



‘The Farm Beneath the Sand’ – an archaeological case study on ancient ‘dirt’ DNA

Hebsgaard, M.B.; Gilbert, T.P.; Arneborg, J.; et.al.

<https://researchportal.murdoch.edu.au/esploro/outputs/journalArticle/The-Farm-Beneath-the-Sand/991005542760107891/filesAndLinks?index=0>

Hebsgaard, M. B., Gilbert, T. P., Arneborg, J., Heyn, P., Allentoft, M. E., Bunce, M., Munch, K., Schweger, C., & Willerslev, E. (2009). ‘The Farm Beneath the Sand’ – an archaeological case study on ancient ‘dirt’ DNA. *Antiquity*, 83(320), 430–444.

<https://researchportal.murdoch.edu.au/esploro/outputs/journalArticle/The-Farm-Beneath-the-Sand/991005542760107891>

Document Version: Published (Version of Record)

‘The Farm Beneath the Sand’ – an archaeological case study on ancient ‘dirt’ DNA

Martin B. Hebsgaard¹, M. Thomas P. Gilbert¹, Jette Arneborg², Patricia Heyn¹, Morten E. Allentoft³, Michael Bunce⁴, Kasper Munch⁵, Charles Schweger⁶ & Eske Willerslev^{1*}

It is probable that ‘The Farm Beneath the Sand’ will come to stand for a revolution in archaeological investigation. The authors show that a core of soil from an open field can provide a narrative of grazing animals, human occupation and their departure, just using DNA and AMS dating. In this case the conventional archaeological remains were nearby, and the sequence obtained by the old methods of digging and faunal analysis correlated well with the story from the core of ancient ‘dirt’ DNA. The potential for mapping the human, animal and plant experience of the planet is stupendous.

Keywords: Greenland, Norse, ancient DNA, AMS dating

Introduction

It is generally known among archaeologists that ancient DNA can be obtained from macrofossil remains such as bones and teeth. It is perhaps less recognised that ancient DNA can also be retrieved directly from ancient sediments, ice and faeces, even in the absence of visible macrofossils (commonly referred to as ancient ‘dirt’ DNA) (e.g. Hofreiter *et al.* 2003; Willerslev *et al.* 2003, 2007; Gilbert *et al.* 2008). For example, it has been shown that ancient DNA deriving from diverse micro-organisms, various plants and vertebrates, including mammoth, horse, bison and musk oxen, can be retrieved directly from small amounts (less than 2g) of sediments both under frozen and non-frozen conditions (Hofreiter *et al.* 2003; Willerslev *et al.* 1999, 2003, 2004a & b; Lydolph *et al.* 2005; Hansen *et al.* 2006; Haile *et al.* 2007; Johnson *et al.* 2007). This has allowed detailed reconstructions of palaeo-ecosystems in Siberia, North America and New Zealand and makes it possible, for

¹ Centre for Ancient Genetics, University of Copenhagen, Universitetsparken 15, DK-2100, Copenhagen Ø, Denmark

² Danish Middle Ages & Renaissance, National Museum of Denmark, Frederiksholm Kanal 12, DK-1220 Copenhagen K, Denmark

³ School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand

⁴ Ancient DNA Research Laboratory, School of Biological Sciences and Biotechnology, Murdoch University, South Street, Perth 6150 Australia

⁵ Department of Integrative Biology, University of California, Berkeley, CA 94720-3140, USA

⁶ Department of Anthropology, University of Alberta, Edmonton, Alberta T6G 2E9, Canada

* Author of correspondence (Email: ewillerslev@bio.ku.dk)

Received: 16 July 2008; Revised: 23 October 2008; Accepted: 14 December 2008

ANTIQUITY 83 (2009): 430–444

the first time, to link past animals and plants in time and space, even in the absence of macrofossil evidence (Willerslev & Cooper 2005). Likewise, DNA obtained directly from 450-800 000 year-old silty ice, stored at the base of the Greenland ice sheet, has revealed the youngest evidence of conifer forest in Greenland (Willerslev *et al.* 2007) and DNA from ancient faeces was recently used to push back the time of the first peopling of North America by more than 1000 years (Gilbert *et al.* 2008). Thus, the 'dirt' DNA approach has proved highly significant in improving our understanding of general evolutionary processes.

Although it has been shown that DNA in sediments can account for up to 10 per cent of extractable phases (Trevors 1996; Turner & Newman 2005), it remains partly unclear how DNA from plants and animals may end up in sediments in sufficient quantities for them to be detected by standard molecular techniques, thousands of years after deposition (Willerslev *et al.* 2004b). Theoretical considerations, coupled with studies on DNA bound to modern soil, points to sloughed off root-cap cells being the major source of plant DNA in sediments (del Pozo & Lam 1998; Willerslev *et al.* 2003). Additional sources may be pollen (mainly nuclear DNA) and leaf-litter. Furthermore, the action of microbial enzymes and pathogens can facilitate the release of DNA into the rhizosphere (Meier & Wackemagel 2003; Poté *et al.* 2005). Experimental evidence suggests that faeces, skin flakes and chitinous material such as hair, feathers and nails are major sources of DNA from vertebrates (Lydolph *et al.* 2005). In all cases the tissue may have disintegrated, releasing DNA to the surroundings, to the extent that no visible macrofossil traces are left behind. Interestingly, for both plant and animals, it has been shown that the 'dirt' DNA (mtDNA and cpDNA) is of regional origin and that long-distance dispersed genetic material seems to be insignificant (Haile *et al.* 2007; Willerslev *et al.* 2007).

Intriguingly, most ancient specimens contain only minor, if any, amplifiable endogenous DNA that additionally is highly degraded (Gilbert *et al.* 2005; Willerslev & Cooper 2005; Binladen *et al.* 2006). This holds for all ancient DNA sources including that of 'dirt'. Therefore, upper time limits exist on DNA survival that is highly dependent on the exact conditions of preservation (Willerslev *et al.* 2004b). This, coupled with the enormous amplification power of the Polymerase Chain Reaction (PCR) used to retrieve ancient DNA sequences, creates a huge risk of obtaining false positive results due to contamination with contemporary DNA (Hebsgaard *et al.* 2005). Thus, the authentication of ancient DNA results demands a heavy burden of proof (Willerslev & Cooper 2005).

