



Challenges in extracting and preserving nuclear DNA from fossils for reviving extinct species

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Abstract. The prospect of extracting nuclear DNA from ancient fossils to reconstruct extinct species has long intrigued both scientists and the public. However, this ambitious goal is hindered by significant scientific challenges. DNA degradation over time, resulting in fragmented and chemically altered sequences, limits the usability of ancient DNA, especially beyond 100,000 years. Modern DNA contamination, chemical modifications such as deamination, and the low yield of nuclear DNA further complicate the process. While mitochondrial DNA is more abundant and better preserved, nuclear DNA is essential for genomic reconstruction but suffers from amplified degradation and bias. Incomplete genomic assembly due to short, non-overlapping fragments and the absence of suitable reference genomes poses additional obstacles. Ethical and practical issues, including low cloning success rates and cross-species cellular incompatibility, further exacerbate the challenges of de-extinction. Despite advancements in genomics and bioinformatics, these limitations underline the complexity of resurrecting extinct species. This paper explores these scientific and ethical barriers, providing a comprehensive overview of the current state of de-extinction research and its future prospects.

Key words: ancient DNA, de-extinction, ethical challenges, genomic reconstruction, nuclear DNA degradation.

Introduction. Paleontology and phylogeny require well-preserved fossils, as well as well-preserved DNA over time, in order to accurately reconstruct the evolutionary history of species (Petrescu-Mag et al 2007; Petrescu-Mag & Oroian 2018). The prospect of extracting nuclear DNA from ancient fossils to reconstruct extinct species has long captured the imagination of scientists and the public alike (Liang et al 2021). However, this ambitious endeavor is fraught with numerous scientific challenges and limitations that make the unaltered extraction of nuclear DNA nearly impossible. Below, we explore the key obstacles grounded in current scientific knowledge.

DNA degradation over time. One of the most significant challenges in working with ancient fossils is the degradation of DNA over time. After an organism dies, its DNA begins to break down due to exposure to environmental factors such as temperature, humidity, and microbial activity. This degradation results in highly fragmented and chemically altered DNA (Figure 1). Studies have shown that the half-life of DNA is approximately 521 years under optimal preservation conditions (Bailleul & Li 2021), meaning that beyond a certain temporal threshold (usually 100,000 years or less), most DNA is too degraded to be usable.

Nucleases play a critical role in the post-mortem degradation of DNA, significantly impacting its preservation in ancient remains. These enzymes, which cleave DNA molecules, are normally regulated within living cells. However, after death, cellular regulatory systems cease to function, and endogenous nucleases become active, initiating the breakdown of DNA. Additionally, exogenous nucleases from environmental microorganisms further accelerate this process. The activity of nucleases is influenced by external factors such as temperature, humidity, and oxygen levels, with higher

temperatures and moisture enhancing enzymatic degradation. This enzymatic activity leads to the fragmentation and chemical modification of DNA, rendering it increasingly difficult to recover intact sequences over time. While nucleases are a major driver of DNA degradation, their activity is minimized in extreme conditions, such as permafrost, where cold temperatures slow enzymatic reactions. Understanding the role of nucleases in DNA degradation is essential for improving methods to recover ancient DNA and for evaluating the preservation potential of fossilized remains.

