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Evidence for the Survival of Ancient DNA in Charred Wheat Seeds from European Archaeological Sites

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The objective of the work reported in this paper was to obtain additional information to support or contradict previous results suggesting that ancient DNA is present in charred wheat seeds. The reproducibility of the previous results was demonstrated by carrying out extractions with replicate samples and performing polymerase chain reactions (PCRs) directed at two separate loci in the wheat nuclear genome. It was discovered that the DNA in charred seed extracts was extensively fragmented and had undergone base degradation, as expected for ancient DNA. Sequence analysis of PCR products revealed novel alleles not present in modern wheats used in the laboratory but which were shown by phylogenetic analysis to represent genuine wheat sequences. Independent, non-PCR evidence for the presence of DNA was provided by hybridisation analysis. It is concluded that polymeric ancient DNA survives in at least some charred plant remains.

Keywords: Ancient DNA, Charred plant remains, DNA–DNA hybridisation analysis, Polymerase chain reaction, Wheat

INTRODUCTION

Preserved seeds and other plant remains are frequently recovered from archaeological sites. Morphological examination of these remains, in conjunction with studies of living plants, has provided information on the wild ancestry of crop plants, the time and pattern of spread of crops during prehistory, and the evolutionary changes of each plant during and after the initial domestication event (Renfrew, 1969; Zohary and Hopf, 1993; Harris, 1996). This conventional approach to archaeobotany depends on the security of morphological indicators as a means of identifying the species or sub-species to which a specimen belongs, and as such has limitations. Some remains are too fragmentary to provide useful morphological information and even when intact structures such as seeds are recovered it may be difficult to make secure identifications because of deformations that have occurred during preservation. Comparisons based on morphology are further complicated by phenotypic variations arising from climatic and other environmental conditions.

Wheat is a case in point. One of the founder crops of European agriculture and a major grain source for prehistoric communities (Zohary and Hopf, 1993), wheat diversified after its initial domestication into a range of economic

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varieties. With several of these varieties the grain morphologies are very similar, which in an archaeobotanical context means that the free-threshing wheats (e.g. *Triticum turgidum*, *Triticum durum* and *Triticum aestivum*) can be impossible to distinguish as they are usually recovered as naked grains, the chaff having been removed by threshing and winnowing after harvesting. Other varieties, such as *Triticum dicoccum*, include such a broad range of morphological forms that serious doubts are entertained about the validity of morphology as an indicator of the underlying genotype.

A possible solution to the problems inherent in conventional archaeobotany is provided by recent advances in the recovery and analysis of preserved biomolecules, especially DNA, from ancient specimens (Brown and Brown, 1994). With plants, the potential of ancient DNA analysis has been illustrated most clearly with desiccated specimens (Rollo *et al.*, 1987; 1991; 1994; O'Donoghue *et al.*, 1996b), but this is of limited general value because of the geographical restrictedness of desiccated remains in the archaeobotanical record. For ancient DNA to have widespread importance in archaeobotany it will be necessary to make use of remains subjected to the commonest form of preservation, charring brought about by exposure to fire or by baking (Fig. 1; Jones *et al.*, 1996). Intuitively one might consider this objective to be unattainable as studies of DNA decay in aqueous solution (reviewed by Lindahl, 1993a) have suggested that polynucleotides will not survive the high temperatures (at least 250°C for several hours: Boardman and Jones, 1990) thought to be involved in the charring process. However, Goloubinoff *et al.* (1993) were able to detect maize DNA in extracts of 600-year-old burnt maize cobs, and we obtained similar evidence for DNA preservation in 2000-year-old charred wheat seeds (Allaby *et al.*, 1994).

