Sexing Viking Age horses from burial and non-burial sites in Iceland using ancient DNA

Heidi M. Nistelberger[a],[*],[1], Albína Hulda Pálsdóttir[a],[b],[**],[1], Bastiaan Star[a], Rúnar Leifsson[c], Agata T. Gondek[a], Ludovic Orlando[d],[e], James H. Barrett[f], Jón Hallsteinn Hallsson[b], Sanne Boessenkool[a],[***]

[a] Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, Postbox 1066, Blindern, 0316, Oslo, Norway
[b] Faculty of Agricultural and Environmental Sciences, The Agricultural University of Iceland, Keldnaholti - Árleyni 22, 112, Reykjavík, Iceland
[c] The Cultural Heritage Agency of Iceland, Suðurgötu 39, 101, Reykjavík, Iceland
[d] Laboratoire d'Anthropobiologie Moléculaire et d'Imagerie de Synthèse, CNRS UMR 5288, Université de Toulouse, Université Paul Sabatier, 31000, Toulouse, France
[e] Centre for GeoGenetics, University of Copenhagen, Øster Voldgade 5-7, 1350K, Copenhagen, Denmark
[f] McDonald Institute for Archaeological Research, Department of Archaeology, University of Cambridge, Cambridge, CB2 3ER, United Kingdom

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ABSTRACT

Horses are the most common grave goods found in Icelandic Viking Age graves. Horse skeletons have previously been sexed based on pelvis shape and the presence of canine teeth in male horses over 4–5 years of age. Morphological data has shown that all horses from Icelandic burials that were amenable to sexing were male. Yet the use of morphological methods to determine sex is problematic since they rely on finding a well-preserved pelvis and/or robust canine teeth. Furthermore, quantitative data underlying the features of the horse pelvis used for sexing is lacking and canine teeth have been reported to occur in mares. In this study we build upon and extend recently developed methodologies to make use of shotgun sequencing of ancient DNA (aDNA) for molecular sexing of Viking Age horse remains. With minimal sequencing effort we identified the sex of the largest collection (n = 22) of Viking Age Icelandic horses studied to date, sourced from both burial (n = 19) and non-burial (n = 3) sites. Our results revealed a male to female sex bias ratio of 18:1 in burial sites, versus 0:3 in non-burial sites. These findings support the significant over-representation of male horses in Viking Age graves in Iceland, yet show that –albeit rare– mares could also be selected for ritual burial in Viking Age Iceland. This cost-effective method provides statistical confidence to allow for sexing of highly fragmented archaeological specimens with low endogenous DNA content.

1. Introduction

Viking Age burials have been the cornerstone of Icelandic archaeology and extensively researched since the late 19th century (e.g. Eldjárn, 1956; Friðriksson, 2004; Leifsson, 2011; Pétursdóttir, 2009; Vésteinsson and Gestsdóttir, 2016; Vigfússon, 1882). These burials (dating from the late 9th to early 11th century AD) are known for their relatively high occurrence of horse remains (Leifsson, 2018, p. 9). Horses are the most common grave good in Viking Age graves in Iceland (Eldjárn, 2016, p. 301). Of the 355 Icelandic Viking Age burials studied as of 2011, 148 were found to contain the remains of over 175 horses. (Leifsson, 2018, p. 229). Most horse remains are clearly associated with a human skeleton (male or female; Leifsson, 2018, p. 298 and Supplementary material) and the burials can be either rich or poor in other grave goods (Eldjárn, 2016; Leifsson, 2011). The horses found in Icelandic graves were slaughtered specifically for burial (Leifsson, 2018, p. 265) with complete, or in a few cases, decapitated carcasses placed in the grave (Leifsson, 2018, p. 254). Despite the conspicuous presence of horses in Viking Age graves, research into Icelandic burial customs has, to date, predominantly focused on artefacts, locations of

* Corresponding author.
** Corresponding author. Faculty of Agricultural and Environmental Sciences, The Agricultural University of Iceland, Keldnaholti - Árleyni 22, 112, Reykjavík, Iceland.
*** Corresponding author.
E-mail addresses: h.m.nistelberger@ibv.uio.no (H.M. Nistelberger), albinap@gmail.com (A.H. Pálsdóttir), sanne.boessenkool@ibv.uio.no (S. Boessenkool).
[1] Contributed equally.

burials and the human skeletons present (e.g. Eldjárn, 2016; Friðriksson, 2009, 2004; Gestsdóttir, 1998; Hreiðarsdóttir, 2005; Maher, 2007; Vésteinsson and Gestsdóttir, 2016).

Determining the sex of the horses used in burials is an important part of interpreting the meaning of horse burial rituals, past horse/human relationships and the status and use of horses in ancient societies (Jennbert, 2014, pp. 183–184, 2011; Lindstrøm, 2012; Ruscillo, 2014). Traditionally, horse remains have been sexed based on morphological characteristics including differences in pelvis shape and the presence/absence of canine teeth (e.g. Daugnora and Thomas, 2005; Lyublyanovics, 2006; Sjøvold and Löfgren, 2013). Based on these characteristics there is a clear bias towards the use of male horses in ritual contexts in the Viking Age (Hufthammer, 2014, p. 55; Nobis, 1961, p. 169, Sjøvold and Löfgren, 2013, p. 185, Svensson et al., 2012, p. 86). In Iceland in particular, out of a total of 175 horses from Viking Age burials, the 46 horses that were amenable to morphological sexing were all identified as males (Leifsson, 2018, pp. 229, 242; Nobis, 1961). Yet there are several problems associated with the use of these morphological methods that may affect their ability to accurately determine the sex of ancient horse remains.

