Knowledge of the Flesh: Using DNA Analysis to Unlock Bibliographical Secrets of Medieval Parchment

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The past century has witnessed enormous advances in the variety and sophistication of tools available to analytical bibliographers for the study of the history and transmission of early printed books and texts. Developments in type studies and the identification and tracing of watermarks, printers' devices, ornaments, and woodcuts, coupled with refinement in the techniques and practices of studying and describing the placement and use of these features in print artifacts, have revolutionized our understanding of the business practices of printers and publishers, deepened and broadened our knowledge of the commercial trade of paper and books printed on it, and facilitated the dating and localizing of the materials comprised in printed books and, in many cases, of the texts these books contain. But the usefulness of the majority of these advancements has largely been restricted to the study of books printed on paper or, in the case of watermarks, to manuscripts

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written on paper as well. The study of manuscripts written on parchment — as most Western medieval manuscripts were — has remained comparatively dormant; codicologists have had no similar series of breakthroughs that greatly augment their abilities to trace the production histories of the codices they study, or to bring a comparable amount of empirical evidence to bear in the analysis of how books were constructed or the study of the circumstances and practices surrounding their creation, trade, and transmission. Recent scientific analysis has shown, however, that such information has lain latent for centuries in parchment manuscripts in the form of the DNA preserved in the animal skins from which the parchment was made and that this data has the potential to increase our knowledge of manuscript production and transmission significantly, as well as to catalyze new paths of inquiry and fields of study.

TERMINOLOGY

This article reports the methodology, results, and potential bibliographical implications of a study of the DNA found in medieval parchment that I conducted in collaboration with C. Michael Stinson, a biologist with expertise in phylogenetics. Because the secondary literature cited here as well as the details of our own research necessitate the use of terminology not commonly used by the bibliographical community, it is best to begin with a review of essential terms and concepts. DNA (deoxyribonucleic acid) is the genetic material for all known cellular life forms; it stores genetic information and provides instructions for the creation of other cellular components. DNA extant in historic specimens and prehistoric materials is known as "ancient DNA" (aDNA), and it survives in and has been extracted from a wide variety of biomaterials. Its survival and availability is affected by many things, including age, whether and how the materials were processed by humans, and the location and conditions of storage in the intervening centuries. The DNA in medieval parchment is subject to a number of such damaging factors:

> Ancient DNA (aDNA) can be detected in historical and prehistoric materials such as bones (Hummel *et al.* 1999; Krings *et al.* 1997), soft tissues (Higuchi *et al.* 1984) or coprolites (Poinar *et al.* 1998). The isolation of aDNA from historical animal specimens is a new approach that also can be applied to all sorts of hide-derived material, such as parchment (Woodward *et al.* 1996), leather, raw hide, pelt, etc. The difference between primary biomaterials, such as bones and teeth, and manufactured artefacts is that the latter have been subjected to physical and chemical treatments, such as defleshing, cooking, etc. and may bear traces of these treatments. And these treatments may have DNA degrading or preserving effects.[1]

There are two types of DNA with which we need to concern ourselves for the purposes of this essay: nuclear DNA and mitochondrial DNA (mtDNA). As is the case with most cells in an animal's body, "most of the cells in an animal skin contain the complete genetic information of the animal." The nucleus "contains the chromosomes, on which almost the complete genetic information lies," i.e., the nucleus contains the combined genetic information passed down to an organism from both parents. A much smaller portion of DNA, meanwhile, "is situated outside the nucleus, in cell organelles called mitochondria."[2] Unlike nuclear DNA, mtDNA contains only the genetic information passed through maternal lineage. Because each cell contains only one nucleus but typically contains many — perhaps even several hundred — mitochondria, there are usually many times more copies of mitochondrial than nuclear DNA found in each cell, and thus in any sample that extracts and amplifies DNA from multiple cells. Both nuclear and mtDNA offer advantages and disadvantages in studies of aDNA: nuclear DNA contains far more information, but may not be as readily or reliably available as mtDNA. Because of the frequently degraded or damaged state of DNA in materials that are old, that may have been stored in nonoptimal conditions, or that have been through a manufacturing process — all of which are usually the case with medieval parchment — most studies focusing on aDNA from such materials, including ours,

