Mitochondrial Ewe - application of ancient DNA typing to the study of domestic sheep (*Ovis aries*) in mediaeval Norway.

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Summary

Around 11000 years ago the first domesticated mammals emerged in the Fertile Crescent. Over time, these animals spread to fertile regions both to the east and west of their domestic centre of origin. By 6000 BP, animal husbandry had reached Northern Europe, and it had become established in Norway by circa 4100 BP. Domestic sheep (*Ovis aries*) was probably among the first domestic species to be introduced to Norway and by the Middle Ages (476-1453 AD) animal husbandry and sheep herding was well established here. Not much is known about the composition of domestic sheep in mediaeval Norway, but it has been suggested that new breeds were introduced from other countries as this was a time of increasing trade.

Four different mitochondrial DNA (mtDNA) haplogroups, known as A, B, C and D, have been observed in domestic sheep, of which mtDNA haplogroup A and B are found in Europe. In this study I analysed genetic data obtained from sheep bones excavated from archaeological sites (Oslo, Bergen and Trondheim) in Norway dated to the Middle Ages (476-1453 AD). I also included a bone sample dated to the Viking Age (800-1066 AD) to see if any genetic variation could be obtained from an older sample. I implemented methods for extracting ancient mtDNA from bones, and aimed to produce reliable extracts that could be authenticated as mediaeval sheep DNA. Authentic mtDNA sequences of mediaeval domestic sheep could provide information that would help answer questions regarding the trading history and composition of breeds at different geographical regions in mediaeval Norway.

Sheep extracts were amplified with sheep specific primers and cytochrome b primers simultaneously. Negative and positive controls, as well as samples of different species were included in the amplifications. PCR amplifications visualised on electrophoresis agarose gels could exclude human and cross-contamination of sheep by the nature of the controls. This suggested that the genetic data were authentic. Of 70 domestic sheep samples, 16 were successfully sequenced, including one from the Viking Age. Two mtDNA haplogroups were observed: A and B. One specimen from Trondheim was allocated to haplogroup A, whereas the remaining samples were haplogroup B. The 16 sequences used in the phylogenetic analyses were short (88 bp), and thus not informative enough to settle questions regarding the trade and composition of breeds in mediaeval Norway. However, it could be concluded that the sheep sequences were authentic, and that two mtDNA sheep lineages were present in Norway in the Middle Ages.

1. Introduction

The domestication of plants and animals, here defined as the selective breeding of species by humans in order to accommodate human needs (Allaby 2009), is one of the most important events in human history. Domestication dramatically changed the world we live in as it facilitated the rise of civilisation, transformed global demography and became the main provider of what we eat and wear (Diamond 2002).

Around 12000 years ago the hunter-gatherer mode of subsistence was replaced by land cultivation and eventually also by the herding of animals in the Fertile Crescent in Western Asia (Smith 1998; Peters *et al.* 1999). The first animals domesticated were goats, sheep, pigs and cattle, and these were later dispersed across the world by trading and migration (Ryder 1984; Zeder 2008). Farming and animal husbandry spread rapidly to the fertile regions both east and west of the Fertile Crescent, but spread inland and northwards in an abrupt pattern with long intervals between regions (Pinhasi *et al.* 2005.; Zeder 2008; Rowley-Conwy 2004; Rowley-Conwy 2009) (Fig. 1).

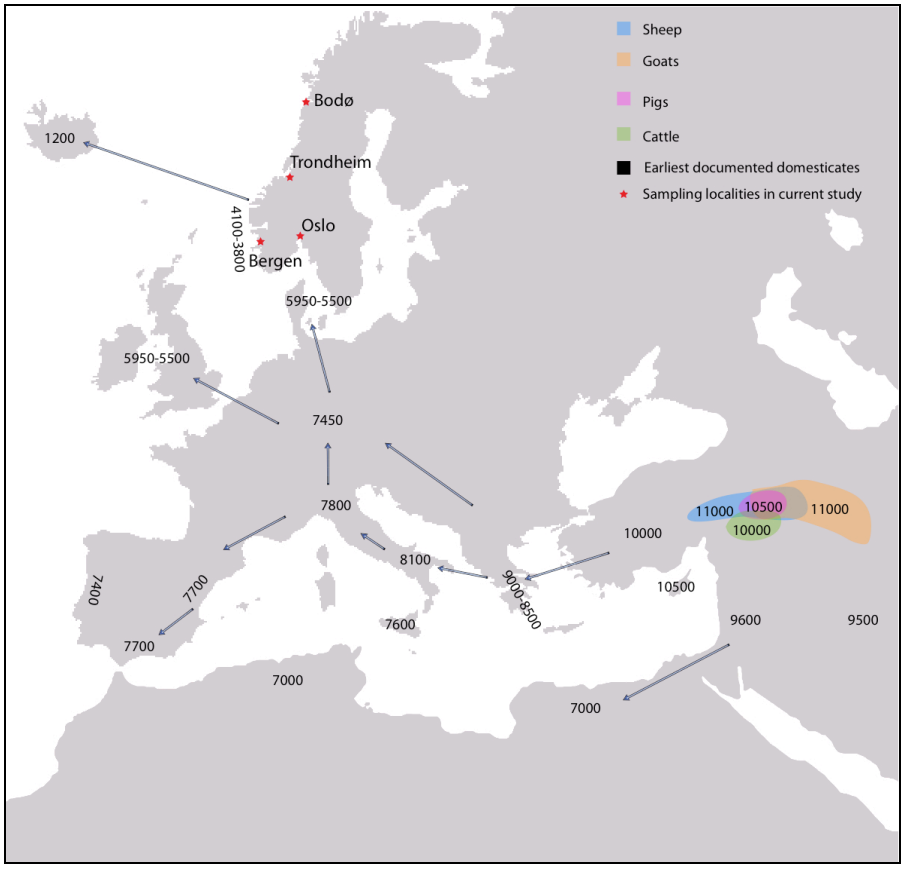


Fig. 1. Origin and dispersal of domestic breeds from the Fertile Crescent to Western Europe. Numbers correspond to years before present (BP), and mark the first evidence of domestic breeds at selected locations. Arrows indicate most likely routes of livestock dispersal through Europe. Coloured fields mark the domestication centres of the different species, in the Fertile Crescent. The sampling localities in the current study are indicated with red stars (Oslo, Trondheim, Bergen and Bodø). The figure is modified from Zeder (2008) and Rowley-Conwy (2004).

Sheep (*Ovis* sp.) were among the first animals to undergo domestication. Based on archaeological evidence it has been assumed that sheep were first domesticated in the Near East 10000-9500 BP. Evidence of a rapid reduction in body size has been used as a criterion for deeming an animal a domesticate. Domestic partnerships with humans have been argued to yield a reduction in size via human predator protection and changes in mobility and adaptations to new terrain (Ryder 1984; Zohary *et al.* 1998). However, others have argued that time estimates based on body size reduction in animals are poorly suited to date the onset of animal domestication, as smaller body size may also result from climate adaptation or sexual dimorphism (Zeder 2006a). Studies based on the relative composition of male and

female skeletal remains, rather than change in morphological characters themselves, have dated the domestication of sheep to circa 11000-10500 BP (Vigne *et al.* 1999; Zeder 2008).

From the Fertile Crescent, sheep spread across Europe and reached Northern Europe by circa 6000 BP. Many Northern European sheep breeds still retain primitive features, such as a thick outer coat which cannot be sheared, coloured coats, short tails and horns in both sexes (Ryder 1981; Dyrmundsson & Niznikowski 2010). Such breeds were probably dominant in Europe until after the mediaeval period, and the modern, improved breeds most common today probably emerged after the Middle Ages (Ryder 1981).

Studies conducted on agriculture and domestication events in Norway have dated the earliest bones from domesticated animals to the Middle Neolithic B (i.e. 4100-3800 BP) (Hjelle *et al.* 2006). There is general agreement that the main establishment of agriculture in Norway occurred in the Late Neolithic (3800-3400 BP) (Bakka & Kaland 1971; Prescott & Walderhaug 1995; Prescott 1996; Myhre 2004; Hjelle *et al.* 2006; Prescott 2007). An expansion of animal husbandry in the Early Bronze Age (3400-2900 BP) facilitated the spread of agriculture from southern and western Norway to the northern and eastern regions of the country (Prescott 2007).

