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How ancient DNA may help in understanding the origin and spread of agriculture

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The origin and spread of agriculture have been central questions in archaeology for the last 75 years and are increasingly being addressed by a multidisciplinary approach involving biologists, ecologists, geographers and anthropologists as well as archaeologists. Molecular genetics has the potential to make an important contribution, especially by enabling the number of times that a crop or animal was domesticated to be determined. Molecular genetics can also assign approximate dates to domestication events, identify the wild progenitor of a domesticate, and provide new forms of evidence relevant to agricultural spread. With wheat, molecular genetical studies of modern plants have suggested that einkorn was domesticated just once but that emmer might have been domesticated more than once. Ancient DNA studies of animal remains have benefited from progress made with equivalent analyses of human bones, and with plant material there have been clear demonstrations of DNA preservation in desiccated seeds. Charred remains have also been shown to contain ancient DNA but this finding is unexpected in view of the high temperatures to which these seeds have supposedly been exposed. Ancient DNA studies of wheat remains have been used in taxonomic identification and in assessment of the possible breadmaking quality of the wheat grown at an Early Bronze Age site in Greece.

Keywords: agriculture; ancient DNA; archaeobotany; archaeology; molecular phylogenetics; plant domestication

1. INTRODUCTION

Since the end of the last Ice Age, some 10 000 years ago, the predominant means of food acquisition for human communities has changed from hunting–gathering to agriculture. This shift was important in an ecological sense, representing a transition from the natural environment in control of humans to humans in control of the natural environment, and has also had far-reaching sociological consequences, enabling a steep increase in human population sizes and the eventual proliferation of complex societies and technologies (Harris 1996). The biological implications of animal and plant domestication as evolutionary processes were recognized by Darwin (1868) and the revolutionary impact of agriculture on prehistoric human communities was first fully expounded by Childe (1928). These seminal works established agriculture as a subject central to biological and archaeological endeavour, and extensive researches over the last 70 years have been devoted to the topic (summarized by Harris 1996). These researches are now taking on an increasingly multidisciplinary flavour—involving ecologists, geographers and anthropologists as well as biologists and archaeologists—but they have predominantly been driven by the archaeological context. As such, ideas regarding agriculture have been influenced by the changing paradigms in archaeology (Harris 1996), the emphasis of the processual era being on 'origins of agriculture' (e.g. Binford 1968), superseded by a post-processual preoccupation with 'domestication systems' (e.g. Hodder 1990), and most recently leading to a reassessment of the influence of climate change as the driving force behind the transition to agriculture, as originally suggested by Childe (e.g. Sherratt 1997).

It is absolutely correct that scholarly thought regarding the transition to agriculture should be centred within archaeology as opposed to any other academic discipline. It is, after all, the remit of archaeology to understand human prehistory and interpretations using the heuristic principles of, for example, evolutionary genetics, must therefore be consistent with archaeological evidence if they are to be meaningful. But it can be argued that the post-processual interpretation of the shift to agriculture as simply a small part of a much broader sociological transition, this view coupled in some cases with an apparent distrust of the applications of molecular genetics in addressing archaeological issues (e.g. Pluciennik 1996), has resulted in the relevance of biological studies being underemphasized. This is unfortunate because two developments in molecular genetics during the last five years have the potential to make an important impact on our understanding of the transition to agriculture. These developments are the increased refinement of molecular phylogenetic techniques, which enable inferences regarding the origins of domesticates to be made by examining the genetic features of living plants and animals, and the gradual elaboration of methodology for gaining meaningful genetic information from ancient DNA preserved in the remains of early domesticates. In this paper, I will illustrate the current achievements and future potential of molecular genetics in studies of agriculture by reviewing work carried out with emmer wheat. First, however, it is important to summarize which

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aspects of agriculture are and are not amenable to study by molecular genetics.

2. UNRESOLVED ISSUES REGARDING THE ORIGIN AND SPREAD AND AGRICULTURE

(a) *Origins of agriculture*

The possible geographical origins of agriculture were first discussed by de Candolle (1882) and Vavilov (1926), the latter identifying 12 'centres of origin' based on the hypothesis that for cultivated plants a simple relationship exists between modern centres of genetic diversity and geographical origins of domestication. This scheme has been extensively criticised (e.g. Zohary 1970) and re-interpreted (e.g. Zhukovsky 1975; Harlan 1971; Hawkes 1983), and its underlying value questioned (Harris 1996), but the assumption that agriculture began at a limited number of discrete locations is still widely held. On a global scale this scenario is defendable, as it is clear that the agricultural system based on wheat, barley and peas originated in South-west Asia, whereas that centred on rice derives from East Asia and that on maize from central America. But the more important question is whether within each centre the transition to agriculture was a chance and unusual advance made by a single group of protofarmers, or a more dispersed event that was an inevitable consequence of the evolving relationship between humans and the environment. Implicit in the former view is the prediction that each crop was domesticated just once, whereas if the latter view is correct then it might be anticipated that at least some of the crops grown by the first farmers were taken into cultivation on more than one occasion at different locations in the same general area. The importance of this question has been emphasized by Harris (1996) who stated: 'If it can be determined whether a particular plant or animal was domesticated once only, or several times in different areas, we can gain important insights into the early history of agriculture and pastoralism'.

Molecular genetics is well suited to determining how many times a plant or animal was domesticated and in fact may be the only way of addressing this question. If a species was domesticated just once then modern domesticates would be expected to display limited genetic diversity, compared with wild specimens, because the domesticated lineage would have passed through a genetic bottleneck at the time of the initial domestication. Molecular phylogenetics therefore has the potential to determine if a plant or animal was domesticated once or on multiple occasions, and to estimate the number of domestications if there were more than one. Comparison between the genetic features of modern domesticates and wild populations can also be used to identify the wild progenitor of each domesticate, though caution has to be exercised in assigning archaeological relevance to the latter information. There is a temptation to equate the current biogeographical range of the wild progenitor with the location of the domestication event (e.g. Heun *et al.* (1997) for einkorn wheat), but this assumes that the biogeography of the species has remained stable since the time of domestication. Also, at least in the case of plants, it has to be established that there was some reason for cultivating a species in a region where it grew in natural abundance, intuition suggesting that domestication has greater value at the edge of a species distribution, or completely outside its range, rather than at its core (Jones *et al.* 1998).

(b) *Spread of agriculture*

The occurrence of domesticates outside of the natural geographical range of the equivalent wild species indicates that after the first appearance of agriculture the novel practices spread to neighbouring geographical areas. On a broad scale, the initial spread of agriculture into new territory can be traced by searching the archaeological record for indicators of agriculture, both artefacts such as quernstones, and biological remains such as preserved seeds and the bones of domesticated animals. This conventional archaeological approach has led to a detailed description of the initial trajectories followed by agriculture from South-west Asia into Europe and Africa (Barker 1985; Bell 1987; Kislev 1992), and has been equally successful in charting expansions in other parts of the world (e.g. Bellwood (1996) for the Indo-Pacific region).