1. Joachim Burger, "Palaeogentics of Parchment" in *Microanalysis of Parchment*, ed. René Larson (London: Archetype Books, 2002), 159. The studies cited by Burger are as follows: S. Hummel et al., "Ancient DNA Profiling by Megaplex Amplifications," *Electrophoresis* 20 (1999): 1717–21; M. Krings et al., "Neandertal DNA Sequences and the Origin of Modern Humans," *Cell* 90 (11 July 1997): 9–30; R. Higuchi et al., "DNA Sequences from the Quagga, an Extinct Member of the Horse Family," *Nature* 312 (1984): 282–4; H. N. Poinar et al., "Molecular Coproscopy: Dung and Diet of the Extinct Ground Sloth Nothrotheriops shastensis," *Science* 281 (1998): 402–6; and Scott R. Woodward et al., "Analysis of Parchment Fragments from the Judean Desert Using DNA Techniques," in *Current Research and Technological Developments on the Dead Sea Scrolls* (*Studies on the Texts of the Desert of Judah*, vol. 20), ed. Donald W. Parry and Stephen D. Ricks (Leiden: E. J. Brill, 1996), 215–38.

2. Ibid.

have targeted the much more readily available mtDNA. The mtDNA is useful both for determining species from which biomaterial is derived as well as for indicating genetic relationships (or lack thereof) of multiple organisms to one another.

Our research (along with most studies of aDNA, including those cited below) employs polymerase chain reaction (PCR), one of the most widely used techniques in molecular biology. PCR can produce many copies of small, targeted sections of DNA, increasing the size of a sample exponentially through replicating it thousands or millions of times. This process creates a sufficient amount of the target DNA to allow analyses that would not have been possible with smaller sample sizes. Although PCR is essential to many types of medical and forensic research, the technique's ability to enable researchers to generate numerous copies of a small, incomplete section of DNA is especially useful in work on aDNA since such DNA is often fragmentary and damaged by a variety of factors, including time, environmental conditions, and manufacturing processes:

> DNA, which is found in large quantities of intact molecules 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 would normally prevent the ability to recover and analyze DNA sequences from ancient tissue. The advent of PCR in 1985 further opened the possibility of isolating DNA sequences in extracts where the majority of molecules are damaged and degraded, to an extent that precluded analysis by other molecular techniques. Theoretically, a single intact copy of a target DNA sequence, which only needs to be on the order of 100–200 base pairs in length, is sufficient for PCR, making it an ideal tool for evolutionary biology, forensic sciences, and aDNA studies.[3]

PCR requires the use of primers, which are short pieces of DNA synthesized to serve as a starting point for the replication of the desired target DNA. Different primers result in the amplification of different segments of the DNA under study. For example, one might design one primer to amplify sequences that distinguish cattle from sheep (keeping in mind that much of the DNA sequence for these two species is identical), and another to distinguish one individual bovine from any other individual.

3. Woodward et al., "Analysis of Parchment Fragments from the Judean Desert Using DNA Techniques," 217.

BACKGROUND OF STUDIES TO DATE

To date, only a few studies have been conducted that have assessed the feasibility of extracting and amplifying DNA from parchment, but early results have been promising. The goals and conclusions of these studies provided a starting point for our work and helped to shape our methodology, and thus are summarized here. In 1996, Woodward et al. published the results of their tests on a number of fragments of the Dead Sea Scrolls and "demonstrated the ability to recover aDNA from parchment on which the Scrolls were written."[4] The authors concluded that two fragments were "likely a wild species of gazelle or ibex," and that "seven other random fragments are derived from goat."[5] The authors also were able "to isolate and amplify DNA from archaeological bones of ibex and goats found at Masada," demonstrating their ability "to recover the necessary genetic information from ancient animal remains that will enable...comparisons between the scroll fragments and the animals from which they were derived."[6] In 1999, a brief report of additional work on this project was published. The authors "obtained three parchment scrolls that simulate the conditions found in the Dead Sea Scrolls and...used these for the development of experimental methods and to demonstrate the power of a molecular approach for identifying unique signatures specific to parchment pages."[7] The studies "demonstrated the ability to identify both the species and individual animal used in the production of the parchment for [the] scrolls," and concluded that one scroll "was produced from one cow...and at least six different sheep," while two others were "both produced from cow skins."[8]

Following these successful early tests, J. Burger et al. demonstrated the feasibility of extracting nuclear and/or mtDNA from a wider variety of hide materials:[9] dried rawhide from "the axe of a war chariot from

4. Ibid., 231.

5. Ibid.

6. Ibid., 228.

7. Scott R. Woodward et al., "Putting the Pieces Together: DNA and the Dead Sea Scrolls," in *The Provo International Conference on the Dead Sea Scrolls (Studies on the Texts of the Desert of Judah*, vol. 30), ed. Donald W. Parry and Eugene Ulrich (Leiden: E. J. Brill, 1999), 30.

