rA and rB but does not possess variants associated with the modern Arab (as with the welsh) or Thoroughbred as with the Spotted Pony and the Irish Draught. Another Fell line clustered at the basal node of the crown group together with the Exmoor and is therefore an ancient line (as explained above). These two Fell lines may therefore be of particular interest for conservation.

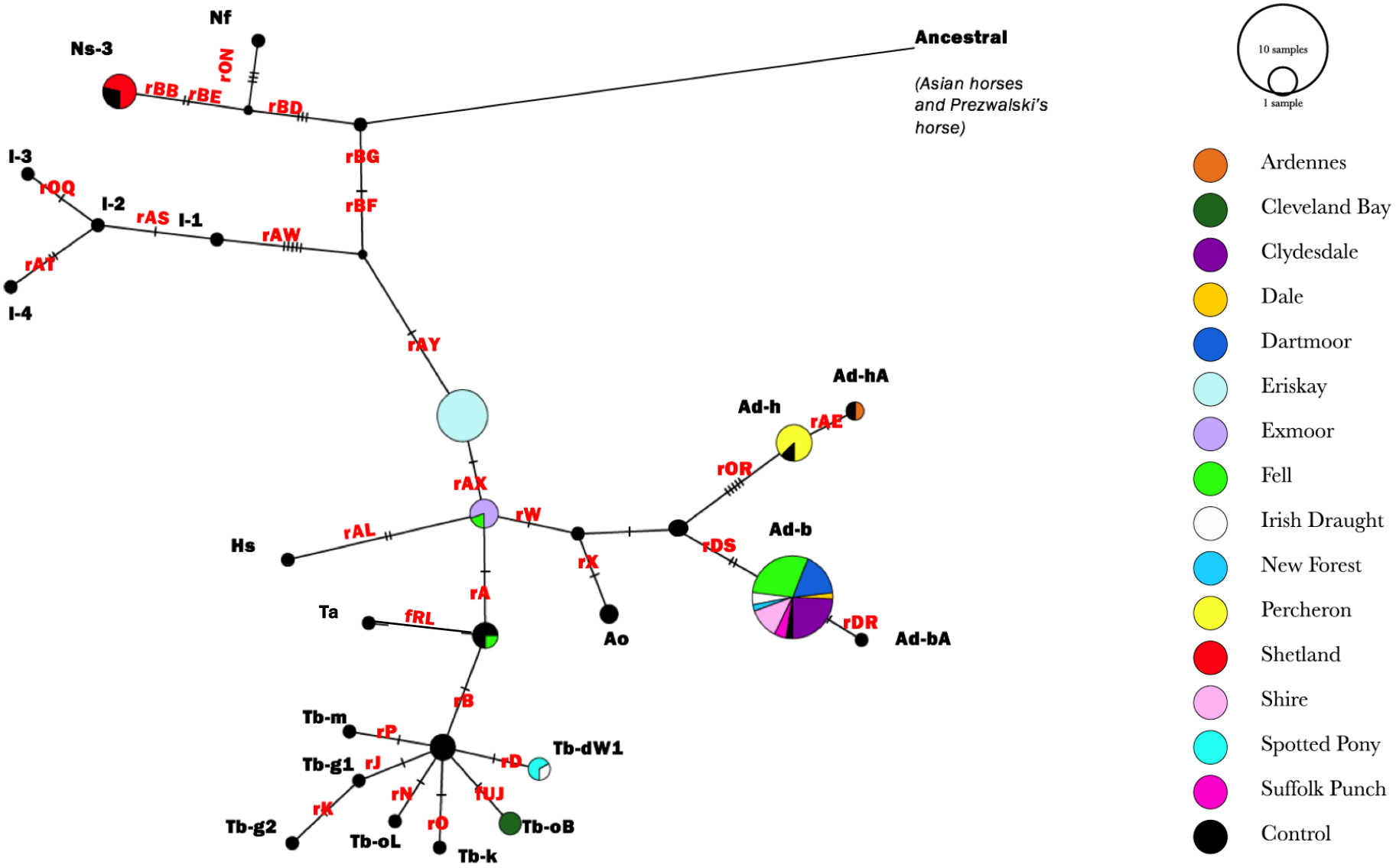

Figure 5: Male Specific region of the Y chromosome haplotype network for breeds analysed in this project. Group names are indicated by black letters and their associated variants are the red letters

7.0 Context and future research

It is not possible from this data to understand why many of our British breeds cluster into the same male lineage (Ad-b) despite having completely different traits. For example, the Clydesdale and the Dartmoor do not look similar but have the same Y chromosome group. Further research using an understanding of each breeds history coupled with statistical simulation of horse migration may be able to explain how this British Ad-b Y chromosome clustering developed. In future studies, identification of Y-chromosomal variants which may be breed specific would also help aid our understanding of the development of the British clustering.

