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Tracing the distribution of Icelandic stallions on the basis of pedigree and Y-chromosomal information

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Declaration of Authenticity

I hereby declare that I have written this thesis independently and that I have not used any other sources and aids other than those listed. All text passages taken from other sources have been cited.

I have carried out the decisive work myself and have listed all contributors with their contributions to the work.

This thesis has not been submitted or published elsewhere.

Vienna, 24.2.2024

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Abstract

When horses were domesticated 4,200 years ago, humans bred them according to their needs, and different breeds evolved over time. With the use of Y-chromosomal markers, the paternal breeding history of horses can be reconstructed. In previous studies, most breeds clustered together into the so called ‘crown group’ of the phylogenetic tree, with only some breeds located outside the crown. The Icelandic horse is one of them. As previous studies showed that the Icelandic horse had no recent influences of modern breeds, we investigated whether this statement was true for the entire stallion population or if certain sublines were missed. Using pedigree information, a fine-scaled Y-chromosomal analysis was conducted. The results verified the previous statement that only Icelandic-specific Y-chromosomal haplotypes can be found in the Icelandic horse. From 12,896 observed stallions, haplotypes could be assigned for 96.6 % of them. Furthermore, the available data extracted from WorldFengur were used to illustrate the distribution of the current Icelandic stallion population and to observe if different sublines are more popular in different countries. As expected, the distribution of sublines throughout countries was fairly even, with no predominant subline in any country. Interestingly, data showed that even though most stallions are currently located outside Iceland, the majority of stallions are born in Iceland. Overall, we verified the absence of modern horse breeds within Icelandic horses and demonstrated the current state of the worldwide distribution of Icelandic stallions.

Zusammenfassung

Als Pferde vor 4.200 Jahren domestiziert wurden, züchtete der Mensch sie nach seinen Bedürfnissen, und im Laufe der Zeit entwickelten sich die verschiedenen heute bekannten Pferderassen. Mithilfe von Y-chromosomalen Markern kann die väterliche Zuchtgeschichte von Pferden rekonstruiert werden. In vorangegangenen Studien wurde gezeigt, dass die meisten Rassen der sogenannten „Krongruppe“ zugeordnet werden können. Nur einige wenige Rassen wurden außerhalb der „Krongruppe“ eingeordnet, das Islandpferd ist eine von ihnen. Bisherige Ergebnisse zeigten, dass kein kürzlicher Einfluss von anderen modernen Pferderassen im Islandpferd nachzuweisen ist. Diese Aussage sollte in dieser Arbeit überprüft werden, des Weiteren sollte analysiert werden, ob bisherige Studien die gesamte Islandpferde Population abdeckten, oder ob es Sublinien dieser Rasse gibt, welche nicht genotypisiert wurden. Daher wurden Proben für die Y-chromosomale Genotypisierung nach ihrer Abstammung ausgewählt. Die Ergebnisse bestätigten die frühere Aussage, dass nur isländisch-spezifische Y-chromosomale Haplotypen im Islandpferd zu finden sind. Von 12.896 inkludierten Hengsten aus der Datenbank, welche die aktuelle Hengstpopulation abdeckt, konnten für 96,6 % Haplotypen zugeordnet werden. Darüber hinaus wurden die bereits bekannten Daten aus WorldFengur verwendet, um die Verteilung der aktuellen isländischen Hengstpopulation zu veranschaulichen und um festzustellen, ob verschiedene Sublinien in verschiedenen Ländern beliebter sind. Wie erwartet, waren die Sublinien in den einzelnen Ländern gleich verteilt, und in keinem Land wurde eine vorherrschende Sublinie gefunden. Im Weiteren, zeigten die Daten, dass die meisten Hengste in Island geboren wurden, obwohl sich die meisten Hengste derzeit außerhalb Islands befinden. Diese Studie zeigte, dass ein Einfluss von den modernen Pferderassen im Islandpferd weiterhin ausgeschlossen werden kann und zeigte des Weiteren den aktuellen Stand der weltweiten Verteilung der Islandpferde Hengste.

List of Abbreviations

FAM	6-Carboxy-Fluorescein
HEX	Hexachloro-Fluorescein
HT	Haplotype
Indel	Insertions and deletions
KASP	Competitive allele-specific PCR
MSY	Male specific Y-chromosomal region
mtDNA	Mitochondrial DNA
NGS	Next generation sequencing
NTC	No template control
RFU	Relative Fluorescence Unit
SNP	Single nucleotide polymorphisms

List of contents

1 Introduction

Human history highly depended on horses as a food source, as helpful farm animals, for transportation, and through their use in the military. Hence, the history of humans and the domestic horse has always been intertwined. It was presumed that the domestication of the horse began 5,000 years ago, but newer studies have found that the domestication of the horse began 4,200 years ago in the West Eurasian Steppe [1, 2]. Knowledge about horse domestication relies on archaeological and genetic information. In addition, the origin of modern horse breeds has sparked interest among scientists and the equine community. To gain insight into the genesis of modern breeds, reconstruction of maternal and paternal lineages using mitochondrial DNA (mtDNA) or Y-chromosomal DNA can be performed [3, 4].

1.1 Origin of modern horse breeds

Most modern horse breeds that we know today are a result of organized breeding strategies throughout the last centuries. In horse breeding, the primary focus is on stallion selection and the import of foreign stallions to breed with local mares is a common practice. In Europe, three importation waves can be differentiated. The first wave, described as the 'Neapolitan' wave, occurred between the 15th and 18th centuries when stallions of Neapolitan and Spanish origin were imported. During the late 18th to the late 19th century, stallions of Turkman and Arabian origin gained popularity and formed the 'Oriental' wave. Breeding with foreign stallions peaked in the 19th and 20th centuries with the common use of English thoroughbred stallions [5].

1.2 Breeding history of Icelandic horses

The breeding history of Icelandic horses is different. The Icelandic horse is the only horse breed found in Iceland. It is stated that the Icelandic horses evolved from Scandinavian horses that arrived on the island with the first humans and other farm animals between 874 and 930 [6].The importance of the horse grew as people needed the horse for transport through the rough terrain of Iceland. Over time, a robust and sure-footed horse breed evolved from the first horses brought to Iceland. Today the Icelandic horse is best known for its friendly demeanour, its compact and sturdy build and its ability to perform two variations of lateral gaits (tölt and pace). Due to the remote and difficult to access areas of Iceland, different lines evolved within the Icelandic breed. These Icelandic horse sublines were purposefully bred later on [7].

The first export of Icelandic horses to other European countries began in the 19th century when Britain began to import the breed to work in coal mines. From there on, other countries started importing Icelandic horses for agricultural purposes as well. After the Second World War, the export of Icelandics as riding horses began to grow. With the establishment of breeding associations in the 20th century, breeding standards were set and lineages purposefully pursued [8]. Five lineages are said to have had the greatest impact on the current Icelandic horse population. These lines are the Svaðastaðir-, the Hornafjörður-, the Hindisvík-, the Stokkhólmi- and the Gufunes line [9]. In recent decades, the Icelandic horse has grown enormously in popularity and has since been exported from Iceland to all over the world.

The Icelandic horse is considered one of the purest horse breeds due to its secluded genesis and Iceland's strict import regulations. The import of horses of any breed to Iceland has been restricted since the first settlements (874-930) and completely prohibited since 1909. In addition, horses can only be classified as Icelandic horses when the maternal and paternal pedigrees originated in Iceland [6].

1.3 Population studies based on mitochondrial and Y – chromosomal DNA markers

Mitochondrial DNA (mtDNA) markers are commonly used to trace maternal family history in animals, and this method was previously performed in horses. The mtDNA, which is inherited only from the mother, is known to show a wide variety in horses, and many maternal ancestors are represented in today's horse breeds. The high mtDNA variety is attributed to the fact that breeding strategies generally focus on stallion selection, meaning that a restricted number of imported stallions were bred with a wide variety of local mares [4]. The recent introduction of Y-chromosomal markers has made it possible to reconstruct paternal heritage and gain a better understanding of the origin of modern horse breeds [3, 10].

1.4 The use of the Y chromosome for reconstructing breeding history in horses

As previously shown in humans, the male specific region of the Y chromosome (MSY) can be used to trace paternal family histories because it is inherited from the father to his sons [11]. Because the MSY is inherited without recombination with the X chromosome, polymorphisms like single nucleotide polymorphisms (SNPs) or insertions and deletions (Indels) form completely linked haplotypes (HTs). Haplotypes are defined by the allelic state at certain loci. The MSY presents a challenge in sequencing because of its highly repetitive structure and low level of variants. Using next-generation sequencing (NGS), a reference sequence of the horse MSY was assembled. The non-repetitive MSY reference sequence contained a length of 1.46 million base pairs (Mbp) [3] and was later updated to represent 6.46 mbp of the horse MSY. This reference (LipY764) was used to define meaningful MSY markers in individual breeds and to designate haplotypes [12, 13].

The relationship between Y-chromosomal HTs within a species can be illustrated using phylogenetic trees. The horse phylogenetic MSY tree showed a clear separation of domestic horses (Equus caballus) and Przewalski horses (Equus przewalskii) and detailed the ancestry of sire lines within domestic horses. When the tree was first conducted in 2013, eight haplotypes (HTs) (six domestic and two Przewalski) were revealed, indicating that the nucleotide diversity was exceptionally low [3]. By increasing the MSY reference sequence and sequencing additional samples, more markers were defined and additional HTs were established. It became

clear that most HTs could be grouped together and formed the so called ‘crown haplogroup’. The ‘crown’ confirmed the historical information on stallion waves and showed that breeds in this group must have had a recent common ancestor of oriental heritage around 700 years ago [10]. The only HTs separate from the crown group were ‘I’ and ‘N’, which were only found in North European breeds. ‘N’ including Shetland ponies and Norwegian Fjords, and ‘I’ only Icelandic horses. HT diversity was found to be higher in those secluded breeds [10]. Updated versions of the tree were published on the basis of a larger reference [14] and included more horse breeds such as remote Asian horse populations and island horses. To date, the equine MSY phylogenetic tree includes 153 HTs defined by 2,966 variants [13]. This study included four Icelandic horses samples in the analysis, and their haplotypes were located on two separate branches outside the crown (‘da3_I’ and ‘da1_Ub-I’) [13]. The refined tree is shown in Figure 1 and represents several branches outside the crown.