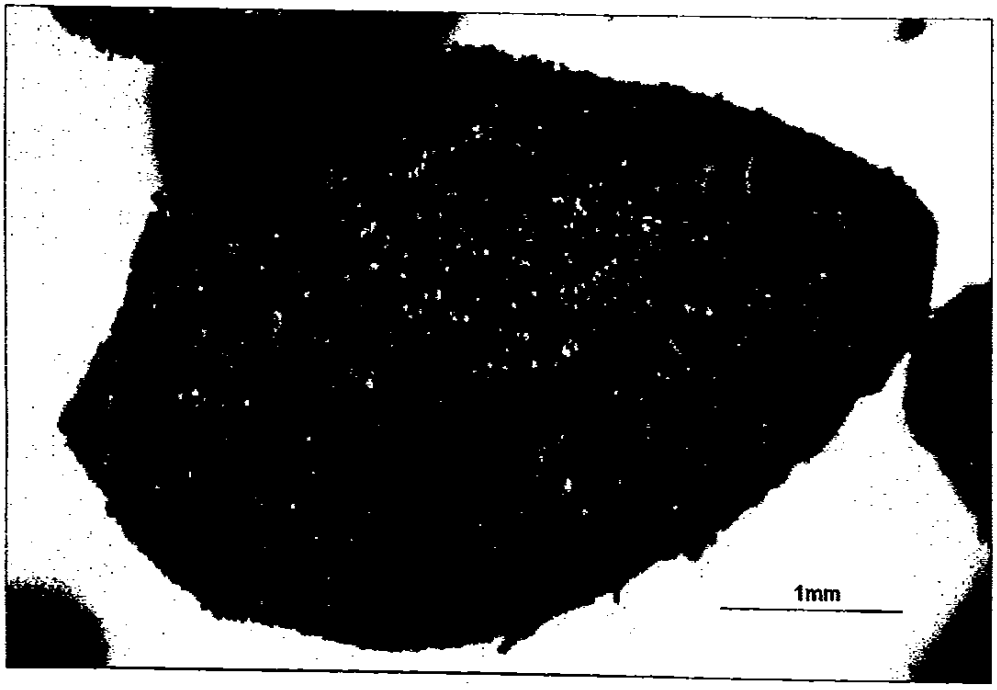

FIGURE 1 Magnified image of a typical charred grain (3300-year-old *T. monococcum* from Assiros, Greece). (See Color Plate I)

Further examination of charred specimens is needed to resolve the apparent contradiction in evidence, the immediate requirement being to assess if the reported DNA detections could be due to contamination with modern sources of DNA rather than the genuine occurrence of ancient DNA. In this paper we describe a more detailed analysis of the DNA present in charred wheat extracts and assess the results with reference to criteria proposed for validation of ancient DNA detections (e.g. Lindahl, 1993b; Handt *et al.*, 1994).

RESULTS

Reproducibility of the Polymerase Chain Reaction (PCR) Results

Our initial report of wheat DNA in charred wheat extracts (Allaby *et al.*, 1994) was based on PCR experiments directed at a 246 bp nucleotide sequence immediately upstream of the high-molecular-weight glutenin genes of wheat nuclear DNA. In these experiments products of the expected size were amplified from extracts of the Danebury *T. spelta* and shown to have nucleotide sequences highly similar to those of glutenin alleles present in modern cultivated wheats. Standard precautions were followed to avoid contamination of the ancient extracts with modern DNA, the results of control experiments suggesting that contamination had not occurred.

The first issue we addressed in the present study was the reproducibility of the published results. Three questions were asked: could the results be reproduced with the same charred sample; could the results be reproduced with other charred samples; and could the results be reproduced with PCR primers specific for different loci of the wheat genome? For all three questions the answer was yes. Three additional extractions were prepared from the Danebury grain studied by Allaby *et al.* (1994) and amplifications carried out with the glutenin primers. Each extract gave a single amplification product of the expected size, although different extracts gave different amounts of amplification product. In each case the accompanying extraction and water blanks gave no amplification products. Attempts were made to reproduce the results with a second sample of charred wheat, 3300-year-old *T. dicoccum* from Assiros. The first extract failed to give an amplification product, but after modifying the procedure by introduction of a ribonuclease step (Allaby, 1995) an amplification product of the expected size was obtained. Once again, the experiments were carried out with accompanying extraction and water blanks that gave no amplification products. Extracts were also prepared from *T. monococcum* from Assiros and the unidentified charred grain from Fiavé, but these failed to give amplification products, either in the initial experiments or when three additional extracts of the *T. monococcum* were tested. Aliquots of a *T. monococcum* extract had no inhibitory effect on the amplification of modern DNA, suggesting that the negative results with this material were due to the absence of DNA rather than the presence of compounds inhibitory to PCR. PCRs were then carried out with primers specific for a second target sequence in the wheat genome, the non-transcribed spacer region of the nuclear rDNA loci (Sallares *et al.*, 1995). An amplification product of the expected size was obtained with the Danebury grain, again with no products seen in the accompanying extraction and water blanks (Fig. 2). The same result was obtained when two additional extractions of the Danebury grain were tested with the rDNA primers.

