Journal of Genetic Engineering and Biotechnology 24 (2026) 100702

Contents lists available at ScienceDirect

Journal of Genetic Engineering and Biotechnology

journal homepage: www.elsevier.com/locate/jgeb

Recovery and analysis of ancient DNA: challenges, methods, and applications in forensic and archaeological science☆

Silverstein Jay [a,*], Chapman Amber [b]

[a] Department of Physical Sciences, Nottingham Trent University, 101 Erasmus Darwin Building, Clifton Lane, Nottingham NG11 8NS, UK
[b] Department of Physical Sciences, Nottingham Trent University, UK

ARTICLE INFO

Keywords:
Ancient DNA
Forensic genetics
Ancestry
Metagenomics
Next-Generation Sequencing (NGS)
Ethical archaeology
Ethical genetics

ABSTRACT

Ancient DNA (aDNA) research has revolutionised archaeology and forensic science by enabling genomic recovery from highly degraded remains. This review explores the biochemical and environmental factors influencing aDNA preservation, alongside methodological advances that have improved data yield and authenticity. Techniques such as next-generation sequencing (NGS), single-stranded library preparation, and hybridisation capture have transformed the field, allowing recovery from ultrashort fragments and challenging contexts such as warm climates. Authentication strategies—including cytosine deamination profiling, fragment length analysis, and rigorous contamination controls—remain essential to ensure reliability.

Applications of aDNA extend beyond ancestry reconstruction and population genetics to include forensic identification, kinship analysis, and pathogen detection. Lessons from forensic genetics, such as stringent validation and contamination mitigation, have informed best practices in archaeological contexts. However, ethical considerations are central to both domains. Issues of Indigenous data sovereignty, consent, repatriation, and culturally sensitive interpretation demand transparent, community-led research frameworks. These principles align with international agreements such as the Nagoya Protocol and emerging guidelines for equitable benefit-sharing.

Despite significant progress, challenges persist, including geographic sampling bias, interpretive uncertainty, and the need for interdisciplinary integration. Future directions emphasise long-read sequencing, metagenomic approaches, and artificial intelligence-driven analytics, alongside robust ethical governance. By combining technological innovation with culturally responsible practices, aDNA research continues to advance our understanding of human history while reinforcing the importance of ethical stewardship in forensic and archaeological science.

1. Introduction

Over the last few decades, the analysis of ancient DNA (aDNA) has transformed the fields of archaeology, forensic science, biological anthropology, and paleopathology. By extracting genetic material from remains, ranging from prehistoric skeletons to mummified tissues, researchers have gained unique insights into ancestry, kinship, population migration, and pathogen evolution. These advances have started to answer questions that cannot be answered through osteological or macroscopic evidence alone.[1,2] Ancestry was once inferred through craniometric analysis, burial context, or artefactual similarity; since, genomic tools have allowed for precise mapping of shared lineages, admixture events, and family ties. This molecular insight has confirmed long-standing archaeological hypotheses, but also overturned others,

Abbreviations: **aDNA**, Ancient DNA; **AI**, Artificial Intelligence; **AMY1**, Amylase 1 gene; **C→T**, Cytosine to Thymine transitions; **DNA**, Deoxyribonucleic acid; **dsDNA**, Double stranded DNA; **DVI**, Disaster Victim Identification; **EDTA**, Ethylenediaminetetraacetic acid; **HEPA**, High Efficiency Particulate Air; **HERC2**, SLC24A5, SLC45A2, Genes associated with pigmentation; **LCT**, Lactase gene; **MPS**, Massively Parallel Sequencing; **mtDNA**, Mitochondrial DNA; **NAGPRA**, Native American Graves Protection and Repatriation Act; **NGS**, Next Generation Sequencing; **PCR**, Polymerase Chain Reaction; **PMI**, Postmortem Interval; **RNA**, Ribonucleic acid; **SNP**, Single Nucleotide Polymorphism; **ssDNA**, Single Stranded DNA; **STR**, Short Tandem Repeat; **UV**, Ultraviolet; **WGS**, Whole Genome Sequencing; **Y-STR**, Y-chromosome Short Tandem Repeat.

☆ This article is part of a special issue entitled: 'Ancient DNA' published in Journal of Genetic Engineering and Biotechnology.

* Corresponding author.
E-mail address: jay.silverstein@ntu.ac.uk (S. Jay).

https://doi.org/10.1016/j.jgeb.2026.100702

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challenging assumptions about cultural continuity, the scale of migrations, and social structure.[3,4]

1.1. Paleopathology and metagenomics

One of the most transformative applications of aDNA research techniques has been in tracing infectious disease through time. Pathogen genomes including *Mycobacterium tuberculosis, Treponema pallidum, Variola virus,* and *Mycobacterium leprae* have been retrieved from ancient human remains, illustrating the long-term evolutionary trajectories of some of the most devastating diseases in human history.[5] Genomic data from American skeletons, for example, show evidence of tuberculosis lineages likely originating from marine mammals, not Europeans as long assumed.[6] The detection of *Yersinia pestis* in Neolithic burials suggests that plagues may have circulated in human populations thousands of years before the historically documented outbreaks in the Roman and Medieval periods.[7] In Europe, genomic analysis of *M. leprae* has shown that the medieval leprosy strains remain remarkably similar to that seen today, indicating low evolutionary turnover, and strong genomic stasis.[8]

New techniques, such as shotgun metagenomics and target enrichment, have expanded the research scope of ancient pathogen detection. Warinner et al (2014) showed that dental calculus can preserve both bacterial DNA and host immune proteins, offering insight into health, diet, and inflammation.[9] Following this, Vågene et al (2018) applied metagenomic sequencing to victims of a 16th century epidemic in Mexico, identifying *Salmonella enterica* DNA as the most likely cause of the Cocoliztli outbreak.[10] These studies demonstrate how aDNA allows researchers to reconstruct past epidemics in molecular detail.