By the Middle Ages (476-1453 AD) animal husbandry was well established in Northern Europe, including Norway (Holm-Olsen 2010; Lopez 1976; Helle 2005). From 950-1200 AD farmers in medieval Europe experienced climatic changes when temperatures increased in the North Atlantic areas, allowing cultivation to expand further north (Hughes & Diaz 1994; Grove 2002). An agricultural revolution in the Middle Ages allowed for an exponential growth in European towns and the trade between them (Allen 2000; Lopez 1976). As trading expanded during the Middle Ages it has been suggested that sheep were imported to Norway from other countries. Historical records suggest England as the most important of Norway's commercial relations in this period, and English merchants may have exported sheep to Norway (Gade 1951; Donkin 1958; Ryder 1984; Albarella 1999; Arnved Nedkvitne, pers.comm.). There is also evidence that English Cistercian monks brought sheep with them when settling in new monasteries in Norway. One such monastery, established in 1207 AD, was located at Tautra, an island in the Trondheim fjord (Gade 1951; Donkin 1958, Ekroll 1996). However, many questions regarding trade still remain unanswered, including whether or not introduced breeds interbred with the native breeds, and if trading of sheep also occurred

within Norway (Arnved Nedkvitne, pers. comm).

Genetic data may complement historical or archaeological evidence on domestication as it provides independent objective data on genetic relationships, and thus the evolutionary history of different domestic lineages. In the current study I analysed mitochondrial genetic data obtained from mediaeval bone samples of *O. aries,* to shed light on the composition of domesticated sheep in mediaeval Norway.

Mitochondrial DNA (mtDNA) (see below) has been used to study the domestication history of many species, and the complete mtDNA sequence of sheep has been determined (16616 base pairs (bp)) (Hiendleder 1998a). Different groups of Eurasian wild sheep have been suggested as the ancestors of, or contributors to, today's domestic sheep (*Ovis aries*). These are the mouflon (*O. musimon* or *O. orientalis*), urial sheep (*O. vignei*) and argali sheep (*O. ammon*) (Ryder 1984). Molecular research comparing mtDNA variation in wild sheep species with *O. aries* has found no close relationship between domestic sheep and the urial or argali sheep. However, a close genetic relationship with mouflon has been inferred, suggesting that it is the ancestor of domestic sheep (Hiendleder *et al.* 1998b, Hiendleder *et al.* 2002).

Studies on extant Northern European breeds such as Soay sheep, Norwegian Spælsau and Shetland sheep have found that these breeds are closely related to the primitive domesticated breeds first introduced to Europe (Dyrmundsson & Niznikowski 2010; Tapio 2006a). Other studies have examined extant domestic breeds from different geographical localities across the globe and found that these breeds contain evidence of four mitochondrial DNA lineages: haplogroup A, B, C and D (Wood & Pua 1996; Hiendleder *et al.* 1998b; Hiendleder *et al.* 2002; Meadows *et al.* 2005; Guo *et al.* 2005; Pedrosa *et al.* 2005; Tapio *et al.* 2006b). It was found that mtDNA lineage B was predominant in sheep located in the Near East and Europe, whereas lineage A existed as a rare form in Europe. In contrast, lineage A was predominant in sheep sampled in Asia, and lineage B has so far not been detected in Asian sheep. Sheep lineage C has been detected in Near-Eastern- and Central Asian sheep breeds, and lineage D has only been found in one sample from the Near East (Hiendleder *et al.* 1998b; Guo *et al.* 2005; Pedrosa *et al.* 2005; Tapio *et al.* 2006b).

Pedrosa *et al.* (2005) calculated that the time of divergence between lineage A and B was approximately 160,000-170,000 years ago, and even earlier for lineage C that diverged from A and B between 450,000 and 750,000 years ago. The time of divergence of haplogroup D has not yet been estimated. As the times of lineage divergence of group A, B and C greatly predate the date of domestication (9000-11000 years ago), it implies that there have been at least three different domestication events of sheep (four when including haplogroup D). Others argue that the existence of four different mtDNA lineages does not necessarily imply four independent domestication events, because the original wild population could have been polymorphic, or there may have been subsequent gene flow from diverged wild populations of sheep into a domestic sheep population (Zeder *et al.* 2006b; Tapio *et al.* 2006b). However, based on the ecology of sheep where males are migratory, and not females, introgression via females seems unlikely (Pedrosa *et al.* 2005). Studies on mtDNA diversity in extant Chinese sheep argue that independent domestication events could also have taken place in other regions than the Near East (Chen *et al.* 2006). Considering the dominance of lineage B in the Near East and archaeological evidence from this region, it is suggested that mtDNA sheep lineage B was first domesticated in the Near East region. More sampling of domestic and wild sheep in a wider geographic region is required to settle the initial domestication location of lineage A, C and D. However, the Fertile Crescent is still considered the most likely place of origin for these lineages as well (Hiendleder *et al.* 1998b; Chen *et al.* 2006).

As the techniques for extracting and analysing genetic data has progressed, methods for investigating even ancient DNA (aDNA) have been developed (Higuchi *et al.* 1984; Pääbo 1985; Pääbo *et al.* 1988; Pääbo *et al.* 1989a; Pääbo *et al.* 1989b). Several studies have successfully extracted DNA from ancient soft tissue materials such as well preserved skin, hair and even human brains (e.g. Pääbo *et al.* 1988; Miller *et al.* 2008; Rasmussen *et al.* 2010). However, after the amplification of mtDNA from archaeological bones and teeth was successfully demonstrated, larger population analyses have been possible, as archaeological bones usually are better preserved and much more abundant than soft tissues (Hagelberg *et al.* 1989; Hagelberg & Clegg 1991; Hänni *et al.* 1990; Barnes *et al.* 2002; Rohland & Hofreiter 2007).

When working with ancient DNA it is particularly important to avoid contamination of samples by present-day DNA, and of course to confirm the authenticity of the obtained data (Cooper & Poinar 2000; Keastle & Horsburg 2002; Poinar 2003; Willerslev & Cooper 2005).

Microbial contamination of ancient specimens has been a major problem in the work with ancient DNA. Early studies of ancient DNA had difficulties in yielding significant amounts of original DNA (Higuchi *et al.* 1984; Pääbo 1985; Golenberg *et al.* 1990). However, the advent of the polymerase chain reaction (PCR) resolved this as only a single intact copy of target DNA is sufficient, thus making it an ideal tool when working with aDNA (Saiki *et al.* 1985; Pääbo *et al.* 1989b). However, a consequence of the large amplifying power of PCR is the increased sensitivity of the method to contamination with modern DNA (Willerslev & Cooper 2002). Thus, obtaining false positives due to intra-laboratory contamination has remained a problem in ancient DNA research. Several previous aDNA studies have later been disproved as a result of modern DNA contamination or not being able to reproduce the results, such as studies thought to have amplified sequences from millions of years old dinosaur bones (Woodward *et al.* 1994) or amber inclusions (Cano *et al.* 1992; DeSalle *et al.* 1992). Thus, several criteria must be fulfilled to render ancient DNA results authentic, as reviewed in Willerslev & Cooper (2002) (Table 1).

Most studies conducted with aDNA have focused on mitochondrial DNA. Mitochondrial DNA is located in the mitochondrion which is an organelle only present in eukaryotic cells. It mutates 5-10 times faster than nuclear DNA, exhibits little or no recombination during meiosis, is maternally inherited, small in size and exists in large quantities in mammalian cells (Cavalli-Sforza *et al.*1994; Jobling *et al.* 2004). These properties combined make mtDNA ideal for studying ancient DNA, as DNA from ancient specimens is often highly degraded and is available in small quantities compared to modern DNA samples. However, as it is maternally inherited, genetic analysis will only reveal the genetic composition of ewes in a domestic sheep population (e.g. Pääbo *et al.* 1989b; Cooper & Poinar 2000: Geigl 2002; Willerslev & Cooper 2002). Also, the effective population size of mtDNA markers is ¼ the effective population size of nuclear markers. Therefore, the coalescent time is on average shorter for mitochondrial markers than for nuclear markers, and hence lineage sorting will on short time scales be complete for mitochondrial, but not for nuclear markers (Hamilton 2009). This means that mtDNA markers allows one to study more recent events than markers based on nuclear sequences, which is useful in the study of domesticated sheep as the evolutionary history of domestic sheep is relatively short (Pedrosa *et al.* 2005)

The control region is of most use when studying ancient DNA samples of closely related lineages such as domesticated animals, as it is the non-coding region with the highest mutation rate in the mitochondrial genome (Jobling *et al.* 2004). Ancient DNA analysis therefore offers a window to the past where genetic links between extinct and extant species can be assessed.