The Physical Nature of the DNA in Charred Wheats

Having established that the PCR results were reproducible we examined the amplification products to assess if the DNA in the charred seed extracts displayed features considered to

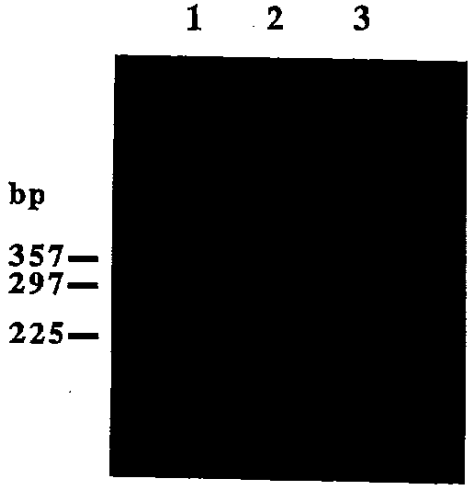

FIGURE 2 Amplification of the non-transcribed spacer region of the wheat nuclear rDNA loci. Lanes: 1, PCR products obtained with DNA from modern *T. aestivum*; 2, products from an extract of the Danebury *T. spelta*; 3, water blank. A ladder of bands (predicted sizes 225, 297, 357, 417, 489, 537, 621, 657, 753, 778, 885, 910, 1017, 1030, 1149, 1151 bp) is seen in lane 1 as the upstream rDNA primer anneals to a repeated sequence (Sallares *et al.*, 1995). Only the smallest of these products was seen after PCR of the ancient extract.

be characteristic of ancient DNA. The most frequently cited of these characteristics is that ancient DNA molecules are fragmented with polynucleotides longer than 300 bp rarely present (e.g. Pääbo, 1989; Hagelberg *et al.*, 1991; Rollo *et al.*, 1991; Handt *et al.*, 1994). Modern contaminating DNA might be expected to include longer molecules. We therefore examined the sizes of the wheat polynucleotides that were acting as PCR templates in the charred seed extracts. This was most readily achieved with the rDNA PCR as the upstream primer in this system anneals to a repeated sequence, so that a series of PCR products from 225–1151 bp are produced, presuming that the template DNA contains molecules of appropriate length (Sallares *et al.*, 1995). As shown in Fig. 2, rDNA PCRs with extracts of the Danebury seeds failed to give amplification products greater than 225 bp in length, suggesting that templates of 297 bp (the length of the second shortest expected product) are not abundant in these extracts. The alternative explanation, that a component of the ancient extracts inhibits PCR so that only short products are synthesised, was tested by adding an aliquot of a charred seed extract into a PCR set up with modern DNA: no inhibition of amplification of the modern template was observed (data not shown).

Further information on the sizes of the DNA molecules in the charred seed extracts was provided by detailed examination of the glutenin PCR products. The sequences of cloned amplification products from a glutenin PCR carried out with the Assiros *T. dicoccum* indicated that the resulting 246 bp product was made up of chimaeric sequences, presumably the result of 'PCR jumping' (Pääbo *et al.*, 1990). From the chimaeric structure of the PCR products (Fig. 3A) it was estimated that the template DNA contained very few, if any molecules of 246 bp, the bulk of the polynucleotides probably being <150 bp with an average length of 50–70 bp (Fig. 3B; Allaby *et al.*, in preparation).