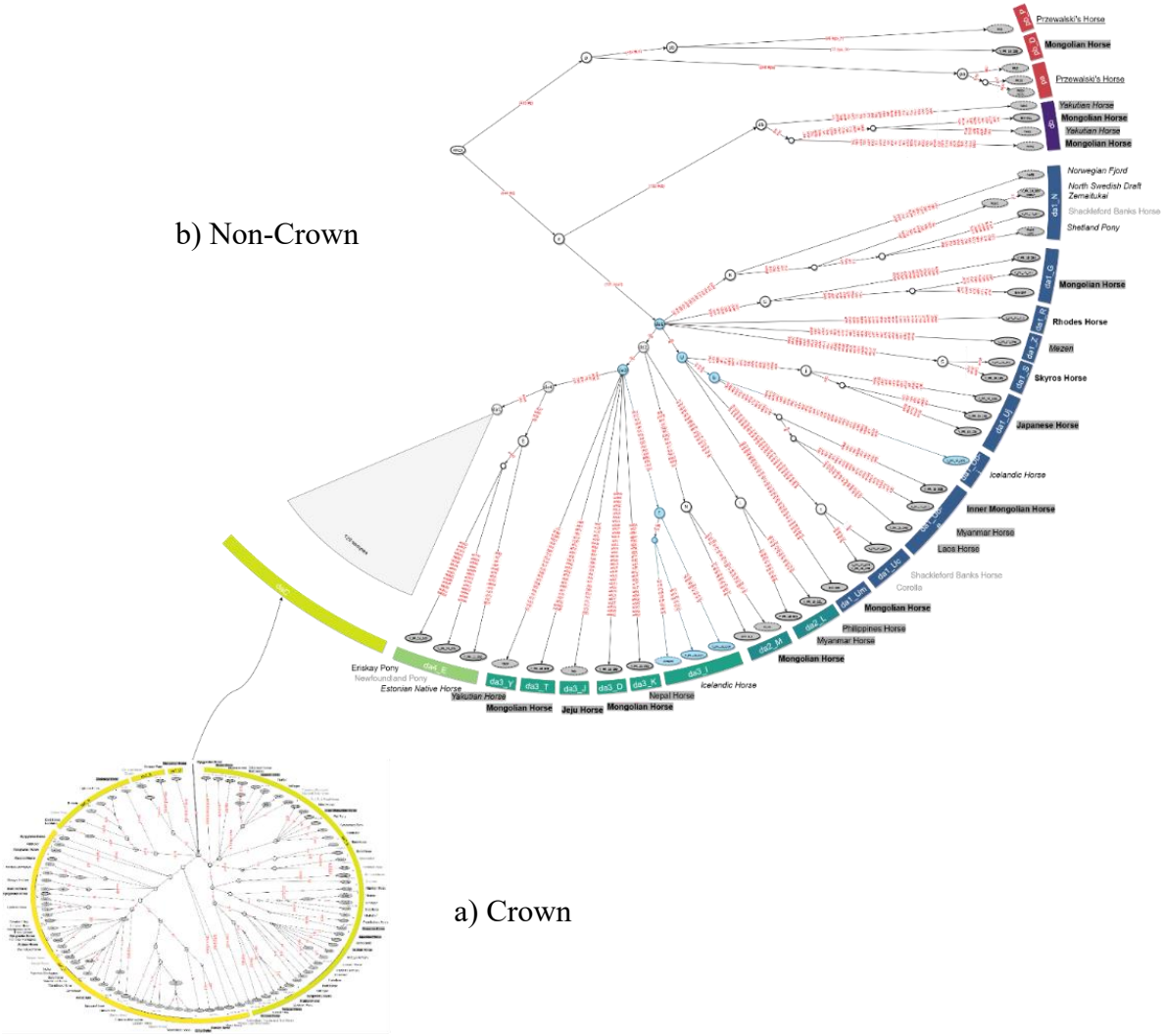


Figure 1 Phylogenetic tree of the horse MSY HTs [13] a) 'Crown '; b) 'Non-Crown'. MSY markers are written in red on the branches with the blue-coloured branches marking the Icelandic haplotypes.

1.5 Aim of this thesis

This thesis aims to investigate the special and remote breeding history as well as the current breeding trends of Icelandic horses based on MSY HT analysis and paternal pedigree information. Icelandic horses are the only horse breed originating in Iceland and are believed to have been unaffected by any other breed, making them genetically interesting and phenotypically unique from other horse breeds. So far, MSY haplotypes found in Icelandic horses were not found in any other breed and no 'crown-HTs' were detected in Icelandic horses, [9, 10, 13] which is in congruence with the remote breeding history. However, previous analyses were not conducted population-wide and not on a fine-scaled MSY HT level. This study will comprehensively investigate previously defined MSY haplogroups [13] in a sire line representative dataset of Icelandic horses to address questions regarding breeding history.

In addition to investigating the paternal MSY history, the worldwide distribution of the current Icelandic stallion population will be explored to observe trends in the breeds since they gained popularity in recent years. With the use of a newly created database from data available at WorldFengur, it will be possible to determine where most Icelandic stallions are currently located. By contrasting data regarding the 'country of residence' and the 'country of birth' of the stallions included in the database, it will be possible to illustrate the distribution and trace the export of Icelandic stallions. Therefore, this information should provide insight into the Icelandic stallion market. Furthermore, the distribution of sire lines is of interest to show breeding trends within the breed. By using the paternal pedigree information of the included stallions, the distribution of different Icelandic 'sublines' can be illustrated throughout the different countries. By addressing these data, it will be possible to determine, if certain countries focus on different sublines. Furthermore, these data will show whether certain sublines are bred only in Iceland or outside Iceland.

Altogether, this thesis aims to investigate the unique breeding history and current breeding trends of the Icelandic horse, with a focus on MSY HT analysis and paternal pedigree information. MSY genotyping was used to validate whether previously conducted studies were representative of the entire Icelandic stallion population. By exploring the worldwide distribution of Icelandic stallions and their sublines, this study will provide insights into the current status of the breed.

2 Materials and Methods

2.1 Analysis of paternal lineages based on pedigree information

2.1.1 Used software and websites

Table 1 Used software and websites

Program/Website	Manufacturer/URL
Excel®	Microsoft Office®
WorldFengur	https://www.worldfengur.com/
R Studio version 4.2.2	Posit Software
Draw.io	https://drawio-app.com/

2.2 Creating a new pedigree database

To collect information on Icelandic stallions, a database was created using Excel®. For this new database, data from all in February 2022 registered Icelandic breeding stallions worldwide were extracted from the database WorldFengur [15] on 26. 02.2022. The data included five categories for each horse: 'FEIF-ID', 'name', 'age' and 'country of residence', as well as data regarding their suitability for breeding using Best Linear Unbiased Prediction (BLUP) values from data available in 2022. The 'FEIF–ID' – for example, AT2010472586 – is a unique identifier for every Icelandic horse and consists of the country code of the country of birth, the year of birth, and a continuous number. In the course of data handling, a separate column for the 'country of birth' was added, by extracting this information from the FEIF–ID. To obtain the proper dataset, certain filters were applied before extracting the data from WorldFengur. These included stallions with an actual age between 260 years in 2022 and with a minimum of one registered offspring. Accordingly, a dataset of 12,896 stallions that met these criteria was created. Next, the male tail line information for each horse was reconstructed as described by Remer in 2022 [16], based on the pedigree information in WorldFengur. The paternal ancestors were added manually in string format for each horse in the Excel® database. The information for the ancestors included the name and the FEIF–ID. The earliest known ancestors, positioned on the far right position in the Excel® database, were designated as 'main founders'. After defining the 'main founders' from the paternal string, influential descendants were classified as 'subfounders'. Accordingly 'subfounders' are the founders of sublines established around 1960-

1980 (Figure 2). An additional column was added to the database, assigning sublines for all horses with pedigree. After finalising the used dataset, Rstudio® was used for descriptive analysis by parsing the database following the research questions.

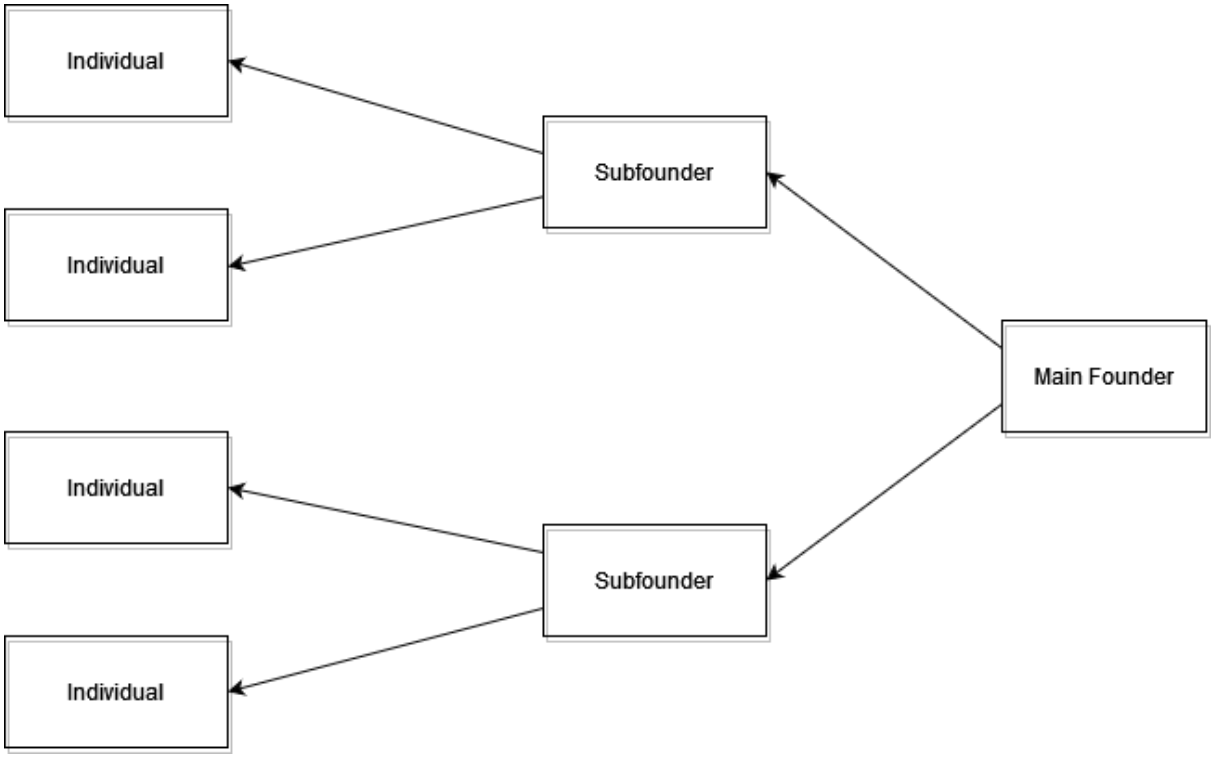


Figure 2 Schematic figure of the path from the 'main founder' to the individual stallion. 'Subfounder' are classified as influential stallions born around 1960 – 1980.

2.3 Y – chromosomal haplotype analysis

2.3.1 Used material

Table 2 Used material

Material	Manufacturer
White 96-Well Plate	Bio-Rad®
Eppendorf Tubes	Eppendorf®
Pipette Tips	Sarstedt AG & Co. KG
Adhesive Foil for 96-Well Plates	Biozym®

2.3.2 Used equipment

Table 3 Used equipment

Equipment	Manufacturer
Pipettes	Gilson®
Centrifuge	Eppendorf®
Vortexer	IKA®
Well Plate Centrifuge	Sigma-Aldrich®
CFX Real-Time System C1000 Touch®	Bio-Rad®

2.3.3 Used samples and chemicals

Table 4 Used samples and chemicals

Sample/Chemical	Manufacturer
2xKASP®Mastermix	LGC Genomics®
KASP®Assaymix	LGC Genomics®
Genomic DNA in solution	Institute of Animal Breeding and Genetics

2.3.4 Used software

Table 5 Used software

Software	Manufacturer
Excel ®	Microsoft Office ®
Bio-Rad CFX Manager 3.1®	Bio-Rad®

2.4 DNA samples for MSY haplotyping

Genomic DNA, with a concentration of 5 ng/µl, isolated from hair root tissue from 72 male Icelandic horses was provided by the Institute of Animal Breeding and Genetics. These samples were originally provided by private Islandic horse owners and were already used in previous projects [9, 10, 13]. Samples were chosen on the basis of the pedigree of the sampled horse to ensure inclusion of the different sublines of Icelandics and to prevent overrepresentation of certain lines. From the defined sublines (Chapter 2.2.), 25 were included in the genotyping dataset (Table 6). In this study, the MSY haplotype was determined for 72 samples by genotyping. The HT values for six previously determined additional samples were provided and included in this study [9].