At the general level, the major questions concerning the spread of agriculture are complex and relate largely to the human activities responsible for the transfer from one location to another. These issues have been addressed by molecular genetic examination of human populations but the problems have not been resolved. For example, the work of Cavalli-Sforza and associates implies that the spread of agriculture from South-west Asia into Europe was accompanied by a large-scale movement of people (e.g. Cavalli-Sforza 1996), supporting the demic diffusion model previously expounded by Renfrew (1987) and others. In contrast, the genetic data of Richards *et al.* (1996) indicate substantial continuity of European human populations since the Palaeolithic, and hence are more consistent with agricultural spread by cultural diffusion, human migration being limited to, at most, a few 'pioneers'.

Genetic analysis of domesticates is unlikely to make any major contribution to these grand issues. Genotype comparisons can certainly be used to trace likely trajectories for a domesticate from one location to another, but it is not possible to determine if the movement of a domesticate was due to it being carried by a group of migrating humans or resulted from trade between two static communities. It is even possible for a domesticate to move in the opposite direction to a human migration, as happened with the potato. Genetic analysis of domesticates can therefore lend support to models of human activity based on archaeological and other evidence, but cannot be used as the sole form of data from which these models are constructed. Wheat genetics, for example, could contribute to our understanding of the spread of agriculture into Italy, but only by testing the alternative hypotheses, based on archaeological researches, that early Italian agriculture was derived from either Greece or the Balkans. This type of analysis is dependent to a large extent on the ability to obtain meaningful genetic information from ancient DNA preserved in archaeological remains. The use of ancient DNA also opens up possibilities for studying changes in farming practices, such as the adoption of new crop varieties at established agricultural

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sites, as has been demonstrated by Schlumbaum *et al.* (1998), who have shown by genetic analysis of charred grains that hexaploid wheats were cultivated in the Swiss alpine foreland at an earlier period than previously suspected.

3. MOLECULAR GENETIC STUDIES OF MODERN DOMESTICATES

(a) *Review of progress to date*

The use of modern and ancient DNA in studies of agricultural origins is the subject of a forthcoming review (Jones & Brown 1998). Here I summarize some salient points to provide the context within which our own studies of emmer wheat, described below, have been carried out.

As in other areas of phylogenetics, the application of genetic data to agricultural origins has passed through three phases. The first of these began in the 1960s when techniques for protein comparisons were developed, based largely on electrophoretic procedures that enabled isozyme variations to be scored. Among the breakthroughs made by studies of this type were identification of the subspecies *Zea mays parviglumis*, a variety of teosinte, as the likely progenitor of domesticated maize (Doebley 1990), and *Aegilops squarrosa* var. *strangulata* as the donor of the D genome of cultivated hexaploid wheats (Nishikawa 1983; Lagudah *et al.* 1993). The development of DNA sequencing techniques in the late 1970s led to the second phase of molecular phylogenetics, in which limited sequence information obtained from cloned DNA fragments was used as the data on which phylogenetic analysis was based. Notable among these studies were comparisons between chloroplast DNA sequences of wild and cultivated barley (Clegg *et al.* 1984; Neele *et al.* 1988) which showed that only one of the three haplotypes found in wild populations is present in cultivated plants, compatible with a genetic bottleneck as expected if this crop was domesticated just once, or at most on only a very few occasions (see also Zohary 1996).

Both the isozyme and cloned DNA approaches to molecular phylogenetics are laborious and neither are able to generate the large amounts of data needed for rigorous comparisons between the genotypes of different organisms. In this field, as in many others, the advent of the polymerase chain reaction (PCR) in the late 1980s has had a major impact. PCR enables genetic information to be obtained rapidly from many different organisms, meaning that a large number of specimens can be included in the analysis and greater confidence can be assigned to any deductions that are made. The development of PCR has been accompanied by the discovery of highly polymorphic regions of plant and animal genomes that provide excellent data for phylogenetic comparisons. The potential of these developments in genetic studies of agricultural origins is still being explored, the two most impressive advances so far being the use of microsatellite sequences to infer relationships between domesticated cattle (MacHugh *et al.*, this issue) and the identification, by amplified fragment length polymorphism (AFLP) fingerprinting, of a wild population of einkorn from the Karacadağ mountains of south-east Turkey that is a likely progenitor of all cultivated einkorns (Heun *et al.* 1997).

(b) *Studies of emmer wheat*

Wild emmer wheat (*Triticum dicoccoides*, nomenclature of Miller (1987)) is currently found in Israel, Jordan, Syria, Lebanon, south-east Turkey, north Iraq and west Iran (Zohary & Hopf 1993). The domesticated form, *T. dicoccum*, first appeared in South-west Asia during the eighth millennium BC (Bell 1987; Kislev 1992; Nesbitt & Samuel 1998) and subsequently spread into Europe, reaching the Balkans by 8500 BP, and then following two routes across Europe, one westwards along the coast to southern Italy, France and Spain, and one through the river valleys of central Europe, reaching the north coast by 6000 BP (Barker 1985). Emmer became the principal cereal of the European Neolithic and Bronze Age and was the progenitor of the free-threshing tetraploids (e.g. *T. durum*) and the hexaploid bread wheats (e.g. *T. spelta*, *T. aestivum*) that subsequently became predominant (van Zeist *et al.* 1991; Zohary & Hopf 1993). It can therefore be argued that understanding the origins of emmer cultivation is a key to a fuller appreciation of the evolutionary dynamics involved in the transition to agriculture in South-west Asia and Europe.

Our studies of the agricultural origins of emmer began as a broader examination of the evolution of the A, B, D and G genomes of cultivated wheats (Allaby *et al.* 1998). For this work we made use of the genes for the high-molecular-weight (HMW) glutenins, which are members of a large group of seed proteins. Two subfamilies of HMW glutenin subunits (x and y) arose by gene duplication before divergence of the A, B, D and G genomes. Single copies of the genes for the x and y subunits occur at two closely-linked loci, *Glu-1-1* and *Glu-1-2* (Thompson *et al.* 1983; Harberd *et al.* 1986), on homologous chromosome 1 (figure 1) (Payne *et al.* 1982). The loci are multiallelic (Payne & Lawrence 1983), the combination of alleles being largely responsible for the breadmaking quality of the wheat (e.g. Dong *et al.* 1991). We used the PCR to obtain short but phylogenetically informative sequences from the DNA regions immediately upstream of the HMW glutenin genes of a range of wheats and constructed a neighbour-joining tree from the sequences of 42 alleles. This tree comprised eight clades, each containing alleles from a different locus (Allaby *et al.* 1998).

