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BRIEF REPORT

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The rise of historical epigenomics and temporal analysis of gene regulation

Clare E. Holleley[1*] and Erin E. Hahn[1]

*Correspondence:
clare.holleley@csiro.au

[1] National Research Collections Australia, Commonwealth Scientific Industrial Research Organisation, Canberra, ACT 2601, Australia

Abstract

Complex diseases driven by gene-environment interactions impose a heavy burden on human and animal health. Addressing these challenges requires innovative research. The emerging field of historical epigenomics offers a promising opportunity to link genotypes with phenotypes using preserved biological material. New methods such as historical chromatin profiling in museum specimens provide valuable insights into vertebrate genome regulation. Building on successful work with formalin-fixed paraffin-embedded (FFPE) samples, we expect growing interest in using historical specimens for biomedical, evolutionary, and ecological research. Applied to historical collections, these tools can provide critical baselines for understanding modern diseases, environmental stressors, and human adaptation.

Keywords: DNA, Formaldehyde, Formalin-fixed, Genome, Museum, Museomics, Epigenetics, Gene expression, Museum epigenomics, Chromatin accessibility

Main

Complex diseases, including conditions such as heart disease and stroke, cancer, Alzheimer's, and type-2 diabetes, where gene-environment interactions (GxE) influence disease progression, are among the leading causes of mortality worldwide [1–3]. These illnesses place an immense burden on society, economically and in terms of social well-being. The SARS-CoV-2 pandemic recently underscored the global challenge posed by emerging diseases, particularly those associated with chronic, complex, long-term symptoms (e.g., "Long Covid" a post-viral syndrome with similarities to chronic fatigue syndrome (ME/CFS) [4–6]. Historical examples, including the 1918 Spanish flu [7], polio [8], and Ebola virus [9], suggest that such post-viral outcomes are not new phenomena [10]. Understanding how past environmental exposures, including pathogens, shape molecular phenotypes may offer retrospective insights into disease vulnerability and gene regulation in affected populations.

A massively underutilised opportunity for exploring links between environmental exposure and disease phenotypes lies in leveraging historical samples to

examine epigenetic indicators of long-term GxE interactions. We use the term epigenetics broadly here to include DNA methylation, histone modifications, chromatin accessibility, and other regulatory features that modulate gene expression without altering the DNA sequence. These molecular traces offer a window into how regulatory states may have responded to historical exposures. Recent methodological advances have improved both the accessibility and scalability of this emerging field of historical epigenomics. This growing body of research may greatly advance our understanding of vertebrate genome regulation, offering a way to more accurately link genotypes and phenotypes by unravelling the specific regulatory mechanisms at play. Notably, the first historical chromatin profiles from museum specimens up to 117 years old have now been recovered [11]. Crucially, these profiles retain biologically informative chromatin architecture, providing novel insights into historical gene expression, complementing and enhancing insights gained through alternative approaches [12–15] (Fig. 1). While this review focuses on molecular insights from historical epigenomics with relevance to disease biology, complementary applications in ecology and conservation are explored in a companion review [16].

While studying historical gene-environment interactions is a newly emerging capability, progress made in related fields, notably the utilisation of formalin-fixed paraffin-embedded (FFPE) samples to generate cancer genome profiles [17, 18], suggests the potential for rapid development in historical epigenomics is high. The success of using FFPE samples shows that with the right methodological advancements, even the most challenging archival materials can yield valuable insights. Significant strides have been made in genome- and epigenome-wide association studies using modern gold-standard clinical tissues and archival FFPE samples [19–23]. These advances demonstrate the value of historical pathological material for establishing retrospective cohorts and expanding sample sizes, particularly for rare diseases where fresh samples are scarce. Over the past 12 years, research using FFPE samples has surged (Fig. 2), with technical advances overcoming challenges posed by paraffin embedding and formalin cross-linking, making these methods widely and commercially viable. Initially focused on recovering and sequencing DNA from FFPE samples [17], studies soon began to yield gene expression and regulation data, as methods evolved to address formalin fixation. These include protocols for RNA sequencing [24], DNA methylation profiling [25], and deep proteomic analyses [26]. More recently, chromatin profiling has also become feasible in FFPE tissue using both chromatin immunoprecipitation (ChIP) and Assay for Transposase-Accessible Chromatin (ATAC) approaches. These include EPAT-ChIP for histone marks [27], FiTAC-seq for enhancer and super-enhancer mapping [28], and FFPE-ATAC for spatially targeted accessibility profiling from as few as 500 nuclei [29]. Complementary low-input protocols like FACT-seq [30] and FFPE-CUTAC [31, 32] have further enabled robust profiling of open chromatin and histone modifications from archival clinical samples. These developments represent a significant leap in our ability to extract biologically meaningful regulatory information from long-term preserved specimens.

