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## THE USE OF ANCIENT DNA IN PALEONTOLOGICAL STUDIES

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**ABSTRACT**—In the past several years the analysis of ancient DNA has become widely used in paleontology and archeology. DNA has been reported to have been isolated from a variety of organisms up to a hundred million years old. The analysis of these DNA molecules has been used to draw conclusions about phylogenetic relationships, migration of populations, and the emergence of pathogens. It has become apparent, however, that many experimental artifacts are associated with the study of ancient DNA and in several cases the DNA purported to have been of ancient origin has been shown to be due to contamination. This review will summarize the sources of ancient DNA, the information contained within it, the methods of analysis, and the limitations of these techniques.

### INTRODUCTION

The development of the field of molecular biology has revolutionized the way we study modern organisms. The techniques of molecular biology also have implications for the study of the past. Scientists have collected the physical remains of plants and animals for well over a hundred years, and have studied the morphology of these organisms. Now it may be possible to unlock information about the genetics and biochemistry of these organisms as well.

While it has been known for some time that ancient tissue can be remarkably well preserved, and demonstrated to contain DNA (e.g., Grimaldi et al., 1994; Rogers and Bendich, 1985; Pääbo, 1985a, 1987; Doran et al., 1986), the first report describing the cloning of DNA from an ancient sample came from the laboratory of Allan Wilson. In 1984, DNA was cloned (described below) from the quagga, an extinct member of the horse family (Higuchi et al., 1984). A piece of dried muscle from a 140-year old preserved quagga was sampled, and a fragment of quagga mitochondrial DNA (mtDNA) was cloned. Analysis of the DNA sequence suggested that the quagga was more closely related to the zebra than to other members of the equine family. At about the same time Svante Pääbo cloned *Alu* repeats, a DNA sequence found in many copies in the human genome, from a 2,400-year old Egyptian mummy (Pääbo, 1985b).

These early successes demonstrated that the techniques of molecular biology could be employed to study ancient DNA. DNA sequence from extinct organisms has been determined and used to help position that organism in a phylogenetic tree. DNA sequence comparisons of current and ancient individuals of extant organisms may allow us to answer questions such as whether a population has contracted or expanded (e.g., Diamond, 1990; Thomas et al., 1990). Specific DNA sequences have been used as molecular tags to follow the migration of specific populations over time (Torrioni et al., 1992; Hagelberg and Clegg, 1993). The development of civilization included the domestication of plants and animals, and it is of interest to know which organisms were first domesticated, and what the effects of domestication were for these species (e.g., Hardy et al., 1994, 1995). Ancient DNA has also been used to study diseases of early populations in order to understand the co-evolution of animals and their parasites (Spigelman and Lemma, 1993; Salo et al., 1994). Examination of plant and animal material found at excavation sites may be able to indicate what the diet of the site's inhabitants consisted of, as has been done

for modern species (Höss et al., 1992; Höss, 1995; Kohn et al., 1995).

The aim of this review is to provide an overview of the field. The sources of ancient DNA and the information gleaned from the analyses of the DNA sequences are described. The techniques used for cloning and analysis of ancient DNA, as well as the limitations associated with these techniques, are also discussed. For a discussion of studies on proteins found associated with ancient material, the reader is referred to one of several reviews on the subject (for example Ambler and Daniel, 1991; Lowenstein and Scheuenstuhl, 1991).

### CLONING OF ANCIENT DNA

The genetic information of an organism is contained in its DNA; this long helical molecule is composed of 4 nucleotides containing the bases Adenine, Cytosine, Guanine, and Thymine, often abbreviated as A, C, G, and T (Fig. 1). The order of the bases specifies the structure of proteins as well as regulatory information. DNA molecules are double helical, and bases from one strand form hydrogen bonds with bases in the other strand. The length of a DNA molecule is measured in base pairs.

Although DNA can be detected in ancient sources (Grimaldi et al., 1994; Rogers and Bendich, 1985; Pääbo, 1985a, 1987; Doran et al., 1986), the amount which can actually be extracted from a sample is too small for analysis. The value of ancient DNA lies in the ability to determine the sequence. This is often accomplished after cloning pieces of DNA. In this case, the term "cloning" refers to isolating the DNA from one or a few genes; it should not be confused with the cloning of organisms, where the transfer of all the DNA contained in the nucleus is performed. A length of DNA is linked into another DNA molecule, called a vector, that will allow the cloned DNA to be maintained and propagated in another organism, such as bacteria (Fig. 2). The first two reports of successful ancient DNA cloning used this direct cloning technique (Higuchi et al., 1984; Pääbo, 1985b). The amount of damage in ancient DNA samples (discussed below) makes it nearly impossible to clone the ancient DNA directly and explains the low efficiency of cloning observed by Higuchi et al. (1984) and Pääbo (1985b).

In 1985 Kary Mullis described the polymerase chain reaction (PCR) (Saiki et al., 1985; Mullis and Faloona, 1987; see also Thomas and Pääbo, 1993), which solved some of the problems associated with ancient DNA (Fig. 3). PCR is an extremely powerful molecular technique that can make many copies of a short specific DNA segment in vitro using a polymerase, an enzyme that copies DNA. The DNA polymerase requires a

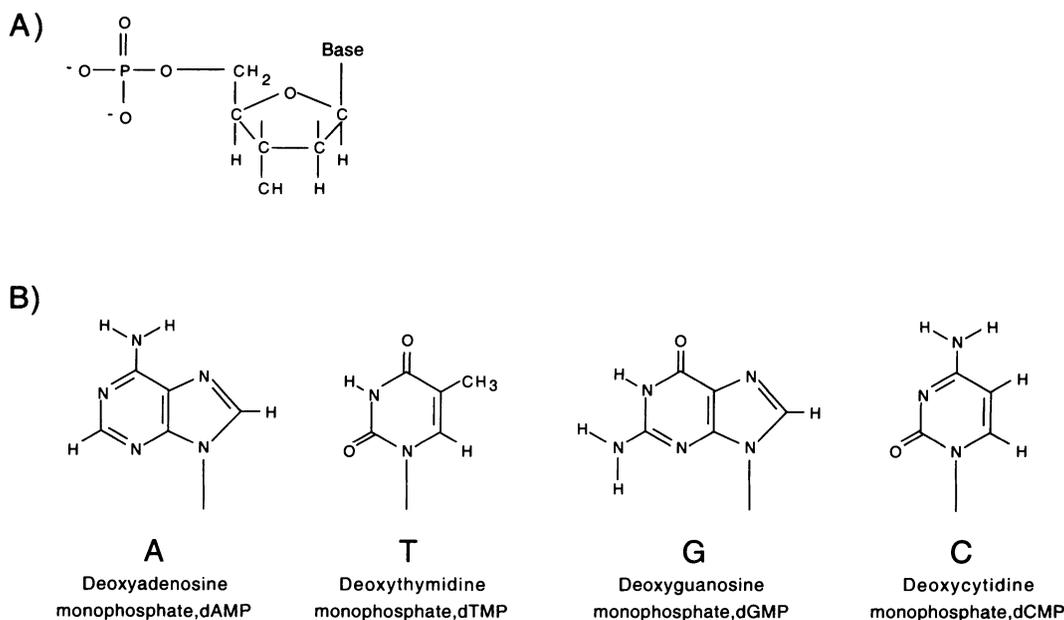


FIGURE 1. Schematic structure of nucleotides. Deoxyribonucleotides contain deoxyribose, which links the phosphate group to the nitrogen base (A). The different nucleotides are defined by the base. The structure of the 4 bases found in DNA are shown (B).

primer (a short piece of DNA); it cannot synthesize DNA without it. Two DNA primers are designed to flank the region of interest (the target). These primers are used to direct the synthesis of target DNA from a small amount of template DNA (the DNA to be copied) (Saiki et al., 1985; Mullis and Faloona, 1987). Repeated rounds of DNA synthesis result in a geometric amplification of the template DNA (Fig. 3).

