Recovery of Mammalian DNA from Middle Paleolithic Stone Tools

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One of the primary goals of archaeology is to understand past human behavior. Although stone tools comprise the vast majority of cultural artifacts for most of the archaeological record, their uses in prehistory are poorly understood. The application of the polymerase chain reaction (PCR) to amplify DNA molecules can help establish a physical link to ancient tool use in processing biological material. Modern experimental stone tools and a sample of stone tools and soils from the Middle Paleolithic site of La Quina, France, dating between approximately 35 and 65 ka, have been examined for the presence of ancient DNA. Extractions from the samples were analyzed using PCR with primers amplifying a small region of the vertebrate mitochondrial cytochrome b gene. Subsequent sequence analysis allowed the identification of some DNAs amplified to family or species of origin. DNA sequences were obtained from tools and associated soil samples. DNA from boar/pig (Sus scrofa), a species represented osteologically at the site, was recovered from one of the tools. Other tools yielded artiodactyl, human, and rabbit-like sequences.

Keywords: ANCIENT DNA, BIOMOLECULAR ARCHAEOLOGY, CYTOCHROME B, POLYMERASE CHAIN REACTION, STONE TOOL FUNCTION.

Introduction

Because stone tools are the most abundant cultural remains for the majority of prehistory, archaeologists rely on them to interpret past human behavior. Archaeologists have attempted to infer the various aspects of ancient tool use through ethnographic analogy, experimental replication and use of tools, and use-wear analysis. Ethnographic and ethnohistoric accounts have been used to find analogues of prehistoric tool function. However, ethnographic observations of tool use cannot be generalized to all tools of similar morphology (Clark & Kurashina, 1981) because one tool morphology can have different uses, or one task can be performed by different tool morphologies. Experimental replication has been used to understand the technological processes of tool manufacture (Crabtree, 1973; Jones, 1980; Totton, 1985) as well as to experiment with the possible range of use-materials and use-actions of a particular tool type (Jones, 1980; Keeley, 1980; Totton, 1985; Anderson-Gerfaud, 1990; Hurcombe, 1992; Shea, 1992). Such experiments can only define plausibility.

As a stone tool is used, its surface is altered through contact with the worked material. Traces of use include edge damage in the form of microflake scars, striations, and changes in the texture and reflectivity of the tool surface (Keeley, 1980; Odell & Odell-Vereecken, 1980; Shea, 1992). Use-wear studies with low-power magnification (× 10–100) can yield functional information through edge damage and striation patterns. The orientation of microflake scars and striations can help determine how a tool was used. For example, striation patterns can reveal that a tool was used for cutting and may indicate in which direction a tool was moved during use (Odell & Odell-Vereecken, 1980). However, edge damage is not a completely reliable predictor of worked material as different worked materials may produce similar damage patterns (Moss, 1983).

The study of microwear polishes at high magnification (× 100–500) held promise for identifying the specific materials on which tools were used because different use-materials (bone, wood, antler, dry hide, wet hide, meat, etc.) formed distinct polishes on stone tool surfaces which could be readily identified (Keeley, 1980). However, it has become clear that the formation
of microwear polishes is a complicated process which is not easy to interpret (Newcomer et al., 1986; Juel-Jensen, 1988). The formation of the polish is related to both the use-material, the composition of that material and to the stone being used. The amount of silica present in either the stone or the worked material affects the rate and amount of polish formation (Fullagar, 1991). Interpretation is problematic when polish is weak or poorly developed, leading to similar polishes from different materials. It is also possible to have similar polishes from different use-materials if the use-duration varies. Polish on a tool used for a short time on antler may be indistinguishable from polish used for a longer time on wood. Identification of specific use-material can therefore be difficult. Further work investigating the mechanisms of polish formation may help eliminate these potential ambiguities.

Physical and chemical traces of use-material can survive on a stone tool surface and have yielded even more direct functional information. Microscopy has identified fragmentary remains of the worked material on stone tools, including blood, wood, various plant tissues, feathers, and hair (Briuer, 1976; Loy, 1983, 1985, 1987, 1993; Anderson-Gerfaud, 1990; Hurcombe, 1992; Loy & Hardy, 1992; Loy et al., 1992). Microscopic observations of the patterning of residue on a tool can provide information about use-action (Anderson-Gerfaud, 1990; Loy & Hardy, 1992). Various chemical techniques have been used to test for the presence of residues. The most widely used methods are immunological, which have been reported to identify the taxon with which the tool is associated (Kooyman et al., 1992; Newman et al., 1993). However, the validity of immunological testing of residues has recently been questioned. Short term experiments in blood residue preservation have failed to replicate earlier results and question the survivability of protein in a form which is immunologically recognizable (Manning, 1994; Eisele et al., 1995).