Table 6 Distribution of sublines throughout sample size. * marks 11 out of 12 most influential subfounders (Figure7)

'Main founder' according to pedigree	**'Subfounder' of the sample**	**Number of samples genotyped**
Blakkur frá Haga	Ljúfur frá Kirkjubæ	2
	Ylur frá Kirkjubæ	3
Blettur frá Vilmundarstöðum	Gáski frá Hofsstöðum *	6
Brúnn frá Árnanesi	Ófeigur frá Flugumýri *	4
	Hrafn frá Árnanesi *	5
	Skotti frá Hesti	1
	Gáski frá	2
Brúnsokki frá Hindisvík	Ófeigur frá Hindisvík	3
Glói frá Hindisvík	Glóblesi frá Hindisvík	3
Skjóni frá Svanavatni	Skjóni frá Svanavatni	1
Jarpur frá Stokkhólma	Hrappur frá Garðsauka *	2
	Ófeigur frá Blönduósi	3
Ísleifs-Gráni frá Geitaskarði	Orri frá Þúfu í	3
	Náttfari frá Ytra-	3
	Kjarval frá Sauðárkróki *	1
	Stormur frá Eiríksstöðum	5
Sörli frá Svaðastöðum	Rauður frá Kolkuósi *	4
	Hylur frá Kirkjubæ *	6
	Léttir frá Vík í Mýrdal	1
	Hörður frá Kolkuósi *	10
	Gráni frá Hólum	2
	Hrafn frá Holtsmúla *	4
	Blesi frá Bólstað	2
	Goði frá Álftagerði	2
	Roði frá Skarði	1

2.5 Markers used for genotyping

Y-chromosomal markers for determining HTs were chosen on the basis of the phylogenetic tree [13]. Figure 3 shows a simplified Y-chromosomal HT tree [13], which was generated on selected 'key-mutations' [17] in preparation. HTs linked to Icelandic horses are marked in blue.

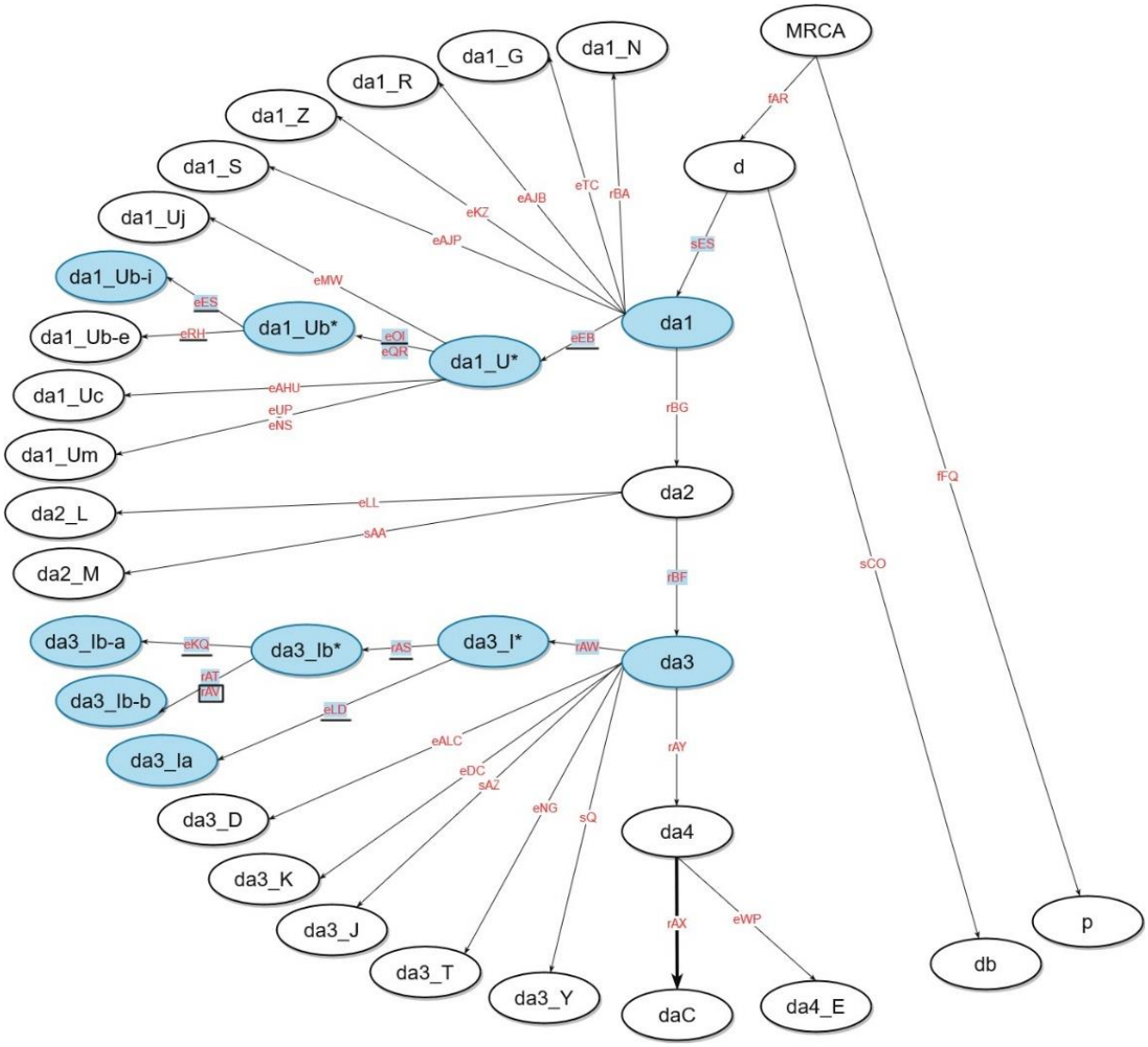


Figure 3 Simplified version of the phylogenetic [13] illustrated by Mag. Lara Radovic. Haplotypes found in Icelandic horses and their determining markers are marked in blue. Underlined markers were genotyped in this thesis. Marker rAV was not genotyped, but samples carrying da3_Ib-b were included from previous studies [7] .

From 75 published Icelandic-specific markers [13], twelve Icelandic markers were chosen for this thesis. The marker eRH, associated with Mongolian, Myanmar, and Laos horses, was included but not expected to be found in Icelandics. Allelic states of the markers used are listed in Table 7. Genotyping of these markers was performed using the KASP® genotyping system, as described in Section 2.6. Markers were genotyped in an order that followed the hierarchical structure of the tree. For example, if a sample carried the derived allele at marker rAW (rAW_C_1), the markers rAS and eLD were genotyped next. In the end, seven different markers were needed to determine the HTs of the 72 samples. Allelic state information at loci not necessarily genotyped were imputed according to the phylogeny. Markers eLD and eKQ were established in the lab for the first time during the course of this thesis (Table 7). Based on the allelic variation, the HT was determined for each sample based on the variant information, as shown in Table 8. The derived allele of the haplotype is assigned '_0' and the ancestral '_01'.

Table 7 Y–chromosomal markers used and their variation. '_1' assigns the derived allele and '_0' the ancestral allele [13].

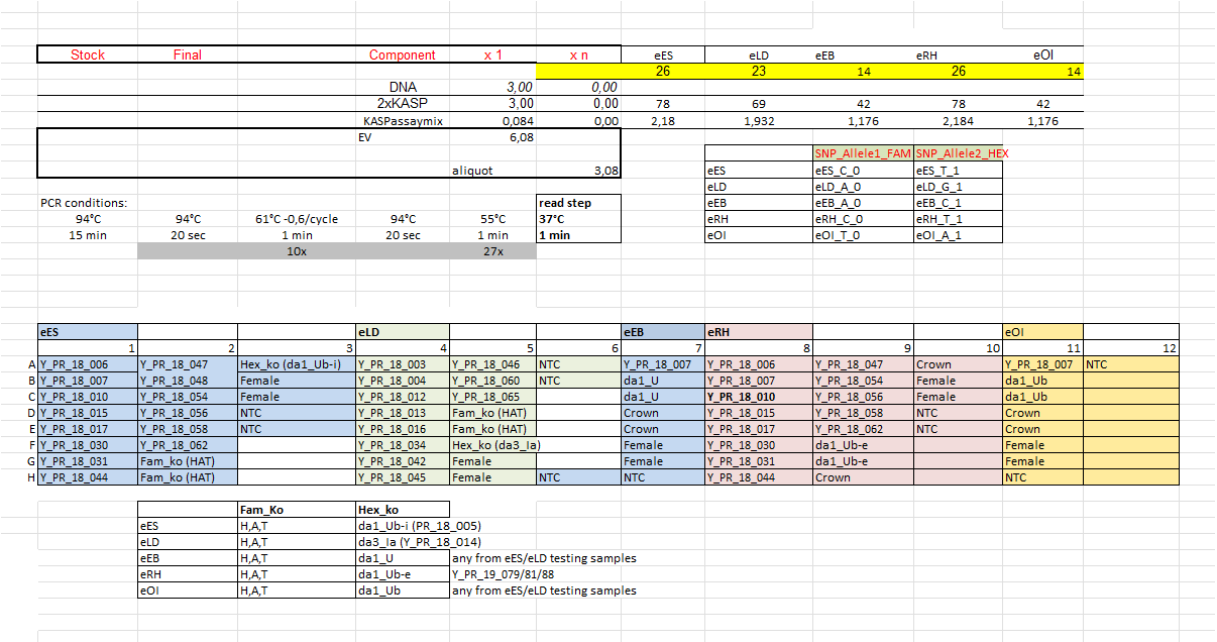
Marker	Ancestral Allele	Derived Allele	Genotyped in this thesis	Number of samples genotyped
rAV	rAV_C_0	rAV_T_1	No – data provided	0
rAW	rAW_T_0	rAW_C_1	No - data provided	0
eEB	eEB_A_0	eEB_C_1	Yes	1
eOI	eOI_T_0	eOI_A_1	Yes	1
eES	eES_C_0	eES_T_1	Yes	14
eRH	eRH_C_0	eRH_T_1	Yes	13
rAS	rAS_A_0	rAS_G_1	Yes	1
eKQ	eKQ_G_0	eKQ_A_1	Yes - assay used for the first time	47
eLD	eLD_A_0	eLD_G_1	Yes - assay used for the first time	11
sES	sES_C_0	sES_A_1	No	0
rBF	rBF_C_0	rBF_T_1	No	0

Table 8 Variant information for Icelandic haplotypes.

Haplotype	Allelic State	Allelic State	Allelic State	Allelic State	Allelic State	Allelic State
da1*	sES_1	eEB_0	eOI_0	eQR_0	eES_0	eRH_0
da1_U*	sES_1	eEB_1	eOI_0	eQR_0	eES_0	eRH_0
da1_Ub*	sES_1	eEB_1	eOI_1	eQR_1	eES_0	eRH_0
da1_Ub-i	sES_1	eEB_1	eOI_1	eQR_1	eES_1	eRH_0
da1_Ub-e	sES_1	eEB_1	eOI_1	eQR_1	eES_0	eRH_1
da3*	rBF_1	rAW_0	rAS_0	eKQ_0	rAT_0	rAV_0
da3_I*	rBF_1	rAW_1	rAS_0	eKQ_0	rAT_0	rAV_0
da3_Ib*	rBF_1	rAW_1	rAS_1	eKQ_0	rAT_0	rAV_0
da3_Ib-a	rBF_1	rAW_1	rAS_1	eKQ_1	rAT_0	rAV_0
da3_Ib-b	rBF_1	rAW_1	rAS_1	eKQ_1	rAT_1	rAV_1
da3_Ia	rBF_1	rAW_1	eLD_1			

2.6 Genotyping Y-chromosomal markers using KASP® technology

After markers and samples were selected as described in Section 2.5, the KASP® genotyping system was used to determine the allelic states of the samples [18]. In addition to the samples used for analysis, positive and negative controls were included for each assay in each run. DNA samples with known allelic states were used as positive 6-Carboxy-Fluorescein (FAM) and Hexachloro-Fluorescein (HEX) controls. DNA samples from female horses were used as negative controls. To validate the lack of contamination, a minimum of one no template control (NTC) per assay was included. To plan the individual KASP® runs on the 96-well plate, a spreadsheet designed and provided by Mag. med. vet. Doris Rigler, from the Institute of Animal Breeding and Genetics, was used.



