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Spines reinforced by the application of animal glues are a distinctive feature of books in later medieval Europe, and one which may reflect a move towards faster, cheaper methods of manufacturing as demand for bound books expanded in the fifteenth century. Despite the significance of glue-stiffened spines for the practices and economics of the late medieval book trade, glues and adhesives

Abstract: Glues, and in particular glued spines, are notable features of late medieval European books, yet little research has been done into how they were sourced, produced, and used. In this article we present preliminary results from using the paleoproteomic methods of Electromagnetic Zooarchaeology through Mass Spectrometry (eZooMS) and Peptide Mass Fingerprinting (PMF) to identify the source species for animal glues used in late medieval books. We first introduce readers to the principal kinds of glue used in medieval craftsmanship and what is known about their use in bookbinding, principally from the discipline of book conservation. We describe the micro-sampling methods of eZooMS, in which a PVC eraser is rubbed gently on the surface of the book. We then describe the process through which we tested and fine-tuned our sampling methods on eight medieval books held in Canadian repositories, addressing some of the challenges we faced, potential further uses or expansions upon the technique, and the benefits of our collaborative approach to such "manuscientific" studies.

Keywords: Adhesives, Glues, Bookbinding, Medieval Bindings, Biocodicology, Peptide Mass Fingerprinting, Mass Spectrometry, eZooMS.

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have not often been the objects of research in codicology and book history. This lacuna is particularly notable given the important role adhesives can play in the wear and tear, preservation, and conservation of medieval books.

At the same time, recent advances in non-destructive sampling techniques, paleoproteomics, and new applications of peptide mass fingerprinting have offered fresh insights into the pages of medieval books, opening up exploration of craft technologies and the use of animals in book production. Our research on adhesives proceeds from the insight that the animal-sourced materials found in historical European books include not only the parchment and leather used for pages and covers, but also hide-based glue. Our team—made up of book historians, librarians, and biochemists—began its work with a question: can proteomic study of the adhesives used in historical bookbindings tell us more about the making of medieval books and the circumstances of their production, and at the same time offer a new avenue for those interested in human and animal interaction through history?

To arrive at some answers, we first needed to test our methods for nondestructive sampling of historical adhesives. In 2019 and 2020, our team collaborated—across long distances, during waves of the COVID-19 pandemic—to analyze suspected adhesives in a small set of medieval European manuscripts using non-destructive eZooMS (electrostatic Zooarchaeology by Mass Spectrometry). We sought to test the potential and the limits of proteomic analysis of medieval glues; we hoped to identify some species of origin for animal glues, to refine the eZooMS method for this use case, and to lay the ground for future research.

Some of our results were positive: it is indeed possible to identify the species of animal used in some books' medieval animal glues, and we report the evidence for this here. But our research was also valuable as it identified the challenges of, and possible improvements to, an approach based in proteomic analysis. In what follows, we describe the problems of sample size and the possible contamination of samples by other animal materials found in the books we were testing. Our account of these challenges can, we believe, help other scholars and scientists refine their own use of non-destructive sampling techniques to study animal materials used in book production. We also offer some new ways forward: alternative techniques for sampling and analysis of adhesives, and expansion of the number and type of books sampled. Testing a larger number of books, made in different locales and in different time periods, would, we argue, enhance understanding of the species commonly used in animal glues. It would also allow for a new kind of historical comparison of textual production and human-animal interaction in different regions of the premodern world.

Finally, this article describes, and argues in favor of, our model of collaborative research, which brought together humanists, librarians, and conservation and natural scientists from multiple institutions. Our research began as a collaboration between several Canadian university libraries (McGill University Library's Rare Books and Special Collections, the University of British Columbia's Rare Books and Special Collections, and Western University Library's Archives and Special Collections), The Book and the Silk Roads research project at the University of Toronto, and Sarah Fiddyment of the European Research Council-funded Beasts to Craft project at the University of Cambridge.[1] As manuscript scholars, this endeavor taught us the benefits of engaging closely with the institutions and experts charged with preserving books. It showed us that a

1. See project websites Beasts to Craft: Biocodicology as a New Approach to the Study of Parchment Manuscripts, accessed 7 November 2022, https://sites.google.com/palaeome.org/ercb2c; and The Book and the Silk Roads, accessed 7 November 2022, https://booksilkroads.library.utoronto.ca/. At a later stage, our collaboration benefited from being joined by Melissa Moreton of the Institute for Advanced Study, Princeton.

diverse, collaborative group can operate successfully across distributed locations, in complex circumstances, with only limited time and resources. The experience confirmed for us what other projects, such as Beasts to Craft, also demonstrated: the tremendous promise of a collaborative interdisciplinary, cross-institutional, international approach to the study of the scattered material remnants of the premodern world.[2]

GLUES AND ADHESIVES IN MEDIEVAL EUROPEAN MANUSCRIPTS

The focus of our initial study was medieval European bookmaking practices, which we defined narrowly to mean books (and their bindings) produced between the twelfth and early sixteenth centuries CE, on the continent as far east as present-day Germany, as far south as Italy, and as far west as the British Isles. Our study thus captured only a small part of the long, global history of early books, but it provided us with a useful frame: a known, chronologically- and geographically-limited, transition point in that larger history. Like other scholars, we assumed that the animals used for glue in European books were most often those also used for parchment and leather making. Such materials have been the focus of a number of previous eZooMS

2. This work was supported by ERC investigator grant no. 787282-B2C and by the Book and the Silk Roads Project (ref. #1802-05532) generously funded by the Mellon Foundation. This project took shape in conversation with Matthew Collins, Fenella France, and others at the 2019 Folger workshop "Biocodicology: The Parchment Record and the Biology of the Book." We would like to thank our colleagues at Cambridge University, McGill University Library, the Thomas Fisher Rare Book Library, UBC Library, and Western University Library for facilitating this research; and our editor Gregory Pass and our blind reviewer for their insightful comments during the revision process.

experiments, so we had more data for animals from this region to assist us with species identification.[3]