The application of molecular biology techniques, particularly the polymerase chain reaction (PCR), to archaeological and paleontological materials has shown that DNA can survive for thousands or even millions of years (Paabo, 1989; Cano et al., 1993; Hagleberg et al., 1994). The use of PCR, which is capable of producing millions of copies from a single molecule of DNA, requires strict precautions against contaminating DNA (Cano et al., 1993; Soltis & Soltis, 1993; Handt et al., 1993). Due to the extreme sensitivity of PCR and the small amounts of ancient DNA template, contamination with modern DNA may occur during excavation, in transport, or in the laboratory (Soltis & Soltis, 1993; Handt et al., 1994). Although it is sometimes possible to distinguish between the contaminant and ancient DNA, methods to reduce modern contamination must be used.

This paper reports the isolation and characterization of DNA from prehistoric stone tools and soils from the Middle Paleolithic site of La Quina, Charentes, France. La Quina is a rock shelter site located in south-west France with archaeological deposits ranging in age from approximately 35,000 to 65,000 years. The deposits are characterized by large numbers of highly fragmented and well-preserved animal bones (Table 1), Neanderthal skeletal remains, and Mousterian stone tools. La Quina has been excavated since the early part of the century by Dr R. Henri-Martin and his daughter, G. Henri-Martin, and originally included approximately 100 m of Mousterian deposits 7 m deep. The early excavations yielded the
fragmentary remains of over 25 hominids. In 1986, A. Debénath and A. J. Jelinek began a new series of excavations (Debénath & Jelinek, 1990). La Quina was the same manner (Hardy, 1994). The tools are drawn taken 2–3 cm away from each tool and were handled in plastic bags. Soil samples (approximately 3–5 g) were recorded, it was placed in a new, clean, self-sealing bag. For DNA analysis was uncovered and its position posing the artefacts. As soon as a stone tool destined for modern DNA contamination to a minimum. Excavators wore new disposable surgical gloves when excavating under controlled conditions in order to reduce the possibility of contamination with DNA from modern sources. The efficacy of the techniques was tested on modern experimental stone tools prior to analysis of the archaeological materials.

Methods

Archaeological samples

Stone tool and soil samples were excavated under controlled conditions in order to reduce the possibility of modern DNA contamination to a minimum. Excavators wore new disposable surgical gloves when exposing the artefacts. As soon as a stone tool destined for DNA analysis was uncovered and its position recorded, it was placed in a new, clean, self-sealing plastic bag. Soil samples (approximately 3–5 g) were taken 2–3 cm away from each tool and were handled in the same manner (Hardy, 1994). The tools are drawn from archaeological layers 6A (Mousterian of Acheulean Tradition), 8 (Typical Mousterian), and M2 (Quina Mousterian). Layers 6A and 8 are approximately 35,000–65,000 years old while level M2 is approximately 60,000–65,000 years old (Debénath & Jelinek, 1990). All of the tools are found in close association with fragmentary animal bones and other stone tools. To date, eight archaeological samples have been examined (see Figure 1). Seven derive from the upper levels (6A and 8) while one comes from the older level (M2).

Modern experimental tools

A series of modern experimental tools were manufactured and used to cut animal tissue. The tools were allowed to dry and some were buried at a depth of approximately 6 inches for up to 3 years. The animal tissue was obtained from the Zooarchaeology Laboratory, Department of Anthropology, Indiana University.

DNA extraction

DNA was extracted by soaking the samples in approximately 100 ml of 4 M guanidine hydrochloride (HCl). Since the extractions were performed by Hardy in the field laboratory at La Quina, guanidine HCl was chosen due to its efficacy as a solvent for DNA and due to the fact that the extracts required no refrigeration. Once transported to the United States, 1–3 ml aliquots of tool and soil extract were placed in dialysis tubing and dialysed overnight in 1 × Tris–EDTA buffer to remove substances which might interfere with the PCR. The dialysed samples were then subjected to PCR analysis without further treatment.

