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Palaeogenetics and cultural heritage. Species determination and STR-genotyping from ancient DNA in art and artefacts

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Abstract

In recent years, a few papers have addressed the palaeogenetic analysis of cultural, historical and archaeological artefacts. We provide an overview of the individual published articles and then describe the results we had in the framework of a palaeogenetic research project involving various historical and prehistoric finds from museums, archaeological excavations, and libraries. We show that ancient DNA can be isolated from most of the various biomaterials (leather, parchment, glue, binding media, crusted organic plant remains in containers). Short pieces of degraded DNA are used, on the one hand, to determine the organic remnant's genus/species of origin, and on the other hand, to create the genetic profile of an individual animal, using STR-typing. This permits us to determine whether two fragments belong to the same find, as well as providing purely biological data on the animal and its population. Moreover, STR-profiles can help to prove the authenticity of data from less variable loci like mitochondrial sequences.

Keywords: Archaeometry; Artefacts; Cultural heritage; Ancient DNA; STR profiling; Mitochondrial DNA

1. Introduction

After the first successful attempt to isolate DNA from ancient specimens [1,2], it did not take long before Saiki et al. [3] reported in 1985 a new technique called polymerase chain reaction (PCR) which would make in vitro amplification of DNA possible, even from minimal number of target molecules and even from short pieces of highly degraded DNA. Since then many scientists have started to examine various old materials to find out whether they contain ancient DNA (aDNA). The examined specimens range from soft tissues of museum specimens [4–8], through amber-enclosed insects [9,10], critically discussed by [11,12], and plant remains [13–15] to coprolites of extinct animals [16]. After the first report of aDNA from bones [17], the examination of skeletal elements soon became the dominant field of interest. In fact, it seems that the mineral matrix of bones and teeth provides the best environment for DNA preservation [18,19]. Moreover, there is also the factor of availability; skeletons make up a major part of our cultural heritage, are available in large numbers from archaeological collections and museums and are of organic origin. Not only can primary biomaterials, like bones, teeth and plant remains contain aDNA; so can secondary products made by man, like consumer goods, handicrafts, utensils, and art objects. Several scientific articles have already been published on aDNA from anthropogenic artefacts.

Abbreviations: STR, short tandem repeats; aDNA, ancient DNA; RT, room temperature

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In 1995, on the III aDNA Meeting in Oxford, Kahila Bar-Gal [20] presented aDNA work done on temple scroll parchments from the Judean desert. PCR and subsequent DNA sequencing of a segment of the mitochondrial cytochrome *b* locus identified goat as the source animal for parchment production. At the same meeting, Hodgins reported the species identification of fish glue [21]. Since glue is a common component in various artefacts, this represents an essential contribution to the analysis of historic material. At the IV aDNA Meeting held in Goettingen in 1997, Marota [22] showed that DNA can be preserved in papyrus. DeReyer et al. [23] presented the identification of organic parts of gold threads from medieval textiles. At the same meeting, Hardy et al. [24] presented a paper which describes the detection of amplifiable DNA from residues from Middle Palaeolithic stone tools from La Quina, France. In the earlier years, Loy et al. [25–30] already stimulated discussion by publishing protein and DNA data obtained from stone tool residues and rock art pigments. Most of the work done by this group has been discussed very critically in various contexts [31–37]. In 1996, Resse et al. [38] reported a bovine sequence obtained from pigment layers of prehistoric Texan rock art.

In the following, we discuss three important groups of materials for which aDNA methods are an additional and enriching approach in the spectrum of material analysis. We refer to data we produced within the scope of an archaeometric project in the last three years. We concentrate on the questions how and why DNA is preserved and what can it be used for.

2. Collagenous material

Besides bones, we address hide-derived material here. Leather has served since prehistory as clothing, housing coverings, and containers. In historical times, parchment became an important factor of cultural activity. Parchment served as writing material and as book coverings up to recent history. Collagen-rich tissue is very likely to contain aDNA if the latter has not been destroyed by aggressive chemicals in the manufacturing process or during diagenesis in an extreme environment. Quick desiccation, a dry and cool environment, neutral pH and the absence of micro-organisms are the main factors leading to DNA preservation [19,39,40]. The parchment and leather samples examined were from 10 to 350 years old; most of them were manuscripts or book leathers stored in libraries. All of the more recent parchments (10–50 years) and about 50% of the historic parchments contained enough endogenous DNA for species identification. For most of them the original manufacturing process cannot be reconstructed, so we can make only theoretical assumptions for the differential states of preservation [41]. However, leather yielded much less positive results (less than 10% success rate), probably because of the chemically aggressive tanning agents that induce acidic hydrolysis of DNA. Fig. 1 shows an example of species identification from a parchment sample. Fig. 2 shows DNA sequences from a 19th century book leather which identify cattle as the source animal.