Existing scholarship on historical European adhesives, while limited, informed our research from the outset. Thanks to the work of earlier scholars, we know that medieval Europeans made use of a variety of adhesives, and that these appear to have been chosen according to their particular properties and fitness for the task at hand. Previous studies also show that adhesives can serve as evidence of the spread of new craft technologies in response to changing economic circumstances.[4]

Medieval animal glues consisted of collagen processed from the skins of animals, including recycled or trimmed edges of parchment. Other adhesives were made from eggs, fish, or milk. Furniture, sculpture, and painted panels, for example, were joined with casein-based "cheese glue," made by combining curdled milk with lime.[5] Egg white, when beaten and then allowed to settle, became glair, a crucial binder for gold leaf.[6] Wheat or other starch paste was used to adhere endleaves to the boards of codices, and to attach

3. Sarah Fiddyment et al., "Animal Origin of 13th-Century Uterine Vellum Revealed Using Noninvasive Peptide Fingerprinting," *Proceedings of the National Academy of Sciences of the United States of America* 112 (2015): 15066–15071, https://doi.org/10.1073/pnas.1512264112; Matthew Teasdale et al., "The York Gospels: A 1000-Year Biological Palimpsest," *Royal Society Open Science* 4 (2017): 170988, https://doi.org/10.1098/rsos.170988.

4. Historical recipes are found in Theophilus, *The Various Arts: De diversis artibus*, ed. and trans. Charles. R. Dodwell, Oxford Medieval Texts (Oxford, 1986); Cennino D'Andrea Cennini, *The Craftsman's Handbook (Il Libro dell' Arte)*, trans. Daniel V. Thompson (New York, 1936); see also Rémy Gug, "Medieval Glues … Sounded Well!" *The Fellowship of Makers and Restorers of Historical Instruments* 1 (Oct 1975): 36–43.

5. Joseph E. Sandford, "Cheese Glue," *Notes and Queries* 158, no. 21 (24 May 1930): 366–67; Cennini, *Craftsman's Handbook*, ch. 112.

6. Cennini, *Craftsman's Handbook*, ch. 131. Egg whites and yolks were also used as binders for pigments in preparing egg tempera.

coverings to those boards—although there is evidence that animal glues were occasionally added to the paste used for this purpose.[7]

Because of their importance for conservation, research on medieval adhesives has often focused on the study of binding media used for paintings and illuminations. Of these, the most commonly discussed are glues made from fish bones and offal or isinglass, a specialized product made from the swim bladders of sturgeon.[8] Appearing first in medical manuals of the Greco-Roman era as an ingredient in their equivalent of plaster casts, fish glues, especially isinglass, allowed for delicate repairs of parchment and the application of gold leaf to surfaces in manuscripts as well as paintings. Isinglass is still used in book conservation today.[9]

Students of medieval musical instruments have also made important contributions to the history of adhesives.[10] In a note for *The Fellowship of Makers and Restorers of Historical Instruments*, Rémy Gug argues that most medieval glues—those from cow skins and fish products—were known from antiquity, but points out that woodworker's "cheese" or casein glue, made with a combination of milk proteins and quicklime, appears to have been a medieval

7. Janos A. Szirmai, *The Archaeology of Medieval Bookbinding* (Aldershot, UK, 1999), 228; see also Anshelmus Faust (1612), *Beschrijvinghe ende onderwijsinghe ter discreter ende vermaerder consten des boeckbinders handwerck / Prescription et enseignementı de la discrète et fameuse science de la manifacture des relieurs de livres*, ed. Georges Colin (Brussels, 1987).

8. Tatyana Petukhova, "A History of Fish Glue as an Artist's Material: Applications in Paper and Parchment Artifacts," *Book and Paper Group Annual* 19 (2000), https://cool.culturalheritage.org/coolaic/sg/bpg/annual/v19/bp19-29.html.

9. John Scarborough, "Fish Glue (Gr. ΙΧΘΥΟΚΟΛΛΑ) in Hellenistic and Roman Medicine and Pharmacology," *Classical Philology* 110, no. 1 (2015): 54–65, https://doi.org/10.1086/678680.

10. Gug, "Medieval Glues," 36–43.

innovation.[11] Gug also describes a fourteenth-century trade in glue, with references to both the international trade in glue products in Italy and amounts paid for glue production in England, hypothesizing that glue-making was a specialized trade.[12]

In early medieval Europe, starch paste appears to have been the common adhesive used for attaching cover materials to books. This was the case through the Romanesque binding period ending in the late fourteenth century when book spines were flat, not rounded. The study of animal glue on books has tended to focus on its role in reinforcing the spines of codices, once binders began rounding them in the late medieval (gothic binding) period.[13] The technique of glue-reinforcement of spines, Janos Szirmai suggests, emerged as a response to the pressures of increased demand. Bookbinders were forced to use poor-quality thread and abbreviated sewing structures as a way of cutting material and labor costs—trade-offs adopted by the sixteenth century to meet the demands of increased production. Glue reinforced these inferior binding methods.[14] Szirmai thus characterizes the introduction of animal glue as among the "most conspicuous changes" seen in gothic binding techniques. "The rounded spine of the heavy volumes was virtually immobilized," he writes, "by the animal glue which became rock hard with time."[15]

11. Ibid., 38.
12. Ibid., 38, 40.
13. Szirmai, *Archaeology of Medieval Bookbinding*, observes (157–59) that animal glue was not found on medieval books through much of the medieval period, and that leather coverings were customarily attached to the wooden boards with starch paste, not animal glue (162).
14. Ibid., 190–92.
15. Ibid., 271, 273.

As glue was integral to these books, it is striking how little attention has been paid to the topic.[16] And such research has its challenges. In particular, it is sometimes difficult to identify a glue as original, or material added during a repair or conservation—a common practice.[17] Glues also break down with age, further complicating their identification. As Szirmai writes:

> [I]t is virtually impossible to determine only with the naked eye whether or not adhesives had been used, since they may have been completely degraded by biological agents: remnants like a powdery residue of starch paste or brittle particles of aged glue may have become lost due to the spine movement.[18]

The proteomic methods we explore in this article might in some cases help address this challenge, by allowing a re-

16. To name only a few examples: Mirjam Foot, *Bookbinders at Work: Their Roles and Methods* (2006); Dorothy Miner, *The History of Bookbinding, 525–1950* (1957); and Philippa Marks, *The British Library Guide to Bookbinding History and Techniques* (1998)—each notes the use of "glue" and "paste," but none of these works include detail about the nature or types of such adhesives.