There is little sexual dimorphism in body size of horses within breeds (Willoughby, 1975, p. 87), and sexing using differences in size and robusticity of long bones has been unsuccessful (Johnstone, 2004, pp. 114–115; Levine, 1979, pp. 78–79; Lyublyanovics, 2006, pp. 244–245). Sex determination based on morphology therefore depends on the accurate determination of distinct characters. Two morphological features are reported as showing sexual dimorphism in horses: the shape of the pelvis –males having a robust pubic tubercle– and the presence of robust canine teeth in males, but not in females. Most publications cite a version of Sisson's *The Anatomy of Domestic Mammals* for sexing based on the pelvis (Getty, 1975, pp. 303–304; Sisson, 1914, pp. 109, 111–112; Sisson and Grossman, 1953, pp. 109, 111–112) and the presence of canines (e.g. Sisson, 1914, p. 399; Sisson and Grossman, 1953, p. 399; St. Clair, 1975, p. 465). Yet these references do not allow for the unequivocal sexing of horses based on these features. For the pelvis there is no quantitative reference data on the variation observed between males and females, and it is poorly understood whether castration and the timing of castration influences the growth and shape of the horse pelvis.

When sexing on the presence of robust canine teeth it is generally assumed that canine teeth are either absent or merely rudimentary in females, but the standard publications cited here do not provide a definition for the difference between "robust" and "rudimentary" canines. Horse canine teeth differ from other horse teeth in that they do not wear down and are considered to be an evolutionary remnant (Clarke, 1884, p. 75). Male horses typically have four fully developed and erupted canine teeth by the age of 4–5 years (St. Clair, 1975, p. 470; Vollmerhaus et al., 2003, p. 215). Yet, anatomical studies of horse dentition show that canines are found in as many as one third of mares analysed. For example, Colyer et al. (1990, p. 121) reported canines in 48 of the 173 female skulls studied (27.8%), and in Ellenberger's (1887) examination of 8000 horses, 2–3% of mares had erupted canines both in the mandible and maxilla while a further 26–37% had erupted canines either in the mandible or maxilla. Visible canines have also been observed in older female horses (e.g. Cornevin and Lesbre, 1894, p. 130; Youatt, 1843, p. 198), suggesting their size may be age related. Finally, canines are not included in the zooarchaeological measurement standard for horse skulls or mandibles (von den Driesch, 1976, pp. 19–23, 52–54) and there is no collection of quantitative data regarding their size and morphology. Based on the above observations, the use of either the pelvis or the presence of canines for sexing horses can in itself be problematic. Yet poor preservation further limits the use of these features in archaeological specimens. First, it is rare to find horse pelvis bones that are complete enough for sexing (e.g. Bökönyi, 1974, pp. 269–270; Johnstone, 2004, p. 111; Lam et al., 1999, p. 346; Leifsson, 2018, p. 242; Lyublyanovics, 2006, p. 242). Second, the occurrence of

loose canines complicates the determination of which specimen they belong to when multiple individuals are present. And finally it is possible that not all bones and teeth were collected –a particular problem for material from old excavations– and the absence of canines may therefore reflect a collection bias rather than a sex bias.

Genetic sexing of archaeological bone remains has been successfully applied to a number of different species with heterogametic sex determination (e.g. XX (female)/XY (male)). Earlier genetic methods used to sex ancient bone material used polymerase chain reaction (PCR) to amplify specific regions that differ between the X and Y chromosomes, such as the amelogenin (AMEL) gene in horses (Götherström, 2002; Hasegawa et al., 2000; Svensson et al., 2012). Nonetheless, PCR-based methods run the risk of allelic dropout and the potential to be affected by contamination (Kim et al., 2013; Skoglund et al., 2013) and the use of whole genome shotgun (WGS) data has become increasingly prevalent. This approach uses the relative proportion of reads mapping to the sex chromosomes and the autosomes, respectively. For instance, by assessing the proportions of reads aligning to the X and Y chromosomes, sex was determined in ancient human remains (Ebenesersdóttir et al., 2018; Skoglund et al., 2013); or, in cases where a separately assembled Y chromosome was absent, by assessing the proportions of reads aligning to the X chromosome compared to an autosome of comparable genomic size such as in the mammoth (Pečnerová et al., 2017) or *Haringtonhippus francisc* (a previously unknown genus of horse; Heintzman et al., 2017). An alternative to such methods compares the relative genomic *coverage* of the mappable fraction of sex and autosomal chromosomes (Schubert et al., 2017), which accounts for possible bias due to differences in the genomic size of chromosomes and their relative fractions of repeated elements. A coverage-based method for X versus mean autosomal chromosome coverage (X:A) has been implemented in the Zonkey pipeline (Schubert et al., 2017), designed specifically for the cost-effective identification of equine species and F1-hybrid individuals from low-coverage data. Nonetheless, a limitation of comparing X:A coverage is that the occurrence of sex-chromosomal imbalance (e.g. Klinefelter syndrome XXY, Ebenesersdóttir et al., 2018) – which is present at a rate of 0.02% in modern horse populations (Kakoi et al., 2005) – may remain undetected. In this study, we modify the method utilised in Zonkey to separately assess both the X:A coverage and the Y:A coverage, in order to confidently sex the largest collection of Viking Age Icelandic archaeological horse specimens studied to date. We compare the genetic sex with the morphologically determined sex where possible, evaluate the earlier observation that male horses were exclusively buried in Viking Age graves in Iceland and compare the sex ratio in burial versus non-burial sites.