The application of aDNA methods in paleopathology also extends to parasitic and congenital disease. Molecular evidence of *Plasmodium falciparum*, the parasite responsible for malaria, has been recovered from Egyptian mummies,[11] as well as the detection of other pathogens such as *Toxoplasma gondii* and *Schistosoma haematobium*.[12] Additionally, whole genome sequencing of 'elite' remains has revealed mutations associated with congenital disorders, including achondroplasia, cleidocranial dysplasia, and familial hypercholesterolaemia.[13,14]

1.2. Genomic insights into human mobility and connectivity

Another significant application of aDNA studies is reconstructing human ancestry and migration patterns. Genomic data from Mesolithic, Neolithic, and Bronze Age individuals has shown that modern European populations are the result of admixture between at least three ancestral sources.[15] These movements, especially the Bronze Age influx from the Steppe, had a major effect on the genetic landscape of Europe and may be linked to the spread of Indo-European languages.[16] In Africa and the Near East, ancient genomes have revealed complex demographic histories. These studies challenge outdated models of isolated regional development and reveal the depth of prehistoric connectivity.

aDNA analysis has enabled kinship reconstruction and phenotypic inference from highly degraded remains. Studies have demonstrated that genetic markers such as mitochondrial DNA (mtDNA), Y-chromosome STRs, and autosomal SNPs can reveal maternal and paternal lineages, confirm family relationships, and predict traits including pigmentation and dietary adaptations. These insights have illuminated genealogical links in historical populations, such as royal Egyptian mummies, and provided evidence of gene–culture coevolution in prehistoric communities. The methodological and interpretive details of these approaches are discussed later in this review.

1.3. Innovations in ancient DNA sequencing

These findings are supported by major methodological advances. For example, traditional PCR was once standard, however, is prone to contamination and artefacts such as allelic dropout. The shift to next generation sequencing (NGS) has enabled whole genome analysis from severely fragmented DNA, improving authenticity and reducing bias. Furthermore, single-stranded library preparation techniques have improved recovery from ultra-short fragments.[17]

Authentication remains a crucial component of aDNA workflows. Characteristic damage patterns, such as cytosine deamination at read ends, are used to differentiate ancient from modern DNA;[18] these are analysed through various bioinformatics tools, like MapDamage and PMDtools, to help assess authenticity and remove likely contaminants.[19] Laboratory protocols involve cleanrooms, bleach and UV sterilisation, dedicated reagents, and negative controls to minimise contamination.[20]

1.4. Ethics, forensics, and genomics

In addition to methodological complexity, aDNA research raises pressing ethical issues. Many studied, ancient populations have living descendant communities, often Indigenous or historically marginalised, who may not have consented to their ancestors' remains being genetically analysed. Garrison et al (2019) and Claw et al (2018) discussed the adoption of Indigenous data sovereignty principles, emphasising collaborative research frameworks that respect cultural values and empower communities to guide interpretation and dissemination.[21,22] Best ethical practices now include minimally destructive sampling, transparent communication, and retuning of results to stakeholder communities. Repatriation of remains, especially in contexts covered by legal frameworks such as NAGPRA (Native American Graves Protection and Repatriation Act), highlights the need for consultation and consent. Research proposals involving human remains are increasingly expected to include ethics statements and community engagement plans.

Forensic applications of aDNA have also expanded. Techniques developed for ancient samples are now applied to disaster victim identification (DVI), cold cases, and the analysis of highly degraded remains in forensic contexts.[23] On the other hand, forensic science has contributed strict validation procedures, such as quantitative thresholds, contamination control, and statistical interpretation, that are increasingly adopted in archaeological genetics. As the field advances, integration with other disciplines continues to expand. Isotopic analysis complements aDNA research by providing information about diet and mobility. When combined with genomic data, this enables refined reconstructions of life history. Similarly, linguistics and archaeology benefit from genetic insights into population movement, especially when explaining language dispersals or cultural transitions.[24]

Despite these advances, significant research gaps remain. Much of the current aDNA literature disproportionally focuses on Eurasian populations, with comparatively fewer genomic studies conducted on African, Oceanic, and Indigenous American remains. This geographic bias limits the understanding of global human diversity and understates the complexity of demographic histories in underrepresented regions. Additionally, pathogen research has primarily targeted bacterial genomes, leaving viral and fungal pathogens underexplored due to their typically lower biomass and greater degradation over time. Challenges remain in recovering nuclear DNA from tropical and arid environments where heat, humidity, and microbial activity accelerate degradation. As climate change accelerates permafrost thaw and exposes archaeological sites, there is increasing urgency to recovery and study vulnerable remains before further degradation occurs.

These factors highlight the current relevance of aDNA research, not only in addressing unanswered historical questions but also in preserving endangered genetic information. Ongoing technological improvements, alongside interdisciplinary and community-based approaches, is essential to overcome existing biases, expand the geographic and taxonomic scope of studies, and ensure that aDNA research progresses both scientifically and ethically.

This review critically examines the methodological and applied dimensions of aDNA research in archaeological and forensic contexts. It evaluates factors influencing DNA preservation, laboratory techniques for extraction and sequencing, and authentication strategies to ensure

data integrity. Applications such as ancestry reconstruction, kinship analysis, pathogen detection, and migration modelling are explored alongside ethical frameworks for working with sensitive remains. By synthesising current advances and challenges, this review aims to provide a comprehensive overview of the scientific value and ethical complexity of aDNA analysis.

2. Review and discussion of the literature

2.1. Preservation and degradation of aDNA

The primary factor of successful aDNA recovery and analysis is its preservation state, which is influenced by a combination of its inherent biochemical instability and a range of post-mortem environmental factors. aDNA is characteristically fragmented, chemically modified, and present in low copy numbers.[25] The most frequent molecular forms of degradation include hydrolytic cleavage of phosphodiester bonds, oxidative damage (e.g., 8-oxoguanine formation), and cytosine deamination, and their presence can be used as key indicators of authenticity.[26,27]

As portrayed in Table 1 below, environmental variables, especially temperature, humidity, pH, oxygen, and microbial activity, significantly affect the rate and extent of degradation.[28] Optimal conditions consist of cold, dry, or anaerobic environments, such as permafrost or desert tombs, and thus typically present the best-preserved samples, with wet and warm conditions yielding the opposite. Despite earlier doubts, recent studies have shown that DNA recovery from warm-climate samples, including Egyptian mummified tissues, is feasible.[29,30] Highlighting the sensitivity of contamination control, Höss and Pääbo (1993) reported DNA preservation in Egyptian mummies,[26] later acknowledging that their findings were compromised by self-contamination.[31]

Table 1
Authentication criteria for aDNA analysis.