Despite the progress with FFPE clinical samples, some techniques that have revolutionized molecular analyses in fresh tissues, such as long-read and single-cell sequencing, remain largely unobtainable in these materials due to persistent fixation-related

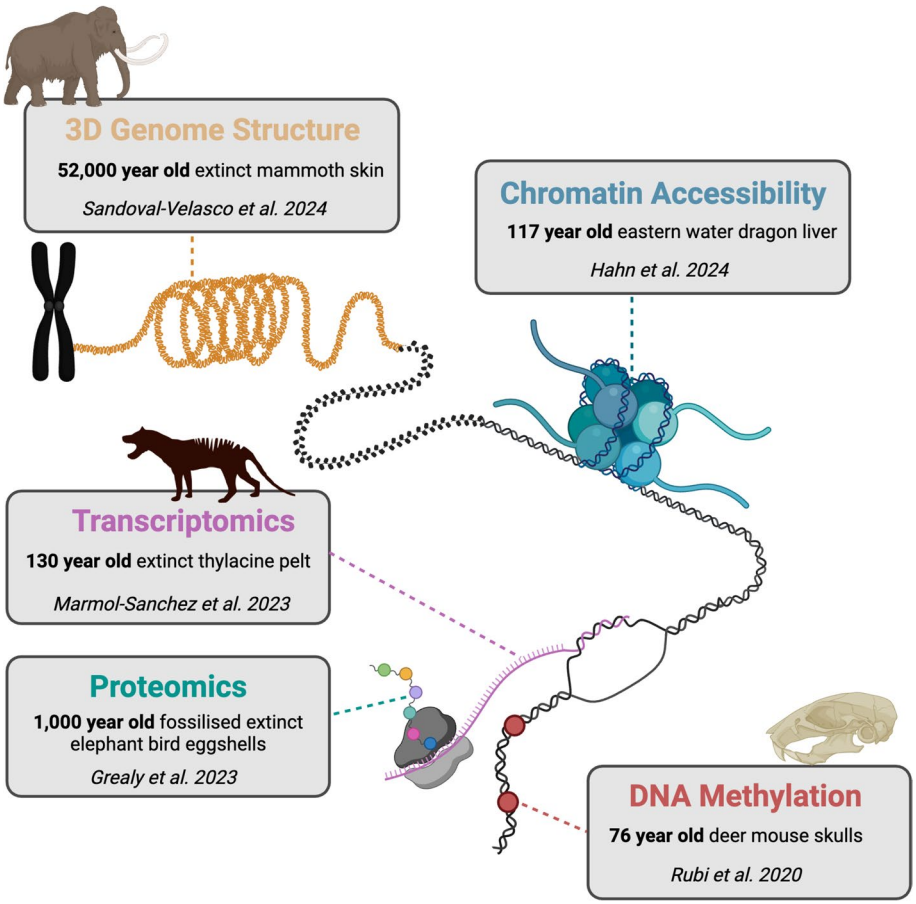

Fig. 1 Characterising historical gene regulation from ancient and historical samples highlights an emerging opportunity. Pioneering studies demonstrate the feasibility of retrieving increasingly detailed gene regulation information from ancient and historical museum specimens using cutting-edge techniques. 3D genome structure was revealed using PaleoHi-C applied to naturally freeze-dried, 52,000-year-old mammoth skin [13]. Chromatin accessibility mapping was successfully conducted on formalin-preserved eastern water dragon liver tissue, preserved for up to 117 years [11]. DNA methylation patterns were characterized from dried deer mouse skulls up to 76 years old [14]. Transcriptome analyses, including of mRNA and microRNAs, were performed on 130-year-old dried muscle and skin tissues of the extinct thylacine [15]. Proteomic studies were conducted on 1,000-year-old fossilized egg fragments of the extinct elephant bird [12]. Created with BioRender.com, released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license. Thylacine icon retrieved from PhyloPic under the Public Domain Mark 1.0 license