2. Materials and methods

2.1. Sampling

Horse bones from Iceland (n = 22) were sourced from 17 archaeological sites (Fig. 1). A total of 19 samples were selected from horse burials, two samples were selected from the Viking Age farm site at Granastaðir in northern Iceland, and a single sample was taken from the 11th century cave site at Leynir in Snæfellsnes peninsula, western Iceland. Viking Age burials are unevenly distributed around Iceland with the majority concentrated in northern and north-eastern Iceland (Eldjárn, 2016, pp. 257–258; Vésteinsson, 2011). Most known Viking Age burials in Iceland are accidental finds and the level of documentation and retention of bone varies widely (Eldjárn, 2016, p. 261; Leifsson, 2018, p. 230). More detail about the sites can be found in the Supplementary material.

Incisors or molars were selected for genetic analysis, except for Leynir cave, where we sampled a calcaneus (Table 1 and Supplementary material, Table S1). To prevent repeated sampling from the same individual, the same tooth type from the same side of the body was sampled within each site, or teeth were selected from different contexts.
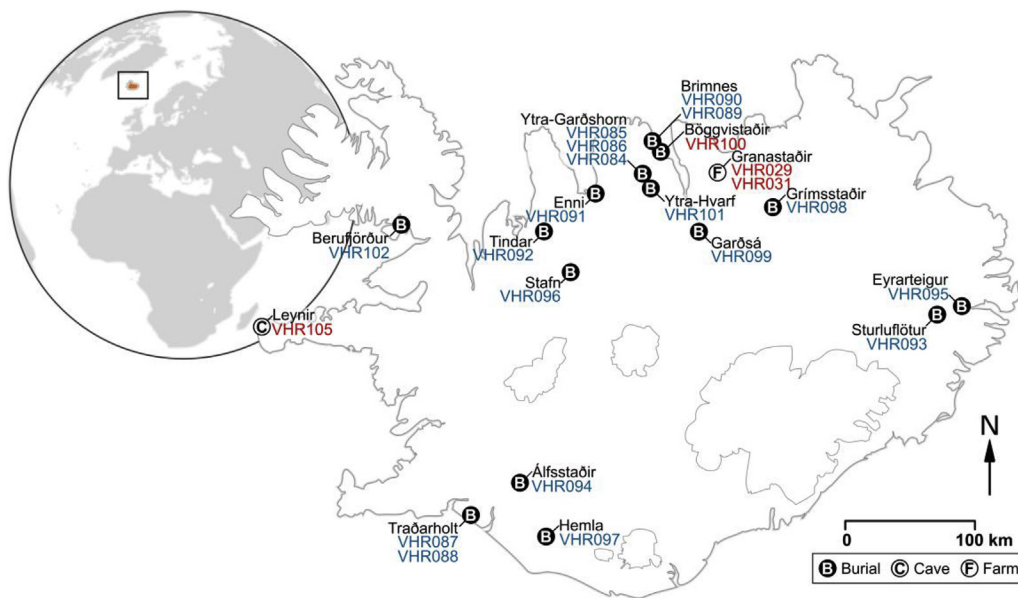
Fig. 1. Map indicating the locations of the samples used in this study. Letters indicate whether archaeological sites were **B**urial sites, or a **F**arm or **C**ave site. Samples are coloured according to genetic sex determination, red for females, blue for males. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

We did not sample any canine teeth or pelvic bones. All samples were obtained from the National Museum of Iceland (NMI).

2.2. Morphological sex determination

Where possible, the horse remains analysed in this study had been morphologically sexed based on the presence of robust canine teeth and/or a robust pubic tubercle, the shape of the ischiatic arch, ischio-acetabular ramus and obturator foramen of the pelvis (Getty, 1975, pp. 296–304; Leifsson, 2018, p. 242; St. Clair, 1975, p. 465). No canines that could be considered rudimentary, or reduced, were found amongst the horse bone retained from Viking Age burials in Iceland (Leifsson, 2018). For detailed information see Supplementary Material Table S1.

Table 1
Characteristics of the 22 Viking Age Icelandic horse samples used in this study including archaeological site; type of site; dating based on typology or radiocarbon; tooth or bone from which DNA was extracted; the numbers of unique reads that mapped post filtering to the EquCab 2.0 genome; the ratio of coverage of the X chromosome to the mean autosome coverage (X:A); the ratio of coverage of the Y chromosome to the mean autosome coverage (Y:A); the percentage of endogenous DNA recovered; sex as determined by genetic or morphological methods (see Supplementary Material Table S1 for detail on morphological diagnostic features). Further details in Table S1.