Category	Feature/ Criterion	Relevance
Molecular damage patterns	Cytosine deamination, C → T transitions at 5′ ends Short fragment lengths, 30–100 bp	Confirms age related damage Reflect postmortem degradation
Contamination control	Extraction and library blanks Independent replication	Detects modern contamination Verifies reproducibility and minimises lab-specific contamination
Taxonomic assignment	Species specific DNA mapping and barcode analysisLow % modern human reads (in non-human samples)	Confirms DNA originates from expected species Absence of human contamination
Laboratory procedures	Dedicated cleanrooms Protective clothing and sterilised equipment	Prevents modern contamination Ensure strict contamination control
Bioinformatic filters	MapDamage or similar damage profiling tools Filtering by length and damage patterns	Quantifies typical damage patterns Ensures authentic molecules retained
Radiocarbon dating	Dating of associated material	Supports authenticity of sample origin
Endogenous DNA content	Proportion of DNA matching target organism	High authenticity if endogenous content reasonable and damage patterns present
Cloning or UDG treatment	Uracil-DNA glycosylase treatment for damage confirmation	Helps distinguish real damage patterns from sequencing artefacts

The type of tissue samples also portrays a significant influence of DNA yield; petrous bone, the densest in the human skeleton, consistently provides the highest concentrations of endogenous human DNA, reportedly up to 100 times more than long bones.[32] Similarly, tooth cementum yields higher mitochondrial DNA (mtDNA) concentrations compared to dentine, due to greater cell density and reduced microbial colonisation.[33] Teeth are also particularly suitable for metagenomic studies of microbial pathogens.[34] Regarding sampling technique, pulverisation of bone material has proven better results than high-speed drilling, which introduces heat, accelerating DNA degradation.[33] Additionally, fragment length is inversely correlated with time since death, with the most recoverable fragments often under 100 bp, validating the need for designing assays that target ultrashort fragments.[26,35]

2.2. Contamination risk and authentication of aDNA

Contamination remains the largest threat to the validity of aDNA studies, especially those involving human remains where modern human DNA may be phylogenetically indistinguishable from target sequences.[25–27] Sources of this contamination include excavation handling, laboratory environments, reagents, and airborne DNA. To mitigate this, dedicated aDNA laboratories implement rigorous contamination protocols including physical isolation from modern DNA workflows, UV-irradiation, bleach sterilisation, and high efficiency particulate air (HEPA) filtration systems.[36] Further, sample pretreatments with sodium hypochlorite and phosphate buffers has been shown to reduce exogenous DNA without significantly compromising endogenous content.[37]

The presence of characteristic molecular damage patterns, specifically terminal cytosine to thymine (C → T) transitions, alongside the dominance of short DNA fragments and reproducibility across independent laboratories, assist in authentication.[27,38,39] Computational tools like MapDamage and PMDtools enable quantitative assessment of deamination rates and provide probabilistic models for authenticating ancient versus modern DNA.[19] In high profile cases, such as the Tutankhamun kinship project, rigorous authentication was achieved using multiple classes of genetic markers (mtDNA, autosomal STRs, Y-STRs) and replicated across independent labs, all exhibiting consistent damage signatures.[40]

2.3. Advances in extraction and sequencing techniques

Early aDNA studies relied on polymerase chain reaction (PCR) amplification of short mitochondrial fragments, but this approach is susceptible to allelic dropout and preferential amplification of contaminants, undermining reliability in degraded samples.[26,38] The emergence of NGS and massively parallel sequencing (MPS) has replaced PCR as the standard, allowing for higher throughput, greater genomic coverage, and authentic sequence reconstruction from ultrashort fragments.[41]

Single stranded DNA (ssDNA) library preparation has demonstrated superior performance over double stranded approaches in recovering highly degraded DNA, especially in cases where double stranded approaches fail.[17] Complementary to this, hybridisation capture methods, which use oligonucleotide probes to target specific genomic regions, have enabled the recovery of exomes, SNP panels, and whole mitochondrial genomes from degraded materials.[42] In the study of a 4000-year-old mummified head, hybridisation capture and shotgun sequencing enabled both mitochondrial genome reconstruction and biological sex determination despite extreme degradation.[43] Such developments highlight the shift from STR focused approaches to SNP based genotyping for phenotypic prediction and kinship analysis. A detailed comparison of these sequencing methods, including their sensitivity, fragment length capacity, and contamination risk, is provided in Table 2. This table highlights the Preparation Techniques and

Table 2
Methods and Applications. Preparation Techniques cover: **PCR amplification**: Targets specific regions (e.g., mtDNA) and amplifies them before sequencing; **Hybridisation capture**: Uses probes to enrich for particular sequences (e.g., pathogen genomes) from a mixed sample; **ssDNA library preparation**: Converts single-stranded fragments into libraries for sequencing—ideal for ultra-degraded DNA; **Ultra-short fragment protocols**: Designed to recover extremely small fragments (<35 bp) from sediments or highly degraded samples.

Prep technique	Sequencing platform	Typical fragment length	Sensitivity to degraded DNA	Typical use case	Example study
PCR amplification	Sanger sequencing	>100 bp	Low	Early mtDNA studies	Neanderthal mtDNA
PCR amplification	Illumina shotgun	60–200 bp	Moderate	Large mammal mtDNA	Mammoth mtDNA
Hybridisation capture	Illumina shotgun	30–100 bp	High	Pathogen genomes	*Y. pestis* genome
ssDNA library preparation	Illumina shotgun	25–80 bp	Very high	Sedimentary aDNA	Cave deposits
Metagenomic library prep	Illumina shotgun	<100 bp	High	Microbiome analysis	Ancient oral microbiome
Nanopore sequencing	Nanopore	>500 bp	Low for short fragments	Experimental long-read trials	Ancient tissue (preliminary)
Linked-read / Long-read	PacBio / Nanopore	>10,000 bp	Very low	Rare in aDNA currently	Limited applications
Ultra-short fragment protocols	Illumina shotgun	<35 bp	Very high	Highly degraded sediment DNA	Sedimentary aDNA

Sequencing Platforms and their initial applications to aDNA studies.

