Amplifying DNA from Archeological Remains: A Meeting Report

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During the half-decade of its existence, the polymerase chain reaction (PCR) has transformed most areas of molecular biology. In the case of the study of DNA from archeological remains, PCR has created an entirely new field. Prior to PCR, only two papers appeared in which DNA sequences from old tissues were presented.[1,2] Since the advent of PCR, at least 10 such papers have been published and several more are currently in review or in the press. The rapid growth of this field was evident at a recent meeting in Nottingham, England (July 8–10) organized by the Natural History Museum and the University of Nottingham—the first meeting ever to be dedicated entirely to the study of ancient DNA. Over 60 investigators convened in a friendly and relaxed atmosphere for presentations dealing with subjects as diverse as extinct mammals and birds, the domestication of plants, and endogenous retroviruses.

MITOCHONDRIAL AND NUCLEAR SEQUENCES

Most work on ancient DNA to date has dealt with the amplification and sequencing of mitochondrial DNA (mtDNA), mainly because mtDNA exists in numerous copies per cell, which are more likely than single-copy nuclear sequences to survive the autolytic degradation of nucleic acids that follows the death of an organism, as well as the effects of damage that accumulate over centuries and millenia. A further advantage of mtDNA is that an individual usually contains only one type of molecule. Thus, when an amplification product is obtained, it can be sequenced directly rather than cloned. This is important because ancient DNA contains large amounts of damage that manifests itself in the form of a high frequency of misincorporated nucleotides[3] and in the formation of in vitro recombinant molecules by means of "jumping PCR."[4] The latter phenomenon is induced by DNA damage that causes the DNA polymerase to stall or fall off its template. The partially extended primers can then "jump" to other alleles and loci during further cycles of amplification. However, since these errors happen more or less randomly, they are averaged out in the direct sequencing and are not detected.[5] For practical reasons, ancient mtDNA sequences are more easily accessible than are other old sequences; thus, it is a fortunate coincidence for the field that the mitochondrial genome is highly informative from a phylogenetic point of view.[6] mtDNA is likely to remain the staple of conversation among molecular archeologists.

However, because animal mtDNA is inherited maternally, it does not reflect the male side of history nor processes that occur in the nucleus. Therefore, it is very stimulating that several groups now report the amplification of nuclear DNA sequences. For example, Lawlor et al.[7] have used conserved primers to amplify major histocompatibility class I heavy-chain genes as well as the single-copy β_2-microglobulin from 7000-year-old human remains from a sinkhole site in Florida. In the case of the highly polymorphic heavy chains, their primers amplified all of the 50 or so genes that exist for these proteins. The amplification product was then cloned and individual clones were sequenced. By comparing large numbers of sequences, the original alleles of the ancient individual could be determined. Since this approach seems too laborious to apply to the large number of samples necessary for work at the population level, Hauswirth and collaborators intend to use allele-specific oligonucleotides to address population genetics questions.

Endogenous retroviruses are similarly being amplified and cloned from ancient Egyptian animal remains by Goudsmid and collaborators.[8] Retroviruses that today are endogenous may not have been so for very long. Indeed, one may speculate that the close coexistence of species that resulted from the domestication of animals accelerated the transfer of viruses between species. Thus, it may turn out to be very interesting to study endogenous as well as exogenous retroviruses in early domesticated species.

AUTHENTICITY

The power of the PCR to amplify a few intact DNA molecules that can be extracted from ancient remains has opened up the field of molecular archeology. However, this power of the PCR also causes severe problems in that the PCR will preferentially amplify any modern undamaged DNA molecules that contaminate an ancient specimen or the PCR. Thus, the problem of contamination has become a spectre that haunts the entire field. Undoubtedly, some of the data that were presented at the meeting, as well as some of the data that have been published, will turn out to be due to contamination. It is important for this young field that investigators try to design projects in which the authenticity of the sequences obtained can be eval-

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uated. One such area is the study of animal remains. For example, when Thomas et al.[9] sequenced a short fragment of the mitochondrial cytochrome *b* gene of the marsupial wolf and showed that this animal belongs in the Australian radiation of marsupials, the sequences inspired confidence because they clearly fell among marsupial animals but were not identical to any extant marsupial studied. At the meeting, Carey Krajewski presented more extensive sequences from a marsupial wolf. These new sequences partly include the previously determined sequences of the cytochrome *b* gene but differ from these at two positions. Thus, they provide a confirmation of an ancient sequence by an independent group. The data by Krajewski futhermore throw new light on the natural history of Australia because they indicate that the marsupial wolf together with the wombat were part of an early radiation of marsupials, later to be eclipsed by a radiation including the Dasyuridae (e.g., the Tasmanian devil and the tiger cat).