With regards to the origins of emmer cultivation, the most intriguing feature of the glutenin tree was the fact that one of the eight clades, for *Glu-B1-1* (the x-subunit gene of the B genome), was split into two segments, the larger (α group) containing seven allele sequences and the smaller (β group) containing two (figure 2). The split displayed 1.87% sequence divergence (corrected for multiple hits using the Kimura two-parameter model). The estimate for synonymous nucleotide change in plant nuclear genes is $5.1–7.1 \times 10^{-9}$ substitutions site^{-1} year^{-1} (Wolfe *et al.* 1989), indicating that the α and β groups diverged 1.3–1.8 million years ago. This relatively ancient divergence time was unexpected as all eight alleles in the *Glu-B1-1* clade are from cultivated emmers or cultivated emmer descendants such as *T. aestivum*. Three different hypotheses can explain the observation that cultivated emmers contain two distinct gene lineages that diverged substantially before the origin of agriculture (Allaby *et al.* 1998). The first is that emmer was domesticated twice, the

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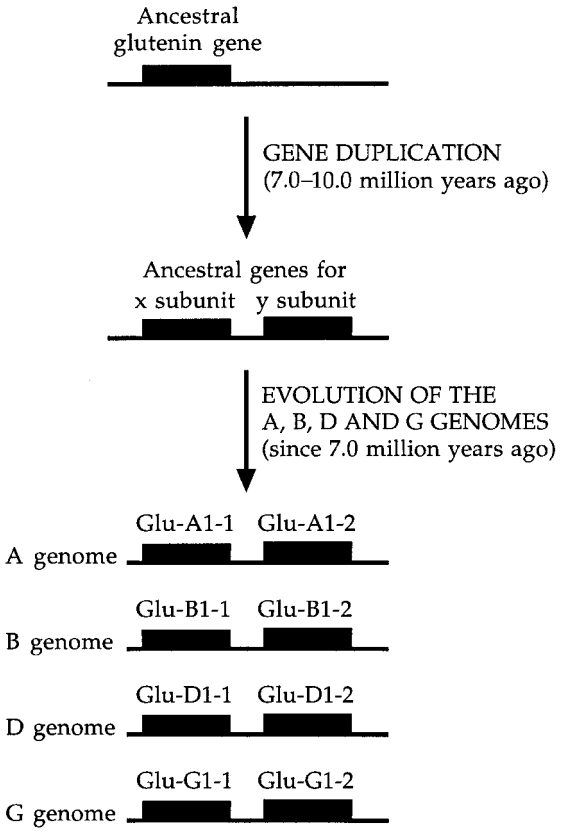

Figure 1. Diagram illustrating the evolution of the wheat glutenin genes and the identity of the present day *Glu* loci on the four wheat genomes. The ancestral glutenin gene, which existed prior to approximately 10 Ma ago (Allaby *et al*. 1998), duplicated to give rise to the genes for the x and y subunits of the modern high-molecular-weight glutenin proteins, present originally on chromosome 1 of the ancestral wheat genome. During the period since 7 Ma ago, this ancestral genome has evolved into the A, B, D and G genomes seen in modern wheats. Each version of chromosome 1 (i.e. chromosomes 1A, 1B, 1D and 1G) has a copy of the x-subunit gene (e.g. *Glu-A1-1*) and y-subunit gene (e.g. *Glu-A1-2*), and there are different allelic versions of each of these genes.

two *Glu-B1-1* subclades present in cultivated wheats reflecting the genotypes of the wild populations from which the domesticates were obtained. The second explanation is that emmer was domesticated only once but the original domesticates included plants with different *Glu-B1-1* genotypes. We discount this explanation because our more recent survey of 262 cultivated emmers has shown that the geographical distribution of modern cultivars with α alleles is drastically different from the distribution of β plants. If there was only one domestication then these non-identical biogeographical patterns could only have arisen if the α plants, which are more broadly distributed, have a selective advantage over β plants throughout the greater part of their range. The distribution patterns are such that no obvious selective pressure can be identified, and as far as we can tell there are no physiological differences between plants with α or β alleles.

The third explanation is that emmer wheat was domesticated once but subsequently acquired a second *Glu-B1-1* subclade by introgression from a wild emmer. This hypothesis is possible even though emmer wheat is predominantly self-pollinating and it can be argued that opportunities for introgression were restricted because the early farmers did not grow domesticated wheats in areas where wild varieties occurred naturally (Flannery 1969; Jones *et al*. 1998). To test if introgression has occurred we are currently analysing other variable loci in cultivated wheats, as the combinations of alleles at unlinked loci enable the extent of cross-hybridization between cultivated and wild wheats to be inferred.

The glutenin tree also enables deductions to be made about the origins of the hexaploid wheats, which arose by hybridization between emmer and the wild grass *A. squarrosa* (DD). As the natural ranges of *T. dicoccoides* and *A. squarrosa* do not overlap, and wild hexaploids are unknown, the emmer partner in this hybridization is assumed to have been a cultivated variety, meaning that all hexaploid wheats have a recent origin, not more than 9000 years ago. This implies that alleles at the *Glu* loci of hexaploid wheats should be relatively non-variable, reflecting the recent genetic bottleneck. This inference holds true for most loci but not *Glu-D1-2*, the latter displaying extensive heterogeneity in hexaploids. We have also discovered that the intergenic spacer regions of the ribosomal DNA loci of the B genome chromosomes of hexaploid wheats display a relatively high degree of nucleotide sequence variability (R. Sallares, unpublished data). Both observations suggest that hexaploid wheats have multiple origins.

4. ANCIENT DNA STUDIES

(a) *Preservation of ancient DNA in plant and animal remains*

The excitement that surrounded the discovery of ancient DNA in plant remains (Rollo *et al*. 1987) and human bones (Hagelberg *et al*. 1989) led to an expectation that ancient DNA studies would become routinely applicable to many questions in archaeology. This initial enthusiasm was dampened by worries that working practices were not always rigorous enough to preclude the possibility that 'ancient' DNA sequences were in fact modern contaminants (Lindahl 1993*b*), and by the realization that ancient DNA, when genuinely discovered, would often be too fragmented to be really useful in phylogenetic analysis (e.g. Rollo *et al*. 1991). The period of re-evaluation that the ancient DNA research community went through in the mid-1990s has resulted in the adoption of a more sophisticated research ethos including repetition of key results in an independent laboratory (e.g. Krings *et al*. 1997), combined with greater efforts to understand the decay processes for DNA in biological materials (Waite *et al*. 1997) and attempts to link these decay processes to other biomolecular changes, such as amino-acid racemization, which are easily measured and might provide a proxy for DNA survival (Poinar *et al*. 1996).