8. Ibid., 31.

9. J. Burger et al., "Mitochondrial and Nuclear DNA from (Pre)historic Hide-derived Material," *Ancient Biomolecules* 3 (2001): 227–38.

ancient Egypt (early 18th Dynasty, approximately 1500 BC; Museo Archeoligica Firenze)"; leather[10] from "[f]ourteen historic bookbinding samples from the Göttingen State and University Library, 12 Celtic leather samples from a site at Glauburg in Germany (5th century BC) and a leather find from the Bronze Age Lichtenstein Cave in Osterode, Germany (800 BC)"; and parchment[11] from "28 early modern and modern samples from various collections," and historical samples "taken from two manuscripts from the Stiftsbibliothek in St. Gall (Switzerland)." Of the two historical samples, one "was obtained from the inner fold of the Hs51 insular script from 720–750 AD," and the other "from the margin of the St. Gall plan from 850 AD." [12] The authors determined that some parchment samples were derived from *Ovis aries* (sheep), and that others matched *Camelus bacrianus*, i.e., the domestic two-humped camel. Two DNA sequences obtained from the book leather meanwhile, closely matched domestic cattle, although DNA proved more difficult to extract from leather, and in other instances no DNA could be extracted and amplified. The authors concluded that "nuclear and mitochondrial DNA can reproducibly be obtained from ancient hide-derived material," and that "[w]hile parchment generally offers good chances of retaining ancient DNA, leather contains endogenous DNA only in sporadic cases."[13]

In 2006, N. Poulakakis et al. published the results of a study that sought to "reveal the genetic signature of the Greek manuscript." [14] Six samples of parchment from three Greek manuscripts dating from the thirteenth to the sixteenth centuries were analyzed, and it was determined that most have "goat-related sequences (*Capra* spp.)."[15] The authors concluded that "DNA of animals whose skins furnished the parchment pages of ancient and medieval books may survive in that parchment," and called for future studies, noting that "[i]t might be possible not only

10. Samples "were considered to be leather if they contained large amounts of tanning agent distributed over the entire fibre network" (Burger, 235).

11. Ibid. Samples were considered parchment if they were "thin, flexible, opaque and showed a smooth surface."

12. Ibid.

13. Burger, 227.

14. N. Poulakakis et al., "Ancient DNA and the Genetic Signature of Ancient Greek Manuscripts," *Journal of Archaeological Science* 20 (2006): 2.

15. Ibid., 1.

to determine the species of the animal from which the skin had been taken, but moreover, it might even be possible to reconstitute the history of the herd from which they originated."[16] Beyond these studies, geneticists have done little research into the DNA contained in medieval parchment, and bibliographers and codicologists have not yet begun to utilize those techniques that have been developed in studies of medieval books.[17] It is with this background that we set out to design a series of tests that would make the techniques and conclusions of scientists engaged in aDNA research available to bibliographers and others who study medieval parchment books and documents.

NEW INVESTIGATIONS

Our investigations were conducted on five disbound parchment leaves purchased from a dealer expressly for the purposes of this study. An analysis of layout, script, and text suggests that these leaves were originally from one manuscript, a fifteenth-century Flemish[18] book of hours. The texts on the leaves are as follows:

MS 15[19]: excerpt from "Obsecro te" beginning *una cum omnibus sanctis dei*

MS 18–19: contiguous excerpts from "O intemerata," beginning *culpa mea culpa mea gravissima culpa* and ending *feci ab ipso filio tuo a quo*

MS 28: excerpt from the annunciation, Gospel of Luke beginning *Ave gratia plena dominus tecum*

16. Ibid., 5.

17. An exception to both of these statements is a study carried out jointly in 2003 by Christopher de Hamel, librarian of the Parker Library, Cambridge, England, and the geneticist Christopher Howe, de Hamel's colleague at Corpus Christi College. Their study also confirmed that DNA can be obtained from medieval parchment and was important in that it was conceived as a collaboration between experts in genetics as well as codicology. The results of the study are unpublished.

18. The author wishes to thank William Noel, Roger Wieck, and James Marrow, who were willing to look at images of a sample leaf and offer opinions on its origin based on the script. The leaves were listed for sale by the dealer as leaves of French, rather than Flemish, origin, and it remains possible that this is correct.