A second characteristic of ancient DNA is the presence of chemically-modified nucleotides (Pääbo, 1989). At least some of the nucleotide modifications expected to arise from hydrolytic and/or oxidative decay processes would result in altered base-pairing properties (Lindahl, 1993a), leading to misincorporation when the ancient polynucleotides are copied during PCR. The predicted outcome is that amplification products derived from ancient DNA will display more sequence errors than products derived from modern templates, assuming the PCRs are carried out under identical conditions. To assess the amount of chemical modification we made a comparison between the sequences of cloned PCR products obtained by amplification of the Assiros *T. dicoccum* with the glutenin primers and equivalent sequences obtained after an identical PCR of modern *T. dicoccum*. The inferred sequence error rates were 8.2×10^{-3}

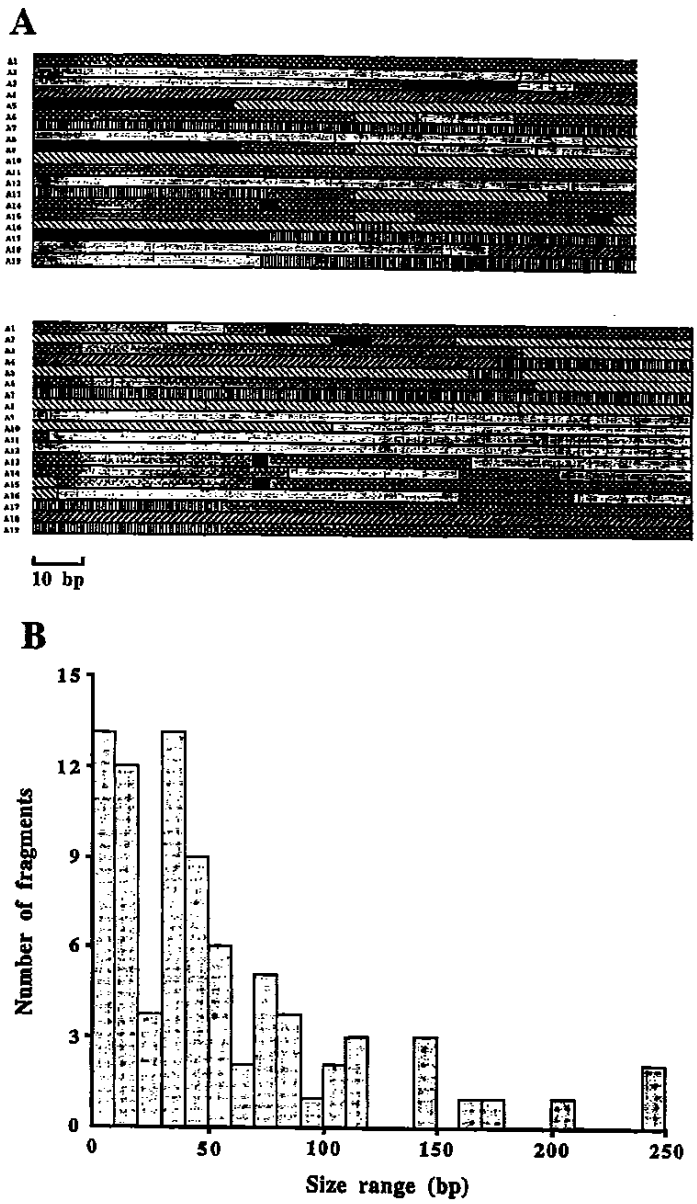

FIGURE 3 Chimaeric structure of the cloned products obtained from a glutenin PCR carried out with the Assiros *T. dicoccum*. A: Diagram illustrating the chimaeric structures of nineteen PCR products. Each bar represents a single sequence (A1–A19), the lower set of bars running directly from the upper set (actual sequences given in Allaby, 1995). Vertical divisions represent the inferred positions of jump points, identified either by the presence of an A insertion (indicative of the position of a PCR jump: Pääbo *et al.*, 1990) or as the junction between two segments of sequence derived from different alleles (positions inferred as described in Allaby, 1995; Allaby *et al.*, in preparation). The different shadings indicate the allele affiliations of the segments. B: Histogram showing the size range of the segments from the chimaeric sequences illustrated in part A. According to our modelling of the events leading to assembly of chimaeric PCR products from fragmented template DNA molecules via PCR jumping (Allaby *et al.*, in preparation), the segment size distribution provides a rough approximation of the size range of template molecules. According to this hypothesis the average length of the DNA molecules in the Assiros extract was 50–70 bp.