Stock	Final	Component	x 1	x n	eES	eLD	eEB	eRH	eOI
					26	23	14	26	14
		DNA	3,00	0,00					
		2xKASP	3,00	0,00	78	69	42	78	42
		KASPassaymix	0,084	0,00	2,18	1,932	1,176	2,184	1,176
		EV	6,08						
			aliquot	3,08					

	SNP_Allele1_FAM	SNP_Allele2_HEX
eES	eES_C_0	eES_T_1
eLD	eLD_A_0	eLD_G_1
eEB	eEB_A_0	eEB_C_1
eRH	eRH_C_0	eRH_T_1
eOI	eOI_T_0	eOI_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	

	eES			eLD			eEB	eRH			eOI	
	1	2	3	4	5	6	7	8	9	10	11	12
A	Y_PR_18_006	Y_PR_18_047	Hex_ko (da1_Ub-i)	Y_PR_18_003	Y_PR_18_046	NTC	Y_PR_18_007	Y_PR_18_006	Y_PR_18_047	Crown	Y_PR_18_007	NTC
B	Y_PR_18_007	Y_PR_18_048	Female	Y_PR_18_004	Y_PR_18_060	NTC	da1_U	Y_PR_18_007	Y_PR_18_054	Female	da1_Ub	
C	Y_PR_18_010	Y_PR_18_054	Female	Y_PR_18_012	Y_PR_18_065		da1_U	**Y_PR_18_010**	Y_PR_18_056	Female	da1_Ub	
D	Y_PR_18_015	Y_PR_18_056	NTC	Y_PR_18_013	Fam_ko (HAT)		Crown	Y_PR_18_015	Y_PR_18_058	NTC	Crown	
E	Y_PR_18_017	Y_PR_18_058	NTC	Y_PR_18_016	Fam_ko (HAT)		Crown	Y_PR_18_017	Y_PR_18_062	NTC	Crown	
F	Y_PR_18_030	Y_PR_18_062		Y_PR_18_034	Hex_ko (da3_Ia)		Female	Y_PR_18_030	da1_Ub-e		Female	
G	Y_PR_18_031	Fam_ko (HAT)		Y_PR_18_042	Female		Female	Y_PR_18_031	da1_Ub-e		Female	
H	Y_PR_18_044	Fam_ko (HAT)		Y_PR_18_045	Female	NTC	NTC	Y_PR_18_044	Crown		NTC	

	Fam_Ko	Hex_ko	
eES	H,A,T	da1_Ub-i (PR_18_005)	
eLD	H,A,T	da3_Ia (Y_PR_18_014)	
eEB	H,A,T	da1_U	any from eES/eLD testing samples
eRH	H,A,T	da1_Ub-e	Y_PR_19_079/81/88
eOI	H,A,T	da1_Ub	any from eES/eLD testing samples

Figure 4 Example of the spreadsheet used to calculate the amount of KASP®Assaymix and KASP®Mastermix and to document the position of samples on the 96-well plate. The sheet also shows the PCR conditions, the control samples used, and the allelic state underlying the FAM and HEX signals for the respective assays.

This spreadsheet was also used to calculate the required volume of KASP®Assaymix and KASP®Mastermix, as well as to provide information on the PCR protocol, control samples, and the allele specificity of the FAM and the HEX signal for each used assay. Figure 4 shows an example of the spreadsheet used. When conducting the analysis, the calculated volumes of KASP®Assaymix and KASP®Mastermix were pipetted together into an Eppendorf tube, vortexed, and aliquoted into the respective positions of a 96-well plate. The genomic DNA from the samples and controls was thawed, and DNA and NTCs were pipetted onto the plate following the layout of the spreadsheet. In the next step, the plate was sealed with an adhesive foil and briefly centrifuged in a microplate spinner. The CFX Real-Time System C1000 Touch® with Bio-Rad CFX Manager® was used for conducting the KASP® Real-Time PCR and for showing the results for each run as a cluster plot (Figure 5). By exporting the data to Excel®, a tabular presentation of the results was created to show the sample ID and variant information (Figure 6). Table 9 provides the PCR protocol used. If insufficient HEX and FAM signals occurred, a recycle step was added, as shown in Table 10.

Table 9 KASP® Real-Time PCR protocol used by the Institute of Animal Breeding and Genetics

Steps	1. Activation	2. Denaturation	3. Elongation	4. Denaturation	5. Elongation
Temperature	94°C	94°C	61°C (-0,6°C per cycle)	94°C	55°C
Time	15 min	20 sec	60 sec	20 sec	60 sec
Repeated cycles per step	1x	10 x		27x	

Table 10 KASP ® Real-Time PCR recycling protocol used by the Institute of Animal Breeding and Genetics

Step	1. Denaturation	2. Elongation
Temperature	94°C	57°C
Time	20 sec	60 sec
Repeated cycles per step	3x	

2.7 Merging haplotypes with tail line information

The paternal pedigree information of the genotyped samples was used to assign haplotypes to their respective sublines. As a next step, the established haplotypes of the 25 genotyped sublines were used to assign haplotypes to the stallions included in the pedigree database that shared the same paternal ancestry. For stallions that did not directly belong to one of the genotyped sublines, HTs could be assigned by relaxing the criteria and linking them to the sublines for which results were obtained. Stallions were linked to sublines with determined HT results if they shared a paternal ancestor up to three generations back from the established ‘subfounder’.

3 Results

3.1 KASP® genotyping results

KASP® genotyping for MSY markers was performed in 72 horses, and results for all samples were obtained. After each KASP® run was completed, the results were presented in a cluster plot in Bio-Rad CFX Manager®. The results of the eLD assay are given as an example in Figure 5. For the marker eLD, samples showing a HEX signal (blue) carried the eLD_G_1 variant, and those presenting a FAM signal (orange) carried the eLD_A_0 variant. Therefore, samples with the allelic state 'eLD_G_1' carry the 'da3_Ia' haplotype (according to Table 8). Genotyping assays for markers eLD and eKQ were successfully established in the laboratory for the first time.

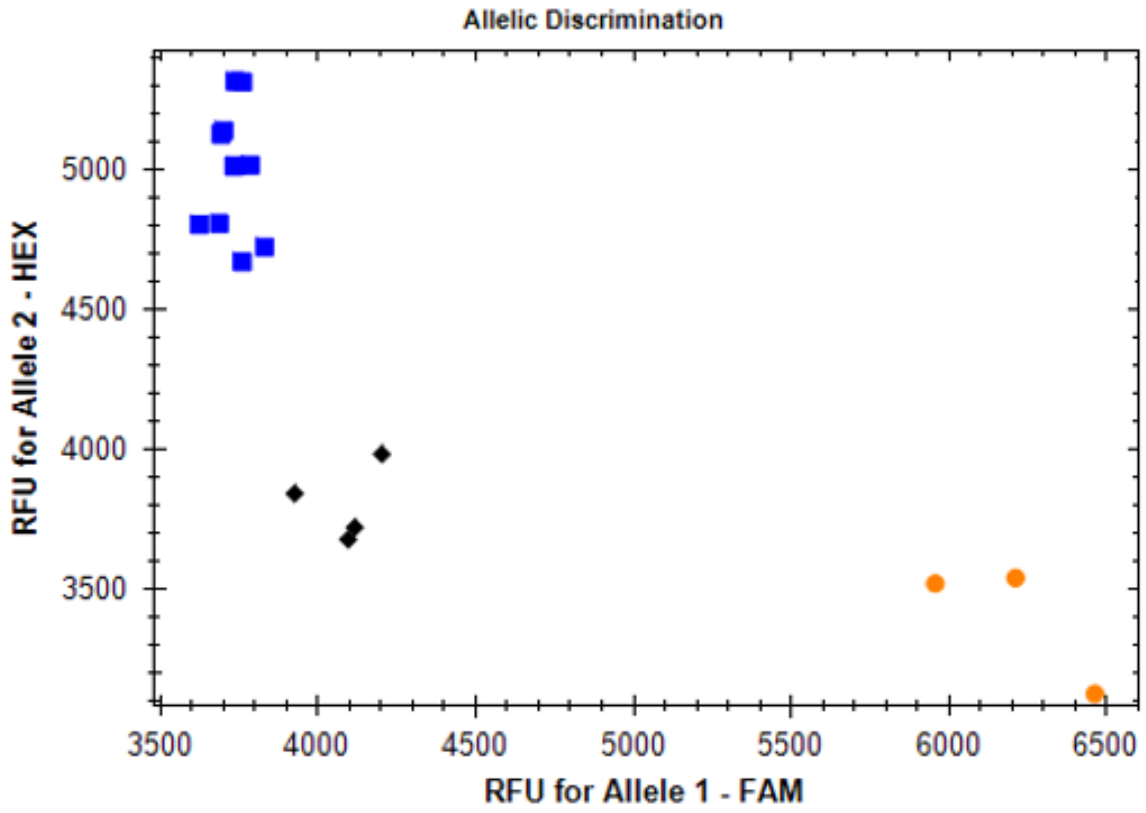


Figure 5 Cluster plot for assay eLD is shown as an example of the results generated by Bio-Rad CFX Manager®.

Well	Sample	Call	RFU1	RFU2	eLD
D05	FAM_ko	Allele 1	5956,47	3519,37	eLD_A_0
E05	FAM_ko	Allele 1	6211,04	3539,44	eLD_A_0
G05	Female	No Call	3925,70	3840,62	No Call
H05	Female	No Call	4094,99	3676,47	No Call
F05	HEX-ko	Allele 2	3700,79	5136,99	eLD_G_1
A06	NTC	No Call	4116,26	3719,58	No Call
B06	NTC	No Call	4202,11	3981,56	No Call
A04	Y_PR_18_003	Allele 2	3759,50	4668,37	eLD_G_1
B04	Y_PR_18_004	Allele 2	3785,19	5013,91	eLD_G_1
C04	Y_PR_18_012	Allele 2	3733,27	5008,55	eLD_G_1
D04	Y_PR_18_013	Allele 2	3686,80	4804,66	eLD_G_1
E04	Y_PR_18_016	Allele 2	3737,29	5312,10	eLD_G_1
F04	Y_PR_18_034	Allele 2	3692,84	5123,35	eLD_G_1
G04	Y_PR_18_042	Allele 1	6462,62	3125,64	eLD_A_0
H04	Y_PR_18_045	Allele 2	3623,65	4801,59	eLD_G_1
A05	Y_PR_18_046	Allele 2	3830,17	4720,92	eLD_G_1
B05	Y_PR_18_060	Allele 2	3738,52	5010,64	eLD_G_1
C05	Y_PR_18_065	Allele 2	3759,99	5310,72	eLD_G_1

Figure 6 Results from the eLD assay as an example of the tabular presentation of results in Excel®. The column 'RFU1' shows the signal strength for the FAM and 'RFU2' for the HEX signal. Depending on the signal strength of 'RFU1' and 'RFU2', column 'Call' gives information on whether allele 1 or allele 2 is present in the sample. The column with the assay name, in this case 'eLD', shows the determined allelic state for each DNA sample (the variant information and whether it carried the ancestral _0 or the derived _1 state; see also section 2.5.).

3.2 MSY haplotypes in genotyped Icelandic horses

Genotyping results for all 72 newly genotyped samples were obtained, and their respective haplotypes were inferred. Five different Icelandic-specific haplotypes were found within the 78 total samples used (72 newly genotyped and 6 with final results from previous studies [9]). A considerable proportion of the samples (40 out of 78) were placed at an inner branching point (da3_Ib*). All haplotypes determined in the 78 samples were previously associated with Icelandic horses, the haplotype 'da1_Ub-e' was not present in the genotyped samples. Markers that determine HT 'da3_Ia' (eLD) and 'da3_Ib-a' (eKQ) were successfully established in the laboratory for the first time. Eleven of the tested samples carried the 'da3_Ia' haplotype and nine the 'da3_Ib-a haplotype. The distribution of samples throughout the haplotypes is shown in Figure 7.