An additional problem faced in 'dirt' DNA studies is the risk of DNA leaching between strata obscuring the temporal context of the data. Although there is strong evidence suggesting that free DNA, in sediments surviving degradation and metabolism by bacterial and fungal exonucleases, will quickly bind to clay, sand, humic substances and organomineral complexes in the sediment (Crecchio & Stotzky 1998), DNA leaching has proved a problem under certain settings. Data have shown that leaching is of less concern under frozen and dry conditions, but can be a problem under warmer and wetter settings (Hansen *et al.* 2006; Haile *et al.* 2007; Willerslev *et al.* 2007; Gilbert *et al.* 2008).

In this study we, for the first time, systematically apply aDNA techniques to anthropogenic sediment layers in order to investigate the potential of this method in archaeology. The site investigated is known as, 'The Farm Beneath the Sand' (GUS) and is located in south-west Greenland on a plain surrounded by low mountains c. 80km east of Nuuk (Figure 1). GUS



Figure 1. The GUS site approximately 80km east of Nuuk, Greenland. The building remains appear in the red circle (photograph by Jette Arneborg 1993).

was occupied by Norse settlers *c.* AD 1000-1400. The building remains were found in 1990 covered by *c.* 1.5m-thick layers of sand and gravel (Andreasen & Arneborg 1992a & b; Schweger 1998: 16-17). Today this area appears as a sandy desert intersected by meandering watercourses draining the icecap. At the time of Norse settlement the scenery must have been quite different, offering grass for fodder production as well as easy access to clean water. The sediment core investigated for DNA was taken beyond the archaeological site, in what were then open fields connected to the farm.

According to AMS dates the GUS farm was established in the first decades of the eleventh century AD (Arneborg *et al.* 1998: 27), and abandoned around 1400. Eight structural phases have been defined. Phases with an absence of synanthropic insects (associated with man or with human dwellings) indicate that the farm was not populated continuously. Still, even in the periods without human occupants, the fossil insect fauna indicates that domesticates from the nearby farms grazed the fields around GUS (Panagiotakopulu *et al.* 2006). The sediment core investigated in this study is from the GUS field (covering the time from establishment to the time the farm was abandoned) and contains no significant macrofossil remains.

The overall goal of the study was twofold: 1) to investigate to what extent DNA is preserved in this kind of anthropogenic sediments from an open field; and 2) to explore

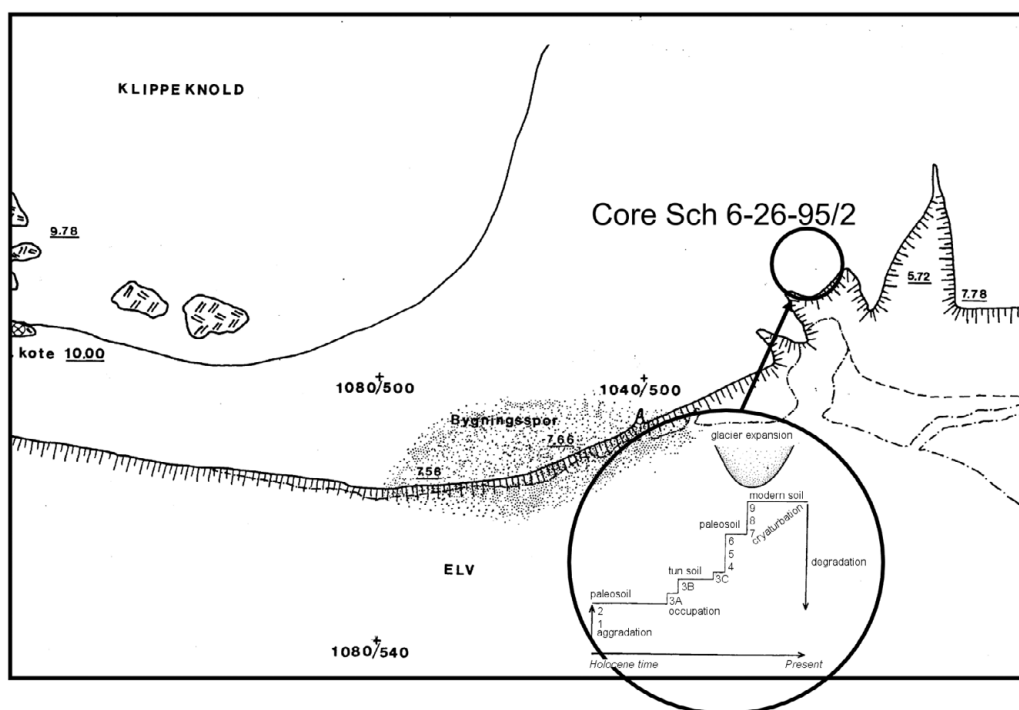


Figure 2. Building remains were located in the dotted area, and the core was taken from the area of the smaller circle. Within the core, stratum 2 represents the landnam surface and stratum 3 is mostly of anthropogenic origin. The strata above strata 3 represent the periodic flooding and sedimentation that took place after the depopulation of the GUS farm (Schweger 1998). Survey by Arneborg, Kapel and Nyegaard 1993. The generalised stratigraphic relationships in the large circle were published by Schweger in Arneborg & Gulløv (ed.) 1998: 14.

if such 'dirt' DNA can be used in a quantitative assay to estimate the relative change of livestock through time and comparing the DNA results to the well-described bone record from the GUS site.