17. Szirmai, *Archaeology of Medieval Bookbinding*, 157–59, observes that Graham Pollard once claimed to have discovered evidence for glue on the spine of a fourteenth-century English manuscript. Unfortunately, Szirmai writes, the binder's note on which Pollard relied refers not to the original manufacture but a later rebinding. Another article that describes later application of glue to a manuscript is Nancy K. Turner, "A Romanesque Binding in the J. Paul Getty Museum: Materials, Craft Technology, and Monastic Reform," *Journal of Paper Conservation* 20 (2019): 213–32, https://doi.org/10.1080/18680860.2019.1763642. These examples show that the presence of animal glue may tell us about the later life and use of manuscripts, as well as their original manufacture; however, it is important to distinguish between these two appearances when making arguments about the adoption of new techniques.

18. Szirmai, *Archaeology of Medieval Bookbinding*, 190–92.

searcher to distinguish an animal glue from a powdery mold or other residue.

Bookbinder's recipes and handbooks also provide valuable resources on the use of glue in medieval European manuscripts. Some of these post-date the first appearance of glue on the spines of European books, but they contain evidence of how glues were used in adjacent periods, and describe the proprietary nature of some recipes. For example, a letter to sixteenth-century bookbinder Maestro Prospero preserves a secret recipe for glue to be applied to the spines of books, which combines animal glue with aloe.[19] Other recipes for glues and adhesives survive in craftsmans' handbooks, but it is unusual to find descriptions for their precise use: this information was likely passed on orally and by demonstration during a bookbinder's training. Dirk De Bray's handbook (1658) demonstrates the application of adhesive, using a glue brush, to a rounded spine held within a lying press. An illustration in the handbook shows a small, steaming glue-pot close at hand, ready for the bookbinder's use.[20]

Maestro Prospero's recipe for glue, praised in its text for its increased flexibility, is interesting given that animal glues are sometimes critiqued for their rigidity. Art historian Bengt Skans has tried to put this belief to rest by showing that the impurities and natural fat content of historical animal glues made them more naturally flexible and longer-lasting, particularly in the face of atmospheric mois-

19. Silvia Pugliese, "When Cover Paper Meets Parchment: A Non-Adhesive Variation of the Limp Parchment Binding," *Journal of Paper Conservation* 20 (2019): 152–57, https://doi.org/10.1080/18680860.2019.1746118.

20. Dirk de Bray, *Kort onderwijs van het boeckenbinden = A Short Instruction in the Binding of Books*, ed. Koert van der Horst and Clemens de Wolf, trans. Harry Lake (Amsterdam, 1977), 9–10; an image of a glue pot in use for gluing up the spine is found on fol. 7r and a glue pot is among an illustration of bookbinder's tools on fol. 41r.

ture, than the animal glues available to artists today.[21] Skans's article is also an early example of the study of glue at the molecular level, using modern scientific techniques. He describes his use of gas chromatography to study animal glues he manufactured, some according to historic recipes. Analyzing the fatty acids that make up the glue, Skans attempts to explain both the superior qualities of older glues and to develop a method for identifying the animals used to produce the glue. His method cannot, however, distinguish between hide or bone glues, or to identify the differences between cattle and sheep products.[22]

Skans's work is one of the few examples of scientific research on adhesives focused on books and bookbinding: most research into the scientific analysis of binders and adhesives has focused on the needs of art conservation.[23] Much of this art-historical research has, like the experiments detailed in Skans's article, used gas or liquid chroma-

21. Bengt Skans, "Analysis and Properties of Old Animal Glues," in *Preprints: 7th International Congress of Restorers of Graphic Art [IADA], 26–30 August 1991*, ed. K. Jonas Palm and Mogens S. Koch (Uppsala, 1991), 1–8.

22. Skans, "Analysis and Properties," 6.

23. One such article, focusing on glues, is Giorgia Sciutto et al., "Single and Multiplexed Immunoassays for the Chemiluminescent Imaging Detection of Animal Glues in Historical Paint Cross-Sections," *Analytical and Bioanalytical Chemistry* 405 (January 2013): 933–40, https://doi.org/10.1007/s00216-012-6463-z. María T. Domenech-Carbo, "Novel Analytical Methods for Characterizing Binding Media and Protective Coatings in Artworks," *Analytica Chimica Acta* 621, no. 2 (July 2008): 109–39, https://doi.org/ 10.1016/j.aca.2008.05.056, offers a comprehensive review of the state of analytical methods in the late 2000s, including a discussion of spectroscopy, mass spectrometry, and chromatography. On the challenges and methods of proteomic research specifically, see Gabriella Leo et al., "Proteomic Strategies for the Identification of Proteinaceous Binders in Paintings," *Analytical and Bioanalytical Chemistry* 395 (2009): 2269–80, https://doi.org/10.1007/s00216-009-3185-y; and Caroline Tokarski et al., "Identification of Proteins in Renaissance Paintings by Proteomics," *Analytical Chemistry* 78 (2006): 1494–1502, https://doi.org/10.1021/ac051181w.

tography in conjunction with mass spectrometry.[24] Other researchers have relied exclusively on forms of mass spectrometry. The present study uses MALDI-TOF mass spectrometry, a process we will now describe in more detail.[25]

A BIOLOGICAL APPROACH TO GLUE: EZOOMS FOR BOOK ADHESIVE SPECIATION

DNA analysis has opened new possibilities for the study of parchment, but it is not possible to carry out this kind of testing on highly-processed materials such as animal glues; the processing denatures the DNA.[26] To research the bio-

24. For example, Maria Perla Colombini et al., "Characterisation of Proteinaceous Binders and Drying Oils in Wall Painting Samples by Gas Chromatography – Mass Spectrometry," *Journal of Chromatography* A 846, nos. 1–2 (June 1999): 113–24, https://doi.org/10.1016/S0021-9673(99)00344-1; Maria Perla Colombini and Francesca Modugno, "Characterisation of Proteinaceous Binders in Artistic Paintings by Chromatographic Techniques," *Journal of Separation Science* 27, no. 3 (February 2004): 147–60, https://doi.org/ 10.1002/jssc.200301625; Luke MacAleese et al., "Protein Identification with Liquid Chromatography and Matrix Enhanced Secondary Ion Mass Spectrometry (LC-ME-SIMS)," *Journal of Proteomics* 74, no. 7 (June 2011): 993–1001, https://doi.org/10.1016/j.jprot.2011.02.009. Another resource is Maria Perla Colombini and Francesca Modugno, *Organic Mass Spectrometry in Art and Archaeology* (Chichester, UK, 2009).