The greater confidence that has emerged during the last two years has enabled ancient DNA studies to make important contributions to our understanding of animal domestication, in particular with cattle (Bailey *et al*. 1996; Turner *et al*. 1998) and horses (Lister *et al*. 1998). These advances have been helped by the fact that considerable experience has been accumulated in the study of bone, both human and animal, and techniques for ancient DNA

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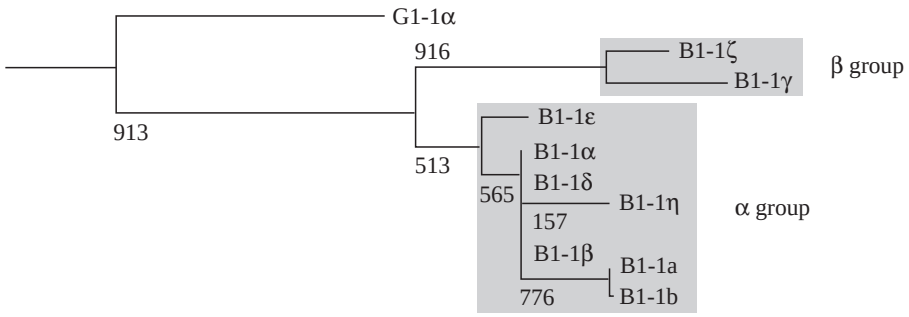

Figure 2. Part of a neighbour-joining tree of 42 glutenin allele sequences, obtained by multiple alignment of a 241–243 bp region immediately upstream of the open reading frame (Allaby *et al*. 1998). The topology of the region containing the alleles at the *Glu-B1-1* and *Glu-G1-1* loci is shown, with the α and β allele groups within the *Glu-B1-1* clade highlighted. The tree was bootstrapped by creating 1000 replicates with the CLUSTAL W program. (For details see Allaby *et al*. (1998).)

analysis with this type of material are now routine. With regard to plants, the methodological problems involved in ancient DNA studies have been more intractable, largely because the forms of preservation for plant remains are very different to those for humans and animals. Original or modified plant remains are frequently found in archaeological and earlier contexts, and can often be recovered in considerable quantities, the four principal modes of preservation being a partial reduction to carbon by oxidation (generally called 'carbonization' or 'charring'), the exclusion of water by desiccation, the exclusion of oxygen in anoxic, usually waterlogged environments, and partial mineralization by calcium phosphate, calcium carbonate or, less commonly in archaeological terms, iron sulphate (pyrites).

In an early paper (Brown *et al*. 1993), we presented evidence for DNA survival in plant remains representing each of these four types of preservation, but only with desiccated and charred remains has there been a sufficient body of subsequent research to confirm our original findings. With desiccated remains it has been clear for some time that there is potential for survival of polymeric DNA for at least several thousand years. This was first reported for mummified maize and cress seeds by Rollo *et al*. (1987, 1991, 1994) who used hybridization analysis and PCR to detect RNA and DNA, the latter less than 100 bp in length in even the best-preserved specimens. O'Donoghue *et al*. (1994, 1996*a*,*b*) extended these results by showing that the amounts of polymeric DNA in 1300-year-old desiccated radish seeds were large enough for the nucleosides and nucleotide bases to be detected by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS), but that despite this abundance, and the remarkable preservation of other biomolecules in these seeds, the DNA was still highly fragmented, the longest PCR products being 169 bp (figure 3). Using sorghum up to 2800 years in age from the same site, Qasr Ibrim in Upper Eqypt, Deakin *et al*. (1998) found similar difficulties in obtaining PCR products longer than 300 bp, but showed that these problems could be at least partially solved by using special techniques such as reconstructive PCR (Golenberg *et al*. 1996).

Ancient DNA studies with desiccated remains could answer some questions regarding plant domestication (e.g. Deakin *et al*. (1998), for sorghum) but the geographical restrictedness of desiccated material limits the impact that this work can have. For ancient DNA to be of general value in understanding plant domestication it will be necessary to make use of charred remains, charring being the commonest form of preservation for archaeological plant specimens with substantial amounts of material available from a broad geographical range. In early papers, Goloubinoff *et al*. (1993) reported the presence of maize DNA in extracts of 600-year-old burnt maize cobs, and Allaby *et al*. (1994) obtained similar evidence for DNA preservation in 2000-year-old charred wheat seeds. In view of the general scepticism surrounding ancient DNA work in the mid-1990s, we set out to obtain additional information to support or contradict the claim that ancient DNA is present in charred wheat seeds (Allaby *et al*. 1997; summarized in table 1). The reproducibility of the original results was demonstrated by carrying out extractions with replicate samples of charred grain, by repeating the work with different accessions, and by targetting more than one locus in the wheat nuclear genome. The physical features of the DNA in charred grain were shown to be consistent with those expected for ancient DNA, the DNA being extensively fragmented (figure 4) and chemically damaged. Genetic evidence was also consistent, charred grains containing alleles that were not present in modern wheats used in the laboratory. Wheat DNA was also detected in charred seed extracts by hybridization analysis, which is much less prone than PCR to false positive results arising from contamination with modern DNA. Our conclusion, supported by more recent work (Brown *et al*. 1998) is that some accessions of charred wheat grains contain ancient DNA, but that not all seeds in these accessions contain DNA, the number possibly being as low as one seed in 20 for 'positive' accessions.

Part of the reason for carrying out such an extensive analysis was the appreciation that DNA is not expected to survive in charred seeds. Plant remains of this type are thought to have undergone burning or baking and hence to have been exposed to high temperatures (possibly 250 °C for several hours: Boardman & Jones 1990) which would be expected to degrade DNA (Lindahl 1993*a*). Attempts to detect polymeric proteins and lipids in charred seeds have been unsuccessful (R. P. Evershed and N. Tuross, personal communication), although the relevance of this observation is questionable as the detection methods for proteins and lipids are much less sensitive than those for DNA and, as yet, no protein or lipid analysis has been carried out with an accession of charred seeds shown to contain ancient DNA. Artificial charring experiments with modern grain show that extensive depolymerization of DNA occurs in wheat seeds

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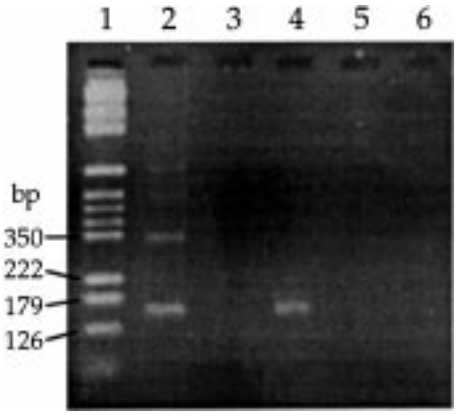

Figure 3. PCR analysis of extracts from 1300-year-old radish seeds, showing that despite the relative abundance of nucleic acids and other preserved biomolecules in these seeds, the DNA molecules are highly fragmented (O'Donoghue *et al*. 1996*b*). PCRs were directed at a 149 bp region of the multi-copy alphoid-like satellite DNA sequences in radish nuclear DNA (Grellet *et al*. 1986). PCR of modern radish seed extracts (lane 2) gave a series of PCR products because the repeat units occur in tandem arrays: the 169 bp band results from amplification of a single repeat unit, the 346 bp band from two linked repeat units, etc. Amplification of the ancient extract (lane 4) yielded only the smallest PCR product. Lane 1 contains size markers; lanes 3 and 5 show the results of PCRs with extraction blanks prepared in parallel with the modern and ancient extractions respectively; lane 6 shows the result of a water blank. Figure reproduced from O'Donoghue *et al*. (1996*b*) with permission of the Royal Society.