Materials and method

Sample acquisition

Charles Schweger, University of Alberta, collected the sample in 1995. The core Sch 6-26-95-2 (Schweger's 6 June 1995 core) was located 51.1m upstream from the datum point established at the archaeological site proper (Figure 2). A small trench behind the exposed face of the steep slope was excavated down close to the black cultural layers to let the permafrost melt and then a pipe was driven down into the sediment 50-200mm behind the exposed face in order to recover uncontaminated material. The pipes were then excavated and removed, the open ends were capped, the pipes labelled and then stored in a cool place. In Nuuk, arrangements were made to ship the cores to Edmonton and University of Alberta. When they arrived 4-6 weeks later they were immediately placed in the freezer. The pipe remained sealed and was sent to the ancient DNA laboratory in Copenhagen on dry ice on 10 February 2004 where it was immediately frozen at -40°C . In Copenhagen the

0.35-0.40m-long core was divided into two. One part was left in the freezer while the other was spiked with recognisable bacterial vector DNA (pCR4-TOPO, Stratagene) on the surface to detect contamination occurring in the sampling and handling process (Willerslev *et al.* 2003, 2004b; Hansen *et al.* 2006). Seven minor cores of 50mm in diameter were drilled from the surface. The outermost 10mm was removed with a sterilised microtome knife to remove material likely to be contaminated. The rest of the samples for DNA processing were examined for the bacterial vector to reveal whether DNA from the outside had penetrated into the sample.

Sample description

The core included the stratigraphic sequences as seen in Figure 2 (and Schweger 1998: 15). On top, a grey sandy layer of medium-coarse sand supposed to have been deposited on top of the anthropogenic soils *after* the Norse farm had been abandoned. Below that, dark sediment of anthropogenic origin is supposed to represent the settlement period from *c.* AD 1000 to *c.* AD 1400 according to AMS-dates from the farm building itself (Arneborg *et al.* 1998; Arneborg 2004: 240). For AMS dating two samples (AAR-10814 and AAR-10704) were taken from the sandy layer on top of the dark organic anthropogenic layers respectively 25mm and 60mm below the surface of the core. Another five samples (AAR-10811, AAR-10812, AAR-10813, AAR-10705 and AAR-10810) are from the anthropogenic soil 100mm, 140mm, 180mm, 225mm and 260mm below surface (Table 1).

Dating

The seven samples were homogenised individually and approximately 2g per sample were sent to dating. Bulk carbon were dated using a fraction of the soil prepared for DNA analyses at Aarhus AMS Dating Centre using a tandem accelerator counting the individual ^{14}C atoms.

DNA extraction and amplification

Pre-PCR work was carried out in dedicated ancient DNA facilities using strict protocols (Hebsgaard *et al.* 2005; Willerslev & Cooper 2005). Primary analyses were performed in the Ancient DNA Laboratory at the Centre for Ancient Genetics, University of Copenhagen, Denmark. Independent replication was completed in the Ancient DNA Research Laboratory, Murdoch University, Australia. DNA was extracted and purified from 0.25g (wet weight) of sediment by using established protocols (Bulat *et al.* 2000; Willerslev *et al.* 2003, 2004a). General 16S mammal primers (Mam1 and Mam2) and a number of more specific ones were used to amplify DNA (Table 2). PCR amplifications were performed in 25 μl reaction volumes with 9 μl of GATC mix (20mM/0.25 μl of dNTPs + ddH₂O), 2.5 μl of each primer, 2.5 μl of MgSO₄, 0.2 μl High Fidelity (HiFi) enzyme (Invitrogen, Carlsbad, CA) with 2.5 μl of HiFi buffer, in addition to 4 μl of BSA. PCR conditions were: 2 min at 92°C initial; 50 cycles (45 sec at 94°C, 45 sec at 55°C, 45 sec at 72°C); and 10 min 68°C final.

Table 1. AMS dates for the samples.

AAR-#	Sample type	Collection site	mm below surface	^{14}C Age (BP)	Calibrated age (1 & 2 sigma ranges)	$\delta^{13}\text{C}$ (‰) VPDB
AAR-10814	Bulk soil	Greenland SCH6.26.95.2	25	398 ± 47	68.2% probability AD 1440(53.9%) AD 1520 AD 1590 (14.3%) AD 1620 95.4% probability AD 1430 (60.8%) AD 1530 AD 1540 (34.6%) AD 1640 (IntCal04)	−25.23
AAR-10704	Bulk soil	Greenland SCH6.26.95.2	60	376 ± 32	68.2% probability AD 1450 (51.2%) AD 1520 AD 1590 (17.0%) AD 1620 95.4% probability AD 1440 (57.6%) AD 1530 AD 1550 (37.8%) AD 1640 (IntCal04)	−25.49
AAR-10811	Bulk soil	Greenland SCH6.26.95.2	100	598 ± 39	68.2% probability AD 1305 (53.6%) AD 1365 AD 1385 (14.6%) AD 1405 95.4% probability AD 1290 (95.4%) AD 1420 (IntCal04)	−26.85
AAR-10812	Bulk soil	Greenland SCH6.26.95.2	140	567 ± 41	68.2% probability AD 1310 (39.7%) AD 1360 AD 1380 (28.5%) AD 1420 95.4% probability AD 1290 (95.4%) AD 1440 (IntCal04)	−27.62
AAR-10813	Bulk soil	Greenland SCH6.26.95.2	185	770 ± 55	68.2% probability AD 1215 (68.2%) AD 1285 95.4% probability AD 1150 (93.3%) AD 1310 AD 1360 (2.1%) AD 1390 (IntCal04)	−27.11
AAR-10705	Bulk soil	Greenland SCH6.26.95.2	225	792 ± 30	68.2% probability AD 1222 (68.2%) AD 1262 95.4% probability AD 1185 (2.4%) 1200 AD 1505 (93.0%) AD 1280 (IntCal04)	−26.50
AAR-10810	Bulk soil	Greenland SCH6.26.95.2	260	903 ± 36	68.2% probability AD 1040 (34.6%) AD 1100 AD 1110 (33.6%) AD 1180 95.4% probability AD 1030 (95.4%) AD 1220 (IntCal04)	−26.75

Table 2. PCR Primer used in this study.