While NGS has transformed aDNA research, the effectiveness of different library preparation methods varies depending on the preservation state of the material. ssDNA library preparation consistently outperforms dsDNA protocols when working with highly degraded samples, often achieving two to three times greater recovery of endogenous DNA.[17,44] This makes it particularly valuable in contexts where DNA is severely fragmented or chemically damaged. However, dsDNA methods remain widely used in cases where preservation is moderate, as they tend to be more cost effective and technically simple. Regarding sequencing strategies, shotgun sequencing offers the advantage of capturing the entire genome without bias but can be inefficient for samples with low endogenous DNA, resulting in lower success rates and higher costs. Alternatively, targeted hybridisation capture enriches specific genomic regions, increasing the proportion of useful data recovered from poorly preserved samples.[42,45] However, this approach depends on prior knowledge of target sequences and may cause bias towards better represented loci, potentially missing rarer variants. Essentially, the choice of method must reflect a balance between sample condition, research aims, and available resources, with many researchers now combining approaches to optimise data recovery and authenticity. An overview of this process, from sample selection through data interpretation, is summarised in Fig. 1 below.

2.4. Human ancestry and population genetics through aDNA

One of the largest contributions of aDNA research is the corroboration of ancient population dynamics. Genomic analyses have proven that modern Europeans descend from at least three ancestral populations: Western Hunter Gatherers, Early European Farmers, and Steppe Pastoralists from the Yamnaya culture.[3] These findings, supported by Reich's large scale population genetic studies,[24] provide evidence of vast Bronze Age migrations that correlate with the spread of Indo-European languages across the continent.

Beyond Europe, aDNA has also revealed unexpected demographic complexity; in the Near East and East Africa, genome analyses suggest extensive admixture between Anatolian, Iranian, and African populations, disproving older models of linear population replacement and

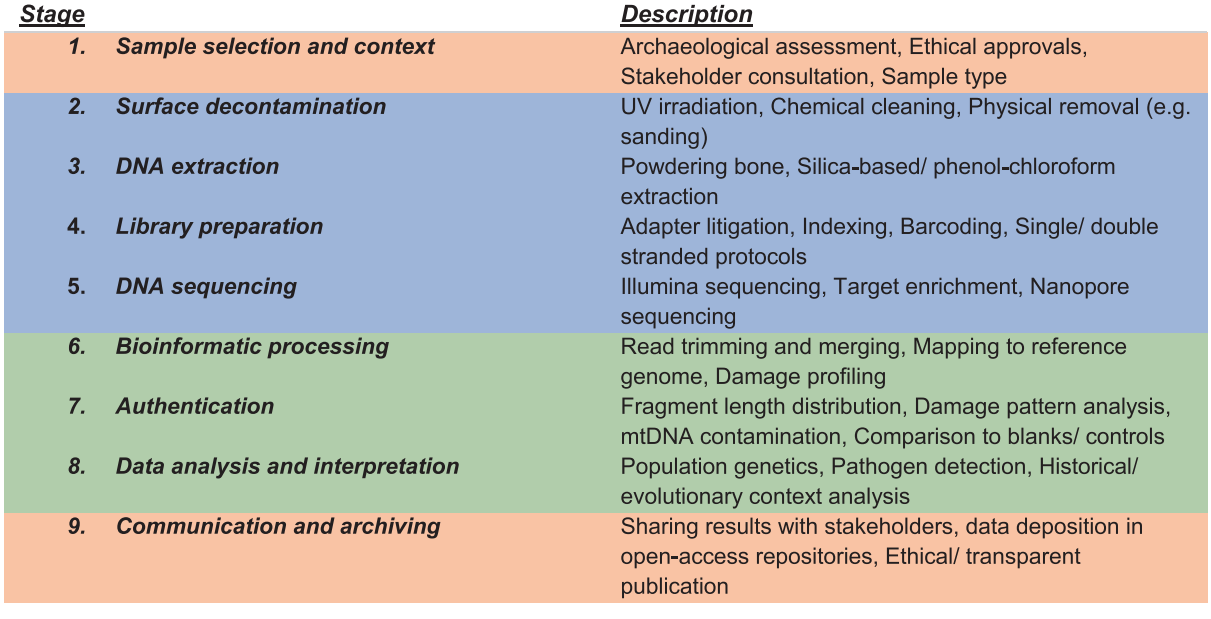

Fig. 1. Workflow of recovery and analysis of aDNA.

instead revealing dynamic and regionally variable patterns of interaction and gene flow.[46] Uniparental markers, such as mtDNA and Y-STRs, are useful in tracking matrilineal and patrilineal descent, particularly where autosomal SNP data is unavailable.[25] Studies in Slovenia have proven that heavily degraded remains can yield STR and SNP profiles sufficient to confirm sibling and parent–child relationships.[47]

In Egypt, Hawass et al (2010) established the genealogical relationships among 18th Dynasty royal mummies, confirming that Tutankhamun was the son of Akhenaten and part of a closely intermarried lineage.[40] Such reconstructions rely on different classes of genetic markers; mitochondrial DNA (mtDNA), inherited maternally, is useful for tracing female lineage and can survive in samples where nuclear DNA is not recoverable. Y-chromosome STRs reveal paternal descent and have been used to confirm patrilineal inheritance in various contexts.

Autosomal SNPs, which cover the entire genome, allow for more detailed kinship analysis and phenotype prediction but require better preservation and higher coverage[33]). SNPs associated with pigmentation (e.g. *HERC2, SLC24A5, SLC45A2* have been used to deduce eye, hair, and skin colour. Early European Farmers likely had darker skin than modern Europeans, despite carrying alleles for lighter eye colour; this phenotype combination is no longer common today.[48] Alleles associated with lactase persistence (*LCT*) and starch digestion (*AMY1*) have revealed how diet and lifestyle has shaped human genomes through gene-culture coevolution.[49]

These varying models, ranging from demographic diffusion to societal dominance, highlight some of the challenges in linking genetic data to historical predictions. On top of this, reliance on sparse or unevenly distributed ancient samples can skew demographic reconstructions, particularly in underrepresented regions such as Sub-Saharan Africa or Southeast Asia, where aDNA studies are significantly less.[24,46] These limitations illustrate the need for caution in drawing definitive conclusions surrounding past mobility, identity, or language spread, and promotes the importance of combining genomic data with archaeological, linguistic, and environmental evidence.