For parchment, we succeeded in obtaining more information about the individual animal through DNA-profiling. PCR amplification of individually polymorphic short tandem repeat (STR) loci makes it possible to establish the individual genetic profile of an animal. An example is given in Table 1 [41]. This technique is used mainly in the museum and restoration area. Isolated fragments of a manuscript can be set in correspondence to the original document. But there is still another reason for using

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                    10         20         30         40         50
                    |          |          |          |          |
Parchment           tgtagttctc tggcgaataa ttttgttatg aaattatcaa agtttagggc taagca
O. cuniculus        ---------- ---------- ---------- ---------- ---------- ------
S. nuttallii        ---------- ------g--- ---------- -------tc- g--------- ------
S. palustris        ---------- ------g--- ---------- g------tt- ---------- ------
S. audobonii        ---------- ------g--- ---------- -------tc- g--------- ------
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Fig. 1. Comparison of a sequence obtained from a parchment with reference sequences from GenBank (20/04/99). The 56 bp sequence falls within the mitochondrial 12S rRNA gene and lies between the primers rR4d and rR4a. Dashes indicate identity to the parchment sequence. The comparison shows that the parchment sequence matches exactly with the rabbit (*Oryctolagus cuniculus*) sequence. The sequences of three closely related species (*Sylvilagus nuttallii*, *S. palustris*, *S. audobonii*) show four mismatches to the parchment sequence each [42].

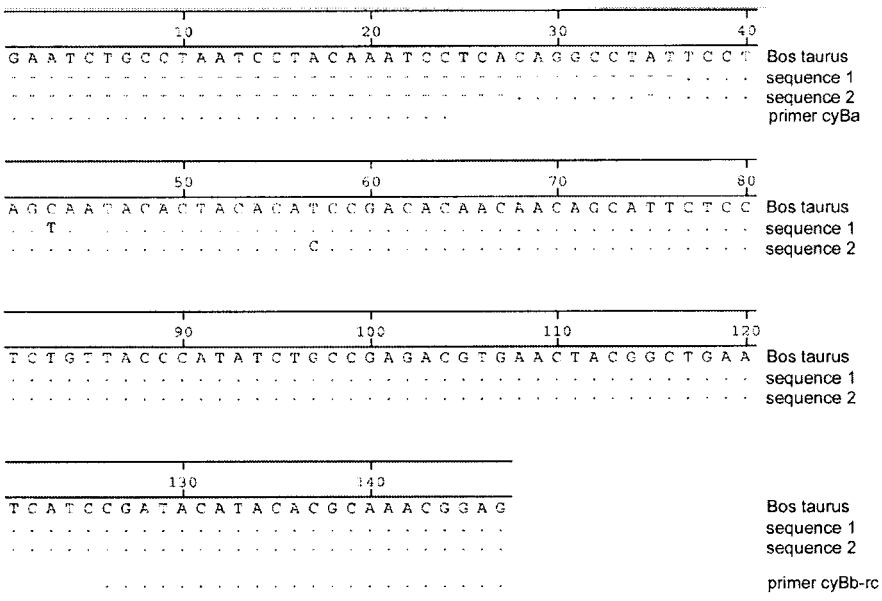

Fig. 2. Comparison of two sequences obtained from a 19th century book leather with a cattle (*Bos taurus*) reference sequence from GenBank. The 147 bp sequence falls within the mitochondrial cytochrome *b* gene and lies between the primers cyBa and cyBb. Dashes indicate identity to the cattle sequence. At one position each sequence obtained from the leather sample is different from the reference sequence, probably due to polymerase errors which occurred in the early cycles of the PCR. Since the changes are not reproducible, they do not affect the establishment of cattle as the species of origin.

individualizable markers like STRs in aDNA studies. DNA-contaminations can interfere with endogenous aDNA and lead to false results. Looking for the animal species of origin of artefacts, human contaminations can be excluded by discriminative primer design. However, possible bovine contaminations originating, e.g. from impure BSA which is used as additive in PCR-buffers, in many cases cannot be distinguished from authentic sequences. Here, bovine STR-profiles (as shown in Table 1) can be used for proofing the authenticity of less polymorphic mitochondrial DNA *sequences*.