Name	Organism	Sequence
Mam 1	Diverse mammals	CGGTTGGGGTGACCTCGGA
Mam 2	Diverse mammals	GCTGTTATCCCTAGGGTAACT
Mam 1b	Diverse mammals	TGGGGTGACCTCGGAGAA
EQ1	Horses	CGTGCATTAAATTTGTTTGCC
EQ2	Horses	CATGGGAGGTGATATGCGTG
Pig 1	Pig	CTTTAAAAACAAAAAACCCATAAAAA
Pig 2	Pig	TTAATGCACGACGTACATAGG
MitMH-732 F	Dog, Fox, Seal	ATGGTTTCTCGAGGCATGGTG
MitMH-878 R	Dog, Fox, Seal	GCCCCATGCATATAAGCATGTAC
L0624	Reindeer	ATCTCACCTAAAATCGCCCCACT
H0682	Reindeer	CCAAATGTATGACACCACAGTTATG
Phoca 975F	Seal	CTGCGTCGAGACCTTTACGG
Phoca 1071R	Seal	GGACTAATGACTAATCAGCC
12So	Diverse mammals	GTCGATTATAGGACAGGTTCTCTA
12Sa	Diverse mammals	CTGGGATTAGATACCCCACTAT

Cloning and sequencing

The PCR products were cloned and sequenced. One to two amplicons per sample were pooled, cloned (Topo TA cloning, invitrogen), purified and sequenced in one direction. The resulting sequences were aligned and investigated for possible recombination as suggested in Willerslev *et al.* (1999).

Sequence identification

DNA sequences were assigned to a taxon using a statistical Bayesian approach (Munch *et al.* 2008). In brief, this method calculates with what probability each sequence belong to a particular clade by considering its position in a phylogenetic tree based on GenBank sequences. In the calculation of these probabilities, uncertainties regarding phylogeny, models of evolution and missing data are taken into account.

Real-time PCR

By using quantitative real-time PCR assay, designed to target a homologous and comparably sized mtDNA fragment of the 16S gene (129bp in each taxa), we directly investigated the amount of comparable fragments of sheep, cow and goat mtDNA in the soil extracts, The qPCR assay was designed to allow simultaneous amplification of mtDNA from all three species for each conserved mammalian primer set (see Figure 3). In the qPCR reaction, 5' labelled MGB probes (Applied Biosystems, Foster City, California), were used, designed to bind specifically to, and differentiate between, DNA from the three species (Table 3). Prior to our assay, the specificity of each probe, under the applied qPCR conditions, was verified using control DNA extracts from each taxa. Each qPCR reaction was performed in triplicate, using a 3-fold dilution series of the template material, with each reaction-series containing only a single probe at a time. To accurately compare and quantify the DNA levels,

Table 3. Probes used for Real-Time PCR.

Name	Sequence
Sheep probe	6FAM-CTCTGAGCGATTTTATAGAG
Cattle probe	6FAM-TCCTCCGAGCGATTTT
Goat probe	VIC-CAAGAGATCTTCCGAGCGA
Sheep standard	TGGGGTGACCTCGGAGAAACAGAAAATCCTCTG AGCGATTTTATAGACTAGACTAACAAGTCAAAC CAAACCAACGGAGTTACCCTAGGGATAACAGC
Cattle standard	TGGGGTGACCTCGGAGAAACAAGAGATCTTCCG AGCGATTTTAAAGACTAGACTTACAAGTCAAAT CAAATTAACGGAGTTACCCTAGGGATAACAGC
Goat standard	TGGGGTGACCTCGGAGAAACAAGAGATCTTCCG AGCGATTTTAAAGACTAGACTTACAAGTCAAATC AAATTAACGGAGTTACCCTAGGGATAACAGC

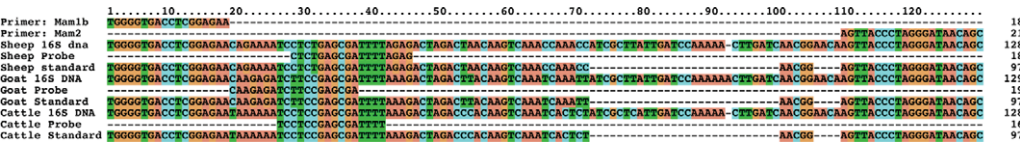


Figure 3. Alignment of the 129bp mtDNA 16s sheep, goat and cow fragment targeted by the qPCR assay, aligned to the species specific probes and 32bp-deletion containing template standards.

a triplicate 10-fold dilution series (10^2 - 10^7 molecules) of artificially synthesised standard DNA molecules was used. Standards were ordered as oligonucleotides designed to mimic the target sequence, and were synthesised by Biomers.net (Ulm, Germany). To prevent contaminating the samples with the concentrated standard molecules, the latter were kept at all times in a separate laboratory from the ancient DNA and qPCR set up. As a further control, the standard was designed to contain a 32bp deletion that rendered it easy to differentiate from the true target using conventional gel electrophoresis. To summarise, a triplicate dilution series of each extract and species was compared to a triplicate 10-fold dilution series of standard.

qPCR reactions were performed in 25 μ l volumes, using TaqMan[®] Universal PCR Master Mix (Applied Biosystems), and containing 1 μ l template or standard DNA, 400 pmoles of each primer, and 300pmoles probe. qPCR was undertaken for 50 cycles with an annealing temperature of 60°C on a Stratagene Mx 3000 qPCR platform. Starting template copies were calculated automatically using Stratagene software, followed by a manual comparison to ensure that the data was not compromised by the presence of PCR inhibition in the reactions. The final template numbers were calculated as the average of the triplicate assay, reflecting the respective number of the 129bp target molecule in the extracts for the 3 species.

Results

Dating

Calibrated AMS results spanned 500 years ranging from AD 1030 to 1530 (Table 1).

The AMS-dates from 25mm and 60mm below the surface of our core confirmed that the bedded sand was deposited after the Norse occupants abandoned GUS in the late fourteenth century, and the layers may correspond with Schweger's units 4 or 5. According to Schweger (1998: 16) the units represent the expansion of the upper valley glacier that resulted in floodplain aggradations and eventual burial of the site (Figure 2).

