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Facts and Artifacts of Ancient DNA

Minireview

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Neandertals, named after the German valley where these fossils were first discovered, were about 30% larger than an average modern man and of great muscular strength. They had low foreheads, protruding brows, and large noses with broad nostrils (Figure 1) and were meat eaters. These individuals became extinct about 30,000 years ago, having lived in Europe and Western Asia for at least 100,000 years. During the last years of this period, the Neandertals coexisted with our direct ancestors, which may well have been a major reason for their subsequent demise. Several monographs on this fascinating topic have appeared recently (e.g., Shreeve, 1995).

A major and hotly disputed question in palaeoanthropology is whether Neandertals were a race of ancient human beings or archaic Homo sapiens falling within the range of variation of human populations and contributing to the human gene pool, or if they represented a distinct and separate species, Homo neanderthalensis, which was displaced by the more recent arrival of Homo sapiens from Africa. The paucity of the fossil record has not allowed a direct resolution of this important problem, although recent morphological studies of the nasal cavity of Neandertals favor the latter alternative (Schwartz and Tattersall, 1996). The small amount of sequence divergence observed in mitochondrial DNA (mtDNA) from different contemporary human populations, especially in Europe, also indicates a relatively recent origin of Homo sapiens without significant admixture of ancient Neandertal sequences, but this conclusion rests on a number of assumptions (Torroni et al., 1994).

DNA Retrieval from a Neandertal Bone

The high mutation rate of mtDNA should be reflected in substantial differences between sequences of Neandertals and modern man, if the former were a distinct species. In a tour-de-force investigation of ancient DNA, Krings et al. (1997 [this issue of *Cell*]) now report on the Neandertal sequence of the 378 base pairs of hypervariable region I of mtDNA, deduced from several short overlapping products of the polymerase chain reaction (PCR). The source of this amplification reaction was an extract of pulverized bone from a Neandertal prototype specimen, recovered in the Neander valley 140 years ago. It apparently took several years of correspondence before a permit could be obtained to sacrifice part of this valuable fossil remain for DNA extraction—this was undoubtedly a blessing in disguise, because there has been great progress in the technical quality of work with ancient DNA in recent years, and it seems quite unlikely that this project could have been carried out as successfully four or five years ago. For example, the 5000-year-old Snow Man from the Tyrol provided some important technical lessons, since initial attempts to recover mtDNA from that great archaeological find resulted in three distinct human sequences, two of which had to be ascribed to contamination with modern human DNA. (A reliable sequence was subsequently produced by independent confirmation of data in two different laboratories, and predictably showed Homo sapiens DNA typical of this region of Europe—a result of only modest interest.) The stringent containment facilities now available in the Pääbo laboratory, together with their extensive expertise in retrieval of ancient DNA, have provided the key to the present astonishing recovery of minute amounts of small but amplifiable DNA fragments of apparent Neandertal origin.

The DNA sequence determined differs markedly from all modern human mtDNA sequences, although it is more similar to human than to chimpanzee DNA. Within the 378 bp region investigated, there were 24 transition mutations, 2 transversions, and 1 single nucleotide insertion in comparison with the human reference sequence; as would be expected, the sequence differences occur at sites that tend to vary between modern human and chimpanzee sequences. In contrast, modern human sequences differ from each other by an average of only 8

Figure 1. Illustration of Neandertal Man
(Reprinted by permission from John Gurche/National Geographic.)

substitutions in the same region. Whereas chimpanzee and man diverged about 4 million years ago, the somewhat limited sequence data now available indicate that the precursor of Homo sapiens, and Homo neanderthalensis, diverged from each other about 600,000 years ago. This event most likely would have taken place in Africa, and a phylogenetic tree with inclusion of the novel Neandertal mtDNA sequences strongly supports a recent (about 150,000 years) African origin of the human mtDNA gene pool.

How reliable is the present DNA sequence of a fragment of Neandertal mtDNA? The field of ancient DNA research has been plagued by accidental contamination of sources with modern DNA, so skepticism about sensational reports is in general highly justified. However, even a critical reader must admit that the evidence provided by Krings et al. (1997) is compelling and convincing. Independent DNA extractions and PCR amplifications have given consistent results, although minority populations of PCR artifacts and contaminating modern human DNA in the initial amplifications strikingly illustrate the technical difficulty of the project. From the initial preliminary sequencing data, discriminative PCR primers could be constructed in the Pääbo laboratory (University of Munich) that allow for amplification of Neandertal DNA but not human DNA. With the aid of such primers, the Neandertal sequences could be independently verified in material from the same bone investigated in the Stoneking laboratory (Pennsylvania State University). The elegant quantitation of the very small number of template DNA fragments, which was on the borderline of what can be reliably amplified, adds further credence to the results. Moreover, an amino acid racemization test showed that the level of hydrolytic decay of macromolecules in the sample used was low enough to be compatible with the survival of short DNA sequences—this is often not the case with ancient bones. A remaining, but highly unlikely, objection is that the core of the Neandertal bone was extensively contaminated in an unspecified way by a single museum attendant during the period of the last 140 years, and that this hypothetical individual would carry exceptional mtDNA highly divergent from that of other human beings. This argument can only be formally refuted by the isolation and amplification of mtDNA from a second Neandertal bone, retrieved from a separate location.