19. The manuscript names are taken from the identifiers given to the loose manuscript leaves by the dealer from whom they were purchased; for example, "MS 15" had "15" penciled in on the bottom of the leaf. The relationship of these numbers to the original foliation of the book to which they belonged is unclear, although the numerical order appears consistent with a customary ordering of texts in a book of hours.

MS 90: hymns from Lauds from the Hours of the Virgin, beginning *descendisti ut salvum faceres genus*

We began with several goals — or sets of goals — in mind. First, we sought to affirm the conclusions of the studies discussed above by proving that DNA is present in medieval parchment — and more specifically the parchment found in Western European codices, which had only been sampled in Burger's work and were not the focus of Poulakakis or Woodward. Second, we sought to use DNA analysis to confirm our visual identification of the parchment as calfskin (*Bos taurus*). Third, we sought to position ourselves to use these first two steps as a basis for demonstrating potential genetic relationships, including herd or population-level relationships, by establishing whether the animals whose skins furnished these leaves were related to one another. As a preliminary step, two parchment samples were sent to the Paleo-DNA Laboratory at Lakehead University, Thunder Bay, Ontario for analysis,[20] which confirmed both the availability of DNA and that our visual identification of the parchment as being derived from *Bos taurus* was correct. Once the species of the samples was known with certainty, three additional samples from the same book were sent to Paleo-DNA Laboratory with instructions to conduct an analysis of all five pieces of parchment (samples can be stored and repurposed for later tests) in order to determine whether and how the organisms were related to one another. Because most Western parchment codices utilize the hides of calves, goats, or sheep, all of which are domestic herd animals, our assumption was that the presence of animals genetically related to one another in one book was not unlikely. The methodology of this analysis is described

20. The laboratory is accredited under the Standards Council of Canada CAN-P-4D and ISO/IEC 17025 (General Requirements for the Competence of Testing and Calibration Laboratories) and CAN-P-1578 (Guidelines for the Accreditation of Forensic Testing Laboratories) and frequently used for research work published in international academic journals. Published examples of their work include identification and subsequent re-analysis of *Mycobacterium tuberculosis* DNA from ancient human remains (M. Spigelman et al., "Confirmation of the Presence of Mycobacterium Tuberculosis-specific DNA in Previously Published Specimens," *International Journal of Osteoarchaeology* 12 [2002]: 393–401), and identification of a single nucleotide polymorphism (SNP) in nuclear DNA taken from ancient (>3000 years) human bones (L. A. Larcombe et al., "Detection of a Single Nucleotide Polymorphism in the IL-6 Promoter Region of Ancient Nuclear DNA," *Infection, Genetics, and Evolution* 5, no. 2 [2005]: 117–122), along with numerous additional projects, including extraction of DNA in various forensic contexts.

below; the first step was conducted by us on site, while the remaining were conducted in the Paleo-DNA laboratory by their technicians:[21]

Acquiring samples:
The work surface was cleaned with bleach and covered with paper. For each sample, new paper, disposable gloves, and disposable scalpels were used. The surface of the parchment was abraded on both sides where the parchment sample was to be taken from the lower margin of the manuscript leaf; the dust produced from the abrasion was collected using a sterile swab. A sample measuring approximately 0.5 × 0.5 cm. was removed with a scalpel and transferred to a sterile test tube using sterilized forceps.

Sample Preparation:
The sections of parchment were removed from the tubes with forceps and cut with a sterile scalpel blade into small pieces for extraction.

Extraction and Purification:
The samples were soaked in a buffer solution of Proteinase K, an enzyme widely used to remove contamination when working with nucleic acids. A Phenol-Chloroform:isoamyl alcohol purification was used to isolate DNA. This was followed by a Microcon column concentration, a step in which the solution is poured into a tube containing a column to which nucleic acids bind.

Primer Design:
The Paleo-DNA Laboratory designed three new primers for this project (using the *Bos taurus* mtDNA reference sequence) and located a fourth extant primer that could be utilized for the desired results. Each of these sequences, listed below, represents a section of *Bos taurus* mtDNA potentially useful for showing population-level relationships and thus revealing potential relationships between members of a herd:

Bos16022F 5′ — GCCCCATGCATATAAGCAAG –3′[22]
Bos16220R 5′ — CGGAGCGAGAAGAGGGAT –3′

21. The methodology reported here is very similar to that used in most investigations utilizing PCR to analyze aDNA, including our preliminary tests that identified the parchment samples as *Bos taurus*. Each of the published studies of parchment cited above includes a detailed account of methodology as well and will serve as a useful basis of comparison for those interested.