The other samples at 100, 140, 185, 225 and 260mm all represent the period of occupation in agreement with the archaeological interpretation (Table 1). The layers are identical to Schweger's unit 3, but Schweger subdivided it into three subunits from bottom to top: subunit 3A consists of humidified allochthonous peat formed from organic rich cultural and agricultural debris. This unit (3A) was only found in the close vicinity of the farm buildings and may not be present in the core. Subunit 3B, consists of peat with both allochthonous and autochthonous (fibrous roots and stems) components and unit 3C, being a thin, *c.* 20-40mm-thick autochthonous peat layer with fibrous roots, well-preserved bryophytes and stems – with no cultural debris (Schweger 1998: 16 and Table 1). Unit 2 is considered the *landnam* layer, i.e. the surface the Norse occupants first settled on (Figure 2). Most probably our samples at 100, 140, 185, 225 and 260mm are identical to Schweger's subunit 3B and represent the period from the middle of the eleventh century to the abandonment of the farm in the late fourteenth century.

Qualitative DNA analysis/taxonomic diversity

The interior part of the sediment samples yielded no DNA from the bacterial vector suggesting that contamination had not penetrated far into the core. However, DNA from humans, cattle, sheep, goat and mouse were obtained from the samples. One query sequence (from a sample 225mm below the surface, which corresponds to AD 1243 ± 38) was found 100 per cent identical to reindeer sequences in Genbank and identified to the subfamily Odocoileinae with 97 per cent likelihood. Within this group only reindeer exists in Greenland. Figure 4 shows the percentage of 294 clones identified using the statistical Bayesian approach (Munch *et al.* 2008). Assignments of clones to the groups; cattle, mouse, sheep and human are generally supported by posterior probabilities above 80 per cent. Most clones assigned to goat are done with posterior probabilities of only 50-60 per cent. However, for each sample in the Norse period settlement, except the oldest (AAR-10810), at least one clone identifies the group with a probability above 80 per cent.

Except for the two youngest samples, the most abundant sequences belong to cattle, sheep, goat and human. The samples also contain mouse and reindeer DNA. The source of the human sequences can be endogenous DNA, but as we only find human sequences in the youngest layers (1450-1520, 1440-1520), representing post-human occupation of the site, it is most likely a result of contamination with modern human DNA (a common problem in ancient DNA studies). To summarise, the results show that cattle, sheep and goat are present throughout the Norse settlement (AD 1040-1365).

Quantitative DNA analysis

Quantification shows approximately 16 times more DNA from cattle than from sheep. Goat DNA was undetectable using Quantitative PCR. The amount of cattle DNA fluctuates but

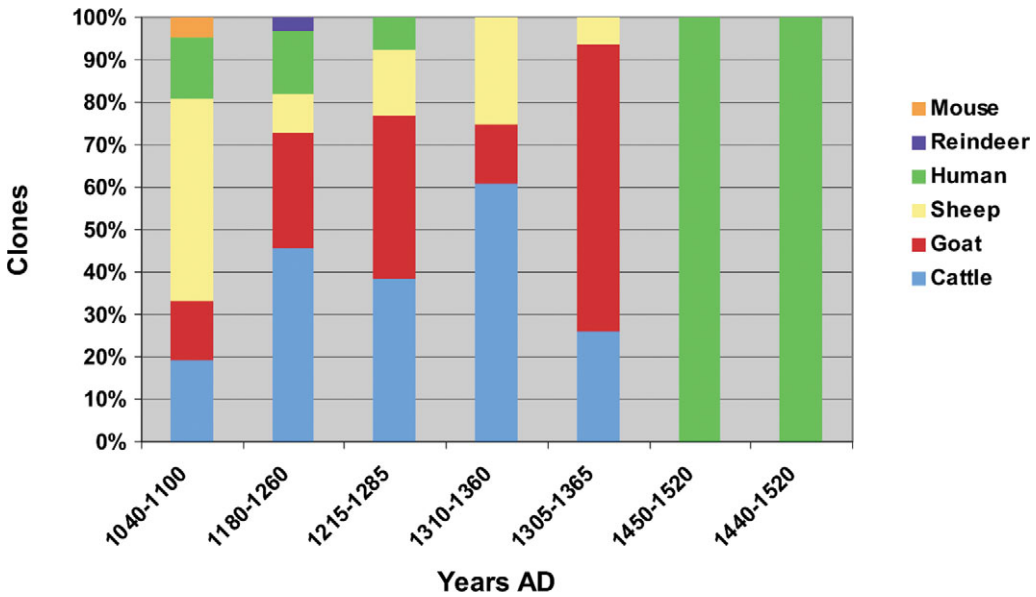


Figure 4. Relative abundance of clones identified with more than 50 per cent posterior probability to a taxonomic group.

shows an overall decline over time towards the Norse abandon of the site while sheep DNA content remains more stable with relatively low template copy numbers, probably reflecting background variation (Figure 5).

Replication

The Ancient DNA Research Laboratory at Murdoch University, Australia, independently confirmed the presence of ancient DNA in the samples, using generic mammal primers designed to target the 12S gene (151bp). The results showed that the 12S gene could be amplified for cattle, sheep and goat in the two samples dated to AD 1215-1285 and AD 1305-1365.

Discussion

The aim of this study was to test the usefulness of aDNA from sediments (ancient ‘dirt’ DNA) in an archaeological context, using the Greenland site, ‘The Farm Beneath the Sand’, (GUS) as a case study. The site was known to have been occupied by Norse from *c.* AD 1000-1400. Specifically, we aimed to investigate to what extent DNA is preserved in sediments of a core taken from the ancient fields around the GUS farm. We wanted to explore if such ‘dirt’ DNA can be used in a quantitative assay to estimate the relative change in livestock through time by comparing the results to the well-described bone record excavated from the GUS farm. Importantly, in comparison to previous ‘dirt’ DNA publications the samples differ in several ways: 1) the deposits are anthropogenic in origin; 2) the sediments have remained wet and unfrozen during prolonged periods of time; and 3) the core was taken at what was then an unprotected open field. This combination has not previously been investigated.