To shed light on the composition of domesticated sheep in mediaeval Norway, I have applied methods for extracting ancient mtDNA from bone samples of domestic sheep. The bones were excavated from sites dated to the Middle Ages, and were obtained from three different localities in Norway: Oslo, Bergen and Trondheim (Fig. 1).A bone dated to the Viking Age from Oslo was also included in the study material. The aims of this study were (i) to produce reliable ancient DNA extracts of mediaeval sheep by implementing established methods for extraction and analysis of ancient mtDNA, (ii) clarify the genetic variation of sheep from different regions in mediaeval Norway. Variation would imply that different breeds of sheep were kept at different locations in Norway, and thus potentially shed light on the trading history of domestic sheep.

Table 1

The nine criteria for authenticity of ancient DNA studies by Cooper and Poinar (2000)
(i) **Physically isolated work areas**: separate samples and extracted DNA from PCR amplified products
(ii)**Negative control extractions and amplifications**: screen for contaminants entering process at any time
(iii) **Appropriate molecular behaviour:** PCR amplification strength inversely related to product size, large DNA fragments should be treated with caution
(iv) **Reproducibility:** multiple PCR and extractions should yield consistent results
(v) **Cloning of products:** assessing damage, contamination and jumping PCR
(vi) **Independent replication:** Separate samples of specimen extracted and sequenced by independent research groups yielding consistent results
(vii) **Biochemical preservation:** assessing amount, composition and relative extent of change in other biomolecules associated with DNA survival
(viii) **Quantification:** competitive PCR or Real-Time PCR to give an indication of the number of starting templates in reaction
(ix) **Associated remains:** assess preservation state and contamination of associated remains

* Modified from Cooper & Poinar (2000) and Gilbert *et al.* (2005).

2. Material and methods

2.1 Ancient DNA samples

A total of 69 bone samples of *Ovis aries* were provided by Anne-Karin Hufthammer at the Museum of Natural History, University of Bergen. The samples were obtained from excavations conducted in Bergen (Dreggalmenningen), Oslo (Mindets tomt) and Trondheim (Bibliotekstomten, Televerkstomten, Erkebispegården) (Fig. 1), and have been stored in cardboard boxes at the Museum of Natural History in Bergen. All excavation sites were dated to the Middle Ages, where Dreggalmenningen is dated to 1170-1332 AD, Mindets tomt 1225-1350 AD, Bibliotekstomten 1225-1475 AD, Televerkstomten 1140-1350 AD, and Erkebispegården 1250-1532 AD. Most samples were supplied in powder form, as technicians at the University of Bergen had drilled bone powder from bone fragments. The other samples were received as intact pieces of bone. Information on the specimens that yielded amplifiable DNA is given in Table 2. For information on all specimens see appendix 1. One bone extract of *O. aries* was provided by Maja Krzewinska at the University of Oslo. DNA extraction of the bone had been performed by Maja Krzewinska using the same methods as described below. The sheep bone was excavated from a site in Rønvik, Bodø, which contained human bones and animal bones. The human bones were dated to the Viking Age (800-1066 AD), and as such the associated faunal remains were also dated to the Viking Age. It had been stored along with the human remains in a box at Schreinerske Samlinger.

All handling of samples and equipment were done using gloved hands to reduce direct contamination from human skin cells. Further, surfaces were carefully cleaned with bleach and ethanol between every step, and equipment was always submerged in water containing biodegradable detergents, and rinsed in distilled water. Equipment was also exposed to UV-radiation on a regular basis to destroy potentially surviving DNA molecules.

This study was conducted in a dedicated ancient DNA facility, where the facility for handling pre-PCR products was physically separated from post-PCR facilities. To detect any contamination, blank controls were included in the study, both in the DNA extraction and DNA amplification process. Extracts were amplified several times, however the extraction process of each bone was only conducted once due to the low amount of starting material. BLAST searches in the NCBI database (GenBank) were conducted to establish the identity of samples, in addition to personally controlling the sequences.

Table 2
Bone samples of *Ovis aries* that yielded amplifiable DNA

#	Bone type	Location (ID number)	City	Bone state	Sequenced
2	Metacarpus	Erkebispegården (JS845)	Trondheim	Fragment	yes
7	Humerus	Erkebispegården (JS845)	Trondheim	Fragment	yes
14	Humerus	Erkebispegården (JS845)	Trondheim	Fragment	no
15	Femur	Erkebispegården (JS845)	Trondheim	Fragment	yes
17	Humerus	Bibliotekstomten (JS765)	Trondheim	Fragment	yes
18	Pelvis	Bibliotekstomten (JS765)	Trondheim	Fragment	yes
19	Sacrum	Bibliotekstomten (JS765)	Trondheim	Fragment	yes
24	Metatarsus	Bibliotekstomten (JS765)	Trondheim	Fragment	yes
26	Radius	Bibliotekstomten (JS765)	Trondheim	Fragment	no
27	Metacarpus	Bibliotekstomten (JS765)	Trondheim	Fragment	yes
29	Pelvis	Bibliotekstomten (JS765)	Trondheim	Fragment	yes
30	Cranium	Bibliotekstomten (JS765)	Trondheim	Fragment	no
31	Humerus	Televerkstomten (JS632)	Trondheim	Fragment	yes
32	Humerus	Televerkstomten (JS632)	Trondheim	Fragment	no
33	Pelvis	Televerkstomten (JS632)	Trondheim	Fragment	no
34	Femur	Televerkstomten (JS632)	Trondheim	Fragment	yes
35	Humerus	Televerkstomten (JS632)	Trondheim	Fragment	no
41	Metatarsus	Dreggalmenningen (JS630)	Bergen	Powder	yes
50	Pelvis	Televerkstomten (JS632)	Trondheim	Fragment	no
51	Humerus	Televerkstomten (JS632)	Trondheim	Fragment	yes
64	Pelvis	Mindets tomt (JS537)	Oslo	Fragment	no
65	Pelvis	Mindets tomt (JS537)	Oslo	Fragment	yes
66	Pelvis	Mindets tomt (JS537)	Oslo	Fragment	no
67	Metatarsus	Mindets tomt (JS537)	Oslo	Fragment	no
68	Metatarsus	Mindets tomt (JS537)	Oslo	Fragment	no
69	Pelvis	Mindets tomt (JS537)	Oslo	Fragment	yes
70*		Bjørvika	Oslo	Extract	yes

* Bone from the Viking Age received as extract from Maja Krzewinska

2.2 Bone preparation

Bone pieces were first cleaned with a micro abrasive blasting unit (Airbrasive 6500 from S. S. White Technologies Inc., NJ, USA) to remove the outer surface of the bone and thus remove potential contamination introduced during excavation and storage. Thereafter the small, intact pieces were crushed to smaller fragments using a hammer that was sterilised with bleach and

ethanol between each sample treatment. Further, small fragments were reduced to fine powder using a freezer mill (Model 6700-230, Spex Industries Inc., Edison, NJ, USA) that was refrigerated with liquid nitrogen. The sample tubes and impactors were carefully cleaned and sterilised between each run in order to prevent cross-contamination: Sample tubes and impactors were first submerged in hot water with a biodegradable detergent (Deconex from Borer Chemie AG, Zuchwil, Switzerland). The sample tubes and impactors were thoroughly polished using a brush, rinsed in distilled water and sterilised using bleach, and finally rinsed with ethanol to remove excess water.