To perform a PCR reaction, polymerase, buffers, and DNA bases are commercially available. Two components of the reaction have to be provided by the researcher; the DNA template, which should be as pure as possible, and the PCR primers, which often are the most critical component for a successful PCR reaction. A detailed consideration of primer design can be found in several papers on the subject (e.g., Innis and Gelfand, 1990; Dieffenbach et al., 1995; Kidd and Ruano, 1995). The considerations most important for ancient DNA work are only briefly summarized here. The primers are designed to recognize one specific region of DNA. By choosing a region of DNA that is unique to the organism of interest, PCR artifacts due to contamination can be minimized. It is also important to design a primer that recognizes a unique region of the organism's DNA; if a DNA sequence is used that appears many times in the genome, many PCR products will be generated from the different regions of the DNA that will not represent the target sequence. PCR has proven to be a very powerful technique in working with tiny amounts of template DNA. This technique has been successful in amplifying DNA from a single cell, and has become a standard technique in forensic science, where samples are typically small and have been subjected to environmental decomposition (sunlight, heat, humidity, etc.) (reviewed in Lee et al., 1994).

It was immediately apparent that PCR could, in theory, circumvent some of the problems associated with the quality and quantity of DNA extracted from ancient tissue. Due to the exquisite sensitivity of PCR, a gene could be amplified if only one or a few intact molecules survived. PCR could be used to generate many copies of the ancient sequence, and the copied DNA could be used in further cloning steps (Fig. 2).

The PCR products can be cloned into a bacterial vector, or sequenced directly (e.g., Thomas et al., 1989). Direct sequencing of PCR products is thought to give a more accurate picture of the ancient DNA sequence (e.g., Sidow et al., 1991). Because ancient DNA is damaged, some of the PCR products will carry an incorrect sequence. This artifact can be minimized, in theory, by sequencing the pool of copied DNA molecules, most of which will have the correct sequence, and a few of which will

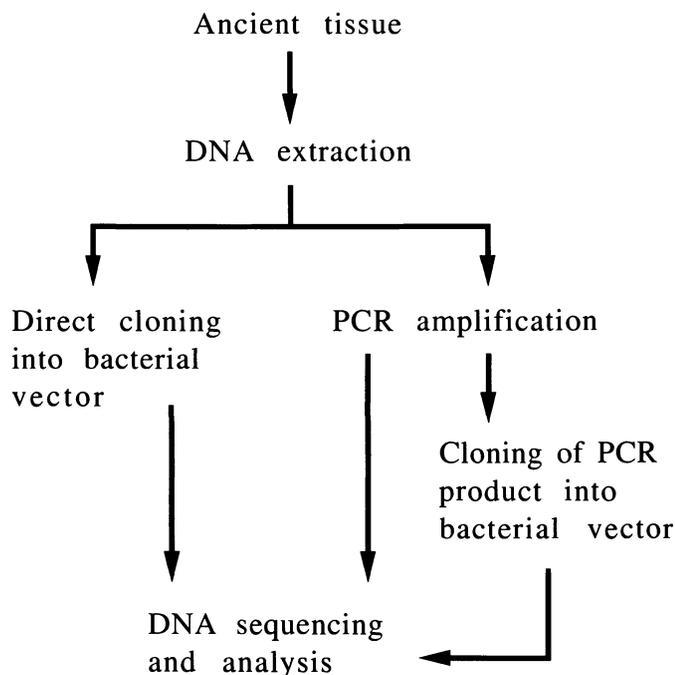


FIGURE 2. General scheme for the use of ancient DNA. Ancient DNA is first extracted from tissue, then that DNA may be used for either direct cloning into a bacterial vector or, more commonly, PCR is used to amplify a particular region of interest, and then the amplified DNA is used both to obtain the DNA sequence of the region and to transfer the region into a bacterial vector. See text for details.

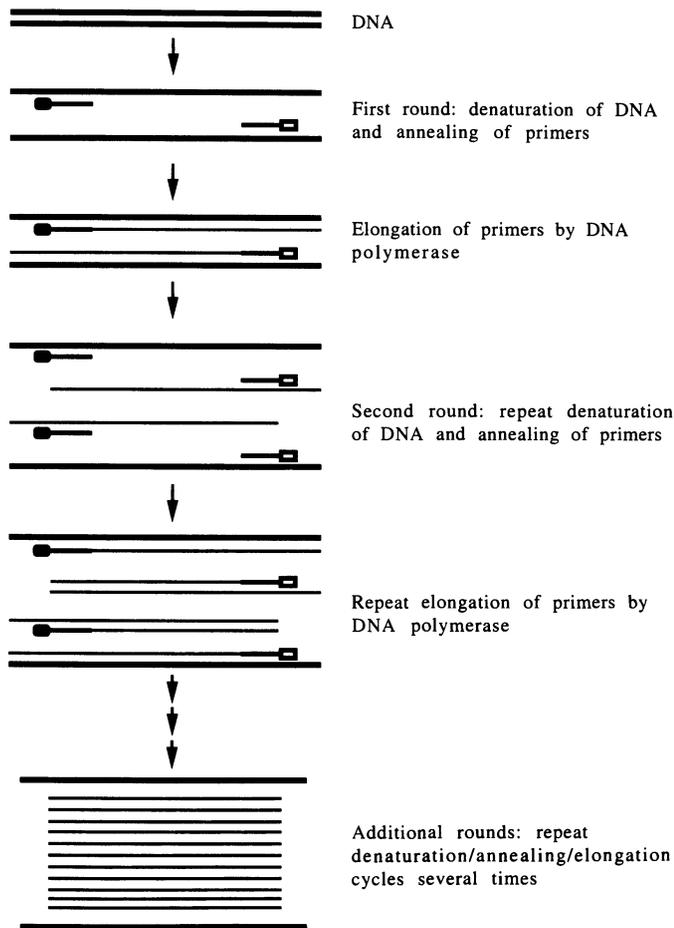


FIGURE 3. Strategy for PCR. A small amount of template DNA is incubated with primers, pieces of DNA designed to flank the region of interest. Addition of an enzyme that can elongate the primers to copy the template DNA results in identical copies of the template DNA. The DNA strands are then separated using heat, and fresh primers can anneal to the DNA. Repeated rounds of copying result in a geometric amplification of the DNA within the region of interest.

have the incorrect sequence but will not be detected by the technique. In addition, PCR is carried out more than once on each aliquot of extracted ancient DNA. These precautions are often taken when working with ancient DNA samples to prevent the cloning of one of the incorrect PCR products. Additional precautions and quantitation of template molecules have recently been suggested to be important in assuring the authenticity of ancient DNA sequences (discussed below).