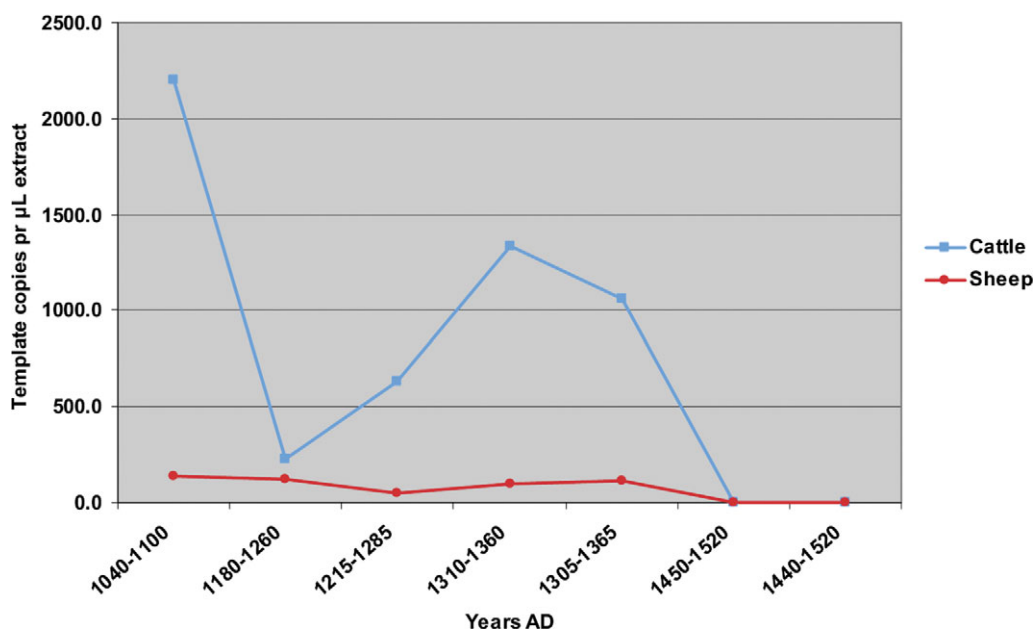


Figure 5. The amount of cattle DNA fluctuates through time with the overall trend being a decline from of 2200 template copies per µL extract to 0µL. Sheep DNA remain relative stable over time starting with 136 template copies per µL extract.

Although a previous study of ancient 'dirt' DNA from wet settings in New Zealand revealed downward molecular movement of sheep DNA between stratigraphic layers (Haile *et al.* 2007), there are several lines of evidence suggesting that DNA leaching is a minor factor at the GUS site. First, the overall relationship between increasing concentration and sediment depth of cattle DNA is directly opposite of what was previously found for downward DNA movement – where concentration decreases with depth (Haile *et al.* 2007). This interpretation is supported by a fairly constant concentration of sheep DNA across strata, a pattern previously associated by 'dirt' DNA stability (Figure 5). Secondly, although an upward movement of DNA could possibly account for the cattle result, this can be discounted by the fact that no DNA from livestock is recovered in the uppermost layers, after the area was abandoned by the Norse (Figure 5). Finally, the DNA results are in overall agreement with the livestock fossil record (see below) including the decrease in cattle DNA AD 1180-1260, which most likely reflects the actual history of the site; in this period the farm was abandoned, but still occasionally used by adjacent farms for grazing of livestock (Panagiotakopulu *et al.* 2006). Thus, the evidence affirms that the DNA preserved in the sediments of the GUS site has remained in place after deposition. This is important, because it suggests that DNA leaching is not necessarily a general feature of wet conditions, even if the sampling site is open and exposed, a conclusion supported by 'dirt' DNA results from lake cores (Rasmussen *et al.* n.d.). In fact it is possible that the previous evidence of DNA leaching results from the extremely high quantity of sheep at the New Zealand site investigated, resulting in DNA 'oversaturation' of the sediments.

The DNA from cattle, goat and sheep shows variable relative abundance over time (Figure 4) with an overall decline of cattle towards the time the site was abandoned

(Figure 5). Excitingly, the result is in agreement with the bone record, suggesting that the number of cattle at the farm decreased during the investigated time period relative to wild animals, (primarily seal) (Enghoff 2003: 89-96). Thus, from about AD 1000-1150, seal bones constitute 28 per cent of all the animal bones found in the farm buildings and 41 per cent from c. AD 1300-1400. In the same period cattle decreases from 15 per cent to 6 per cent whereas caprine (sheep and goat) increases from 27 per cent to 33-35 per cent (Enghoff 2003: 89-90). Interestingly, the presence of reindeer DNA speak in favour of open grass fields close to the farmhouse, with reindeer grazing in late summer/early autumn. According to the animal bone record, sheep and goats made up the majority compared to cattle (Enghoff 2003: 87). The opposite pattern in DNA distribution may be explained by the hypothesis that cattle for milking, and perhaps a few milking sheep, were kept close to the farmhouse, whereas the majority of the sheep – kept primarily for the wool – grazed the distant fields. It may, however, also be explained by the fact that only the cattle, and very few sheep and goats, were stabled during the winter – producing less sheep and goat manure to be spread in the grass fields.

Overall, the conformity between the cattle DNA and fossil data is significant, given that the samples of animal bones came from excavations inside the farm building (Enghoff 2003), while the core samples in the present study are from the anthropogenic soil accumulated around the site. Domestic animals living at the farm and the surroundings are fossilised just like the seals, as dead animal parts. However, the amount and distribution of DNA is very different. For example, cow and sheep living at and around the farm leave traces of DNA through their dropping and urine all year round, while what the seal cadavers leave behind only contributes to the ancient DNA pool what is not eaten or otherwise utilised. So, in principle, the archaeological excavations and the DNA samples from the anthropogenic soil represent two independent sources of evidence, telling different parts of the same story and although the two approaches sample the site very differently, the independent results support each other. Importantly, however, several aspects can influence the quantitative DNA analysis. The specificity of the probes used in the study could be different and hence affect the reactions. Though an effort was made to eliminate the effects of inhibition in the PCR reactions, it could still play a role.

