Am. J. Hum. Genet. 57:1259–1262, 1995

INVITED EDITORIAL
Ancient DNA: How Do You Know When You Have It and What Can You Do with It?

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Few aspects of molecular anthropology (i.e., the application of molecular genetic methods to questions of anthropological interest) have excited as much public or professional interest as the recovery and analysis of DNA from ancient remains. For example, the initial demonstration in 1984 that DNA could be retrieved from an extinct species, the quagga (Higuchi et al. 1984), soon led to tabloid articles heralding the resurrection of dinosaurs by the military establishment for defense purposes (Clifton 1984). In another example of how professional and public enthusiasm for ancient DNA can become intertwined, ancient DNA techniques were a centerpiece of Michael Crichton's popular novel *Jurassic Park,* which was subsequently reviewed in professional journals (Weishampel 1991).

To be sure, there are other indications besides public notoriety that ancient DNA has "arrived" as a legitimate field of inquiry. Both manuscripts and grant applications that deal with ancient DNA are on the rise, as any reviewer for either the relevant journals or the relevant funding agencies can attest. Additional evidence includes several international meetings, at least one book (Herrmann and Hummel 1994), a thriving ancient DNA discussion list on the Internet, and plans to launch a journal (*Ancient Biomolecules*). However, there is also a good deal of cynicism surrounding the field. It is, of course, the development of PCR, more than anything else, that has made it feasible to analyze the minuscule amounts of highly degraded DNA that are all that can typically be recovered from ancient specimens. Yet, this same exquisite sensitivity of PCR also means that the process is highly susceptible to contamination from modern DNA; how can one be certain that the source DNA that was the template for the PCR product one ends up analyzing is of truly ancient and not modern origin? Furthermore, the bulk of the ancient DNA work done to date deals with single individuals, or with remains that are widely separated in space and/or time, and hence does not represent true populations. Even if one can be confident that the DNA is indeed authentically ancient, of what possible use or interest is a DNA sequence from just one or a few ancient specimens? The report by Béraud-Colomb et al. (1995) in this issue of the *Journal,* claiming to have characterized DNA polymorphisms from the β-globin gene region from specimens up to 12,000 years old, is instructive in considering these two troubling issues that confront the ancient DNA field.

Received October 5, 1995; accepted for publication October 5, 1995.

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This article represents the opinions of the author and has not been peer reviewed.

0002-9297/95/5706-0001$02.00

The Authenticity of Ancient DNA

Béraud-Colomb et al. (1995) describe in some detail the precautions taken to minimize the potential for extraneous DNA contamination, including (1) DNA extractions carried out in a laboratory where human DNA had not previously been analyzed; (2) physical separation of the laboratory rooms where pre- and post-PCR procedures were carried out; (3) sterilization of all buffers by both autoclave and filtration; (4) continuous monitoring of reagents for DNA contamination by performing PCR on random aliquots in the absence of added DNA; and (5) use of dedicated pipettors that were sterilized between use via UV irradiation and use of aerosol-resistant, plugged pipette tips. Another potential problem, surface contamination of the bones, was avoided by removing the outer layer of bone prior to DNA extraction. Mock extraction reactions, consisting of all reagents and extraction steps but without added bone, were carried out in parallel with each real extraction, and these were analyzed via PCR along with PCR reaction blanks, as a further check that the reagents were DNA free. Finally, multiple independent extractions were carried out from each sample, so that concordance of the results from different extracts served as an additional check on the authenticity of the results.

These exhaustive procedures appear to meet all of the informal guidelines suggested by the ancient DNA community for avoiding contamination (Pääbo et al. 1989). Indeed, a particularly nice feature of this paper is that their laboratory procedures are presented in such painstaking detail, and representative examples of the mock extraction and PCR controls are included in the gel photographs. This may seem like overkill to the uninitiated, but the ancient DNA community tends to be a rather suspicious lot and likes to see some evidence that people are paying attention to the concerns that have been raised about avoiding contamination and authenticating results.

About the only thing the authors did not do was to have another laboratory analyze the remains independently as yet another means of verifying their results. Most laboratories engaged in human genetic studies would bristle at the suggestion that such independent verification should be a prerequisite for publication, yet this very idea has been raised in the ancient DNA community. My own opinion is that, while it would certainly make sense for ancient DNA laboratories to make their own collaborative arrangements for independent analysis of ancient remains, to *require* such independent analysis would cause more problems than it would solve. For example, in many cases there are restrictions on sending samples outside of the laboratory engaged in the primary analysis of the ancient remains. Independent verification would also mean additional destruction of what are usually quite valuable remains, and the logistics of arranging such collaborations would undoubtedly add considerably to the already lengthy time it takes to obtain and analyze ancient DNA. Furthermore, it is difficult enough to obtain funding for a single laboratory, let alone two or more, to conduct a careful and thorough DNA analysis from a particular set of ancient remains. It would seem that careful attention to the sorts of precautions taken by Béraud-Colomb et al. (1995), in particular carrying out multiple independent extractions from each sample, should suffice to alleviate most concerns about DNA contamination.

There is one other check that could and should be done to help verify the authenticity of putatively ancient DNA, and this is that the resulting DNA type should make "phylogenetic sense." This criterion differs, depending on whether one is analyzing nonhuman versus human remains. If one is analyzing nonhuman remains, then the ancient DNA type should not be of human origin and furthermore should make some sense in terms of what is known about the evolutionary relationships of the creatures being studied. While this seems rather obvious, failure to heed this principle led recently to the "dinosaur DNA debacle," in which a purported dinosaur DNA sequence (Woodward et al. 1994) that was not analyzed phylogenetically was shown, on such analysis, to most likely be of human origin (Hedges and Schweitzer 1995; Zischler et al. 1995).