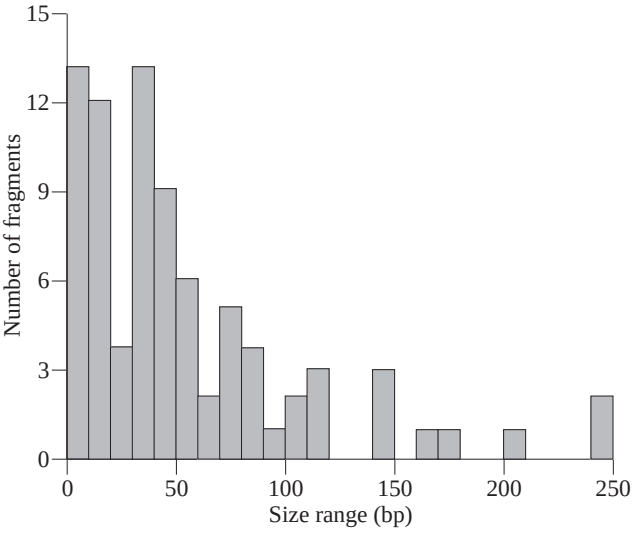

Figure 4. Histogram showing the estimated sizes of the DNA molecules in the 3000-year-old charred wheat from Assiros (Allaby *et al*. 1997). The fragment sizes were estimated by examining the sequences of cloned PCR products obtained after amplification of the HMW glutenin loci. These sequences were chimaeric, being made up of segments derived from different alleles linked together by PCR jumping (Allaby *et al*. 1998). According to our modelling of the events leading to the assembly of chimaeric PCR products from fragmented template DNA molecules via PCR jumping (Allaby & Brown 1999), the segment size distribution provides a rough approximation of the size range of the template molecules. According to this hypothesis the average length of the DNA molecules in the Assiros grain was 50–70 bp.

heated to 250 °C for longer than 30 min, but that decay is substantially less rapid at 200 °C and below (Threadgold & Brown 1999). It has not yet proved possible to devise artificial charring conditions that reproduce the range of morphological and ultrastructural features seen in archaeological remains. At present, the most likely explanation for the presence of ancient DNA in charred grains is that the few seeds in an accession that contain DNA have undergone sufficient transformation to preclude extensive microbial decay of the remaining biomolecules, but have not been exposed to temperatures high enough to cause total biomolecular depolymerization. An important unresolved question is whether the assumption that 'charred' grains have been exposed to high temperatures is correct, or if the transformation displayed by archaeological remains could result from a long-term, low-temperature oxidative process.

(b) *Ancient DNA in studies of wheat domestication*

Despite the early doubts about the survival of DNA, it has proved possible to use ancient DNA analysis of charred grains in studies relevant to the origin and spread of wheat cultivation. The main initial success has been in using genetic analysis in taxonomic identification. Cultivated wheats include a number of different species (in the archaeobotanical if not genetic sense) and although morphological features can be used to distinguish between these when intact plants are examined, taxonomic identification is often less easy when only fragments or explants such as dehusked grain are available. In particular, agriculturally important varieties such as *Triticum turgidum*, *T. durum* (tetraploid wheats) and *T. aestivum* (a hexaploid) (nomenclature of Miller (1987)) are often impossible to distinguish in the archaeological record. Hexaploids do not exist in the wild and the origins, evolution and changing importance of these forms are central questions in prehistoric agriculture (e.g. Sallares 1991), questions that cannot be answered without secure identifications of archaeobotanical remains.

The main genetic distinction between tetraploid and hexaploid wheats is that the latter contain two copies of the D genome, derived from *A. squarrosa*. Comparison between DNA sequences for D genome loci and those on the A and B genomes revealed differences in the intergenic spacer regions of the ribosomal DNA regions (Sallares *et al*. 1995), but attempts to use these differences as a means of identifying charred grains of hexaploid wheats proved fruitless (Brown *et al*. 1998) because the DNA in charred grains was too fragmented to give unambiguous results in the genetic fingerprinting method that was employed. Greater success was achieved when a second approach was adopted, based on the presence or absence of the HMW glutenin alleles characteristic of the D genome. With archaeological remains this was first applied successfully to taxonomic identification of a 3000-year-old assemblage of charred grain from the Early Bronze Age site of Assiros, Greece (Brown *et al*. 1998), and subsequently to Neolithic wheats from Switzerland, the latter project showing that hexaploid wheats were cultivated in the Swiss alpine foreland earlier than had previously been thought (Schlumbaum *et al*. 1998).

The taxonomic analysis of the Assiros grain also provided some additional interesting results. Five HMW

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Table 1. *Evidence for the presence of ancient DNA in charred wheat seeds* (*Allaby* et al. *1997*)

form of evidence	details
reproducibility	1. Some assemblages regularly give positive results when tested for the presence of ancient DNA (e.g. 2000-year-old *T. spelta* from Danebury, UK; 3000-year-old mixed grains from Assiros, Greece).
	2. Some assemblages regularly give negative results (e.g. 3000-year-old *T. monococcum* from Assiros; 3000-year-old wheat from Fiavé, Italy).
	3. Positive assemblages give positive results when PCRs are directed at different genetic loci.
	4. Equivalent results have been obtained in other laboratories (e.g. Dr A. Schlumbaum, University of Basel).
physical features	1. DNA molecules in charred grains are fragmented: e.g. in the Assiros grain the bulk of the polynucleotides are less than 150 bp with an average length of 50–70 bp (figure 4).
	2. DNA molecules in charred grains are chemically damaged: e.g. sequences of cloned PCR products from the Assiros grain contained approximately 12 times as many unexpected base changes as sequences of cloned PCR products from modern wheat.
genetic features	DNA from charred wheats contain genetic alleles that are not present in the modern wheats that we use in the laboratory: e.g. the Assiros grain contained two HMW glutenin alleles not previously reported in modern wheats studied at UMIST or elsewhere.
use of methods other than PCR	DNA can be detected in charred wheat extracts by hybridization analysis.

glutenin alleles were identified in this accession, three of which had been previously identified in modern bread wheats. One of these glutenin alleles, called *Glu-D1-1b*, is thought by plant biotechnologists to code for a glutenin protein with a highly helical structure (i.e. a very springy protein) that confers superior viscoelastic properties on the dough. The *Glu-D1-1b* allele is therefore associated with good breadmaking quality and was not expected to be common in prehistoric wheats as these are not thought to give rise to particularly good quality bread, although it has been suggested that Linear B texts from Bronze Age Greece include early references to the existence of bakers. Possibly the presence of *Glu-D1-1b* in the Assiros grain is a chance discovery and the allele is indeed uncommon in prehistoric wheats. It is, however, an interesting demonstration of the type of information that can be obtained by ancient DNA analysis of plant remains.