25. As seen in Stepanka Kuckova, Radovan Hynek, and Milan Kodicek, "Identification of Proteinaceous Binders Used in Artworks by MALDI-TOF Mass Spectrometry," *Analytical and Bioanalytical Chemistry* 388, no. 1 (2007): 201–206, https://doi.org/10.1007/s00216-007-1206-2. For a review of mass spectrometry methods as they respond to the needs of proteomic research, see Timothy P. Cleland and Elena R. Schoeter, "A Comparison of Common Mass Spectrometry Approaches for Paleoproteomics," *Journal of Proteome Research* 17 (2018): 936–45, https://doi.org/10.1021/acs.jproteome.7b00703.

26. For example, Joachim Burger, Susanne Hummel, and Bernd Herrmann, "Palaeogenetics and Cultural Heritage: Species Determination and STR-Genotyping from Ancient DNA in Art and Artifacts," *Thermochimica Acta* 365 (2000): 141–46, https://doi.org/10.1016/S0040-

logical sources of medieval glues used in bookbinding, we required a non-invasive method that could use molecules that survive the glue-making process. The paleoproteomic technique called Electrostatic Zooarchaeology by Mass Spectrometry (eZooMS), an offshoot of Zooarchaeology by Mass Spectrometry (ZooMS), serves both these needs.[27]

ZooMS was developed by Mike Buckley and Matthew Collins in 2010. In this process, microscopic samples of animal material, such as bone, are put through a mass spectrometer to identify the protein fragments (peptides) that make them up. A process known as Peptide Mass Fingerprinting (PMF) is then used to identify the species of animal the sample came from. As each species will have produced slightly different combinations of collagens and peptides, the mass spectrometer produces a peptide profile

6031(00)00621-3; Timothy Stinson, "Knowledge of the Flesh: Using DNA Analysis to Unlock Bibliographical Secrets of Medieval Parchment," *Papers of the Bibliographical Society of America* 103 (2009): 435–53, https://doi.org/10.1086/pbsa.103.4.24293890; Michael Campana et al., "A Flock of Sheep, Goats, and Cattle: Ancient DNA Analysis Reveals Complexities of Historical Parchment Manufacture," *Journal of Archaeological Science* 37 (2010): 1317–25, https://doi.org/10.1016/j.jas.2009.12.036; Timothy Stinson, "Counting Sheep: Potential Applications of DNA Analysis to the Study of Medieval Parchment Production," in *Kodikologie und Paläographie im digitalen Zeitalter 2 = Codicology and Palaeography in the Digital Age* 2, eds. Franz Fischer, Christiane Fritze, and Georg Vogeler (Norderstedt, 2010), 191–207; Matthew Teasdale et al., "Paging through History: Parchment as a Reservoir of Ancient DNA for Next Generation Sequencing," *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 370, no. 1660 (2015): 20130379, https://doi.org/10.1098/rstb.2013.0379; Tomasz Lech, "Ancient DNA in Historical Parchments: Identifying a Procedure for Extraction and Amplification of Genetic Material," *Genetics and Molecular Research* 15 (2016), https://doi.org/10.4238/gmr.15028661.

27. A recent review article offers a comprehensive overview of the state of Paleoproteomics as a field: Christina Warinner, Kristine Korzow Richter, and Matthew J. Collins, "Paleoproteomics," *Chemical Reviews* 122 (2022): 13401–446, https://doi.org/10.1021/acs.chemrev.1c00703.

or "fingerprint" that, when compared to a library of collagen fingerprints from known samples, can be used to identify the species from which the sample was taken.[28]

eZooMS uses the same methods of analysis, but replaces the destructive sampling used for ZooMS with samples taken with PVC erasers, widely used in library conservation for cleaning and conservation of heritage objects. Eraser crumbs from a routine cleaning would usually be thrown away; however, because the rubbing of the eraser across the surface of a book or document generates a faint electrostatic charge, proteins from the surface are attracted to the eraser and bond to the eraser crumbs, meaning they can be used to provide microscopic samples for peptide mass fingerprinting without damage to the object being studied.[29] The crumbs from the eraser are incubated in a saline solution and processed to extract any proteins attracted to the faint electrostatic charge created by rubbing. These proteins are then analyzed through mass spectrometry, providing a "fingerprint" that can be compared to entries in a database, identifying the species used. eZooMS is a kind of microsampling technique, in that the method removes, in many cases, only a microscopic amount of the material to be analyzed. We do not know of any experiments with techniques such as DART, used in forensic science for detecting and analyzing chemicals on surfaces. We have entered into some very preliminary conversations with colleagues in en-

28. Krista McGrath et al., "Identifying Archaeological Bone via Non-Destructive ZooMS and the Materiality of Symbolic Expression: Examples from Iroquoian Bone Points," *Scientific Reports*, 9:1 (2019): 11027, https://doi.org/10.1038/s41598-019-47299-x; Michael Buckley et al., "High-Throughput MicroCT and ZooMS Collagen Fingerprinting of Scombrid Bone from the Marquesas Islands," *Journal of Archaeological Science* 136 (2021): 105475, https://doi.org/10.1016/j.jas.2021.105475.

