

HAIR, BONE PLATES AND COPROLITES: ANCIENT DNA RESEARCH ON RARE ARCHAEOLOGICAL MATERIALS

ELENA A. NIKULINA

Abstract

The article describes the possibilities and limitations of ancient DNA research on organic materials that are usually not the focus of archaeogenetics, because of their low preservation potential and thus rareness in archaeological settings. These are remains from fish and birds, animal hairs, and coprolites. Several of the author's own key studies from different regions and time periods are presented.

Key words: ancient DNA, archaeozoology, animal hairs, fishbone, *Acipenser*, *Pelecanus*, coprolites, paleofaeces.

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Introduction

Although today many questions of ancient DNA (aDNA) analyses are palaeoecological, cultural and historical, the large majority of aDNA laboratories, however, are still departments of forensic science, anthropological, or molecular biological institutions. As they are organised as parts of natural science institutes, archaeological or archaeozoological approaches or research questions often have to be of secondary importance.

Against this background, the Centre for Baltic and Scandinavian Archaeology (ZBSA) at Schloss Gottorf in Schleswig (Schleswig-Holstein, Germany) established in 2009/2010 a specialised laboratory exclusively for archaeologically relevant genetic research on animal remains (Nikulina, Schmölcke 2010). From the beginning, it has been an essential part of our self-image to invite archaeologists and archaeozoologists from all of Scandinavia and the Baltic Sea region for cooperation and common projects. In fact, the aDNA-laboratory at the ZBSA is the first molecular-genetics institute that specifically deals with investigations of ancient animal DNA from archaeological contexts, and that officially aims to clarify archaeological and historical questions relevant to disciplines of the historical humanities. Consequently, our first studies dealt for instance with the history of domestic species (Trixl et al. 2013), as well as with hunted animals (Schmölcke, Nikulina 2015; Nikulina, Meadows 2013).

However, not only do we organise and conduct archaeogenetic projects like these, but also undertake analyses in different fields of basic research. This includes work with organic materials rarely found at archaeological excavations, such as animal fibres and

coprolites, or with subjects that are rarely the focus of archaeogenetic research, such as remains from fish and birds. In this paper, the potential and possibilities, but also the limits, of these materials in aDNA research will be presented and illustrated with key studies from different regions and time periods.

Archaeogenetic analyses of animal hairs

The preservation of animal hairs or textiles needs optimal sediment conditions, in particular they must have been hermetically sealed since the time of their embedding. Preserved fibres provide insights into the fabrication of former clothes. Ancient animal hairs could also be of interest for archaeogenetics, for example if there is a question of species identification. However, there are generally several reasons why animal hairs are difficult to use for genetics. Due to their structure, even recent hairs contain no nuclear DNA, and only a relatively small amount of mitochondrial DNA. This limits the extraction and amplification of the DNA. Due to size and form, hairs are highly exposed to external influences, such as humidity, oxidation and temperature extremes. All these factors disintegrate the DNA molecules inside a hair. Moreover, hairs are soft, and consequently vulnerable to microorganisms. This is the reason why they are not normally preserved in archaeological find layers. All these parameter are also true for DNA in modern hairs, but they make the analysis of ancient DNA of hairs hundreds or thousands of years old extremely difficult. For conventional analyses of ancient DNA, about one gram of material is necessary to get enough fragments of molecules. But in most cases, the weight of archaeological hair samples is much

smaller. Most archaeological finds are heavily tainted by humic acids, which are strong inhibitors of PCR, the basis of all genetic research. Finally, it is difficult to clean the surface of hairs from external contamination by foreign DNA, since the use of generally accepted techniques such as bleaching or UV-light could easily destroy the hairs, or at least the native DNA.

By reason of the rareness of this archaeological find group, and the complexity of DNA extraction, archaeogenetic studies of animal hairs are uncommon. Together with the department of Functional Morphology and Biomechanics at the Zoological Institute of Kiel University (Germany), the ZBSA conducted an experimental study combining the microscopic and archaeogenetic identification of animal hairs. The finds derived from a dung layer excavated at Castle Lenzen, a Slavic settlement on the River Elbe in Central Europe dated to the tenth century AD. Our joint study had three main aims: to test the extraction method, to test the aDNA preservation, and at the end to identify the species. As a DNA marker, we used the mitochondrial control region, and finally it was possible to obtain a 156 bp long fragment of ancient cattle DNA from all three studied samples (Nikulina et al. 2015). Since it was not clear if the DNA fragments in fact originated from the hairs or rather from the circumfluent dung (Linseele et al. 2013), light and electron microscopy were suc-

cessfully used to verify the species identification from aDNA analyses. One conclusion from this study is that hairs from archaeological excavations can be a useful source of aDNA, but on account of the complexities mentioned, the above verification by additional methods such as microscopy seems to be necessary.

