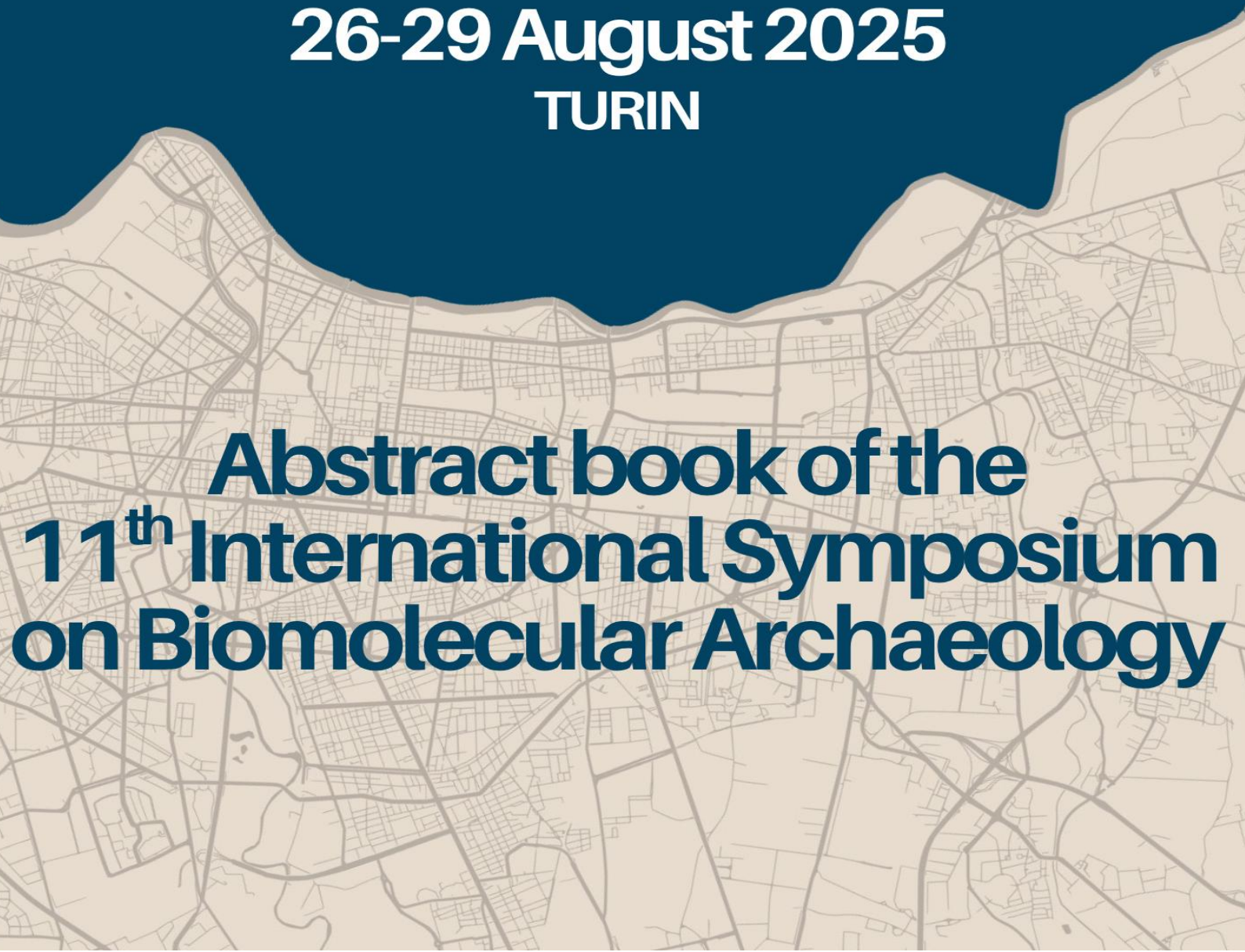




ISBA11

26-29 August 2025
TURIN



**Abstract book of the
11th International Symposium
on Biomolecular Archaeology**

WELCOME LETTER

Dear Colleagues,

Welcome to the 11th International Symposium on Biomolecular Archaeology, and welcome to Torino!

We are delighted to be the first Italian institution to host the Symposium. It has been both an honour and a challenge to organise such an important event for our community in a location away from the more traditional centres of archaeological science. We have (perhaps over-) thought every single aspect of the conference, but of course, what you see reflects our own approach and the limits of what we could achieve within the realities we face. We hope you will enjoy the experience, embrace its uniqueness, and forgive the inevitable glitches.

Torino is a complex city, with a proud past as the royal capital, followed by a long period as an industrial hub, and now an increasingly multicultural centre for culture and tourism. The University and the Museums are at the heart of Torino's new identity, so it seemed fitting that ISBA11 should reflect this reality and the close collaboration between these institutions. Accordingly, the conference will take place at the University for the podium presentations and flash (aka "espresso") talks, while the posters will be displayed in the courtyard of the Palazzo Reale at the Musei Reali di Torino (MRT). We have chosen to experiment by allowing citizens and visitors to experience first-hand the variety of our work. Our students and volunteers will be on hand, but if you wish, you are warmly invited to present your research to both your peers and the general public.

The program you are about to read is diverse: not only in terms of the methodologies employed, but, crucially, in the range of fundamental historical, archaeological, and biological questions being asked. This variety reflects the intellectual vitality of our field and the many ways in which biomolecular approaches continue to transform our understanding of the past. All contributions were reviewed blindly by our scientific committee, and we are deeply grateful for their dedication to a fair, inclusive, and rigorous evaluation process. For the first time at ISBA, we are running three parallel sessions, a decision driven by our commitment to give as many researchers as possible, particularly our Early Career colleagues, the opportunity to present their work.

We thank the ISBA Society, the Affiliate communities, and our institutional and commercial sponsors and supporters for enabling us to bring our vision for ISBA11 to life as a melting pot of ideas, a safe and inclusive space, and an occasion to reflect on our discipline (including the lingering traces of "academic feudalism") and on the role of our community in the wider world. We hope you will enjoy your time here as much as we have enjoyed preparing to host this new edition of the International Symposium on Biomolecular Archaeology.

The Organising Committee

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ORGANISING COMMITTEE

University of Turin

*Department of Life Sciences and Systems
Biology*

Beatrice Demarchi

Rosa Boano

Sergio Favero Longo

Sarah Sandron

Maria Codlin

Rivka Chasan

Jan Dekker

Camilla Mazzucato

Katerina Carlotta Koukzelas

Carmen Dominguez-Castillo

Meaghan Mackie

Marlisa Mazzola

Ramazan Parmaksiz

Department of Historical Studies

Rosina Leone

Alessio Palmisano

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Dominique Scalarone

Monica Gulmini

*Department of Molecular Biotechnology
and Health Sciences*

Federica Dal Bello

Serena Arpaia

Department of Earth Sciences

Evdokia Tema

Department of Physics

Alessandro Lo Giudice

Musei Reali di Torino

Elisa Panero

Alessia Monticone

SCIENTIFIC COMMITTEE

The scientific committee is composed of a diverse cohort of scientific experts divided into sub-section related to different themes that will be explored at ISBA 11.

Methodological advances in biomolecular archaeology: Hannes Schroeder (Co-chair); Camilla Speller (Co-chair); Christina Warriner (Co-chair); Benjamin Vernot (Co-chair); Frido Welker (Co-chair); Melanie Roffet-Salque (Co-chair); Christophe Snoeck (Co-chair); Muriel Gros-Balthazar; Laura Epp; Nicola Alexandra Vogel; Ainara Sistiaga; Shevan Wilkin; Samantha Brown; Takumi Tsutaya; Timothy Cleland; Lisette Kootker; Klervia Jaouen; Simon Hammann; Lea Drieu

Integrating ancient biomolecules and archaeological theory: Tom Booth (Chair); Sarah Inskip; Cate Frieman; Maanasa Raghavan; Anna Davies-Barrett; Carl Heron

Authentication of reliability of ancient molecular datasets: Matthew Collins (Chair); Carli Peters; Zandra Fagernäs; Pete Heintzman; Ricardo Fernandes; Yun Chiang

Environmental change, sustainability, and ancient ecosystems: André Colonese (Chair); Aurélie Manin; Courtney Hofman; Eric Guiry; Niklas Hausmann; Francisca Santana Sagredo

Evolution of human and non-human animals, plants, and microbes: Claudio Ottoni (Chair); Elisabetta Cilli (Co-chair); Kristine Korzov-Richter; Martin Sikora; Magdalena Krajcarz; Michael Westbury; Jorune Sakalauskaite

Interactions between humans and non-human animals, plants, and microbes: Ludovic Orlando (Co-chair); Giedre Motuzaite Matuzeviciute (Co-chair); Aida Andrades Valtueña (Co-chair); Allowen Evin; Victoria Mullin; Laurent Frantz; Greger Larson; Shinya Shoda; Pooja Swali; Meriam Guellil; Maria Lopopolo; Frank Maixner; James A. Fellows Yates; Anita Radini

Migration, mobility, and translocation: Federico Lugli (Chair); Guido Gneocchi Ruscone; Hannah James; He Yu

Diet and foodways: Ester Oras (Chair); Alice Toso; Miranda Evans; Christine Hastorf; Miriam Cubas; Andrew Millard; Harry Robson; Lisa Marie Shillito

Embodied identities in life and death: Hannah Koon (Chair); Julia Beaumont; Effrosini Vika; Noemi Procopio; Stefania Vai; Steve Buckley

Ancient biomolecules and museums: Ashley Coutu

“Academic feudalism”: Research ethics and sustainability in biomolecular archaeology: Rita Peyroteo Stjerna; Mari Tõrv

VENUE INFORMATION

Conference: Aldo Moro, University of Turin, Via Giuseppe Verdi, 10123 Turin TO.

Lunch and poster session: Musei Reali di Torino, Piazzetta Reale, 1, 10124 Turin TO

Conference dinner: Museo Nazionale dell'Automobile, Corso Unità d'Italia, 40, 10126 Turin TO

How to get to the Musei Reali di Torino from Aldo Moro:

On foot: The Musei Reali can be reached easily on foot in 12 minutes by either walking along the covered streets of Via Po or along Via Giuseppe Verdi or by taking **line 13 or 15** from Sant'ottavio to Castello.

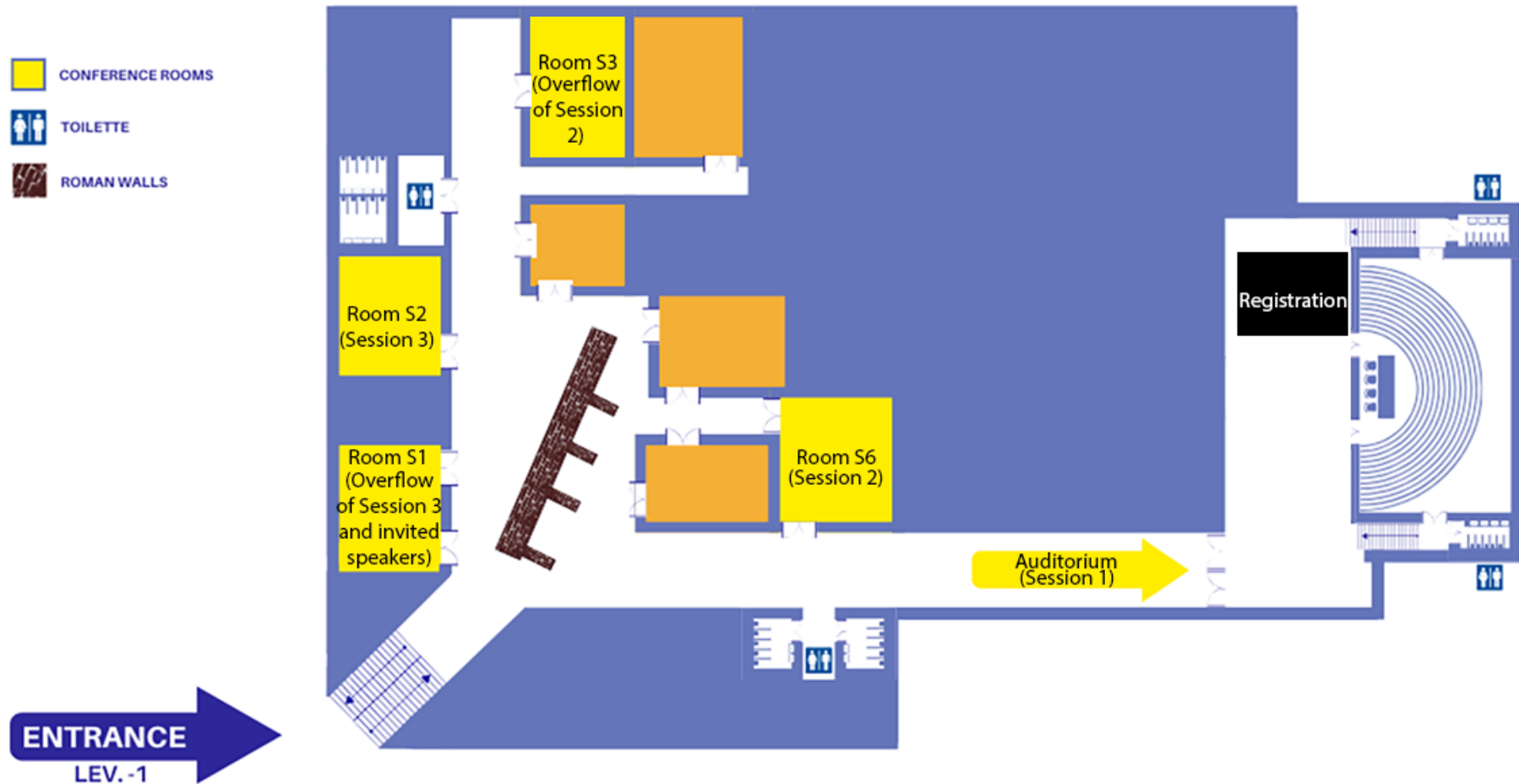
How to get to the Museo Nazionale dell'Automobile:

Arranged transport: A bus from the city center will be organized to take the participants to and from the Museo Nazionale dell'Automobile.

Bus: If individuals do not wish to take the organized bus, the museum can be reached by taking **Bus line 18** to the stop: Finalmarina. The bus back to the city center departs from the same stop.

Metro: If individuals do not wish to take the organized bus, the museum can be reached by taking the **Metro** to the stop: Lingotto. The metro back departs from the same stop.

ISBA11 - Conference map



ORGANISING INSTITUTIONS



Musei Reali Torino

UNIVERSITÀ
DI TORINO



MINISTERO
DELLA
CULTURA

The following departments from the University of Turin are involved:



ARCHEOLOGIA
GEOGRAFIA
STORIA
STORIA DELL'ARTE
STORIA DEL LIBRO
E DEL DOCUMENTO

DIPARTIMENTO DI
**STUDI
STORICI**



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COMPACT PROGRAMME

26 August	
9:00-17:30	Meetings of the affiliate communities
13:00-17:30	Registration
18:00	Welcome cocktail

27 August	Session 1 (auditorium)	Session 2 (S6)	Session 3 (S2)
8:30-8:45	Welcome speech		
8:45-9:30	Invited talk – Joanna Brück		
9:30-10:30	Methodological advances in biomolecular archaeology (part 1)	Embodied identities in life and death (part 1)	Migration, mobility, and translocation (part 1)
10:30-11:00	Coffee break		
11:00-12:40	Interactions between humans and non-human animals, plants, and microbes (part 1)	Embodied identities in life and death (part 2)	Espresso talks
13:00-15:00	Lunch at the Musei Reali and poster session		
15:30-16:15	Invited talk - Christina Valdiosera		
16:15-18:15	Ancient biomolecules and museums	Integrating ancient biomolecules and archaeological theory	Migration, mobility, and translocation (part 2)

28 August	Session 1 (auditorium)	Session 2 (S6)	Session 3 (S2)
8:45-9:30	Invited talk – Shinya Shoda		
9:30-10:30	Methodological advances in biomolecular archaeology (part 2)	Espresso talks	Evolution of human and non-human animals, plants, and microbes (part 1)
10:30-11:00	Coffee break		
11:00-12:40	Interactions between humans and non-human animals, plants, and microbes (part 2)	Diet and foodways (part 1)	Evolution of human and non-human animals, plants, and microbes (part 2)
13:00-15:00	Lunch at the Musei Reali and poster session		
15:15-16:00	Invited talk - Enrico Cappellini		
16:00-16:15	Talk by Thermo Fischer Scientific		
16:15-18:15	Migration, mobility, and translocation (part 3)	Diet and foodways (part 2)	Evolution of human and non-human animals, plants, and microbes (part 3)
19:30	Conference dinner (Museo Nazionale dell'Automobile)		

29 August	Session 1 (auditorium)	Session 2 (S6)	Session 3 (S2)
9:00-9:45	Invited talk – Judy Sealy		
9:45-10:45	Espresso talks	Methodological advances in biomolecular archaeology (part 3)	Environmental change, sustainability, and ancient ecosystems (part 1)
10:45-11:15	Coffee break		
11:15-12:35	Migration, mobility, and translocation (part 4)	Methodological advances in biomolecular archaeology (part 4)	Environmental change, sustainability, and ancient ecosystems (part 2)
12:45-14:45	Lunch at the Musei Reali and poster session		
15:00-16:40	“Academic feudalism”: Research ethics and sustainability in biomolecular archaeology	Methodological advances in biomolecular archaeology (part 5)	Authentication of reliability of ancient molecular datasets
16:40-17:30	Annual general meeting		
17:30-18:30	Final remarks and prize ceremony		