Things get a little more complicated if human remains are being analyzed, since it is then hard to imagine circumstances under which the resulting DNA type would be nonhuman. With human remains, ideally the ancient DNA types should differ from the DNA types of any of the people who have handled the remains, and the actual genotype(s) should make some sense in the context of what is known about the geographic distribution of the assayed polymorphism in contemporary populations. Béraud-Colomb et al. (1995) attempt to address this issue by sequencing a small fragment of mtDNA from two of the remains. Unfortunately, one of the ancient sequences matched the senior author, but it is not clear to what extent this casts doubt on the results, since this particular sequence is also quite common in Europeans; perhaps in the future only people with rare genotypes should be allowed to work with ancient DNA! The other ancient mtDNA sequence fortunately did differ from the author's mtDNA sequence, and in fact it differs from any mtDNA sequence so far reported in any of the databases, although it does appear to be most closely related to some sub-Saharan African sequences (H. Soodyall, T. Jenkins, and M. Stoneking, unpublished data).

The relevant β-globin genotypes of the people who worked with the samples are not reported, so that information cannot be used to judge the authenticity of the putative β-globin genotypes from the ancient remains. However, since different genotypes could be reproducibly obtained from different ancient specimens, contamination from a single contemporary DNA source cannot explain their results. It therefore seems likely that they have indeed managed to retrieve ancient DNA genotypes from these remains.

The Utility of Ancient DNA

In light of, as described above, all of the exhaustive and laborious procedures that must be followed to obtain and verify the authenticity of ancient DNA while avoiding the ever-present specter of contamination, the ancient DNA community understandably gets a little testy whenever the question is raised of what can actually be done with the resulting information. After all, isn't it a neat enough trick to show that DNA can indeed be obtained from ancient specimens? Alas, if ancient DNA is to become a legitimate field of scientific inquiry, then the answer must be no. "Because it is there" may be sufficient justification for climbing mountains, but, when it comes to grinding up valuable specimens, one ought to spend a little time beforehand considering just what one can expect to learn from an analysis of ancient DNA.

In principle, there is much that could be learned from a careful analysis of ancient DNA variation. Archeologists are traditionally interested in the same sorts of questions about their skeletal populations that human population geneticists are generally interested in when surveying their contemporary populations, questions such as: Who were these people? Where did they come from? How long have they been here? How did they get here? How much variation is there in this population? How are they related to surrounding populations? Is there any tendency for males or females to marry into or out of the community? If there are recognizable social classes in the population, do they tend to be structured along kinship lines? It is readily apparent that ancient DNA analysis ought to be very informative for many of these issues (which is why there is so much enthusiasm for ancient DNA), but it is also readily apparent that addressing these population-level questions requires the analysis of many samples from discrete, well-defined populations.

Yet, to date, analyses of ancient DNA have largely focused on just one or a few specimens that are either from widely separated geographic locations or from time periods spanning hundreds to thousands of years. (I [modestly] point out that one exception that I am aware of is our study of the pre-Columbian Oneota of Illinois [Stone and Stoneking 1993]; another is the work in the Pacific by Hagelberg [Hagelberg and Clegg 1993; Hagelberg et al. 1994].) The study by Béraud-Colomb et al. (1995) is fairly typical in this respect: they analyzed a total of 10 specimens that ranged in age from 12,000 to 620 years old that were from Morocco, the Sahara, Sudan, Ethiopia, Italy, Sardinia, and France. Beyond the fact that they were (probably) successful in getting β-globin gene framework genotypes from remains up to 12,000 years old, the only other conclusion of note is that they can state that some of these polymorphisms must be at least 12,000 years old. However, since these polymorphisms are widely distributed in human populations, it is likely that they originated before the diversification of human populations, and hence this is hardly an unexpected or unanticipated finding.

I stress that this rather harsh summary of the Béraud-Colomb et al. (1995) study can be equally applied to the majority of the papers in the field. Even the main contribution of the recent analysis of mtDNA from Ötzi the iceman (Handt et al. 1994) was essentially that Ötzi was indeed of European origin and not an Egyptian mummy that had been fraudulently placed in the Tyrolean Alps; while this study attracted widespread interest and attention, this finding is not exactly a great leap forward for ancient DNA! I further hasten to add that from a technical standpoint, the demonstration by Béraud-Colomb et al. (1995) that nuclear DNA (and not just mtDNA) can apparently be obtained from human remains as old as 12,000 years is indeed a significant contribution. And certainly, there are instances where a DNA type from a single specimen would be quite valuable. For example, an authentic mtDNA sequence from a Neanderthal ought to settle once and for all the issue of whether or not Neanderthal mtDNAs were ancestral to modern human mtDNAs (the danger in this analysis, of course, is that a contemporary-appearing mtDNA sequence will be obtained and proclaimed to represent authentic Neanderthal mtDNA, when in fact it is the product of contamination). Demonstrating the presence of a particular pathogen at a particular geographic location and time in the past, such as the presence of tuberculosis in the New World prior to Columbus (Salo et al. 1994) could also be accomplished with just one sample.

Nevertheless, we now have had a decade of papers describing that DNA could be obtained from one or a few remains and then extolling the promise of what could be done with ancient DNA; if ancient DNA is to be more than just a technological curiosity, then we don't need any more such papers. Instead, for the real anthropological potential of ancient DNA to be realized, we need to see more studies analyzing the sorts of populations and addressing the sorts of questions that anthropologists are interested in. Only then will we be able to really assess the merits of ancient DNA, and determine whether, as I for one suspect, it really is worth all the trouble one must go to.

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