challenges. Likewise, other forms of preserved historical materials remain underutilized for molecular research. These include non-paraffinized formalin-fixed biopsy material and formalin-fixed museum specimens, which may include whole organs and whole organisms. This is particularly true for vertebrate models and non-model species alike, where such resources have yet to be fully tapped for their research potential. In part, this reflects long-standing assumptions about the irretrievability of meaningful molecular signals from formalin-fixed samples, assumptions that are now being overturned by methodological advances [16]. This lag in archival specimen-based research is also driven by the variability in fixation conditions, which make it difficult to develop protocols for non-standardized input, and the often low quality of the biological material caused by decay and age. Unlocking the potential of these

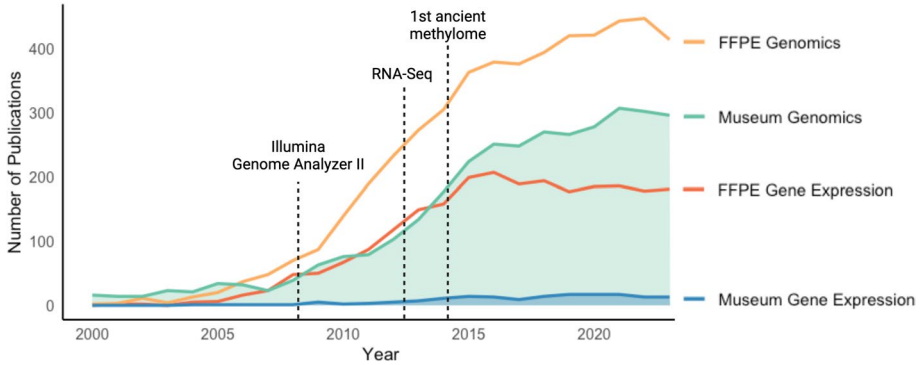

Fig. 2 Publication trends in clinical and museum genomics and historical gene expression analysis (including epigenomics). This graph displays the number of publications per year from 2000 to 2023 that present genomic or epigenomic and gene expression data from FFPE or museum specimens. The rise in publications featuring museum specimens mirrors that of FFPE histological material, just at a lower volume. Publications featuring epigenetic or gene expression data make up a large portion of the overall FFPE-focused publications (44% in 2023) and just a small proportion of museum specimen-focused publications (4% in 2023). Search queries are provided in the Supplementary Methods. Created with BioRender.com, released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license

extra-challenging historical specimens required a shift in perspective, a new approach that linked the historical tissue-preservation process of formalin fixation with modern chromatin biology techniques. We now know that formalin preservation not only preserves the anatomy and cellular structure of the specimen but also preserves chromatin architecture and the 3D structure of the genome in situ. This structure can now be mapped using various approaches including DNase hypersensitivity [33], micrococcal nuclease digestion [34], chromosome conformation capture (3C) [35], and formaldehyde-assisted isolation of regulatory elements (FAIRE) [36]. To retrieve these signals, Hahn and colleagues [11] adapted modern epigenetic assays to overcome challenges posed by extreme specimen age and chemical treatment, developing two complementary assays optimized for historical chromatin analysis.

This archival approach, inspired by modern chromatin biology assays, offers novel insights into the temporal dynamics of chromatin regulation evolution from formalin-preserved historical and museum specimens. A key advantage of this method is that it does not require antibodies, making it more universally applicable across a range of organisms. This approach has been validated in distantly related vertebrates from both genetically diverse wild populations and genetically uniform laboratory model species (wild house mice, C56Blk mice, wild Australian water dragon lizards). The ability to draw comparisons across such diverse species is crucial for identifying functional and causal variants and offers a broader context for evolutionary studies [37]. Coupled with advances in recovering RNA expression data [15] and RNA viruses [38, 39], these transform historical specimens into rich resources of environmental response data.

Perhaps the most important aspect of this new capability is the ability to gain a temporal context for modern epigenomic diversity. It is now possible to retrospectively conduct impact studies following natural disasters, diseases, or environmental stressors. For example, museum specimens collected before and after known events, such

as the chytrid fungal outbreak in amphibians, the intense bushfire seasons recently observed across Australia and North America, or the spread of DDT and other contaminants, provide natural experiments for assessing vertebrate regulatory response to environmental change. The 2019–2020 Australian "Black Summer" fires alone impacted over 30 million hectares and more than 800 vascular plant species [40] and 832 vertebrate species [41], many with broad biogeographic ranges and varying fire responses, underscoring the importance of historical baselines in assessing recovery and resilience. These comparisons can be extended across species and time periods to reveal shared or divergent modes of gene regulation.