Sample	Site name	Site type	Date	Tooth or bone element	Mapped reads	X/autosome ratio	Y/autosome ratio	Endogenous DNA (%)	Genetic sex	Morphological sex features
VHR029	Granastaðir	Farm	Viking Age	Incisor	100292	1.003	0	3.3	female	not present
VHR031	Granastaðir	Farm	895–1025[a]	Molar	2180600	1.045	0	33.9	female	not present
VHR084	Ytra-Garðshorn	Burial	920–1000[b]	Molar	102431	0.451	0.337	3.5	male	not present
VHR085	Ytra-Garðshorn	Burial	Viking Age	Molar	328916	0.453	0.325	11.6	male	not present
VHR086	Ytra-Garðshorn	Burial	Viking Age	Incisor	19291	0.453	0.394	0.8	male	not present
VHR087	Traðarholt	Burial	960- > 1000[b]	Molar	211270	0.477	0.341	6.4	male	male
VHR088	Traðarholt	Burial	960- > 1000[b]	Molar	12497	0.466	0.326	0.4	male	not present
VHR089	Dalvík (Brimnes)	Burial	Viking Age	Molar	1206752	0.443	0.335	39.8	male	male
VHR090	Dalvík (Brimnes)	Burial	950-1000[b]/978–1027[c]	Molar	24944	0.442	0.427	0.6	male	fragmented
VHR091	Enni	Burial	Viking Age	Molar	22564	0.490	0.241	0.9	male	fragmented
VHR092	Tindar	Burial	Viking Age	Molar	33094	0.473	0.504	1.5	male	male
VHR093	Sturluflötur	Burial	960- > 1000[b]	Molar	320403	0.490	0.448	10.4	male	not present
VHR094	Álfsstaðir	Burial	891-1016/960- > 1000[b]	Molar	135751	0.521	0.335	5.6	male	male
VHR095	Eyrarteigur (Þórisá)	Burial	955-1000[b]/935–1015[d]	Incisor	2798	0.435	0.075	0.8	male	male
VHR096	Stafn	Burial	Viking Age	Incisor	4960	0.612	0.298	2.4	male	male
VHR097	Hemla	Burial	Viking Age	Molar	20401	0.539	0.494	5.0	male	male
VHR098	Grímsstaðir Suður-þingeyjarsýslu	Burial	Viking Age	Molar	1010	0.522	0	0.6	male	male
VHR099	Garðsá	Burial	Viking Age	Molar	30202	0.497	0.364	1.0	male	male
VHR100	Dalvík (Böggvisstaðir)	Burial	Viking Age	Molar	331120	1.013	0	14.2	female	uncertain
VHR101	Ytra-Hvarf Burial I	Burial	Viking Age	Molar	38595	0.482	0.316	1.6	male	not present
VHR102	Berufjörður 1898	Burial	890–1015[a]	Molar	1828639	0.510	0.341	31.7	male	not present
VHR105	Leynir	Cave	983-1151[a]/1022–1155[a]	Calcaneus	2123366	0.997	0	29.5	female	not present

[a] Dating based on ^{14}C analyses cal. AD (2σ), details in Supplementary Material Table S2.
[b] Dating based on typology from Leifsson (2018, p. 291).
[c] Previously reported radiocarbon date of human bone calibrated with IntCal/Marine04 (1σ) (ARR-5860) (Sveinbjörnsdóttir et al., 2010, p. 686).
[d] Previously reported radiocarbon date of horse bone cal. AD (1σ) (Eldjárn, 2016, p. 231).

2.3. Radiocarbon dating

We obtained radiocarbon dates for three of the analysed samples. Two further horse bones from Leynir cave had been dated previously as part of the investigation of the site by The Cultural Heritage Agency of Iceland. All radiocarbon dating was done at SUERC Radiocarbon Laboratory (Glasgow University; for detailed information see Supplementary Material Table S2). All other samples could be confidently dated to the Viking Age through associated artefacts and burial context.

2.4. Ancient DNA extraction

Sample processing, DNA extractions and library preparations were carried out in the dedicated aDNA laboratory facility at the University of Oslo, which adheres to strict standards for contamination control and data authentication (e.g. Cooper and Poinar, 2000). Tooth or bone specimens were treated with UV on all sides for 10 min prior to use and were subsequently drilled and milled as described in Boessenkool et al. (2016). DNA was extracted from 200 mg of tooth or bone powder using a modified double digest protocol (Damgaard et al., 2015; Gamba et al., 2016) as described in Boessenkool et al. (2016) (See Supplementary material for details).

2.5. Library builds, sequencing and read processing

Blunt-end Illumina libraries (Meyer and Kircher, 2010) were built following Schroeder et al. (2015) as described in Star et al. (2017). Sequencing was carried out on an Illumina HiSeq 2500 at the Norwegian Sequencing Centre. The read data were processed with the PALEOMIX pipeline v. 1.2.4 (Schubert et al., 2014) as per Star et al. (2017) which utilises ADAPTERREMOVAL v.2.1.7 to remove adapters, collapse and trim reads, with collapsed reads < 25bp in length discarded (Schubert et al., 2016). The backtrack algorithm implemented in BWA v.0.5.10 was used to align reads to the EquCab 2.0 genome (Wade et al., 2009) and the Y-chromosome (Wallner et al., 2017) with seeding disabled in PALEOMIX. Post-mortem DNA damage patterns were investigated using mapDamage v.2.0.6 (Jónsson et al., 2013). Unmapped reads, PCR duplicates and reads with a MapQ lower than 25 were excluded from subsequent analyses.