2.3 DNA extraction

DNA was extracted from bone tissue by the silica method A described by Rohland & Hofreiter (2007). This method involves extracting DNA from bone powder using a buffer of EDTA and proteinase K, and adding silica for DNA binding and purification. The protocol was followed except for a minor modification: during the DNA purification and elution step 100 µl H_20 was used instead of 50 µl TE buffer. The amount of bone powder used in each extract was 0.3 g for the freezer-milled samples, and 0.03 g for the samples that was received as powder. Extraction solutions were adjusted proportionally. Extractions were always performed with four sheep samples at a time in order to minimise loss of material if an error should occur. A blank extract was included in every extraction to monitor cross-contamination.

2.4 DNA amplification

Two sets of overlapping primers designed by Cai *et al.* (1997) from reference sequence AF010406 (GenBank) were used to amplify a 271 bp mtDNA fragment. This fragment contains the partial $tRNA^{pro}$ and control region sequence between nucleotide positions 15391 and 15661 (Table 3 and Fig. 2). As a control for contamination, a fragment of the cytochrome b (cyt b) region (309 bp) was also amplified in all samples, as this is a conserved region with low variation among species. The low variation allows cyt b primers to amplify homologous segments across species, and human or cross-contamination to be detected in controls. Sheep samples were always amplified along with the blank extraction, a negative PCR control

containing H_2O (mqH_2O) purified in a water purification system (Millipore Corporation, MA, USA), a positive PCR control of human tibia bone, and an extract of a different species than sheep (chiefly cattle or goat), to control for contamination and the authenticity of the samples.

Table 3
Primers for PCR amplifiactions

Primer	mtDNA position	Primer sequence	Fragment length (bp)	Ref.
L15391[a]	$tRNA^{Pro}$ -	5'CCACTATCAACACCCAAAG'3	144	1
H15534	HVR1	5'AAGTCCGTGTTGTATGTTTG'3		
L15496[a]	HVR1	5'TTAAACTTGCTAAAACTCCCA'3	166	1
H15661		5'AATGTTATGTACTCGCTTAGCA'3		
L14841	Cytochrome B	5'AAAAAGCTTCCATCCAACATCTCAGCATCATGAAA'3	309	2
H15149		5'AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA'3		

a The numbers give the 5'-end of the primers according to AF010406.
L refers to the light strands, H refers to the heavy strands.
1 Cai *et al.* 2007
2 Kocher *et al.* 1989

The extracted DNA was amplified using the polymerase chain reaction (PCR) (Saiki *et al.* 1985; Pääbo *et al.* 1989b). The PCR amplifications were performed in 25 µl reactions containing 14.4 µl mqH_2O, 2.5 µl 10 x PCR buffer (Applied Biosystems, Foster City, CA, USA), 2.5 µl 2.5 µM $MgCl_2$, 2µl 2.5µM dNTPs, 1 µl bovine serum albumin, 0.75 µl 10µM forward and reverse primer, 0.125 µl 1.25 U/µl Taq polymerase (AmpliTaq Gold DNA polymerase, Applied Biosystems, Foster City, CA, USA) and 1 µl DNA. A positive and a negative PCR control were also included in every reaction. A master-mix was prepared for the number of samples processed each run, of which 24 µl was added to each reaction, in addition to 1 µl DNA, to a final volume of 25 µl.

The following PCR-program was used: initial denaturation at 95 ºC for 5 min, followed by 40 cycles of denaturation at 95 ºC for 45 s, primer annealing at 56 ºC for 45 s, extension at 73 ºC for 45 s and a final extension step at 73 ºC for 7 min. The amplifications were performed in a Gene Amp PCR System 9700 Thermocycler (Applied Biosystems, Foster City, CA, USA).

2.5 Gel electrophoresis

To establish the yield and size of any DNA segments the PCR products were run on 1.5% agarose gels, where 1µl 6x Promega Loading Dye (Promega Corporation, WI, USA) was added to 5 µl PCR product. A 100bp ladder (GeneRuler 100bp DNA Ladder from MBI Fermentas, Hanover, MD, USA) was added in separate wells. The gels were made in $^{1}/_{2}$XTBE buffer and stained with ethidium bromide to visualise the DNA fragments under ultraviolet light. Gels were run at 70V for 45 min.

2.6 DNA sequencing

PCR products were cleaned using ExoSAP-IT (USB Corporation, OH, USA). ExoSAP-IT contains two hydrolytic enzymes that remove unconsumed dNTPs and primers that can interfere during sequencing. The manufacturer's protocol was followed, but products were diluted 1:10 with upH_2O when strong bands of DNA were evident on the gel. Sequencing was performed on an ABI 3730 high-throughput capillary electrophoresis instrument (Applied Biosystems, Foster City, CA, USA), using the specific sheep PCR primers. Sequencing of the cytochrome b fragments was not conducted as it was only amplified to control for contamination.

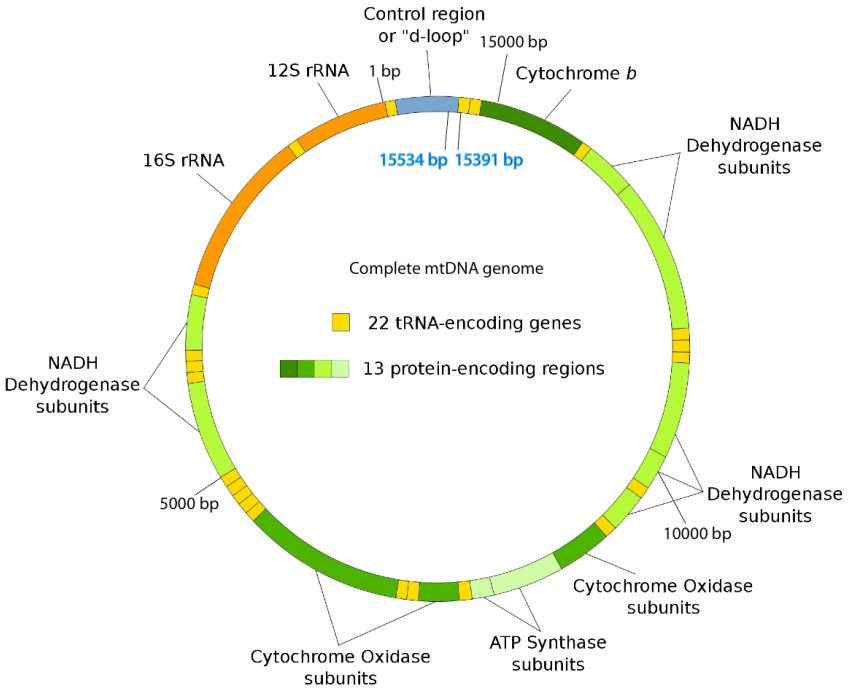


Fig. 2. The complete mitochondrial genome depicting tRNA-encoding genes, protein-encoding genes and the control region. The region amplified in this study using sheep-specific primers is highlighted in blue; ranging from 15391 bp-15534 bp.

2.7 Data analysis

The sequences obtained were edited by eye and aligned using SEQUENCHER VER 4.8 (Gene Codes Corp., MI, USA). A neighbour-joining tree (Saitou & Nei, 1987) was constructed in MEGA VER 4.0.2 (Tamura *et al.* 2007) to illustrate differences within and between mtDNA haplogroups in *Ovis aries*. The trees were drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.* 2004) and were in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). Bootstrap support was calculated as percentages among 2000 replicates. Reference sequences of different haplogroups of domestic sheep were obtained from GenBank (Table 4). Reference sequence selection was based on collecting known complete

mtDNA sequences of haplogroups A, B and C, in addition to the partial D haplogroup sequence. Other reference sequences were carefully chosen to represent different breeds from different regions. A second neighbour-joining tree was constructed using the same GenBank sequences in addition to the ancient *O. aries* sequences obtained in this study, to resolve which mtDNA haplogroups these were most closely related to. The trees were constructed using default settings. As neighbour-joining phylogenies are based on distance methods, trees based on optimality criteria were also constructed to check if similar results were obtained. Maximum Parsimony trees (Eck & Dayhoff 1966) and Minimum Evolution trees (Rzhetsky & Nei 1992) which are based on optimality criteria were constructed in MEGA VER. 4.0.2 using default settings.