29. Fiddyment et al., "Animal Origin."

gineering about the use of scanning probe microscopy to gather data about the material components of books.[30]

Over the course of this project, we used eZooMS to process samples from eight medieval manuscripts and early printed books originating in France, Germany, Italy, and Spain. The earliest of these books dates to the twelfth century, although its binding could be as late as the sixteenth century; the others are all from the fifteenth or sixteenth centuries. Of course, many early books held in Canadian collections have been rebound in the modern era. Accordingly, our samples were limited to those from books, both manuscript and printed, which retained medieval (if not original) bindings—that is, bindings that, like the books, had features dateable to the fifteenth or sixteenth centuries, and which showed no obvious signs of modern repair. We also selected books where potentially adhesive-bearing surfaces were exposed, often by unrepaired damage, making them easily accessed for sampling. Canadian collections of medieval European books are relatively small, and these criteria meant that, on first pass, we found just one to three suitable books in each repository. The sampled books share a place and period of origin, but vary in their size and content. Three are large musical manuscripts with heavy wooden boards and tooled leather covers with bosses. Four are philosophical tracts—two manuscript and two printed—and one is a small Book of Hours from fifteenth-century France.

Sampling was conducted by researchers based at each institution, following shared eZooMS protocols and using

30. DART is "direct analysis in real time," an ion source technique that can be used for rapid, noncontact analysis of materials at ambient pressure. See Robert B. Cody, James A. Laramee, and H. Dupont Durst, "Versatile New Ion Source for the Analysis of Materials in Open Air under Ambient Conditions," *Analytical Chemistry* 77:8 (2005): 2297–2302, https://doi.org/10.1021/ac050162j.

testing kits provided by Beasts to Craft; where feasible, a University of Toronto team member travelled to observe and document the sampling process. Working in this distributed way allowed us to build and strengthen a new network between curators and conservators of each collection and interested scientists and scholars. The samples were submitted at different times and results produced in a staggered fashion. This too had benefits, allowing us to catch limitations in our results and refine our sampling processes over time.

In total, non-invasive eZooMS analysis was carried out on twenty-seven samples from areas of suspected adhesive on medieval European bookbindings.[31] We expected collagen-based animal glues, and so we used the standard eZooMS extraction protocol. Briefly, the area that contained adhesives (generally the spine) was gently wiped using pieces of PVC eraser and the resulting eraser crumbs collected for analysis. The eraser crumbs were then incubated in a saline solution and the extracted collagen digested using trypsin. The resulting peptides were analyzed using PMF via MALDI-TOF mass spectrometry to identify the species of origin.

Of the twenty-seven samples analyzed, six produced poor spectral resolution, and the species could not be identified (see Table 1). Of the twenty-one remaining samples, eight were identified as sheep or likely sheep. Two additional samples were identified as sheep or goat, but the resolving identifying marker was not detected. Eight were identified as calf or likely calf, and three had markers for both calf and sheep.

31. The data and metadata generated over the course of the project have been collected and ingested into the Scholar's Portal Dataverse platform for public use. The datasets all have a CC BY-NC-SA 4.0 licence.

Table 1.
Results of eZoomMS Testing for Animal Glues in Medieval European Books

SAMPLE NO.	SAMPLE AREA	RESULT
London, ON, Western University, Archives and Special Collections, MS M2150 (Spain, Granada or Burgos? s.xvi, Choir Book)[32]		
BSRP 01	On exposed spine, just above sewing support nearest headband	Sheep?
BSRP 02	On exposed spine, just above tailband	Sheep
BSRP 03	Interior of right board, upper right turn-in, 6 mm. from top of board; rubbing from adhesive on wooden board, adjacent to mitered edge of turn-in	No ID
BSRP 04	Exterior of left board, where clasp strap was attached (now missing)	No ID
Toronto, ON, Thomas Fisher Rare Book Library, MSS 05321 (France?, ca. 1153, De claustro animae; poss. s.xvi Italian binding)[33]		
BSRP 07	Interior of left cover, white substance	Sheep?

32. This manuscript is siglum CDN-Lu M2150 in the CANTUS Database, which has the most substantial description of the manuscript; see Debra Lacoste, Inventory of "London (Canada), University of Western Ontario – Music Library, M2150," in Cantus: A Database for Latin Ecclesiastical Chant—Inventories of Chant Sources, University of Waterloo, https://cantus.uwaterloo.ca/source/123619 (accessed 12 January 2023). For Western M2150's peptide mass fingerprint datasets, see Sarah Fiddyment, 2021, "MS M2150 Peptide Mass Fingerprinting," https://doi.org/10.5683/SP3/9RBSIK, Borealis, V1, UNF:6:cNhkEpiC/2+mJr6RWZ/BYg== [fileUNF].

33. See Fisher MSS 05321's catalogue entry and digitization at https://fishercollections.library.utoronto.ca/islandora/object/fisher2%3A164 (accessed 12 January 2023). For Fisher MSS 05321's peptide mass fingerprint datasets, see Fiddyment, 2021, "Fisher MSS 05321, Peptide Mass Fingerprinting," https://doi.org/10.5683/SP3/RL2647, Borealis, V1, UNF:6:qLY5MKIkZLDoEhlJSCO4zg== [fileUNF].

SAMPLE NO.	SAMPLE AREA	RESULT
BSRP 08	Interior of left cover (control sample)	Sheep
BSRP 10	Parchment spine lining (fragment from a Hebrew manuscript), interior of left cover at spine. Top of lining is parallel with line on first folio of book-block beginning "est regnum celorum," 12 lines from bottom	Calf
Toronto, ON, Thomas Fisher Rare Book Library, MSS 03245 (France, Arras, s.xv, Book of Hours)[34]		
BSRP 05	Interior of left board, near left leather turn-in	Calf
BSRP 06	Parchment pastedown now detached from interior of right board, verso, mid-page (control sample)	Calf
BSRP 09	Interior of left board, on lower leather turn-in (control sample)	Calf?
Toronto, ON, Thomas Fisher Rare Book Library, MSS 05242 (Italy, Siena, 1407, Questiones in Aristotelis De caelo et mundo)[35]		
BSRP 11	Interior of right board, under paper pastedown	No ID
BSRP 12	On spine, under loose spine covering	Sheep (+Calf?)