Another study about ancient animal fibres was recently conducted in cooperation with our colleagues H. Jöns and A. Siegmüller from the Lower Saxony Institute for Historical Coastal Research in Wilhelmshaven (Germany). The focus of this project were accumulations of Iron Age sheep's wool excavated at the North Sea marshland site Feddersen Wierde (Roman Period, northern Germany) (Fig. 1). Sheep played an important economic role for the people of the Feddersen Wierde, and therefore many bones and teeth from sheep were found during the excavations. These bones were already integrated into a comprehensive aDNA-study about the history of sheep husbandry running at the ZBSA (Nikulina 2014, 2015), and their aDNA should be compared with the DNA of the wool to detect similarities or differences that would show the effects of local wool production or textile import. It was possible to apply successfully the method routinely used in our laboratory for the study of ancient sheep remains, and hence we tried to amplify all four aDNA fragments of the mitochondrial control region utilised in the other

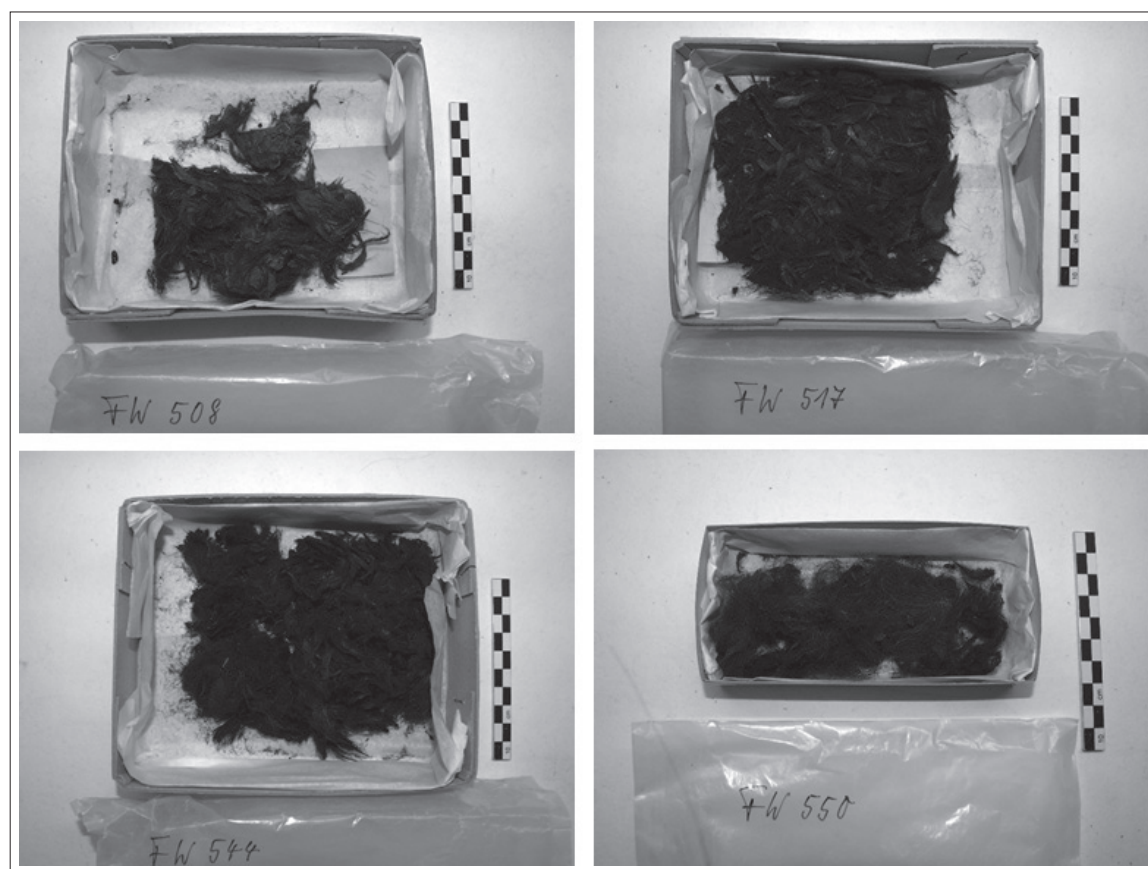


Fig. 1. Wool samples from Feddersen Wierde (northern Germany, Iron Age).