The interpretation that the 'dirt' DNA comes from faeces and urine, rather than cadavers and bones of dead animals, is supported by the fact that we were not able to identify any seal DNA in the anthropogenic soil, and except for reindeer and mouse, we recorded no DNA from wild animals at all. It is likely that urine and faeces was deposited in a byre and then removed during 'mucking out', or directly from animals near the farm (compared with Schweger's allochthonous components in subunit 3B). Seals would have arrived at the site as dead carcasses or butchered lumps of meat only to be cooked and consumed by occupants of the farm, whereas reindeer could have utilised fields around GUS, especially if the farm had been vacated for any period of time. According to traditional Norse field management (e.g. Bruun 1928), domestic animals were byred through the winter and fed on fodder collected from pastures adjacent to the farm. With spring, byred animals were released. Animals for milking were kept close to the farm, while the rest were moved to more distant outfields or possibly even to saetters/shielings, which were distant summer farms. Not to jeopardise the fodder yield, domestic animals would only have been kept on

the pastures after the grass was cut for winter fodder in late summer. The GUS byre and sheep/goat stables were 'mucked out' annually and the waste deposited on the grass fields to ensure their continued high fertility (Schweger 1998). The anthropogenic soil at GUS was formed from this annual increment of manure, and while we know it was spread over at least 450m, we have no idea how far it may have extended into the old floodplain (Schweger 1998: 16). Byre/stable manure added to the grass fields would have deposited DNA of the domestic livestock directly on to the field.

Conclusion and implications

Previous studies on ancient 'dirt' DNA have focused on 'natural' permafrost settings (Willerslev *et al.* 2003; Lydolph *et al.* 2005; Hansen *et al.* 2006) or wet or dry cave/rock shelter sites (Hofreiter *et al.* 2003; Haile *et al.* 2007). Our results show that ancient 'dirt' DNA can be preserved in anthropogenic sediments at least on a historical timescale, even if the site has remained open, unprotected and non-frozen for extensive periods. Importantly, the data also suggests that the effects of DNA leaching are not prohibitive for all wet and unfrozen settings. In an archaeological context, ancient 'dirt' DNA proved highly informative and our research has refined a series of previous interpretations of the Norse life by adding information not revealed to the naked eye. Our DNA identifications have gone further than previous biomolecule (lipid based) analyses, which separated herbivorous from omnivorous and made distinctions within the omnivorous class, but was not able to make distinctions within the herbivorous class (e.g. Bull *et al.* 1999). In the future, ancient 'dirt' DNA has the capacity to be used more vigorously to investigate the diet of past cultures and possibly the genetic composition of the inhabitants, even in cases where no human and animal remains are preserved. However, we do advocate for more comparative investigations, in which bone records and DNA results are compared, before one can be confident in archaeological interpretations on ancient 'dirt' DNA results. Given the benefits of the molecular data in understanding 'The Farm Beneath the Sand' we urge archaeologists to consider sterile sampling of sediments in and around archaeological sites for ancient DNA profiling.

Acknowledgements

MBH, MTP and EW were financially supported by the National Danish Research Council. MB acknowledges the support of the Australian Research council (DP0771971). The authors would like to thank Cornelis Grimmelikhuijzen for the use of the qPCR machine. The archaeological investigations at 'The Farm Beneath the Sand' were carried out by the Greenland Nationalmuseum & Archives in Nuuk.

References

- ANDREASEN, C. & J. ARNEBORG. 1992a. Gården under Sandet. Undersøgelserne 1991. *Tusaat/Forskning i Grønland* 1/92: 10-18.
- 1992b. Gården under Sandet: Nye nordboarkæologiske undersøgelser i Vesterbygden 1991. *Grønlandsk Kultur og Samfundsforskning* 92: 11-49.
- ARNEBORG, J. 2004. Det europæiske landnam. Nordboerne i Grønland, in H.C. Gulløv (ed.) *Grønlands forhistorie*: 220-78. Copenhagen: Gyldendal.
- ARNEBORG, J., J. HEINEMEIER, N. RUD & A. SVEINBJÖRNSDÓTTIR. 1998. AMS dates from the hall (XVII), in J. Arneborg & H.C. Gulløv (ed.) *Man, culture and environment in ancient Greenland*: 27-30. Copenhagen: Danish National Museum & Danish Polar Center.