Amplification

PCR was performed using primers L15684 (5’ CTC-CATCATACTTAACTTC 3’) and H15760 (5’ TGTTCGACCTGGTTGCTTCC 3’), for a portion of cytochrome b, which amplify a fragment 116 bp long (including the primers) in mammals between positions 15664 and 15780. PCR reactions were performed in 25 µl volumes using 250 µM dNTPs, 10 µM concentrations of primers, and 0.125 units of Taq polymerase in each reaction. Next, 2 µl of template were added and the reaction was carried out in a MJC PTC-100 thermocycler (30 cycles, 92°C–1 min, 55°C–1 min, 72°C–1 min). A negative (no DNA) control reaction was included in each set of PCR reactions. PCR products were visualized on 1–1.5% agarose gels or 4% metaphor agarose gels stained with ethidium bromide. The expected product size was 116 bp.

Cloning and sequencing of PCR products

PCR products were cloned into Invitrogen TA cloning vector pCR® II and cleaned with QIAGEN plasmid kit (QIAGEN, Inc.) prior to double-stranded sequencing with 35S–dATP using the SEQUENASE (U.S. Biochemicals) protocol. Sequencing was also performed using Licor automated sequencing protocols. Because we suspected that multiple DNAs might be present in a single sample, we cloned the DNA prior to sequencing in order to increase our chances of finding rare DNAs in a sample. Furthermore, direct sequencing of a PCR product containing two or more DNAs would result in multiple banding and ambiguities at some sites in the sequence data. In order to avoid possible sequence errors in the cloning, we sequenced multiple times from different clonings. Phylogenetic analysis of sequences was conducted using Phylogenetic Analysis Using Parsimony (PAUP) Version 3.1 (Swoford, 1993).

Cytochrome b sequence database

DNA sequences obtained from cloned PCR products were compared with a database of known sequence for species identification. The database of cytochrome b sequence currently contains more than 100 different extant vertebrate species for comparison. The database is expanded as new sequences are deposited in Genbank, or when we sequence new species of archaeological importance (performed in laboratories separate from the archaeological analysis). The large size of the database increases the chances that a DNA will match a known species or that a closely related species will be present. Furthermore, the large database aided in the identification of potentially chimeric molecules caused
by PCR jumping. Phylogenetic computer programs allow for identification of unknown sequences.

Precautions against contamination by modern DNA Due to the extreme sensitivity of PCR, contamination with modern DNA may occur in the choice of site, during excavation, in transport, or in the laboratory. Some ancient DNA studies suffer from a lack of stringency in the handling of specimens resulting in the detection of DNA of modern origin (e.g. Golenberg et al., 1990). While it is sometimes possible to distinguish between the contaminant and ancient DNA (Handt et al., 1994), methods to reduce modern contamination must be used.

Precautions against contamination included: (a) choice of sites in primary, undisturbed context; (b) excavation with new disposable surgical gloves; (c) immediately placing and sealing excavated specimens in new self-sealing plastic bags; (d) use of brushes with synthetic bristles (if using brushes for excavation); (e) sampling of surrounding soils and non-artefactual remains; (f) work in a laboratory where related modern DNAs have not been previously studied (to prevent
cross-over contamination); (g) use of positive displacement pipettes dedicated to ancient DNA work (UV irradiated periodically); (h) UV irradiating solutions to ensure that they are DNA-free; (i) aerosol resistant or positive displacement tips (to prevent aerosoling of DNA between reactions); (j) negative (no DNA) reactions to detect contaminating DNA; (k) running PCR reactions with different PCR reagents and machines to check for possible cross-over from earlier PCR products; and (l) limitation of modern control DNAs to species which do not occur at the site.

Results

DNA was successfully extracted and amplified from modern experimental tools and tissue as well as archaeological stone tools and soil samples. To date, eight stone tools from La Quina and their corresponding soil samples have been analysed and five have yielded DNA. If a DNA sequence was recovered from a tool, but not in the soil sample taken from a few cm away, it supports the argument that the DNA from the tool is related to its use. In all PCR reactions, a negative (no DNA) reaction was included to check for possible modern laboratory contamination.

