Putting the Pieces Together: DNA and the Dead Sea Scrolls

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A number of questions concerning the origin and production of the Dead Sea Scrolls may be addressed using DNA analysis. These documents were for the most part written on what is thought to be goat- or sheepskin parchment. Based on radiocarbon and other analyses, these manuscripts date between the mid–second century BCE and the first century CE.[1] Under most conditions it would be remarkable that organic material, like parchment, would survive intact after such a long period of time. However, some of the material is remarkably well preserved because of the unique climate and storage conditions at Qumran. Because these parchments were produced from animal skins, they may contain remnant DNA molecules. Within the last decade, new techniques in molecular biology have been developed that have made it possible to recover DNA from ancient sources. The molecular analysis of ancient DNA (aDNA) from the Judean desert parchment fragments would enable us to establish a genetic signature unique for each manuscript. The precision of the DNA analysis will allow us to identify the species, population, and individual animal from which the parchment was produced.

Background

The ability to recover biomolecules, most importantly DNA, from ancient remains has opened new research that has many implications.[2] Access to aDNA provides the opportunity to study the genetic material of past organisms and identify individual and population histories. Unfortunately, the DNA recovered from archaeological specimens is of such a degraded nature that the usual techniques associated with DNA fingerprinting cannot be used. However, modifications of the traditional procedures that involve using something called polymerase chain reaction (PCR) and short segments of unique DNA from the mitochondria and flanking short simple repeats from nuclear DNA can be used to identify the origin and identity of biological materials such as preserved skins or parchments.[3]

In 1984 the first reports on the retrieval of informative DNA sequences from an extinct animal appeared,[4] followed by the cloning of DNA from the skin of an ancient Egyptian mummy dated 2400 BP (before present).[5] The rapid degradation of biomolecules begins immediately following death. Except in unusual circumstances, this process continues unabated until the molecules return to a native state. DNA, found in large quantities in living tissue, degrades rapidly after death, and in most instances only small amounts of short DNA molecules can be recovered from dead tissue. This normally prevents recovery and analysis of DNA sequences from ancient tissue. However, the advent of PCR[6] in 1985 further opened the possibility of isolating DNA sequences in extracts in which the majority of the molecules are damaged and degraded. Theoretically, a single intact copy of a target DNA sequence, which only needs to be on the order of one hundred to two hundred base pairs in length, is sufficient for PCR, making it an ideal tool for aDNA studies. PCR products can be sequenced directly from a sample (this is preferable), or after cloning, making DNA sequence comparisons an extremely useful tool for the study of kinship relationships between individuals and populations. The amplification of mitochrondrial DNA (mtDNA) from ancient bones and teeth dated from 750 to 5450 years BP has been accomplished recently by a number of investigators.[7] aDNA has also been used in sex identification of skeletal remains.[8] PCR has been successfully applied to the analysis of ancient mtDNA from a variety of soft tissue remains, including a seven-thousand-year-old human brain,[9] an extinct marsupial wolf,[10] and—particularly relevant to this study—the preserved museum

skins of over thirty kangaroo rats.[11] Numerous reports document the successful extraction and amplification of aDNA from museum skins and field-collected specimens,[12] including both naturally preserved (mummified) and actively treated skins from a wide variety of organisms, especially birds and mammals.[13] Some of these skins have been subjected to the same conditions that we expect to exist in the scroll parchments, and the extraction procedures for such specimens are not substantially different from those we have used in previous studies of aDNA.

Methodology

Although there are many successful studies employing aDNA analysis, numerous difficulties and methodological problems still arise. The PCR technology is extremely sensitive and can be easily affected by contamination from nonrelevant DNA material. The source of contamination may be other personnel working in the field and laboratory or microorganisms such as bacteria. Another problem is the presence of inhibitors of unknown origin in aDNA extracts that interfere with the PCR reaction.[14] In our laboratories, all work is routinely carried out using rooms, equipment, and reagents kept only for aDNA analysis. All personnel wear masks and sterile gloves to minimize contamination, and extensive controls are routinely used in all stages of DNA extraction and amplification. Specimens are thoroughly cleaned before sampling, and only sterile instruments that have been exposed to ultraviolet light to destroy DNA are used. Approaches have been developed to overcome the inhibitor effect, either through dilution of the inhibitor prior to PCR[15] or alternate purification techniques. Contamination by contemporary human DNA will not pose a serious problem to this study because it is easy to differentiate the contaminating human DNA from the animal DNA obtained from the parchments.

The aDNA obtained from the parchment fragments may help answer some interesting questions like the following:

What species of animals were used for parchment production?

Currently it is thought that most of the scrolls were written on goat- or sheepskins, but variations in texture, color, thickness, and follicle number and distribution in the surviving parchments may indicate that other skins were also used. On the basis of microscopic examination of the distribution of hair follicles remaining in the parchment fragments, Ryder[16] was able to determine four different groups that could have been the possible species of origin for twenty samples of parchment from the Dead Sea area. He determined that one sample group derived from calf, one from a fine-wooled sheep, one from a medium-wooled sheep, and one from a hairy animal that could have been either a sheep or a goat. However, the exact species identification is impossible using only microscopic examination.