2.6. Genetic sex determination

We used several approaches to genetically determine the sex of the ancient horse remains. First, we determined the sex of our individuals using the X:A coverage ratio, expecting a ratio of 1.0 for female specimens and 0.5 for male specimens. Then we repeated this method using the Y:A coverage ratio to allow detection of chromosomal abnormalities such as Klinefelter syndrome (XXY), expecting a ratio of 0.0 for females and 0.5 for males. In addition, for both the X:A and Y:A coverage ratios respectively, we investigated how many reads were required to reliably determine sex when limited numbers of endogenous reads (i.e. less than 20 000 reads) were obtained. For the X:A and Y:A approaches, subsets of reads were randomly down-sampled from those specimens (*n* = 17, Table 1) with more than 20 000 endogenous reads (and for which genetic sex could be reliably determined), from 500 reads up to 20 000 reads, in increments of 500 reads. For each increment and individual, the random resampling procedure was iterated 20 times to obtain a bootstrapped confidence interval (script available in Supplementary material). The sex-coverage ratios of those samples with less than 20 000 reads (*n* = 5) were compared to the randomly obtained probability distributions of the sex ratios of the other specimens. Details on the analysis of extraction blanks and the potential for cross-sample contamination are addressed in the Supplementary material Note 1.3 and Table S3.

3. Results

3.1. Morphological sex determination

Of the 19 horses sampled from burial contexts, nine had been morphologically identified as male (Leifsson, 2018), nine were not sexed because neither the pelvis nor the area of the mandible or maxilla where the canines are found were preserved, and one (from the Dalvík Böggvisstaðir burial site) was classified as uncertain. At the latter site it is unknown whether the molar used in aDNA analysis originated from one of the individuals that were morphologically identified as male or from an individual that could not be sexed (Supplementary material). The two bones sampled from the Granastaðir farm site represent disarticulated bones from several horses and could not be morphologically sexed. The single horse deposited in the Leynir cave site could not be morphologically sexed as neither canines nor the pelvis were collected at the site.

3.2. Sequence data

Between 170 433 (VHR 098) and 7 021 861 (VHR105) collapsed reads were obtained from the sequenced DNA libraries (Supplementary material, Table S1). The preservation of the samples was variable, with the percentage of endogenous DNA ranging from 0.4% (VHR088) to 39.8% (VHR089), with a mean of 9.34% (Table 1). Post-filtering, this produced from 1010 reads (VHR098) to 2 180 600 unique reads (VHR031) mapping to the EquCab 2.0 reference genome (Table 1). Mapped reads indicated post-mortem damage consistent with the fragmentation and nucleotide mis-incorporation patterns expected for aDNA (Jónsson et al., 2013) (Supplementary material, Fig. S1). On average, molars yielded higher percentages of endogenous DNA (mean = 9.93%) than incisors (mean = 1.79%) although the difference was not statistically significant (Mann-Whitney $U = 20$, n1 = 4, n2 = 17, $P > 0.05$ one tailed). The only bone sample analysed – a calcaneus from Leynir cave – yielded 29.5% endogenous DNA (VHR105).

3.3. Genetic sex determination

Using the Zonkey pipeline, all samples were confirmed to originate from *Equus caballus* and not asses or F1 hybrids (data not shown). Four of the 22 (18%) ancient horse remains were identified as female (Table 1). Two of these originated from the Granastaðir farm site (VHR029 and VHR031), one from Leynir cave (VHR105) and one from the Dalvík (Böggvisstaðir) burial site (VHR100). This sex ratio is significantly biased towards males for burial site contexts compared to non-burial site contexts ($p = 0.0026$, two-tailed Fisher exact test).

We investigated the minimum number of reads required to confidently assign genetic sex using the X:A and Y:A coverage ratios. By randomly subsampling a variable number of reads, we found that the range of probability distributions of female and male X:A coverage ratios fully differentiate the sexes with a minimum of 2000 mapped reads (Fig. 2). Although sample VHR098 had just 1010 mapped reads, this sample falls outside the observed X:A ratio range of female specimens in the dataset (X:A ratio = 0.052, Fig. 2, Table 1), hence this sample was identified as a male. The range of probability distributions of female and male Y:A coverage ratios fully differentiate the sexes with a minimum of 18 000 reads (Fig. 2). We were unable to confidently determine the sex of VHR098 using the Y:A method alone, but samples VHR095 and VHR096 with less than 5000 reads were identified as male, with ratios falling outside the range determined for the female horses in the dataset (Fig. 2). No indications of chromosomal abnormalities were observed in any of the horse specimens. Finally, we confirmed that males with low endogenous DNA (< 2.4%) and number of reads (< 20 000) were not misclassified due to cross-sample contamination (Supplementary material note 1.3, Table S3, Fig. S3).