Table 4
mtDNA sequences (control region) of *Ovis aries* used in this study from GenBank

Breed	GenBank accession numbers	Country/region sample was obtained from
German-Merino	AF010406	Germany
	AF010407	
	AF039577	
Kazakh Fat-rumped	AY829388	China
	AY829385	
Tibetan	AY829418	China
	AY829421	
Merino	Z35228	New Zealand
Polled Dorset	AY829377	Australia
	AY829382	
Akkaraman	AY091497	Turkey/Central Anatolia
Texel	AY829406	Netherlands
Karachai	DQ242212	North Caucasus

3. Results

3.1 Amplification and authenticity of ancient DNA sequences

Of 69 extracted samples of mediaeval domestic sheep, 25 samples yielded evidence of amplified mtDNA on the agarose gel electrophoresis. Of these, 15 samples were successfully sequenced (for details see table 2), and identified as sheep. However, only one of the specific sheep-primer sets worked: L15391 – H15534 (Table 3 and Fig. 2). The obtained sequences ranged from 88 bp to 140 bp depending on the quality of the product. The samples that were amplified with cytochrome b primers were run on agarose gels, but were not sequenced. The Viking domestic sheep bone extract was also successfully amplified and sequenced with primers L15391 – H15534. In addition to the successfully amplified samples, 8 samples showed signs of contamination (blank controls yielded amplified DNA) after multiple PCR-reactions and were consequently discarded from the study. The remaining 36 samples did not amplify any DNA.

Gel pictures verified the authenticity of the samples when the following two conditions were met: (i) all sheep samples, positive controls and second species extracts were successfully amplified using cytochrome b- primers, whilst the negative controls remained negative, and (ii) specific sheep primers only amplified sheep extracts, whereas all other samples remained negative. An example of successfully amplified PCR products of sheep mtDNA and controls is illustrated in Fig.3.

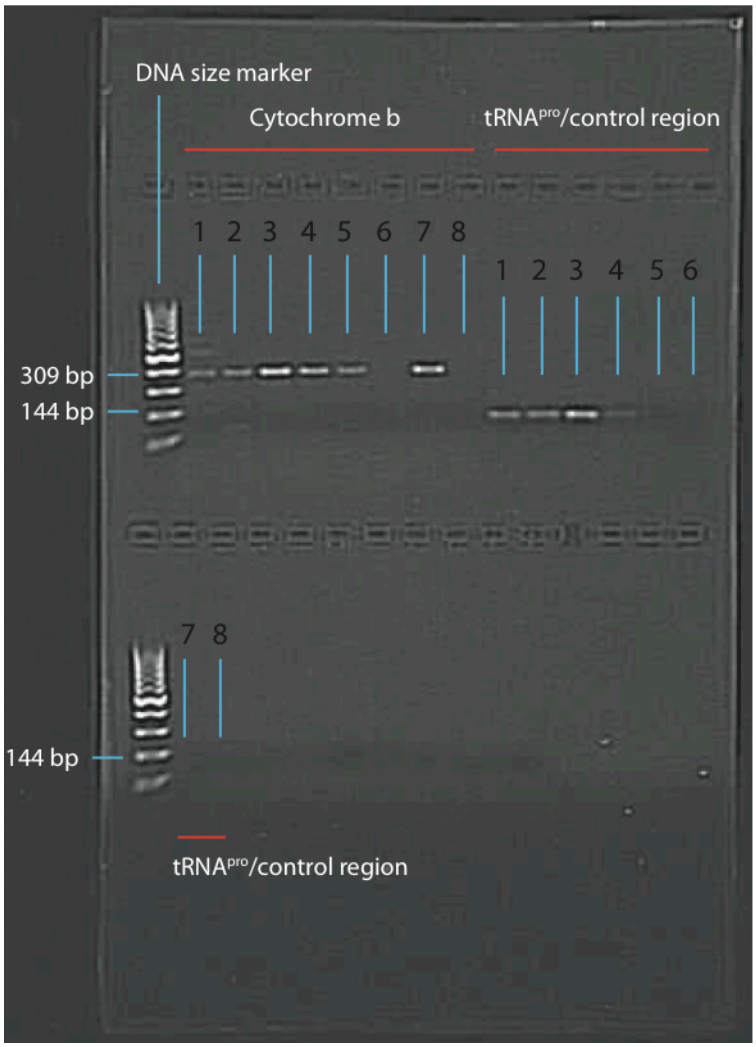


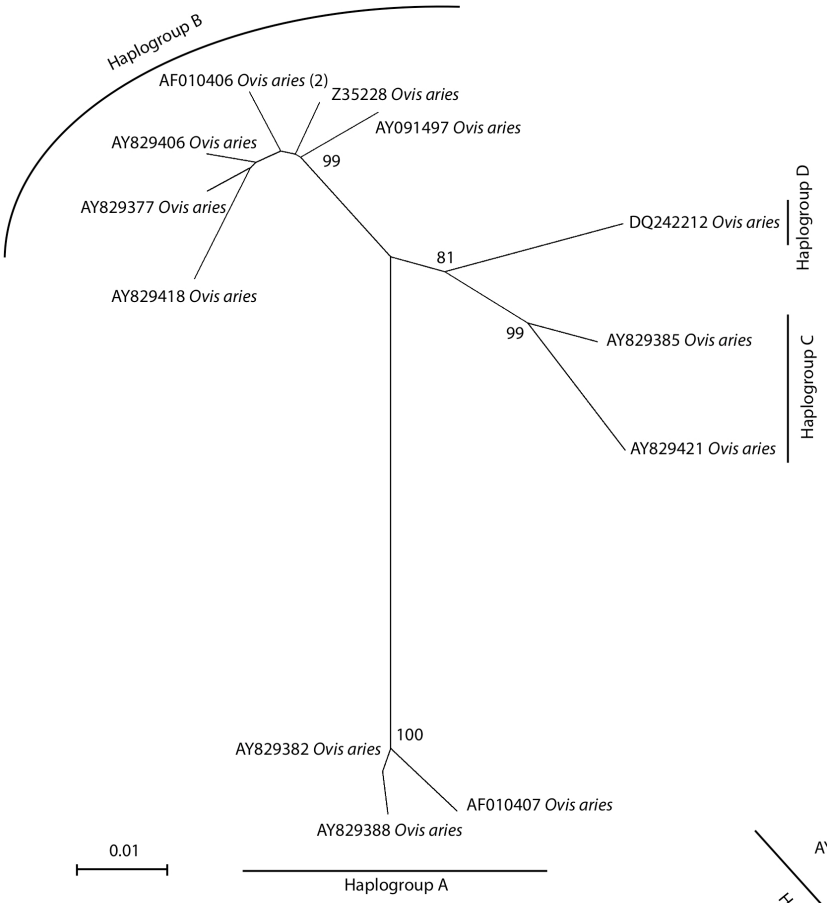
Fig. 3. PCR amplifications from agarose gel electrophoresis of mtDNA samples numbered 1-8, where: (1-4) DNA from mediaeval sheep bones; (5) mtDNA from cattle bone (6) Blank extraction control containing no bone; (7) Positive PCR control containing DNA from modern human tibia; (8) Negative PCR control containing H_2O. The amplified products were of cyt b of 309 bp (mtDNA bases 14841-15149) and partial $tRNA^{pro}$ region and control region of 144 bp (mtDNA bases 15391-15534).