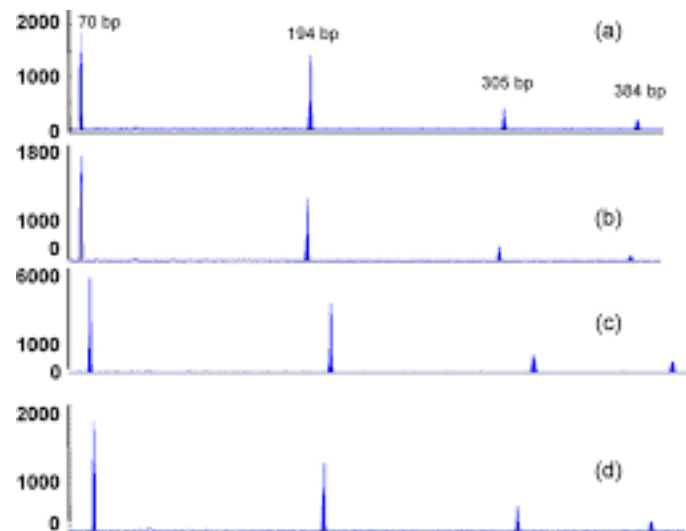


Figure 1. The graph shows electropherograms derived from DNA samples extracted from soft muscle tissue of fragmented and whole rabbit carcasses, measured at 13 and 112 accumulated degree-days (ADD) (Nazir et al 2011). Panel (a) illustrates the DNA profile from body fragments at 13 ADD. At this early stage post-mortem, a full 4-plex DNA profile is observed, indicating minimal degradation. Panel (b) shows the DNA profile from body fragments at 112 ADD. At this later stage, significant degradation is evident, with a reduced ability to generate complete DNA profiles. Panel (c) depicts the DNA profile from whole bodies at 13 ADD. Similar to the fragmented bodies, the DNA remains intact enough to yield a full 4-plex profile. Panel (d) represents the DNA profile from whole bodies at 112 ADD. Degradation is also evident, mirroring the results seen in body fragments. The figure highlights that while DNA integrity is initially preserved (13 ADD), degradation becomes progressively more pronounced as ADD increases, correlating with the environmental accumulation of time and temperature. This reinforces the importance of early DNA sample collection in forensic contexts, as degradation may impede DNA profiling after prolonged intervals (see details in Nazir et al 2011).

Contamination from modern DNA. Another major difficulty is contamination from modern DNA. Fossils are often handled by humans, exposed to laboratory equipment, and sometimes even treated with conservation materials that introduce foreign DNA (Arning & Wilson 2020; Peyrégne & Peter 2020). Additionally, environmental microbes and other organisms that have interacted with the fossil over millennia further complicate the extraction of pure ancient DNA (Gaeta 2021). Distinguishing between endogenous ancient DNA and exogenous contaminants is an intricate process that requires advanced bioinformatics tools.

Chemical modifications to DNA. Over time, DNA undergoes chemical modifications such as deamination, which converts cytosine to uracil, leading to erroneous sequences when the DNA is analyzed. These post-mortem changes make it challenging to reconstruct the original genetic code accurately. Advanced techniques like uracil-DNA glycosylase can address some of these issues (Rohland et al 2015), but they do not fully restore the original sequence fidelity.

Low yield of nuclear DNA. While mitochondrial DNA (mtDNA) is often more accessible due to its abundance and circular structure, nuclear DNA is typically present in much smaller quantities and is more prone to degradation (Mundorff & Davoren 2014). This

disparity makes it difficult to recover sufficient nuclear DNA for meaningful reconstruction efforts. Additionally, the extraction process often preferentially amplifies mtDNA, further complicating the recovery of nuclear DNA.

Lack of suitable fossil preservation conditions. Fossils found in temperate or tropical regions are particularly challenging for DNA preservation due to higher temperatures and humidity levels, which accelerate degradation. In contrast, permafrost or other extreme cold environments provide better conditions for DNA preservation. However, even in optimal conditions, DNA survival is limited to a few hundred thousand years at best, far shorter than the timespan since most extinct species, such as dinosaurs, disappeared.

Incomplete genomic reconstruction. Even if fragments of nuclear DNA are successfully recovered, assembling these fragments into a complete genome is an extraordinary challenge. Ancient DNA fragments are often short and lack the overlap needed for accurate genome assembly (Kearney & Clark 2003). Additionally, the absence of a closely related reference genome can make it nearly impossible to fill in the gaps, leading to incomplete or erroneous reconstructions.

Ethical and practical concerns in cloning. Reconstructing an extinct organism's genome is only one part of the challenge. Even if a complete genome is obtained, creating a viable organism requires advanced cloning techniques, such as somatic cell nuclear transfer (SCNT). This process has a low success rate, even with living species, and faces additional complications when using ancient or synthetic genomes. Ethical questions also arise regarding the implications of reviving extinct species, especially if they have no natural habitat in the modern world (Cottrell et al 2014).

Cross-species cellular compatibility. To clone an extinct organism, a compatible egg cell from a closely related species is required. However, genetic and epigenetic differences between species often result in developmental failures. For example, the cellular machinery of a host species may not correctly interpret the regulatory sequences of the extinct species' DNA, leading to non-viable embryos.

Conclusions. While the dream of resurrecting extinct species through nuclear DNA extraction from fossils is scientifically fascinating, it is hindered by formidable challenges, including DNA degradation, contamination, chemical modifications, and the difficulty of reconstructing complete genomes. Current advancements in genomics, bioinformatics, and cloning technologies provide hope for overcoming some of these barriers, but many fundamental limitations remain. As research progresses, a deeper understanding of these obstacles will be essential for determining whether de-extinction is a feasible goal or remains within the realm of science fiction.

Conflict of interests. The authors declare that there is no conflict of interest.

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