Many island populations often have unique Y chromosome sequences due to their isolation as they have not had the opportunity to be influenced by other breeds. This is true for the Norwegian Fjord, Shetland and Icelandic horses which branch off from a more ancestral node in the Y chromosome tree, see figure 4 above. It is expected that the Eric line of the Eriskay as well as the Exmoor and Fell lines that cluster at the bottom of the Crown group also have unique variants specific to their breeds which would cause unique branches as in the Norwegian Fjord, Shetland and Icelandic horses. Further sequencing of whole Y chromosome is needed to achieve this.

8.0 Limitations

As stated before, the Y chromosome is inherited directly from father to son and is therefore a perfect marker to trace recent introgression. But this part of the genome is very tiny, containing only a small proportion of genetic information and does not change except for the odd mutation. Because of this, the most recent cross breeding is the one that is reflected in the Y chromosome. For example, the grandsire maybe a Belgium Draught but if the sire is a Barb it is the Barb Y chromosome that gets passed on to the son and the Belgium draught Y chromosome is lost for that horse. Therefore, only the most recent history is reflected in the Y chromosome.

Y-chromosomal analysis cannot show to which extent a sire has influenced your horse. For example, if you mate a Shetland pony stallion with pure Clydesdales by the third generation the descendant will only be 12.5% Shetland but will have the SAME Shetland pony Y

chromosome as the Shetland great- great grandsire. To put this into context (figure 4), if you had a Shetland pony sire in your Clydesdale 30 generations ago then your colt will be 0.000000009% Shetland pony but will still have the SAME Shetland pony Y chromosome.