It is likely that scrolls destined to contain religious writings were produced from ritually clean animals. According to Maimonides, "A scroll of the Law or phylacteries written on skins not expressly tanned for those purposed, is unfit for use."[17] Evidence from biblical sources and from at least one of the Judean desert scrolls (*Temple Scroll*) shows that very strict requirements were placed on the purity of animal skins. In particular, the skins brought into the temple or the temple city had extra requirements placed on their origin and preparation. According to Yadin, these skins had to be not only pure but "entirely holy and pure."[18] In the *Temple Scroll* this requirement is stressed:

> Skin, even if it was made from the hide of a clean animal, unless the animal had been sacrificed in the Temple [should not be brought to the Temple city]. Such ordinary skins are, indeed, clean for the need of

all labour in other cities, but "into the city of my temple they shall not bring [them]."[19]

Some of the parchments used at Qumran may have had less strict requirements for cleanliness and purity applied to them. It was therefore possible to use skins from species of animals that were clean, but not necessarily ritually pure and used for sacrifice in the temple. These clean, but not temple-city-worthy animals could have included a number of animal species such as gazelle, ibex, dishon, or deer. By identifying the species of animal used for the production of a specific parchment, it may be possible to postulate a hierarchy of importance for the different manuscripts. Some would have been intended for use in the temple or synagogue and other important sites within the temple city or community, and others may have had lesser religious significance.

How many different manuscripts are represented in the collection of fragments at the Rockefeller and Israel museums?

Unfortunately, most of the recovered parchment material is quite fragmented, making it difficult to establish physically contiguous pieces of manuscripts. It is estimated that the approximately ten thousand fragments can be grouped into perhaps eight hundred different manuscripts, and it would be of tremendous value to be able to determine exactly which fragments belong together. Obtaining DNA signatures unique to each manuscript will make it possible to sort out the physical relationships of scroll fragments. Such information should prove particularly useful in sorting out the huge number of small fragments that cannot be confidently grouped on the basis of fragment shape, style of handwriting, or text, and it could provide unique insights into the subsequent interpretation of the scrolls.

Which fragments can be grouped together as originating from the same manuscript because they are from identical or closely related parchments?

Because individual animals can be identified by their unique genetic signature, it is theoretically possible to identify the unique origin of each of the parchment fragments based on their genetic information. Using the techniques of aDNA analysis, fragments belonging to the same or closely related skins can be grouped together. This could assist both in the reconstruction of manuscripts and in the verification of assemblies already made.

Did more than one scribe work on a single document, or did different scribes use parchment originating from the same source for different manuscripts?

There are examples in which two or more scribes worked on the same manuscript, as was the case with the *Temple Scroll*, *Thanksgiving Scroll*, and several other scrolls. If more than one scribe participated in the production of a single scroll, which was then subsequently damaged and is today quite fragmented, the critical analysis based only on paleography could falsely identify separate origins of the text.

Because of their size, some of the scrolls (i.e. the *Isaiah Scroll*, the *Manual of Discipline*, and the *Temple Scroll*) are composed of parchments produced from a number of different animals. The *Temple Scroll* is written on nineteen separate sheets of parchment, each one thirty-seven to sixty-one centimeters in length.[20] It is probable that no more than two or four sheets were derived from the same animal. Analysis of fragments from each section of these scrolls will allow us to determine the degree of relatedness of the parchments in a single scroll and whether they are derived from identical or closely related animals. This analysis could also be applied to repair patches that would give us information about where a scroll was when it was patched. Is the parchment for the patch from the same herd as the original manuscript? Does the patch represent a herd from a different region, reflecting mobility

of either the original scroll or the herd? Perhaps parchment was a trade item that was brought from one or a number of different sources. The resulting data, revealing the level of relatedness of the parchment from a single scroll, will establish benchmarks valuable for the subsequent interpretation of the genetic data obtained by analysis of the aDNA from the fragments.

Does the collection represent a library from a single locality, or is it a collection representing contributions from a wide region?

Comparing DNA fingerprints recovered from the parchments and those obtained from archaeological remains of animals found in ancient sites throughout Israel can determine the origins of the parchment. In the ancient populations of domestic animals in Israel certain alleles (forms of a gene) likely became fixed by inbreeding in local herds. This is especially true if a group such as that at Qumran was isolated and closed.[21] Biblical examples of the importance of separating flocks and herds are reflected in Genesis 13:5–9, when Abram and Lot separate their herds to different locales, and again in Genesis 30:40, when Jacob separates his herds from those belonging to Laban.