2.5. Phenotypic and health inference from genetic markers

aDNA not only reveals ancestry but can also predict physical traits and health profiles of past populations. As mentioned above, SNPs associated with pigmentation have enabled the reconstruction of phenotypic traits.[44] SNP multiplex analyses of Bronze Age Siberian remains revealed individuals with light skin pigmentation and blue/green eye colour, illustrating the power of aDNA to infer external appearance.[45]

Genomic studies of dietary adaptations have revealed alleles linked to lactase persistence and amylase copy number variation reflecting gene-culture coevolution in response to dairy and starch-rich diets.[50] Moreover, non-communicable diseases have also been detected in ancient remains; in particular, Egyptian mummies have shown molecular evidence of conditions such as sickle cell disease, atherosclerosis, and achondroplasia, highlighting the long-standing presence and heritability of certain pathologies in human populations.[51]

Despite the increasing use of SNPs for phenotypic prediction, this area of research remains widely questioned.[52] Predictive models are often generated using recent genome-wide association studies (GWAS), which may not accurately reflect the genotype-phenotype relationships in ancient populations.[44] Variables such as gene-gene interactions, environmental modulation, and epigenetic effects are rarely accounted for, potentially leading to overconfident or anachronistic interpretations of appearance and disease risk. Additionally, the growing trend of reconstructing faces from genetic data, especially when amplified through media, has raised ethical and epistemological concerns. Such representations risk reducing individuals to genetic caricatures and may reinforce deterministic assumptions regarding identity, ignoring the inherent uncertainty and socio-cultural dimensions of appearance.[45,53]

2.6. Detection of ancient infectious and parasitic diseases

Pathogen genomics has become a major subfield of aDNA research, offering unprecedented insight into the origins, evolution, and historical impact of infectious diseases. Ancient pathogen DNA, such as *Yersinia pestis, Mycobacterium leprae,* and *Salmonella enterica,* has been recovered using metagenomic and targeted approaches.[5] These studies not only bring light to past pandemics but also broaden the understanding of pathogen evolution and human-microbe interactions. Notably, Vågene et al (2018) identified *S. enterica* as the cause of a 16th century Mexican epidemic using shotgun metagenomics, revising historical assumptions.[10] Similarly, tuberculosis DNA has been found in ancient Egyptian remains, providing molecular confirmation of palaeopathological diagnoses,[54] presenting direct evidence of disease presence long before written medical records. A more detailed overview of pathogens identified through aDNA analysis, including their temporal range, geographical context, and detection methods, is provided in Table 3.

Parasitic pathogens, such as *Plasmodium falciparum, Toxoplasma gondii,* and *Schistosoma* species, have also been identified in Egyptian mummies,[51,55] contributing to studies regarding historical disease burdens and epidemiological patterns. These studies reveal not only ancient disease burdens but also potential routes of transmission, such as the zoonotic origin of malaria and toxoplasmosis through close animal contact, thus shaping modern infectious disease dynamics.

2.7. Lessons from aDNA and their application in forensic genetics

The methodological stringency developed in aDNA workflows has become increasing relevant in forensic science practices, particularly in cases involving degraded skeletal remains.[41] Key lessons from aDNA studies include prioritising high-yield tissues (e.g. petrous bones), avoiding high-speed drilling to minimise heat induced degradation, and using EDTA-based extractions for short fragment recovery,[33,56] all of which have proven directly transferable to forensic applications.

SNP-based genotyping, now a standard approach in ancient studies, is also being adopted in forensic investigations, especially where conventional STR typing fails due to extensive DNA degradation.[23] A Slovenian case study demonstrated that identity-informative SNPs, analysed by MPS, provided definitive sibling identification where STR analysis yielded inconclusive results.[42] The integration of MPS and hybridisation capture techniques has further expanded forensic capabilities, allowing the production of comprehensive genetic profiles, including ancestry inference, maternal and paternal lineage analysis, and phenotypic predictions, from trace or compromised samples.[23,42] Table 4 provides a summary of influential aDNA studies that illustrate these methodological innovations and their diverse applications. Table 5.

3. aDNA and ethical considerations

The recovery and analysis of aDNA, especially from human remains, raises important ethical questions. Issues regarding consent, cultural sensitivity, and community engagement have become increasingly emphasised, particularly when working with Indigenous or marginalised groups.[21,57] Current practices now include stakeholder consultation, ethical review boards, minimally destructive sampling, and dissemination of results in accessible formats. Garrison et al (2019) proposed a six principal framework for ethical genomic research, prioritising Indigenous data sovereignty, transparency, and co-governance throughout the research process.[21] In museum and archaeological contexts, these principles align with wider efforts towards the repatriation of remains and the decolonisation of scientific practice.[53,58] Recent research on Egyptian mummies now incorporates clear legal and cultural protocols, reflecting evolving attitudes surrounding ownership, consent, and the ethical responsibilities of scientific enquiry.[30,51]

Ethical and practical concerns demand a structured framework for

Table 3
Summary of pathogens detected through aDNA.

Pathogen	Species	Age (years)	Region	Sample type	Detection method
Plague	*Yersinia pestis*	~500–5000	Eurasia	Teeth, bones	Targeted capture and NGS
Tuberculosis	*Mycobacterium tuberculosis*	~9000	Americas, Europe, Africa	Bone (spine, ribs)	PCR, metagenomics
Leprosy	*Mycobacterium leprae*	~1000–2000	Europe, India	Bones (facial, limbs)	Target capture
Hepatitis B virus	*HBV (genotypes A-D)*	~4500	Europe, Asia, Africa	Teeth, petrous bones	Shotgun sequencing, targeted capture
Syphilis (treponemal)	*Treponema pallidum*	~500	Mexico, Europe	Teeth, bone lesions	PCR, hybridisation capture
Smallpox	*Variola virus*	~3000–300	Eurasia	Mummified tissue, teeth	Shotgun and targeted capture
Brucellosis	*Brucella* spp.	~800	Italy, Middle East	Calcified nodules, bones	Metagenomic analysis
Malaria	*Plasmodium falciparum*	~1500–3000	Egypt, Mediterranean	Mummified tissue, bones	PCR, shotgun sequencing
Helicobacter pylori	*H. pylori*	~1000–5000	Europe, Asia, Americas	Stomach tissue, dental calculus	Metagenomics, targeted capture

Table 4
Summary of key studies in aDNA research.