errors/nucleotide position for the Assiros sample (based on examination of nineteen 241–243 bp sequences) and 0.67×10^{-3} errors/nucleotide position for the modern wheat (twenty 241–243 bp sequences).

Genetic Analysis of the DNA in Charred Wheats

A further indication of the veracity of an ancient DNA preparation can be obtained by analysis of the sequences obtained from the PCR products (e.g. Höss and Pääbo, 1993; Höss *et al.*, 1994; Hagelberg *et al.*, 1994). Authenticity is indicated if two requirements are met: first, a sequence from ancient DNA should not be identical to an equivalent sequence from a modern specimen used in the same laboratory; and second, it should be possible to infer (e.g. by phylogenetic analysis) that an ancient DNA sequence could indeed be an authentic sequence from the ancient specimen.

Relevant information is obtainable from the glutenin PCRs as the wheat glutenin genes are multicopy and multiallelic with the alleles present at different loci displaying distinctive sequence features. When the sequences of the glutenin alleles present in the DNA amplified from an Assiros *T. dicoccum* extract were reconstructed from the chimaeric sequences of the cloned PCR products (Allaby, 1995; Allaby *et al.*, in preparation), five alleles were identified, two of which were identical to alleles known to be present in modern wheats including those used in our laboratory. The three other alleles were novel, being absent from the published dataset and absent from the three wheats used extensively in our laboratory as reference specimens during the course of this project. A neighbour joining tree was constructed from the sequences of the five alleles from the ancient extract and 13 alleles from modern wheats (eight previously published plus five determined by us). The tree displayed six clades, each clade containing the alleles specific to a single glutenin locus. Each of the five alleles from the ancient extract affiliated with a clade in the tree, with each allele showing equable group distances compared with the modern alleles in the clade (Table I; Allaby *et al.*, in preparation). This indicated that each of the three novel alleles present in the ancient extract had sequence features consistent with a genuine glutenin allele.

Analysis using Methodology other than PCR

A major problem with authentication of ancient DNA results is that most published reports depend entirely on PCR. The extreme sensitivity of this technique means that a false-positive amplification signal might be expected if an ancient specimen is contaminated with very few, perhaps just one, modern DNA molecules with appropriate primer annealing sites. It is therefore desirable to use a less sensitive, non-PCR method to obtain independent evidence for the presence of DNA in an ancient specimen.

Hybridisation analysis is a suitable alternative to PCR as it is able to detect sub-nanogram amounts of species-specific DNA. We analysed extracts of the Danebury *T. spelta* by dot blot hybridisation with a 21-mer oligonucleotide probe (oligonucleotide 3 in Sallares *et al.*, 1995) specific for the multicopy rDNA loci on the wheat B and D genomes. As shown in Fig. 4, the ancient extract gave a hybridisation signal. This experiment was carried out at a level of hybridisation stringency previously shown to discriminate between the rDNA loci on the A and B wheat genomes, which differ at just three nucleotide positions within the target sequence for the probe (Sallares, unpublished). This high stringency suggests that the hybridisation signal in Fig. 4 is due to the presence of wheat DNA, rather than microbial or any other DNA, in the ancient extract.

TABLE I Pairwise differences between modern glutenin allele sequences and five sequences obtained from the Assiros *T. dicoccum*.