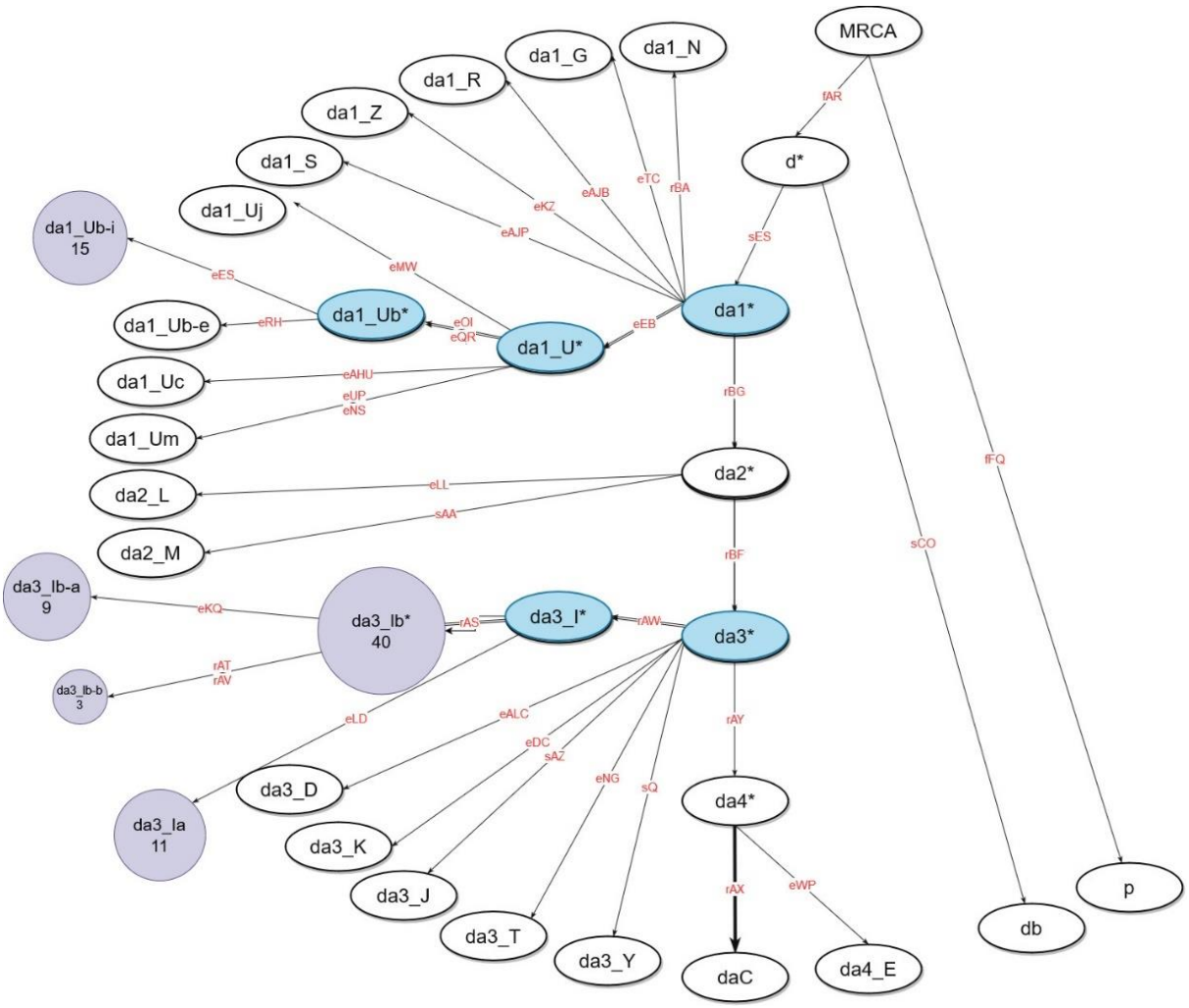


Figure 7 Genotyping results for 78 samples presented in the phylogenetic tree. Haplotypes associated with Icelandic horses in previous studies are coloured. HTs for which results were obtained are shown in violet, with circle sizes proportional to the number of samples. The remaining Icelandic HTs are marked in blue.

3.3 Pedigree and sublines

Of the 12,896 stallions included in the pedigree database, the male tail lineage information could be reconstructed from WorldFengur pedigree information for 12,816 stallions. For the remaining 80 stallions, the paternal pedigree could not be reconstructed because of the lack of pedigree information, and these were set as ‘unknown’ in the database. The oldest known ancestor, placed on the far right position of the paternal string, was classified as the ‘main founder’. The 12,816 stallions could be traced back to 89 ’main founders’. ‘Main founders’ were born around 1900 to 1940. 295 stallions born around 19601980 were chosen as ‘subfounders’, and for 12,816 stallions, their ‘subline’ was added accordingly to their pedigree. From the 295 defined sublines, 66 were marked with ‘short pedigree’ meaning, that the ‘main founder’ was also used as the ‘subfounder’. Eighty-three sublines received the remark ‘one horse’ meaning, that only a single horse out of the 12,816 stallions was assigned this subline. Not only stallions born in Iceland were designated as ‘subfounders’; 18 sublines were founded by a stallion born outside Iceland.

If a closer look at the various sublines is taken, it became evident that only a few sublines contain most of the stallions in the database. The subline after Orri frá Þúfu í Landeyjum (1986) was the most frequent in the dataset with 2,493 members. The subline with the second most members was after Hrafn frá Holtsmúla (1968); 2,253 stallions were attributable to him. The third most influential ‘subfounder’ was Ófeigur frá Flugumýri (1974), with 1,573 stallions as descendants (Figure 8). Overall, 83.8% of the dataset was explained by twelve ‘subfounders’ as shown in Figure 8. Smaller sublines with less than 100 individuals per line were not listed individually; the cluster ‘sublines with less than 100 individuals’ consisted of 133 different sublines.

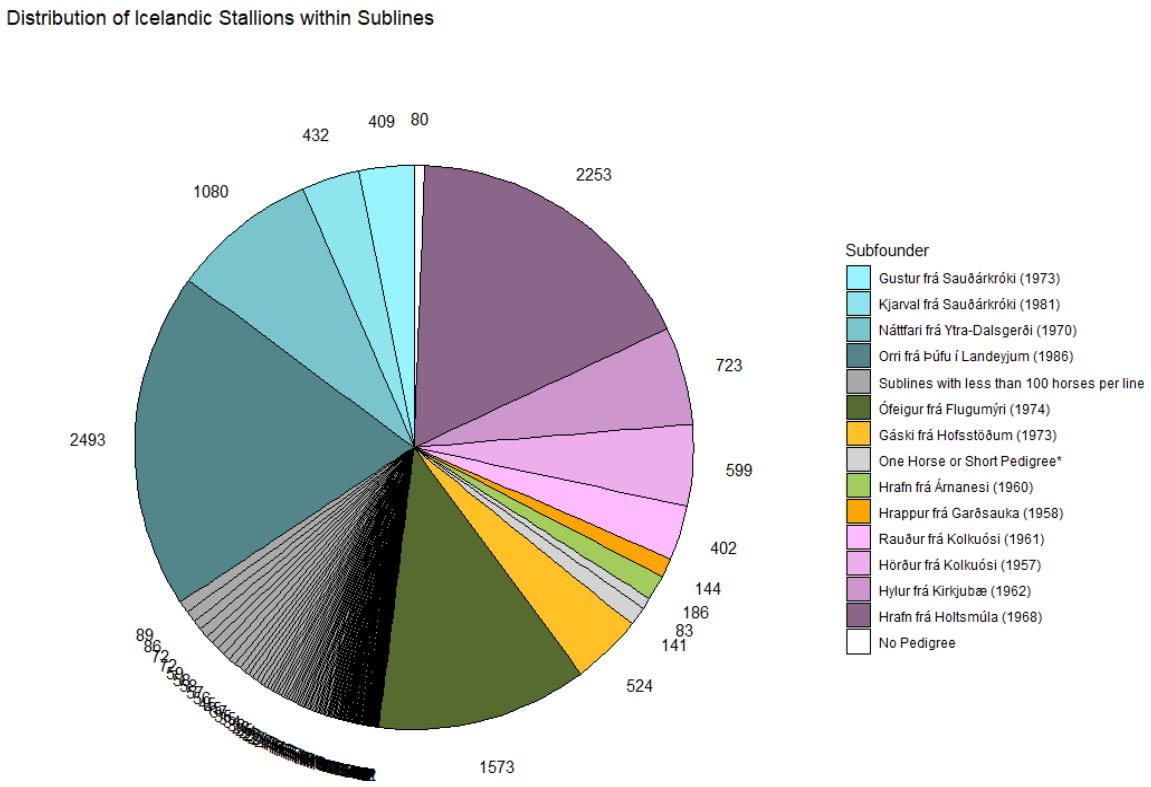


Figure 8 Distribution of sublines within the 12.896 stallions included in the pedigree database. The 133 sublines with less than 100 individuals are shown in grey. Lines with one individual per subline are shown as a single group 'one horse', (83 stallions), and horses with a short pedigree are also shown as a group 'short pedigree' (141 stallions). 'No pedigree' marks the 80 stallions for which paternal line information was not available.

Going further back in the paternal tail line, the pedigree becomes narrower as popular sublines trace back to only very few 'main founders' (Figure 2). Twenty-one 'main founders' are listed in Figure 9. Overall, 11,661 stallions (90.42%) can be traced back to the three most influential 'main founders', Brúnn frá Árnanesi (~1908), Ísleifs-Gráni frá Geitaskarði (~1915) and Sörli frá Svaðastöðum (1908) (Figure 9). Noticeable is that all descendants of the 'main founder' Blettur frá Vilmundarstöðum (1946) in the dataset are via the subline of Gáski frá Hofsstöðum (1973).

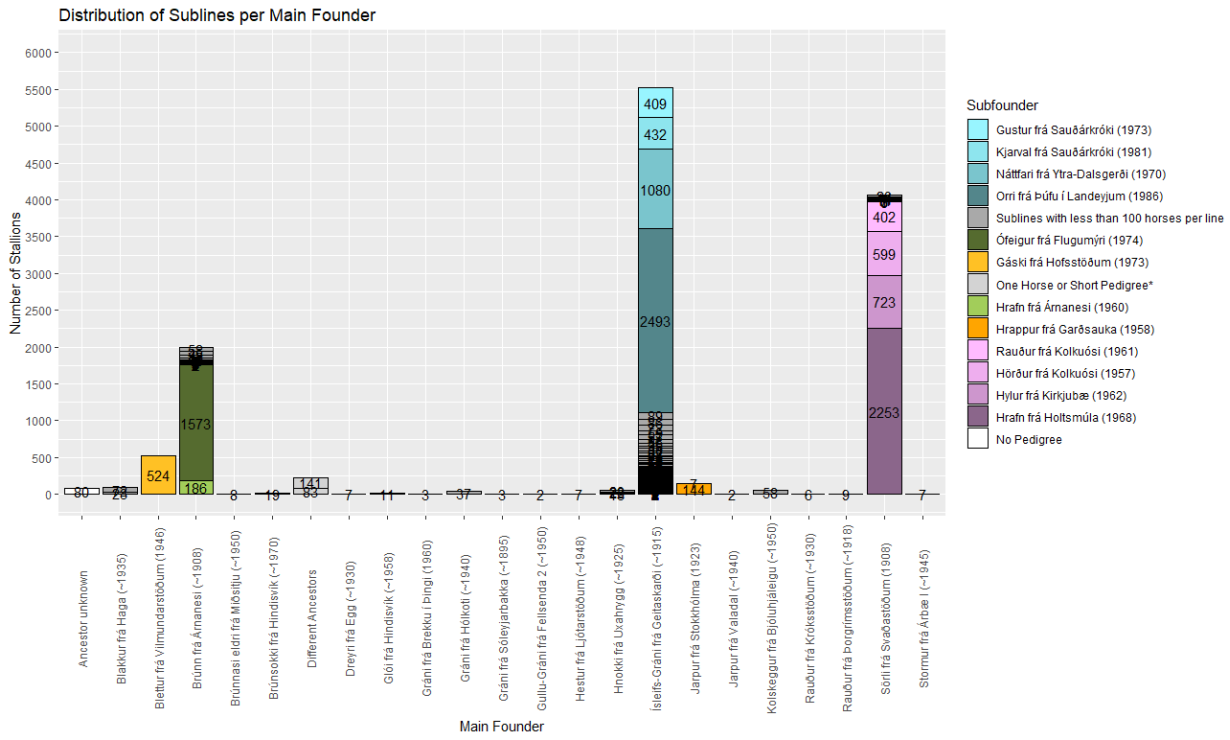


Figure 9 Sublines according to their 'main founders'. Horses with the remark 'short pedigree' or 'one horse' are clustered together into the category 'different ancestors' which include 68 'main founders'.