34. See Fisher MSS 03245's catalogue entry and digitization at https://fishercollections.library.utoronto.ca/islandora/object/fisher2%3A157 (accessed 12 January 2023). For Fisher MSS 03245's peptide mass fingerprint datasets, see Fiddyment, 2021, "Fisher MSS 03245, Peptide Mass Fingerprinting," https://doi.org/10.5683/SP3/MGCoSC, Borealis, V1, UNF:6:k1l+VxLHgXX2ncIUQ9CmOw== [fileUNF].

35. See Fisher MSS 05242's catalogue entry and digitization at https://fishercollections.library.utoronto.ca/islandora/object/fisher2%3A143 (accessed 12 January 2023). For Fisher MSS 05242's peptide mass fingerprint datasets, see Fiddyment 2021, "Fisher MSS 05242, Peptide Mass Fingerprinting," https://doi.org/10.5683/SP3/DO7IOQ, Borealis, V1, UNF:6:47QZyAmo8omCk3VB316OEg== [fileUNF].

SAMPLE NO.	SAMPLE AREA	RESULT
BSRP 13	On spine, leather cover (control sample)	Sheep
BSRP 14	Parchment fol. 71v (final leaf) (control sample)	Sheep?
Montreal, QC, McGill Library Rare Books and Special Collections, MS 73 (Italy, s. xiii$^{3/4}$–s.xvi$^{1/4}$, s.xv, Choir Book; parchment endleaves including pastedowns s.xii)[36]		
BSRP 15	Interior of right board, shiny substance on pastedown	Sheep/ Goat
BSRP 16	Interior of right board, surface of wood board, with abundance of dark residue	No ID
BSRP 17	Interior of right board, upper right leather turn-in under detached pastedown	No ID
BSRP 19	On spine, spine threads between second and third sewing supports from head of book, close to left board	Sheep?
BSRP 42	Interior of left board, on lower leather turn-in (control sample)	Sheep

36. See digitization and catalogue description at Archive.org https://archive.org/details/McGillLibrary-rbsc_ms-medieval-073-18802/page/n1/mode/2up (accessed 12 January 2023). This manuscript is siglum CDN-Mlr MS Medieval 0073 in the CANTUS database; see Alessandra Ignesti, Inventory of "Montréal, McGill University – Rare Books and Special Collections – Manuscript Collection, MS Medieval 0073," in Cantus, https://cantus.uwaterloo.ca/source/680970 (accessed 12 January 2023). For McGill MS 73's peptide mass fingerprint datasets, see Fiddyment, 2022, "McGill MS 73, Peptide Mass Fingerprinting," https://doi.org/10.5683/SP3/MEDVXE, Borealis, V1, UNF:6:sD68Q74Yu5emEW/8PwAbmQ== [fileUNF].

SAMPLE NO.	SAMPLE AREA	RESULT
Montreal, QC, McGill Library Rare Books and Special Collections, MS 234 (Germany, s. xvi$^{3/4}$, Choir Book)[37]		
BSRP 20	On spine, exposed spine lining, below lowest sewing support near left board	Calf + Sheep?
BSRP 21	Gutter between interior left cover and fol. 1r, where adhesive is visible	Calf + Sheep?
BSRP 22	Parchment fol. 1r, from area near sample BSRP 21 (control sample)	Calf
BSRP 41	Interior of left board, lower corner of leather turn-in (control sample)	Calf
Vancouver, BC, UBC Library Rare Books and Special Collections, PA3892.A2 J6 1480 (Venice, 1480 [Printed Book])[38]		
BSRP 35	Exterior of right board, from wooden board at hinge under leather covering	No ID
BSRP 36	Interior of left board, at centre hinge	Calf
BSRP 37	On spine, at headband	Sheep / Goat

37. See McGill MS 234's digitization and brief description at https://archive.org/details/McGillLibrary-rbsc_ms-medieval-234-21233 This is CDN-Mlr MS Medieval 0234 in the Cantus Database; see also Jennifer Bain et al, Inventory of "Montréal, McGill University – Rare Books and Special Collections – Manuscript Collection, MS Medieval 0234," in CANTUS, available at https://cantus.uwaterloo.ca/source/678936 (accessed 12 January 2023). For McGill MS 234's peptide mass fingerprint datasets, see Fiddyment, 2021, "McGill MS 234, Peptide Mass Fingerprinting," https://doi.org/10.5683/SP3/GIJOUN, Borealis, V1, UNF:6:i-UcZDs5OqbVZemM3TlB6xw== [fileUNF].

38. See UBC PA3892.A2 J6 1480's catalogue entry and digitization at https://open.library.ubc.ca/collections/manuscripts/items/1.0361552. For UBC PA3892.A2 J6 1480's peptide mass fingerprint datasets, see Fiddyment, 2021, "UBC PA3892 A2 J6 1480, Peptide Mass Fingerprinting," https://doi.org/10.5683/SP3/IKKMYL, Borealis, V1.

SAMPLE NO.	SAMPLE AREA	RESULT
Vancouver, BC, UBC Library Rare Books and Special Collections, BX1749.T52 1484 (Venice, 1484 [Printed Book])[39]		
BSRP 38	On spine, at second sewing support	Calf

The first book we studied, London, ON, Western University, Archives & Special Collections, MS M2150, is a sixteenth-century antiphonal perhaps from a monastery in Burgos, Spain.[40] It was ideal for our purposes because its spine is exposed in two areas and the leather covering is loose around the turn-ins, allowing us to collect samples from two areas in which we expected to find adhesive without disrupting or damaging the manuscript.[41] A Rare Books Librarian at Western University took four samples, collecting eraser rubbings from the exposed areas on the spine, as well as from locations both on and under the turn-ins.

39. See UBC BX1749.T52 1484's catalogue entry and digitization at https://open.library.ubc.ca/collections/manuscripts/items/1.0364177?o=0 ; (accessed 12 January 2023). For UBC BX1749.T52 1484's peptide mass fingerprint datasets, see Fiddyment, 2021, "UBC BX1749.T52 1484, Peptide Mass Fingerprinting," https://doi.org/10.5683/SP3/IQA2QV, Borealis, V1, UNF:6:BFXsmxV+1bnRsEhGcrP40A== [fileUNF].