studies. However, the amplification success from four studied samples was relatively low, and the sequencing conducted at the Institute of Clinical Molecular Biology at Kiel University confirmed that the ancient DNA was extremely fragmented. Also, the reproducibility of the results was low. In the end, it became evident that all haplotypes obtained from the wool samples had already been obtained from sheep bones from Feddersen Wierde. Accordingly, wool and bones might originate from sheep of the same population, even if the same haplotypes are also known from other contemporaneous settlements and populations. However, several problems have to be taken into consideration. The biggest problem is the high heterogeneity of the samples, i.e. from each sample a mixture of haplotypes have been obtained. There are three explanations for this result: first, the wool in one wool accumulation originated from different sheep; second, the wool was contaminated during the life of the sheep by body contact with other sheep, with foreign dung etc; and third, the sampled wool was contaminated by foreign sheep DNA after the excavations during the curation process or conservation. Since in particular cases it cannot be ascertained which kind of contamination was responsible for the heterogeneity of the aDNA from hair samples, the results of this study have only a low significance. They confirmed our suspicion that in comparison with bones and teeth, it is very difficult to remove the surficial contamination of hairs. The routinely used UV-light and bleaching methods cannot be applied in hairs, because of the danger of destroying the target DNA.

Archaeogenetic analyses of coprolites

Faeces are generally a poor source of DNA, because digestive enzymes and intestinal flora activities destroy the DNA. However, due to the promising research questions connected with this material, the first attempts to analyse the ancient DNA of coprolites from archaeological sites go back to the onset of archaeogenetic research (Fricker et al. 1997; Poinar et al. 2001). Even if the methods have been optimised since those days, until now it remains difficult to extract the aDNA of the defecator. A major difficulty is the high risk of recent contamination of ancient samples, especially in the case of potential human palaeo faeces (Gilbert et al. 2009), and therefore such investigations are rarely done (Kuch, Poinar 2012).

A well-preserved coprolite was excavated recently at the Beregovaya 2 site, one of the rare Stone Age bog sites in the Urals region with excellent preservation conditions for organic material. The ZBSA gene lab conducted a study to determine the species of origin



Fig. 2. Coprolite from the Beregovaya 2 site (the Urals, Stone Age, about 7500 BC) (photograph by Sönke Hartz, Archaeological State Museum, Schleswig).

(Zhilin et al. 2014) (Fig. 2). Prior to this analysis, the coprolite was investigated archaeozoologically, based on bones in the coprolite the producer's final meal was fish (perch), and directly radiocarbon dated to 8480 ± 40 BP (about 7500 cal. BC; Poz 46389).

The genetic analyses undertaken at the ZBSA had three aims: to test the individually designed extraction method, to test the DNA preservation in the coprolite, and finally to identify the species. At first, it was necessary to create a special bar-coding marker system, which allows the animal species identification of the defecator, and not of the animal species consumed (cf. Poinar et al. 2001). We developed and applied a special DNA extraction method for coprolites, and we subsequently designed a method allowing genetic identification of *Canidae* (dog, wolf and fox). This limitation stems from the archaeozoological observation of relatively large but unchewed fish vertebrae inside the coprolite that indicates its non-human but potentially canid origin. In the end, the aDNA extraction was successful, but the DNA preservation was poor, and only short fragments were amplifiable, in our case 100 bp. Even if the length was not enough for further implication or comparisons with data of other studies (e.g. Germonpré et al. 2009; Druzhkova et al. 2013), it was enough for a species identification, and shows that the Beregovaya coprolite was in fact from a dog. Therefore, this ca. 9,500-year-old DNA is among the oldest records of dog DNA ever sequenced.