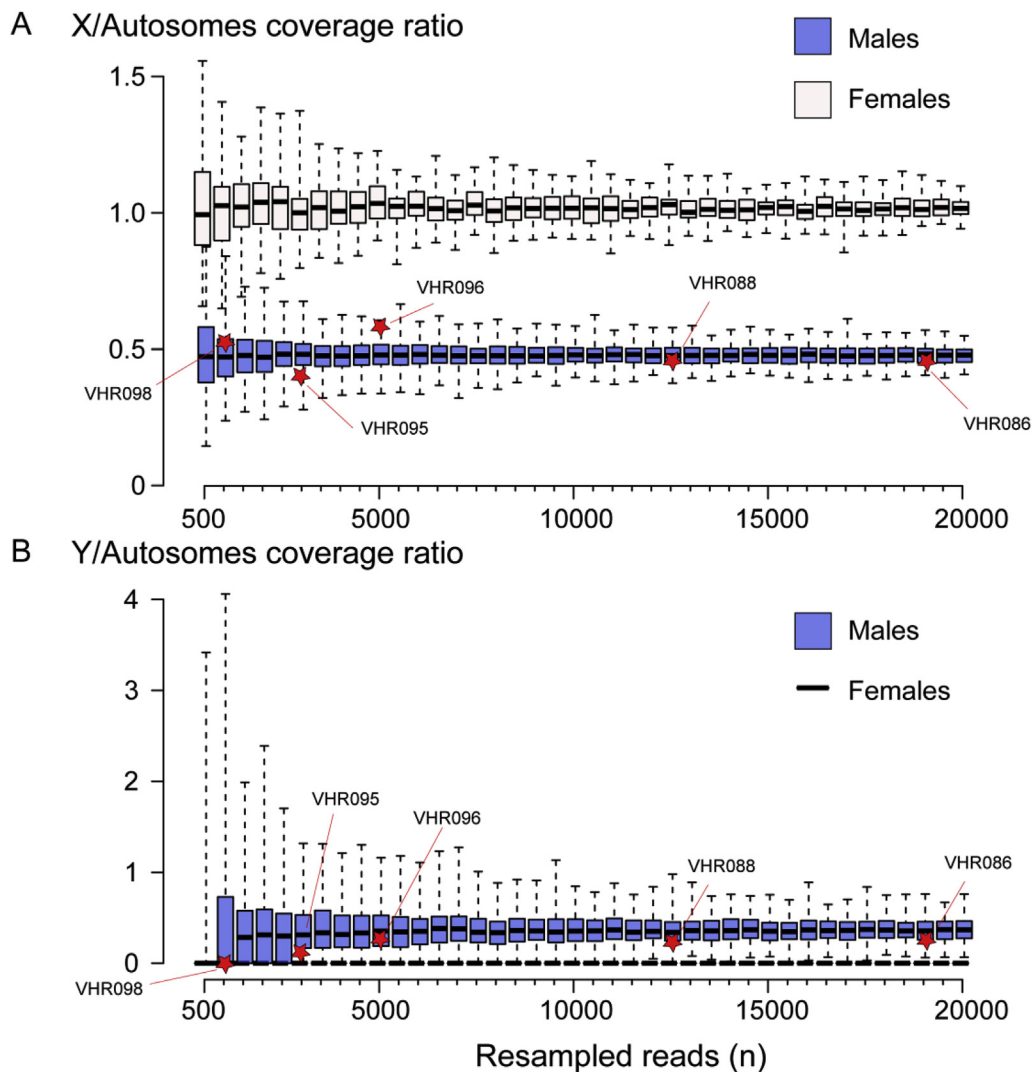## A   X/Autosomes coverage ratio

Fig. 2. Ratio of (A) X chromosome to mean autosome coverage (X:A) and (B) Y chromosome to mean autosome coverage (Y:A) generated by down-sampling BAM files from 20 000 to 500 reads in 500 reads increments. At each interval, and for each individual, the down-sampling is iterated 20 times to generate a confidence distribution. Box plots represent the median (*solid line*), the 25th and 75th percentiles (*box*) and the entire ratio range (*dotted lines*) at each read interval bin. Five individuals (*red stars*) did not have sufficient reads to allow down-sampling to 20 000 reads. Their ratios are calculated from original, complete BAM files. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

B   Y/Autosomes coverage ratio

3.4. Morphological versus genetic sex

We were able to compare morphological and genetic sex of the same specimen for a total of nine horses. The genetic results for these individuals were concordant with the morphological sex determination. All these individuals were from burial contexts and all were identified as male.