In this paper, we report the recovery of animal DNA. This is not because we assume that the stone tools were used exclusively on animals instead of plants. We decided to concentrate initially on animal DNA for the following reasons: (1) the range of animal species present at the site was known while the range of plant species was not; (2) a gene (cytochrome b) with a large database of known sequences was available for animals; and (3) the primers for this gene would amplify a wide range of species. We chose to look at the cytochrome b gene of animals for several reasons: (a) because it is mitochondrial, the gene would be present in many more copies than an nuclear gene, thus increasing the chance of its survival and subsequent detection; (b) a large database of known sequence was available for the gene (e.g. Irwin et al., 1991) pertinent species are listed in Table 1; (c) the small size of the fragment is consistent with previous observations of the size of ancient DNA fragments (<500 bp); and (d) the primers targeted highly conserved regions surrounding a variable region which would allow species identification from sequence data. In the future, we hope to use a similar gene (such as the rbcL gene) to examine plant DNAs from prehistoric stone tools.

Modern controls

DNA from modern controls was first amplified and sequenced in order to test the reliability of our technique and to confirm that we could identify species based on sequence data (see Figure 2). Four control DNAs were used: (1) human; (2) pronghorn antelope (Antilocapra americana); (3) wallaby (Macropus rufogriseus); and (4) raccoon (Procyon lotor). Except for human, which was unavoidable in the laboratory, these control species were chosen for their availability and because they do not occur osteologically at La Quina. Contamination between reactions by the control DNAs could therefore be easily recognized. We successfully matched the published sequences of human and pronghorn antelope to DNA that we amplified from modern tissue. Modern stone tools were used to cut wallaby tissue and were then dried. DNA was extracted from the surfaces of two experimental tools and yielded the same sequence as that obtained directly from wallaby tissue. Several modern stone tools were used to cut raccoon tissue and allowed to dry for 1 h. The tools were then buried behind CRAFT Human Origins Research Center in Bloomington, Indiana, at a depth of approximately 6 inches. The tools remained buried for 3 years prior to extraction, during which time they were subjected to the precipitation and temperature fluctuations of the area. Mean average annual temperature ranges from a high of 29.6°C in July to a low of −9.3°C in January with an average annual precipitation of 1351 mm. DNA was successfully amplified from three of four tools tested. DNA sequence obtained from one buried tool showed one bp difference from the fresh modern tissue (Figure 2). The bp difference may be due to the fact that the modern tissue and the tissue cut by the tools were from two different individuals. It could also be an error resulting from PCR amplification, possibly due to a damaged site in the DNA. Two soil samples collected 2–3 cm away from the raccoon tools failed to yield DNA.

Eliminating contamination

As stated previously, a negative (no-DNA) control was included in each set of PCR reactions. Occasionally, a band of the appropriate size was observed in the no-DNA lane. These contaminated negative reactions were cloned and sequenced and proved to be human and wallaby DNA. The wallaby contamination was eventually traced back to a 100 mM stock of dNTPs. The contamination of the stock most likely came from an aerosol from a pipettor. The human DNA most likely derived from individuals in the laboratory. The use of new reagents eliminated this problem.

Avoiding inhibition

Several modern experimental tools used to cut raccoon tissue which were buried for several years appeared to contain an inhibitory substance. Extracts from them did not amplify with either one or two rounds of PCR. Furthermore, addition of a small aliquot of one of these samples into a positive control (wallaby tissue DNA), inhibited amplification. It was necessary to dilute the samples 1:100 in water in order for amplification to occur. At this dilution, the amount of inhibitory substance was insufficient to interfere with the reaction. The extraction of the La Quina tools in a
<table>
<thead>
<tr>
<th>Modern controls</th>
<th>#Clones Sequenced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wallaby tissue</td>
<td></td>
</tr>
<tr>
<td>Wallaby tool</td>
<td></td>
</tr>
<tr>
<td>Pronghorn</td>
<td></td>
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<tr>
<td>Pronghorn 2</td>
<td></td>
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<tr>
<td>Raccoon tissue</td>
<td></td>
</tr>
<tr>
<td>Raccoon tool</td>
<td></td>
</tr>
<tr>
<td>Archaeological samples</td>
<td></td>
</tr>
<tr>
<td>Pig (boar)</td>
<td></td>
</tr>
<tr>
<td>Sequence #1</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td></td>
</tr>
<tr>
<td>Sequence #2</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td></td>
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<tr>
<td>Sequence #3</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td></td>
</tr>
<tr>
<td>Sequence #4</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Comparison of cytochrome b sequences from controls and archaeological samples used in this study.
Table 2. Summary of DNA recovered from La Quina tools