HUMAN REMAINS

Results that may also lend themselves to controls against contamination are the attempts to determine the sex of human skeletal remains by the use of Y-chromosome-specific sequences. This topic is relevant to physical anthropology because morphometrics provide very limited help for sex determination for skeletons of nonadults as well as skeletal fragments. Hummel[10] showed that in some cases Y-chromosome-specific repeated sequences are amplifiable from archeological bones. This system offers the possibility of controlling for the frequency of false-positive signals by the use of skeletons that are morphologically shown to be female. A study of a large series of such bones, performed in a blind manner, would do much to increase the confidence in work from ancient human remains in general.

With the exception of the Y-chromosome sequences, the area in which the problem of contamination is most vexing is the study of human remains. The reason for this is that the most likely source of contamination is human DNA and that the sequences themselves are unlikely to give indications of their authenticity. Controls such as amplifications from extracts devoid of tissue samples and amplifications without extract, as well as multiple extracts from a specimen, can, and should, always be performed to control for contamination.[11] However, a very small amount of contaminating DNA may go undetected in such controls, perhaps because the molecules become bound to the walls of the reaction tubes. What is disturbing in this regard is our recent observation (A. Cooper, M. Höss, S. Pääbo, unpubl.) that ancient extracts often contain molecules that will act as carriers, causing small amounts of contaminating DNA to be amplified in the extract amplifications while no visible amplification products appear in the controls. Such a product may then be sequenced and considered to be ancient. Bryan Sykes pointed out at the meeting that contaminants may, for example, also manifest themselves as an animal sequence carried over to a human bone extract from previous amplifications.

DOMESTICATED SPECIES

In view of these difficulties, it seems ironic that most work performed on ancient DNA is devoted to ancient human remains. Our anthropocentricity probably makes this unavoidable. We should not forget, though, that the history of humans can be followed, not only by the study over time of our own genes, but also of those of our companion species, particularly domesticated animals and plants. The first results of this endeavor were presented at the conference by Franco Rollo and Pierre Goloubinoff, who both study ancient maize. Goloubinoff has amplified and reconstructed alleles for the nuclear alcohol dehydrogense 2 (*adh 2*) gene from Peruvian and Chilean maize specimens that go back 4500 years in time, i.e., more than half-way toward the domestication of maize that took place in Middle America around 7000 years ago. One of the enigmas of maize biology is the vast morphological diversity that has been generated over this very short history. This seems to be paralleled by a large amount of diversity at the DNA sequence level, manifested, for example, among modern *adh 2* alleles. Both of these phenomena sometimes have been claimed to be due to an acceleration of the rate of DNA sequence evolution. However, the 4500-year-old maize alleles are as different from each other as are modern alleles from each other, and some ancient alleles are very closely related to modern alleles. Therefore, the data clearly refute claims of an acceleration of the evolution at the primary sequence level in maize. The genetic basis for explosive morphological evolution of maize must therefore be sought elsewhere, for example, in the activities of transposable elements.

The potential of the maize work for elucidating the history of humans is great. Once the different ancient maize variants have been thoroughly characterized by DNA sequence analysis, they can be used to study the spread of agriculture and subsequent trade connections. This will most likely be successful in the Americas where a dry climate in many regions facilitates corn cob preservation. One hopes that attempts to embark on the same route using Old World crops will also prove fruitful.