4. Discussion

Using ancient DNA, we determined the sex of 22 Icelandic Viking Age horse remains sourced from 17 archaeological sites. In nine sites horse sex was also determined using morphological characteristics. The X:A coverage ratio provided the greatest diagnostic power, was robust to observed levels of cross-sample contamination and allowed for sex identification with as little as 1000 endogenous reads and less than 0.5% endogenous DNA content. Moreover, it enabled the identification of a specimen with no coverage of the Y chromosome (VHR098) as a male. Although both the X:A and Y:A ratio methods were useful at delineating between the sexes, the Y:A ratio method was not as powerful as the X:A ratio. The poorer performance of the Y:A ratio is likely due to the repetitive, heterochromatic nature of the horse Y chromosome (Raudsepp et al., 2004) that results in poor read mapping (Treangen and Salzberg, 2012) and the lower number of sites available for the Y-chromosomal assembly (~1.6 million sites) compared to the

X-chromosomal assembly (~121 million sites). The likelihood of confidently identifying aligned reads against the X chromosome is therefore considerably larger than against the Y chromosome, especially when sequencing efforts are limited. Overall, sexing ancient bone material using the WGS coverage approach is straightforward, and robust to samples with low endogenous DNA content where allelic dropout is likely to heavily influence PCR of genes located on the Y chromosome (Kim et al., 2013). Although WGS has been applied for sexing archaeological bone in previous studies (e.g. Ebenesersdóttir et al., 2018; Pečnerová et al., 2017; Skoglund et al., 2013), we here extend these methods to improve their ease of application and provide statistical confidence by combining the X:A and Y:A ratio methods into a single, straightforward resampling approach that can be readily applied to any species with heterogametic sex determination. This includes many economically important species such as sheep, goats, cattle and dogs whose bones are common in archaeological faunal assemblages but are often hard to sex morphologically.

Our data identified (in concordance with previous morphological findings) that there is an over-representation of male horses in Icelandic Viking Age burials (late 9th to early 11th century), with a male to female ratio of 18:1 in our dataset. A bias towards the use of male horses in burials has been noted in other time periods and locations from the earliest horse burials at Berel' 2300 years BP (Librado et al., 2017), and is not unique to the Viking culture (Ambros and Müller, 1980, pp. 11–12; Bökönyi, 1974, p. 268; Cross, 2011; Daugnora and Thomas,

2005; Fern, 2005; Lepetz, 2013; O'Connor, 1994). Below, we discuss whether the male bias we observed could be co-incidental, reflecting an underlying sex bias in the use of horses, or whether male horses were actively selected for use in Viking Age burials in Iceland.

4.1. An underlying sex ratio bias?

Horse bones are relatively rare in non-burial contexts in Iceland and Northern Europe and often consist of single elements, which cannot be morphologically sexed (e.g. Dobney et al., 1996, p. 46; Johnstone, 2004; Leifsson, 2018, p. 263; Levine, 1990; McCormick and Murray, 2007, p. 96; McGovern, 2009, p. 220; O'Connor, 1994, p. 31; Rackham, 1995; Sundkvist, 2004, p. 243). Sexing data has not previously been published for any Icelandic Viking Age horses from non-burial contexts. Here we present the first sexing of such horses using aDNA and identified all three specimens as female. These three horses originated from the Granastaðir farm site and Leynir cave. The horse bones from Granastaðir were interpreted as having been butchered for food (Amorosi and McGovern, 1995) and the Leynir cave individual is believed to have been carried into the cave as a source of food (pers. comm, R. Leifsson). These findings suggest female horses at least, had a role as a food source in Viking Age Iceland. Further documented evidence on the specifics of horse use and herd management in Iceland from the late medieval period until the early 19th century is scant, but suggests that both female and male horses (castrated and uncastrated) were used interchangeably in multiple roles (Júlíusson, 2013, pp. 142–143). There is therefore no indication that there was a true underlying male bias in the source population that would make males more available for ritual sacrifice and burial in Viking Age Iceland.

4.2. Selection of male horses for burials

Horses have been included as grave goods in several well-known Viking Age burials in Scandinavia, and horse sex has been reported for some of these. Notably, in the Oseberg Viking Age ship burial from Norway all of the horses that could be morphologically sexed have been identified as male (Hufthammer, 2014, p. 55; Nobis, 1961, p. 169). In Sweden three Viking Age burials have been analysed and all reflected a bias towards males. In Ultuna2, a 3:1 ratio of male:female horses was observed in the areas of the site connected to ritual activity, whereas such a bias was not present in the food refuse site Ultuna1 (Svensson et al., 2012, p. 86). At Valsgärde, two horses were identified as males (two others could not be sexed; Sjøvold and Löfgren, 2013, p. 185), and at the fortified village Eketorp in Sweden (500–1100 CE) five male horses and a single mare were found in contexts relating to ritual activities. Finally, in horse burials at the upper class boat cemetery at Vendel, Sweden (600–1000 CE), a bias towards males was also present, although not as pronounced (Götherström, 2002). To date, all analyses of Viking Age horse burials in Scandinavia therefore reflect preferential sacrifice of male horses over females. These observations agree with our horse sexing results in Icelandic Viking Age burials and strengthen the notion of an active selection of male horses.