22. This primer was designed by Troy et al. See "Genetic Evidence for Near-Eastern Origins of European Cattle," *Nature* 410 (26 April 2001): 1088–91.

Bos16139F 5′ — CCTTACCATTAGATCACGAGC –3′
Bos00010R 5′ — AGCCATTAGTCCATCGAGAT –3′

Polymerase Chain Reaction:
The primers listed above were used to conduct a polymerase chain reaction. The primers targeted hypervariable regions of the mtDNA, i.e., those segments that have the greatest variance from individual to individual and thus are most likely to provide data that distinguish one individual from another.[23]

Gel Electrophoresis:
The results of the PCR were visualized using gel electrophoresis,[24] a technique in which molecules placed in a gel are separated through the application of electric current. Due to the polarity of DNA molecules, they move through the gel in response to the application of the electric charge to the gel. Larger molecules move more slowly than smaller ones, resulting in a series of separate, distinct groups, one for each different size of DNA molecule present in the sample. Once stained, these groups appear as separate bands, and sequence differences between samples that produce different DNA molecule sizes thus produce different banding patterns.

Sequencing:
The products of the PCR process were purified and then sequenced, a process in which the identity and order of the bases in a molecule of DNA are determined and recorded.[25] For example, the series of letters in the primers above denote DNA sequences.

When compared with previous research on bovine genetics, the resulting data from this process were sufficient to determine both the haplogroup and haplotypes of the animals whose skins furnished the parchment tested. "Haplogroup" is a designation used to distinguish groups of organisms within a species that share a common variation in one nucleotide (represented as *C*, *G*, *A*, or *T* in the primers above) in the

23. A standard Platinum Taq DNA Polymerase PCR reaction was done using primers specific for the *Bos taurus* mtDNA hypervariable region 1 (HVI base pairs 16022–16338). Amplicon targets were ~200 bp sizes. Each sample was amplified multiple times. A dilution series on each sample was performed for the amplifications.

24. The PCR reactions were run on a 6 percent Polyacrylamide Gel stained with ethidium bromide.

25. Any PCR product obtained was purified with Applied Biosystems (AB) recommended purification protocols, direct sequenced with AB Big Dye Chemistry and run on the ABI 3100 Genetic Analyzer.

DNA sequence; each haplogroup comprises a number of haplotypes, which occur together on one chromosome and thus are frequently inherited together and occur in closely related individuals.

TABLE 1
Analysis of Haplogroups

Sample Name	Haplogroup
MS 15	T_3
MS 18	T_3
MS 19	T_3
MS 28	T_3
MS 90	T_3

As shown in Table 1, all five samples belong to *Bos taurus* haplogroup T_3. This is to be expected since 96 percent of all *Bos taurus* throughout Europe are part of this haplogroup[26] and the specimens were taken from leaves formerly belonging to a fifteenth-century Flemish codex, as described above.

TABLE 2
Mitochondrial DNA Results

Sample	mtDNA Nucleotide Position 16122
BRS	T
MS 15	C
MS 18	C
MS 19	T
MS 28	C
MS 90	T

Table 2 demonstrates variations in mtDNA that show that, of the five samples submitted, samples MS 19 and MS 90 share one common maternal lineage, and MS 15, MS 18, and MS 28 share another common maternal lineage. This was determined by comparing a particular nucleotide, in this case nucleotide 16122 in the genomic sequence, against a

26. The distribution of *Bos taurus* haplogroup T_3 in Europe was provided in the final report submitted by the Paleo-DNA Laboratory.

Bos taurus reference sequence ("BRS" in the table) as well as against the other samples. We can conclude from this that at least two different individuals, from two different maternal lineages, are represented, and that those with the shared nucleotide are either the same individual or a group of individuals with a shared maternal ancestry.

INTERPRETATION OF DATA

Perhaps one of the most promising aspects of this study to date is the demonstrated mutual potential of genetics and bibliographical studies, including an understanding of the historical practices of parchment production, to provide evidence that expands the possible conclusions that can be drawn from studies in either field. It is true, of course, that the conclusions described above could be pursued further through genetics alone. An analysis of nuclear DNA, for example, might make it possible to demonstrate conclusively the identity of individual organisms as well as the animals from which they descended. (As has already been noted, mtDNA indicates maternal lineage, but even hypervariable regions do not always change from one generation to another and, for the purposes of mapping herd populations and genetic relationships, mtDNA does not offer the same level of precision as nuclear DNA.) Usable segments of nuclear DNA may be more difficult to locate than mtDNA, however, and perhaps impossible to locate in some circumstances. It is significant, then, that these conclusions can also be augmented with contextual evidence available through a historical knowledge of how parchment was produced and how books were made from it. A combination of two facts — that cattle tend to give birth to single offspring, and that only calves were used in the production of parchment — lead to a number of conclusions, some more certain than others, that would not be available using DNA analysis alone. Although we do not have data on the number of twin births in domesticated medieval cattle populations, the naturally occurring rate is quite low in modern cattle; for example, in 1981 the "twinning rate in cattle ranged from about 0.5% in British beef breeds, to 1 to 2% in the Continental breeds, and to 4% in some dairy breeds."[27]