	D1-2b	*D1-2a*	*B1-2b*	Gal-ii	*B1-1a*	*B1-1b*	Gal-iii	Ass-iii	Ass-iv	*D1-1a*	*D1-1b*	Gal-i	Ass-i	*A1-1b*	Gal-iv	Ass-ii	*A1-2a*	Gal-v
Glu-D1-2a	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Glu-B1-2b	16	19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Gal-ii	15	18	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Glu-B1-1a	26	30	28	27	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Glu-B1-1b	25	29	27	26	3	—	—	—	—	—	—	—	—	—	—	—	—	—
Gal-iii	21	26	23	22	6	5	—	—	—	—	—	—	—	—	—	—	—	—
Ass-iii	21	26	21	21	8	7	2	—	—	—	—	—	—	—	—	—	—	—
Ass-iv	23	28	23	23	10	9	4	6	—	—	—	—	—	—	—	—	—	—
Glu-D1-1a	26	31	24	24	24	23	18	19	19	—	—	—	—	—	—	—	—	—
Glu-D1-1b	24	29	22	22	22	21	16	17	17	2	—	—	—	—	—	—	—	—
Gal-i	24	29	22	22	22	21	16	17	17	2	0	—	—	—	—	—	—	—
Ass-i	24	29	22	22	22	21	16	17	17	2	0	0	—	—	—	—	—	—
Glu-A1-1b	27	32	29	29	27	26	21	22	22	14	12	12	12	—	—	—	—	—
Gal-iv	27	32	29	29	27	26	21	22	22	14	12	12	12	0	—	—	—	—
Ass-ii	27	32	29	29	27	26	21	22	22	14	12	12	12	0	0	—	—	—
Glu-A1-2a	17	21	16	16	32	31	27	28	27	28	26	26	26	32	32	32	—	—
Gal-v	19	23	18	18	33	32	28	30	29	30	28	28	28	34	34	34	4	—
Ass-v	17	21	16	16	32	31	27	28	27	28	26	26	26	32	32	32	2	4

Glu-D1-2a etc., are published allele sequences (Thompson *et al.*, 1983; Forde *et al.*, 1985; Sugiyama *et al.*, 1985; Halford *et al.*, 1987; Anderson *et al.*, 1989), Gal-i to Gal-v are modern allele sequences determined by us (accession numbers X98583–X98587; Allaby *et al.*, in preparation), and Ass-i to Ass-v are the five allele sequences from the charred extract (accession numbers X98588–X98592; Allaby *et al.*, in preparation). The sequences that were compared were 241–243 bp in length and were amplified from the region immediately upstream of the glutenin open reading frame (see Materials and Methods). Each boxed area contains the pairwise differences between alleles affiliated with a single clade. The data suggest that the three novel sequences obtained from the Assiros sample (Ass-iii, Ass-iv and Ass-v) are genuine glutenin allele sequences as each displays pairwise differences that are comparable with the pairwise differences shown by the modern members of its clade.

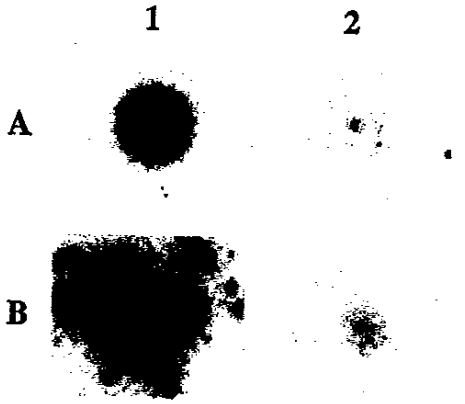

FIGURE 4 Dot blot hybridisation analysis with an oligonucleotide specific for the wheat rDNA sequences. Row A: modern wheat DNA (1, *T. dicoccum*; 2, *T. monococcum*), each dot is the extract from 0.5 mg seeds. Row B, dot 1, extract from 1.25 g of the Danebury *T. spelta*. Row B, dot 2, extraction blank. The probe (oligonucleotide 3 of Sallares *et al.*, 1995) is specific for the rDNAs of the B and D wheat genomes and so should hybridise to *T. dicoccum* (AABB) and *T. spelta* (AABBDD) DNAs but not to *T. monococcum* (AA). The dots shown are taken from a single autoradiograph. The scanned image was manipulated to reposition the dots but the relative intensities were not changed.