If an entire gene is cloned, that gene can produce the protein it codes for. If a non-coding region or a fragment of a gene is isolated, the DNA sequence can be compared with sequence obtained from other members of the species, or other species. This sequence information can be used to infer phylogenetic relationships. There are several such regions commonly used in these studies, as thousands of sequences have been gathered from many species and have been organized into databases; these regions include portions of the mitochondrial cytochrome *b* gene, ribosomal RNA genes, nuclear *Alu* repeats, the chloroplast *rbcl* gene, and others (e.g., Horai et al., 1991; Epplen et al., 1992; Soltis et al., 1992; Goloubinoff et al., 1993; Poinar et al., 1993; Cooper, 1994a; Arcot et al., 1995; Béraud-Colomb et al., 1995; Hardy et al., 1994, 1995). The DNA sequences may be analyzed using one of several com-

puter algorithms available (for examples see <http://evolution.genetics.washington.edu/phylip/>).

#### QUALITY OF ANCIENT DNA

Despite the early success in cloning ancient DNA, problems were apparent that would continue to plague the field. The condition of the DNA in old samples became suspect; in each of the earliest reports the cloning was noted to have been extremely difficult and very inefficient (Higuchi et al., 1984; Pääbo, 1985b).

Examination of DNA from old sources showed that it had been degraded into small fragments only one to several hundred base pairs long (e.g., Pääbo, 1986; Hagelberg and Sykes, 1989; Golobinoff et al., 1993), in contrast to DNA extracted from fresh tissue, which can be obtained in long fragments measuring tens of thousands of base pairs long. DNA damage begins almost immediately after death. Bases are modified so that they no longer resemble the normal bases of DNA. Long strands of DNA are chopped into short fragments. DNA molecules become unnaturally crosslinked to each other. Studies have shown that much of the postmortem damage is due to oxidation and hydrolysis of the DNA molecules (Höss et al., 1996b; reviewed in Lindahl, 1993a). In order to preserve DNA, then, rapid removal of oxygen and/or water would be critical. For example, if desiccation were to occur rapidly after death, as would be the case in mummification, where the bodies were dried in salts, or in natural mummification, which can occur in dry climates, the DNA damage would be reduced. Besides the exclusion of water and oxygen, high salt concentrations, low temperature and neutral pH are factors that also slow down the DNA degradation process (Lindahl, 1993a).

Some postmortem autolytic damage seems to be inevitable. However, examination of old tissue suggests that once desiccation occurs, the DNA remains relatively stable for thousands of years (Kelman and Moran, 1996, and references therein). Extremities of mummies are particularly well preserved, and have been shown to contain intact cells and reasonably well-preserved DNA (Pääbo, 1987, and references therein). It has been suggested that some degradation may be due to cleavage of the DNA at the linker sequences between nucleosomes (the basic packaging unit of chromosomes) (Kelman and Moran, 1996). DNA is presumably protected because it is wrapped around nucleosome proteins. The unprotected "linker sequences" between adjacent nucleosomes, if cleaved, would yield fragments of approximately the size (100–200 base pairs) found in old samples.

Using a degradation rate obtained for DNA in aqueous solution it was calculated that DNA molecules the size of the human genome will degrade into short pieces in several thousand years under moderate conditions of temperature, pH, and salt concentration (Pääbo and Wilson, 1991; Lindahl, 1993a; see also DeSalle and Grimaldi, 1994). The racemization of amino acids has also been used as an indicator of DNA degradation, and also suggests the limit of survival of DNA molecules is several thousand years (Poinar et al., 1996). These theoretical calculations are for hydrated samples under moderate conditions. The time for DNA degradation may be extended in different environments. These calculations, however, raise skepticism when putative ancient DNA is retrieved from paleontological finds that are millions of years old (Table 1).

#### LIMITATIONS

Although PCR and molecular biology techniques represent advances in the study of ancient DNA, there are limitations to the techniques that should be considered when the data are analyzed.

Because ancient DNA is damaged, cloning is not practical

unless the DNA is first amplified using PCR. A drawback of this is that the primers (ends of the sequence to be amplified) must be provided. This complicates the recovery of novel regions of DNA. Many of the other limitations of using molecular techniques to analyze ancient DNA are due to the artifacts associated with PCR.

### Contamination of the Sample

Contamination is a major problem encountered when dealing with ancient DNA. Ancient specimens may be contaminated with ancient microorganisms. During the recovery and routine handling of artifacts, modern microorganisms may affix themselves to ancient tissues. Because PCR is routinely used to amplify DNA extracted from ancient samples, “contamination” would include the minute amounts of DNA that become associated with ancient tissue. This problem is a direct consequence of the sensitivity of the technique. PCR can amplify sequence from only one or a few intact template molecules, meaning that contaminating DNA from bacteria or other soil microorganisms present on the specimen (Herrmann and Hummel, 1994a), or from people who handled a specimen can act as a template in a PCR reaction (Handt et al., 1994a; Stoneking, 1995). This problem is especially acute in the recovery of ancient DNA, since so much of the old DNA is damaged. A very small amount of modern DNA will be preferentially amplified because it is undamaged. Special precautions must be taken when dealing with samples that contain ancient DNA (discussed below). It is not uncommon to find that non-human tissue has become contaminated with human DNA (e.g., van der Kuyl et al., 1995). Many specimens, particularly those from museums and private collections, have been handled extensively. Of course, a most difficult type of contamination to detect is that of modern human DNA (from handlers or researchers) on ancient human samples.

The sample itself is not the only source of contamination, however. Molecular biology laboratories contain a lot of DNA, which may contaminate samples. Special care must be taken to insure that no modern DNA is present to contaminate old samples in an effort to prevent a form of contamination called “PCR carryover,” which can occur if the DNA product of a previous PCR amplification is present in a laboratory. PCR generates large numbers of DNA molecules, and these molecules would be ideal templates for future PCR reactions. Another source of contamination in PCR was shown to occur as a result of bovine DNA present in the bovine serum albumin (BSA) that is used as an enzyme stabilizer in PCR reactions. It was demonstrated that by UV irradiation of the BSA solution this problem can be minimized (Taylor, 1996).

There is another artifact associated with the use of PCR in the analysis of ancient DNA. A curious consequence of the technique, called “jumping PCR,” could allow for the *in vitro* reconstruction of genes from fragmented DNA (Fig. 4). During PCR, if a damaged base is present on the template strand or if the DNA is fragmented, the polymerase stalls, or pauses. As the next cycle begins, this “incomplete” molecule will dissociate into two single strands. The “incomplete” DNA molecule can then associate with, or “jump” to, another template strand (Pääbo et al., 1990; DeSalle et al., 1993). The second template may also have DNA damage, but the likelihood of damage occurring at the same base in many template molecules is low. During this cycle, synthesis using a different template will bypass the damage of the first molecule. In theory, “jumping PCR” can amplify intact genes from badly damaged DNA, accurately synthesizing longer pieces of DNA than are actually present in the sample. Jumping PCR, then, seems like a beneficial artifact of the technique that would allow the analysis of ancient DNA despite degradation.