Archaeogenetic analyses of fish bones

Until now, fish remains have rarely been used for ancient DNA analyses, even though they can be found at archaeological sites in high numbers, and their potential in palaeoecological and palaeoeconomical questions has occasionally been demonstrated (Arndt et al. 2003; Speller et al. 2012; Shirak et al. 2013;

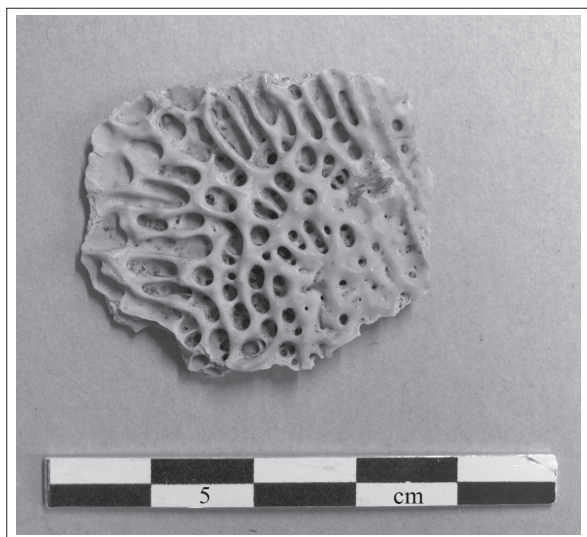


Fig. 3. Sturgeon bone (*Acipenser oxyrinchus*) from Neustadt LA 56 (northern Germany, Stone Age, about 4000 BC).

Hutchinson et al. 2015). In the Baltic Sea region, during the last decade, it was sturgeon that came into the focus of genetic research. Only a short while ago, it was believed that the European sturgeon *Acipenser sturio* was the only sturgeon species in north European waters (Holčík et al. 1989; Williot et al. 2002). In contrast, today, as a result of studies of archaeological remains and modern specimens, it has been clarified that the Atlantic sturgeon *Acipenser oxyrinchus* has been the only, or at least by far dominant, sturgeon species in the Baltic Sea (Popović et al. 2014). Recent archaeogenetic analyses at the ZBSA of the oldest known sturgeon bone fragment from the Baltic Sea (Fig. 3) shows the presence of this species already 6,000 years ago, and suggests immigration into the Baltic basin soon after the formation of the Baltic Sea (Nikulina, Schmölcke 2016, forthcoming). This result corresponds well with a reconstruction of the prehistoric distribution of the Atlantic sturgeon in the North Sea: the aDNA extracted from more than 40 bony plates from several archaeological sites along the coastline conducted at the ZBSA proves that the Atlantic sturgeon was the dominant species in that area during prehistoric and historic times. The occurrence of the European sturgeon ranged only from south European waters up to approximately the mouth of the Rhine (Nikulina, Schmölcke, 2015a; Nikulina, Schmölcke in preparation). However, since, on the other hand, previously published genetic data from about 100 to 200-year-old museum specimens show a strong dominance of the European sturgeon in the North Sea area (Ludwig et al. 2002), *A. oxyrinchus* must have been replaced by this species some time in the course of the second half of the last millennium.

In summary, genetic analyses of archaeological bone plates show the Holocene distribution of sturgeons



Fig. 4. Pelican bones (*Pelecanus crispus*) from the Rosenhof Mesolithic site in northern Germany (about 4600 BC).

in the northeast Atlantic had a complex pattern. They demonstrate that aDNA analyses of fish remains are not only of archaeological interest, but, as in the case of sturgeons, they can be of special importance for conservation and restoration programmes.

Archaeogenetic analyses of bird bones

It is non-controversial in the scientific community today that ancient mammal bones are an appropriate and suitable source for DNA. This view is not shared for fishbones (see above), or for bird remains. Potentially due to the difficulties connected with the relatively thin-walled bones, and consequently low preservation success, only very few aDNA studies about birds have been published so far. Most of these studies discuss aspects of the evolution, taxonomy, or distribution of larger bird species of the Pacific and Australian region, such as moa (Cooper et al. 2001), giant eagle (Bunce et al. 2005), or geese (Paxinos et al. 2002). For Europe, there was a lack of research in this field. At the ZBSA, we combined recent archaeozoological data with genetic analysis of bones from pelicans from archaeological sites in northern, western and central Europe, to verify the mid-Holocene distribution of the species Dalmatian pelican (*Pelecanus crispus*) in the North Sea area (Nikulina, Schmölcke, 2015b). For that, we designed and applied several universal PCR

primers for the genus *Pelecanus* to amplify three fragments of mitochondrial COX1, and one of 12S rRNA genes. The ca. 6,600-year-old bone sampled from the Mesolithic site Rosenhof in northern Germany (Fig. 4) provided reproducible amplifications in all four tested primer systems, and showed in all cases identity between the obtained sequence and the relevant gene fragments of the Dalmatian pelican. Thereby, the study gives evidence for the presence of a typical bird species for the eastern Mediterranean and Black Sea area far to the north. We demonstrated that between 7,400 and 5,000 years ago, the number of Dalmatian pelican records in the west Baltic Sea area is so numerous that they probably do not originate from vagrants, but point to a regular expansion of the species range (Nikulina, Schmölcke 2015b). Very probably, these changes in distribution ranges were caused by the warm climate during the mid-Holocene Atlantic period. In this perspective, the Dalmatian pelican is an example that investigations of the way bird species have responded to climate changes in the past can be relevant for models about their potential reaction to current climate developments (Stewart et al. 2010).