POPULATION STUDIES

One of the most stimulating developments in the field has been the demonstration by Hagelberg and others[12–14] that bones in many instances may yield amplifiable DNA. Thus, one can hope to retrieve old DNA sequences in regions of the world where soft tissues are not preserved; also increased is the prospect of retrieving DNA sequences from many individuals. As a result, it may become possible not only to study a few representatives of extinct species but to follow populations over substantial time periods. To date, the most extensive such study involves contemporary and museum populations of kangaroo rats.[15] This work illustrates both the great potential and the problems of this approach. Thomas et al. found that for populations where one mitochondrial lineage dominates—generally small populations—it is possible to determine whether a certain population is the descendant of another population. In contrast, a larger rodent population exhibited such an amount of mitochondrial diversity that samples on the order of some dozens of individuals were not able to confirm or reject the hypo-

thesis that the populations living in the area had changed over the last 60 years.

These studies point to a general problem of all studies that try to use ancient DNA to address population genetic questions; that is, we will often be unable to obtain representative samples of ancient populations. Even when large numbers of individuals are preserved, such as in the sinkhole sites in Florida, we do not know whether the samples come from individuals that are related to one another, if they are separated in time by several generations, or if gene flow into the population has occurred. For humans, this problem is exacerbated by the demonstration that even small tribal groups may contain large amounts of genetic diversity.[16] The only way out of this dilemma may be to perform extensive studies of present-day populations to determine exhaustively their genetic variability. With such background information, we may then be able to make use of even a fragmentary sampling of an old population.

If this becomes possible, a number of questions concerning linguistic and cultural change in relation to population history may be addressed. In archeology, a very frequent question is whether or not a change in material culture detected by artefacts exhumed at excavations represents a replacement of one people by another or whether it simply reflects a change in economic structure or even fashion of, for example, pottery making. The current trend in archeology is to stress continuity of populations, whereas earlier, replacement was stressed (see, e.g., ref. 17). Such issues may in some cases be resolved by molecular biology (see, e.g., ref. 18). This will not only be of great interest to historians but may also prove medically important. For example, the spread of disease alleles such as those for sickle cells must be understood as a result of selection as well as of population movements. However, without an understanding of the extent of past population movements, it is impossible to distinguish these two factors from one another. Furthermore, if ancestor–descendant relationships between ancient and modern populations can be demonstrated in the New World, effects of selection on MHC alleles from European infectious diseases could potentially be followed.

FOSSIL DNA

The result that has caused the most excitement in the field since the initial work showing that it was at all possible to go back in time is the report last year of amplification of a 790-bp DNA fragment from a *Magnolia* leaf compression fossil that is 17–20 million years old.[19] This finding is particularly intriguing because the presence of water in the deposit would seem to indicate that spontaneous depurination should have degraded the DNA after some tens of thousands of years.[20] Also, the high-molecular-weight DNA that can be seen in many extracts from the Clarkia fossil bed specimens can be shown to be of bacterial origin.[21] Dr. Golenberg announced that other groups have been able to determine sequences from Clarkia leaf fossils, verifying that DNA can survive for millions of years. These results are eagerly awaited because they will determine if the field of ancient DNA will fulfill the recent promise of being able to go back over geological time.

ALLAN WILSON

The only circumstance casting a sombre atmosphere over this otherwise pleasant conference was the news of Allan Wilson's disease. Since the meeting, we have been saddened by Allan Wilson's death. No other individual scientist has done more to establish the study of ancient DNA. The large number of contributions by people who presently or previously worked in his laboratory is a testimony to his seminal influence. In particular, Russell Higuchi's talk on his pioneering work in Allan Wilson's laboratory with the quagga and his long-time involvement with the mammoth provided a beautiful history of the field. Allan Wilson's absence will be painfully felt for many years to come.

CONCLUSION

In the few decades since biologists have learned to manipulate plasmids and restriction enzymes, many areas of biology have converged at the molecular level. Thus, biologists interested in such diverse areas as behavior, intermediary metabolism, or cell motility today find themselves studying the molecular structure of genes. Now that the PCR has made the retrieval of ancient DNA a feasible task if not an easy one, it is possible to envision that in 10 years archeologists, anthropologists, and paleontologists will all have come together at the same inevitable meeting point—the double helix.

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