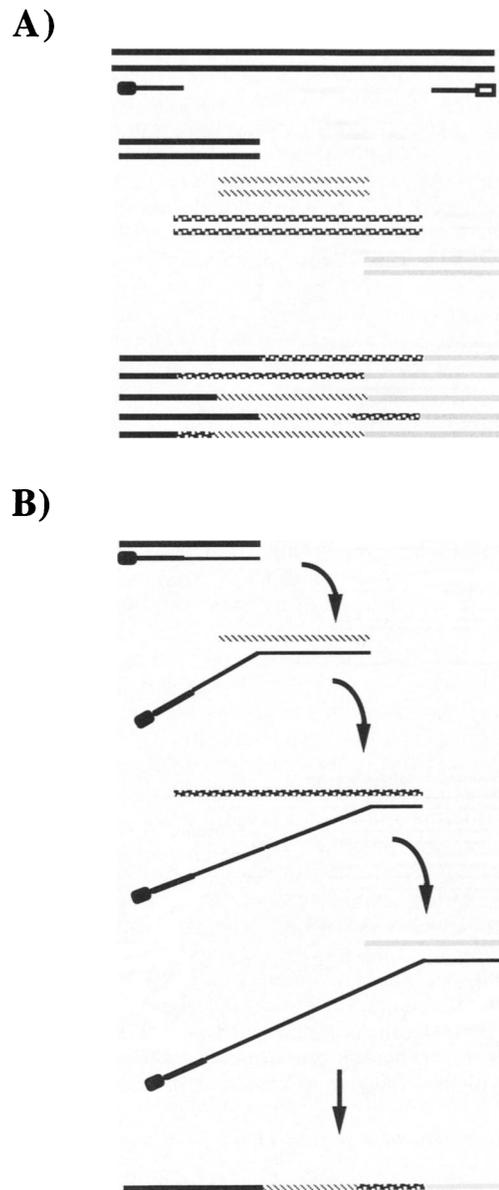


FIGURE 4. Schematic representation of “jumping PCR.” The full-length template is shown, along with four hypothetical degraded template molecules (each shaded differently) (A). Primers are shown below full-length template. The lower part of Panel A shows several possible jumping PCR products produced using the four partial templates. Mechanism of “jumping PCR”; only one example is shown (B). The primer is extended to the end of the partial template (or to a damaged base on a DNA molecule). After the denaturation step (see text and Figure 3), the partially completed PCR product can anneal to a different damaged DNA template. The PCR product will again be extended until the end of this template molecule. Additional rounds of denaturation and annealing will allow the extension of the PCR product, theoretically allowing for reconstruction of the entire target region of DNA. For simplicity, only one primer and one possible outcome are shown. The reconstructed full-length PCR product can serve as a template during the additional PCR cycles, allowing for amplification of the entire template sequence.

In practice, however, there is a serious drawback to jumping PCR, which becomes important when amplifying nuclear DNA. Diploid organisms have two alleles of each autosomal gene in the nucleus (for example, humans receive one allele of each gene from the mother and one from the father). Alleles often

differ by small variations in DNA sequence. The consequence of amplifying two alleles using jumping PCR would be to generate composite molecules containing sequence from both of the alleles. The resulting molecules would not be exact copies of the target genes, but artifacts of the amplification technique. This would complicate the interpretation of ancient DNA sequences. In order to avoid this ambiguity, many groups are now studying chloroplast DNA or mitochondrial DNA (mtDNA) (e.g., Pääbo et al., 1988; Golenberg et al., 1990; DeSalle et al., 1993; Handt et al., 1994b; Villablanca, 1994; Krings et al., 1997; Yang et al., 1997), since these organelles have their own DNA genomes that replicate independently of the nuclear DNA. The amplification of ancient mtDNA is currently more feasible than studying nuclear DNA for several reasons. mtDNA is maternally inherited, that is, only the mother's mtDNA is passed along to offspring. This means that there is only one allele (one sequence of DNA) for each mitochondrial gene, so PCR should generate accurate copies of target sequences. Also, there are many copies of the mitochondrial genome in each cell, making it more likely that some mtDNA molecules will survive relatively intact. Of course, "jumping PCR" remains a concern if a sample is contaminated with DNA from two or more sources, or in rare cases of heteroplasmy, where individuals contain two or more types of mitochondrial DNA.

A finding has cast doubt on several studies and urged caution for future analyses of mtDNA. It was shown that the nuclear DNA of anthropoid primates (monkeys and hominoids) contains some mitochondrial sequences, likely due to a gene transfer event some 30 million years ago (Nomiya et al., 1985; Kamimura et al., 1989; Collura and Stewart, 1995; van der Kuyl et al., 1995; Perna and Kocher, 1996). Presumably these regions of DNA were not essential, underwent high rates of mutation, and currently look similar but not identical to genuine mtDNA sequences (Collura and Stewart, 1995; van der Kuyl et al., 1995; Perna and Kocher, 1996). These regions, then, which are present in modern human DNA, could be mistakenly amplified by PCR and identified as "ancient" DNA. This information has caused the re-evaluation of old studies which relied on cytochrome *b* analysis; in some cases it is likely that modern human DNA from researchers or other humans was isolated rather than genuine ancient sequences (Perna and Kocher, 1996).

### The Authenticity of Ancient DNA

During the years in which ancient DNA has been studied several criteria and control measurements for DNA extraction and analysis have been suggested (Pääbo et al., 1989; Lindahl, 1993b; Handt et al., 1994a; Hummel and Herrmann, 1994a; Stoneking, 1995; Austin et al., 1997b). These criteria are summarized below.

1. Laboratories where the ancient DNA is extracted and analyzed must be physically separated from laboratories where other DNA studies are performed. This is particularly important in laboratories involved in the analysis of human DNA. There should also be separate rooms for pre- and post-PCR procedures.
2. A dedicated set of equipment should be used during ancient DNA studies. All solutions should be sterilized and the room and equipment should be irradiated after every set of experiments to destroy any DNA molecules left from the previous experiment. The people performing the work should take extra precautions such as wearing gloves, hair coverings, and masks, and not talking to limit the possible contamination by human DNA.
3. Contamination should be monitored for by performing mock extractions, a control where every procedure is followed with the exception that the ancient tissue is not added. The extractions will be used in parallel with the PCR reactions.

Any PCR product resulting from the mock extraction will indicate contamination which is not due to the ancient tissue (for example due to contamination by the researcher's DNA or that contained in buffers).

4. At least two DNA extractions should be performed from the same tissue on two different occasions to confirm the results.
5. Ancient DNA is degraded into small fragments (discussed above). Therefore, an inverse relationship is expected between the length of the target DNA and the amplification efficiency. The amount of amino acid racemization or other methods can also be used to determine if the sample is likely to contain ancient DNA (Poinar et al., 1996; Krings et al., 1997). It has been suggested that less important artifacts, such as animal bones found near a well-preserved skeleton, should be sampled before damaging or destroying other fossils (Cooper et al., 1997; Krings et al., 1997).
6. The putative ancient DNA sequence that is obtained from the ancient sample should make phylogenetic sense. It is expected that the DNA isolated from an extinct insect, for example, should closely resemble that of extant insects, and less closely resemble that of mammals or plants. Computer alignment programs as well as phylogenetic algorithms have been used to evaluate the relationships among DNA sequence obtained from organisms.
7. If possible, another laboratory should independently analyze the specimen to show reproducibility of the results. The issue of reproducibility is a particularly difficult one for ancient samples. Most of the artifacts are unique, which precludes reproducibility in the strictest sense, and the extraction of DNA is destructive. Many of the specimens are fragile and the movement to another laboratory may mean further destruction of the remains as well as raise issues of ownership and authorship. Further advances in technique may make sampling less of an issue in the future.

It is important to note that of the work done to date, only one study, that of Krings et al. (1997), met all of these criteria.

There are other approaches which may help to determine the authenticity of the DNA. Several computer alignment algorithms have been developed to distinguish between sequences from different species (e.g., Pietrokovski et al., 1990; White et al., 1993; Pietrokovski, 1994). Such computer algorithms may be useful in verifying whether the isolated DNA is authentic to the species it was putatively isolated from.