Case study	Region	Age (years)	Target DNA	Method	Findings
Cheddar Man Genome	England	~10,000	Nuclear genome	Whole genome sequencing	Revealed early Britons had dark skin, blue eyes Insights into Mesolithic population genetics
Dog domestication	Eurasia	~14,000–4000	Canine mtDNA, nuclear DNA	Hybrid capture, NGS	Provided hypothesis for dog domestication in East and West Eurasia
Ancient bison hybridisation	Siberia, North America	~20,000–10,000	Bison mtDNA	PCR, sequencing	Identified hybridisation between bison species during Pleistocene
La Braña 1	Spain	~7000	Human genome	Illumina sequencing	Demonstrated blue eyes/ dark skin phenotype Lacked genes for lactose intolerance
Ancient Maize domestication	Mexico	~5000	Maize nuclear DNA	Target enrichment, NGS	Traced spread and genetic selection in early domesticated maize
Permafrost-preserved Pleistocene DNA	Siberia	~30,000–50,000	Plant, mammal environmental DNA	Shotgun sequencing	First demonstration of DNA survival in permafrost soils Origin of sedaDNA research
Greenland Norse diet study	Greenland	~500–1000	Animal and plant DNA from midden	Metabarcoding	Revealed marine-heavy diet of Norse settles
Kennewick man genome	Washington state	~9000	Human nuclear genome	Whole genome sequencing	Linked Kennewick man to Native American tribes
Early Andean DNA	Peru, Chile	~8000–500	Human genome	Targeted enrichment, shotgun sequencing	Showed genetic continuity and migration patterns in ancient Andrean populations

Table 5
SNP chips vs whole-genome sequencing.

Feature	SNP Chip	Whole-Genome Sequencing (WGS)
Coverage	~600 k–700 k SNPs (~0.02% of genome)	~3.2 billion base pairs (100%)
Cost	$50–$150	$300–$1,000+
Resolution	Limited to pre-selected SNPs	Captures all variants (SNPs, indels, structural)
Rare Variant Detection	Poor	Excellent
Population Bias	High (ascertainment bias)	Low
Imputation Needed	Yes	No
Structural Variants	Not detected	Fully detected
Accuracy for Ancestry	Good for broad estimates	Excellent, fine-scale resolution

aDNA analysis to ensure integrity throughout the process: planning → consultation → data stewardship → dissemination. Skipping these steps risks undermining the project's scientific and ethical goals.

- **Planning** involves defining the research rationale, applying scientific rigour to hypothesis formulation, and selecting appropriate methods.
- **Consultation** requires engaging with descendant communities and stakeholders who may have legal, cultural, or financial interests in the samples.
- **Data stewardship** focuses on designing secure curation and protecting sensitive information, with clear communication to stakeholders before analysis begins.
- **Dissemination** ensures that data use aligns with agreed terms and respects stakeholder concerns.

Fig. 2 summarizes these practices, presenting a framework for responsible aDNA research from project design to stakeholder communication and long-term stewardship.

International attention to ethics and genetic material is reflected in the Nagoya Protocol (2010), which establishes a legal framework to ensure that aDNA research respects the rights of source communities and nations. It requires prior informed consent, transparency, and fair distribution of benefits—principles that are particularly relevant when working with human remains and culturally sensitive samples. Complementing this, the UNESCO Recommendation on Open Science, adopted in 2021, places responsibility on researchers and states to

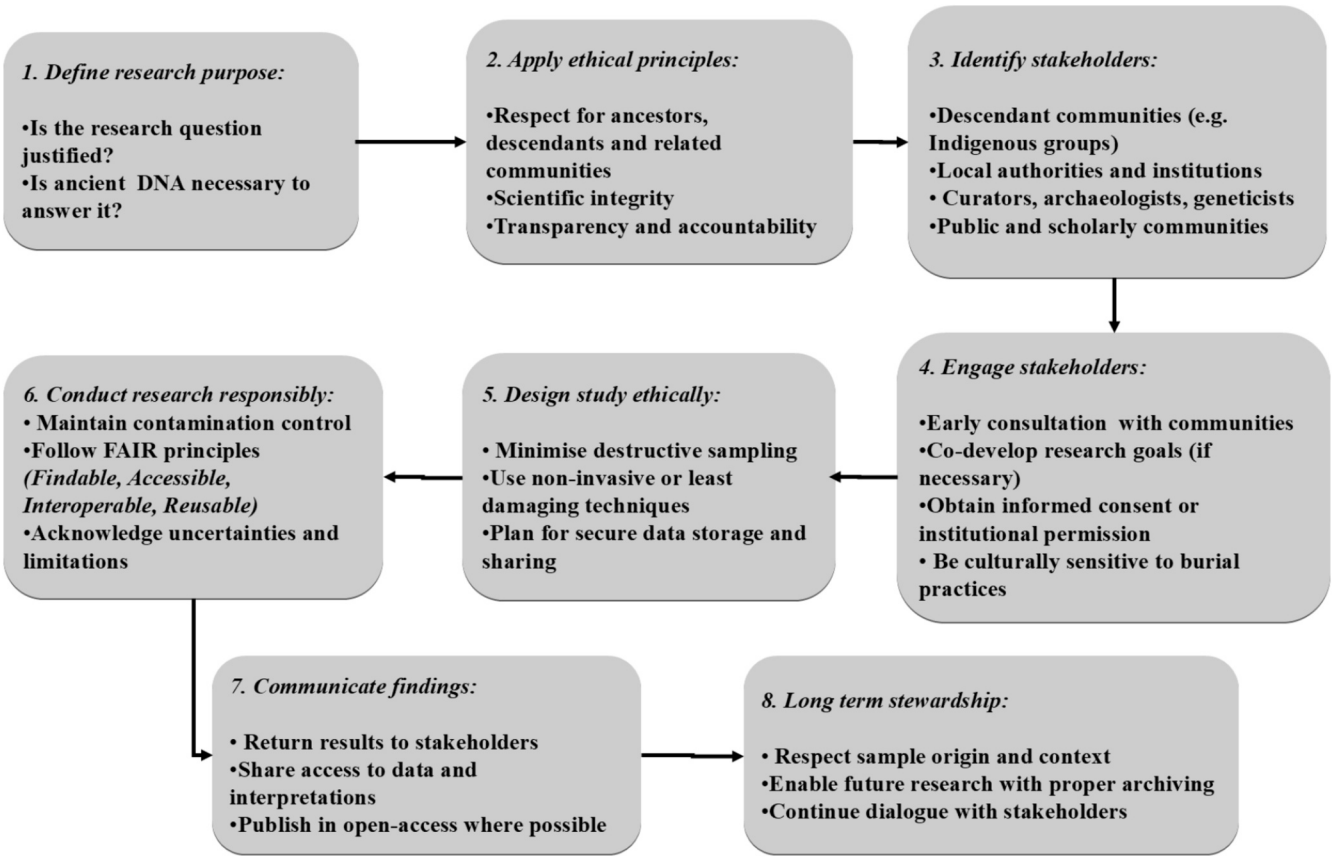

Fig. 2. Flowchart demonstrating ethical consideration in aDNA research.

involve all stakeholders in shaping research that aligns with their concerns and goals.