The Neandertal DNA successfully isolated in the present study is not the oldest DNA ever recovered. Over the last couple of years, several groups have reported on the retrieval of short sequences of mtDNA from 50,000- to 100,000-year-old mammoths discovered in the Siberian permafrost region. These DNA sequences are similar to those of Asian elephants, an observation in support of the excellent fossil record in this case. The low temperature of preservation undoubtedly has greatly retarded the inevitable decay of this ancient DNA. A comprehensive study of DNA recovery from 10,000- to 20,000-year-old bones of the extinct South American giant ground sloth (Höss et al., 1996a) perhaps provides a better parallel with the present Neandertal bone data. In that case, only 2 out of 35 bones yielded amplifiable DNA fragments, and successful results were only obtained with bones from a cold climate region.

The Fiasco of DNA from Insects in Amber

In early attempts at retrieval of ancient DNA, several excited reports appeared in leading scientific journals on the apparent recovery of DNA from 100-million-year-old insects in amber, as well as from dinosaur bones and very ancient leaves. The popular "Jurassic Park" book and movie gave an impetus to those studies that was hardly scientifically motivated. In retrospect, the work appears somewhat naive and lacking in the rigorous controls to exclude contamination with modern DNA that have become routine in more recent investigations of ancient DNA. A major problem was the general lack of reproducibility of data, with anecdotal published reports on a single PCR amplification of a valuable disintegrated fossil. A careful and extensive investigation of the possibility of retrieval of DNA fragments from insects entombed in ancient amber has now appeared from a group at the Natural History Museum in London (Austin et al., 1997). This museum has a very large collection of such specimens, so a higher number of fossil insects have been investigated than in all previous published studies on this topic combined. Several different DNA extraction methods and PCR conditions were evaluated, including the use of different nested primers and a variety of amplification protocols. Occasionally, a DNA sequence could be amplified from amber, apparently independent of the presence or absence of a fossil insect, but those results were not reproducible and the DNA sequences were unrelated to the insects investigated. In an important control that should have been carried out long ago by other workers in this field, Austin et al. show that much younger insects preserved in copal also were useless as potential sources of amplifiable DNA. The sticky pine resin in which the insects were originally trapped solidifies into copal, which is then converted to amber over a time period of about four million years. The inescapable conclusion from the paper by Austin et al. is that the previous reports on recovery of very ancient DNA from insects in amber can be disregarded as experimental artifacts. Amber is permeable to gases, so DNA would be grossly degraded by oxidation over a time span of millions of years. The oxidative decay of six-membered pyrimidines to five-membered hydantoin rings in DNA is particularly troublesome, because the Taq DNA polymerase used in PCR reactions (or any polymerase) cannot copy these damaged residues, which slowly accumulate as a function of time and oxygen exposure (Höss et al., 1996b). The failure of DNA recovery from insects in copal indicates that the polymer cross-linking conditions in the resin actually offer a poor environment for the retention of amplifiable DNA, although macroscopic insect morphology is impressively preserved.

Perspectives

The present recovery of Neandertal DNA represents a landmark discovery, which is arguably the greatest achievement so far in the field of ancient DNA research. The mtDNA sequence data offer strong support for the displacement model, in which Neandertals did not contribute significant genetic information to modern man during their coexistence for many thousands of years in ancient Europe. The major remaining experimental problem, as almost always in studies on ancient DNA,

is that of reproducibility. It is now of great importance to attempt to verify the present results with a second Neandertal bone from a different location. The information already obtained might simplify this task, although the administrative, scientific, and ethical problems involved should not be underestimated. As shown by Krings et al. (1997), it is possible to devise PCR primers that serve to amplify Neandertal DNA but not human DNA; using several sets of such primers, it might be possible to retrieve relevant material even from Neandertal bones where only trace amounts of very short mtDNA fragments may remain. As soon as the present sequence has been confirmed, it seems much less clear that continued destruction and pulverization of rare and irreplaceable Neandertal bones for DNA extraction should proceed, since the scientific gains would be rapidly diminishing. Similarly, it seems extremely unlikely that any useful DNA can ever be extracted from Homo erectus and other very old African fossils, so attempts would just be wasteful.

With the currently available Neandertal material described by Krings et al. (1997), it should be possible to greatly extend the amounts of mtDNA sequence available. It would certainly be a satisfactory achievement to complement the human mtDNA sequence determined 16 years ago by Sanger, Barrell, and their collaborators with a complete, or almost complete, sequence of the 16,500 base pairs of Neandertal mtDNA! Recovery of some short repeated sequences of nuclear DNA might also be possible, although they are likely to be less informative and distinctive than the mtDNA because of the lower mutation rate. In contrast, as noted by Krings et al., the DNA decay that has already occurred in the Neandertal bone effectively precludes any realistic attempts at recovery of unique nuclear DNA sequences. The dubious achievement of having caused the extinction of the Neandertals can probably be claimed by our forefathers, and it is not within our power as descendants to undo this damage, or even to retrieve the great majority of lost genetic information.

Selected Reading

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