27. S. E. Echternkamp and K. E. Gregory, "Reproductive, Growth, Feedlot and Carcass Traits of Twin Versus Single Births in Cattle," USDA, ARS Roman L. Hruska U.S. Meat Animal Research Center. <http://www.fass.org/fass01/pdfs/Echternkamp.pdf> (9 January 2009).

Furthermore, in the medieval era, as now, only calves were used to produce parchment, a claim commonly made in discussions of medieval parchment that has been confirmed for the purposes of this study by two current authorities on parchment making. Christopher Clarkson, former conservator with the Library of Congress, the Walters Art Museum, and the Bodleian Library, notes that calfskins (and not those of adult cattle) "were and are only used for parchment text-blocks. Even a large, rather heavy, parchment membrane, such as the Mappa Mundi at Hereford, is still a calfskin, c. 11 months old."[28] Jesse Meyer, a practicing parchmenter and owner of the tannery and parchment-making studio Pergamena, confirms this; he reports that adult cattle skins used to produce leathers can weigh as much 70 pounds, but that parchments are produced from calfskins weighing as little as 5 pounds and a skin in the range of 12–15 pounds represents the upper possible limit for parchment making.[29] When combined, these facts support a number of conclusions that serve to contextualize the data available from DNA analysis:

1. Any organism identified through DNA analysis will not be found to be the ancestor of any other organism found in subsequent analyses of other parchment samples. This is the case because animals used for parchment are slaughtered before they are old enough to reproduce.
2. Similarly, we should not expect to find direct ancestors of any organism identified through DNA analysis in subsequent analyses of other parchment samples. This is the case because animals old enough to give birth are too old to furnish skins for parchment production.
3. Any parchment samples used contemporaneously in one codex, derived from calfskin, and shown to share mtDNA, as those in our study do, are unlikely to be siblings. As noted above, twins are statistically unlikely, and the only other alternative — i.e., that a calf would be rendered into parchment, that another cycle of pregnancy and gestation from the same mother would follow (during which the skin of the first calf was stored), and that a sibling would be born and also rendered into parchment a year or so later before being used in the same book as the offspring from the previous year — is clearly not the most economically practical or scientifically feasible explanation of such test results. In cases of parchment derived from sheep or

28. Personal correspondence to author, 22 December 2008.

29. Personal correspondence to author, 4 December 2008.

goats, meanwhile, such conclusions will have to be modified due to the fact that twin births are the norm for these animals.

4. A simple explanation for test results indicating shared mtDNA, such as those reported here, is that the samples are derived from the same organism. It is also possible that the results could be explained through other family relationships (e.g., identical results could also occur in the case of animals whose mothers were siblings). In many cases, the data provided by mtDNA could provide strong circumstantial evidence regarding the local origins and date of parchment; if for example, a sample is revealed to share particular mtDNA with a number of other samples from a known time and place, such evidence would provide strong independent (i.e., nontextual) evidence regarding the possible origins of a book and, perhaps, copies of texts within it. If more exact information is needed, an attempt could be made to extract nuclear DNA. If nuclear DNA is not available, other evidence from the codex in question might be useful as well; for example, if the total surface area of leaves exhibiting shared mtDNA is greater than one would expect from a typical calfskin, this implies closely related organisms rather than one organism. In all of these scenarios, it is significant not only that codicologists are gaining a new tool, but that genetic and bibliographic analysis together permit us to conclude far more than we could with either discipline alone, and that each serves as a means to confirm, understand, and contextualize the independent conclusions of the other discipline more fully.

RELATIONSHIP TO BIBLIOGRAPHY/POTENTIAL BENEFITS

Having demonstrated that DNA survives in the parchment of Western medieval codices and explained the methodology for extracting and analyzing it, I will turn now to some of the potential practical applications of this technique to the field of bibliography. We have identified four areas where this study has the potential to contribute to manuscript studies; these benefits range in scope from critical debates surrounding single volumes to a database containing DNA datasets involving thousands of manuscripts and organisms.