3.4 Y-chromosomal haplotypes in Icelandic sublines

Based on the genotyping results, haplotypes were added to the stallions of the pedigree database based on their subline. The 78 samples genotyped for their Y-haplotypes carried five Icelandic-specific haplotypes and represented 25 sublines. In some sublines, more than one haplotype was observed. For 9,594 (74.40%) of the 12,896 stallions in the pedigree database, haplotypes could be added directly based on the stallions' subline, which was represented in the genotyping dataset. In the next step, stallions from not genotyped sublines were linked by commonly shared ancestors to genotyped sublines when possible. Haplotypes were only linked if the common ancestor of the genotyped and not genotyped subline was not more than three generations back from the 'subfounder'. Altogether, HTs were allocated to 12,462 (96.6%) stallions. The remaining 434 (3.4%) stallions consisted of 80 stallions without pedigree information and 354 individuals for which the haplotypes could not yet be determined or linked. Of the 354, 140 had the remark 'short pedigree', the remaining 214 stallions came from 25 sublines.

3.5 Geographic distribution and age of the stallions included in the database

Because of the Icelandic horses' interesting breeding history and recent gain in popularity, we used the data already extracted from the pedigree database to examine the worldwide distribution of Icelandic stallions. By addressing the three parameters 'country of birth', 'country of residence' and 'age', we aimed to determine the movement of Icelandic stallions, as well as the age distribution of stallions in different countries.

The 'country of birth' was extracted from the FEIF-ID, and the 'country of residence' was defined as the country in which the horse was registered when the data were extracted from WorldFengur (February 2022). To illustrate where Icelandic stallions were born in contrast to where they are located now, the 'country of birth' and the 'country of residence' were contrasted. If the 'Country of residence' was not registered in WorldFengur, the location was set to 'country not assigned' in the pedigree database (160 stallions). As shown in Figure 10, 7,458 (57.83%) of the 12,896 stallions were born in Iceland, followed by 2,679 (20.75 %) in Germany, Austria, and Switzerland. In contrast, although more than half of the stallions were born in Iceland, only 3,551 (27.58 %) stallions are still located there. With 4,158 (32.24%) individuals, most stallions are currently registered in Germany, Austria, and Switzerland. This shows that most horses born in Iceland are exported to other countries.

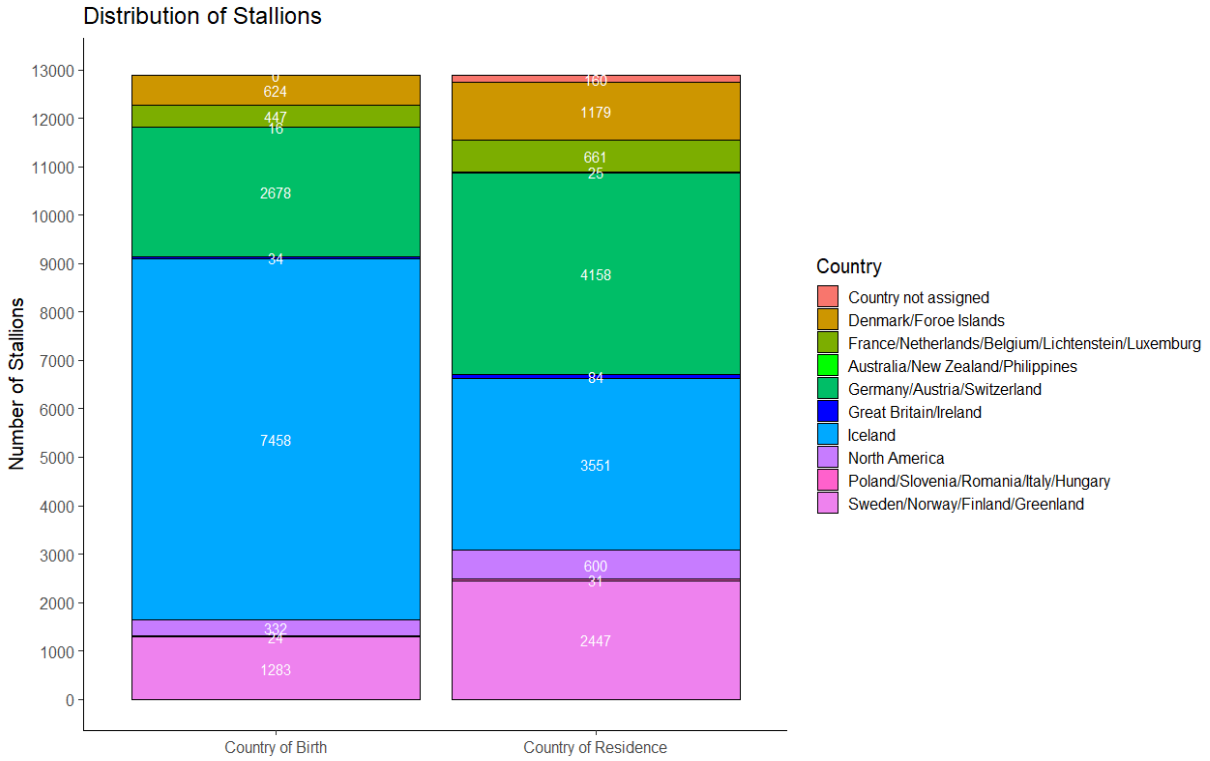


Figure 11 Comparison between the 'country of birth' and the 'country of residence' of the 12,896 Icelandic stallions included in the pedigree database.

The analysis of age in relation to the 'country of residence' can provide more information about trends in breeding and export. The oldest horse in the dataset was born in 1963 and the youngest one in 2019. The number of very old horses probably amounts to nominal members of the database. Most horses in the dataset used were born between 2005 and 2010 (Figure 11).

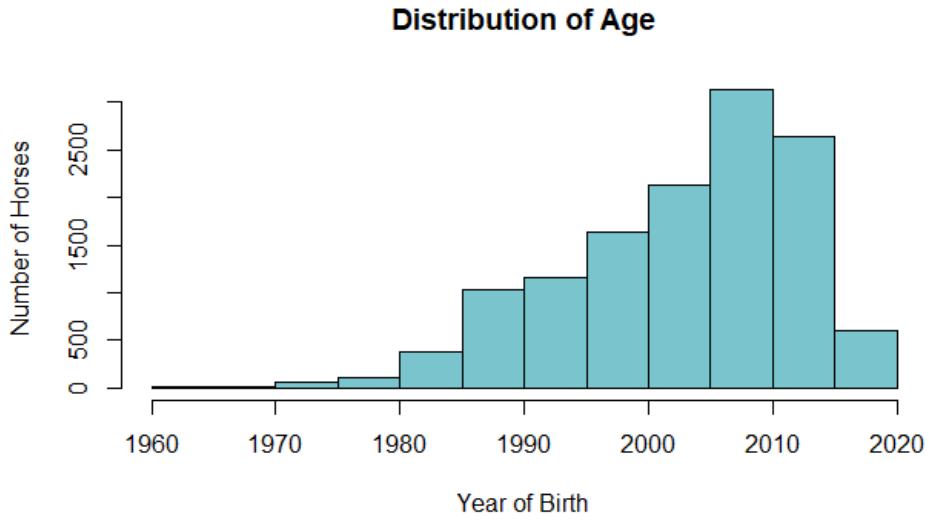


Figure 10 Histogram showing the distribution of the age of all 12,896 stallions. Most stallions were born between 2005 and 2010.

To gain a better understanding of the whereabouts of younger stallions, the analysis of age in relation to the ‘country of residence’ was conducted for the horses born between 2000 and 2019. This subset included 8,800 of 12,896 stallions. The 8,800 stallions were grouped into four age groups, born between '20002004’, ‘20052009’, ‘20102014’, and ‘20152019’, respectively (Figure 10). In the oldest age group, which comprised 1,825 stallions, 33,9 % remained in Iceland, second most horses in this group were found in Scandinavia (26,1%) and only 12,4% in German-speaking countries. Group ‘2005-2009’ and ‘2010-2014’ showed a similar distribution. The majority of these groups were found in Germany/Austria/Switzerland, followed by stallions in Iceland (approximately a third of the stallions). Among the youngest group, the highest proportion is still registered in Iceland, with 47,5% of the 1,088 stallions. This showed that most recently bred stallions still originate from Iceland, although in general most Icelandic stallions are located outside Iceland (Figure 12).

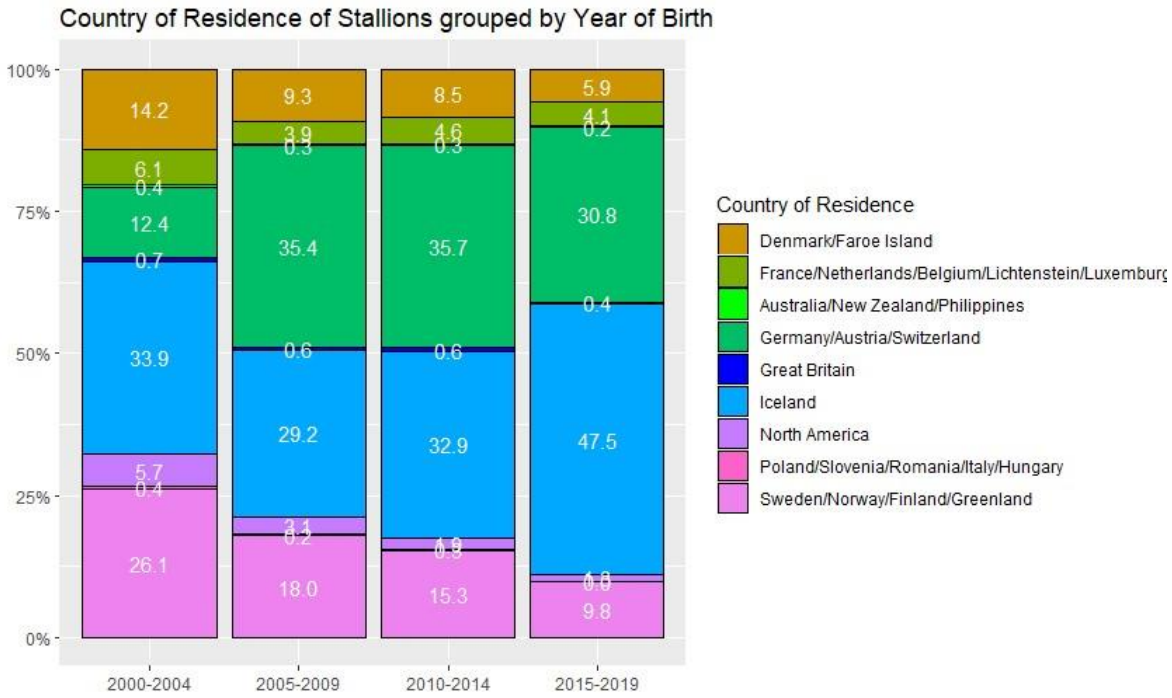


Figure 12 ‘Country of residence’ in relation to the ‘year of birth’ of 8,800 stallions in the pedigree database. Stallions were grouped into four age groups. The percentage is given in white numbers within the bars and the total number of stallions per group on top of the bars.