FULL PROGRAMME



27/08

08:30 - 08:45

WELCOME

08:45 - 09:30

INVITED TALK - Joanna Brück

Decolonizing European prehistory

METHODS I

Room: Auditorium

Chair: Christina Warinner

IDENTITIES I

Room: S6

Chair: Hannah Koon

MIGRATION I

Room: S2

Chair: Ludovic Orlando

09:30 - 09:50

Barbara Huber

Metabolomics-guided Identification of Ancient Tars: Differentiating plant products and production processes

09:30 - 09:50

Sandra Lösch

Bioarchaeological analysis of Neolithic Burials from the Western Alpine Region

09:30 - 09:50

Gisela Kopp

The making of a God: Tracing early Egyptian contact with baboons

09:50 - 10:10

Joanna H. Bonczarowska

Unearthing Ancient Parasites: Screening of Metagenomic Samples from Medieval Europe for the Presence of Parasitic Helminths

09:50 - 10:10

Núria Puig i Riera

Patrilineality and female exogamy in a Late Chalcolithic mountain community at the Eastern Pyrenees

09:50 - 10:10

Jolijn Erven

Migration and Mobility: Exploring Identity-By-Descent (IBD) across ancient livestock

10:10 - 10:30

Florian Exler

Density Separation as a Decontamination Method for Ancient DNA Extraction from Sediments

10:10 - 10:30

Eleftheria Orfanou

Lifeways of Late Bronze Age communities in central Germany: Insights from ancient DNA, isotopes, and archaeology

10:10 - 10:30

Katerina Carlotta Koukzelas

Chronological mismatch between Bronze Age contexts and biomolecular traces of exotic landfowl in Crete

10:30 - 11:00

COFFEE BREAK

AUTHENTICATION

Room: Auditorium

Chair: Matthew Collins

IDENTITIES II

Room: S6

Chair: Scott Haddow

ESPRESSO TALKS

Room: S2

Chair: Maria Codlin

11:00 - 11:20

Camilla Speller

Biomolecular Archaeology and Indigenous Data Sovereignty in the Age of UNDRIP

11:00 - 11:20

Balázs Gyuris

Social Relations in Avar-Period Southern Transdanubia: Multidisciplinary Insights from the Kölked-Feketekapu Cemetery

11:00 - 11:05

Amr Khalaf Omran

Resilience and Adaptation: Isotopic Reconstruction of the Impact of Climate Change and Human Interventions on the Nile River, Plant Biodiversity and Ancient Egyptian Foodways

11:05 - 11:10

Aurélie Manin

Slices of teeth, slices of life: serial stable isotopes and palaeoproteomics for the study of ancient Mesoamerican populations

11:10 - 11:15

Lauren T. Clark

A Sequence of Southern Sweden: Sedimentary Ancient DNA Analysis of Environmental Change on the Kullen Peninsula

11:15 - 11:20

Dilek Koptekin

Out-of-Anatolia Migrations during the Neolithization of Western Eurasia

AUTHENTICATION

11:20 - 11:40 Lucas Anchieri
Strong batch effect when co-analyzing 1240K capture and Whole Genome Shotgun sequencing data

11:40 - 12:00 G. Arjen de Groot
Towards an archaeological workflow for sedaDNA sample collection: methods and best practices for minimizing surface contamination

12:00 - 12:20 Clarissa Dominici
Monitoring changes in human activities at the end of the Upper Palaeolithic: A comprehensive approach for the critical assessment of ancient organic residues

12:20 - 12:40 Miranda Evans
The Demodifier: a tool for screening modification-induced alternate peptide taxonomy in palaeoproteomics

IDENTITIES II

11:20 - 11:40 Miren Iraeta Orbegozo
Mass violence, age and gender in the Early Iron Age of the Carpathian Basin

11:40 - 12:00 Caterina Zaggia
Unlocking Ancient Craftsmanship: A Paleoproteomic Analysis of Glues and Binders from Pakepu's Coffin Set (680–664 BCE, Thebes, Egypt)

12:00 - 12:20 Lucy Koster
New biomolecular insights into Pictish identity

12:20 - 12:40 Emmanuelle Casanova
Libation rituals in Iron Age Iran? Unveiling the function of funerary pottery vessels at Sialk (Kashan) and Ghalekuti (Dailaman) through typology and organic residue analysis

ESPRESSO TALKS

11:20 - 11:25 Question Time

11:25 - 11:30 Aikaterini Glykou
Cultural and Economic Responses in Prehistoric Baltic Coastal Societies During the 8200 cal BP Cold Event

11:30 - 11:35 Michael James Boyle
Ancient DNA preserved in archaeological sediments from Stone Age Mongolia

11:35-11:40 Youssef Tawfik
Promises and Limitations in Reconstructing Ancient Methylomes

11:40 - 11:45 Xinyi Ouyang
Stable isotope insights into horse management and human-horse relationships in Bronze Age China (ca. 1300-770 BCE)

11:45 - 11:50 Question Time

11:50 - 11:55 Sebastián López-Núñez
Evaluating Agricultural Practices in the coast and valleys of the Atacama Desert (1000 BC – AD 1450): An approach to crop management using carbon, nitrogen and strontium isotopes

11:55 - 12:00 Meret Häusler
DORIAN: A damage-aware genome reconstruction tool to improve the analysis of ancient samples

12:00 - 12:05 Zara Evans
Eliminating the Gap: new radiocarbon dates and aDNA identification suggest chronological overlap between humans and the extinct Mallorcan Dwarf-Goat

12:05 - 12:10 Erkin Alacamli
Region-specific reference panel for Genotype Imputation in ancient human genomes from Eastern Europe

12:10 - 12:15 Question Time

12:15 - 12:20 Xuekai Qi
Evaluation of Resin Impregnation for Preserving Organic Residues in Archaeological Soils

12:20 - 12:25 Jens Blöcher
The upper Palaeolithic roots of European Mesolithic Hunter-Gatherers

12:25 - 12:30 Mariana Escobar Rodriguez
An Archaeogenetic Perspective on the Impact of European Colonization on the Formation of Mendoza City, Argentina

12:30 - 12:35 Meaghan Mackie
Towards more Sustainable Analysis of Archaeological Dental Calculus

12:35 - 12:40 Question Time

13:00 - 15:00

**LUNCH BREAK AND POSTER SESSION
AT MUSEI REALI**

15:30 - 16:15

INVITED TALK - Cristina Valdiosera

Biomolecular Echoes: The Unequal Footprint of Human History

MUSEUMS

Room: Auditorium

Chair: Ashley Coutu

THEORY

Room: S6

Chair: Tom Booth

MIGRATION II

Room: S2

Chair: Christophe Snoeck

16:15 - 16:20 Sarah Stark
Excavation to Archive: A Balancing Act in Biomolecular Ethics

16:20 - 16:25 Eleanor Green
Rats at the Museum

16:25 - 16:30 Ulla Nordfors
Communicating biomolecular research in a museum exhibition – Experiences from Finland

16:30 - 16:35 Jess Thompson
Biomolecular research on human remains in museums: balancing knowledge acquisition and future proofing?

16:35 - 16:40 Valeria Nicolini
The genomic identity of unknown museum specimens

16:40 - 16:45 Luciana da Costa Carvalho
The invisible residues preserved in copper corrosion from a taverna in Pompeii

16:45 - 16:50 Alessia Monticone
Bones and Biomolecules: Biomolecular Approaches to the Archaeological Collections at the Musei Reali di Torino

16:50 - 16:55 Mélanie Pruvost
The Foissac cave: Integrating paleogenomics and preservation in a prehistoric site of unique fragility

16:55 - 18:15 ROUND TABLE

Panelists:

Paola Buscaglia
Oliver Craig
Elisa Panero
Chiara Ricci
Francisca Santana-Sagredo
Rebecca Stacey

16:15 - 16:35 Kent Johnson
Elucidating and integrating theoretical approaches in archaeogenetic kinship studies

16:35 - 16:55 Hannah Moots
Moving Beyond Migration: Using Archaeogenetics to Study Diverse Modes of Mobility in the Ancient Mediterranean

16:55 - 17:15 Aleksa Alaica
Conviviality and Multispecies Relationality: What can guinea pig isotopes tell us about human social interactions in the Andes?

17:15 - 17:35 Anthi Tiliakou
Social Belonging at the Rise of the Greek City-State: Halieis Case Study

17:35 - 17:55 Katherine Kanne
Biomolecular beasts & becomings: integrating contemporary theory with scientific approaches to archaeological animals

17:55 - 18:15 Rebecca Gerdes
Biomolecular assemblages: Exploring organic residue analysis and archaeological interpretation through assemblage theory

16:15 - 16:35 Sandra Oliveira
The origin and expansion process of early European farmers

16:35 - 16:55 Hugh McColl
Steppe Ancestry in Western Eurasia and the Spread of the Germanic Languages

16:55 - 17:15 Martyna Molak
Genetic (dis)continuity in the Vistula and Oder River basins

17:15 - 17:35 Sandra Penske
Long-distance genetic relationships connect prehistoric Central Europe

17:35 - 17:55 Marco Milella
"Celts" up the Alps: Territorial Mobility and Funerary variability in Late Iron Age Switzerland

17:55 - 18:15 Maura De Coster
The Bioarchaeology of a Borderland: military communities in the Roman Period through the lens of Sr-O-C isotopes and aDNA analysis

08:45 - 09:30

INVITED TALK - Shinya Shoda

Expanding collaboration and social engagement through biomolecular archaeology

METHODS II

Room: Auditorium

Chair: Beatrice Demarchi

ESPRESSO TALKS

Room: S6

Chair: Jan Dekker

EVOLUTION I

Room: S2

Chair: Claudio Ottoni

09:30 - 09:50 **Ragnheiður Diljá Ásmundsdóttir**

Composition variations in archaeological human bone proteomes

09:30 - 09:35 **Rivka Chasan**

Unwrapping past embalming practices through the multidisciplinary analysis of Egyptian mummified heads

09:30 - 09:50 **Konstantina Cheshmedzhieva**

A genetic perspective on the relationships between ancient Papua New Guinea Highlanders and modern populations worldwide

09:35- 09:40 **Ester Oras**

Biomolecular Humanities: moving on and beyond

09:40 - 09:45 **Karine Taché**

What Lies Behind Li Tripods? Nuancing the Shang-Zhou Transition Through a Multifaceted Approach to Ceramics

09:45 - 09:50 **Mailys Turini**

5 A Day: the role of plant substances in the ancient diet of Corsica during Protohistory

09:50 - 10:10 **Katerina Douka**

Testing a unified, minimally-invasive protocol for the analyses of ancient bone remains

09:50 - 09:55 **Christina M. Carolus**

The Early Spread of Rice (*Oryza sativa*) in Iron Age Central Asia: New Paleoproteomic Evidence from the Mongolian Steppe (c. 250 BC-150 AD)

09:50 - 10:10 **Kirsten Bos**

Evolutionary history of the treponematoses: ancient DNA evidence for an American origin of syphilis, yaws, and bejel

09:55 - 10:00 **Question Time**

10:00 - 10:05 **Alison Beach**

Medieval Work and Disability as Embodied Experience: The Case of ELS215 from Bone to Biomolecule

10:05 - 10:10 **Alice Dobinson**

Bioarchaeological perspectives on horse-human relationships in Bronze and Iron Age Britain

METHODS II	ESPRESSO TALKS	EVOLUTION I
10:10 - 10:30 Tina Lüdecke Expanding the Isotopic Toolbox: Nitrogen Isotopes in Fossil Tooth Enamel and First Data of Early Hominins	10:10 - 10:15 Frankie Tait A multidisciplinary approach to fragmented bone from the late Neanderthal site of Cioarei Boroșteni (Romania)	10:10 - 10:30 Mario Apata Ancient Genomic History and Oral Health in Pre-Columbian Coastal Populations of the Atacama Desert
	10:15 - 10:20 Guido Alberto Gneccchi-Ruscione Population shifts in the 5th & 6th centuries CE in central Europe. Integrating, historical, archaeological and archaeogenomic evidences from whole cemeteries	
	10:20 - 10:25 Lisa Briggs The Last Supper on a Phoenician Ship: Organic residue analysis of Archaic cooking pots and transport amphorae from the Xlendi wreck, Malta	
	10:25 - 10:30 Question Time	

10:30 - 11:00

COFFEE BREAK

INTERACTIONS I	FOODWAYS I	EVOLUTION II
Room: Auditorium Chair: Aida Andrades Valtueña	Room: S6 Chair: Ester Oras	Room: S2 Chair: Muriel Gros-Balthazard
11:00 - 11:20 Annette Oertle (presented by Laura van der Sluis) Human presence and subsistence in the New Guinea Highlands over the Holocene	11:00 - 11:20 Andrea Czermak A Tooth for All Seasons: prehistoric subsistence strategies uncovered by refined sequential dentine microsampling & isotope analysis	11:00 - 11:20 Shuya Zhang Archaeogenomics of maize evolution in the South American Andes
11:20 - 11:40 Yichen Liu Ancient metagenomics reveal human- microbial interactions over evolutionary timescale	11:20 - 11:40 Hannah James Reconstructing past diets: expanding the role of strontium isotopes through experiments and multi-isotope comparisons	11:20 - 11:40 Ryan Paterson Unveiling Equidae Evolution through a Rich Dental Proteome of an Arctic Pliocene (3.9 Ma) Hipparionine Horse
11:40 - 12:00 Ruairidh Macleod Lethal Plague Outbreaks in Lake Baikal Hunter–gatherers 5500 Years Ago	11:40 - 12:00 Diego Mayorga Dietary histories in the Atacama Desert during the Formative Period (1000 BC- 900 AD) through incremental hair stable isotope analysis	11:40 - 12:00 Choongwon Jeong Ancient genomes illuminate the origins and dynamic history of East Asian cattle
12:00 - 12:20 Mohamed Sarhan De-novo assembly and reconstruction of an ancient <i>Streptococcus pyogenes</i> genome from a pre-Columbian mummy: Insights into the evolution of a human adapted pathogen	12:00 - 12:20 Nadia Neff Tracing Diet Quality and Nutritional Stress using Amino Acid Isotope Analysis: Insights from a Forager-to- Farmer Transition in Southern Belize	12:00 - 12:20 Deon de Jager An extinct wildebeest lineage identified by morphology and ancient DNA of a 42,000-year old molar from high-altitude Ethiopia
12:20 - 12:40 Elizabeth Nelson Paleogenomic Insights into the History of Tuberculosis in South America Across Diverse Cultural Contexts	12:20 - 12:40 Michelle Alexander Reconstructing resource landscapes of early Swahili towns: a multi-isotopic approach to urban ecology in Zanzibar	12:20 - 12:40 Xiaolei Lei Paleogenomic study reveal the origin and expansion history of the woolly rhinoceros in Late Pleistocene

13:00 - 15:00

LUNCH BREAK AND POSTER SESSION AT
MUSEI REALI

15:15 - 16:00

INVITED TALK - Enrico Cappellini

Palaeoproteomics: what's going on and what's next

Room: Auditorium
16:00 - 16:15 Thermo Fischer Scientific

New dimensions in isotope ratio analysis of amino acids with Orbitrap IRMS technology

MIGRATION III

Room: Auditorium
Chair: Federico Lugli

FOODWAYS II

Room: S6
Chair: Rivka Chasan

EVOLUTION III

Room: S2
Chair: Benjamin Vernot

16:15 - 16:35 Pablo Carrion
Tracing Mediterranean Interconnection in South Iberia During Antiquity and Medieval Ages

16:15 - 16:35 Marcello A. Mannino
Marine resources in Mediterranean hunter-gatherer subsistence and diet

16:15 - 16:35 Maria Lopopolo
Uncovering pre-European contact leprosy in the Americas and its enduring persistence

16:35 - 16:55 Claudia Gerling
Challenging traditional Migration Period narratives with a multi-proxy approach

16:35 - 16:55 Sam Leggett
Finding the Fish Event Horizon? Identity and Dietary Transitions in (early) Medieval Scotland

16:35 - 16:55 Meriam Guellil
2500 Years of Human Betaherpesvirus 6A and 6B Evolution

16:55 - 17:15 Joachim Burger
Population Dynamics and Cultural Continuity in Post-Roman Central Europe

16:55 - 17:15 Julia Becher
Evidence for secondary vessel use? Investigating subsistence patterns in Late Neolithic, Britain, with a multidisciplinary organic residue approach

16:55 - 17:15 Iseult Jackson
Evolutionary history and convergent host adaptation in ancient *Salmonella enterica*

17:15 - 17:35 Luca Traverso
Keszthely, Sirmium, Singidunum: genetic insights into the end of Roman cities at the limes

17:15 - 17:35 Joannes Dekker
Trial by fire, testing the taxonomic and tissue identifications of proteomic analysis with experimental foodcrust

17:15 - 17:35 Somnath Chindhe
Genomic footprints of adaptive responses to past epidemics in the Finnish population

17:35 - 17:55 Eirini Skourtanioti
Archaeogenetic insights into 1st millennium BCE Athenian Society

17:35 - 17:55 Lilly Olet
Lipid residue analysis and compound-specific radiocarbon dating to characterise vessel use in Wales, Orkney and Ireland ca. 3600-2500 cal BC

17:35 - 17:55 Ian Light-Maka
Bronze Age *Yersinia pestis* genome from sheep sheds light on hosts and evolution of a prehistoric plague lineage