Chemical analysis of the specimen has been used as another approach to confirm whether a specific specimen contains authentic ancient DNA (Lindahl, 1993b; DeSalle and Grimaldi, 1994). The identification of polysaccharides and proteins within the specimen is a good indication that DNA will be present as well. The age and method of preservation of bone, as well as the use of preservatives, has been shown to affect the amount of protein that can be isolated, and may reflect the variable yields of DNA (Tuross et al., 1988, 1989; Tuross, 1993; Tuross and Fogel, 1994). Amino acid racemization was also shown to correlate with the extent of DNA degradation (Poinar et al., 1996). Furthermore, if intact organelles such as chloroplasts and mitochondria can be observed in the specimen with electron microscopy, it may indicate that the DNA contained in the sample is also preserved (e.g., Herrmann and Hummel, 1994a; Poinar et al., 1994). Thus it may be possible to select specimens more likely to be good sources of DNA based upon chemical composition and histological properties.

The suggestion that the DNA sequence should make phylogenetic "sense" has been problematic because, while there may be expectations that a particular extinct species be related to particular extant organisms, demanding that this be the case as part of the criteria that this sequence be accepted as genuine has a circular logic to it. Several groups have criticized this

criterion, and have suggested that more sequence be collected from extant taxa before analysis of ancient DNA be continued, and that more stringent criteria, such as attempting to reproduce results and implementing more controls, be applied in the analysis of ancient DNA (Lindahl, 1993b; DeSalle and Grimaldi, 1994; Stoneking, 1995).

### Avoiding Sample Contamination

There are a few general guidelines that can be followed during sample collection to minimize the chance that samples will become contaminated. Because bones and teeth preserved under desiccating conditions have proven to be good sources of amplifiable DNA, these guidelines are most applicable to these samples; special care must be taken with samples preserved in wet or humid conditions, and, as the methods used to extract DNA from sources continue to improve, so, no doubt, will the recommended guidelines for collecting samples. If wet preserved samples are to be fixed, 95% or 100% ethanol is suggested (Thomas and Pääbo, 1993). In general, water, oxygen, and ultraviolet (UV) light are the worst enemies of DNA, and samples should be kept under conditions that minimize exposure to these agents.

Particular caution must be taken when dealing with ancient human artifacts since contamination with modern human DNA is a major confounding factor in ancient DNA studies. The ideal specimen for PCR amplification would not have been touched by people during excavation or sample preparation. While contamination with modern human DNA is a less critical factor when dealing with other animal samples, care should still be taken. Ideally, DNA extraction should take place as soon as possible on a sample that has been minimally handled.

1. Sterile gloves and a mask should be worn while excavating and handling samples. If it is feasible, long sleeves and a hairnet could be worn to further reduce the chance of contamination.
2. Ideally, only one person should handle an artifact. If this is not possible, records should be kept detailing each person who came into contact with the artifact. DNA samples from these people can then be used as controls for contamination if the artifact is later used in ancient DNA studies.
3. Samples should be protected from further sources of contamination, i.e., people, insects, animals, bacteria and fungi. Other artifacts should also be considered sources of contamination, so samples should be separated. Small artifacts can be placed into sterile tubes; larger ones should be kept under desiccating conditions (if preserved under desiccating conditions).
4. Any obvious signs of bacterial or fungal contamination of artifacts should be noted.
5. Small animal bones or teeth from the area should also be collected under sterile conditions to serve as controls for the preservation of DNA in more valuable artifacts.
6. If artifacts are considered to be good candidates for DNA extraction (well preserved artifacts less than 100,000 years old, for example), it might be worthwhile to arrange for DNA preparation to begin at the collection site. This will minimize the chance of contamination and/or further breakdown of the DNA.

### SOURCES OF ANCIENT DNA

DNA has been extracted and sequenced from a variety of prokaryotic and eukaryotic sources that were preserved in different conditions. Here we describe the different preservation conditions and give selected examples of the organisms preserved under these conditions and the information contained in the DNA extracted from them (summarized in Table 1).

TABLE 1. Representative examples of sources reported to contain ancient DNA (see text for references).

Preservation condition	Organism	Estimated age (years before present)	
Dry	Human	600–5,000	
	Quagga	100	
	Horse	35,000–45,000	
	Kangaroo Rat	60–90	
	Ground Sloth	13,000	
	New Zealand Wren	40	
	Maize	500–5,000	
	Wheat	700–2,000	
	Wet	Human	6,000–10,000
		Frozen	Human
Teeth	Mammoth	10,000–50,000	
	Bone	Grass	4,500
Amber	Human	1,200–2,000	
	Human	200–12,000	
	Rabbit	300–11,000	
	Mammoth	50,000	
	Neandertal	<100,000	
	Weevil	$120 \times 10^6$ – $135 \times 10^6$	
	Bee	$23 \times 10^6$ – $40 \times 10^6$	
	Termite	$25 \times 10^6$ – $30 \times 10^6$	
	Angiosperm	$35 \times 10^6$ – $40 \times 10^6$	

### Fossils

Although fossils are the most common remains of the most ancient life forms, there must be some biological material associated with the fossil if DNA is to be found (bones and teeth are considered below). Some of the fossilized remains of plants and animals have been demonstrated to contain DNA, but the quality has been variable (reviewed in DeSalle, 1994; Golenberg, 1994; Austin et al., 1997b). Some of the more controversial studies of ancient DNA have involved sequences putatively recovered from the partially fossilized remains of plants (Golenberg et al., 1990; Sidow et al., 1991; Soltis et al., 1992).

A 16 million year old *Magnolia* leaf preserved in the Clarkia fossil beds of Idaho yielded chloroplast DNA sequences that reportedly showed this extinct species is related to modern *Magnolia* species (Golenberg et al., 1990; Soltis et al., 1992). The DNA extracted from this water-saturated tissue was reportedly in remarkably good condition; the researchers in this case extracted the DNA from the tissues within minutes of collecting the samples. This study, however, raised several concerns. The observation that the sample was saturated with water made it very unlikely that long DNA fragments were able to survive for 16 million years (Pääbo and Wilson, 1991; Lindahl, 1993a, b). Also, study of the preservation of other biopolymers in fossils found at the same location failed to detect any proteins or polysaccharides (Logan et al., 1993), and extensive racemization of amino acids, used as an indicator for DNA degradation, was observed (Poinar et al., 1996). Furthermore, analysis of fossil leaves found in the same Clarkia deposit, by an independent laboratory, showed that the only detected long DNA fragments were from bacteria and no plant DNA sequences could be recovered (Sidow et al., 1991). The controversy regarding the authenticity of these DNA fragments has not yet been resolved and only further analysis of different genes may help to determine the source of the retrieved DNA.