Table 1
The early modern samples parchment 1 and parchment 2 (PAI, PAII) were disrupted in fragments (1–4) [41][a]

Microsatellites (bp)								
Parchment	Fragment	MTG 4B	TGLA 122	TGLA 227	TGLA 53	BM 2113	BM 1824	ETH 225
PAI	1	134	143/(155)	93/97	156/158	129/137	182/190	(144)/148
	2	134	143/(155)	93/97	156/158	129/137	182/190	144/148
PAII	3	134/144	139/151	89/91	164/180	139/141	178/(188)	142/150
	4	134/144	139/151	89/91	164/180	139/141	178/(188)	142/150

[a] MTG4B, TGLA122, etc. are the names of STR loci. STRs consist of a variable number of tandemly repeated core units of 2–6 DNA bases. Here, we used dinucleotide-repeats, i.e. repeat units of two bases. The more repeat units are present at a certain locus the longer the DNA-fragment becomes, for example, PAI is homozygous on locus MTG4B and yielded a fragment length of 134 bp, PAII is heterozygous for the same locus and shows a fragment of 134 bp and one of 144 bp, the latter being five repeat units longer than the 134 bp fragment. The STR-profile, i.e. the combination of various loci, was obtained from each fragment. The results show that the parchments can be distinguished and each fragment can be set in correspondence with its original parchment.

3. Contents of pots, vessels and other containers

We analysed Aztec vessels from the Berlin ethnographic collection which still contain organic encrustations. We applied various DNA extraction methods and amplified a part of the *rbc*L-gene of the plant chloroplast genome by PCR. From the vessel shown in Fig. 3, we obtained a DNA sequence which could be attributed to the genus *Martinella*, one of the climbers. The species *Martinella obovata*, which gave the closest match with the sequence obtained from the vessel, is a big woody vine and forms 60–100 cm long capsules. The overall distribution extends from Belize to Brazil. The capsules contain a pharmacological agent that is still used by American Indians as an eye salve today [42].

Another example is the so-called sausage-end from the Celtic (Latène A) site Duerrnberg/Austria [43]. The find is probably one end of a prehistoric container made of animal skin. One sample was taken by scraping off the inner surface of the find near the knot where small clumps of the surmised earlier contents have been preserved. The DNA sequences obtained from this match the genus *Salvia* (sage) reference sequences in GenBank. However, the ancient sequence is closer to wild species of sages (*S. divinorum*, *S. uliginosa*, *S. hispanica*) than to the garden sage (*S. officinalis*) [42].

The last two examples show how aDNA techniques can be used to identify (pre)historic foodstuff or pharmacological substances. In the context of questions of cultural heritage, the results provide information about the usage and function of finds.

Fig. 3. Aztec vessel (IV CA 1855) from the Berlin Ethnographic Museum.

4. Binders, glues, and oils

Binders and glues are used in the production of books, paintings, furniture, instruments, stucco marble, etc. We were able to confirm the feasibility of the method presented by Hodgins [21] on isinglass and obtained a sequence from a commercial fish glue, allegedly isinglass. Isinglass is supposed to be made from the swimbladder of sturgeons. Isinglass is a high quality product that cannot be replaced by cheaper products made from swimbladders of other fishes. In this case, the glue was not produced from a sturgeon species but from the species *Rhodeus ocellatus*, a bitterling. The possibility to isolate endogenous DNA from ca. 10 year old isinglass encourages to pursue the goal to identify various historical glues from objects of art. The same is true for oils; DNA extraction of ca. 5 year old walnut oil resulted in DNA sequences which identified the source plant at the genus level. Oils and protecting media are often applied to organic material as wood or leather. Here, we face the common problem that artefacts consist of mixtures of differing organic materials. Theoretically, there are two technical solutions for this: species-specific PCR primers can be designed to amplify only one certain species but not another. For this, a preliminary hypothesis must be made to know what species one is looking for. The other, more promising way is DNA sequencing of cloned PCR products. Under optimal conditions, each source species of a mixture would appear in the same proportion in the clones as represented in the sample. Thus, complex biological mixtures in historical artefacts can be identified on the species level and historical recipes, production techniques and material treatments can be reconstructed.

5. Conclusion

The application of aDNA techniques provides several new possibilities for the material analysis of (pre)historic artefacts. Table 2 gives an overview on the materials examined so far. The main biological question, what animal/plant a find derives from can be answered on a taxonomic level. But even the individual animal can be identified. DNA-profiling shows whether two finds belong together (i.e. were made

Table 2
List of materials examined by palaeogenetic methods so far

Material	Refs.
Stone tools	[24,27]
Glue	[21]
Manuscripts: parchment	[20,41,44]
Rock art	[38]
Wall paintings	[45]
Textiles (gold threads)	[46]
Manuscripts: papyrus	[22]
Glass	[46]
Contents of containers	[42]
Book bindings: leather	[41]

from the same individual animal), but at the same time, it can tell us where this animal came from. The comparison of the genetic status of a historical animal or plant with other historical or recent data will increase our knowledge not only about the material itself but also about domestication, cultivation, planting and herding practices. Here we experience how basic material analysis, matters of restoration and preservation, and biological information interact and how each points beyond itself in the sense of a reconstruction of human and environmental history.

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