1. Localizing herds.

Once a substantial number of manuscripts were tested and the results entered into databases, we would have the potential to localize both

herds and manuscripts. Working from manuscripts with known dates and provenance,[30] we might be able to construct models showing the likely family descent and local origin of animals and parchment, thereby equipping scholars with a new tool for determining the origins of manuscripts that supersedes our current reliance upon evidence gleaned from paleography, scribal dialect, and contextual clues provided by texts, a possibility that has been raised by Poulakakis et al. following their experiments.[31] Such a process might enable the simultaneous dating not only of books, but of herd animals and texts, thereby generating evidence useful in fields of study as diverse as literary history, codicology, and the history of animal husbandry in medieval Europe.

2. Studying the parchment trade.

Little is known about the medieval parchment trade, a deficit that this project could begin to remedy. It is generally assumed that medieval monasteries engaged both in copying texts and in raising herd animals for meat, hide, and wool likely used their own herd animals to produce parchment, and that the earlier a manuscript is the more likely that this is the case. DNA tests on manuscripts showing localized, closely related herd animals would confirm these assumptions. Commercial production of parchment in the later medieval era, meanwhile, likely involved trade routes and the mixing and redistribution of skins in both market towns and in workshops producing and selling parchment in large cities and university towns such as London, Paris, and Oxford. Such practices would complicate the study of both herd populations and the origins of individual skins, but would provide data for tracking the movement of herd animals and skins in the parchment trade. Although no research has yet been completed on this topic, J. Burger et al. recognized the potential of discovering "patterns of distribution, and trade routes of parchment" after their successful efforts to identify parchment samples at the species level.[32]

30. A good starting point for such research would be standard reference works listing dated and datable manuscripts such as P. R. Robinson, *Catalogue of Dated and Datable Manuscripts c.888–1600 in London Libraries* (London: British Library, 2003), 2 vols.; and Andrew G. Watson, *Catalogue of Dated and Datable Manuscripts c. 435–1600 in Oxford Libraries* (Oxford: Clarendon Press, 1984), 2 vols.

31. Poulakakis et al., 1.

32. Burger et al., 235.

3. Analyzing the construction of codices.

Although parchment is quite durable, as are the books created from it, many manuscripts have of course deteriorated substantially due to poor handling, intentional disbinding for financial or other motives, fire, moisture, overzealous binding, and misguided conservation efforts. This has often resulted in the loss of codicological information necessary to understand how the manuscript was initially constructed. Because bifolia were originally one piece of skin, DNA identification to the level of the individual organism might allow scholars to deduce the original gatherings and how they were combined to create a codex. An example of such an instance may be seen even in our preliminary investigations (which were not designed to reveal such data), as we know our MSS 18 and 19 to be textually contiguous, but written on the skins of different animals. As such, we know they cannot have formed the inner bifolium of a gathering. Beyond such cases when components of a disbound manuscript remain together, DNA matches might not only suggest how a book was initially put together, but could alert researchers to potential relationships between leaves that are currently widely dispersed. This technology has the potential to redefine the practices of codicology, which currently relies on scribal marks and visible indications of how gatherings were ordered and sewn to understand how books were constructed, the very types of evidence often lost to the hazards listed above. Furthermore, since the gathering was the basic unit of work for scribes copying texts and many manuscript codices contain booklets added decades apart, information available through DNA analysis would be useful to historians and literary scholars interested in dating a variety of texts that might be contained in one book. Similar work has already been very fruitful in studies of paper manuscripts where watermarks (as opposed to DNA sequences) have made such advances possible.[33]

4. Resolving debates concerning individual manuscripts.

Scores of puzzles and debates surrounding single codices might be resolved (or at least one position in these debates substantiated) through such analysis. A good example of such, and one that has been previously

33. For a thorough list of such studies, see "Part 5: Paper" of G. Thomas Tanselle's *Introduction to Bibliography: Seminar Syllabus* (Charlottesville, VA: Book Arts Press, 2002), especially "Section G. Bibliographical Analysis," 188–93.

raised by Christopher de Hamel,[34] is the famous Bury Bible, one of the treasures of the Parker Library that dates to the twelfth century. The *Gesta Sacristarum*, a late thirteenth-century history of the Bury St Edmunds abbey where the Bury Bible was made, states that the parchment used for the Bible's illustrations was a special, expensive lot brought in "from regions of *Scotia*" because Master Hugo, the illuminator, could find no local parchment to suit him.[35] This story is seemingly borne out by the fact that the illuminations are all rendered on individual leaves of parchment glued to ones beneath them that are sewn into the book, but scholars disagree on whether *Scotia* refers to modern-day Scotland or Ireland. An analysis of the origins of this book combined with comparisons to parchment from other codices could augment our understanding of a medieval historical text (the *Gesta*), add important context to our understanding of how a medieval Latin place name was used, and go far towards solving a modern scholarly debate about this important manuscript.