17:55 - 18:15 Stefania Sasso
Genetic and Social Stratification in Medieval Estonia: Insights from Urban and Rural Burial Practices

17:55 - 18:15 Shevan Wilkin
Proteomics of symbolic objects from eastern and sub-Saharan Africa

17:55 - 18:15 Louis L'Hôte
Ancient DNA reveals 4000 years of Sheeppox virus evolution through Eurasia

19:30

CONFERENCE DINNER AT MAUTO (Museo dell'Auto)



29/08

09:00 - 09:45

INVITED TALK - Judy Sealy

A view from the South: Integrating biomolecular and archaeological approaches in South Africa

ESPRESSO TALKS

Room: Auditorium
Chair: Meaghan Mackie

METHODS III

Room: S6
Chair: Melanie Roffet-Salque

ENVIRONMENT I

Room: S2
Chair: Camilla Speller

09:45 - 9:50 Lindsey Paskulin
Clear as milk: Challenges in authenticating proteins identified in ancient pottery

09:45 - 10:05 Samuel P. Johns
Historical Records of Climate Preserved Within Parchment Lipids

09:45 - 10:05 Wenxi Li
Ancient Beluga Genomes: Unraveling Long-Term Climate Impacts on Arctic Marine Mammals

09:50 - 09:55 Shahar Silverman
Applying Ancient DNA Techniques to Formaldehyde-Fixed Samples: Reexamination of Landmark Case Auguste Deter

09:55 - 10:00 He Yu
Palaeogenomic study of the extinct *Bubalus mephistopheles* shed light on the evolutionary history of the Bubalus Genus and domestic water buffaloes

10:00 - 10:05 Arve Lee Willingham Grijalba
Investigating Past Human Mobility and Interactions in the Southern Cone of the Americas using Phylogenomics of Ancient Commensal Microbes

10:05 - 10:10 Davide Bozzi
Analysis of a 5,500-year-old *Treponema pallidum* genome from Sabana de Bogotá, Colombia

10:05 - 10:25 Elena Armaroli
Combined time-resolved Sr-O-C isotopes and proteomics unravel the ecology of extinct straight-tusked elephants at Neumark-Nord (~125 ka, Saxony-Anhalt, Germany)

10:05 - 10:25 Ambra D'Aurelio
Phenotypic and Genotypic Diversity of Brown Trout During the Last Glacial Maximum

10:10 - 10:15 Question Time

10:15 - 10:20 Frank Maixner
The 3,300-year-old *Yersinia pestis* genome of an ancient Egyptian mummified individual

10:20 - 10:25 Minna Maunula
Antibiotic Resistance and Microbial Communities in the Environment of an 18th-century Slaughterhouse

ESPRESSO TALKS

10:25 - 10:30 **Gabriel Yaxal Ponce Soto**
High-resolution oral microbiome analyses by mapping metagenomic data on a new curated genomic database of oral microbes

10:30 - 10:35 **Aida Andrades Valtueña**
Mycobacterium leprae genomes from Central Europe provide clues into the past diversity of the leprosy bacterium

10:35 - 10:40 **Flore Wijnands**
Arctic Marine ecosystem reconstruction using sedaDNA: a comparison of shotgun metagenomics and metabarcoding

10:40 - 10:45 **Question Time**

METHODS III

10:25 - 10:45 **Kirsty Penkman**
Smaller, faster, better? Microfluidics & UHPLC for ancient amino acids

ENVIRONMENT I

10:25 - 10:45 **Sanne Boessenkool**
Arctic faunal phylogeographic history and environment reconstructed from 75000 year old bulk-bone

10:45 - 11:15

COFFEE BREAK

MIGRATION IV

Room: Auditorium

Chair: Katerina Carlotta Koukzelas

11:15 - 11:35 **Mehmet Somel**
A comparison of genetically visible mobility patterns in Southwest Asia and East Asia through the Holocene

11:35 - 11:55 **Nicolas Rascovan**
Origins and decline of a farming population in the Southern Andes

11:55 - 12:15 **Judith Ballesteros Villascán**
Genetic relationships of ancient Caribbean individuals from the Dominican Republic

12:15 - 12:35 **Éadaoin Harney**
Unearthing historical migrations and individual identities via identical-by-descent (IBD) connections to 17th century St. Mary's City, Maryland

METHODS IV

Room: S6

Chair: Carmen Dominguez Castillo

11:15 - 11:35 **Ismael Rodríguez Palomo**
The role of the ceramic fabric in the preservation and recovery of proteins

11:35 - 11:55 **Zandra Fagernäs**
Optimization of dental enamel demineralization for palaeoproteomics

11:55 - 12:15 **Lou Spanneut**
Exploring an integrative approach: Metabolomics of Paleolithic faunal remains for dietary reconstruction and radiocarbon dating

12:15 - 12:35 **Carina T. Gerritzen**
The Potential of $\delta^{88}\text{Sr}$ – a journey through Mass Spectrometry and Application

ENVIRONMENT II

Room: S2

Chair: André Colonese

11:15 - 11:35 **Anastasia Papadogianni**
Palaeoproteomic investigations in Palaeolithic Siberia; the case of Ust'-Karakol

11:35 - 11:55 **Lea Frank**
From forests to fire: Reconstructing Holocene forest ecosystems and Nordic Bronze Age human cave activity at Solvikhola, Northern Norway

11:55 - 12:15 **André Carlo Colonese**
Tales of prestige seekers and mud hunters: dietary variability among pre-colonial Sambaquis revealed through stable carbon, nitrogen, and sulfur isotope analysis

12:15 - 12:35 **Jeanne Marie Geiling**
Ungulate Ethology and Late Pleistocene Human Ecodynamics. A Multiproxy Analysis of Prey Species at El Mirón Cave (Cantabria, Spain)

12:45 - 14:45

LUNCH BREAK AND POSTER SESSION AT MUSEI REALI

ACADEMIC FEUDALISM		METHODS V	INTERACTIONS II
Room: Auditorium Chairs: Rita Peyroteo Stjerna & Mari Torv		Room: S6 Chair: Elisabetta Cilli	Room: S2 Chair: Giedre Motuzaite Matuzeviciute
15:00 - 16:40	ROUND TABLE	15:00 - 15:20	Marco Rosario Capodiferro
	Panelists:		Archaic ancestry inference in imputed ancient human genomes
	Beatrice Demarchi		
	Selina Carlhoff		
	Matthew Collins		
	Andre Carlo Colonese		
	Katerina Douka		
	Audrey T. Lin		
		15:20 - 15:40	Adam B. Rohrlach
			Analysing Networks to uncover and compare Genetic - and Cultural - relatedness in Connected Ancient Cemeteries
		15:40 - 16:00	Maria C. Codlin
			Novel avian protein sequences improve resolution of palaeoproteomic approaches to taxonomic identification and reveal widespread intraspecies variability
		16:00 - 16:20	Fei Yang
			Refining Deamidation Estimation in MALDI-TOF Data: development and application of the MALDI Deamidation Score (MDS)
		16:20 - 16:40	Emilio Mármol-Sánchez
			Ancient RNA expression profiles from the extinct woolly mammoth
		15:00 - 15:20	Natalia Przelomska
			Plant medicine, stimulant and cultural keystone: applying a herbariomics approach to explore the adoption of the coca plant by humans
		15:20 - 15:40	Rodrigo Barquera
			Once upon a time in the Americas: How 16th Century epidemics reshaped Indigenous immunogenetic diversity
		15:40 - 16:00	Zhihan Zhao
			Ancient genomics reveal Chinese pig domestication history associated with human migration and management
		16:00 - 16:20	Pedro Morell Miranda
			Describing early domestic genetic diversity using ancient sheep genomes from Anatolia
		16:20 - 16:40	Lydia Furness
			Manatees in the Margins: Population fragmentation and ecological differentiation of African manatees revealed by ancient DNA and stable isotopes

16:40 - 17:30

17:30 - 18:30

ANNUAL GENERAL MEETING

FINAL REMARKS AND PRIZES

POSTER PROGRAMME

27/08

Ancient biomolecules and museums	
Author	Number and Title
Anne Marie Høier Eriksen	[1] Extracting microbial DNA from waterlogged archaeological wood
Claire-Elise Fischer	[6] A Collaborative Approach to (Biomolecular) Archaeological Research: The Tromsø University Museum Framework
Shaymae Iken	[7] ZooMS and Isotopic analysis of El Hammar and El Hattab II caves
Kostja Junglas	[12] Unprovenanced bronze ritual vessels from museums reveal contents through bottom-up proteomics
Nicola Rambaldi Migliore	[13] Genomic insights into <i>Hippopotamus amphibius</i> through museomics
Martina Pasino	[18] Tracing time's footprints: exploring feeding ecology and historical changes of Mediterranean common dolphin (<i>Delphinus delphis</i>) over two centuries (presented by Elisabetta Cilli)
Danijela Popović	[19] Genetic Check-Mate: Unlocking the Secrets of the Sandomierz Chessmen
Sara Tomei	[24] A glimpse into the Prehistoric Qatari Society

Embodied Identities in life and death	
Author	Number and Title
Beatriz Amorim	[25] Early colonial interactions and indigenous enslavement in the Spanish colonized Caribbean through an archaeogenomic lens
Ana Arzelier	[30] Embodied Identities in Megalithic Funerary Spaces: Revisiting the Aveyron Dolmens through Archaeo-Anthropology and Palaeogenomics
Alison Beach	[31] Medieval Work and Disability as Embodied Experience: The Case of ELS215 from Bone to Biomolecule
Rosa Boano	[36] Cloistered lives: the case of S. Maria della Stella, Saluzzo (Italy)
Maxime Bami	[37] A Genomic Transect through the Later Prehistory of Luxembourg
Elisabetta Canteri	[42] Exploring the link between human mobility changes and spatiotemporal variations in burial rite practices
Selina Carlhoff	[43] Archaeogenetics in the Traisen Valley: Mobility and Social Structure during the Iron Age in northeast Austria
Rivka Chasan	[48] Unwrapping past embalming practices through the multidisciplinary analysis of Egyptian mummified heads
Veronika Csáky	[49] Romans versus Barbarians: a 3-5th century genomic study from the Carpathian Basin
Toni de-Dios	[54] Ancient DNA exposes the presence of <i>Yersinia pestis</i> in Copper Age Italy
Corinne Feuillatre	[55] Bringing up Baby: evaluating the physiology/diet interplay on isotope composition

Elizabeth Garnett	[60] Ancestry and kinship in a Romano-British Cemetery at Poundbury Camp, Dorset (UK)
Büşra Katırcıoğlu	[61] Insights into Neolithization in Upper Mesopotamia: Çayönü Tepesi
Mariia Makarova	[66] Detecting osteoporosis in past populations - a bioarchaeological approach
Aurelie Manin	[67] Slices of teeth, slices of life: serial stable isotopes and palaeoproteomics for the study of ancient Mesoamerican populations
Mackenzie Masters	[72] Solving the Mystery of M141: A multi-disciplinary re-assessment of commingled human remains outside the city walls of Romano-Byzantine Ibida (3rd-5th c. CE) (Romania)
Elisha Meadows	[73] Redressing the Balance: Exploring personhood in Roman-period York using a multi-isotope approach
Iñigo Olalde	[78] Archaeogenetic evidence of a Late Bronze Age population crash in the Cantabrian region (Northern Iberia)
Andaine Seguin-Orlando	[79] Ritual murder of women: biomolecular insights into the Middle Neolithic case of Saint-Paul-Trois-Châteaux (Rhône Valley, France)
Marina Silva	[84] Genetic histories of kinship and ancestry in Roman Britain
Shahar Silverman	[85] Applying Ancient DNA Techniques to Formaldehyde-Fixed Samples: Reexamination of Landmark Case Auguste Deter
Franceska-Cristina Ştirbu	[90] Understanding Mediaeval Wallachian Diet, Health and Mobility through a Stable Isotopes and Paleopathology Analysis
Anna Szécsényi-Nagy	[91] Genomic signs of community organisations and social changes in the Early Copper Age of the Eastern Carpathian Basin
Cristina I. Tica	[96] Out of the mouth of babes: Dental enamel sex estimation and oral pathology in Sarmatian children on the Great Hungarian Plain
Anna-Lena Titze	[97] Archaeogenetic insights into a Megalithic Burial Monument in Southern France
Laura Viñas Caron	[102] Untangling identity in the Danish Viking Age through proteomics analyses of fur potentially used as power dressing
Maria Teresa Vizzari	[103] The origin of social inequalities in Northern Italy: clues from ancient genomes
Eren Yüncü	[108] Female lineages and changing mortuary practices in Neolithic Çatalhöyük: Insights from genomic and archaeological data (presented by Mehmet Somel)
Brina Zagorc	[109] Genetic and Social Complexities of Late Roman Emona, Slovenia

Evolution of human and non-human animals, plants, and microbes	
Author	Number and Title
Paolo Abondio	[114] Detecting evolutionary and functional signatures through archaic and ancient polymorphic transposable elements under selection in modern humans
Aida Andrades Valtueña	[115] <i>Mycobacterium leprae</i> genomes from Central Europe provide clues into the past diversity of the leprosy bacterium

Miguel Baltazar-Soares	[120] Searching ancient genomes for footprints of human self-domestication hypothesis
Natalia Berezina	[121] Invisible diseases of pastoralists: a complete genome of <i>Erysipelothrix rhusiopathiae</i> recovered from Early Medieval human remains in the North Caucasus
Jens Blöcher	[126] The upper Palaeolithic roots of European Mesolithic Hunter-Gatherers
Celine Bon	[127] Paleogenetic analysis of the Saint-Memmie (Marne, France) hypogeum from the Late Neolithic
Katia Bougiouri	[132] Limits and best practices for local ancestry inference in imputed ancient genomes
Davide Bozzi	[133] Analysis of a 5,500-year-old <i>Treponema pallidum</i> genome from Sabana de Bogotá, Colombia
Miriam Bravo-Lopez	[136] Paleogenomic reconstruction of pathogens from the 19th-century in Tlatelolco, Mexico
Barbara Bujalska	[139] Characterization of Late Pleistocene European small hamsters (subfamily Cricetinae) based on aDNA analysis
Keri Burge	[142] Phylogenetic and functional insights from ancient oral archaea across time and space
Elisabetta Cilli	[145] Ancient wolf population dynamics and ancestry of dogs in Southern Europe
Isabella Cowan	[148] Coprolite Insights: Ancient Homo GI Microbiome
Tatiana Fioravanti	[151] Genetic identification of cetacean remains from the archaeological site of Grotta dell'Uzzo (North West Sicily, Italy)
Aurore Galtier	[154] Assembly of ancient and modern oral metagenomes reveals new insights into red-complex bacteria.
Katherine Hearne	[157] Dynamics of resistance and susceptibility to pathogenic <i>Yersinia</i> species in domestic and wild taxa
Alina Hiss	[162] Mineralised Tissue Formations as an alternative source for human and pathogen DNA
Alexander Hübner	[163] Studying evolution through the de novo reconstruction of microbial diversity from ancient DNA
Andrey Kritsky	[168] Highly accurate and efficient diagnostics of tuberculosis in ancient human remains (presented by Natalia Berezina)
Audrey Lin	[171] A legacy of genetic entanglement with wolves shapes modern dogs
Frank Maixner	[173] The 3,300-year-old <i>Yersinia pestis</i> genome of an ancient Egyptian mummified individual
Amanda Meuser	[177] Evolutionary dynamics of extinct and extant moose in North America
Caitlin A. Mitchell	[179] Genomic evidence of <i>Yersinia pestis</i> from Iron Age Romania
Carolina Padilha	[183] Population History of Pre-Colonial Maritime Societies Of São Paulo State (Brazil): An Archeogenetic Analysis of the Piaçaguera Shellmound
Emily Pigott	[185] Palaeoproteomic analyses of bone fragments from Palaeolithic Crimea reveals two new hominins

Alise Akermane-Pokšān	[189] Preliminary exploration of genetic data of an Iron Age skull from Latvia with multiple lytic bone lesions
Patrícia Santos	[191] Oral bacterial genome evolution during the Neolithic transition
Eleni Seferidou	[195] Genetic research into pre-colonial dogs from the Caribbean region using ancient DNA
Emelie Ståhl	[197] Tracing <i>Gephyrocapsa huxleyi</i> in Arctic Ocean sediments through time and space
Anne Stone	[201] <i>Mycobacterium tuberculosis</i> diversity in the post-European contact Americas
Vincent F.D.F. Thygesen	[203] Characterization of the ancient human oral microbiota recovered from teeth
Giacomo Villani	[207] Deciphering the genomic history of the Rendena Valley people
Arve Lee Willingham Grijalba	[209] Investigating Past Human Mobility and Interactions in the Southern Cone of the Americas using Phylogenomics of Ancient Commensal Microbes.
He Yu	[213] Palaeogenomic study of the extinct <i>Bubalus mephistopheles</i> shed light on the evolutionary history of the Bubalus Genus and domestic water buffaloes
Teresa Zeibig	[215] Reconstructing the evolutionary history of Parvovirus B19