Conclusions

The value and significance of ancient DNA analyses of animal remains from archaeological sites has been demonstrated repeatedly, amongst other regions, also in the area of the Baltic Sea. The focus of the research has been several wild mammal, bird, reptile and fish species (e.g. Sommer et al. 2009; Fraser et al. 2012; Bray et al. 2013; Herman et al. 2014; Horn et al. 2014; Nikulina, Schmölcke 2015a, 2015b), as well as domestic cattle, pigs and dogs (Nikulina, Schmölcke 2008, 2015; Scheu et al. 2008; Krause-Kyora et al. 2013; Zhilin et al. 2014; Niemi et al. 2015). In particular, the genetic analysis of well-preserved teeth and bones to identify the species, the so-called DNA bar-coding, is reasonably priced and can be undertaken, for instance, in cooperation with the Centre for Baltic and Scandinavian Archaeology (ZBSA) in Schleswig, Germany, with its specialised laboratory for ancient animal DNA (Nikulina, Schmölcke 2010). However, as the present paper demonstrates, not only teeth and bones, but also rare and exceptional archaeological find groups, or those with a quite low content of DNA, can be used successfully to answer special cultural-historical or ecological research questions. We showed that single hairs from archaeological layers, for instance, can be identified to species level (Nikulina et al. 2015), and that they can provide information even at the population level. Ancient DNA can also help to distinguish between closely related animal species with nearly

identical morphological bone characteristics. This has been demonstrated in fish species (Nikulina, Schmölcke 2015a) and in birds (Nikulina, Schmölcke 2015b). In both cases, it was the ancient DNA analysis that finally allowed the reconstruction of the former distribution of the investigated species.

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Elena A. Nikulina
Zentrum für Baltische und Skandinavische Archäologie,
Stiftung Schleswig-Holsteinische Landesmuseen
Schloss Gottorf
24837 Schleswig, Germany
E-mail: elena.nikulina@schloss-gottorf.de

PLAUKŲ, KAULINIŲ PLOKŠTELIŲ IR IŠKASTINIŲ FEKALIJŲ LIEKANOS – RETŲ ARCHEOLOGINIŲ RADINIŲ SENŲJŲ DNR TYRIMAI

ELENA A. NIKULINA

Santrauka

Archeologinių tyrimų metu rastų gyvūnų liekanų DNR analizės vertė ir svarba labai greitai išryškėjo įvairiose šalyse, taip pat ir Baltijos jūros regione. Tyrimo objektai yra laukiniai žinduoliai, paukščiai, ropliai ir žuvis, taip pat naminiai galvijai, kiaulės ir šunys (1–4 pav.). Pavyzdžiui, iš gerai išlikusių dantų ir kaulų liekanų nustatoma rūšis, aprašoma vadinamoji tipinių genomų regionų trumpoji DNR. Šie tyrimai atliekami Šlezvige (Vokietija), Baltijos ir Skandinavijos archeologijos centre, specialioje senosios gyvūnijos DNR laboratorijoje. Šioje laboratorijoje tiriami net tik dantys ir kaulai, bet ir reti bei unikalūs ar turintys labai mažą kiekį DNR tyrimams reikalingos medžiagos archeologiniai radiniai, kurie gali būti sėkmingai naudojami ieškant atsakymų į kultūrinių-istorinių ar ekologinių tyrimų klausimus. Net iš vieno plauko, rasto archeologiniame sluoksnyje, galima nustatyti ne tik gyvūno rūšį, bet įmanoma gauti ir informacijos apie rūšies paplitimą. Senosios DNR taip pat gali padėti atskirti artimas gyvūnų rūšis, kurių kaulų morfologijos charakteristikos yra beveik identiškos. Tai matome iš žuvų ir paukščių liekanų tyrimų. Abiem atvejais DNR tyrimai leido rekonstruoti buvusį tiriamų rūšių paplitimą.