The interpretation of aDNA findings is shaped not only by molecular data but also by the assumptions and analytical frameworks applied during research. A major concern is the sampling bias that pervades current literature: the majority of published aDNA studies originate from European contexts, reflecting better preservation conditions, funding priorities, and geopolitical accessibility.[32] This has led to a disproportionate focus on Eurasian prehistory, overshadowing equally significant narratives from Africa, Oceania, and the Americas. As a result, global human diversity, and its complex evolutionary history, is often underrepresented in palaeogenomic reconstructions.

Research in these underrepresented regions demonstrates the potential of aDNA to transform our understanding of human history. For example, in Sub-Saharan Africa, Wang et al. analysed 28 Pleistocene samples (80,000–20,000 BP) to correlate gene flow patterns with archaeological evidence.[59] Likewise, Lipson et al. examined 20 sequences dating from approximately 172 BP to 3359 BP to infer changes in subsistence strategies.[60] In Oceania, Skoglund et al. studied sequences from three individuals from Vanuatu and one from Tonga, dating to around 3000 BP, and concluded that initial migration likely originated from East Asia rather than Papua.[61]

These examples illustrate the need to invest research efforts into ancient population studies in underrepresented regions. Although sample sizes remain limited, they have been used to propose significant conclusions. Modern genomes and archaeological data should not be the sole sources for reconstructing ancient migrations; aDNA plays a critical role in building consilience across models of migration and cultural evolution

Choice of method also affects interpretation; population structure models such as admixture and principal component analysis rely on reference datasets that may be incomplete or unrepresentative, introducing statistical artefacts or reinforcing simplified population categories.[24] These models often imply discrete ancestry components; however, gene flow is continuous and context dependant. Such simplifications risk concepts like ‘purity’ and ‘origins’ in ways that are not biologically meaningful and may inadvertently bring about outdated racial typologies. Addressing these challenges requires not only improved sampling and methodological refinement, but also a developed awareness of how data is communicated within and beyond academic settings.[36]

3.1. Caveats from the evolution of forensic genetics

Forensic genetics and aDNA research share core molecular techniques—such as sequencing and contamination control—but differ fundamentally in purpose and context. Forensic genetics aims at individual identification for legal or criminal investigations, including criminal cases, disaster victim identification, and paternity testing, requiring rapid and legally defensible results under strict chain-of-custody standards.[62] In contrast, aDNA research reconstructs population histories, migrations, and evolutionary processes from highly degraded samples, often thousands of years old, within archaeological and anthropological frameworks.[53]

Both fields rely on sequencing technologies and statistical interpretation, yet their goals diverge: forensic genetics prioritises individual certainty, while aDNA seeks population-level insights. Although forensic samples are usually recent, real-world cases such as the Lockerbie bombing, the 9/11 World Trade Center attack, military remains treated with formaldehyde, and recoveries from acidic soils show that forensic DNA can face severe degradation challenges similar to those in aDNA research.[63–65] Ethical frameworks also differ: forensic genetics operates under legal and privacy standards, whereas aDNA research is guided by archaeological ethics and international agreements such as the Nagoya

Protocol.

Insights from forensic genetics highlight the dangers of overconfidence in statistical certainty. Early reliance on SNP-based analysis often created an illusion of precision, leading to exaggerated match probabilities and misinterpretation in court, particularly when population databases were limited or biased.[66,67] While DNA evidence is powerful for exclusion, its use for identification remains probabilistic and vulnerable to stochastic variation, especially with degraded samples or low-template DNA.[68]

Interpreting DNA—whether for individuals or populations—requires contextual support. Consumer genealogy services such as AncestryDNA® illustrate this challenge: their SNP-based genotyping uses ~ 700,000 markers, only 0.02% of the human genome.[69] While cost-effective for broad ancestry estimates, SNP chips have major limitations:

1. Ascertainment bias from non-random marker selection.[70]
2. Loss of rare variants.[71]
3. Distorted genetic metrics (e.g., $F_{ST} = \frac{\text{Variance among populations}}{\text{Total genetic variance}}$).[72]
4. Incomplete linkage disequilibrium coverage.[73]
5. Inability to detect structural variants.[74]
6. Dependence on imputation, which introduces errors if reference populations are unrepresentative.[75]

Whole-genome sequencing (WGS), though more expensive, provides a complete and unbiased view of the genome, capturing rare and structural variants.

These limitations—particularly ascertainment bias and incomplete variant detection—highlight why DNA evidence must be interpreted within a holistic evidentiary framework, where genetic findings are weighed alongside artefacts, historical context, and osteological data. Over-reliance on DNA alone has led to miscarriages of justice, as seen in early forensic cases.

Early applications of forensic DNA analysis were not immune to interpretive errors, particularly in the statistical weighting of match probabilities. In the late 1980s and early 1990s, several cases revealed how reliance on small, non-representative population databases inflated claims of certainty. For example, in *People v. Castro* (1989), the court excluded DNA evidence after finding that the laboratory's statistical methods were flawed and its population frequency tables inadequate.[76] Similarly, in *State v. Schwartz* (1989), concerns over database limitations and lack of standardisation led to judicial scepticism of DNA evidence.[77] These cases underscore the dangers of the "prosecutor's fallacy" and the tendency to overstate the probative value of DNA evidence when statistical assumptions are poorly understood.[78] The lessons from these early controversies remain relevant: genetic evidence must be interpreted within a robust statistical framework and contextualised alongside other evidentiary sources to avoid misleading conclusions.