3.2 Haplogroup allocation of ancient mtDNA sequences from domestic sheep

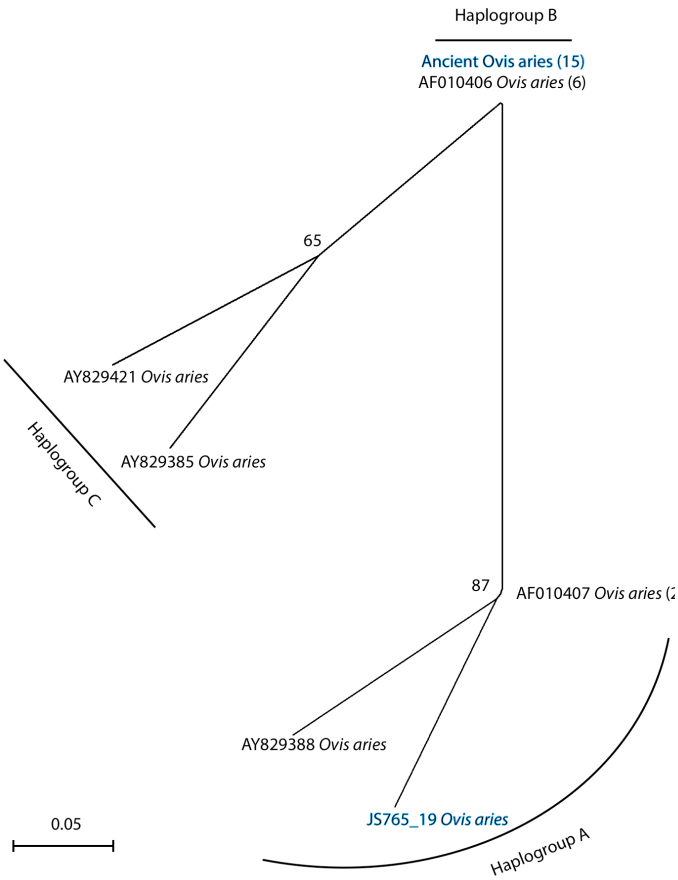
Sixteen ancient mtDNA sequences of domestic sheep from mediaeval Norway were analysed together with published mtDNA sequences of modern domestic breeds to reveal their haplogroup affinities. Neighbour-joining (NJ), maximum parsimony and minimum evolution methods revealed identical branching patterns, thus only NJ is shown here (Fig 4). The NJ analysis containing only modern domestic breeds clearly shows a splitting into four

haplogroups in domestic sheep: A, B, C and D (Fig. 4a). As seen from Fig. 4a, haplogroup A and B contain both European and Asian breeds, whereas haplogroup C contains only Asian breeds. Haplogroup D consists of one individual from North Caucasus (see Table 4 for details). The final data set which was analysed consisted of 635 bp, and yielded a tree with good resolution and high bootstrap support.

In the NJ analysis that included the 16 ancient sheep samples, 15 individuals were allocated to haplogroup B, whereas one individual was included in haplogroup A. As these ancient sheep samples only yielded informative sequences of 88 bp, a tree with lower resolution was obtained with a decrease in bootstrap values (Fig. 4b). The individual that was placed in haplogroup A (JS765_#19) was excavated from Bibliotekstomten in Trondheim. The remaining samples from Trondheim, Bergen and Oslo (including the Viking bone), were all allocated to haplogroup B.



4 (a)



4 (b)

Fig. 4 (a). Neighbour-joining tree (Saitou & Nei 1987) illustrating the differences between and within the four domestic sheep (*Ovis aries*) mtDNA haplogrups: A, B, C and D. The tree is based on mtDNA control region sequences from GenBank, with the accession numbers identifying the samples (Table 4). The number of sequences included in identical haplotypes is indicated in brackets. Bootstrap support numbers for the main branches are indicated at the nodes as percentages among 2000 replicates. **(b).** Neighbour-joining tree (Saitou & Nei 1987) illustrating the haplogroups of the ancient *Ovis aries* sequences in the current study (blue colour). The tree is based on sequences of the mtDNA control region from GenBank (Table 4) and the 16 sequences of ancient domestic sheep mtDNA sequences in this study (Table 2). The number of identical haplotypes is indicated in brackets, and bootstrap numbers are indicated at the nodes as percentages among 2000 replicates.

When aligning reference sequences from GenBank of known mtDNA haplogroups A, B and C with the sequences obtained in this study, polymorphisms along the 88 bp sequence were evident (Table 5). This fragment represents only a part of the control region (site 15447 – 15534), and not any portion of the $tRNA^{pro}$ region. Sites where one or more of the other sequences differ from the Haplotype A reference sequence is indicated with red letters signifying the site of mutation

The ancient sample JS765 #19 was identical to the haplogroup A reference sequence at all sites, except for a Guanine (G) base at site 15462, instead of Adenin (A). The remaining ancient samples were identical to the Haplogroup B reference sequence, and differed from Haplogroup A at sites 15460 and 15485. Finally, haplogroup C differed from A at sites 15451, 15460, 15485 and 15510, and from haplogroup B at sites 15451 and 15510. Haplogroup C was not detected in any of the ancient samples. Haplogroup D was not used in the alignment as the reference sequence is only partial and does not contain the region obtained in this study.

Table 5

Comparison of Haplogroups A,B and C using reference sequences and ancient samples (88 bp from site 15447-15534) from control region (mtDNA)

Haplo-group	Name	Sequence
A	AF010407*	CAACGATACTTATTAATATATTTCCAAAAATATAAAGAACCTCTCCAGTATTAAACTTGCTAAAACTCCCAAACATACAACACGGACT
A	JS765_#19"	..............G..
B	AF010406*	............C.A.....................G.....
B	15 ancient"	............C.A.....................G.....
C	AY829421*	....A........C.A.....................G.....................G.....
C	None"	

* Sequences obtained from GenBank

" Sequences obtained in current study

Red letters indicate site of polymorphism between sequences

4. Discussion

4.1 Is the mtDNA of mediaeval domestic sheep authentic?

Studies of ancient DNA face problems of contamination and the authenticity of their results. Based on the implemented routines to prevent contamination and the amplified DNA visualised on the agarose gels after electrophoresis, I argue that the sequences obtained in this study are of sheep from the Middle Ages (476-1453 AD) and the Viking Age (800-1066) (Fig. 3).

Bone samples which are excavated from archaeological sites are often handled by several people before they are properly stored or delivered for analysis. The sheep bones in this study were therefore always thoroughly cleaned with a micro abrasive blasting unit to reduce the chance of external contamination. Another source of contamination could come from the environment from which the study material was excavated. Studies extracting aDNA from different layers of sediments have proved that vertical migration of aDNA across strata do occur, and can possibly contaminate adjacent material (Haile *et al.* 2007). However, more research is needed to reveal the extent of such contamination, and thus far it is concluded that vertical migration of DNA poses an unlikely threat to the authentication of aDNA results. Given these precautions and considerations, I do not consider pre-laboratory contamination to pose a significant threat to the current study, although it cannot be entirely ruled out.

Intra-laboratory contamination is another problem encountered in aDNA studies. The current study has implemented methods for reducing the chance of contaminating the samples, as described in the material and methods section. Based on the obtained results I consider the mtDNA data as sequences of authentic ancient domestic sheep. First, the amplified PCR products coincide with the expected product size of 309 bp for the cyt b region and 144 bp for the specific sheep-primers targeting the control region. The amplified pcr-products did not yield strong bands, which are unusual in ancient DNA samples due to the low amount and degradation of DNA in these, thus the PCR amplification strength was inversely related to product size. This is as expected, and fulfils one of the criteria proposed to authenticate ancient DNA results (Table 1) (Cooper & Poinar 2000; Willerslev & Cooper 2005). Furthermore, the blank extract and negative PCR controls remained negative, which indicates absence of introduced contamination and cross-contamination, as any presence of DNA would have been amplified. If the blank controls were contaminated with human DNA,

the cyt b-primers would have amplified these, as they did amplify the positive PCR control of human DNA. If the blank controls were cross-contaminated, both cyt b-primers and the sheep-primers targeting the control region would have amplified the sheep DNA in these controls. Moreover, cross-contamination can be ruled out as the control containing mtDNA from cattle or goat bones did amplify with cyt b-primers, but not with the specific sheep-primers. If samples were cross-contaminated, the sheep primers would have amplified the cattle extract as well, since it would have contained sheep DNA. It is possible that the sheep primers were not specific enough and also amplified human DNA. However, I see this as unlikely as the sheep-specific primers did not amplify the human DNA control, whereas the cyt b-primers did, thus confirming that DNA was present in that sample.

Cross-contamination of samples in this study thus seems unlikely, and samples which were successfully amplified, sequenced and identified as sheep, can be regarded as mtDNA sequences representing different individuals of mediaeval and Viking age domestic sheep. Further, I exclude the possibility that the samples consist of modern sheep DNA, as no material of modern sheep has been introduced to the laboratory facilities, nor have any persons associated with the lab been in contact with such material. Moreover, if the samples were contaminated with modern sheep DNA this would most likely have been detected in the blank controls.