Archaeological evidence indicates that ritual horse killing in Icelandic Viking Age burial customs was practiced in a highly structured way. Although the horses span a wide range of ages, animals in their prime (5–15 years) seem to have been preferred, followed by immature animals (younger than 5 years; Leifsson, 2018, pp. 236–242). The horses were commonly bridled and saddled when killed and buried, suggesting they were first and foremost seen as riding animals (Leifsson, 2018, pp. 254–260). The pathologies seen on older animals indicate repetitive strain injury due to load bearing or excessive riding and suggests horses were tamed as soon as they could carry a human, though burying very young horses with riding gear was likely a symbolic act (Leifsson, 2018, pp. 254, 262). Killing methods and arrangement of horses in the grave followed set protocols and this structured nature of the rituals reveals the importance placed on the ceremony

itself (Leifsson, 2018, pp. 265–269, 292–296). Horses were killed by bashing in heads and cutting of throats and the resulting *bloodiness* and *theatrics* were likely an important feature of the ceremony (Leifsson, 2018, p. 309; Price, 2014, 2010). The sex ratio and age distribution of the killed horses suggests that there was a semantic structure behind the rituals in which the chosen animals acted as symbolic representatives (Leifsson, 2018, p. 318). The conscious choice of males was perhaps linked with the characteristics of stallions; virility and aggression could have been a strong symbolic factor. Furthermore, the theatrics of the act and the violent and visceral drama may have helped the animal killing rituals to become quickly popular in Iceland (Leifsson, 2018; see also Lucas and McGovern, 2007). The rituals were actively used in the developing society of the 10th century to affirm Norse, non-Christian identity and to construct status. The archaeological remains of the buried animals can thus be regarded as *materialized* expressions of cultural politics in a new society under formation (Leifsson, 2018, pp. 322–327).

Although the number of samples from non-burial sites is limited, by including the sex of horses from two non-burial sites, we were able to identify a highly significant relationship between horse sex and site context with a male to female ratio of 18:1 for burial sites and 0:3 for non-burial sites. In the future it would be valuable to increase the number of specimens from non-burial sites to further explore the (lack of) males in such sites in Iceland from this time period. The single female horse found in a burial context was at the site of Dalvík (Böggvisstaðir). This boat burial was excavated in 1937, but it had been opened previously and all the human bone had been removed and the horse bones (from 3 to 4 individuals) disturbed. Unfortunately, the possible ritual role of this female horse is difficult to interpret, because it comes from a poorly documented site that was excavated over 80 years ago. The original excavator did not note that the burial contained multiple horses (Leifsson, 2018, pp. 131–132). This is not unusual as animal bones were often poorly documented prior to the 1960s and often only part of the animal bone found in archaeological excavations was curated (Leifsson, 2018; Ruscillo, 2014). Taphonomically all the horse bones and teeth from Dalvík (Böggvisstaðir) look similar and there is nothing to indicate that any of the horse remains recovered at the site might have come from a non-burial context. Our finding of a female horse in a burial context indicates that mares may have also served a ritual funerary purpose in Viking Age Iceland in rare instances.

4.3. Geldings or stallions?

Detecting castration of animals archaeologically is problematic (Binois-Roman, 2016, p. 136) and we are unable to determine whether the male horses found in Viking Age burials were castrated (geldings) or uncastrated (stallions). The castration of horses adds to the complexity of morphological sexing, with the timing and method of castration influencing the effects on the skeleton. Specifically, castration can affect bone fusion times, skeletal proportions and the robustness of bones and sexual characteristics (Binois-Roman, 2016, p. 136), though very little data are available regarding these effects in horses specifically (Johnstone, 2004, p. 112). Castration is not known to affect the presence and development of canine teeth (Cornevin and Lesbre, 1894, p. 129; Vollmerhaus et al., 2003; Youatt, 1843, p. 196), but it has been suggested that it influences the morphology of the pelvis by giving the pelvic surface a concave shape in geldings similar to that found in females (Sisson, 1914, p. 109). Such similarity between the pelvis of females and castrated males may lead to incorrect sexing based on the pelvic morphology. A systematic study of the impact of castration on the skeletal morphology of horses, and specifically on the pelvis is needed to clarify this uncertainty. Moreover, development of a method to identify castrates in the archaeological record would enable a greater understanding of the role of geldings in past cultures.

4.4. Sexing based on morphological versus genetic methods

Morphological sexing of horse remains relies on the recovery of a well-preserved pelvis and/or the presence of robust canine teeth. Even if such elements are recovered, however, there is little quantitative data underlying the features of the horse pelvis used for sexing, and canine teeth have also been reported in mares. In this study the use of genetic methods allowed us to confidently identify the sex of all 22 sampled individuals, highlighting the strength of genetic sexing of archaeological specimens with a heterogametic sex determination system. We were able to directly compare the morphological and genetic sex for nine of the analysed individuals in sites where only a single horse was found. In all these cases morphological and genetic sex were concordant, with all nine individuals identified as males. Nevertheless, the near exclusive presence of male horses in Viking Age burial contexts limits the power for such comparative analyses. The described uncertainties of current morphological sexing methods therefore remain. Further work is needed to clarify the strengths and limitations of morphological sexing in horses. Until then, we recommend that any sexing based on canine teeth includes quantitative measurements and photographic evidence.

5. Conclusion

Using a simple, cost-effective genetic method we have revealed the sex of the largest collection of Icelandic Viking Age horses studied to date, showing a significant bias towards the use of male horses in burials. The use of this method enabled the sexing of a suite of archaeological specimens that would not have been possible using morphological methods alone due to a lack of intact bone material. This method applies equally well to any animal with a heterogametic sex determination system, providing a statistically robust framework within which to determine the sex of archaeological specimens - even in circumstances of poor endogenous DNA content.

Data availability

All ancient read data are available at the European Nucleotide Archive (ENA, www.ebi.ac.uk/ena) under study accession numbers PRJEB28294 and PRJEB29661.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jas.2018.11.007.

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