<table>
<thead>
<tr>
<th>Tools and corresponding soils</th>
<th>DNA recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tool QM 5–4950 (percőir)</td>
<td>Sequence #1 boar (Sus scrofa)</td>
</tr>
<tr>
<td>Soil QM 5–4950</td>
<td>Sequence #2 probable artiodactyl</td>
</tr>
<tr>
<td>Tool QM 5–4881 (flake)</td>
<td>Sequence #2 probable artiodactyl</td>
</tr>
<tr>
<td>Soil QM 5–4881</td>
<td>Sequence #2 probable artiodactyl</td>
</tr>
<tr>
<td>Tool QM 5–5004</td>
<td>Sequence #2 probable artiodactyl</td>
</tr>
<tr>
<td>Soil QM 5–5004</td>
<td>No amplification</td>
</tr>
<tr>
<td>Tool QM 5–5008</td>
<td>Sequence #2 probable artiodactyl</td>
</tr>
<tr>
<td>Tool QM 5–5093 (flake)</td>
<td>Sequence #4 mammalian</td>
</tr>
<tr>
<td>Soil QM 5–5093</td>
<td>Sequence #2 probable artiodactyl</td>
</tr>
<tr>
<td>Tool QN 5–2708 (flake)</td>
<td>No amplification</td>
</tr>
<tr>
<td>Soil QN 5–2708</td>
<td>No amplification</td>
</tr>
<tr>
<td>Tool QN 5–2972</td>
<td>No amplification</td>
</tr>
<tr>
<td>Tool QE5–507 (scraper)</td>
<td>Sequence #2 probable artiodactyl</td>
</tr>
<tr>
<td>Soil QE5–507</td>
<td>No amplification</td>
</tr>
<tr>
<td>Non-artefactual samples</td>
<td>No amplification</td>
</tr>
<tr>
<td>Limestone 1</td>
<td>No amplification</td>
</tr>
<tr>
<td>Limestone 2</td>
<td>No amplification</td>
</tr>
</tbody>
</table>

Large volume of guanidine HCl (100 ml) may be responsible for the lack of inhibition observed in these samples.

La Quina sequences

DNA analysis of archaeological samples comprised eight stone tools and their corresponding soil samples. DNA was obtained from both tool and soil samples (Table 2). The non-artefactual limestone listed in Table 2 was taken from the rock shelter wall at La Quina. The limestone was tested to determine if DNA was present on non-artefactual lithic material. No DNA was recovered from these samples. The identity of the sequences recovered is discussed in terms of percentage identity (Table 2) and PAUP (Figure 3).

Tool QM 5–4950, a perçoir, has yielded two different mammalian sequences. Sequence #1 matches 100% to Sus scrofa (boar or pig), a species represented osteologically at the site (Figure 2). Boar/pig sequence has only been found in tool sample #1 (see Table 2). It does not occur in the corresponding soil sample or in the other tool or soil samples. It has not been found in any modern sample. A large number of clones from both tool and soil samples were digested with the enzyme SpeI to test for the presence of boar/pig (Figure 4). An SpeI restriction site is present in only two of the sequences in our database; boar/pig and rhinoceros. Rhinoceros were present in Pliocene Europe, but are not represented osteologically at the site. Of the 30 clones tested from tool QM 5–4950, 50% were cut by SpeI and were taken to be boar/pig sequence along with the four clones that were sequenced. The 30 clones from tool QM 5–4950 were taken from two separate sets of dialysis, PCR, and cloning. None of the clones from the other samples (Soil #1, 20 clones; Tool QM 5–5093, 20 clones; Soil #2, 20 clones) were cut by SpeI.