CONCLUSION

Bibliography has a long tradition of leveraging technology to study books as physical objects, including beta-radiography, particle induced x-ray emission (PIXE), cyclotron particle accelerators, and multi-spectral, x-ray fluorescence, and digital imaging.[36] But these developments have disproportionately benefited studies of paper more than studies of parchment (the Archimedes Palimpsest Project's use of multi-spectral

34. "DNA — Genetic Fingerprinting of Medieval Manuscripts," University of Cambridge, Corpus Christi Alumni News, September 2003.

35. *Memorials of St. Edmund's Abbey*, ed. Thomas Arnold. *Rerum Britannicarum Medii Aevi Scriptores* (Rolls Series) no. 96 (London: Eyre and Spottiswoode, 1890–96) ii, 290.

36. For a substantial list of studies using beta-radiography and cyclotrons, see Tanselle's *Syllabus*, sections K5 (340–1) and K8 (342–3), respectively. For an example of PIXE technology applied to bibliography, see Thomas A. Cahill, Bruce H. Kusko, and Richard N. Schwab, "Analyses of Inks and Papers in Historical Documents through External beam PIXE Techniques," *Nuclear Instruments and Methods* 181 (1981): 205–8. The Archimedes Palimpsest Project provides a prominent example of the use of multi-spectral and x-ray fluorescence imaging to study books; for a discussion of this technology and bibliography of associated publications, see http://www.archimedespalimpsest.org/.

and x-ray fluorescence imaging being a notable exception). Scholars studying paper have also profited from the development of systematic approaches to identifying and utilizing physical features of paper that have no analogues in the study of parchment, such as Allan H. Stevenson's landmark studies of watermarks[37] and David Vander Meulen's study of paper without watermarks in editions of Pope's *Dunciad*.[38] Finally, researchers working with parchment have had no reference works such as those by Briquet, Churchill, and Heawood, which provide tools to assist those attempting to date and localize specimens of paper. The investigations reported here, which are hopefully the beginning of ongoing work in this field, were designed to address these needs in the study of parchment by demonstrating that valuable and quite specific data is present in most parchment books, and that advances in DNA analysis have provided us with tools and techniques for analyzing this data that can yield bibliographical information of a level of specificity and usefulness currently available only in studies of paper. Furthermore, these tools have the potential to provide the information necessary to create valuable reference works in the form of databases containing known DNA sequences from parchment linked to dates, localities, and contents of parchment books and documents. The techniques of such analysis still need to be developed and refined — it is particularly crucial that we devise a means to obtain similar results using less invasive tests that do not visibly damage or alter the parchment being tested — but the information yielded in these early tests indicates that such a goal is well worth pursuing, and that the results could reshape the study of parchment books. In a recent article entitled "How to Read Book History," Vander Meulen, writing about Sir W. W. Greg, notes that in formulating a definition of bibliography Greg "wanted to stretch the bounds as far as possible...But at the same time he recognized the value of boundaries."[39] A quotation from Greg follows: Greg states that the

37. Stevenson's studies include "New Uses of Watermarks as Bibliographical Evidence," *Studies in Bibliography* 1 (1948–9): 151–82; and "Watermarks Are Twins," *Studies in Bibliography* 4 (1951–2): 57–91.

38. David Vander Meulen, "The Identification of Paper without Watermarks: The Example of Pope's *Dunciad*," *Studies in Bibliography* 37 (1984): 58–81.

39. David Vander Meulen, "How to Read Book History," *Studies in Bibliography* 56 (2003–4): 172. Vander Meulen quotes W. W. Greg, "What is Bibliography?" *Transactions of the Bibliographical Society* 12 (1911–13): 45.

field "attends to the preparation of vellum," but is "indifferent to the breeding of calves." Perhaps today we might stretch Greg's boundaries a bit further still — and stretch them in a way that he could not have possibly imagined when he wrote those words almost a century ago — by uncovering and utilizing bibliographical data encoded in genetic information generated by nothing other than the breeding of calves.