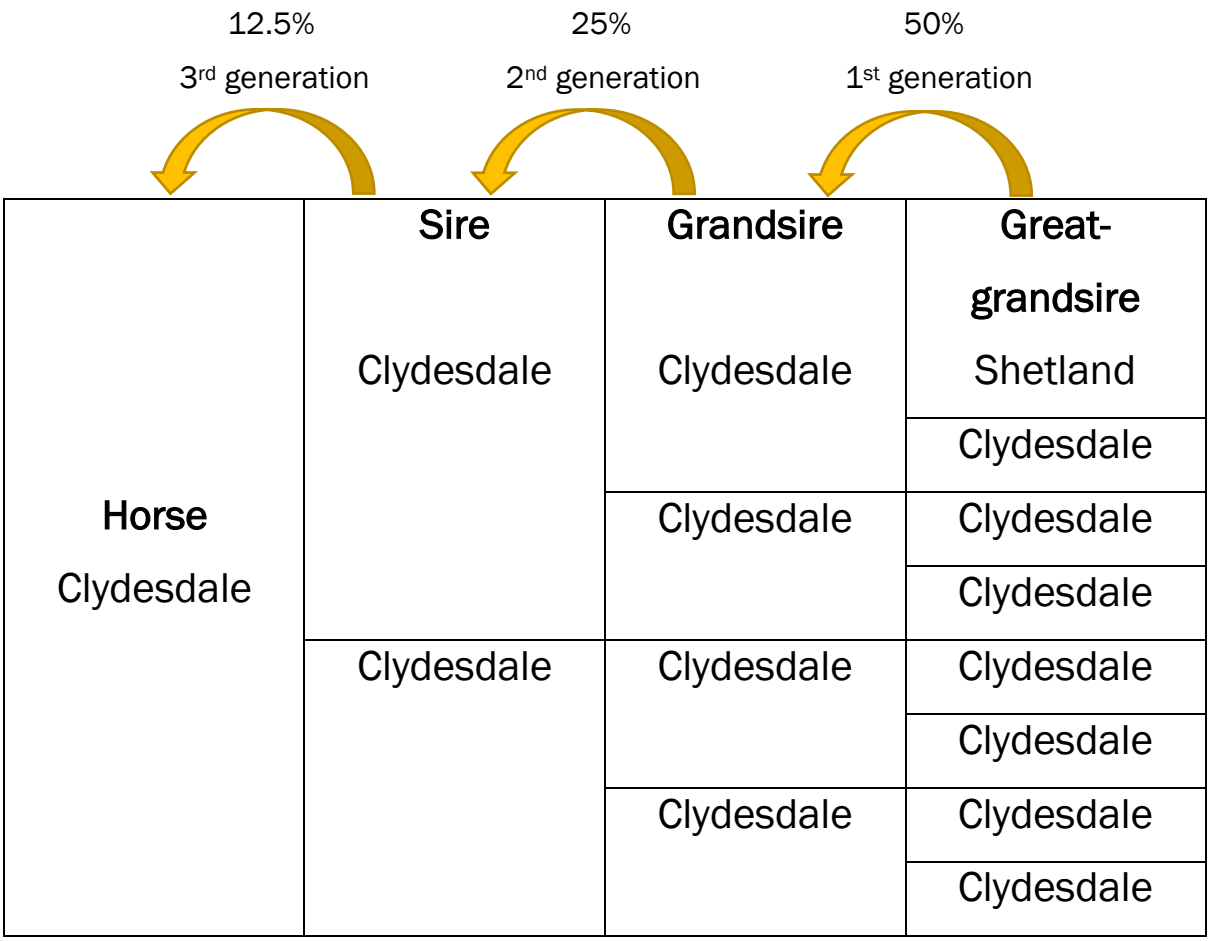

Figure 6: Pedigree tree showing the genetic contribution between generations

9.0 Conclusion

Using existing Y chromosome variants, it has been possible to uncover that British horses and ponies predominantly cluster into the Y chromosome group Ad-b regardless of the extreme trait diversity seen between native UK breeds. We identified that the Eriskay Eric line, the Exmoor and a Fell line cluster into unique Y chromosome groups which have avoided oriental influence representing the UK's most ancient male lineages along with the Shetland pony. These lines have been identified as lines which are potentially of interest for conservation. Further research is required to understand why British breeds form such a clustering.