The case of Charles McAllister provides a modern example of how institutional and legal presumptions of genetic primacy can obstruct scientifically sound identifications. Despite overwhelming concordance across multiple lines of evidence—including archaeological context, artefactual associations, osteological analysis, and genetic data—the U. S. Defense POW/MIA Accounting Agency refused to certify the identification, citing the commonality of the mtDNA haplogroup and statutory interpretation.[79] This bureaucratic stance delayed legal recognition for years, illustrating how an over-dependence on DNA, rather than weighting it within the full evidentiary context, can lead to unjust outcomes.[80]

By contrast, ethical challenges in aDNA research rarely involve legal certification but centre on cultural sensitivity, consent from descendant communities, and equitable benefit-sharing. Both fields demonstrate that genetic evidence alone is insufficient; however, while forensic cases risk politicisation and bureaucratic inertia, aDNA research faces scrutiny over colonial legacies and the rights of source communities. In both domains, a multidisciplinary approach that integrates genetic, contextual, and stakeholder considerations is essential to uphold scientific integrity and social responsibility.

3.2. Future directions

The future of aDNA research lies in expanding the scope of genomic analysis across various temporal, geographic, and biological contexts. Technological advances, such as single cell sequencing, long-read platforms (e.g. Oxford Nanopore), and improved library preparation tailored to ultrashort fragments, will further enhance the recovery of genetic material from previously inaccessible samples. There is growing potential in metagenomic and sediment DNA approaches to reconstruct not only individual genomes but entire microbial communities and environmental landscapes, providing insight into past ecosystems and public health dynamics.

Future research is also expected to focus on ancient RNA, which could present direct evidence of gene expression, viral infections, and host-pathogen interactions in ancient populations. Expanding the scope of ancient pathogen genomics would provide critical insight into zoonotic mixture events and inform contemporary models of infectious disease emergence. Additionally, the integration of aDNA with proteomics, stable isotope analysis, and AI-assisted phenotype reconstruction will facilitate increasingly multidimensional reconstructions of past lives, encompassing both biological and cultural information. Ethically, future studies must prioritise collaborative, community-led frameworks, particularly when working with Indigenous or historically marginalised remains. A globally inclusive sampling strategy is also imperative, as current literature remains heavily skewed towards European datasets. Addressing these gaps will ensure that future aDNA research is both scientifically rigorous and ethically accountable.

Another promising avenue for future research is the application of artificial intelligence (AI) to the interpretation of aDNA datasets.[81] Given the complexity and volume of genomic data, AI tools can uncover subtle patterns and correlations that traditional statistical approaches may overlook. For example, machine learning algorithms support population clustering, admixture modelling, and the detection of previously unrecognised genetic substructures.[69,82] Deep learning models are also being explored for phenotypic prediction using metagenomic data from both ancient and modern genomes.[70,71,83] These approaches can integrate aDNA with isotopic, proteomic, and environmental metadata, enabling more holistic reconstructions of past lives and ecosystems.

Recent collaborations between RNA biologists and computer scientists highlight the potential of AI in sequencing and functional analysis,[63,64] a trend mirrored by advances in ancient RNA (aRNA) research. We are on the cusp of applying AI to the specific challenges of reconstructing highly degraded RNA. Similarly, the field of paleoproteomics has emerged over the past decade, extending the temporal reach of biomolecular analysis to proteins dating back millions of years.[65] Continued improvements in bioinformatic tools promise to support the reconstruction of complex and deep phylogenetic relationships.

However, the use of AI introduces new ethical and epistemological challenges; algorithms may inadvertently reinforce existing biases if trained on unrepresentative datasets or obscure the interpretive transparency of genetic conclusions.[71] Therefore, it is essential that future implementations of AI in aDNA research be transparent, reproducible, and guided by rigorous validation protocols. Collaborations between computational scientists, bioinformaticians, archaeogeneticists, and ethicists will be key to ensuring that AI tools are responsibly integrated into the research pipeline. With these developments, aDNA research may become even more data-rich, interdisciplinary, and capable of illuminating key historical events in increasingly precise and meaningful ways.[72,84]

4. Conclusions

The recovery and analysis of aDNA have critically reshaped the fields

of archaeology, bioanthropology, and forensic science; by enabling the extraction of genetic material from both skeletal and mummified remains, researchers can now address once unanswerable questions through reconstructing ancestry, identifying kinship, inferring phenotypic traits, and tracing the emergence and spread of infectious diseases. This review has critically analysed the biochemical, environmental, and methodological factors influencing the success of aDNA recovery, with particular emphasis on sample selection, contamination control, and sequencing techniques.

Significant advancements in molecular biology, particularly the shift from PCR to NGS, the development of ssDNA library preparation, and the application of hybridisation capture, have drastically increased the resolution and reliability of aDNA studies. These innovations have allowed researchers to work with highly degraded samples and recovery meaningful genomic data from previously inaccessible contexts such as tropical climates and ancient museum specimens. Authentication methods like damage profiling, cytosine deamination analysis, and rigorous lab controls have further improved the credibility of results.

Beyond this, aDNA has proven to be a crucial tool in understanding human history. Population genetic studies have revealed migration patterns, admixture events, and evolutionary adaptations that have redefined conventional narratives of prehistory. From identifying the genetic legacy of Steppe pastoralists in Europe to detecting tuberculosis and malaria in ancient Egyptian remains, the insights gained are both broad and profound. Additionally, aDNA research has made significant contributions to forensic science, especially in cases involving degraded or historical remains where conventional short tandem repeat (STR) analysis is insufficient.

However, the expanding capabilities of aDNA analysis demands clear ethical frameworks. As researchers work with human remains, often of Indigenous or marginalised origin, there is a continuous pressing need for community collaboration, transparent communication, and respect for cultural values. Ethical considerations must be embedded into all stages of research, from transparent project design to open publication of results. Future research would benefit from emerging technologies such as long-read sequencing, single cell genomics, and ancient RNA analysis. There is also increasing potential in sediment and metagenomic approaches for reconstructing entire ecosystems and pathogen landscapes, yet these scientific opportunities must be matched by efforts to address current geographic and cultural biases in sampling. Only through globally inclusive, ethically sound, and methodologically robust practices can the full potential of aDNA be acknowledged.

5. Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the authors used Microsoft 365 Copilot in order to aid with literature summaries, reword texts, and correct grammar. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Funding

This work was supported by the Nottingham Trent University School of Science and Technology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are especially grateful to the Forensic Science Staff at Nottingham Trent University, particularly Jay Silverstein, Robyn Adams, and Ryan Austin, whose passion for the subjects discussed first inspired the idea behind this paper and sparked Amber Chapman's interest in the topic. We would like to thank Stacey Bagdi, Yehia Gad, Valeria Puddu for their support, advice, and encouragement in the preparation of this article. We also wish to thank the editorial reviewers from JGEB whose insights greatly improved this work.

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