### Bones and Teeth

Bones and teeth are the most common vertebrate artifacts. The condition of these calcified tissues varies greatly due to a number of factors, and so the condition of the DNA contained in them is also highly variable (Tuross, 1993, 1994; Merriwether et al., 1994). Ancient human bones have been studied

by many groups (e.g., Pääbo, 1986; Hagelberg et al., 1989, 1991; Hagelberg and Clegg, 1991, 1993; Kurosaki et al., 1993; Stone and Stoneking, 1993; Tuross, 1993; Merriwether et al., 1994; Tuross and Fogel, 1994; Béraud-Colomb et al., 1995). Human teeth have also yielded putative ancient DNA (Kurosaki et al., 1993; Merriwether et al., 1994; Woodward et al., 1994a). Studies have also been conducted on bone from other animals such as rabbits (Hardy et al., 1994, 1995), mammoth (Höss et al., 1994; Hagelberg et al., 1994) the saber-toothed cat (Janeczowski et al., 1992), an extinct species of horse (Höss and Pääbo, 1993), and an extinct ground sloth (Höss et al., 1996a). A report of DNA sequence obtained from 80 million year old bone fragments (Woodward et al., 1994b) has been hotly debated, and the sequence appears to be most likely of human origin (discussed below).

The recent determination of Neandertal mtDNA sequence from a humerus by Krings et al. (1997; discussed below), has been hailed as the biggest breakthrough in work done on ancient DNA, and will likely serve as the benchmark study against which future work will be compared (Kahn and Gibbons, 1997; Lindahl, 1997; Ward and Stringer, 1997).

### Desiccated Specimens

Desiccated sources of animal tissue include mummified remains, museum skins and preserved specimens. Desiccated plants and seeds have been collected at many archeological sites, and the charred remains of plants have also been found. These dry tissues are relatively plentiful, and generally contain DNA that can be detected and analyzed (see Brown et al., 1994; Cooper, 1994b; Ellegren, 1994; Rollo et al., 1994a; Pääbo, 1986). The initial reports of the cloning of ancient DNA by Higuchi et al. (1994) and Pääbo (1985b) used dried muscle from a museum specimen and dried mummy tissue, respectively. Other studies have utilized ancient human remains (e.g., Pääbo, 1986; Rogan and Salvo, 1994; Salo et al., 1994; Handt et al., 1996; Parr et al., 1996). Examples of ancient DNA isolated from dry animal sources include the thylacine or Tasmanian wolf, an extinct marsupial (Krajewski et al., 1992; Krajewski et al., 1997), the kangaroo rat (Thomas et al., 1990), several species of wolf (Roy et al., 1994), the moa (Cooper et al., 1992), and New Zealand wrens (Cooper, 1994a). In addition, several groups have reported the isolation of ancient DNA from dried plant sources, including maize (Rollo et al., 1988, 1991; Goloubinoff et al., 1993) and wheat seeds (Brown et al., 1994). Herbarium specimens contain DNA that can be extracted and analyzed (reviewed in Taylor and Swann, 1994).

### Wet Specimens

Some wet preserved tissues, such as those found in peat bogs and sinkholes, appear to be in good condition, but the state of the DNA depends to large degree on the pH of the water. Only sites with a neutral pH, such as Florida's sinkholes, have yielded tissues with preserved DNA (Doran et al., 1986; Pääbo et al., 1988; Lawlor et al., 1991; Hauswirth et al., 1994a). The peat bogs of Europe, unfortunately, do not seem to contain remains with well-preserved DNA. Museum specimens preserved in formaldehyde or ethanol contain DNA in reasonable condition (Cano and Poinar, 1993). Ancient DNA sequence has also been obtained from asphalt-preserved specimens of Rancho La Brea (Janeczowski et al., 1992).

### Frozen Specimens

Frozen specimens are relatively rare, but tend to be very well preserved. Frozen mammoths have been studied (Hagelberg et al., 1994; Yang et al., 1997), and the 5,000 year old "Tyrolean Ice Man" find, discovered in 1991, consisted not only of the

frozen remains of a man, but leather, wood, and woven grass artifacts in an exceptional state of preservation (Handt et al., 1994b; Rollo et al., 1994b). Human remains buried in the Arctic permafrost have yielded DNA sequence for analysis (Nielsen et al., 1994). As more artifacts are recovered from the permafrost, more DNA sequence will likely be retrieved (Cooper and Wayne, 1998).

### Amber Inclusions

Amber inclusions are a rich source of ancient tissue in relatively good condition. Many examples of insects, spiders, and fungi in amber exist. Less commonly, crustaceans, mites, and bacteria have been preserved in amber, and a few examples of protozoa, mosses, liverworts, gymnosperms, nematodes, annelids, mollusks, scorpions, and frogs in amber have been found (reviewed in Poinar, 1994). To date, the only parts of birds that have been found preserved in amber are feathers, and, until 1996, only hair from mammals had been found in amber, but the 1996 discovery of mammalian bones (MacPhee and Grimaldi, 1996) may suggest that more specimens exist. Since a single hair has been shown to be a source of DNA amplifiable by PCR (Higuchi et al., 1988), and DNA sequence has been obtained from feathers (Ellegren, 1994), much information may be contained in the existing amber inclusions. Ancient DNA sequence has been reportedly obtained from plants (Poinar et al., 1993), insects (DeSalle et al., 1992, 1993; Cano et al., 1993, 1994), and bacteria (Cano et al., 1994) embedded in amber. The specimens are often in a remarkable state of preservation (e.g., Poinar and Hess, 1982; Grimaldi et al., 1994). The oldest organism that has been reported to yield DNA sequence to date is a 120–135 million year old weevil preserved in amber (Cano et al., 1993). Indeed, organisms preserved in amber have yielded putative ancient DNA sequence from specimens three orders of magnitude older than those preserved in other manners (Table 1).

Recently, the existence of ancient DNA in amber has been challenged by Austin et al. (1997a; see also Walden and Robertson, 1997), who attempted to extract DNA from amber-preserved insects using the same methods reported to have yielded successful results by other groups. The researchers could not reproduce any of the previous DNA sequences, isolated only sequences that resulted from contamination, and concluded that DNA cannot survive millions of years in amber (Austin et al., 1997a). This result is in concordance with the conclusion reached by Lindahl (1993a) and Höss et al. (1996b), who calculated that DNA could not survive more than 100,000 years or so.

### THE INFORMATION CONTAINED IN ANCIENT DNA

When authentic ancient DNA sequence can be obtained from ancient sources, the data can be used for a variety of studies. A selection of the applications and findings will be presented below.

### Phylogenetics

DNA analysis can be used in the construction of phylogenetic trees (reviewed in Felsenstein, 1988). DNA sequence from a quagga was used to compare this extinct organism to extant species (Higuchi et al., 1984). Sequence comparisons can be used to determine the relationships between organisms. The large quantity of well-maintained museum collections has allowed several studies and analysis of museum skins and live animals has led to the suggestion that the red wolf may be a hybrid of the gray wolf and the coyote (Roy et al., 1994). In New Zealand, the examination of DNA from museum samples of New Zealand wrens has suggested there may be a previously

unsuspected relationship between rifleman, rock and bush wrens that challenges the current taxonomic relationship (Cooper, 1994a). Another unexpected finding concerns the moa, an extinct flightless bird. Several species are known to have existed, with very different morphologies; however, DNA analysis suggests that these species are more closely related to each other than had been previously thought, and only distantly related to the modern kiwi (Cooper et al., 1992). Mammoth DNA sequences have been obtained from 9,000 to 50,000 year old samples of frozen tissue and dried bone. Analysis has shown that the mammoth is indeed related to the modern elephant, but has also suggested that mammoths were a more diverse group of organisms than previously suspected (Hagelberg et al., 1994; Höss et al., 1994).

Ancient DNA sequence is an additional criterion that can be used in the construction of phylogenetic trees, and will not replace the analysis of morphological characteristics, but rather will complement these studies. In order to be valuable, several regions of DNA should be sequenced, and the regions chosen for analysis should make sense for the organism being studied (DeSalle and Grimaldi, 1994). As more DNA sequence information becomes available on extant taxa, the DNA sequence obtained from ancient organisms will become more informative.