Interaction between humans and non-human animals, plants, and microbes	
Author	Number and Title
Kevin Daly	[219] Building Ovicaprine Bridges: Combined Palaeogenomic and Palaeoproteomic Analysis for the Study of Caprinae Early Domestication
Alice Dobinson	[221] Bioarchaeological perspectives on horse-human relationships in Bronze and Iron Age Britain
Carmen Domínguez Castillo	[225] Identification of ancient Equids with shotgun proteomics. Revisiting biomarkers and biomolecular aging.
Gunilla Eriksson	[227] Tracing early hop use through stable isotope analysis
Zara Evans	[231] Eliminating the Gap: new radiocarbon dates and aDNA identification suggest chronological overlap between humans and the extinct Mallorcan Dwarf-Goat
Yoko Fukuhara	[233] Reconstruction of Oral Environments of Ancient Hunter-Gathers in Japan Using Palaeogenomics and Palaeoproteomics
Jakob Hansen	[237] Raw material selection strategies in leather production: <i>Equus</i> sp. and cultural dissonance in Copenhagen's Medieval Period and Renaissance
Percy Hei Chun Ho	[239] Genomic evidence for the dispersal of early domestic sheep into Neolithic Central Asia
Sarah Johnston	[243] Strength in Numbers: DNA Preservation, Sex Ratios, and Species Identifications in >1,300 Ancient Canids
Tabea Koch	[245] Divine Creatures: new molecular research on the Egyptian animal mummies at the British Museum
Magdalena Krajcarz	[249] Dietary strategies in medieval urban cat populations through multi-proxy stable isotope analyses of bone collagen

Liam Lanigan	[251] Pathological dental calculus?: exploring the oral microbiome in individuals with leprosy
Alice Lee	[255] Exploring the Impact of Anthropogenic Environmental Factors on Non-Human Mammalian Oral Health: A Study of Dental Calculus from Smithsonian Institution Collections
Yi Hsien Lin	[259] Tracing the Emergence of Millet Agriculture in Northern China: Radiocarbon Chronology and Stable Isotope Insights from the Xinglong Site
Sebastián López - Núñez	[261] Evaluating Agricultural Practices in the coast and valleys of the Atacama Desert (1000 BC – AD 1450): An approach to crop management using carbon, nitrogen and strontium isotopes
William Marsh	[263] Biomolecular analysis of Pleistocene canids indicates widespread connectivity and close human-dog association across Western Eurasia
Minna Maunula	[266] Antibiotic Resistance and Microbial Communities in the Environment of an 18th-century Slaughterhouse
Megan Michel	[268] Exploring spatiotemporal variation in pathogen detection rates using deep sequencing data
Sofía Minniti Nogueras	[270] Exploring human-parasite interactions: A palaeoparasitological study of human coprolites from the Aragón-1 site, Atacama Desert (3300 BCE – 530 CE)
Betty Mouraud	[272] Paleogenomic insights into the dispersal of domestic cats into Europe and selection patterns over time.
Gonzalo Oteo-Garcia	[274] Ancient DNA from the tiny Mediterranean archipelago of Columbretes: a deadly crossroads of extinct snakes and pirates
Xinyi Ouyang	[276] Stable isotope insights into horse management and human-horse relationships in Bronze Age China (ca. 1300-770 BCE)
Ramazan Parmaksız	[278] All your eggs in a network: the case of Çatalhöyük
Valentina Rovelli	[279] The genomic make-up of ancient wild and domestic cats from the Levant and North Africa
Rikai Sawafuji	[280] Past human activities affected microbial communities in ancient archaeological sediments from the Dalverzin Site, Uzbekistan
Patrizia Serventi	[282] Genomic and isotopic insights into cat dispersal and diet from Viking to medieval northern Germany
Gene Shev	[283] Expanding dietary and ecological insights of archaeofauna at Aqaba Castle, Jordan, using non-exchangeable hydrogen isotope ratio ($\delta^2\text{H}$) analysis
Rosa Soler	[284] Optimising sheep reproductive strategies in Roman Iberia: The case study of the villa of Els Tolegassos (Catalonia).
Madeleine Thirolle	[285] Metagenomic and proteomic studies of Stone Age anthropogenic birch tar samples.
Leire Torres-Iglesias	[286] The sex(y)-ing part of enamel: estimating sex ratios in hunted ungulates from the Late Pleistocene

Authentication and reliability of ancient molecular datasets	
Author	Number and Title
Anders Bergström	[3] Improving data archiving practices in ancient genomics
Guillermo Carrillo	[4] Deinotherium teeth samples reveal enamel protein sequence preservation in the Iberian Peninsula across the Miocene
Pere Gelabert	[9] Bias in the recovery of human aDNA from sediments and bones using TWIST genome-wide In-Solution Target Enrichment
Sofia Paasikivi	[10] Protein preservation through time and space - Comparative analysis between study type and archaeological context
Lindsey Paskulin	[15] Clear as milk: Challenges in authenticating proteins identified in ancient pottery
Surabhi Ranavat	[16] Benchmarking workflows for ancient DNA analysis of polyploid crop species (poster presented by Nathan Wales)
Ilaria Serafini	[21] Evaluation of dyes and protein survival in charred textiles: the case of Pompeii and Greek area samples
Christine Ta	[22] Changepoint: Reference-free authentication of ancient DNA
Diet and foodways	
Author	Number and Title
Adrià Breu Barcons	[27] Four-lugged pots and the dairying revolution in northwestern Anatolia: lipid residues from Barcın Höyük (6600-6000)
Carla Bica	[28] The role of fish in the diet of pre-Columbian mound builders of the Patos-Merín Lagoon system through stable isotope analysis
Anne-Julie Bilodeau	[33] Evolving Lipid Evidence: Decoding Contributions of Aquatic Fat in Early Pottery of the Southeastern United States
Annie Bones	[34] Examining childhood diet in Iron Age Orkney using compound specific isotope analysis
Arnau Brosa I Planella	[39] Exploring the evolution of Atlantic Fish Trade in Western Mediterranean through stable isotope analysis of Medieval and Early Modern fish remains
Daniel Brown	[40] Lifting the Lid on the Hebridean Neolithic: Using Organic Residue Analysis to Reconstruct Foodways in the Hebridean Social Landscape
Christina M. Carolus	[45] The early spread of rice (<i>Oryza sativa</i>) in Iron Age Central Asia: new paleoproteomic evidence from the Mongolian Steppe (c. 250 BC-150 AD)
Jingchao Chen	[46] What's on the Menu? Feasting on the Frontier Steppe of Medieval Mongolia
Margaux L. C. Depaermentier	[51] Adjusting carbon isotope threshold values for tracing C4 plant consumers across Europe
Marina Ellis	[52] Archaeogenomic Investigation of Stratified Domestication of Maize in South America using Brazilian Archaeological Maize
Emanuele Grotto Maffiotti	[57] Early Iron Age foodways: revisiting South African faunal assemblages using ZooMS

Aurore Hars	[58] Identifying animal management practices at Treby Borg using stable isotopes
Jay Hilsden	[63] Flatfish in focus: Developing a ZooMS reference database to identify archaeological Pacific flatfish
George Janzen	[64] Dietary practices of a rural society in Western Germany under Rome: Lipid analysis of pottery covering major historical transitions (1st to 4th century CE)
Mathieu Leclerc	[69] Lipids, Lapita, and Lore
Louise Martignac	[70] Characterization of the fish products lipidic signatures in ethnographic pots for a better interpretation of archaeological remains
Dario Monaco	[75] Food in Medieval Northern Italy: Paleoproteomic Evidence from a Rural Village
Amr Khalaf Omran	[76] Resilience and Adaptation: Isotopic Reconstruction of the Impact of Climate Change and Human Interventions on the Nile River, Plant Biodiversity and Ancient Egyptian Foodways
Brett Ostrum	[81] Integrating Early Neolithic dietary data and kinship practices at Hazleton North
Estelle Praet	[82] Towards a culinary archaeology of cacao-based recipes in El Salvador and Isla de Sacrificios, Mexico
Maxime Rageot	[87] Dairy, Fruit, and Beyond: Consumption Practices and Culinary Activities in the Third-Millennium South Caucasus
Huiyun Rao	[88] Protein Preservation Mechanisms in Cooked Pottery and Optimization of Protein Extraction Methods
Francisca Santana-Sagredo	[93] Raw fish, maize and quinoa? Evaluating long-term culinary practices in the Atacama Desert (450 BC-AD 1450) through lipid residue analysis of pottery
Siddhant Sarkar	[287] From Hearth to Castle: Dietary Insights and Social Dynamics Between Botolphs and Bramber Castle in Sussex, England (950-1300 AD) (presented by Dan Brown)
Sangyu Shen	[94] Dietary Variability and Inequality at Trinity Burial Ground, Kingston-Upon-Hull: Results from Incremental Dentine Isotope Analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$)
Akshyeta Suryanarayan	[99] Contents of early vessels in the eastern Gulf: organic residue analysis of sixth-fifth millennia BC imported Mesopotamian vessels in south-eastern Arabia
Karine Taché	[100] What Lies Behind Li Tripods? Nuancing the Shang-Zhou Transition Through a Multifaceted Approach to Ceramics
Mari Törv	[105] Stable isotope analysis of amino acids: comparison of GC-c-IRMS and LC-MS results
Mailys Turini	[106] 5 A Day: the role of plant substances in the ancient diet of Corsica during Protohistory
Ayla Türkecul	[111] A Comparison of Organic Residue Analysis on Pottery Vessels from Layer 7 Aşağı Pınar with Results from Other Layers
Lizzie Wright	[112] Evidence for intensified dairying at the Corded-Ware cultural horizon in Central and Northern Europe using lipid residue analysis and zooarchaeology

Ruitong Yao	[117] Dryland Agriculture in Early Neolithic Northern China: Organic Residue Evidence from the Yumin Culture
Giulia Zampirolo	[118] Shell middens as sedaDNA archives: expanding perspectives on Mesolithic and Neolithic dietary practices in the North Sea region
Zhiyan Zhang	[123] Dairying Sustained Seasonal Transhumance in the Bronze Age Western Tianshan Mountains

Environmental change, sustainability, and ancient ecosystems

Author	Number and Title
Michael James Boyle	[124] Ancient DNA preserved in archaeological sediments from Stone Age Mongolia
Weronika Cieszyńska	[129] Uncovering the Exploitation of Animal Resources in Early Medieval Pohansko Using ZooMS Analysis
Lauren T. Clark	[130] A Sequence of Southern Sweden: Sedimentary Ancient DNA Analysis of Environmental Change on the Kullen Peninsula
Asier Garcia-Escarzaga	[134] Stable oxygen isotope composition ($\delta^{18}\text{O}$) of freshwater apple snails (Pomacea) as a proxy for reconstructing seasonality, subsistence strategies, and settlement patterns of Amazonian pre-Columbian foragers
Aikaterini Glykou	[137] Cultural and Economic Responses in Prehistoric Baltic Coastal Societies During the 8200 cal BP Cold Event (presented by Markus Fjellström)
Bin Han	[140] Pottery residue analysis revealed dairying sustained prehistoric southern Tibetans in adapting the worsening climate after 3,000 BP
Teri Hansford	[143] Palaeoenvironmental reconstruction of Bouldnor Cliff, a submerged Mesolithic Forest of Cultural Significance
Hannah Koon	[146] Rewilding humans: evaluating the dietary changes recorded in the hair of foragers
Meaghan Mackie	[149] Towards more Sustainable Analysis of Archaeological Dental Calculus
Taru-Marja Mäkinen	[152] Genetic History of Bacteria in Human-Impacted Environments
Johanna Muurinen	[155] Microbial seed banks – another window to the past
Linda Reynard	[159] Hydrogen isotopes in Pleistocene fauna and environmental change
Melissa Ritchey	[160] Localized management of novel crops in the mountains of Central Asia during the Bronze and Iron Ages
Bacara Ashleigh Spruit	[165] ZooMS identifies fragmented fauna from MIS 2 to 1 at Boomplaas Cave
Freya Steinhagen	[166] Metagenomic analysis of sedaDNA from Palaeolithic cave sites in Serbia
Gillian Taylor	[169] Microbial dynamics of ancient peatlands: Can this help understand current rapid changes?
Rachel Winter	[172] Historical ecology of elasmobranchs in the early modern period from proteomics and marine shagreen
Christina Youngpeter	[174] Stable Carbon Isotopes of Lost Crops of Eastern North America
Maria Zicos	[175] Sedimentary ancient DNA from the Middle Palaeolithic Cave site Ararat-1, Armenia

Migration, mobility, and translocation	
Author	Number and Title
Corey Alwell	[178] Genomic Insights into an Irish Hunter-Gatherer Community
Charlotte Antoine	[180] Investigation of admixture between Neanderthal, Denisova and Central and Southeast Asian populations
Sara B. Armas-Quintana	[181] San Marcial de Rubicón: paleogenomic analysis of the first European city in the Canary Islands
Elisa Bella	[184] Mobility and social structure in Late Antique Italy at the edge of Longobard and Byzantine territories
Lisa Briggs	[186] The Last Supper on a Phoenician Ship: Organic residue analysis of Archaic cooking pots and transport amphorae from the Xlendi wreck, Malta
Catherine Butt	[187] A genetic exploration of Bronze Age connectivity in northwestern Europe
Valentina Coia	[190] Reconstruction of the ancestry and genetic structure of ancient Alpine groups in the Iceman's territory over ~5000 years (from Late Mesolithic to Middle Bronze-Age)
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Nicolas Delsol	[193] Looking for Champlain's Cows: Cattle Origins, Movement, and Management in Colonial New France
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INVITED TALKS ABSTRACTS

27 AUGUST 2025

Decolonizing European prehistory

Joanna Brück

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In our troubled contemporary world, as archaeological sites are deliberately targeted in ongoing conflicts, it is impossible to ignore the link between archaeology and politics. In contrast, European prehistory has for me always seemed like a safe space – a place where we don't need to worry about the kinds of injustices that Indigenous archaeologists have called out in other parts of the world, and where archaeological science can remain untroubled by the legacies of colonialism. In the past few years, however, I have come to realize that colonial thinking has had a profound impact even in my own specialist area, the Bronze Age of northwest Europe. The categorical distinctions we draw between culture and nature, between subject and object, have had major implications for our interpretations of European prehistory. In this talk, I will explore how this is evident firstly in our approaches to animals (which we tend to frame as food, wealth or symbols) and secondly in our understandings of kinship and the body (in which the social is often seen as secondary to the biological). I will consider how the insights provided by archaeological science can be productively combined with other forms of knowledge, such as Indigenous perspectives on agency, personhood and relatedness, to understand how social relations (including relations with non-human others) were created in prehistory.

Biomolecular Echoes: The Unequal Footprint of Human History

Cristina Valdiosera

CENIEH, University of Burgos, Burgos, Spain

Addressing inequalities using ancient biomolecular research provides a crucial window into the past, challenging simplistic narratives of human history. For example, ancient DNA research can illuminate the genetic inequalities of the past and present. This is more than just reading ancient genomes; it is interpreting the echoes of ancient lives, and those echoes carry the weight of social structures, power dynamics, and inequalities. These interpretations are empowered utilising multiple sources of evidence, emphasizing the necessity for multidisciplinary approaches and the alliance of local social and academic expertise. Understanding these ancient inequalities is crucial for contextualizing present-day disparities and fostering more inclusive and responsible scientific practices.

28 AUGUST 2025

Expanding collaboration and social engagement through biomolecular archaeology

Shinya Shoda

Nara National Research Institute for Cultural Properties, Nara, Japan / BioArCh, Department of Archaeology, University of York, York, United Kingdom

Biomolecular archaeology, which uses cutting-edge science and technology to unravel the mysteries of the past, is also a powerful tool for promoting collaboration among researchers in diverse academic fields and broader social engagement. In this lecture, the speaker will introduce his unique history of collaboration, taking examples of cooperation not only with field archaeologists and laboratory researchers across different countries, but also with non-academic experts such as professional chefs, food industry specialists, and even Japanese armour collectors. Diverse outreach activities for social participation will also be introduced, such as communications with researchers at police forensic institutes and restaurant owners. Based on the speaker's experience, it is a fact that various issues can arise due to a lack of mutual understanding when collaborating with people from different backgrounds. However, this lecture aims to serve as an opportunity for the audience to rediscover the richness of biomolecular archaeology's diverse applications and to celebrate its potential.

Palaeoproteomics: what's going on and what's next

Enrico Cappellini

Globe Institute, University of Copenhagen, Copenhagen, Denmark

Palaeoproteomics has rapidly matured from a proof-of-concept technique into a cornerstone of biomolecular archaeology. Its established power lies in retrieving deep-time molecular information, often succeeding where ancient DNA fails. Recent applications contributed to: (i) reconstruct the phylogeny and diversity of extinct taxa, (ii) determine the sex of fragmentary fossil remains from tooth enamel peptides, and (iii) push the timeline for the recovery of genetic information beyond ten million years. Looking forward, the next phase of palaeoproteomics promises to move beyond simple protein identification towards more detailed biological narratives. The major frontiers include the systematic analysis of even older material, the recovery of richer proteomes, and the integration of machine learning for more sophisticated data interpretation. As analytical sensitivity improves, palaeoproteomics could address increasingly complex questions about human and animal evolution, human-environment interactions and more.

29 AUGUST 2025

A view from the south: Integrating biomolecular and archaeological approaches in South Africa

Judy Sealy

Department of Archaeology, University of Capetown, Capetown, South Africa

In southern Africa, biomolecular (mainly stable isotope) work has made a substantial contribution to reconstructing past diets and by extension, landscape use and social organisation. Most work has focussed on the Holocene, but some involves fossil hominins several million years old. This research extends over several decades, and I will offer some comments on how (whether?) our work can be linked to developing theoretical perspectives in archaeology. These impact very differently on different areas of archaeology; at the more recent end of the time scale, they are instrumental in framing research questions. How successful have we been in linking important research questions with appropriate biomolecular tools for answering them? What are the implications of inherent biases in the data we produce? The interplay between biomolecular and archaeological approaches highlights significant strengths and limitations of both.

