Cell 181, June 11, 2020 (https://doi.org/10.1016/j.cell.2020.05.031)

Unroll Please: Deciphering the Genetic Code in Scrolls and other Ancient Materials

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The unrelenting development of ancient DNA methods now allows researchers to obtain archaeogenetic data from increasingly diverse sources. In a new study, researchers apply the latest DNA technologies to unravel the mysteries of the Dead Sea Scrolls, one of the world's most famous and influential sets of ancient parchments.

A series of breakthroughs in ancient DNA technology has in recent years allowed scientists to redefine our understanding of human history and prehistory. Even though most archaeogenetic work has been focused on analyzing DNA from human bones and teeth (see (Slatkin and Racimo, 2016), the remains of ancient humans are sometimes not available for sampling, or do not exist at all. Therefore, geneticists need to take alternative, more imaginative, routes to study our history. For example, some recent studies have made use of less obvious sources of ancient human DNA, such as human coprolites and cave sediments (Gilbert et al., 2008; Slon et al., 2017). However, there is also a more indirect way to study human history, especially when ancient human remains are absent: animal DNA from artifacts manufactured by humans, such as tools, clothing, ornaments and parchments. In this issue of *Cell*, Anava et al. (2020) combine the full power of modern ancient DNA laboratory methods with state-of-the-art bioinformatic analyses to explore the origin of a set of parchments with fundamental historical, religious and linguistic significance: the Dead Sea Scrolls.

Identification of ancient animal DNA recovered from parchments was early recognized as a promising source of human socio-cultural information, providing clues on historic practices in manuscript production and knowledge transmission (reviewed in Bower et al., 2010). This is because parchment made of dried animal skin (typically goat, sheep or cattle) was the most common writing material for religious and formal legal documents and records until the 16th Century. However, it was not until modern sequencing technologies bloomed, and thus allowed the recovery of good quality ancient DNA, that more in-depth studies of the characteristics and origin of parchments became possible (Teasdale et al., 2015).

The Dead Sea Scrolls (DSS) are a collection of thousands of fragments and scraps of manuscripts dated to between the third century BCE and the first century CE found in the Judean Desert, which contain religious literature highly influential for both Judaism and Christianity. Early efforts of obtaining DNA from the DSS were already tailored at discerning which animals had been used for parchment production in the region, and sorting out which fragments could be grouped into a manuscript by identifying similar DNA signals between them (Woodward et al., 1996).

In Anava et al. (2020), the authors extracted ancient DNA and used high-throughput DNA sequencing to recover vast amounts of data from multiple samples of DSS parchments. The results show that most of the DSS fragments were made of sheepskin and only a few of cowskin. By comparing the sheep haplotypes recovered from the parchments, they were also able to pinpoint that the geographical origin of these animals was consistent with Qumran, the region where the DSS fragments were found. However, it seems that the cow-made parchments do not originate from the Judean Desert at all, since cows were not present in that area at that time. Thus, at least some parts of the DSS may have been written somewhere else and later imported into the region of Judea, which in turn has implications regarding the textual pluriformity of the DSS.

Fig. 1. *Recovery of DNA from ancient parchments.*

As is the case for most ancient DNA research, the shadow of contamination is an ever-present issue. In the case of parchments such as the DSS, several forms of contamination are possible, including microbial DNA from the soil matrix that the fragments were found in, human contamination from the numerous people handling of the fragments since their discovery, and cross-contamination between fragments made from different species due to leakage during storage or the original production of the parchment (Campana et al., 2010). In addition, DNA from domestic and laboratory animals such as sheep, cow and pig sometimes occur in commercial reagents (Leonard et al., 2007), which could lead to contamination during laboratory work. To address these issues, Anava and colleagues spent a considerable amount of effort using computational methods to rule out the effects of contamination, both on species identification and when trying to resolve which fragments belonged to the same manuscript. They also included negative controls during DNA extraction, and sequenced libraries built from these in order to monitor for contamination. In most cases, this enabled the authors to disentangle and alleviate the effect of contamination. In one case, however, goat DNA was identified in a parchment fragment from a scroll that otherwise was made of sheepskin, and the authors were unable to validate whether this was real or just some form of contamination.

It should however be noted that non-endogenous DNA from the parchments could also provide additional insights into the history of the DSS. For example, both ancient microbiomes and DNA from plants recovered from the parchments could yield information about the environment of the time the scrolls were written. Also, it is intriguing to think that DNA from the DSS's original authors may be hiding somewhere among the 2.6 billion DNA sequences generated by Anava et al. (2020). Unfortunately, separating the authentic ancient microbial and plant DNA, as well as the authors' DNA sequences, from modern contamination could be exceedingly difficult, if not outright impossible.

In general, as ancient DNA methods and computational techniques develop further in the future, we expect that researchers will be capable of obtaining valuable archaeogenetic data from new, and even more imaginative, untapped sources. For example, maybe it will be possible to obtain a glimpse into ancient trade routes through DNA analysis of tools, figurines and other items manufactured by Paleolithic humans using the bones, teeth and ivory from the Pleistocene Megafauna. And perhaps residues from the surface of well preserved pottery items can yield genetic information of the animal origin of the foods that were stored in it? What about tissue and blood residues on stone arrow tips or other hunting tools? Some of these sources were unsuccessfully explored in the past with more rudimentary ancient DNA methods (see Green and Speller, 2017), but could potentially soon be sequenced and read like an open book, enabling further tidbits of history to unroll before our eyes.

Acknowledgements
D.D.d.M. is funded from the Tryggers Foundation (CTS 17:109), and L.D. acknowledges funding from the Swedish Research Council (grant 2017-04647). The authors would also like to thank Erik Ersmark for providing the illustration in Fig. 1.

Figure caption
Ancient DNA allows for species-identification of the animal skins that parchments of the Dead Sea Scrolls were made from (*illustration by Erik Ersmark*).

Declaration of Interests
The authors declare no competing interests.

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