- BINLADEN J., C. WIUF, M.T.P. GILBERT, M. BUNCE, G. LARSON, R. BARNET, A.J. HANSEN & E. WILLERSLEV. 2006. Assessing the fidelity of ancient DNA sequences amplified from nuclear genes. *Genetics* 172: 733-44.
- BRUUN, D. 1928. *Fortidsminder og nutidshjem paa Island*. Copenhagen: Gyldendal.
- BULAT, S.A., M. LÜBECK, I.A. ALEKHINA, D.F. JENSEN, I.M.B. KNUDSEN & P.S. LÜBECK. 2000. Identification of an UP-PCR derived SCAR marker for an antagonistic strain of *Clonostachys rosea* and development of a strain-specific PCR detection assay. *Applied and Environmental Microbiology* 66: 4758-63.
- BULL, I.D., I.A. SIMPSON, P.F. VAN BERGEN & R.P. EVERSLED. 1999. Muck n'molecules: organic geochemical methods for detecting ancient manuring. *Antiquity* 73: 86-96.
- CRECCHIO, C. & G. STOTZKY. 1998. Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subsp. *kustuki* bound to humic acids from soil. *Soil Biology & Biochemistry* 30: 463-70.
- DEL POZO, O. & E. LAM. 1998. Caspases and programmed cell death in the hypersensitive response of plants to pathogens. *Current Biology* 8: 1129-32.
- ENGHOFF, I.B. 2003. *Hunting, fishing and animal husbandry at 'The Farm Beneath the Sand', western Greenland: an archaeozoological analysis of a Norse farm in the Western Settlement* (Meddelelser om Grønland, Man & Society 28). Copenhagen: Danish Polar Center.
- GILBERT, M.T.P., L. RUDBECK, E. WILLERSLEV, A.J. HANSEN, C. SMITH, K.E.H. PENKMAN, K. PRANGENBERG, C.M. NIELSEN-MARSH, M.E. JANS, P. ARTHUR, N. LYNNERUP, G. TURNER-WALKER, M. BIDDLE, B. KJØLBYE-BIDDLE & M. COLLINS. 2005. Biochemical and physical correlates of DNA contamination in archaeological human bones and teeth excavated at Matera, Italy. *Journal of Archaeological Science* 32: 785-93.
- GILBERT, M.T.P., D.L. JENKINS, A. GOTHERSTROM, N. NAVERAN, J.J. SANCHEZ, M. HOFREITER, P.F. THOMSEN, J. BINLADEN, T.F.G. HIGHAM, R.M. YOHE, R. PARR, L.S. CUMMINGS & E. WILLERSLEV. 2008. DNA from pre-Clovis human coprolites in Oregon, North America. *Science* 320: 786-9.
- HAILE, J., R. HOLDAWAY, K. OLIVER, M. BUNCE, M.T.P. GILBERT, R. NIELSEN, K. MUNCH, S.Y.W. HO & E. WILLERSLEV. 2007. Ancient DNA chronology within sediment deposits: are paleobiological reconstructions possible and is DNA leaching a factor? *Molecular Biology Evolution* 24: 982-9.
- HANSEN, A.J., D.L. MITCHEL, R. RØNN, C. WIUF, L. PANIKER, J. BINLADEN, T.B. BRAND, D.A. GILICHINSKY & E. WILLERSLEV. 2006. Crosslinks rather than strand breaks determine access to ancient DNA sequences from frozen sediments. *Genetics* 2: 1175-9.
- HEBSGAARD, M.B., M.J. PHILLIPS & E. WILLERSLEV. 2005. Geologically ancient DNA: fact or artefact? *Trends in Microbiology* 13: 212-20.
- HOFREITER, M., J.I. MEAD, P. MARTIN & H.N. POINAR. 2003. Molecular caving. *Current Biology* 13: R693-5.
- JOHNSON, S.S., M.B. HEBSGAARD, T. CHRISTENSEN, M. MASTEPANOV, R. NIELSEN, K. MUNCH, T.B. BRAND, M.T.P. GILBERT, M.T. ZUBER, M. BUNCE, R. RØNN, D. GILICHINSKY, D. FROESE & E. WILLERSLEV. 2007. Ancient bacteria show evidence of DNA repair. *Proceedings of the National Academy of Sciences USA* 104: 14401-5.
- LYDOLPH, M.C., J. JACOBSEN, P. ARCTANDER, M.T.P. GILBERT, D.A. GILICHINSKY, A.J. HANSEN, E. WILLERSLEV & L. LANGE. 2005. Beringian plaeoecology inferred from permafrost preserved fungi DNA. *Applied Environmental Microbiology* 71: 1012-17.
- MEIER, P. & W. WACKEMAGEL. 2003. Monitoring the spread of recombinant DNA from field plots with transgenic sugar beet plants by PCR and natural transformation of *Pseudomonas stutzeri*. *Transgenic research* 12: 293-304.
- MUNCH, K., W. BOOMSMA, J.P. HUELSENBECK, E. WILLERSLEV & R. NIELSEN. 2008. Statistical assignment of DNA sequences using Bayesian phylogenetics. *Systematic Biology* 57: 750-7.
- PANAGIOTAKOPULU, E., P. SKIDMORE & P. BUCKLAND. 2006. Fossil insect evidence for the end of the Western Settlement in Norse Greenland. *Naturwissenschaften* 94(4): 300-06.
- POTÉ, J., P. ROSSÉ, W. ROSSELLI, V. VAN TRAN & W. WILDI. 2005. Kinetics of mass and DNA decomposition in tomato leaves. *Chemosphere* 61: 677-84.
- SCHWEGER, C.E. 1998. Geoarchaeology of the GUS site: a preliminary framework, in J. Arneborg & H.C. Gulløv (ed.) *Man, culture and environment in ancient Greenland*: 14-18. Copenhagen: Danish National Museum & Danish Polar Center.
- TREVORS, J.T. 1996. Bacterial biodiversity in soil with an emphasis on chemically contaminated soils. *Water, Air and Soil Pollution* 34: 125-30.
- TURNER, B.L. & S. NEWMAN. 2005. Soil phosphorus compounds in subtropical wetlands: the importance of phosphate diesters. *Journal of Environmental Quality* 34: 1921-9.

'The Farm Beneath the Sand' – an archaeological case study on ancient 'dirt' DNA

- WILLERSLEV, E. & A. COOPER. 2005. Ancient DNA. *Proceedings of the Royal Society of London, Series B, Biological Sciences* 272: 3-16.
- WILLERSLEV, E., A.J. HANSEN, B. CHRISTENSEN, J.P. STEFFENSEN & P. ARCTANDER. 1999. Diversity of Holocene life forms in fossil glacier ice. *Proceedings of the National Academy of Sciences USA* 96: 8017-21.
- WILLERSLEV, E., A.J. HANSEN, T.B. BRAND, J. BINLADEN, T.M.P. GILBERT, B. SHAPERIO, M. BUNCE, C. WIUF, D.A. GILICHINSKY & A. COOPER. 2003. Diverse plant and animal DNA from Holocene and Pleistocene sedimentary records. *Science* 300: 792-5.
- WILLERSLEV, E., A.J. HANSEN, T.B. BRAND, R. RØNN, I. BARNES, C. WIUF, D.A. GILICHINSKY, D. MITCHELL & A. COOPER. 2004a. Long-term persistence of bacterial DNA. *Current Biology* 14: R9-10.
- WILLERSLEV, E., A.J. HANSEN & H.N. POINAR. 2004b. Isolation of nucleic acids and cultures from fossil ice and permafrost. *Trends in Ecology and Evolution* 19: 141-7.
- WILLERSLEV, E., E. CAPPELLINI, W. BOOMSMA, R. NIELSEN, M.B. HEBSGAARD, T.B. BRAND, M. HOFREITER, M. BUNCE, H.N. POINAR, D. DAHL-JENSEN, S. JOHNSEN, J.P. STEFFENSEN, O. BENNIKE, S. FUNDER, J.-L. SCHWENNINGER, R. NATHAN, S. ARMITAGE, J. BARKER, M. SHARP, K.E.H. PENKMAN, J. HAILE, P. TABERLET, M.T.P. GILBERT, A. CASOLI, E. CAMPANI & M.J. COLLINS. 2007. Ancient biomolecules from deep ice cores reveal a forested southern Greenland. *Science* 317: 111-4.