ORAL PRESENTATION ABSTRACTS

27 AUGUST 2025

Ancient Biomolecules and Museums

Excavation to Archive: A Balancing Act in Biomolecular Ethics

Sarah Stark ⁽¹⁾ - Simon Mays ⁽¹⁾

Historic England, Investigative Science, Portsmouth, United Kingdom ⁽¹⁾

Keywords: Destructive sampling, Pre-deposition stewardship, Developer-funded projects

Increasingly, biomolecular studies are being applied not only in research council-funded projects but also in post-excavation phases of fieldwork projects in the threat-led (mainly developer-funded) commercial archaeological sector. Whilst the extension of biomolecular work into this sector is, in principle, welcome, it poses significant ethical challenges. Time constraints and limited expertise in biomolecular science in this sector, have often led to data-driven rather than hypothesis-driven sampling programs. This approach yields unsatisfactory results, discourages stakeholders, and causes excessive destruction of remains. As a result, skeletal archives may already be significantly depleted in their research potential upon their deposition in a museum.

To address these challenges, common ethical frameworks must be applied consistently across the sector. Post-excavation work from fieldwork projects should align with the standards of research council-funded projects. Destructive analyses should prioritize hypothesis-driven research whilst minimising destruction, helping preserve collections for future study.

We discuss a model which might help address these challenges, drawing on a process proven successful in a related field of archaeology – radiocarbon dating. This model involves engaging our staff with expertise in biomolecular archaeology with project teams at an early stage in major site-based fieldwork. Liaison with post-excavation teams and biomolecular laboratory staff, enables identification of site-specific research questions suited to biomolecular analysis. If appropriate, then the costing of hypothesis-driven research and sampling programs can be designed collaboratively. This process helps ensure that biomolecular research in fieldwork projects is problem-driven, and balances the generation of new knowledge with the ethical stewardship of human remains.

Rats at the Museum

Eleanor Green ⁽¹⁾ - Michelle Feider ⁽¹⁾ - Nathan Wales ⁽¹⁾ - David Orton ⁽¹⁾

University of York, Archaeology, York, United Kingdom ⁽¹⁾

The consideration of research ethics has become a standard expectation of funding bodies and collaborators spurred by legislative measures such as the NAGPRA regulations and the Nagoya Protocol. Additionally, a renewed focus on ethical archaeology has catalysed the advancement of minimally invasive, destructive sampling approaches. While substantial improvements have been made to minimise the impact of DNA testing on human remains, less effort has been extended to archaeofaunal remains, even less so for microfauna.

As part of ongoing research for the RATTUS project, we are investigating the spread of the brown rat (*Rattus norvegicus*) from its native range of North-East Asia into Europe. This expansion, thought to have occurred rapidly within the last 300 years, overlaps with the establishment of many European Natural History Collections. As such, we are working with archaeology and zoology curators to identify suitable samples for our phylogenetic assessment of migrations of *Rattus norvegicus*. The “micro” nature of microfauna means that size does matter - firstly, as microfauna are not always well represented in assemblages and secondly, an average rat femur weighs < 500 mg, which is rapidly accounted for in multiproxy projects like ours. So, we have been investigating the suitability of sampling different tissue types (i.e., bone, teeth, skin, tail, osteocrusts, toepads) and sampling weights to minimise invasiveness.

Here, we briefly discuss our experience with Nagoya Protocol compliance and the findings from our varied sampling approaches across archaeological and historical specimens to improve the ethical sampling of archaeological microfauna.

Communicating Biomolecular Research in a Museum Exhibition – Experiences from Finland

Ulla Nordfors ⁽¹⁾ - Sanni Peltola ⁽²⁾ - Ronan James O'Sullivan ⁽³⁾ - Sofia Paasikivi ⁽⁴⁾ - Elina Salmela ⁽³⁾ - Päivi Onkamo ⁽³⁾

Museum Centre Vapriikki, University of Turku, Tampere, Finland (1) - Museum Centre Vapriikki, University of Helsinki, Helsinki, Finland (2) - University of Turku, Department of Biology, Turku, Finland (3) - University of Turku, Department of Archaeology, Turku, Finland (4)

Keywords: Exhibitions, Biomolecular Archaeology, Science communication

In 2022, the Vapriikki Museum Centre in Tampere, Finland, launched a research project in preparation for an ancient DNA exhibition. The project began with an archaeological excavation at Pälkäne Rauniokirkko, a burial site used between the 13th and 19th centuries. Previous excavations provided a well-documented historical context, allowing for a carefully designed, interdisciplinary investigation. A wide range of samples was collected for scientific analyses, including aDNA, proteins, isotopes, pollen, insects, and microscopic hair and textiles. The research was exceptional in Finland as no previous excavation had integrated such a broad combination of methods and research questions, let alone in the context of an exhibition plan. The exhibition opened in June 2025. The scientific findings are being published in peer reviewed formats for the scientific community, while the exhibition translates the results to the general public. However, transforming scientific data into an engaging and accessible museum experience presents challenges. This presentation examines the questions faced during the project: How can complex topics be communicated to a general audience? How do public expectations differ from those of the academic community, and how these expectations were addressed in the project? The exhibition has been open for two months at the time of ISBA 2025, enabling us to present initial visitor feedback and discuss the lessons learned in bridging the gap between research and public engagement.

Biomolecular Research on Human Remains in Museums: Balancing Knowledge Acquisition and Future Proofing?

Trish Biers ⁽¹⁾ - Jess Thompson ⁽²⁾ - Sarah-Jane Harknett ⁽³⁾

Duckworth Laboratory, University of Cambridge, Department of Archaeology, Cambridge, United Kingdom ⁽¹⁾ - National Museum of Scotland, Scottish History & Archaeology, Edinburgh, United Kingdom ⁽²⁾ - Museum of Archaeology and Anthropology, University of Cambridge, Cambridge, United Kingdom ⁽³⁾

Keywords: Human remains, Curation, Ethics, Sampling

Over the past two decades, there has been a substantial increase in the destructive analysis of human remains. Since 2010, >10k ancient human genomes have been sequenced and more recently there has been an upsurge in sampling for novel techniques. While providing exciting new insights into past lives, these techniques challenge curatorial objectives to preserve the integrity of human remains. As a result, recent works have begun to address the need for widely-followed ethical guidelines across multiple geographical regions. This paper showcases results from a survey investigating destructive sampling which was circulated to collections managers and practitioners working predominantly on collections in the UK and Ireland. Alongside crucial ethical considerations are a range of practical issues faced by those responsible for collections access, many of which were repeatedly highlighted in the survey results. We outline some of these core issues as they relate to different stages of the sampling process. Drawing on survey respondents' and our own experience as curators and researchers working with global archaeological human remains collections, we suggest areas for improvement in the processes and protocols involved in sampling human remains. We aim to highlight opportunities for effective collaborations between biomolecular researchers and museum/university staff, while also centring collections integrity.

The Genomic Identity of Unknown Museum Specimens

Valeria Nicolini ⁽¹⁾ - Nicola Rambaldi Migliore ⁽¹⁾ - Nicola Carrara ⁽²⁾ - Giacomo Villani ⁽¹⁾ - Anna Tommasi ⁽¹⁾ - Nataliia Kozak ⁽¹⁾ - Elisabetta Moroni ⁽¹⁾ - Anna Olivieri ⁽¹⁾ - Massimo Mezzavilla ⁽³⁾ - Luca Pagani ⁽³⁾ - Alessandro Achilli ⁽¹⁾

University of Pavia, Department of Biology and Biotechnology "L. Spallanzani", Pavia, Italy ⁽¹⁾ - Museo della Natura e dell'Uomo, University of Padua, Padua, Italy ⁽²⁾ - University of Padua, Department of Biology, Padua, Italy ⁽³⁾

Keywords: Museomics, Genetic history, Ancient DNA

Over the past two decades, ancient DNA has become a cornerstone in the study of human history, with new advancements in genomic analysis enabling the study of archival specimens through the emerging field of “museomics”. This study aims to emphasize the value of 23 human individuals from the “Museo della Natura e dell’Uomo” (Padova, Italy). These belong to a historical collection of skulls, for which minimal context is available, except for a few fragmentary information on geographic origin or cultural affiliation. After several attempts of DNA extraction from teeth, we managed to recover genomic data, achieving an endogenous content ranging from 2.63 to 44.58%. Preliminary allele frequency-based analyses confirmed the biogeographic origins recorded by the museum labels for the entire set, except for one African individual labelled as Indian. In the upcoming step, we are focusing on two different subsets: 1) two individuals, labeled as members of the Botocudo culture, one male and one female, who appear to be related. These genomes provide a modest but valid addition to the genetic history of this Southern American population. 2) a group of eight individuals, labelled as Saracens, who exhibit a significant mitochondrial diversity without evidence of genetic relatedness. This investigation could supply a deeper understanding of the historical and genetic context of this heterogeneous group. The purpose of this work is to underline the significant contribution of museomics to the renewal of lost museum collections and the importance they might gain in the discovery of our past.

The Invisible Residues Preserved in Copper Corrosion from a Taverna in Pompeii

Luciana da Costa Carvalho ⁽¹⁾ - Elisabete Pires ⁽²⁾ - Stephanie Ward ⁽³⁾ - Miriam Orsini ⁽³⁾ - Kelly Domoney ⁽³⁾ - Gabriel Zuchtriegel ⁽⁴⁾ - James McCullagh ⁽²⁾ - Mark Pollard ⁽⁵⁾

University of Oxford, Dep Chemistry/School of Archaeology, Oxford, United Kingdom ⁽¹⁾ - University of Oxford, Department of Chemistry, Oxford, United Kingdom ⁽²⁾ - University of Oxford, Ashmolean Museum, Oxford, United Kingdom ⁽³⁾ - Parco Archeologico di Pompei, General Director, Pompei, Italy ⁽⁴⁾ - University of Oxford, School of Archaeology, Oxford, United Kingdom ⁽⁵⁾

Keywords: Organic residues, Mass spectrometry, Corrosion

In 2019, the Ashmolean Museum in Oxford received copper vessels excavated from a taverna in Pompeii for exhibition. Long kept in storage unconserved, these objects presented a unique opportunity to investigate organic residues preserved within their corrosion—a material often discarded during conservation. We utilized a multi-analytical protocol to analyse corrosion from a bowl, a pot and a dish - all were identified as copper carbonate hydroxides via X-ray diffraction.

Mineralized insects were found on the bowl, its corrosion yielding fatty acids, cholesterol derivatives and fish proteins. Peptides recovered from a mineralized larvae were matched to flesh-eating fly bacteria, suggesting the bowl once held food scraps. Corrosion from the pot yielded carboxylic acids, lactose, maltitol, and fungal sterol, while proteomics detected desmin, caseins, wheat fungi and yeasts linked to meat and cereal processing. In the dish corrosion malondialdehyde and 3-methoxyphenylacetic acid (human metabolites excreted in urine), Staphylococcus proteins and nitrogen with binding energy consistent with urea suggested the dish may have been used as a chamber pot.

Corrosion is often removed from objects for display, erasing valuable information about past life and culture. Our findings not only extend the field of organic residue analysis in archaeology but also advocates for conservation practice that preserve corrosion as a vital source of historical evidence.

Bones and Biomolecules: Biomolecular Approaches to the Archaeological Collections at the Musei Reali di Torino

Alessia Monticone ⁽¹⁾ - Marlisa Mazzola ⁽²⁾

Musei Reali Torino, Museo di Antichità, Torino, Italy ⁽¹⁾ - *Università di Bologna, Dipartimento di Beni Culturali, Bologna, Italy* ⁽²⁾

Keywords: Biomolecular archaeology, Museum collections, ZooMS

Omics-based methodologies are transforming the study and valorization of archaeological collections within museum contexts. Among these, paleoproteomics—specifically Zooarchaeology by Mass Spectrometry (ZooMS)—has proven instrumental in taxonomic identification and the detection of historical restoration treatments, such as collagen-based adhesives.

Since 2019, the Musei Reali di Torino has been engaged in biomolecular research through a collaborative initiative with the ArchaeoBiOmics lab (Department of Life Sciences and System Biology, University of Turin). This partnership, formalized under the “Bones” project within two doctoral research programs, aims to explore the potential of biomolecular approaches in enhancing archaeological interpretation and museum curation strategies. The study focuses on three case studies: a single artifact, a complex assemblage of Longobard osseous artifacts, some of which originate from contexts well known in biomolecular research¹, and a large-scale faunal study from a prehistoric site. The latter integrates traditional morphological analysis with biomolecular techniques, including collagen and ancient DNA (aDNA).

Minimally invasive sampling techniques—eraser-based, bag-rubbing, and microchip removal (<1 mg)—successfully retrieved collagen, enabling species identification and revealing previously undocumented conservation treatments. Notably, unexpected raw materials, such as bovid rather than deer antler in high status longobard objects, were identified. Within the MRT collections, the eraser-based method proved to be a highly effective balance between analytical efficiency and low invasiveness.

These findings underscore the potential of biomolecular techniques in museum-based archaeology, offering new insights into the biographies of curated artefacts. As a result, ZooMS and related approaches will be further integrated into future research at the Musei Reali di Torino, reinforcing the role of biomolecular archaeology in reinterpreting cultural heritage.

References

1. Amorim, C. E. G. *et al.* Understanding 6th-century barbarian social organization and migration through paleogenomics. *Nat. Commun.* **9**, 3547 (2018).

The Foissac Cave: Integrating Paleogenomics and Preservation in a Prehistoric Site of Unique Fragility

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Keywords: Burial cave, Neolithic, In-situ sampling

The prehistoric cave of Foissac (Aveyron), in France, offers a rare and exceptional insight into Neolithic life due to its extraordinary state of preservation. Sealed approximately 4,500 years ago by the collapse of its porch, the cave offers an extraordinary time capsule, preserving footprints in clay, storage areas, clay extraction sites, and funerary deposits. Four very well-preserved individual burials lie on high terraces bordering the underground river, flooding annually during the winter season.

Listed as a historic monument in 1978, the site is now open to the public, with archaeological features preserved in situ. This decision enhances its uniqueness but also creates significant challenges for research, particularly for biomolecular studies requiring careful sampling in this delicate environment. As part of the LINK project in 2024, an interdisciplinary operation addressed these challenges through the systematic reassessment of artifacts, high-resolution 3D modeling of the site, and advanced paleogenomic analysis of the human remains. In partnership with the Foissac museum team, a specific sampling protocol was therefore developed in order to minimize contamination risks with maximum adaptation to cave conditions and a particular attention to the preservation of the remains. This study presents initial results from the LINK project, emphasizing the integration of paleogenomics with conservation strategies. The Foissac cave highlights how fragile, publicly accessible heritage sites can advance the study of ancient societies, offering valuable insights while demanding innovative methodologies to balance research and preservation.

Authentication and reliability of ancient molecular datasets

Biomolecular Archaeology and Indigenous Data Sovereignty in the Age of UNDRIP

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Keywords: Indigenous data sovereignty, Cultural heritage, UNDRIP

The principles of FAIR (Findable, Accessible, Interoperable, and Reusable) data and open-access policies are becoming increasingly central to biomolecular archaeology, driven by their potential to advance data validation and authentication, and promote collaborative research. However, these principles may conflict with Indigenous rights as outlined under the UN Declaration on the Rights of Indigenous Peoples (UNDRIP) and assertions of Indigenous data sovereignty through OCAP (Ownership, Control, Access, and Possession) and CARE (Collective Benefit, Authority to Control, Responsibility, and Ethics) frameworks, especially in North American contexts. This tension is particularly evident when research involves Indigenous cultural heritage, where open-access policies may unintentionally expose sensitive data to misuse or erode community control over their knowledge systems. This talk will examine the challenges of aligning the demands of national funding agencies for open-access data with ethical obligations toward Indigenous communities in North America. Using case studies from biomolecular archaeology, this talk will explore ethical tensions, as well as strategies to foster equitable research partnerships while respecting Indigenous data sovereignty. By reflecting on these challenges, this talk aims to contribute to a deeper understanding of the interplay between transparency, data validation, and Indigenous rights in biomolecular archaeology.

Strong Batch Effect when Co-Analyzing 1240K Capture and Whole Genome Shotgun Sequencing Data

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Keywords: Methods, Genomics, Data generation

Over the past decade, the amount of publicly available ancient human genome-wide data has increased exponentially. Most of it was generated either by whole genome shotgun sequencing (WGS) (~4,000 genomes) or in-solution enrichment targeting ~1.24 million SNPs (1240K capture) (~8,800 genomes). Yet, as the molecular approaches used to generate either dataset differ significantly, batch effects are a recurrent concern when co-analyzing WGS and 1240K data. In this work, we investigate potential biases by relying on publicly available data and standard population genetic approaches. Specifically, we combine individuals sequenced by both strategies to generate a reference panel typical of ancient DNA studies. We then explore the dataset through multidimensional scaling (MDS), admixture clustering, D-statistics, and f-statistics to assess the potential biases that can arise when combining WGS and 1240K data in standard population genetic analysis. We show a clear effect for MDS, where individuals cluster by data type already in the third dimension. Similarly, ADMIXTURE allocates a specific cluster to 1240K individuals starting at K=5. Finally, we find that a well-established signal of steppe-like gene flow in western European Bronze Age individuals using D-statistics disappears when combining WGS and 1240K data. Overall, our results suggest that these biases can have a profound effect on the results and the interpretation of ancient DNA data. Finally, we also show how some measures, such as restricting the analyses to a set of SNPs shown to reduce the bias (Rohland et al. 2022), do not fully resolve the problem.