5. CONCLUSIONS

I have argued that genetic studies of modern and archaeological specimens can make a significant contribution to research into the origin and spread of agriculture. The potential will be fully realised only if the issues being addressed are carefully defined and data resulting from molecular genetics are interpreted within the archaeological context. This is particularly true for studies involving ancient DNA, as the difficulties in obtaining authentic results combined with the shortness of ancient DNA sequences means that results must always be backed up with other forms of evidence. The potential of molecular genetics as a means of providing novel information critical to central issues in archaeology is illustrated by recent results from studies of modern plants that suggest that einkorn wheat was domesticated just once but that emmer wheat may have been domesticated at least twice, and by ancient DNA studies which have revealed unexpected genetic features in Bronze Age grain.

The work of the UMIST group on emmer wheat has been carried out by Robin Allaby, Robert Sallares, Kerry O'Donoghue, Monica Banerjee, Jayne Threadgold, Lisa Nencioni, Sarah Lindsay, Victoria James and Sara Horton and has benefited greatly from collaborations with Martin Jones (University of Cambridge), Glynis Jones (University of Sheffield), Richard Evershed (University of Bristol), Angela Schlumbaum (University of Basel) and Keri Brown (UMIST). Martin Jones, Robin Allaby and Keri Brown are thanked for critical reading of this paper, though the opinions expressed are those of the author. The UMIST group has received funding from NERC, BBSRC, the Royal Society and the Leverhulme Trust.

REFERENCES

Allaby, R. G. & Brown, T. A. 1999 Use of multigene PCR to assess DNA fragmentation. (In preparation.)

Allaby, R. G., Jones, M. K. & Brown, T. A. 1994 DNA in charred wheat grains from the Iron Age hillfort at Danebury, England. *Antiquity* **68**, 126–132.

Allaby, R. G., O'Donoghue, K., Sallares, R., Jones, M. K. & Brown, T. A. 1997 Evidence for the survival of ancient DNA in charred wheat seeds from European archaeological sites. *Ancient Biomolec.* **1**, 119–129.

Allaby, R. G., Banerjee, M. & Brown, T. A. 1998 Evolution of the high-molecular-weight glutenin loci of the A, B, D and G genomes of wheat. *Genome*. (In the press.)

Anderson, O. D. & Greene, F. C. 1989 The characterization and comparative analysis of high-molecular-weight glutenin genes from genomes A and B of a hexaploid bread wheat. *Theor. Appl. Genet.* **77**, 689–700.

Bailey, J. F., Richards, M. B., Macaulay, V. A., Colson, I. B., James, I. T., Bradley, D. G., Hedges, R. E. M. & Sykes, B. C. 1996 Ancient DNA suggests a recent expansion of European cattle from a diverse wild progenitor species. *Proc. R. Soc. Lond.* B **263**, 1467–1473.

Barker, G. 1985 *Prehistoric farming in Europe*. Cambridge University Press.

Bell, G. D. H. 1987 The history of wheat cultivation. In *Wheat breeding—its scientific basis* (ed. F. G. H. Lupton), pp. 31–49. London: Chapman & Hall.

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Bellwood, P. 1996 The origins and spread of agriculture in the Indo-Pacific region: gradualism and diffusion or revolution and colonization? In *The origins and spread of agriculture and pastoralism in Eurasia* (ed. D. R. Harris), pp. 465–498. London: UCL Press.

Binford, L. R. 1968 Post-Pleistocene adaptations. In *New perspectives in archaeology* (ed. S. R. Binford & L. R. Binford), pp. 313–341. Chicago, IL: Aldine.

Boardman, S. & Jones, G. 1990 Experiments on the effects of charring on cereal plant components. *J. Archaeol. Sci.* **17**, 1–11.

Brown, T. A., Allaby, R. G., Brown, K. A. & Jones, M. K. 1993 Biomolecular archaeology of wheat: past, present and future. *World Archaeol.* **25**, 64–73.

Brown, T. A., Allaby, R. G., Sallares, R. & Jones, G. 1998 Ancient DNA in charred wheats: taxonomic identification of mixed and single grains. *Ancient Biomolec.* (In the press.)

Cavalli-Sforza, L. L. 1996 The spread of agriculture and nomadic pastoralism: insights from genetics, linguistics and archaeology. In *The origins and spread of agriculture and pastoralism in Eurasia* (ed. D. R. Harris), pp. 51–69. London: UCL Press.

Childe, V. G. 1928 *The most ancient East: the oriental prelude to European prehistory.* London: Kegan Paul, Trench, Trubner.

Clegg, M. T., Brown, A. H. D. & Whitfield, P. R. 1984 Chloroplast DNA diversity in wild and cultivated barley: implications for genetic conservation. *Genet. Res.* **43**, 339–343.

Darwin, C. 1868 *The variation of animals and plants under domestication*, vol 1. London: John Murray.

Deakin, W. J., Rowley-Conwy, P. & Shaw, C. H. 1998 Amplification and sequencing of DNA from preserved sorghum of up to 2800 years antiquity found at Qasr Ibrim. *Ancient Biomolec.* **1**, 27–41.

de Candolle, A. 1882 *Origine des plantes cultivés.* Paris: Germer Baillière.

Doebley, J. 1990 Molecular evidence and the evolution of maize. *Econ. Bot.* **44** (suppl.), 6–28.

Dong, H., Cox, T. S., Sears, R. G. & Lookhart, G. L. 1991 High molecular weight glutenin genes: effects on quality in wheat. *Crop Sci.* **31**, 974–979.

Flannery, K. 1969 Origins and ecological effects of early domestication in Iran and the Near East. In *The domestication and exploitation of plants and animals* (ed. P. J. Ucko & G. W. Dimbleby), pp. 73–100. London: Duckworth.

Golenberg, E. M., Bickel, A. & Weihs, P. 1996 Effect of highly fragmented DNA on PCR. *Nucl. Acids Res.* **24**, 5026–5033.

Goloubinoff, P., Pääbo, S. & Wilson, A. C. 1993 Evolution of maize inferred from sequence diversity of an adh2 gene segment from archaeological specimens. *Proc. Natn. Acad. Sci. USA* **90**, 1997–2001.

Grellet, F., Delcasso, D., Panabieres, F. & Delseny, M. 1986 Organization and evolution of a higher plant alphoid-like satellite DNA sequence. *J. Molec. Biol.* **187**, 495–507.

Hagelberg, E., Sykes, B. & Hedges, R. E. M. 1989 Ancient bone DNA amplified. *Nature* **342**, 485.