### Migration

Questions regarding human movements in prehistoric times are being addressed by studying ancient human remains. Genetic information can be used to supplement the linguistic and archeological evidence. For example: the so-called "fast train" model for the origin of modern Polynesians states that Southeast Asians with the navigational skills to bypass the Melanesian islands populated the Polynesian islands relatively recently. DNA sequence data obtained from ancient human remains contradict at least part of this theory, because bones found in Fiji and Tonga dating to a time soon after the putative "fast train" migration (2,700–1,600 years before present) have the same mtDNA sequences as are found in Melanesia, while more recent remains have the Southeastern Asian mtDNA sequences (Hagelberg and Clegg, 1993; Hagelberg, 1997). These data suggest that people from neighboring Melanesia predated the Southeastern Asian migration to Polynesia.

Sequence information from ancient DNA is also being used to address the question of how the New World was settled. Analysis of mtDNA sequences suggest that there may have been only one or two migrations from Asia rather than the three migrations supported by linguistic studies (Torroni et al., 1992; Merriwether et al., 1994). Other groups are studying ancient rat bones, since rats migrated along with the ancient humans (Matisoo-Smith et al., 1997)

Many of the current studies on human migration focus on mtDNA, which is maternally inherited (discussed above). The human Y chromosome is paternally inherited and present in one copy in males, making it a candidate for PCR amplification in ancient samples as well as a complementary technique to the mtDNA studies. Regions of the Y chromosome have been used to determine the sex of specimens, and may be used in other studies in the future (Brown and Brown, 1994; Hummel and Herrmann, 1994b; Nielsen et al., 1994; Dorit et al., 1995; Pääbo, 1995). The recent finding that the Cohanim, male Jewish priests who are designated as such because they are the sons of other priests, have similar Y chromosome alleles suggests that all Cohanim may indeed share a common ancestor, as suggested by biblical account (Skorecki et al., 1997).

### Population Studies

Populations can be studied to determine patterns of past migration, or if expansion or contraction of the population occurred. Thousands of mummified human remains exist in Egypt and Peru; these specimens span a time period from 4,000 BC to the eighteenth century (reviewed in Pääbo, 1986). Many more skeletalized remains exist. Study of these remains would provide a large sample of DNA sequences that could be compared with samples found in other parts of the world, and may yield information about patterns of migration. Other studies of genetic diversity would also be possible. For example, it would be of interest to follow disease markers to investigate claims of increased resistance to some illnesses (for example, evidence suggesting that carriers of sickle cell anemia have increased resistance to malaria). DNA analysis has been used to show that the human remains buried at the Windover site, a shallow pond located in Florida that was used as a burial ground from 6,000 to 5,000 BC, represent a single human population (Hauswirth et al., 1994a, b). Studies to address questions of genetics can now be proposed for this well-preserved population.

Other examples of non-human populations also exist. Large museum collections of flora and fauna can be used to compare past and present populations to detect changes in gene frequencies, or to document the loss of genetic diversity in populations (e.g., Diamond, 1990; Thomas et al., 1990; Taylor and Swann, 1994; Rosenbaum et al., 1997).

### Cultural/Behavioral Studies

Analysis of ancient DNA may also supplement studies of ancient human behavior. For example, human skeletons were found buried either alone or in groups at 5th century burial sites at Hirohata and Hanaura, Japan. It was suggested that families might be buried together. Kurosaki et al. (1993) used DNA analysis to show the presence or absence of kinship among these remains. The 1995 discovery of the enormous tomb of Ramesses II has raised hopes that the mummified remains of his 52 sons may be found. DNA analysis of the remains, if found, may conclusively answer the question of whether marriages between pharaohs and their sisters were consummated.

### Agriculture and Domestication

DNA sequence analysis may offer a new perspective on early domestication of plant and animal species. Wheat was grown in the "Fertile Crescent," and domesticated species were cultivated and carried with early human populations. Some wheat species cannot be positively identified morphologically, making DNA sequence analysis necessary to positively identify ancient seeds (Brown et al., 1994). Ancient DNA sequences have been obtained from the charred or desiccated remains of wheat and maize (Goloubinoff et al., 1993; Allaby et al., 1994). An interesting application of the study of ancient plant DNA to the development of agriculture may allow conclusions to be drawn about the migration of human populations by identifying the domesticated wheat carried with them. DNA sequence collected from ancient plant material can be used to reconstruct trade routes, or to identify groups of people who had contact with each other on the basis of which strains of crop plants they had. Maize was domesticated and cultivated by early humans living in Central America, and is similarly being studied. Evidence against a theory that maize experienced high rates of mutation during domestication has been found (Goloubinoff et al., 1993).

European rabbits were domesticated and transported throughout the world by humans. The effect of man's intervention on rabbit populations is being studied using the numerous rabbit bones that are commonly found along with human artifacts at archeological sites (Hardy et al., 1994). After comparing

mtDNA sequences isolated from rabbit remains found throughout Europe with those obtained from current domestic rabbits, it was suggested that rabbits were domesticated approximately 1,500 years ago (Hardy et al., 1994, 1995).

### Mutation Rate Determination

As we collect DNA from a variety of sources, we can compare the sequences from loci known to mutate at fast or slow rates to attempt to directly determine the rate of mutation. Several groups have begun to measure mutation rates for mitochondrial hypervariable sites; plant studies have looked at the chloroplast *rbcL* gene, whose product, the large subunit of ribulose biphosphate carboxylase, is found in all plants (Handt et al., 1994b; Golenberg et al., 1990). Data from these studies can be used to supplement the knowledge we have gained from examining extant organisms (reviewed in Golenberg, 1994; Villablanca, 1994).

### Diet

It has been shown that PCR analysis of fresh faeces can reveal information not only about the organism through shed intestinal cells, but also about the diet of the organism, through DNA of plant matter (Höss et al., 1992; Höss, 1995; Kohn et al., 1995). Excrement analysis was used to study a threatened bear population because this technique did not disturb the animals, and could be used to screen a large area for a small number of animals. It was possible to determine the approximate number of male and female bears present, and identify a fruit that was a dominant component of the bear's diet (Höss et al., 1992; Höss, 1995; Kohn et al., 1995). At least one group is investigating ancient human droppings (Spigelman, 1994). Rhodes et al. (1998) reported finding Mastodon remains including preserved intestinal tissue including incompletely digested food. The isolation and identification of DNA from food remains may allow us to learn about the diet of different animals.

### Symbiosis

Molecular techniques have been used to study symbiotic relationships. The intestinal flora of modern bees is known to include several bacterial species, and analysis of the stomach contents of an extinct bee preserved in amber has yielded evidence of *Bacillus* DNA, suggesting that a similar symbiotic association existed in ancient species (Cano et al., 1994).

Bacterial spores were isolated from a fossil bee, and were reported to have germinated and grown after having been encased in amber for 25 million years (Cano and Borucki, 1995). Common molecular biology techniques were used to analyze the DNA from these "extinct" bacteria. A comparison of the DNA sequence of one of the ribosomal RNA genes of the bacterium to that of extant bacteria convinced the authors that they had indeed revived an extinct species. This astonishing discovery elicited several criticisms in response, suggesting that the bacteria isolated must represent contamination by an extant species and not an ancient one (Beckenbach, 1995; Priest, 1995; Yousten and Ripper, 1997).