The main proportion of extracted samples in this study was not successfully amplified, as only 26 of 70 samples yielded amplified DNA products. This could be explained by the varying degree of degradation of ancient DNA in specimens, as *post-mortem* DNA quickly suffers destruction by hydrolysis and oxidation, and ancient material therefore seldom contain amplifiable endogenous DNA (Pääbo 1989b; Höss *et al.* 1996). The low proportion of amplified products is probably also due to the fact that most samples used in this study consisted of low amounts of bone powder (approximately 0.03 grams). In fact, only one sample in powder form amplified successfully, whereas the remaining amplified products were of freezer-milled bone pieces which yielded bone powder of several grams (Table 1). Because DNA exists in such low amounts in ancient specimens, it is likely that the chances of obtaining any useable DNA fragments increase with increasing amounts of study material.

All of the nine criteria of the authentication of ancient DNA described by Cooper and Poinar (2000) were not fulfilled in the current study (Table 1). For instance, the results were

not replicated in another independent research facility to completely discount intra-laboratory contamination. However, most ancient DNA studies have not applied all nine criteria, but still defend the reliability of their data. A strict adoption of the nine criteria does not guarantee authenticity of the obtained genetic data, for instance following these nine criteria would not detect contamination of samples obtained prior to analyses (Gilbert *et al.* 2005). It is therefore sensible to take a more cognitive approach when assessing the reliability of results, and evaluate the results on a case-to-case basis. But the nine criteria should still be regarded important, and used as a guide-line to assess the data. Based on these considerations I argue that the results obtained in the current study are reliable based on the nature of the controls and the fact that the results make sense. However, as the mtDNA sequences obtained were short, I do not rely on them providing enough data to fully support any conclusions drawn from phylogenetic analyses (Fig. 4b).

4.2 Phylogenetic analyses of mediaeval domestic sheep mtDNA

In the current study 25 sequences of mtDNA were amplified from domestic sheep bones excavated from sites dated to the Middle Ages (476-1453 AD), and one sequence of sheep dated to the Viking Age (800-1066 AD). Of these 26 amplified PCR products, 16 were successfully sequenced and used in phylogenetic analyses. Only one set of the primer pair targeting the $tRNA^{pro}$ and control region worked, thus yielding a much shorter sequence (88 bp) than originally intended.

Phylogenetic analyses allocated 15 out of 16 sequences of mtDNA from ancient domestic sheep to mtDNA haplogroup B, whereas one sequence was allocated to haplogroup A. However, due to the short sequence of 88 bp obtained in the study, the actual variation in Norwegian mediaeval domestic sheep has most likely not been fully captured. Also, the captured genetic variation is biased as the mitochondrial genome is maternally inherited, hence the mtDNA sheep sequences only capture genetic variation in the ewes of the population (Jobling *et al.* 2004).

The fact that only 16 of the amplified products were successfully sequenced in this study, might be a result of degraded DNA in samples. For instance, the DNA could be amplified but was too damaged to yield informative sequences. Decaying DNA has been

shown to have strand breaks, miscoding lesions and cross links, which again can lead to sequencing artefacts or the amplification of undamaged contaminating DNA (Pääbo 1989a; 1989b; Lindahl 1993; Willerslev & Cooper 2005).

The domestic sheep sequence (JS765_19) allocated to haplogroup A was obtained from a site in Trondheim. This sequence displayed a polymorphism (Guanine instead of Adenine) at site 15462 which was not observed in any of the other haplogroups. This polymorphism could be explained by an AT→GC transition. Studies have shown that aDNA amplification products often exhibit sequencing artefacts such as GC→AT or AT→GC transitions as a consequence of *post-mortem* degradation in ancient DNA (Hansen *et al.* 2001; Gilbert *et al.* 2003). Nevertheless, it is likely that sequence JS765_19 belongs to haplogroup A as it is identical to the reference sequence at all sites, including sites 15451, 15460, 15485 and 15510. Sites 15451, 15460, 15485 and 15510 were the informative sites detected in the 88 bp long fragment of the control region in domestic sheep, as polymorphisms existed here between haplogroups A, B and C (see table 5). These informative sites were most likely not a result of sequencing artefacts, as they corresponded to reference sequences from GenBank. Taken together, the results therefore indicate that there were at least two different mtDNA haplogroups of domestic sheep in mediaeval Norway.

The results of the current study are in accordance with other studies that have found mtDNA lineage A and B in extant domestic sheep from Northern Europe. Since sheep lineage A has not been revealed in samples from Eastern Europe, it has been proposed that this mitochondrial lineage arrived in northern Europe from the Near East across Russia (Tapio *et al.* 2006b). Chen *et al.* (2006) found evidence of two central founder types within haplogroup A. It was concluded based on the pattern of genetic variation that two independent domestication events had occurred in haplogroup A. However, the initial domestication centres of these domestication events are yet to be resolved. One can therefore not conclude with certainty that haplogroup A originated in the Near East, and spread to Norway from this region. The current study does however suggest that the introduction of two mtDNA haplogroups in Norway (haplogroup A and B) must have occurred in, or prior, to the Middle Ages.

This study also reveals that haplogroup B was present in Norway in the Viking Age, as the sample dated to the Viking Age was identical to the haplogroup B reference sequence at

the surveyed sites. However, there is not enough information in the sequences to infer whether different locations in mediaeval Norway were associated with different breeds of sheep since there were not enough informative sites obtained to infer differences on the level of sheep breeds. It was therefore not possible to reveal evidence of whether or not domestic sheep introduced to Norway in the Middle Ages interbred with native breeds. The domestic sheep sample allocated to haplogroup A may be an example of an introduced breed, but this question cannot be settled by the current data. Low differentiation, weak phylo-geographic structure and overlapping genetic similarity have been revealed in domestic sheep. This can be explained by their short evolutionary history and high levels of gene flow between populations (Meadows *et al.* 2005; Kijas *et al.* 2009). These factors have probably contributed to the genetic similarity revealed among the domestic sheep samples investigated in this study.

5. Conclusion

I argue that the sequences of mtDNA from mediaeval domestic sheep and Viking Age sheep are authentic based on the implemented methods for reducing contamination. The information yielded from these sequences can therefore be regarded as authentic information on mediaeval sheep breeds in Norway. Phylogenetic analyses showed that all sheep samples were allocated to mtDNA haplogroup B, except one which was allocated to haplogroup A. This reflects studies which have revealed that mtDNA haplogroups A and B are present in domestic sheep in Northern Europe, and it can be concluded that these two haplogroups were present in Norway in the Middle Ages.

However, as the sequences used in the analyses were short (88bp), they most likely revealed only a small part of the actual genetic variation among the mediaeval sheep samples. Hence this study did not produce enough reliable data to answer questions regarding the trade of domestic sheep in mediaeval Norway. Further research should be concentrated on designing primers which could amplify a longer, more informative fragment of the control region of these domestic sheep. Longer mtDNA sequences may reveal more variation, and allow one to distinguish between different sheep breeds, and thus shed more light on questions regarding the trading history of sheep in the Middle Ages.

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7 Appendix

A list of all samples of *Ovis aries* samples provided to this study by the University of Bergen.