Harberd, N. P., Bartels, D. & Thompson, R. D. 1986 DNA restriction-fragment variation in the gene family encoding high molecular weight (HMW) glutenin subunits of wheat. *Biochem. Genet.* **24**, 579–596.

Harlan, J. R. 1971 Agricultural origins: centers and noncenters. *Science* **174**, 468–474.

Harris, D. R. 1996 Introduction: themes and concepts in the study of early agriculture. In *The origins and spread of agriculture and pastoralism in Eurasia* (ed. D. R. Harris), pp. 1–9. London: UCL Press.

Hawkes, J. G. 1983 *The diversity of crop plants.* Cambridge, MA: Harvard University Press.

Heun, M., Schäfer-Pregl, R., Klawan, D., Castagna, R., Accerbi, M., Borghi, B. & Salamini, F. 1997 Site of einkorn wheat domestication identified by DNA fingerprinting. *Science* **278**, 1312–1314.

Hodder, I. 1990 *The domestication of Europe.* Oxford: Basil Blackwell.

Jones, M. K. & Brown, T. A. 1998 Agricultural origins: the evidence of modern and ancient DNA. *Holocene.* (In the press.)

Jones, M. K., Allaby, R. G. & Brown, T. A. 1998 Wheat domestication. *Science* **279**, 302–303.

Kislev, M. E. 1992 Agriculture in the Near East in VIIth millennium BC. In *Préhistoire de l'agriculture: nouvelles approches expérimentales et ethnographiques* (Monographie 6, Centre de Recherches Archéologiques) (ed. P. C. Anderson), pp. 87–93. Paris: Editions du CNRS.

Krings, M., Stone, A., Schmitz, R. W., Krainitzi, H., Stoneking, M. & Pääbo, S. 1997 Neandertal DNA sequences and the origin of modern humans. *Cell* **90**, 19–30.

Lagudah, E. S., Appels, R., McNeil, D. & Schachtman, D. P. 1993 Exploiting the diploid D genome chromatin for wheat improvement. In *Gene conservation and exploitation, 20th Stadler genetics symposium* (ed. J. P. Gustafson, R. Appels & P. Raven), pp. 87–107. New York: Plenum Press.

Lindahl, T. 1993*a* Instability and decay of the primary structure of DNA. *Nature* **362**, 709–715.

Lindahl, T. 1993*b* Recovery of antediluvian DNA. *Nature* **365**, 700.

Lister, A. M., Kadwell, M., Kaagan, L. M., Jordan, W. C., Richards, M. B. & Stanley, H. F. 1998 Ancient and modern DNA in a study of horse domestication. *Ancient Biomolec.* (In the press.)

Miller, T. E. 1987 Systematics and evolution. In *Wheat breeding—its scientific basis* (ed. F. G. H. Lupton), pp. 1–30. London: Chapman & Hall.

Neele, D. B., Saghai-Marrof, M. A., Allard, R. W., Zhang, Q. & Jorgensen, R. A. 1988 Chloroplast DNA diversity in populations of wild and cultivated barley. *Genetics* **120**, 1105–1110.

Nesbitt, M. & Samuel, D. 1998 Wheat domestication: archaeobotanical evidence. *Science* **279**, 1433.

Nishikawa, N. 1983 Species relationship of wheat and its putative ancestors as viewed from isozyme variation. *Proc. 6th Int. Wheat Genetics Symp., Kyoto, Japan,* pp. 53–63.

O'Donoghue, K., Brown, T. A., Carter, J. F. & Evershed, R. P. 1994 Detection of nucleotide bases in ancient seeds using gas chromatography/mass spectrometry and gas chromatography/mass spectrometry/mass spectrometry. *Rapid Comm. Mass Spectrom.* **8**, 503–508.

O'Donoghue, K., Brown, T. A., Carter, J. F. & Evershed, R. P. 1996*a* Application of high performance liquid chromatography—mass spectrometry with electrospray ionisation to the detection of DNA nucleosides in ancient seeds. *Rapid Comm. Mass Spectrom.* **10**, 495–500.

O'Donoghue, K., Clapham, A., Evershed, R. P. & Brown, T. A. 1996*b* Remarkable preservation of biomolecules in ancient radish seeds. *Proc. R. Soc. Lond.* B **263**, 541–547.

Payne, P. I., Holt, L. M., Worland, A. J. & Law, C. N. 1982 Structural and genetic studies on the high-molecular-weight subunits of wheat glutenin. 3. Telocentric mapping of the subunit genes on the long arms of the homeologous group 1 chromosomes. *Theor. Appl. Genet.* **63**, 129–138.

Payne, P. I. & Lawrence, G. J. 1983 Catalogue of alleles for the complex gene loci, *Glu-A1*, *Glu-B1* and *Glu-D1* which code for high molecular weight subunits of glutenin in hexaploid wheat. *Cereal Res. Commun.* **11**, 29–35.

Pluciennik, M. 1996 Genetics, archaeology and the wider world. *Antiquity* **70**, 13–14.

Poinar, H. N., Höss, M. M., Bada, J. L. & Pääbo, S. 1996 Amino acid racemization and the preservation of DNA in the archaeological and palaeontological record. *Science* **272**, 864–866.

Reddy, P. & Appels, R. 1993 Analysis of a genomic DNA segment carrying the wheat high-molecular-weight (HMW)