Appendix 1: List of references

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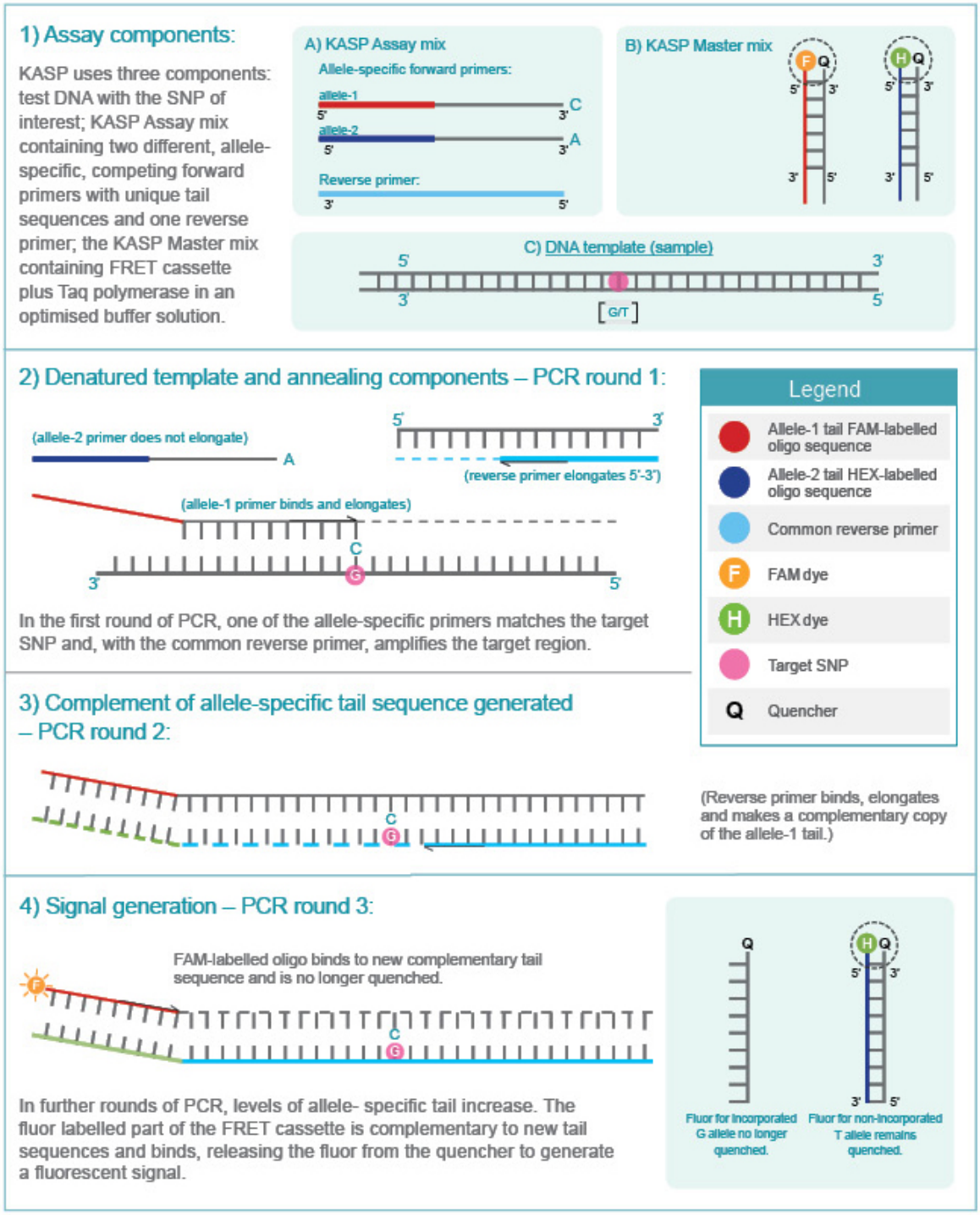
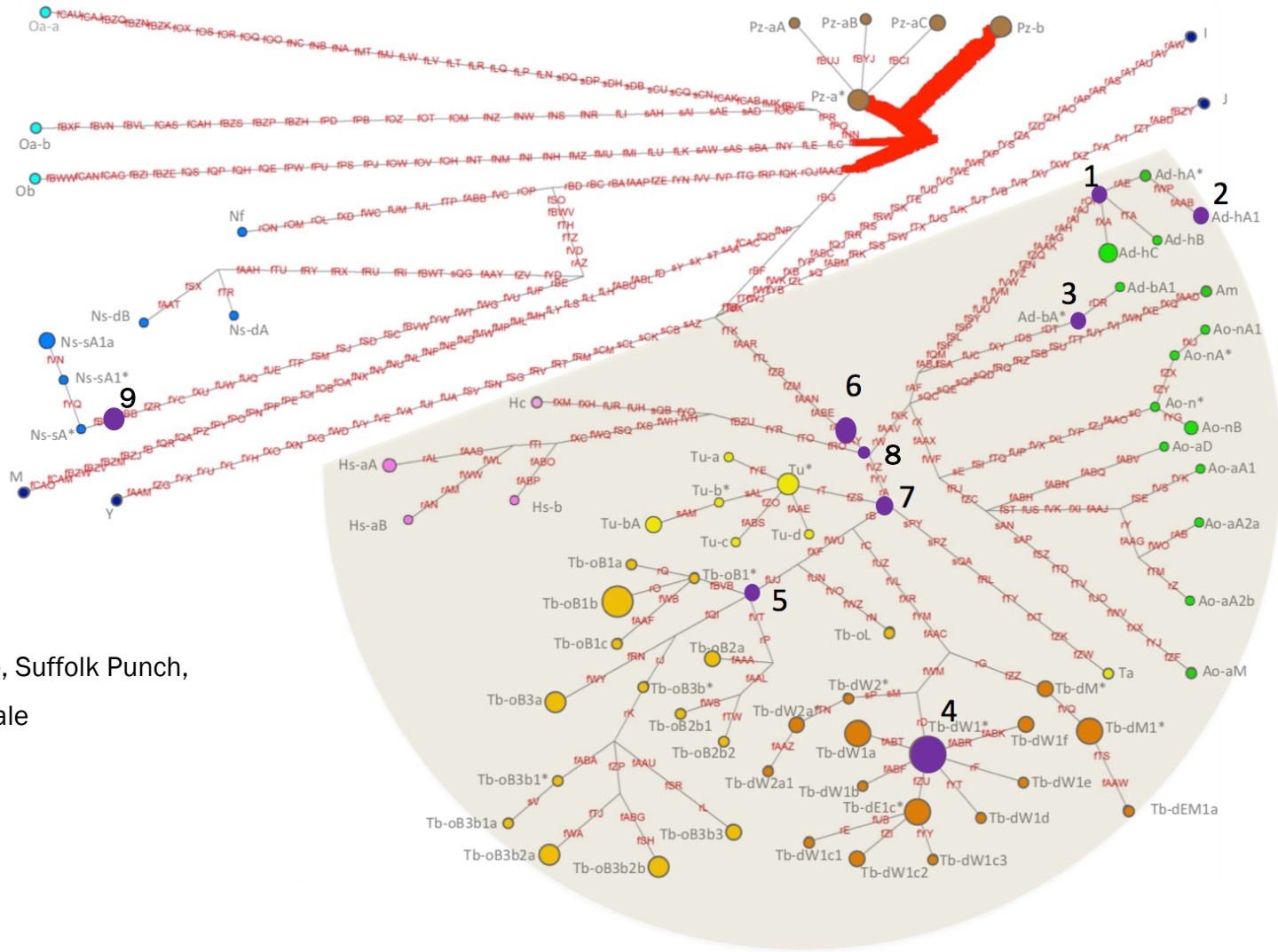
Appendix 2: KASP technique



Appendix 3: Full MSY haplotype network for horses analysed in this project adapted from Felkel et al., (2019)



1: Percheron

2: Ardennes

3: Irish Draught, Clydesdale, Shire, Suffolk Punch, Dartmoor, New Forest, Fell and Dale

4: Irish Draught and Spotted Pony

5: Cleveland Bay

6: Eriskay

7: Fell

8: Exmoor and Fell

9: Shetland