It was apparently critical that animals for the production of skins to be used in Jerusalem, the temple city, were derived from flocks and animals that were "known to their ancestors."[22] This suggests that flocks and herds were carefully observed and may have been guarded against "contaminating" crossbreeding. Such patterns of husbandry would effectively produce closed breeding groups with predictable genetic consequences. Fixed allele patterns would establish specific markers in the population that could be used to identify and differentiate local herds. Analysis of aDNA extracted from goat bones excavated at Qumran and other archaeological sites within present-day Israel could reveal any fixed allele patterns and will be compared to the alleles found in the parchments. An aDNA analysis will determine if the sampled parchments were produced locally at Qumran or collected from different locations. A test of the sensitivity of this procedure could be performed comparing genetic fingerprints from scrolls that were likely composed at Qumran, such as the *Rule of the Community* (1QS), and others that were possibly brought to Qumran from another location in Palestine, such as the *Isaiah Scroll* (1QIsa^{a}).[23] Another potential source of information about the origin of manuscripts is a comparison of DNA sequence with "autograph" documents, several of which may now have been identified in the Qumran collections.[24] These autographs may be considered to have been authored by the people at Qumran and would provide a genetic fingerprint of the parchment used by these individuals.

The molecular identification of parchment fragments involved a number of complex steps. We first demonstrated the ability to isolate and amplify aDNA from parchment on "modern parchment," animal skins that have been treated in a similar way to that which we believe was practiced anciently. To extract the DNA, the skin fragments were pulverized in liquid nitrogen, dissolved and lysed in a highly chaotropic solution and the DNA recovered by collection on silica beads. We have extracted DNA from museum skins of rabbits and commercially prepared deer and sheep skins. These fragments were sequenced and shown to be specific for rabbit, deer, and sheep, respectively, and these procedures were then used to obtain aDNA from the ancient parchment.

After we demonstrated that it was actually possible to obtain DNA from treated skins, the next step was to identify in modern goats—both domestic and wild—and other potential parchment sources the appropriate DNA sequence changes, or polymorphisms, capable of differentiating individual, herd, or species. DNA was isolated from modern domestic goats, wild goats, sheep, ibex, and other animals possibly used for parchment production

and then amplified using the polymerase chain reaction (PCR). From our preliminary results it is clear that unique DNA regions will be identified that will give good differentiation at the species and herd level.

Results

We have begun to extract aDNA from small portions of parchment fragments of the Dead Sea Scrolls, amplify biologically active DNA using the polymerase chain reaction (PCR), obtain DNA sequences, and identify unique genetic signatures of the fragments. This has shown that the process is feasible and can be used to reestablish the physical relationships of scroll fragments that may help clarify the translation and interpretation of the scrolls.

We have extracted DNA from eleven small pieces (approximately 0.5 cm^2) of parchment from the area and time period corresponding to the Dead Sea Scroll parchments. DNA from these fragments has been successfully amplified and sequenced. The sequence of six of these fragments is most closely related to, but not identical with, that of both wild and domestic goats. It is significantly different from the human sequence, demonstrating that the parchment material was not contaminated by human DNA either in the handling of the parchment during collection or during the laboratory manipulations. The number of differences between the aDNA and the contemporary goat DNA is greater than is generally expected because of the accumulated normal evolutionary mutations over the two-thousand-year interval. The aDNA is probably not from the same species as the contemporary goat samples. However, fewer differences occur between the ancient sample and the modern goat than between the ancient sample and either sheep or cow. This suggests a closer relationship to a goatlike animal than to a cow or sheep. We then compared the first two of the eleven fragments with sequences that we have determined for the modern ibex and gazelle. These comparisons indicate the possibility that these fragments derived from either a gazelle or ibexlike animal.

We have also examined six fragments from five different sheets of the *Temple Scroll*. These have all been shown to be derived from goat. For these fragments, no difference exists between ancient and modern goats at this gene locus. We are currently in the process of identifying individual DNA polymorphisms in those fragments to determine the degree of relatedness of the animals used to produce the parchment in the scroll.

We have also been able to isolate and amplify DNA from archaeological bones of ibex and goats found at Masada. In most of the instances, horn cores that have been identified by species have been used as the source of DNA. This demonstrates our ability to recover from ancient animal remains the necessary genetic information that will enable us to compare the scroll fragments with the animals from which they were derived. This will allow geographical localization of the parchment sources.

In conclusion, we have demonstrated the ability to recover aDNA from the parchment on which the Dead Sea Scrolls were written. We have also shown that it is possible to recover authentic sequence from this material and use it to make comparisons with other sequences. Our early results indicate that the skins from which the first two ancient fragments were derived are not domestic or wild goat, but are likely a wild species of gazelle or ibex. We have also determined that seven other random fragments are derived from goat; six of these fragments come from the *Temple Scroll*. These analyses differ from the classifications made using microscopic analyses of similar parchment fragments from the same area by Ryder.[25] We have not yet identified any parchment from a species of sheep.

This project is the beginning of a fruitful collaboration that will continue over the next few years. We hope that the analysis of DNA from parchment fragments will add a new level of critical analysis to scroll scholarship.

Notes

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1. See K. Thomas et al., “Spatial and Temporal Continuity,” 101–12.
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3. See K. Thomas et al., “Spatial and Temporal Continuity.”
4. See Hagelberg and Clegg, “Isolation and Characterization,” 45–50; Pääbo, “Ancient DNA: Extraction.”
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8. Yadin, *Temple Scroll,* 1:309.
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0. See ibid., 1:9–10.
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4. See Golb, “The Problem of Origin,” and Golb, “Who Hid the Dead Sea Scrolls?”
5. See Ryder, “Remains Derived from Skin.”