40. Debra S. Lacoste, "A Late-Medieval Antiphoner at the University of Western Ontario," in *Chant and its Peripheries: Essays in Honour of Terence Bailey*, ed. Bryan Gillingham and Paul Merkley (Ottawa, 1998), 310–19.

41. We use the standardized terminology of the Language of Bindings Thesaurus (https://www.ligatus.org.uk/lob/), developed by the Ligatus research group for working with and describing historic books. One point that may be new to some readers is the use of "left/right" for covers, boards, endleaves, etc., instead of "front/back" or "upper/lower." This is meant to avoid confusion about which board or leaf is the front. Whether in Latin, Syriac, or Arabic, when the book is open in front of the user, left and right remain consistent.

When the samples were analyzed, the eZooMS method was unable to identify any animal proteins on the turn-ins. Although it is possible that this represents problems with the sample, it is more likely that the adhesive used on the turn-ins was wheat paste, not animal glue, which would be in keeping with known binding practices in the region and the period.[42] Both spine samples, in contrast, had protein markers indicating the use of sheep-based materials. At this point, however, we reached a revealing limit of our method. Clearly the markers could have been from a glue; we would expect to find an animal glue in this position on the book, and we were careful to take the sample from what appeared to be remnants of an adhesive. All in all, the finding of identifiable animal proteins here suggests a sheep animal glue. But other components of the spine were sourced from animals, and we cannot rule out contamination of the sample: the lining of the spine is parchment, the cover leather. Both components might also be sheep in origin.

Accordingly, we adjusted our methods to include control samples from areas without adhesive, in order to compare control-area proteins with those from the sampled areas and to rule out proteins that may have come from other components of the codex. This was the approach we took with our next set of samples, three manuscripts from the Thomas Fisher Rare Book Library in Toronto: MSS 03245, a fifteenth-century Book of Hours from Arras; MSS 05321, a mid-twelfth-century academic manuscript, possibly of French origin, in a later, possibly sixteenth-century, Italian binding; and MSS 05242, an early fifteenth-century Italian university manuscript in a fifteenth-century binding.

All samples were taken by an author, with Fisher librarians and conservators observing. From MSS 03245—the Book of Hours—a sample was taken from an area of adhesive on the interior of the left board near the left turn-in

42. Szirmai, *Archaeology of Medieval Bookbinding*, 162.

of the book's leather cover, along with control samples from the leather cover and parchment pastedown (now detached). All three revealed bovine protein markers, suggesting the use of cow, ox, or calf products in the components. This probably included the glue, but we could not distinguish those components' origin with certainty.

From MSS 05321, a twelfth-century copy of Hugh of Fouilloy's *De claustro animae* in a sixteenth-century Italian limp binding, we sampled the interior of the left cover in two areas, where a white substance was visible, and where it was not. We also took a sample from one of several Hebrew manuscript fragments used in the binding as spine linings. The parchment fragment gave us a bovine protein marker result, while both the control sample and the white substance on the interior of the leather cover gave us protein markers associated with sheep products. Because the markers from both the control sample and the potential adhesive were from the same animal, we again were not able to say definitively whether we had identified proteins from adhesive residue.

We had a clearer outcome with MSS 05242, a copy of Albert of Saxony's *Quaestiones in libros Aristotelis de caelo et mundo* that can be dated by colophon and owner inscriptions to 1407, Siena, Italy. The catalogue entry describes MSS 05242 as having "beech boards partially covered with pigskin, with raised bands; with remains of clasps," and offers a fifteenth-century, possibly French origin for this binding.[43] One board has detached completely from the book, allowing us interior access for the spine for sampling. Samples were taken from the interior of the right board underneath a paper paste-down, and from beneath the loosened cover of the spine. Control samples were taken from

43. Thomas Fisher Rare Book Library, catalogue entry for MS 05242, "Questiones in Aristotelis De caelo et mundo," accessed 21 February 2022, https://fishercollections.library.utoronto.ca/islandora/object/fisher2:143.

the leather cover and from the final parchment leaf. We were unable to identify protein markers from beneath the paper paste-down, suggesting the binder used wheat paste or other non-animal adhesive. The leather cover of the spine and the parchment both contain sheep-related proteins, meaning the catalog's description of the leather as pigskin is not correct—a useful, albeit incidental, finding. Under the loose spine cover, we had our first success clearly identifying an adhesive when our sample revealed protein markers for multiple species. Samples with multiple species usually arise in areas that have multiple components (i.e., parchment page, leather cover, and adhesive). This sample yielded calf and sheep markers. By comparing this result to the control samples from the parchment and leather, we were able to attempt a formal identification. We identified the control samples from the nearby parchment and the leather cover as sheep. This suggests the adhesive was bovine in origin (although a mixture of calf and sheep glue cannot be completely ruled out).

Our third group of samples was taken from two books in McGill University Library's Rare Books and Special Collections. Both are choir books: MS 73 is Italian, with gatherings written between the late thirteenth and fifteenth centuries, and MS 234 is German, of the late sixteenth century. Samples were taken by a McGill University Rare Books Librarian. We investigated five samples from McGill MS 73: three from the interior of the right board—from a shiny material on the parchment paste-down, from a loose dark residue on the wooden surface, and from an area of the leather turn-in where the paste-down was formerly adhered; one sample from spine threads between the second and third sewing supports (counting from the head); and an additional control sample from the lower leather turn-in on the left board. We found no identifying protein markers in the samples from the dark residue on the wood or from the adhered surface of the leather turn-in of the right board. The reflective material found on the paste-down revealed sheep or goat markers. The spine threads appear to have some sheep proteins, and the control sample of the leather turn-in from the left cover was analyzed as sheep. Once again, it is not possible to say definitively that we identified traces of adhesive on the spine threads; however, it was useful to confirm that the shiny spill on the paste-down was indeed an animal product

We had more conclusive results from the German antiphonal, McGill MS 234. From this manuscript, we took samples from the exposed spine lining and from an area of adhesive visible along the gutter of fol. 1r (a leaf original to the manuscript), along with control samples from an adjacent area of fol. 1r and from the leather turn-in at the lower corner of the left cover. Here, our results were the opposite of the Fisher samples in which we found evidence of adhesive. In this manuscript the control samples from the neighbouring parchment and the leather cover were both identified as bovine, and we found protein markers for multiple species—sheep as well as cow—in our samples from the spine and gutter. Although we cannot rule out that there is also calf glue, or glue from a combination of species, present, we can say that the glue does contain sheep collagen and the additional bovine markers are likely cross-contamination from the surrounding parchment.