In contemporary research, it can be difficult to access appropriate negative controls, particularly for those studying the impact of ubiquitous and pervasive gene-expression-altering contaminants like microplastics, BPAs, and PFAS [42–44]. In these cases, historical collections offer unique opportunities to establish impact-free historical baselines to anchor our modern understanding of these impacts. Natural history museums house an estimated 2.5–3 billion specimens [45, 46], including millions of vertebrate specimens, with well-documented spatial and temporal metadata. Digital metadata repositories such as the Global Biodiversity Information Facility (GBIF; https://www.gbif.org) and the Atlas of Living Australia (ALA; https://www.ala.org.au) provide searchable, open-access databases that allow researchers to explore available specimens and estimate sample sizes across taxa and time periods. In many taxa, hundreds to thousands of specimens from distinct timepoints or regions may be available, offering sufficient sample sizes for population-level inference. In parallel, formalin-fixed archives from hospitals and research biobanks retain preserved samples from well-characterised human disease cohorts. These often include metadata on exposures, treatment outcomes, and demographics, enabling detailed case–control or longitudinal epigenomic comparisons. This dual potential, to study both natural and clinical systems, positions historical epigenomics as a powerful framework for understanding environmental, evolutionary, and disease-related regulatory change.

The field of historical epigenomics is at an inflection point, with a growing suite of tools and analytical pipelines making specimen-based research increasingly feasible. Estimating historical gene expression is no longer restricted to very specific and rare preservation conditions, which up until now could offer only temporally sparse, geographically restricted insight (e.g., mammoth permafrost—essentially a naturally "frozen analogue" [13]). Ancient specimens that survive the test of time do not necessarily preserve the tissue-specific expression patterns relevant to the phenotype of interest or disease presentation. For example, methylation estimation from ancient bone specimens has been possible since 2010 [47–49] but it is very difficult to generalise this information and infer expression profiles in ancient human soft tissue traits [50]. We anticipate that historical specimens will experience a renewed utility for research in biomedical, genome evolution, and genome function, much like FFPE samples have in the last decade (Fig. 2). Just as ancient DNA sequencing revolutionised our understanding of modern genomic variation in human populations [51, 52], historical epigenomics allows us to look backward to estimate how gene expression has changed over the last century.

Crucially, gene expression changes may reflect either evolved differences in regulatory architecture or plastic responses to past environments. Environmental shifts have

driven selection on gene regulation in diverse taxa, including urban songbirds adapting to urbanisation [53, 54], amphibians evolving pathogen resistance [55], and estuarine killifish populations responding to industrial pollutants [56]. These systems are often supported by museum specimens with known sampling histories, making them ideal for retrospective comparisons. Although regulatory evolution within a single century is expected to be rare and likely restricted to systems experiencing strong selection, historical sampling nonetheless provides a unique opportunity to disentangle plastic environmental responses from underlying heritable regulatory change [57, 58]. This enables estimation of the magnitude, mode, tempo, and directionality of regulatory change in response to documented environmental stressors [59], particularly in well-characterised cohorts with ecological, clinical, or demographic metadata.

The potential applications of historical epigenomics reach beyond a single specific research focus and may have profound implications for various fields, such as biomedicine, conservation biology, ecology, and evolution. Museums and biobanks may hold the key to combatting future immunological and environmental challenges. By studying past environmental events, we will gain insights into managing the ongoing and future challenges posed by human disease and environmental stressors. Through careful application of cutting-edge technologies to old samples, we will unlock previously inaccessible baselines, gaining valuable insights not only into other species' responses but also into our own species' adaptation to the enduring legacy of human environmental impact.

Supplementary Information

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Additional file 1: Supplementary material.

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Authors' contributions
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Authors' BlueSky handles
Clare Holleley: @clareholleley.bsky.social.
Erin Hahn: @erinhahn.bsky.social.

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Data availability
Methods describing our publication trend search presented in Fig. 2 are available in Supplementary Information.

Declarations

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The authors declare no competing interests.

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