Sequence #2 is present in all of the archaeological samples (tool and soil) analysed so far that show DNA amplification. It does not match any of the fauna in our database but appears to be mammalian in origin. Table 3 shows the species with the closest match to sequence #2. The closest match is to Ovis aries (see Figure 2). For this region of cytochrome b, species of the same genus typically exhibit 90–95% identity, within the same family usually share 85–95% identity, and within the same order usually share between 80 and 90% identity. Below 80% identity, the diversity includes different orders. While these percentages are not strict divisions, the list in Table 3 suggests that sequence #2 is most likely an artiodactyl, possibly a
bovid or a cervid. PAUP (Figure 3) supports our assignment of this taxa to the family Artiodactylae. Two tools, QE5–507 and QM5–5004, yielded sequence #2, but their corresponding soil samples did not amplify. This would normally imply that sequence #2 is related to the use of these tools. However, due to its prevalence in other samples at the site, it is more likely that sequence #2 is present on these tools as part of its general pattern at the site. The presence of sequence #2 in all of the samples amplified suggests that it may be a “background” sequence, i.e. one which is scattered throughout the site. The fact that three tools and five soil samples did not amplify argues against the possibility that sequence #2 is a systematic contaminant. Sequence #2 has not been recovered from any modern samples. Of the species represented osteologically at La Quina, there is one artiodactyl for which we do not have sequence, *Bison priscus*, which is extremely common at the site. Work is currently under way to obtain bison sequence for comparison with sequence #2.

Sequence #3 has only been found in the soil sample SQM 5–4950. It does not match any species in the database, but is clearly cytochrome b and appears to be mammalian in origin. The best match is with Canis simensis (Ethiopian wolf) at 80·3% identity. While there are canids present at the site (*Canis lupus*), an identity of 80·3% is insufficient to provide an identification (see Figure 3). PAUP (Figure 3) indicates that this sequence is most closely related to *Canis simensis* or *Oryctolagus cuniculus* (rabbit). Oryctolagus cuniculus has only 71% identity with sequence #3. We are unable to identify this species positively beyond the mammalian level at this time. Further expansion of the sequence database may aid in identification of this sequence.

Sequence #4 shows 99% identity with modern human (see Figure 2). There is one bp difference between the two sequences. The one bp difference has been observed in several different clones. Sequence #4 has only been found from tool QM 5–5093. While it is difficult to rule out the possibility of modern human contamination, this sequence was obtained from PCR reactions with clean no-DNA negative control reactions. This, along with the fact that longer fragments of DNA did not amplify (see below) suggests the possibility that this DNA may be ancient. However, this is only a possibility. Further analysis will be necessary before any conclusion can be drawn. Primers for portions of the mitochondrial control region have been used to document population variation among modern humans and have also been used with ancient samples to establish ethnicity (Handt et al., 1994). Further analysis with control region and other primers will be necessary before we can confirm that ancient human DNA is present at La Quina. In summary, a total of eight tool and corresponding soil samples have been tested from La Quina. Five of the eight tools and three of the eight soils have yielded DNA. DNA from boar/pig is only found on one tool and is not present in the soil. This DNA is most likely use-related. The same may be true for the human DNA on tool QM 5–5093. The remaining DNAs are more difficult to interpret (see below).

The results reported here have been repeated several times in our laboratory from different aliquots of
extracted samples. We will provide aliquots of samples for independent corroboration by other researchers.

Size of DNA recovered

It is generally agreed that DNA from ancient sources is only present in fragments <500 bp long (e.g. Higuchi et al., 1984; Paabo, 1985, 1989; Rogers & Bendich, 1985; DeSalle et al., 1992; Lindahl, 1993; Soltis & Soltis, 1993; Handt et al., 1994). In order to test this hypothesis, primer L15162 (5′ GCAAGCTTCTACCATGAGGACAAATATC 3′) described in Irwin et al. (1991), was used in combination with primer H15760 from this study to obtain a product of 618 bp. Primer L15162 is also designed to amplify a wide range of vertebrate species. The 618 bp fragment was successfully amplified from human genomic DNA prepared from modern tissue culture. No 618 bp products were obtained from any of the archaeological samples. We were also unable to obtain 618 bp fragments from wallaby and pronghorn antelope DNA prepared from muscle tissue. The tissue used to prepare this DNA was obtained after the animals had been dead for an unknown period of time (several hours to several days). These results suggest that degradation to small fragment size occurs very rapidly (see Tuross, 1994). However, the lack of amplification of large products from the archaeological samples suggests that the DNA does not derive from modern lab contamination and is therefore consistent with an ancient origin.

Discussion

Is the DNA recovered related to tool use?

The recovery of DNA from the surfaces of stone tools does not necessarily mean that it is related to the use of the tool. In order to establish that DNA recovered from stone tools was related to their use, we took the following measures: precautions against contamination by modern DNA (as described above), testing of soil samples taken in close proximity to the tools to establish that DNA is isolated on the tool, testing of non-artefactual materials to see if DNA is scattered throughout the site, and attempting to amplify large fragments (>500 bp) of DNA which would not be characteristic of ancient material.