3.6 Popularity of sublines in certain countries

To evaluate if there are countries in which certain sublines are more dominant, the distribution of sublines in relation to the 'country of residence' was conducted. The results showed that the distribution of sublines was similar in every county and that there were no predominant sublines. Of the 12 most popular sublines, descendants of the subline of Hrappur frá Garðsauka (1958) originating from Jarpur frá Stokkhólma (1923) were found only outside Iceland, with most stallions of this subline located in Germany (Figure 13).

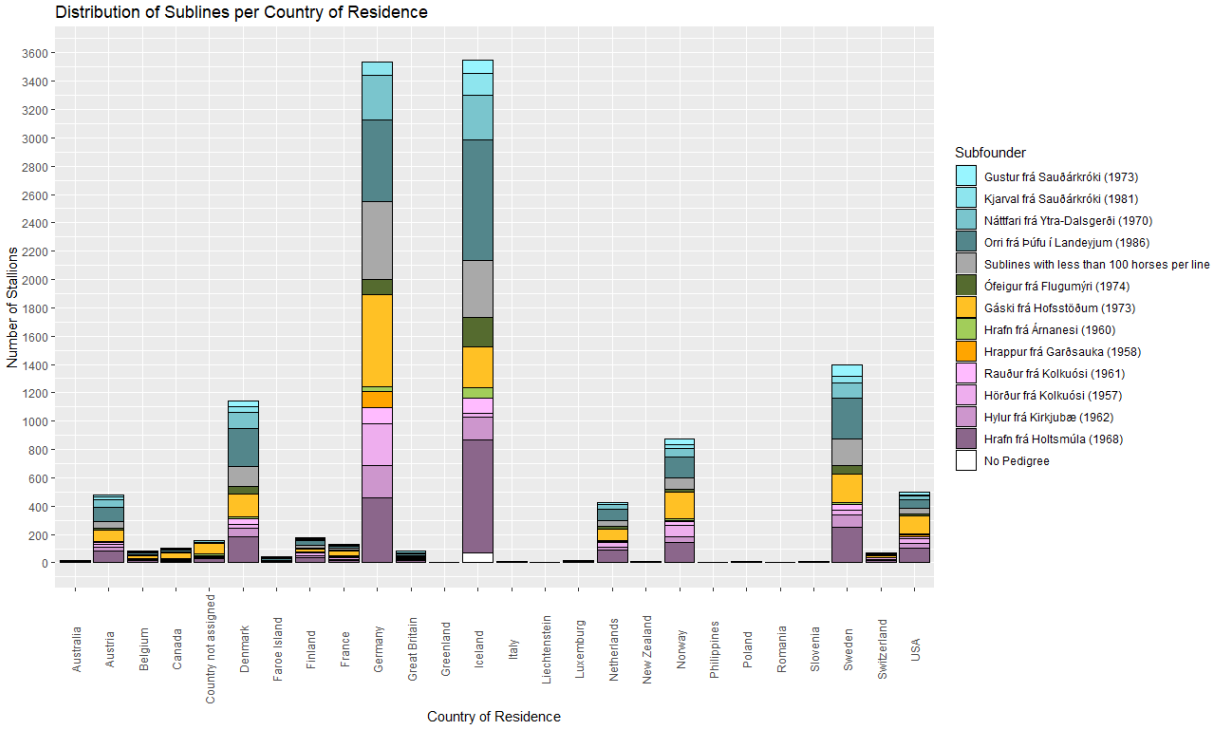


Figure 13 Distribution of the twelve most popular Icelandic sublines throughout different countries. Sublines with less than 100 stallions per line are shown in grey.

4 Discussion

The secluded genesis of the Icelandic horse and its strict breeding policy makes the breed said to be one of the purest in the world. Reconstruction of paternal [9, 10, 13] and maternal heritage [19] has been conducted in the past. Focusing on the evolution of the paternal line, MSY markers can be used to detect Y–chromosomal haplotypes and show the relationship between horse breeds using phylogenetic trees. So far, the Icelandic horse has harboured its own branches outside of the ‘crown group’ in the MSY phylogenetic tree, meaning that no Icelandic haplotypes have been found in other breeds [9, 10, 13].

To evaluate whether the results of previous MSY HT studies represented the entire Icelandic stallion population, a pedigree-based haplotype analysis was conducted. The created pedigree database based on WorldFengur provided the basis for sample selection by illustrating the distribution of sublines within the breed. The sample set consisted of 78 DNA samples from 25 different Icelandic sublines, and as expected, only Icelandic-specific HTs were detected within the genotyped samples. Icelandic samples were also tested for the ‘da1_Ub-e’ haplotype that was previously associated with Mongolian, Myanmar, and Laos horses [13]. This haplotype is located on the same branch as the Icelandic haplotype 'da1_Ub-I'. It is assumed that the Mongolian horse, played a role in the origin of Norwegian breeds due to human and livestock migration under Attila and Genghis Kahn [20]. Later the Icelandic horse evolved out of these Norwegian horses brought to Iceland by Viking settlers [6]. However, the haplotype ‘da1_Ub-e’ was not detected in any Icelandic sample.

Taking the paternal pedigrees of the genotyped Icelandic horses into account, we can infer that the tested Icelandic HTs represented the male tail lineages of 96.6% of the 12,869 stallions active in 2022. If it is assumed that the pedigree information for the Icelandic stallions is correct, only 3.4% of the stallions could carry HTs that were not yet tested. These stallions would be considered interesting for future testing to validate the true absence of HTs from other breeds within Icelandics. On the basis of the results of this study and previous studies [9, 13], recent introgression of other breeds into Icelandic was not found. The obtained Y-HT results further underline the uniqueness of the Icelandic stallion population.

Although Icelandic HTs were obtained for all genotyped samples, in some cases it was not possible to assign only one Icelandic HT to the genotyped subline. Due to the uniparental

heritability of the Y–chromosome, it is not possible to observe different HTs within one subline when the pedigree is correct. It was already shown that the pedigree data of Icelandics may not always be accurate, as similar results were obtained previously [9]. Various HTs within one paternal subline were also found in other breeds such as the Lipizzan [21], the English Thoroughbred [12], Arabians [16], as well as in Haflingers and Norikers [22]. In the case of Icelandics, errors in the pedigree may be due to the lack of pedigree documentation in the breeds' earliest history. When WorldFengur was founded in the 20th century, available ancestry records were integrated or possibly reconstructed if no pedigree information was available. Therefore, conflicting HT results can be expected, but this observation does not weaken the main argument that only Icelandic HTs were present within the samples. This study used previously collected samples of privately kept geldings located in Austria and Germany with suitable pedigrees, If precise haplotype results for Icelandic sire-lines and their sublines want to be obtained, samples should be collected from breeding stallions representing the subline of interest. Furthermore, genotyping readouts are still limited by the number of used MSY markers tested. The twelve selected MSY markers were sufficient to obtain results for each sample, but a respective number of samples clustered on the internal node 'da3_Ib*' in the phylogenetic tree. This shows that the definitive HTs of the samples have not yet been identified and should be genotyped with additional Y-chromosomal markers in the future or sequenced in order to determine HTs that have not yet been identified.

In addition, the created pedigree database was used to trace the current worldwide distribution of Icelandic breeding stallions. Because of the breeds recent gain in popularity, the distribution of stallions and especially their paternal sublines was of interest. As of today, the Icelandic horse can be found in over 30 countries, with the majority of horses outside of Iceland. Of the estimated 250,000 Icelandic horses worldwide, approximately 175,000 horses are found outside Iceland and around 65,000 in Iceland [8]. A similar distribution of stallions was found within the used dataset of 12,896 active breeding stallions in 2022, with more than 70% of stallions outside of Iceland and only a third of stallions in Iceland (Figure 10). In contrast, more than 50% of all active stallions in 2022 were born in Iceland. Even though there are more active breeding stallions outside of Iceland, the shift from the 'country of birth' to the 'country of residence' showed that Iceland is still the main distributor of stallions. This argument is supported by the 2019 'WorldFengur – Annual Report' [23], which states that, even though

most Icelandic horses can be found in Germany today, the average number of Icelandic horses born between 2010-2019 was 6,445 in Iceland and only 2,369 in Germany. This does not come as a surprise as the report also shows where Icelandic horses were exported to, with Germany importing the vast majority of Icelandic horses from 2011 to 2019 (Figure 13) [23].

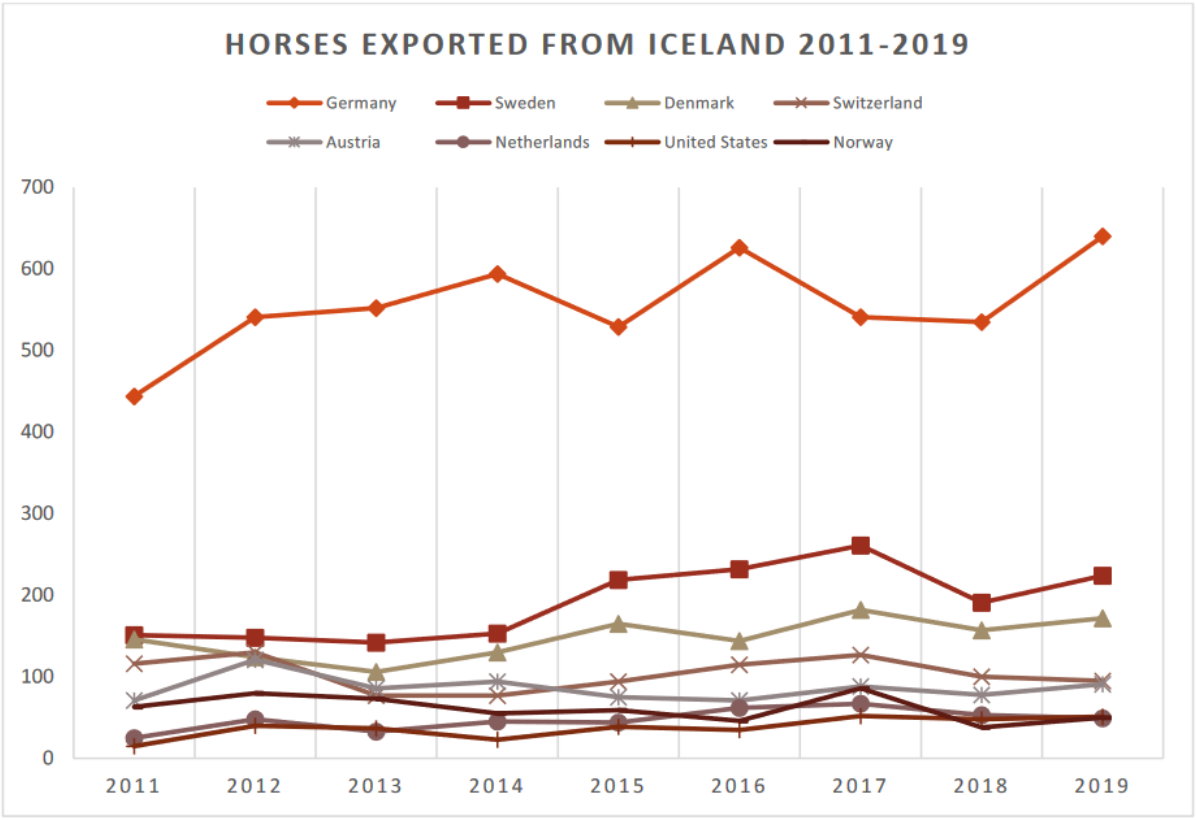


Figure 14 Distribution of exported Icelandic horses from Iceland between 2010 – 2019 taken from the 'WorldFengur – Annual Report 2019' [23].