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glutenin Bx17 subunit and its use as an RFLP marker. *Theor. Appl. Genet.* **85**, 616–624.
Renfrew, C. 1987 *Archaeology and language, the puzzle of Indo-European origins*. London: Jonathan Cape.
Richards, M., Côrte-Real, H., Forster, P., Macaulay, V., Wilkinson-Herbots, H., Demaine, A., Papiha, S., Hedges, R., Bandelt, H.-J. & Sykes, B. 1996 Paleolithic and Neolithic lineages in the European mitochondrial gene pool. *Am. J. Hum. Genet.* **59**, 185–203.
Rollo, F., LaMarca, A. & Amici, A. 1987 Nucleic acids in mummified plant seeds: screening of twelve specimens by gel electrophoresis, molecular hybridization and DNA cloning. *Theor. Appl. Genet.* **73**, 501–505.
Rollo, F., Venanzi, F. M. & Amici, A. 1991 Nucleic acids in mummified plant seeds: biochemistry and molecular genetics of pre-Columbian maize. *Genet. Res.* **58**, 193–201.
Rollo, F., Asci, W. & Sassaroli, S. 1994 Assessing the genetic variation in pre-Columbian maize at the molecular level. In *Conservation of plant genes II: utilization of ancient and modern DNA* (ed. R. P. Adams, J. S. Miller, E. M. Golenberg & J. E. Adams), pp. 27–35. St Louis: Missouri Botanical Garden.
Sallares, R. 1991 *The ecology of the Ancient Greek World*. London: Duckworth.
Sallares, R., Allaby, R. G. & Brown, T. A. 1995 PCR-based identification of wheat genomes. *Molec. Ecol.* **4**, 509–514.
Schlumbaum, A., Neuhaus, J.-M. & Jacomet, S. 1998 Coexistence of tetraploid and hexaploid naked wheats in a neolithic lake dwelling of Central Europe. Evidence from morphology and ancient DNA. *J. Archaeol. Sci.* (In the press.)
Sherratt, A. 1997 Climatic cycles and behavioural revolutions: the emergence of modern humans and the beginning of farming. *Antiquity* **71**, 271–287.
Thompson, R. D., Bartels, D., Harberd, N. P. & Flavell, R. B. 1983 Characterisation of the multigene family coding for HMW glutenin subunits in wheat using cDNA clones. *Theor. Appl. Genet.* **67**, 87–96.
Threadgold, J. & Brown, T. A. 1999 Decay of DNA in artificially charred wheat seeds. *J. Archaeol. Sci.* (In preparation.)
Turner, C. L., Grant, A., Bailey, J. F., Dover, G. A. & Barker, G. W. W. 1998 Patterns of genetic diversity in extant and extinct cattle populations: evidence from sequence analysis of mitochondrial coding regions. *Ancient Biomolec.* (In the press.)
van Zeist, W., Wasylikowa, K. & Behre, K.-H. (eds) 1991 *Progress in Old World palaeoethnobotany*. Rotterdam: Balkema.
Vavilov, N. I. 1926 *Studies on the origin of cultivated plants*. Leningrad: Institut Botanique Appliqué et d'Amélioration des Plantes.
Waite, E., Child, A. M., Craig, O. E., Collins, M. J., Brown, T. A. & Gelsthorp, K. 1997 A preliminary investigation of DNA stability in bone during artificial diagenesis. *Bull. Soc. Géol. Fr.* **168**, 547–554.
Wolfe, K., Sharp, P. M. & Li, W.-H. 1989 Rates of synonymous substitution in plant nuclear genes. *J. Molec. Evol.* **29**, 208–211.
Zhukovsky, P. M. 1975 *World gene pool of plants for breeding. Mega-genecenters and endemic micro-genecenters*. Leningrad: N. I. Vavilov Institute of Plant Industry.
Zohary, D. 1970 Centers of diversity and centers of origin. In *Genetic resources in plants—their exploration and conservation* (ed. D. H. Frankel & E. Bennett), pp. 33–42. Oxford: Blackwell Scientific.
Zohary, D. 1996 The mode of domestication of the founder crops of Southwest Asian agriculture. In *The origins and spread of agriculture and pastoralism in Eurasia* (ed. D. R. Harris), pp. 142–158. London: UCL Press.
Zohary, D. & Hopf, M. 1993 *Domestication of plants in the Old World*, 2nd edn. Oxford Scientific Publications.

Discussion

L. Biek (*London, UK*). Have you tried to extract DNA from seeds buried under anoxic waterlogged deposits which have in my experience always shown to be remarkably well-preserved?

T. A. Brown. Waterlogged seeds might be good sources of preserved biomolecules but we have not yet examined this type of material.

D. R. Harris (*University College London, UK*). I would like to congratulate you on a most interesting paper, make a general comment, and ask one question. The comment concerns your repetition of what is a widespread misapprehension, often stated in the literature on 'agricultural origins', that agriculture originated at approximately the same time in South-west Asia (the 'Fertile Crescent'), East Asia (more specifically the middle and lower Yang-tse Valley in China), and the Americas (principally southern Mexico). The radiocarbon chronology now available, which is increasingly based on direct AMS dating of plant remains from archaeological sites, does not support this generalization. The evidence from South-west Asia (for wheat and barley domestication) is at least two millennia earlier than the earliest evidence from Mexico (for cucurbit and later maize domestication), and the evidence from East Asia (for rice domestication in the Yang-tse Valley—which it has been suggested may be as early as wheat and barley in South-west Asia) is as yet inconclusive.

My question relates to your data on the α and β emmer lineages in Europe. Might not the contrast in their distributions that your maps showed reflect, partly at least, different habitat preferences and local environmental variation, rather than two chronologically separate diffusions of emmer from South-west Asia?

T. A. Brown. I thank David Harris for correcting my error concerning the latest dating evidence for agricultural origins. With regards to the α and β subclades of the *Glu-B1-1* locus, we have observed that modern emmers of the α lineage are found in all areas of Europe, North Africa and West Asia, whereas the β lineage is restricted to Azerbaijan, Turkey, the Balkans, south-eastern and central Europe, and Italy, with the bulk of the specimens in central Europe and the north and central regions of Italy. We propose that these non-identical distributions of the two lineages could indicate that there were two expansions of emmer cultivation into Europe, one involving the α lineage and one involving the β lineage. Different habitat preferences and local environmental variations could also explain the data, but I feel that this is a less likely possibility for two reasons. First, although the genetic differences between the α and β lineages are profound they involve mutations that are almost certainly neutral and we therefore believe that α and β plants do not differ in any significant biochemical or physiological respect. Therefore their responses to the environment should be identical. Second, the nature of a preferred environment for the β plants is difficult to deduce from their distribution pattern as they are common in disparate regions (e.g. both north and south of the Alps), but not uniformly present in regions where the conditions might be thought to be similar (e.g. common in central Italy but absent from Iberia).

F. Rollo (*Università degli Studi di Camerino, Italy*). How do you explain that you find similar patterns of DNA degradation in desiccated and charred plant seeds?

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T. A. Brown. One would indeed expect the DNA in charred seeds to be more degraded than that in desiccated seeds. In fact, the data that I have presented probably underemphasize the differences. With 3000-year-old charred seeds, we have carried out a detailed analysis of the sizes of the ancient DNA molecules and concluded that their average length is 50–70 bp. With 1300-year-old desiccated seeds, all we have shown is that a PCR product of 169 bp is obtainable, whereas one of 350 bp is not. The DNA in the desiccated seeds could therefore have an average length >200 bp. Also, our GC/MS and LC/MS experiments suggest that, regardless of fragment length, the absolute amount of DNA is greater in desiccated, compared with charred, seeds.

R. P. Evershed (*University of Bristol, UK*). I was very interested in the abrupt weight loss that you see when heating seeds at around 200 °C. Could this be due to the loss of volatile Maillard products? It would be interesting to look at the kinetics of the reaction and see whether there is an acceleration in the rate of reaction at around that temperature. Also, it would be interesting to know whether or not the involatile compounds play any part in the preservation of DNA.

T. A. Brown. Volatilization of Maillard products is quite possibly the explanation of the weight loss that occurs at 200 °C. When we run DNA samples on agarose gels, we see a reduction in the blue-fluorescing material at this temperature, which might also indicate volatilization of Maillard products. We will investigate this issue further.

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