References

1. Rohland, N. *et al.* Three assays for in-solution enrichment of ancient human DNA at more than a million SNPs. *Genome Res.* **32**, 2068–2078 (2022).

Towards an Archaeological Workflow for SedaDNA Sample Collection: Methods and Best Practices for Minimizing Surface Contamination

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Keywords: Contamination, Non-experts, Sampling

Sedimentary ancient DNA (sedaDNA) has been successfully recovered from various contexts, enabling researchers to address both paleoenvironmental and archaeological questions. However, its application in archaeology has been limited and primarily focused on the study of human DNA. The sedaDNA record holds important information on a wider biodiversity that might inform us on key questions concerning human lifestyle, such as dietary practices. One of the first steps to integrate sedaDNA into standard workflows in archaeology, is to find ways to realize sound sample collection by archaeologists involved in relevant excavations.

Despite its potential, the reliability of sedaDNA data depends heavily on proper sampling and handling practices. Contamination remains a significant challenge in ancient DNA research, with profound implications for data integrity and interpretation. Numerous studies have highlighted the importance of minimizing contamination levels. Yet a challenge is realizing this in practice, as a specialized DNA expert is not always available to perform sampling. Here, we will present tailored workflows for the execution of two widely used sedaDNA sampling methods by archaeologists without sedaDNA-expertise.

We evaluated their effectiveness in reducing surface contamination, comparing results for samples taken by either a sedaDNA-expert or non-experts. In this respect, we compared results between samples taken by archaeologists with and without sedaDNA expertise. Our results demonstrate that clean sampling protocols significantly minimize surface contamination, regardless of prior sampling experience. Additionally, our findings suggest that subsampling sediments with minimal surface contamination from previously collected materials is feasible, emphasizing the potential of archived sediment collections for sedaDNA analysis.

Monitoring Changes in Human Activities at the End of the Upper Palaeolithic: A Comprehensive Approach for the Critical Assessment of Ancient Organic Residues

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Keywords: Residue analysis, Machine learning, Dataset reliability

Residue analysis is crucial for reconstructing the technical expertise and adaptability of Palaeolithic hunter-gatherers. However, the field currently lacks standardized practices for collecting, interpreting, discussing and sharing data due to the diverse analytical methods adopted by different research groups. This study integrates three **analytical approaches (AAs)** to analyse a single dataset of high-resolution infrared (IR) spectra obtained from archaeological residues found on 23 backed pieces from the Epigravettian (Late Upper Palaeolithic) levels of Grotta della Cala (Marina di Camerota, Italy). These are:

- **AA1** – qualitative analysis.
- **AA2** – chemometrics.
- **AA3** – *predictive modelling*, using four machine learning algorithms trained on a collection of known reference materials relevant to Palaeolithic residues.

Spectroscopic data were primarily collected at the SISSI-Bio beamline of the Elettra Sincrotrone Trieste, mainly in microscopy. We compare the results from each AA, advocating for: a) a comprehensive, community-wide effort to describe comprehensively the spectral features observed in each analysis (building on **AA1**); b) the development of statistically-driven methods to enhance objectivity in data interpretation (drawing from **AA2**); and c) the establishment and collaborative expansion of shared reference libraries, for the interpretation of archaeological residues (using **AA3** as foundation). The presented predictive model is scalable and flexible and we anticipate its expansion with additional spectroscopic data and integration with highly specific biomolecular information, such as that provided by spectrometric techniques.

The Demodifier: A Tool for Screening Modification-Induced Alternate Peptide Taxonomy in Palaeoproteomics

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In palaeoproteomics, accurate taxonomic detection is crucial, as research questions often hinge on which species were utilised by ancient people. However, protein modifications such as deamidation and pyro-glutamic acid formation can alter peptide sequences, leading to spurious taxonomic matches and inaccurate archaeological interpretations. While a handful of examples of this phenomenon have been reported, the issue is more widespread than currently appreciated. In reality, any peptide containing a deamidated glutamine/asparagine residue, an unmodified glutamic/aspartic acid residue, or a pyroglutamic acid modification can yield sequence permutations of equal mass to the input peptide, that may match real species. Thus, whenever these residues or modifications are detected, the sequence—and potentially its taxonomy—may be incorrect.

While some researchers manually check for modification-induced alternate peptide taxonomies, this is not yet standard practice and can be labour-intensive, especially for peptides with numerous permutations. To address this, I present **The Demodifier**: a fast, open-access tool that screens for these alternate peptide taxonomies, enabling researchers to make informed interpretations. To assess its utility, The Demodifier was tested against an archaeological dataset containing all unique, readily accessible peptides from palaeoproteomic studies of dental calculus and food vessels. The results reveal that modification-induced alternate peptide taxonomies are severely underreported, occurring 15% more frequently than previously recognised in the literature. These taxonomic shifts can change species identifications or alter taxonomic specificity.

By rapidly identifying peptides with alternate sequences and potentially unsound taxonomies, **The Demodifier** helps prevent misinterpretations in archaeological research, improving the reliability of palaeoproteomic studies.

Embodied identities in life and death I

Bioarchaeological Analysis of Neolithic Burials from the Western Alpine Region

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Keywords: Neolithic Burials, Western Alps, Osteology, Stable isotopes, Radiocarbon, Ancient genomes

We investigated Neolithic burials from the western Alpine region in the framework of a bioarchaeological interdisciplinary project. Our focus is on changes in burial practices, in particular the development from single to multiple burials, and later to megalithic burials, and their connection to the social structure of Neolithic communities.

In addition to dating, our goal was to gain insights into their social stratification, nutrition, mobility, kinship relationships, population genetics and phenotypes.

The analysis of the human remains revealed radiocarbon-dates from the Middle to Late Neolithic. In addition, we analysed the stable isotopes $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$ and ancient genomes.

Nitrogen and carbon isotope ratios were relatively homogeneous for males and females within the sites. Therefore, we assume a similar diet and thus egalitarian burial groups. In general, their diet was rich in carbohydrates, which is also reflected in the intensity of caries. The sulfur isotope ratios indicate local mobility compared to regional animal data. We found first- and second-degree genetic relationships within the sites. Within one burial site, the dolmen of Oberbipp, we postulate a patrilocal community.

Our results confirm the arrival of ancestry related to Late Neolithic pastoralists from the Pontic-Caspian steppe in the region as early as 2860–2460 calBCE. However, our findings suggest a prolonged and complex genetic turnover, spanning nearly a millennium and involving highly structured populations. This could be due to the specific geographic and topographic location in the western Alpine region.

Patrilineality and Female Exogamy in a Late Chalcolithic Mountain Community at the Eastern Pyrenees

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Keywords: Archaeogenetics, Late Chalcolithic, Iberia

Roc de les Orenetes (Queralbs, north-eastern Spain) is a burial cave in the high Eastern Pyrenees mountains (1840 metres above sea level) containing a collective inhumation. At least two differentiated phases of use between the 3rd-2nd millennia BCE have been described according to archaeological, anthropological and radiocarbon analyses. In this study, we generated genome-wide data for 65 exceptionally well-preserved individuals belonging to the Late Chalcolithic phase (2500-2300 BCE). We were able to reconstruct a pedigree with 38 closely related individuals, organised around five sub-families likely descending from five brothers and following a strong patrilineal structure, with the remaining individuals connected to the main pedigree via distant familiar relationships. There seems to be a particular funerary treatment for women, brought to light by the significant sex ratio imbalance in favour of male individuals (3.75:1) and the absence of daughters from the clan in the cave, even before reaching reproductive age. This could be partially explained by the practice of female exogamy and the exclusion of subadult girls from the burial rite. The absence of recent endogamy in the genome of these individuals, thought to be isolated due to their peculiar location, suggest that they were part of a broader highland community. Despite being contemporaneous to the earliest presence of steppe-associated ancestry in Iberia, the Roc de Les Orenetes community lacked this ancestry component, highlighting that social organization around patrilineality and female exogamy were ongoing before the large-scale demographic change that took place in the Iberian Peninsula during the Early Bronze Age.

Lifeways of Late Bronze Age Communities in Central Germany: Insights from ancient DNA, Isotopes, and Archaeology

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Keywords: Late Bronze Age, aDNA, Mobility

The Late Bronze Age (LBA; 1300–800 BCE) of Central Europe has been proposed as a period of major population movements, socioeconomic changes, and cultural connections. However, reconstructing the impacts of these dynamics on different populations has been hindered by the fact that cremation was the dominant mortuary practice, limiting the use of biomolecular approaches. Here, we apply ancient DNA, oxygen and strontium isotope analyses, and osteoarchaeological analyses to rare inhumation burials from the sites of Kuckenburg and Esperstedt in central Germany. These sites present varied mortuary practices such as settlement burials, cemetery burials, skull-only burials, and multi-stage funerary rites, allowing us to investigate the community's burial choices and societal perceptions of the individual. The analysis of 36 LBA individuals reveals regional genetic continuity over time with subtle differences between early and late LBA individuals. Comparison with 14 LBA individuals from Neckarsulm and published data from southern Germany shows a clear ancestry dichotomy: southern individuals have more Early European Farmer-related ancestry than those from central Germany. However, oxygen and strontium isotope analyses suggest most individuals from central Germany, including those who were cremated, were local, with limited exceptions. Our findings suggest that the diverse inhumation practices at Kuckenburg and Esperstedt were culturally and socially motivated, reflecting local traditions and decisions rather than the influx of new groups with substantially different ancestries and/or “non-local” individuals.

Embodied identities in life and death II

Social Relations in Avar-Period Southern Transdanubia: Multidisciplinary Insights from the Kölked-Feketekapu Cemetery

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Keywords: aDNA, Isotopes, Early medieval social structure

Kölked-Feketekapu, with its more than 1,300 graves—including both smaller burial groups and larger cemeteries—is one of the most significant archaeological sites of the Avar period Carpathian Basin. The cemetery was in use from the mid-6th century to the late 8th century, and its diverse material culture—encompassing Late Antique, Merovingian, and Eastern nomadic elements—stands out as exceptional within both the region and the period. Recent advancements in scientific methods have opened unprecedented opportunities for studying the lives and social structures of such communities. Archaeogenetics, which examines ancient DNA sequences, is now capable of not only uncovering the origins of individuals but also reconstructing kinship relationships among them. Isotope analysis provides insights into the dietary habits (C/N) and mobility (Sr) of individuals. For the Kölked site, we have analysed DNA sequences from 360 individuals, strontium (Sr) isotope data from 212 individuals, and carbon-nitrogen (C/N) isotope data from 75 individuals. We identified newcomer communities coexisting with distinct local groups in the site's history. Differences in dietary habits further emphasize cultural distinctions, demonstrating how the integration of bioarchaeological methods can shed light on the lives and histories of both individual communities and broader regions. Our findings, when combined with previous archaeological and anthropological research, offer a novel perspective on the formation and daily life of this burial community.

Mass Violence, Age and Gender in the Early Iron Age of the Carpathian Basin

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Keywords: Age and gender, Mass violence, Interdisciplinarity

This study presents the bioarchaeological findings of 77 individuals and the biomolecular analysis of 25 individuals from a 9th-century BCE mass grave at Gomolava in the Carpathian Basin, Southeast Europe. The site is located at the crossroad of complex socio-spatial relations, divergent cultural traditions, and competing ideologies of landscape use. Excessive lethal violence enacted mostly on women and children suggests a selective demographic bias. Employing a multidisciplinary framework, we suggest a model for the social context in which the violent events surrounding the death of those buried in Gomolava took place. We explore the cause of death through osteological analysis, discern mobility patterns and diet habits through isotope analyses, reconstruct their genetic ancestry through genomic analyses, ascertain the age and sex distribution across the complete assemblage using a combination of osteological examination, genetic sexing and enamel peptide analysis and establish the age and time frame of the burial event using radiocarbon dating and micro-CT scanning. These findings shed new light on the socioeconomic roles and significance of women and young individuals in later European prehistory and aim to serve as a model for integrating genetic data across disciplines to construct meaningful, context-rich narratives.

Unlocking Ancient Craftsmanship: A Paleoproteomic Analysis of Glues and Binders from Pakepu's Coffin Set (680–664 BCE, Thebes, Egypt)

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Keywords: Collagen, MALDI-TOF, LC-MS/MS

Paleoproteomics is a rapidly advancing field that integrates molecular biology and archaeology to uncover detailed insights into past societies and their environments. Recently, this approach has been applied to structural materials such as plasters and mortars, revealing information about organic binders, their sources, and their preparation methods.

This study investigates the use of organic binders in the inner and intermediate coffins of Pakepu (E.2.1869), part of the Fitzwilliam Museum collection and dated to approximately 680–664 BCE. Calcium carbonate-based pastes mixed with glue were used as gap-fillers and ground layers for painted decoration. Notably, the intermediate coffin features simple paste layers, while the inner bivalve coffin exhibits a complex cartonnage-like layering, built atop a thick layer of ‘fibrous glue’ directly applied to the wooden surface.

Optical and electron microscopy, combined with Fourier-transform infrared spectroscopy, confirmed the presence of collagen in both the pastes and the fibrous glue. These findings were further analyzed using paleoproteomic techniques, including MALDI-TOF MS and LC-MS/MS, to investigate the production processes and identify the animal species from which the materials were derived. The resulting data were processed using two different software platforms, Novor Cloud and MaxQuant, to demonstrate the challenges of paleoproteomics, as variations in software can lead to differing interpretations.

This research seeks to determine whether the organic binder in the pastes differs from the fibrous glue or if similarities suggest shared production methods. Additionally, it aims to examine the potential symbolic significance of the animals used in glue production within this funerary context.

New Biomolecular Insights into Pictish Identity

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Keywords: Picts, aDNA, Isotopes

The self-identity of the Picts, the group who inhabited the north and north-east of Scotland between ~300 and 900 CE, has been a topic of debate in archaeology since the 1950s as a result of the lack of historical and archaeological evidence from this period in Scotland. Considerable research has been conducted in particular over the past decade or so on the history, archaeology, and bioarchaeology of the Picts, which has provided greater insight into the nature of this archaeological group. However, because of the scarcity of human remains and the lack of Pictish written sources, the popular imagination still draws heavily on mythical narratives from contemporary historical accounts. This paper presents insights into Pictish life histories and social organisation by combining novel and published isotopic and ancient DNA analyses. These data show high levels of mobility over varying distances, genetic heterogeneity, and a lack of close relatedness in cemetery populations. Biomolecular analysis therefore provides important insights alongside historical and archaeological research into the emergence of a later cohesive group identity amongst the Picts.

Libation Rituals in Iron Age Iran? Unveiling the Function of Funerary Pottery Vessels at Sialk (Kashan) and Ghalekuti (Dailaman) through Typology and Organic Residue Analysis

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Keywords: Funerary ceramics, Organic residue analysis, Libation ritual

Pottery vessels often comprise major burial goods, providing valuable information for reconstructing the past mortuary practices. However, because of the uncertainty of its function, which has been interpreted mostly through typological studies alone, the analytical potential of funerary pottery has not been fully exploited.

This study applies organic residue analyses to funerary pottery vessels of the Necropolises of Sialk excavated in the 1930s and of Ghalekuti excavated in the 1960s. At Ghalekuti results demonstrated that the vessels retained remains of botanical and animal origin¹. In particular, jars with tubular spouts, were likely specialised for botanical products. Interestingly, both carcass and dairy products from ruminant animals were processed in short-neck jars and bowls, including spouted bowls, suggesting their use in a liquid state. At Sialk, lipids were primarily identified as ruminant adipose fats. Dairy fats were found in only one Iron Age long-spouted pottery vessel, a type distinctive of the Iron Age period².

The typology of ceramics combined with sampling strategies in specific places (spout or neck opposing the handle) permits to establish that commodities of botanical and animal origin were used as a liquid state and poured before the deposition of the vessels in the graves. Considering some types of funerary ceramics are very refined and unknown from domestic assemblages, they were likely not made for daily use but rather had a function dedicated to funerary rituals. These results indicate that pottery vessels likely played a role in the mortuary rituals of Iron Age Iran.

References

- 1 Casanova, et al, 2024 Unravelling the Function of Funerary Pottery Vessels of the 2nd-1st Millennia BC in the Dailaman Province (Iran) through Typology, Petrography, and Organic Residue Analyses. *PLOS ONE* 19, 9: e0306647. <https://doi.org/10.1371/journal.pone.0306647>.
- 2 Casanova, et al, 2024 Unveiling the function of long-spouted ceramics at Sialk, Iran: Insights from organic residue analysis. *Archaeological Research in Asia* 40: 100570. <https://doi.org/10.1016/j.ara.2024.100570>.

Integrating ancient biomolecules and archaeological theory

Elucidating and Integrating Theoretical Approaches in Archaeogenetic Kinship Studies

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Keywords: Archaeogenetics, Kinship, Theory

Advances in archaeogenetic research facilitate reconstructions of extensive pedigrees among individuals who lived thousands of years ago, seemingly unlocking new insights into kinship in the past. While impressive, such studies can reify a biocentric approach to kinship common within Western discourses of relatedness. In many societies kin relationships are created through shared experiences like commensality, collective labor, and co-residence, not given at birth. In these contexts, archaeogenetic approaches may conceal the diverse ways ancient peoples forged kin relationships and networks. Despite recent calls to incorporate social theoretical approaches in archaeogenetic kinship research, little has changed. I suspect this is because critiques have not thoroughly assessed the theory informing archaeogenetic studies. This project reviews recent archaeogenetic kinship literature to examine the theoretical frameworks explicitly and implicitly used to investigate kinship and explores ways to more effectively integrate archaeogenetic approaches and archaeological theory.