The location of stallions is of particular importance in cases where the stallions from a subline are only located outside of Iceland. In such cases, the subline cannot be continued in Iceland because of its import ban. The results showed that out of the 12 sublines examined in more detail, the subline after Hrappur frá Garðsauka (1958) can only be found outside of Iceland. The subline of Hrappur frá Garðsauka (1958) traces back to Jarpur frá Stokkhólma (1923) and has only one other subline that amounts to the shared 'main founder'. The two samples of the subline of Hrappur frá Garðsauka (1958) that were included for genotyping both carried the 'da3_Ib*' haplotype, placing the samples on an inner node in the phylogenetic tree. Because the definitive HT of this subline is not known, it is not possible to say what consequence the

loss of this subline has for Iceland. Future studies should determine the final MSY HT for the subline and investigate autosomal markers to see if those horses have a special genetic composition that is not found in Iceland anymore. Altogether, the general distribution of sublines throughout the countries appeared to be even with no dominant subline found in any countries.

In conclusion, the results supported the argument that Icelandic horses were not recently influenced by paternal lineages from other breeds. The findings also showed that previous studies were already representative of the Icelandic stallion population. Further studies can focus on genotyping stallions that could possibly carry non-Icelandic HTs (3,4 % of the stallions). This thesis has contributed to the understanding of the genetic paternal heritage of Icelandic horses and the worldwide distribution of their sublines.

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Figure index

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Supporting Information

Sample	Last_variant_typed	Assay	rAW	eLD	rAS	eKQ	rAV	eEB	eOI	eES	eRH
		Haplotype	da3_J	da3_Ia	da3_Ib	da3_Ib-a	da3_Ib-b	da1_U	da1_Ub	da1_Ub-i	da1_Ub-e
Icelandic horse 1	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_dr		rAS_A_1_dr	eKQ_G_0_LW_20230220	rAV_C_0_dr				
Icelandic horse 2	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 3	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 4	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 5	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 6	eLD_1	da3_Ia	rAW_C_1_mf	eLD_G_1_LW_20230222	rAS_G_0_mf		rAV_C_0_mf				
Icelandic horse 7	eLD_1	da3_Ia	rAW_C_1_mf	eLD_G_1_LW_20230222	rAS_G_0_mf		rAV_C_0_mf				
Icelandic horse 8	eES_1	da1_Ub-i	rAW_T_0_mf		rAS_G_0_mf		rAV_C_0_mf	eEB_C_1_dr_20171217	eOI_A_1_dr_20171217	eES_T_1_LW_20230222	eRH_C_0_LW_20230222
Icelandic horse 9	eES_1	da1_Ub-i						no call_LW_20220222	no call_LW_20220222	eES_T_1_LW_20230222	eRH_C_0_LW_20230222
Icelandic horse 10	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_dr		rAS_A_1_dr	eKQ_G_0_LW_20230220	rAV_C_0_dr				
Icelandic horse 11	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_dr		rAS_A_1_dr	eKQ_G_0_LW_20230220	rAV_C_0_dr				
Icelandic horse 12	eES_1	da1_Ub-i						eEB_C_1_dr_20171217	eOI_A_1_dr_20171217	eES_T_1_LW_20230222	eRH_C_0_LW_20230222
Icelandic horse 13	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_dr		rAS_A_1_dr	eKQ_G_0_LW_20230220	rAV_C_0_dr				
Icelandic horse 14	eLD_1	da3_Ia	rAW_C_1_dr	eLD_G_1_LW_20230222	rAS_G_0_mf		rAV_C_0_mf				
Icelandic horse 15	eLD_1	da3_Ia	rAW_C_1_dr	eLD_G_1_LW_20230222	rAS_G_0_mf		rAV_C_0_mf				
Icelandic horse 16	eES_1	da1_Ub-i	rAW_T_0_mf					eEB_C_1_dr_20171217	eOI_A_1_dr_20171217	eES_T_1_LW_20230222	eRH_C_0_LW_20230222
Icelandic horse 17	eLD_1	da3_Ia	rAW_C_1_dr	eLD_G_1_LW_20230222	rAS_G_0_mf		rAV_C_0_mf				
Icelandic horse 18	eES_1	da1_Ub-i	rAW_T_0_mf		rAS_G_0_mf		rAV_C_0_mf	eEB_C_1_dr_20171217	eOI_A_1_dr_20171217	eES_T_1_LW_20230222	eRH_C_0_LW_20230222
Icelandic horse 19	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 20	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 21	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 22	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 23	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 24	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 25	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 26	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 27	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 28	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 29	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 30	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 31	eES_1	da1_Ub-i	rAW_T_0_mf		rAS_G_0_mf		rAV_C_0_mf	eEB_C_1_dr_20171217	eOI_A_1_dr_20171217	eES_T_1_LW_20230222	eRH_C_0_LW_20230222
Icelandic horse 32	eES_1	da1_Ub-i	No Call		rAS_G_0_mf		rAV_C_0_mf	eEB_C_1_dr_20171217	eOI_A_1_dr_20171217	eES_T_1_LW_20230222	eRH_C_0_LW_20230222
Icelandic horse 33	eKQ_1	da3_Ib-a	rAW_C_1_mf		rAS_A_1_mf	eKQ_A_1_LW_20230220	rAV_C_0_mf				
Icelandic horse 34	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 35	eLD_1	da3_Ia	rAW_C_1_mf	eLD_G_1_LW_20230222	rAS_G_0_mf		No Call				
Icelandic horse 36	eKQ_1	da3_Ib-a	rAW_C_1_mf		rAS_A_1_mf	eKQ_A_1_LW_20230220	rAV_C_0_mf				
Icelandic horse 37	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 38	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 39	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 40	eKQ_1	da3_Ib-a	rAW_C_1_mf		rAS_A_1_mf	eKQ_A_1_LW_20230220	rAV_C_0_mf				
Icelandic horse 41	eKQ_1	da3_Ib-a	rAW_C_1_mf	eLD_A_0_LW_20230222	rAS_G_1_LW_20230302	eKQ_A_1_LW_20230302	rAV_C_0_mf				
Icelandic horse 42	eES_1	da1_Ub-i	rAW_T_0_mf		rAS_G_0_mf		rAV_C_0_mf	eEB_C_1_dr_20171217	eOI_A_1_dr_20171217	eES_T_1_LW_20230222	eRH_C_0_LW_20230222
Icelandic horse 43	eLD_1	da3_Ia	rAW_C_1_mf	eLD_G_1_LW_20230222	rAS_G_0_mf		rAV_C_0_mf				
Icelandic horse 44	eLD_1	da3_Ia	rAW_C_1_mf	eLD_G_1_LW_20230222	rAS_G_0_mf		rAV_C_0_mf				
Icelandic horse 45	eES_1	da1_Ub-i	rAW_T_0_mf		rAS_G_0_mf		rAV_C_0_mf	eEB_C_1_dr_20171217	eOI_A_1_dr_20171217	eES_T_1_LW_20230222	eRH_C_0_LW_20230222
Icelandic horse 46	eES_1	da1_Ub-i	rAW_T_0_mf		rAS_G_0_mf		rAV_C_0_mf			eES_T_1_LW_20230222	
Icelandic horse 47	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 48	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 49	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 50	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 51	rAS_1, eKQ_rAV_0	da3_Ib*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 52	eES_1	da1_Ub-i	rAW_T_0_mf		rAS_G_0_mf		rAV_C_0_mf	eEB_C_1_dr_20171217	eOI_A_1_dr_20171217	eES_T_1_LW_20230222	eRH_C_0_LW_20230222

Icelandic horse 53	rAS_1, eKQ_rAV_0	da3_lb*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 54	eES_1	da1_Ub-i	rAW_T_0_mf		rAS_G_0_mf		rAV_C_0_mf	eEB_C_1_dr_20171217	eOI_A_1_dr_20171217	eES_T_1_LW_20230222	eRH_C_0_LW_20230222
Icelandic horse 55	rAS_1, eKQ_rAV_0	da3_lb*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 56	eES_1	da1_Ub-i	rAW_T_0_mf		rAS_G_0_mf		rAV_C_0_mf	eEB_C_1_dr_20171217	eOI_A_1_dr_20171217	eES_T_1_LW_20230222	eRH_C_0_LW_20230222
Icelandic horse 57	rAS_1, eKQ_rAV_0	da3_lb*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 58	eLD_1	da3_la	rAW_C_1_mf	eLD_G_1_LW_20230222	rAS_G_0_mf		rAV_C_0_mf				
Icelandic horse 59	rAS_1, eKQ_rAV_0	da3_lb*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 60	eES_1	da1_Ub-i	rAW_T_0_mf		rAS_G_0_mf		rAV_C_0_mf	eEB_C_1_dr_20171217	eOI_A_1_dr_20171217	eES_T_1_LW_20230222	eRH_C_0_LW_20230222
Icelandic horse 61	eKQ_1	da3_lb-a	rAW_C_1_mf		rAS_A_1_mf	eKQ_A_1_LW_20230220	rAV_C_0_mf				
Icelandic horse 62	eLD_1	da3_la	rAW_C_1_mf	eLD_G_1_LW_20230222	rAS_G_0_mf		rAV_C_0_mf				
Icelandic horse 63	rAS_1, eKQ_rAV_0	da3_lb*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 64	eKQ_1	da3_lb-a	rAW_C_1_mf		rAS_A_1_mf	eKQ_A_1_LW_20230220	rAV_C_0_mf				
Icelandic horse 65	eKQ_1	da3_lb-a	rAW_C_1_mf		rAS_A_1_mf	eKQ_A_1_LW_20230220	rAV_C_0_mf				
Icelandic horse 66	rAS_1, eKQ_rAV_0	da3_lb*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 67	rAS_1, eKQ_rAV_0	da3_lb*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 68	rAS_1, eKQ_rAV_0	da3_lb*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 69	rAS_1, eKQ_rAV_0	da3_lb*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 70	rAS_1, eKQ_rAV_0	da3_lb*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 71	rAS_1, eKQ_rAV_0	da3_lb*	rAW_C_1_mf		rAS_A_1_mf	eKQ_G_0_LW_20230220	rAV_C_0_mf				
Icelandic horse 72	eKQ_1	da3_lb-a	rAW_C_1_mf		rAS_A_1_mf	eKQ_A_1_LW_20230220	rAV_C_0_mf				
Icelandic horse 73	eKQ_1	da3_lb-a	rAW_C_1_NGS		rAS_A_1_NGS	eKQ_A_1_NGS	rAV_C_NGS				
Icelandic horse 74	eLD_1	da3_la	rAW_C_1_NGS	eLD_G_1_NGS	rAS_G_0_NGS		rAV_C_0_NGS				
Icelandic horse 75	eES_1	da1_Ub-i	rAW_T_0_NGS		rAS_G_0_NGS		rAV_C_0_NGS	eEB_C_1__NGS	eOI_A_1__NGS	eES_T_1_NGS	eRH_C_0_NGS
Icelandic horse 76	rAV_1	da3_lb-b					rAV_T_1_mf				
Icelandic horse 77	rAV_1	da3_lb-b					rAV_T_1_mf				
Icelandic horse 78	rAV_1	da3_lb-b					rAV_T_1_mf				