The final books we sampled were two fifteenth-century early printed Venetian books from the collections of the University of British Columbia. The first of these, Vancouver, UBC Library Rare Books and Special Collections, PA3892.A2 J6 1480, was printed in 1480 by printers Johann von Köln and Johann Manthen. It is an edition of Jean de Jandun's commentary on Aristotle's *De anima* and measures 315 mm. high by 220 mm. wide. The binding has notable damage, including a detached right board, scratches on the exposed left board, missing endleaves, torn outside joins, and exposed sewing cords. We studied three samples: one from the wooden right board, at the hinge, under the leather cover; one from the center hinge at the interior of the left board, and one from the interior spine, at the head band. We were not able to identify any species from the first sample. The second sample, of the interior hinge, was identified as calf, while our sample from the interior spine was either sheep or goat. We concluded that two animal species were sources of the components of this early book, and it is possible that the sample from the interior spine reflects the use of an animal glue.

Our last sample was a single sample taken from Vancouver, UBC Library Rare Books and Special Collections, BX1749.T52 1484, a 1484 printing on paper of the first part of Thomas Aquinas's *Summa theologicae* printed by Antoninus de Strata. It is made up of 215 leaves and measures 300 mm. high by 225 mm. wide. Our sole sample from this codex is taken from an exposed cord in the spine, the second from the top. It was identified as bovine; however, we did not have other samples to which we could compare this result.

CONCLUSIONS

The results of this preliminary and experimental project suggest that the animal glues used in medieval books do yield collagen peptide fingerprints, and that these are detectable and in some cases identifiable by species, through eZooMS. Usefully, our results also reveal limits to the identification of animal glues using eZooMS. First, if the species used in manufacturing the animal glue is the same as the species used in producing the parchment or leather that is being adhered, the results of sampling may be suggestive, but they cannot be definitive: it is not possible to tell which protein markers belong to the skins used in the book, and which to the adhesive made from skins, or whether an adhesive is being detected at all. Second, when a mix of species is detected, we can say that there is likely an adhesive contributing to the collagen fingerprint—a single piece of parchment cannot be from both a sheep and a cow. But we cannot confirm that a glue is from a single species that differs from the surrounding animal components (in which case we would be picking up proteins from multiple components), or whether it was made from multiple animals of different species.

Encountering these limits and working within them allowed us to see some new ways forward. We think that broader sampling from a greater variety of manuscripts might allow us to refine how we identify, sample, and read the analysis of potential adhesives, and would be beneficial, given how little research has been done on the use of animal glues in manuscripts. With a broader sample set, we could see how often the parchment animal and the adhesive animal differ from one another. Was it more convenient to use the same animal for both, particularly in a bookbinding shop in which parchment scraps were being processed in-house? Economic records show that glue-making developed as a specific, separate craft: can we see evidence of new "trade" glues, and do they differ in the species used? How do animal glues change when books are printed on paper: did parchment scraps disappear from bookshops? Is there any discernible geographic variation in specific animals used for adhesives across Europe?

We could also broaden the scope of our inquiry to include a wider range of materials. Our research focused on animal glues, the principal glue used on late medieval book spines. More extensive proteomic analysis could potentially identify other protein sources, like aloe or wheat, through a comprehensive proteomic study using LC-MS/MS. We think it would be particularly interesting to explore this question in conjunction with looking at non-Western codices, in which adhesive was used under covered spines from an earlier date than in Western book production.[44] We started our research hoping to compare the glues of Western medieval bookbinding to those found in books from other regions, particularly given the movement of books

44. Georgios Boudalis, personal communication, 18 February 2022.

and technology around the Mediterranean, and we hope that future research into this question will be made possible by an increase in the number of collaborating institutions and researchers as well, perhaps, as an expansion of the collagen fingerprint library to include new species.

Finally, we hope that our initial tests will increase awareness and interest in this work among conservators, librarians, and book historians. Our preliminary results underscore the advantage of simple, cheap, non-destructive sampling processes that can be expanded to test a large number of books and that offers a new source of information to researchers and conservators. This is, in itself, a contribution to the emerging field of "biocodicology" or "manuscience."[45] Our project also suggested one way for that field to advance: through ongoing, accretive projects which build databases, collections of results, and collaborative relationships over an extended period of time and in different stages.

University of Toronto (AHS, JL, AG)
University of Cambridge (SF)
University of British Columbia (AL)
Western University (DMW)
Institute for Advanced Study, Princeton (MM)
McGill University (LW)

45. Domenico Pangallo, Katarina Chovanova, and Alena Makova, "Identification of Animal Skin of Historical Parchments by Polymerase Chain Reaction (PCR)-Based Methods," *Journal of Archaeological Science* 37, no. 6 (2010): 1202–1206, https://doi.org/10.1016/j.jas.2009.12.018; Sarah Fiddyment et al., "So you want to do biocodicology? A Field Guide to the Biological Analysis of Parchment," *Heritage Science* 7, no. 35 (2019), https://doi.org/10.1186/s40494-019-0278-6; Guadalupe Piñar et al., "Decoding the Biological Information Contained in Two Ancient Slavonic Parchment Codices: An Added Historical Value," *Environmental Microbiology* (2020): 3218–33, https://doi.org/10.1111/1462-2920.15064; "Manuscience: From Technology to Text," website, accessed 23 February 2022, https://www.manuscience.com.