Review of the literature reveals that few archaeogenetic kinship studies explicitly incorporate social theory. Instead, they implicitly rely on theories of inheritance and population genetic processes rooted in the modern synthesis of evolution. These are difficult to reconcile with the agency-based theoretical perspectives that characterize archaeological kinship research. To effectively incorporate social theory, archaeogenetic kinship studies must 1) conceptualize kinship as a complex biosocial process, 2) embrace contemporary evolutionary concepts like plasticity and inclusive inheritance that are more amenable to synthesis with theories of agency and materiality, and 3) apply this analytical framework to diverse biomolecular (e.g., genomic, epigenomic, isotopic, microbiotic) and archaeological datasets.

Moving Beyond Migration: Using Archaeogenetics to Study Diverse Modes of Mobility in the Ancient Mediterranean

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Keywords: Ancient DNA, Rome, Mobility

Migration has often been invoked as the primary mechanism of genetic change in archaeogenetic studies. While this term has frequently been used as an umbrella term for any type of human movement, mobility occurs and has occurred in many diverse patterns. This project examines how archaeogenetic studies can explore various modes of mobility, beyond migration, especially through interdisciplinary collaborations and the integration of historical, archaeological and bioanthropological sources. We identify challenges in the study of mobility patterns - such as equifinality that arises from working with small, geographically heterogeneous datasets and the spectre of culture-historical archaeology's fixation on migration - and suggest possible solutions, such as developing a shared terminology across disciplines. To illustrate the theoretical points raised above, we reanalyze archaeogenetic data from port cities of the Mediterranean in the context of complementary information from allied disciplines. Port cities are an excellent arena to shed light on the diverse patterns of mobility, as they are important sites of interaction between the maritime world and the hinterlands beyond, between local and diasporic populations. The primary case study will be insights gained from archaeogenetic and mobility isotope data for 20 individuals from Isola Sacra, the necropolis for Rome's primary trading port.¹⁻³ The complementary nature of these two analyses, combined with insights from archaeological and textual sources, provides a complex picture of mobility in the Roman world which could not have been fully understood from either source alone.

References

Prowse, T. et al. Isotopic evidence for age-related immigration to imperial Rome. *Am J Phys Anthropol.* 132(4):510-9 (2007).

Antonio, M., Gao, Z., Moots, H.M., et al. Ancient Rome: A genetic crossroads of Europe and the Mediterranean. *Science* 366, 708-714 (2019).

Antonio, M., Weiss, C., et al. Stable population structure in Europe since the Iron Age, despite high mobility. *eLife* 13, e79714 (2024).

Conviviality and Multispecies Relationality: What can Guinea Pig Isotopes Tell Us about Human Social Interactions in the Andes?

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Domesticated guinea pigs (*Cavia porcellus*) are food sources in the Andes. Archaeological evidence attests to their role in human diet for millennia. They also hold important sociopolitical, economic and cultural value as trade items, medicinal ingredients, and ceremonial offerings. Biomolecular work in the Andes have focused on South American camelids (SAC), while minimal analyses have focused on guinea pigs to understand their trophic position as a commensal species. In this paper, we examine spatial differences in guinea pig isotopic variability from Cocahuischo, a Late Nasca site (450-750CE) in Peru. We employ the concept of conviviality, a framework that centers the relationships and interdependencies between humans and nonhumans to co-create the world we live in together.

We posit that guinea pig isotope data can shed meaningful light on the strategies people are employing to raise these animals within their households but also that they can serve as a proxy for how guinea pigs are shared in different private and public contexts for social and political reasons. Guinea pig isotopic variability at Cocahuischo suggests these animals were raised through heterogenous strategies and individuals controlling wealthy house contexts may have held political influence over diverse groups to obtain guinea pigs for their use in feasting events. The ability for some groups at Cocahuischo to leverage political control over others involves a network of relationships between both human and nonhuman agents. Thus, employing the framework of conviviality in conjunction with a biomolecular technique enables robust place-based interpretations about human social interactions in the past.

Social Belonging at the Rise of the Greek City-State: Halieis Case Study

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Keywords: Archaic-Classical Greece, Ancient DNA, Bioarchaeology, Kinship, Biological relatedness, Identity formation

Whereas “kinship” or “family” have long been understood as biologically determined institutions, recent research has shown that these concepts are highly dynamic and vary greatly depending upon cultural contexts, with biological and social aspects crucial for their understanding. When it comes to the Archaic-Classical Greek communities (ca. 750-479/479-323 BCE), scholars seem to mostly examine kinship ties through an evolutionary prism that connects the formation of identities to the emergence of the polis and an increasingly structured social context, with its focal point being the “oikos” (i.e., household). In the current study, we explore the genetic relatedness within a group of burials from a part of the Halieis necropolis situated in the rural landscape of the Greek Argolid. Considering the sound level of preservation and documentation, Area 3 was selected, containing 26 graves with 30 burials, from which 23 individuals were sampled for ancient DNA analysis. Biological relatedness analyses and the finding of a multigenerational pedigree suggest that Area 3 might represent an extended family that used the same burial plot for a long time and favored consanguineous unions in consecutive generations. Furthermore, the variability of mtDNA haplogroups poses the question of marital practices and whether Area 3 was spatially organized along particular lines of descent. Our questions, therefore, address whether genetic ancestry and biological kinship might have weighed upon the formation of individual/group identities, marital ties, and consanguineous relationships and how these might be reflected in the spatial organization of the burial grounds and the preference of certain burial practices/goods over others.

Biomolecular Beasts & Becomings: Integrating Contemporary Theory with Scientific Approaches to Archaeological Animals

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Keywords: Theory, Animal-human relations, Domestication

The biomolecular revolution in archaeology has led to massive transformations in how we understand animal domestication and prehistoric animals. Critiques of biomolecular approaches used to bolster outdated theory or ‘grand narratives’ have radically improved our interpretations of the human past. However, interpretations of the animal past often remain conservative: staunchly anthropocentric, techno-deterministic, and economic focused. Employing a multispecies, ethological approach, this paper centres the archaeological animal with examples drawn from European prehistory to explore how an ever-increasing corpus of biomolecular results can be better harnessed to understand the central roles of animals in spurring the emergence of new lifeways, and in configuring and transforming human societies.

Biomolecular Assemblages: Exploring Organic Residue Analysis and Archaeological Interpretation through Assemblage Theory

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Keywords: Assemblage theory, Organic residue analysis, Science communication

This paper explores assemblage theory as a way to think about interpretation in biomolecular archaeology, focusing on organic residue analysis. Biomolecules have become indispensable molecular-scale “ecofacts” in archaeological assemblages. Assemblage theory, as a branch of the “relational turn,” has been used in archaeology to explore how the processes and relationships between parts of an archaeological assemblage converge to make something greater than the sum of their parts: an inference about the past.¹ Considering both how organic residues are combined toward an interpretation (e.g. suites of lipid biomarkers) and the processes that produce residues as part of the broader archaeological assemblage draws attention both to past processes (taphonomy as well as human actions in the past) and to how archaeologists’ actions in the present impact interpretations. I revisit the interpretation of a 30-year-old organic residue study and its implications for theories about the economic organization of Late Bronze Age Cyprus, to explore how residues become archaeologically meaningful evidence for cuisine, diet, etc. through researchers’ sampling, extraction, analysis, interpretation, and citation activities. This paper considers how assemblage theory might serve as a science communication tool to integrate interpretation of organic residues and other biomolecular techniques conceptually into broader archaeological interpretative frameworks and might also improve the utility and accessibility of our interpretations for wider archaeological narratives.

Reference

¹ Lucas, G. *Understanding the Archaeological Record*. Cambridge University Press (2012).

Methodological advances in biomolecular archaeology I

Metabolomics-guided Identification of Ancient Tars: Differentiating Plant Products and Production Processes

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Keywords: Metabolomics, Molecular Markers, Thermochemical Processing

The production of 'synthetic' organic substances dates back to at least the Middle Palaeolithic, as demonstrated by Neanderthals' transformation of wood into tar. Unlike natural plant exudates such as resins, which oozes from trees, tars are thermochemically produced through processes like dry distillation or pyrolysis, requiring controlled heating to extract them from organic materials. When resins, tars and essential oils originate from the same organism, they often share overlapping molecular signatures, making their differentiation in archaeological contexts challenging, especially when degradation further complicates identification.

Recent advancements in the field of metabolomics provide a comprehensive approach to distinguishing such complex archaeo-organic materials. By analyzing complete metabolite profiles, new metabolomics data-processing workflows facilitate the differentiation of closely related plant products and substances.

This study presents a novel GC/MS-based metabolomics approach for identifying molecular markers that distinguish tars, resins, and oils. Applied to modern *Cedrus atlantica* samples, this method identified diagnostic compounds characteristic of these substances and their production processes. These markers were then used to analyze archaeological samples, determining their composition, origins, and methods of production.

Our results highlight the potential of metabolomics as a powerful tool for distinguishing plant-derived materials, and then significantly improving molecular identification in archaeological contexts. Furthermore, this research advances our understanding of ancient technologies and material use, offering a robust framework for biomolecular archaeology and heritage science.

Unearthing Ancient Parasites: Screening of Metagenomic Samples from Medieval Europe for the Presence of Parasitic Helminths

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Keywords: SedaDNA, Helminths, ParasiteMT

In recent years, ancient DNA (aDNA) research has provided relevant insights into past health crises and the evolution of human pathogens, with a particular focus on bacteria and viruses. However, multicellular pathogens such as helminths have received comparatively less attention due to challenges in identifying and isolating their DNA from ancient samples.

To address this challenge, we developed a methodological pipeline for isolating and analyzing ancient helminth DNA from diverse ancient environmental sources. We designed a targeted capture probe panel (ParasiteMT) that covers mitochondrial genomes of 133 parasite helminth species. We then applied it to Next-Generation Sequencing (NGS) aDNA libraries from medieval soil, sediment, and latrine samples collected in the Czech Republic. Previous microscopic and basic molecular examination of these samples provided evidence of helminth eggs and DNA, respectively, in several samples. Our molecular approach enabled the detection of ancient helminth DNA even in samples lacking visible eggs and facilitated species-level identification of parasite communities.

Subsequent genomic and phylogenetic analyses offered new insights into evolutionary changes in these species, enhancing the resolution of existing helminth phylogenies. As helminths co-evolved with humans, exerting significant selective pressures on our genomes, our results highlight the potential of aDNA to study helminth evolution. The new approach for ancient helminth DNA detection, identification, and analysis, can aid in elucidating the evolutionary dynamics between humans and their parasites, the environmental conditions that may have facilitated the spread and persistence of parasitic diseases, and the broader human-environment interactions over time.

Density Separation as a Decontamination Method for Ancient DNA Extraction from Sediments

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Keywords: Sediment Ancient DNA (sedaDNA), Decontamination, Inhibition Removal

A key challenge in the field of sediment ancient DNA (sedaDNA) is the co-extraction of modern DNA contamination and other organic molecules that inhibit downstream applications. Both factors have the potential to reduce the genetic information recovered from a sample, complicating or even preventing downstream analysis. In this study, we aim to address these issues by reducing contaminants prior to DNA extraction. To achieve this, we developed a sediment density separation protocol using a heavy liquid to selectively isolate ancient DNA-enriched phases from those with little or no ancient DNA. Our results demonstrate that this method significantly reduces the impact of co-existing inhibitory substances on ancient DNA extraction from sediments and downstream analysis. Additionally, modern DNA contamination present in significant excess in a bulk sample could be drastically reduced or nearly completely separated using this method. This approach is particularly useful for heavily contaminated samples, which are often difficult or even impossible to process using standard methods. The amount of sediment that can efficiently be processed in one batch is often limited by the amount of inhibitory substances. This method offers the potential for processing larger quantities of material in a single batch, thereby optimizing resource efficiency for the gain of sedaDNA data.

Migration, mobility, and translocation I

The Making of a God: Tracing Early Egyptian Contact with Baboons

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Keywords: Baboons, aDNA, Sahara

Recent discoveries on the Egyptian-Libyan border have thrown a spotlight on Ennedi, a remote plateau in north-east Chad. Sometimes referred to as the Eden in the Sahara, Ennedi is not only prominent for its unique landscape, extraordinary archaeological sites, and iconic desert-dwelling fauna, but is also home to a relict population of baboons, isolated for millennia on this rock island in a sea of sand from their conspecifics further south. The discovered rock art and hieroglyphic inscriptions have energized debate on the location of Yam, a mysterious name of an unknown region in ancient Egyptian culture and the vital role of the Wadi Howar/Yellow Nile corridor during the desertification of the Sahara. Our aims are twofold: to determine (1) the phylogeographic history of baboons living in Ennedi today and (2) the geographic provenance of baboons imported to Egypt 6,000 years ago, which fueled a fascinating worship of baboons in ancient Egyptian culture. We answer these questions by combining approaches from non-invasive wildlife genetics and stable isotope analyses to track the geographical origins of baboon individuals and situate these findings with historical and archaeological evidence from Egyptology. Our overarching objective is to uncover the ancient pathways that connected people and animals living in the eastern Sahara during the Holocene.

Migration and Mobility: Exploring Identity-By-Descent (IBD) across Ancient Livestock

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Keywords: Identity-By-Descent (IBD), Migration, Cross-species comparison

Migration played a crucial role in the Neolithization process, driving the spread of agriculture and transformed human communities across diverse landscapes. Identity-By-Descent (IBD) analysis is a powerful genetic tool for investigating migration, mobility and kinship by identifying segments of DNA inherited from a common ancestor. Here we exploit the successful imputation of ancient low-coverage genomes to explore IBD patterns in two species in parallel: cattle and goat, shedding light on migration, trade networks, and cultural exchanges. We will discuss how the demographic characteristics of cattle and goats – such as effective population size, mating structure and generation time – differ from those of humans, and how this influences IBD sharing and interpretation. We explore two case studies: the migration of Neolithic cattle-focused communities into Europe via the Danubian migration route, using shared IBD of ~115 ancient imputed genomes, and the migration/exchange of goats in Eurasia, based on shared IBD of ~40 ancient imputed genomes. Both case studies examine the broader migration patterns as well as more regional local dynamics, and reflect how the interconnected histories of human and livestock populations can be revealed using IBD analyses.

Chronological mismatch between Bronze Age contexts and biomolecular traces of exotic landfowl in Crete

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Keywords: Human-avifauna interactions, Translocations, Palaeoproteomics

During the rise of the Minoan civilization in the Eastern Mediterranean (Bronze Age, ~3,100–1,050 BCE), urbanization, agricultural intensification, and integration into long-distance trade networks facilitated the translocation of non-local materials and species. It is possible that exotic birds, such as chickens and peacocks, would have been introduced during this time. Indeed, scant but intriguing zooarchaeological and iconographic evidence seems to suggest that these Asian landfowl species were known and perhaps even present on Crete and other islands within the Minoan world. Here we report on ongoing research conducted as part of the ERC Consolidator project “AviArCh - Avifauna in Archaeoecological Networks,” which investigates legacy environmental samples from systematic excavations of the ancient town of Kydonia (Khania). Our research focuses on bird eggshell and bone remains to reconstruct the range of avifaunal species present in this significant urban center. The contexts we examine include buildings of probable domestic use, considered part of the Kydonia Palatial Center on Kastelli Hill, dating to the Late Minoan period (1420/10–1075/50 BCE). We employ a range of methods to clarify the biological origins of the remains, demonstrating that Zooarchaeology by mass spectrometry (ZooMS) and palaeoproteomics can reveal the taxonomy of eggshell and bone fragments. These findings provide novel insights and begin to illuminate avian-human interactions during the transition from the final palatial to the post-palatial periods, offering a unique lens to study the legacies of human-environment interactions on islands.

Migration, mobility, and translocation II

The Origin and Expansion Process of Early European Farmers

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Keywords: Neolithic, Demographic expansion, Ancient DNA

The spread of farming into Europe has been linked to a demographic expansion of people from Anatolia. While genetic studies show that Neolithic farmers experienced low levels of admixture with local hunter-gatherer groups in central and western Europe, recent demographic reconstructions suggest that the ancestors of early Anatolian farmers carried substantial amounts of Western Hunter-Gatherer (WHG) ancestry. To further refine the demographic history of early farmers and hunter-gatherers in Europe and investigate modes and timings of dispersals across the Aegean, Balkans, and central and western Europe, we analyzed 89 high-coverage ancient genomes, 40 of which were newly sequenced. We inferred divergence times between ancient populations, their effective sizes, and admixture events, providing new insights into the geographic and genetic origin of early European farmers. Additionally, we used local ancestry inference to identify genomic regions enriched for WHG ancestry and investigated whether this ancestry has been under selection in early farmers. Our results suggest that the Neolithic expansion into Greece and along the Danubian corridor occurred through a mixture of short- and long-distance dispersal events, rather than through a linear process in space. This mode of diffusion led to patterns of genomic diversity that depart from theoretical predictions of a classical and progressive range expansion, for instance, by contributing to the maintenance of high levels of diversity among early European farmers.