Location ID	Sample #	Location	City	County	Species	Bone type	Archeological data	Bone form	Weight
845	1	Erkebispegården	Trondheim	Sør-Trøndelag	Ovis aries	Metacarpus	N-169515, K5c	Powder	
845	2	Erkebispegården	Trondheim	Sør-Trøndelag	Ovis aries	Metatarsus	N-169515, K5c	Piece	3 gr
845	3	Erkebispegården	Trondheim	Sør-Trøndelag	Ovis aries	Metacarpus	N-170764, I 4 c, lag 1168	Powder	
845	4	Erkebispegården	Trondheim	Sør-Trøndelag	Ovis aries	Metacarpus	N-170764, I 4 c, lag 1168	Powder	
845	5	Erkebispegården	Trondheim	Sør-Trøndelag	Ovis aries	Metatarsus	N-170764, I 4 c, lag 1168	Powder	
845	6	Erkebispegården	Trondheim	Sør-Trøndelag	Ovis aries	Metatarsus	N-170764, I 4 c, lag 1168	Powder	
845	7	Erkebispegården	Trondheim	Sør-Trøndelag	Ovis aries	Humerus	N-170764, I 4 c, lag 1168	Piece	2,6 gr
845	8	Erkebispegården	Trondheim	Sør-Trøndelag	Ovis aries	Cranium	N-170764, I 4 c, lag 1168	Powder	
845	9	Erkebispegården	Trondheim	Sør-Trøndelag	Ovis aries	Metatarsus	N-167564, H6D	Powder	
845	10	Erkebispegården	Trondheim	Sør-Trøndelag	Ovis aries	Metatarsus	N-167564, H6D	Powder	
845	11	Erkebispegården	Trondheim	Sør-Trøndelag	Ovis aries	Metacarpus	N-167564, H6D	Powder	
845	12	Erkebispegården	Trondheim	Sør-Trøndelag	Ovis aries	Radius	N-167564, H6D	Powder	
845	13	Erkebispegården	Trondheim	Sør-Trøndelag	Ovis aries	Ulna	N-167564, H6D	Powder	
845	14	Erkebispegården	Trondheim	Sør-Trøndelag	Ovis aries	Humerus	N-167564, H6D	Piece	3,2 gr
845	15	Erkebispegården	Trondheim	Sør-Trøndelag	Ovis aries	Femur	N-167564, H6D	Piece	2,3 gr
765	16	Bibliotekstomten	Trondheim	Sør-Trøndelag	Ovis aries	Calcaneum	N-85483, FJ 140	Powder	
765	17	Bibliotekstomten	Trondheim	Sør-Trøndelag	Ovis aries	Humerus	N-85483, FJ 140	Piece	3,7 gr
765	18	Bibliotekstomten	Trondheim	Sør-Trøndelag	Ovis aries	Pelvis	N-85483, FJ 140	Piece	2,6 gr
765	19	Bibliotekstomten	Trondheim	Sør-Trøndelag	Ovis aries	Sacrum	N-85483, FJ 140	Piece	2,4 gr
765	20	Bibliotekstomten	Trondheim	Sør-Trøndelag	Ovis aries	Metatarsus	N-89982, FN 623	Powder	
765	21	Bibliotekstomten	Trondheim	Sør-Trøndelag	Ovis aries	Femur	N-85920, FJ 140	Powder	
765	22	Bibliotekstomten	Trondheim	Sør-Trøndelag	Ovis aries	Radius	N-85920, FJ 140	Powder	
765	23	Bibliotekstomten	Trondheim	Sør-Trøndelag	Ovis aries	Metacarpus	N-85920, FJ 140	Powder	
765	24	Bibliotekstomten	Trondheim	Sør-Trøndelag	Ovis aries	Metatarsus	N-85920, FJ 140	Piece	3,1 gr
765	25	Bibliotekstomten	Trondheim	Sør-Trøndelag	Ovis aries	Metatarsus	N-87545, FN 386	Powder	
765	26	Bibliotekstomten	Trondheim	Sør-Trøndelag	Ovis aries	Radius	N-93262, FN 707	Piece	3,1 gr
765	27	Bibliotekstomten	Trondheim	Sør-Trøndelag	Ovis aries	Metacarpus	N-83130, FJ 90	Piece	2,9 gr
765	28	Bibliotekstomten	Trondheim	Sør-Trøndelag	Ovis aries	Calcaneum	N-84328, FN 325	Powder	
765	29	Bibliotekstomten	Trondheim	Sør-Trøndelag	Ovis aries	Pelvis	N-92566, FN 707	Piece	2,7 gr
765	30	Bibliotekstomten	Trondheim	Sør-Trøndelag	Ovis aries	Cranium	N-85483, FJ 140	Powder	

632	31	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Humerus	N-49890, VB 404	Piece	3,0 gr
632	32	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Humerus	N-49890, VB 404	Piece	3,1 gr
632	33	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Pelvis	N-49890, VB 404	Piece	2,5 gr
632	34	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Femur	N-49889,VB 404	Piece	2,1 gr
632	35	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Humerus	N- 48124, VA 150 Fase 6, 150	Piece	2,5 gr
630	36	Dreggalmenningen	Bergen	Hordaland	Ovis aries	Metatarsus	Fase II, B 291	Powder	
630	37	Dreggalmenningen	Bergen	Hordaland	Ovis aries	Metatarsus	Fase II, B 281	Powder	
630	38	Dreggalmenningen	Bergen	Hordaland	Ovis aries	Metatarsus	Fase II, B 70	Powder	
630	39	Dreggalmenningen	Bergen	Hordaland	Ovis aries	Metatarsus	Fase II, B 273	Powder	
630	40	Dreggalmenningen	Bergen	Hordaland	Ovis aries	Metatarsus	Fase II, B 273	Powder	
630	41	Dreggalmenningen	Bergen	Hordaland	Ovis aries	Metatarsus	Fase II, B 295	Powder	
630	42	Dreggalmenningen	Bergen	Hordaland	Ovis aries	Metatarsus	Fase II, A 197	Powder	
630	43	Dreggalmenningen	Bergen	Hordaland	Ovis aries	Metacarpus	Fase II, B 287, G-1	Powder	
630	44	Dreggalmenningen	Bergen	Hordaland	Ovis aries	Metacarpus	Fase II, B 273	Powder	
630	45	Dreggalmenningen	Bergen	Hordaland	Ovis aries	Metacarpus	Fase II, A 225	Powder	
630	46	Dreggalmenningen	Bergen	Hordaland	Ovis aries	Metacarpus	Fase II, B 252, G+1	Powder	
630	47	Dreggalmenningen	Bergen	Hordaland	Ovis aries	Metacarpus	Fase II, B 65	Powder	
630	48	Dreggalmenningen	Bergen	Hordaland	Ovis aries	Metacarpus	Fase II, B 273, F+1	Powder	
630	49	Dreggalmenningen	Bergen	Hordaland	Ovis aries	Metacarpus	Fase II, B 66	Powder	
632	50	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Pelvis	N-49835, F 4b, F404	Piece	2,3 gr
632	51	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Humerus	N-49835, F 4b, F404, Dp 58 mm	Piece	4,9 gr
632	52	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Metacarpus	N-49835, F 4b, F404	Powder	
632	53	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Pelvis	N-48169, Fase 6, 172	Powder	
632	54	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Femur	N-48169, Fase 6, 172	Powder	
632	55	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Femur	N-48159, Fase 5, 253	Powder	
632	56	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Radius	N-48159, Fase 5, 253	Powder	
632	57	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Ulna	N-48159, Fase 5, 253	Powder	
632	58	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Metatarsus	N-49269, Fase 4a, 300	Powder	
632	59	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Calcaneum	N-49269, Fase 4a, 300	Powder	
632	60	Televerkstomten	Trondheim	Sør-Trøndelag	Ovis aries	Humerus	N-49266, Fase 4b, 415	Powder	
537	61	Mindets tomt	Oslo	Oslo	Ovis aries	Femur	Q 20, under brann 6 over koksteinslag	Powder	
537	62	Mindets tomt	Oslo	Oslo	Ovis aries	Femur	Q 20, under brann 6 over koksteinslag	Powder	
537	63	Mindets tomt	Oslo	Oslo	Ovis aries	Femur	Q 20, under brann 6 over koksteinslag	Powder	
537	64	Mindets tomt	Oslo	Oslo	Ovis aries	Pelvis	Q 20, under brann 6 over koksteinslag	Piece	3,5 gr

537	65	Mindets tomt	Oslo	Oslo	Ovis aries	Pelvis	Q 20, under brann 6 over koksteinslag	Piece	2,9 gr
537	66	Mindets tomt	Oslo	Oslo	Ovis aries	Pelvis	Q 20, under brann 6 over koksteinslag	Piece	2,6 gr
537	67	Mindets tomt	Oslo	Oslo	Ovis aries	Metatarsus	Q 20, under brann 6 over koksteinslag	Piece	2,6 gr
537	68	Mindets tomt	Oslo	Oslo	Ovis aries	Metatarsus	Q 20, under brann 6 over koksteinslag	Piece	2,3 gr
537	69	Mindets tomt	Oslo	Oslo	Ovis aries	Pelvis	Q 20, under brann 6 over koksteinslag	Piece	2,1 gr