DISCUSSION

This project started with the supposition that it was unlikely that DNA would survive in charred plant remains. Intuitively, one would expect the high temperatures involved in the charring process (Boardman and Jones, 1990) to depolymerise the DNA in the specimens. The initial results (Allaby *et al.*, 1994) were therefore surprising and demanded that a more detailed investigation be carried out, this investigation taking into account criteria for authentication of ancient DNA results (Lindahl, 1993b; Handt *et al.*, 1994). According to these criteria, a PCR product from an ancient specimen can be considered 'authentic' if the following apply: negative controls including extraction blanks should not give amplification products; the PCR result should be reproducible with replicate samples; it should be possible to demonstrate that short degraded molecules predominate in the extraction; the sequence of a PCR product from the ancient specimen should be novel and compatible with what is known about the genetics of the specimen; and it should be possible to obtain independent, non-PCR evidence for DNA or general biomolecular preservation.

Our results appear to satisfy all these criteria. The reproducibility of the original results was demonstrated by carrying out extractions with replicate samples of the Danebury grain, by repeating the work with the Assiros *T. dicoccum*, and by targeting a second locus in the wheat nuclear genome. In all cases amplification products of the expected sizes were obtained from the charred seed extracts, with no amplification products seen in the accompanying extraction and water blanks. Extracts of two charred samples, the *T. monococcum* from Assiros and the unidentified Fiavé grain, consistently failed to give amplification products. The latter results are relevant as our ability repeatedly to prepare charred seed extracts that contained no amplifiable DNA suggests that our basic laboratory procedure was able to prevent contamination of specimens and extracts with modern DNA.

The physical features of the DNA in the Assiros *T. dicoccum* extract were consistent with the proposition that the DNA was of ancient origin. Extensive fragmentation of the *T. dicoccum* DNA was indicated by the inability to obtain PCR products > 250 bp in length and by the PCR jumping that was revealed when cloned products from the glutenin PCR were sequenced. These sequences contained twelve times as many errors as seen after PCR of modern *T. dicoccum*. Although some of these errors were probably artefacts of jumping PCR, the indication is that the DNA in the ancient extract had undergone a certain amount of base degradation. The genetic evidence also supported the hypothesis that the Assiros *T. dicoccum* contained ancient DNA, the glutenin PCR products including novel sequences that were not present

in modern wheats used in the laboratory but which nonetheless were genuine glutenin alleles.

Independent, non-PCR evidence for the presence of DNA in the charred seed extracts was provided by hybridisation analysis, an extract of the Danebury *T. spelta* giving a signal when probed with a wheat-specific oligonucleotide. We also used the mass spectrometric techniques described by O'Donoghue *et al.* (1994, 1996a) to examine hydrolysates of charred seed extracts for nucleosides and nucleotide bases. These resulted in clear detections of 2'-deoxyadenosine, 2'-deoxycytidine and 2'-deoxyguanosine in the Assiros *T. monococcum* (the only sample examined for nucleosides) and tentative identifications of cytosine and thymine in the Danebury *T. spelta* and the Assiros *T. dicoccum* and *T. monococcum* (the only samples examined for nucleotide bases). These results are not presented as evidence for the presence of ancient DNA as we are unable to confirm that the charred seeds are free from microbial DNA, the only indication of their sterility being provided by visual examination (e.g. Fig. 1).

We believe that the evidence presented in this paper can be interpreted in only one way: that polymeric ancient DNA survives in at least some charred plant remains. It is clear that DNA survival is not uniform as some accessions have failed to give positive results despite repeated attempts. Furthermore, extractions performed with individual grains of the Assiros *T. dicoccum* have been successful with only five out of 100 seeds tested so far. We are well aware that the presence of ancient DNA in charred seeds is contrary to the widely accepted descriptions of DNA decay (Lindahl, 1993a) which state that complete depolymerisation of DNA should occur at the high temperatures involved in the charring process. We note that various researchers, such as Collins (1995), have proposed mechanisms by which a small proportion of the biopolymers in a decaying specimen could be protected from degradation. One of these proposed mechanisms involves adsorption of a positively charged biopolymer such as DNA to an inorganic surface, such as might occur in the high-carbon environment within a charred seed. To further resolve the issue we intend carrying out experiments using the conditions described by Boardman and Jones (1990) with the objective of defining the decay pathway for DNA during artificial charring of modern seeds.