There are three possible patterns of DNA distribution to interpret:

1. DNA is isolated on the tool. In this case, the DNA most probably derives from tool use. This pattern is found with the boar/pig DNA;
2. DNA is found on the tool and in the surrounding soil. The DNA may be present because of tool use or it may be incidental to tool use. This pattern is found with the probably artiodactyl sequence;
3. DNA is found throughout the site, including on non-artefactual materials. The DNA most likely comes from sources not related to tool use.

The first two of these patterns have been found on the tools from La Quina. The first pattern shows a high probability that the DNA is related to tool use. The second pattern has two potential sources. Because our current understanding of the taphonomy of DNA at archaeological sites is limited, we cannot say whether this DNA is related to or incidental to tool use. Further investigation and experimentation into the survival and movement of DNA in an archaeological environment will be necessary to understand the source(s) of DNA found on tools and in soil.

Loy (1993) has previously reported the identification of bovine satellite DNA on one 2200-year-old tool from British Columbia on the basis of nested PCR. This study has examined a sample of tools and their surrounding soils. We have found DNA from several tools and soil samples and have used sequence data, rather than PCR, to identify species of origin. The discovery of boar/pig DNA on tool QM5–4950, but not from other samples, suggests that the DNA is related to the use of the tool. The tool which yielded boar/pig DNA is classified typologically as a perçoir or awl. If the DNA and tool were associated with one another through a mechanism other than the use of the tool, we would expect the DNA to be more widespread than it is. The same is true for the occurrence of human DNA, although the possibility that the human DNA is of modern origin is always high and cannot be ruled out here.

The preliminary data reported here clearly illustrate that DNA survives on buried modern tools and can also be detected on materials from La Quina. The identification of species of origin of the DNA can be made from sequence data. This is a level of identification previously unavailable to the archaeologist. The discovery of DNA in the soil from La Quina indicates that each archaeological site may have a distinct DNA profile. Further analysis of tools and soils from La Quina taken in combination with evidence from more traditional archaeological approaches could provide one of the most detailed pictures of Neanderthal behaviour to date. If Neanderthal DNA is present, its sequence can be contrasted with the range of modern human DNAs. Such data would cast light on the debate concerning the origins of modern humans.

Conclusions

Stone tool function at La Quina

Interpretations of stone tool function are often made on the basis of close physical association between stone artefacts and faunal remains. However, close physical association does not always imply a functional relationship. Amplification and identification of DNA from stone tools helps archaeologists to establish more direct links to tool function. The data presented here indicate that one of the tools from La Quina was used in processing boar/pig. This species is represented
osteologically at the site, but its remains are not common. Without the DNA results, boar/pig remains could have been interpreted as incidental to the activities at the site. The DNA results, however, indicate that boar/pig was related to Neanderthal behaviour at the site, at least on one occasion. Although it is clear from the faunal remains at the site that Neanderthals at La Quina were exploiting animal resources, DNA analysis can provide associations of specific artefacts with specific animal species.

Archaeological importance of DNA analysis of stone tools
DNA analysis of residues on stone tools will potentially allow archaeologists to: (1) test the assumption that associated stone tools and animal bones are associated due to use and not by chance; (2) test hypotheses linking specific tool types with specific uses; (3) test for multiple uses of tools; (4) detect behaviour which occurred away from the site (if tools were returned to the site, but the material processed was not); (5) investigate paleoenvironmental data through ancient biomolecules; (6) investigate the mechanisms of preservation of ancient DNA; (7) detect processing of small animals whose remains may be under-represented at a site; (8) identify plants which were used in prehistory; (9) investigate molecular evolution (including humans); and (10) provide evidence for stone tool function.

Further work in ancient DNA analysis of stone tools will include the sequencing of additional modern species to increase the cytochrome b database, both from tissues of modern animals and possibly from bone or tissue of extinct animals. Increasing the sample size of tools analysed from La Quina will allow us to address such archaeological questions as the functional validity of typological categories and whether or not the association of animal bones and stone tools accurately reflects the activities at the site. Furthermore, it may be possible to reconstruct broader patterns of subsistence and possibly even recover Neanderthal DNA. Archaeologists are urged to consider the possibility of DNA analysis when excavating sites and to modify their excavation procedures accordingly to assure the collection of uncontaminated artefact samples as well as sediment samples for potential DNA residue analysis.

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