In a more recent study, 38 different types of bacteria were cultured from the preserved small and large intestines found in a 12,000 year old Mastodon (Rhodes et al., 1998). Enteric bacteria were not found in the surrounding soil, and the varieties of bacteria found associated with the Mastodon tissue were found to correlate well with the expected taxa (Rhodes et al., 1998).

### Co-evolution/Disease

Another use of ancient DNA is to elucidate the evolutionary relationships between organisms and their parasites. Cano et al. (1994) reported the isolation of *Bacillus* DNA from the abdominal tissue of extinct bees preserved in Dominican amber. The same kinds of analysis may allow us to better understand modern disease. Data from deer ticks collected in the 1940s in New York suggest that Lyme disease was present at that time, more than 20 years before the disease was formally recognized (Persing et al., 1990). If ancient disease-causing organisms can be isolated, sequence analysis may allow for a better understanding of their modern counterparts. It would be of interest, for example, to look for evidence of retroviral DNA in ancient human remains. A study such as this might show that some diseases thought to be modern in origin may have much older roots. Prior to the use of molecular biological techniques, infectious diseases could only be recognized if they produced distinct morphological changes. For example, evidence that tuberculosis existed in the pre-Columbian Americas was considered controversial because the skeletal changes that have been used to identify tuberculosis victims are not specific to that one cause. Two groups reported the identification of *Mycobacterium tuberculosis* DNA from ancient sources; in a lung lesion of a 1,000-year old Peruvian mummy (Salo et al., 1994), and from pre-Columbian skeletons (Spigelman and Lemma, 1993), providing more evidence that tuberculosis pre-dated the European colonization of the Americas. Evidence of *M. leprae* infection is similarly being sought to enhance our knowledge of the history of leprosy (Rafi et al., 1994).

### Information Regarding Neandertals

Recently, the laboratories of Svante Pääbo and Mark Stoneking collaborated on a project that has re-energized the field of ancient DNA studies. It was announced that mtDNA sequence had been recovered from the first Neandertal skeleton ever discovered (Krings et al., 1997). The authors conducted near-exhaustive controls to insure that the sequences isolated were not due to modern human contamination and could be accepted as genuine Neandertal sequence. To this end, bone sample was independently analyzed in Germany and the U.S. to show that the results were reproducible (Krings et al., 1997). In addition, PCR primers were designed that could amplify DNA sequence from Neandertal but not modern human sources (Krings et al., 1997). The authors concluded that the sequence obtained was indeed endogenous to the Neandertal bone, and found that the sequence fell outside the normal range of variation of modern humans. Phylogenetic analysis showed that the Neandertal sequence was more closely related to that of modern humans than to chimpanzees. The evidence lends support to the hypothesis which states that modern humans arose in Africa as a distinct species, and did not interbreed with Neandertals, who eventually became extinct. It was estimated that Neandertals and modern humans diverged approximately 550,000 to 690,000 years ago (Krings et al., 1997). The authors and others are quick to caution, however, that information from one Neandertal individual does not settle the question of whether Neandertals and European human ancestors could interbreed (Clark, 1997; Kahn and Gibbons, 1997; Krings et al., 1997; Lindahl, 1997; Ward and Stringer, 1997).

### Information About Dinosaurs?

One of the areas where molecular biology may contribute in the future is in the study of dinosaurs. To date, no group has claimed to have isolated dinosaur DNA, although a cautiously worded report claimed to obtain DNA sequence from 80-million year old bones found in an area rich in dinosaur bones

(Woodward et al., 1994b). The DNA sequences of cytochrome *b* isolated from the samples was related to, but did not match, any extant sequence, evidence that it may have come from an ancient source. After the initial excitement generated by this discovery, further analysis strongly suggested that these sequences are probably not from dinosaurs. The analysis of the phylogenetic relationship (“phylogenetic sense”) between the sequences and cytochrome *b* sequences obtained from other organisms suggested that the DNA obtained may have resulted from contamination by a human source (Allard et al., 1995; Hedges and Schweitzer, 1995; Zischler et al., 1995). An advanced DNA sequence analysis performed using the putative dinosaur sequences also revealed that they are probably of mammalian origin (Allard et al., 1995; Henikoff, 1995).

### CONCLUDING REMARKS

When the first reports describing the cloning and analysis of ancient DNA were published, there was a great deal of excitement and anticipation surrounding the field of molecular archeology. This early enthusiasm tempered as more problems started to emerge. Much of the early promise of molecular biology techniques, such as cloning extinct genes, has not been realized due to the fact that PCR must be used to obtain enough DNA to work with. The amplified DNA is suspect, mainly because of the chance of contamination, which would result in the isolation of DNA that did not originate from the ancient source. It became apparent that very careful analysis of putative ancient DNA has to be performed to determine whether the DNA is genuinely old or a result of contamination. If genuine ancient DNA is isolated, however, it would contain useful information for different studies. Researchers should be aware that ancient samples are subject to contamination, and should take special precautions if there is any chance that a specimen may be used to extract DNA. At the least, researchers should not directly handle samples, and the number of researchers who contact a sample should be kept to a minimum and documented. If contamination is later suspected, the researcher’s DNA can be sampled and amplified to determine if the sequences match. It should also be noted that ancient samples may also contain DNA from other ancient organisms such as bacteria.

Austin et al. (1997a) could not reproduce the work of other groups that purported to isolate ancient DNA sequences from amber-preserved insects, and concluded that DNA cannot survive millions of years in amber. They further suggest that amber fossils should be preserved for their morphological characteristics and not be destroyed in an attempt to isolate small fragments of DNA; indeed, it is worth weighing the value of any archeological specimen against the possible gain from using a destructive method to examine it. In light of the fact that most of the work done on ancient DNA has not been reproduced, this sort of analysis might be best used with specimens of more recent origin, for example, from museum collections, where more individuals of a species can be obtained and where the DNA seems to be of better quality.

What can we expect in the future? New molecular techniques may reduce the number of PCR-induced artifacts that currently plague the field, or allow better resolution of artifactual and authentic sequence. Better methods of DNA extraction and amplification may allow for better analysis of nuclear genes. DNA sequence information provided by the human and other genome projects will make more meaningful any sequence obtained from old sources. Developments of new techniques to better analyze biomolecules other than DNA, such as RNA, proteins, polysaccharides, and lipids, may complement DNA analysis (Venzani and Rollo, 1990; Ambler and Daniel, 1991; Lowenstein and Scheuenstuhl, 1991; Logan et al., 1993). Until these

problems are resolved, however, unique artifacts may be better studied using non-destructive methods.

Molecular techniques allow the transfer of genes between organisms, for example from human to yeast, in order to study the function of a protein. It may be possible, then, to extract ancient DNA and to transfer a gene from an extinct organism into a modern one. We may then study the function of an extinct protein in a modern organism, and this may give us new information about proteins that no longer exist. Although currently unfeasible, if the future allows for the isolation of extinct genes, this might allow us to study, for example, a dinosaur gene (perhaps a protein that is not found in any extant organism) in a chicken or a lizard to try to discern its function.

Molecular biology, therefore, is an additional tool to be used in the study of ancient organisms. DNA sequence information can supplement our current knowledge, and the analysis of ancient DNA can add to our understanding of the past. Until more work is done, however, any DNA sequence reported must be examined with a critical eye. Only the future will tell if these techniques can truly reveal the past.

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