MATERIALS AND METHODS

Archaeological Plant Material

The charred specimens were 2000-year-old *T. spelta* from Danebury, UK (Jones, 1984; previously used for DNA studies by Allaby *et al.*, 1994), 3300-year-old *T. monococcum* and *T. dicoccum* from Assiros Toumba, Greece (Jones *et al.*, 1986), and 3000-year-old unidentified *Triticum* seeds from Fiavé, Italy. The Danebury samples were part of material excavated by B. Cunliffe (Jones, 1984), taken from well-sealed assemblages that were removed *en bloc* and immediately enclosed within aluminium foil. The Assiros material was excavated by K.A. Wardle (University of Birmingham, UK) and that and the Fiavé grains were provided by G. Jones, University of Sheffield, UK. The typical appearance of a charred grain is shown in Fig. 1.

DNA Extraction and Analysis

Nucleic acids were prepared from 500 mg samples of modern and ancient wheat specimens by the method previously described by O'Donoghue *et al.* (1996b). Extracts dissolved in 100 µl water were further purified by electroelution at 6.66 $V\,cm^{-1}$ for 1 h after fractionation in a 1.3% agarose gel (Towner, 1991) or by silica adsorption (Höss and Pääbo, 1993). Extracts of the charred *T. dicoccum* were incubated at 37°C for 2 h with 0.01 vol 10 $mg\,ml^{-1}$ DNase-free RNase A. All extracts were finally reprecipitated with 2 vol ice-cold absolute ethanol,

washed with 70% (v/v) ethanol, and redissolved in 100 µl water.

PCRs used the 'hot-start' strategy (Erlich *et al.*, 1991). PCRs directed at the glutenin loci (Allaby *et al.*, 1994) were carried out in 100 µl reaction mixes containing 10 µl extracted nucleic acid (approximately 10 ng for modern samples), 10 µl buffer (Boehringer-Mannheim), 50 µM each dNTP, 200 ng each primer and 2.5 units *Taq* DNA polymerase (Boehringer-Mannheim), using the primers described by Allaby *et al.* (1994) either with or without 5′ extensions (5′CTCTGGATCC-) containing a restriction site for cloning purposes, and using the cycle conditions described by Allaby *et al.* (1994). PCRs directed at the non-transcribed spacer regions of the wheat rDNA loci were carried out as described by Sallares *et al.* (1995). PCR products (25 µl aliquots) were fractionated in 3% NuSieve or MetaPhor agarose gels (FMC BioProducts), and cloned and sequenced as described by O'Donoghue *et al.* (1996b). Sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994) and subjected to principal coordinate analysis (Higgins, 1992). Neighbour-joining trees were obtained using CLUSTAL W and the topology verified by the maximum likelihood method with PHYLIP 3.57 (Felsenstein, 1989). Dot blot hybridisation analysis was carried out as described by Sallares *et al.* (1995).

Precautions were taken to minimise the risk of contaminating ancient material with modern plant DNA: ancient extracts were prepared in a separate room not used for handling modern plant DNA or PCR products; PCR mixes were set up in a third laboratory in a laminar flow cabinet (HEPA filter, Class 100, conforming to BS 5295 and 5726) used for no other purpose; the procedure included standard precautions regarding pipette types, sterilisation of solutions by autoclaving and ultraviolet irradiation; control experiments were run to check for contamination of solutions used in DNA extractions and in PCRs; and all ancient DNA amplifications were accompanied by an extraction blank (extraction carried out with no seeds) and a water blank (PCR set up with no DNA).

Acknowledgements

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