Steppe Ancestry in Western Eurasia and the Spread of the Germanic Languages

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Today, Germanic languages, including German, English, Frisian, Dutch and the Nordic languages, are widely spoken in northwest Europe. However, key aspects of the assumed arrival and diversification of this linguistic group remain contentious. By adding 712 new ancient human genomes we find an archaeologically elusive population entering Sweden from the Baltic region by around 4000 BP. This population became widespread throughout Scandinavia by 3500 BP, matching the contemporaneous distribution of Palaeo-Germanic, the Bronze Age predecessor of Proto-Germanic. These Baltic immigrants thus offer a new potential vector for the first Germanic speakers to arrive in Scandinavia, some 800 years later than traditionally assumed. Following the disintegration of Proto-Germanic, we find by 1650 BP a southward push from Southern Scandinavia into presumed Celtic-speaking areas, including Germany, Poland and the Netherlands. During the Migration Period (1575–1375 BP), we see this ancestry representing West Germanic Anglo-Saxons in Britain, and Langobards in southern Europe. We find a related large-scale northward migration into Denmark and South Sweden corresponding with historically attested Danes and the expansion of Old Norse. These movements have direct implications for multiple linguistic hypotheses. Our findings show the power of combining genomics with historical linguistics and archaeology in creating a unified, integrated model for the emergence, spread and diversification of a linguistic group.

Genetic (Dis)continuity in the Vistula and Oder River Basins

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Keywords: Poland, West Slavs, Autochtony

The fast development of laboratory and analytical methods and the ever-growing body of genomic data generated from ancient individuals continuously increases the resolution of archaeogenomics-based inference of human history. In the Vistula and Oder basins region – currently territory of Poland – such inference is, however, largely hampered by lack of skeletal material caused by the widespread custom of cremating the dead across many archaeological periods and cultures, as well as by very low divergence levels between spatiotemporal groups, obscuring the inference of direction and timing of gene flow processes. The recently published genome-wide data for Iron Age and Medieval individuals from Poland¹ have been claimed by the authors to confirm long-term genetic continuity in the region, i.e., autochtony of West Slavs, but this interpretation of the obtained results has been widely criticized among the academic community and already has been undermined by a subsequent study².

Here, we present novel data for nearly 200 samples representing spatiotemporal transect for the territory of what is now Poland. With sampling spanning between the Neolithic and Modernity and across the whole geographical region we are exploring the long-term population dynamics. We are further testing hypotheses of genetic continuity, with particular focus on the Bronze Age – Iron Age – Middle Ages transitions, as well as the affinity to external groups, by applying state-of-the-art high-resolution analyses like twigstats and IBD analyses. Our hitherto results, i.e., confirm continental-scale mobility in the Gothic cultural circle group from Eastern Poland, as well as find familial connections between distant populations.

References

1. Stolarek, I. *et al.* Genetic history of East-Central Europe in the first millennium CE. *Genome Biol.* **24**, 173 (2023).
2. Speidel, L. *et al.* High-resolution genomic history of early medieval Europe. *Nature* **637**, 118–126 (2025).

Long-distance Genetic Relationships Connect Prehistoric Central Europe

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Keywords: Population genetics, Genetic relatedness, Identity-by-descent, Neolithic, Bronze Age

The transition from a foraging to a farming lifestyle in Europe ~7000-6000 BCE was accompanied by a major genetic turnover. A second genetic transformation began ~3300 BCE triggered by the expansion of steppe pastoralists from eastern Europe and accompanied by the spread of the so-called ‘steppe-related ancestry’. These cultural and genetic changes are well attested in regions of Central Europe. Analyzing new genome-wide data from 633 prehistoric individuals from France, Germany, Poland and Czechia, we fill spatial and temporal sampling gaps and provide detailed genetic ancestries of individuals associated with various prehistoric archaeological cultures, resulting in a more nuanced narrative of the genetic changes from the Neolithic to the Early Bronze Age. Culturally connected settlement hubs in Moravia, Bohemia, southern Poland and Central Germany highlight mutual genetic influences in the form of shared genetic affinities and close biological relationships, which mirror the observed cultural similarities between the regions. Furthermore, using established and new methods, we identify numerous close genetic intra-site relationships but also many relationships between sites and different regions, and even between different cultural associations. We utilize this unique dataset to estimate effective population sizes and timing of mixture events/pulses to gain insight into the underlying demographic processes that lead to the characteristic genetic profiles of individuals from each time period and associated cultural groups.

"Celts" up the Alps: Territorial Mobility and Funerary Variability in Late Iron Age Switzerland

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Keywords: Late Iron Age, Mobility patterns, Funerary practices

The Late Iron Age in Europe (5th–1st centuries BCE) was marked by profound economic, social, and cultural shifts, including the expansion of trade and interaction networks. These aspects provide a valuable framework for exploring human mobility and its demographic and social implications. This study examines the extent and characteristics of mobility during this period within the core of the La Tène cultural sphere, focusing on present-day Switzerland. Additionally, it explores potential cultural factors shaping mobility patterns. To assess residential mobility, we analyze strontium ($^{87}\text{Sr}/^{86}\text{Sr}$), oxygen ($\delta^{18}\text{O}$), and sulfur ($\delta^{34}\text{S}$) isotopic ratios from dental enamel and bone collagen in a sample of human remains ($\text{N } ^{87}\text{Sr}/^{86}\text{Sr} = 101$; $\text{N } \delta^{18}\text{O} = 90$; $\text{N } \delta^{34}\text{S} = 121$) from various Swiss sites dating to the last five centuries BCE. These results are interpreted in relation to faunal and environmental baselines and examined for demographic and funerary trends. Results show considerable spatial variation in the frequency of nonlocal individuals, suggesting that both short- and long-distance mobility was influenced by regional political and economic factors. No correlation was observed between mobility and sex, implying that specific residential rules were not systematically applied. Isotopic variability does not correlate with funerary treatment, suggesting that geographic origin played a minor role in social identity compared to factors such as gender, socioeconomic status, and community affiliation. These results contribute to a broader understanding of demographic dynamics in Iron Age Switzerland and underscore the complexities of reconstructing past mobility through isotopic analysis.

The Bioarchaeology of a Borderland: Military Communities in the Roman Period through the Lens of Sr-O-C Isotopes and aDNA Analysis

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Keywords: Roman Period, Isotope analysis, aDNA analysis

The Dutch city of Valkenburg, situated on the coast along the Lower Germanic Limes, hosts one of the largest Roman-period cemeteries in the Netherlands, containing more than 600 graves. While the majority of graves are cremation burials (n=520), 134 inhumation graves have also been excavated. Since the 1980s, this cemetery has been studied extensively; however, questions of who these individuals were and where they originated remain unanswered.

These questions underpin this multidisciplinary study, which combines Sr-O-C isotope and aDNA analyses to explore mobility, dietary practices, genetic ancestry, and phenotypic predictions shedding light on the lives of those interred in Valkenburg. A first and outdated explanation of why the buried individuals were treated differently from the rest of the community was that they were not of local descent, in contrast to the cremated population. However, this was quickly debunked by the isotope analysis, which revealed that both the interred as well as the cremated individuals showed high variability, indicative of mobility regardless of the way they were treated after death. The aDNA analyses of the inhumations further clarified and enforced this idea of a highly diverse community, showing significant genetic variation.

The study's findings offer unprecedented insights into the population dynamics of Roman Valkenburg, specifically about the communities that were associated with military presence.

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Diet and foodways I

A Tooth for All Seasons: Prehistoric Subsistence Strategies Uncovered by Refined Sequential Dentine Microsampling & Isotope Analysis

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Keywords: Dentine micro-sampling, Stable carbon and nitrogen isotope analysis, Hunter-gatherer

Stable isotopic analysis of sequential dentine samples has redefined the study of past human dietary patterns, providing chronological insights into early-life nutrition. Here, we present easily applicable and straightforward methodological improvements that enhance precision and temporal resolution: (1) We cut longitudinal sections from the tooth centre and utilise biopsy punches for sequential micro-sampling, thereby reducing the averaging effect of sampling overlapping incremental layers; and (2) we carefully consider the anatomical location of the samples and account for age-related changes in root growth velocities to accurately assign approximate ages and time spans covered by each sample. Together, this approach not only allows for tracking life histories at sub-annual resolution but also reduces material loss to preserve enough dentine for complementary analyses.

Applying our refined micro-sampling method to analyse carbon and nitrogen isotope profiles from prehistoric Siberian hunter-gatherers, we reveal significant seasonal changes in dietary resources, reflecting shifting reliance on distinct food types. Such patterns of dietary shifts highlight the dynamic interaction between humans and their environment and elucidate how resource availability and subsistence strategies were influenced by seasonal cycles. Our findings underscore the importance of seasonal patterns in shaping human survival and cultural practices, providing a more nuanced understanding of lifeways, adaptations, and subsistence strategies in prehistoric hunter-gatherer communities.

Reconstructing Past Diets: Expanding the Role of Strontium Isotopes through Experiments and Multi-Isotope Comparisons

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Keywords: isotopes, diet, experimental archaeology

Understanding ancient diets is a vital part of studying past human populations. However, not all dietary practices are visible in the archaeological record, as only certain aspects of diet are identifiable through techniques such as stable isotope analysis. This presentation explores how traditional isotopic analyses, like carbon and nitrogen, can be complemented by the use of strontium isotopes—both radiogenic ($^{87}\text{Sr}/^{86}\text{Sr}$) and stable ($\delta^{88}\text{Sr}$)—along with strontium concentrations ($[\text{Sr}]$), to potentially uncover new dimensions of dietary behaviour.

Radiogenic strontium isotopes ($^{87}\text{Sr}/^{86}\text{Sr}$) are typically used to understand mobility, but as these signatures represent food consumed they can be influenced by, and therefore identify, the consumption of non-local or marine foods, or alteration by food processing. In addition, stable strontium isotopes ($\delta^{88}\text{Sr}$) show promise in distinguishing dietary elements (such as marine vs terrestrial), and strontium concentrations ($[\text{Sr}]$) offer clues about practices such as salt consumption.

This presentation will highlight the steps taken to develop strontium as a dietary proxy. Firstly, through the creation of a comprehensive database of strontium values in foods to understand variation introduced by specific dietary components. Then through conducting experiments how strontium isotope ratios shift during processes like stock preparation, fermentation, brewing, and decomposition, is investigated. Lastly, by comparing strontium with traditional dietary isotopes the contribution to the archaeological understanding of diet can be evaluated. By connecting the influence of dietary practices on strontium signatures, we can broaden our understanding of ancient dietary practices and also challenge how we interpret mobility patterns in archaeological contexts.

Dietary Histories in the Atacama Desert during the Formative Period (1000 BC-900 AD) through Incremental Hair Stable Isotope Analysis

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Keywords: Stable isotopes, Hair, Atacama Desert

The Formative Period was a key moment in the South-Central Andes (since 1000 BC), characterized by an intensification of sedentism, transition to agriculture, animal domestication, and the emergence of social inequality. Tarapacá-40, one of the most representative cemeteries of this period in the middle of the Atacama Desert (1000 mosl), provides valuable evidence of these transitions.

This inland site presents a unique opportunity to assess how these socio-cultural changes influenced dietary practices. The aim of this work is to evaluate the diet composition of 22 adult individuals (including males and females) from the Tarapacá-40 cemetery, through incremental hair stable isotope analysis ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$). Hair was cut every 1 cm, with total hair lengths varying between 5 and 26 cm. A total of 251 hair samples were analysed in order to achieve more detailed information about their diet during their last months of life.

Stable isotope values indicated that males exhibited a homogeneous diet based on terrestrial C_3 resources, while females showed significantly greater variability by incorporating coastal resources, with higher $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values. Results show very marked intra-site variability not previously observed in other archaeological sites. We propose that agricultural development and new resources did not imply a substantial change in the diet. Instead, C_3 plants like *algarrobo* (*Neltuma* sp.) were the principal sustenance, unlike maize. Consumption of coastal resources by females could be related to the role of women in patrilocal and exogamic kinship system between the interior and the coast of the region.

Tracing Diet Quality and Nutritional Stress using Amino Acid Isotope Analysis: Insights from a Forager-to-Farmer Transition in Southern Belize

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Keywords: Amino acid isotope analysis, Diet quality, Nutritional stress

Studying past diets, diet quality, and nutritional stress in archaeological populations provides critical insight into how selective pressures influenced individual health outcomes, well-being, and fecundity. Traditional methods of identifying nutritional states, like examining skeletal markers of physiological stress, are constrained by their focus on developmental or extreme adult stress and are further complicated by the osteological paradox—healthier individuals live long enough to develop skeletal markers while less healthy individuals may die before lesion formation. $\delta^{13}\text{C}$ Amino Acid Isotope Analysis (AAIA) coupled with $\delta^{13}\text{C}$ bioapatite offers a novel approach to evaluate nutritional stress by assessing the proportion of *de novo* nonessential amino acid synthesis integrated into skeletal tissues during the last 7-15 years of life. Furthermore, combining $\delta^{13}\text{C}$ AAIA and bioapatite data with $\delta^{15}\text{N}$ AAIA enables the detection of differential physiological stress within populations, likely reflecting chronic nutritional challenges.

We present the results of AAIA-bioapatite diet quality and physiological stress models applied to a neotropical forager-to-farmer population in southern Belize, spanning 9,500 to 1,200 BP. Preliminary results indicate a decline in diet quality and increased nutritional stress during the agricultural transition, likely driven by increased consumption of carbohydrates. By leveraging the macromolecular origins and cycling of carbon in bone bioapatite and nitrogen and carbon in bone collagen using intraindividual level data, this methodology is applicable across diverse dietary contexts, even in the absence of baseline isotopic data. When integrated with archaeological evidence, this approach holds significant potential to illuminate the health consequences of social and environmental selective pressures.

Reconstructing Resource Landscapes of Early Swahili Towns: A Multi-Isotopic Approach to Urban Ecology in Zanzibar

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Keywords: Compound-specific, Isotope, Africa

The emergence of Swahili stone towns along the East African coast (late first and early second millennia CE) marks a period of significant socio-economic change. Understanding the relationship between these urban centers and their resource landscapes is crucial. This study investigates diachronic shifts in resource exploitation in the Zanzibar archipelago, comparing the Iron Age settlement of Unguja Ukuu (7th–10th centuries CE) with a later site on Tumbatu island (11th–15th centuries CE). By integrating detailed zooarchaeological analysis with a cutting-edge multi-isotopic approach, we aim to reconstruct the resource landscape and dietary practices of these coastal populations.

Zooarchaeological data reveal distinct subsistence strategies across time and location, reflecting adaptations to environmental and social changes. Our bulk carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$) (n=320), and sulfur ($\delta^{34}\text{S}$) (n=50) stable isotope analyses establish the first detailed resource isoscape for a Swahili coastal urban population. This isoscape illuminates the use of C_3 and C_4 terrestrial resources, differentiating browser and grazer exploitation. Isotopic signatures of marine turtles and fish reveal diverse foraging strategies, reflecting varied exploitation of aquatic environments.

Bulk and serial isotope analyses of human remains from Unguja Ukuu provide insights into the dietary life histories of early urban inhabitants, shedding light on resource access. Compound-specific amino acid isotope analysis of human and faunal remains (n=20) offers high-resolution insights into trophic levels and resource consumption, enriching our understanding of urban ecologies. This integrated approach provides an enhanced understanding of human-environment interactions during the formative stages of Swahili urban development.

Diet and foodways II

Marine Resources in Mediterranean Hunter-Gatherer Subsistence and Diet

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Coastal adaptations by hunter-gatherers in the Mediterranean are debated, even though in the last decade biomolecular methods have improved our understanding of them. To investigate similar aspects of subsistence, it is essential to establish the degree to which humans relied on marine resources. This can only be done through an interdisciplinary approach that accounts for: (i) the food refuse (e.g. faunal remains) left behind by the groups in question, and (ii) the isotopic composition of inhumated hunter-gatherers, to reconstruct the contribution of terrestrial vs. marine protein in their diets, and the position of the protein consumed in the trophic chain. Here, we review the Upper Palaeolithic and Mesolithic zooarchaeological and isotopic databases for the Mediterranean and then focus on the case study of Grotta dell'Uzzo. New taphonomic data show how cetaceans were exploited by the hunter-gatherers who occupied the site during the 8.2 cal. BP event. Ancient DNA analyses taxonomically reassign specimens of Mysticeti and Odontoceti that proved difficult to identify on morphological grounds. Bulk ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$) and compound-specific ($\delta^{13}\text{C}_{\text{AA}}$, $\delta^{15}\text{N}_{\text{AA}}$) isotope analyses on bone collagen of human and faunal specimens suggest that the last hunter-gatherers at Grotta dell'Uzzo consumed cetaceans and allow us to establish the role of marine resources in Mesolithic and Neolithic diets. Combining the different sources of evidence, we reconstruct the role of shellfish, fish and marine mammals in the subsistence and diet of humans living at the center of the Mediterranean Sea during the early Holocene.

Finding the Fish Event Horizon? Identity and Dietary Transitions in (early) Medieval Scotland

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Keywords: Scotland, Fishing, Biomolecular

This paper presents the findings of the Leverhulme Trust funded project “ArchaeoFINS: Medieval Archaeology of Fishing in the Irish and North Seas”. ArchaeoFINS is centred around an old and still unresolved archaeological question of when, why and where people began to eat fish again after the introduction of farming to Europe, which brought about a decline in fish consumption c. 5-10,000 years ago in favour of cereal crops and dairying. In essence we are interrogating the “Fish Event Horizon”, using Scotland as the core study region as it has previously been identified as a watershed region for the re-introduction of fish consumption in western Europe.

This paper presents new isotopic and organic residue data from approx. 500 samples across five sites in Scotland: Upper Scalloway in Shetland, Iona, Finlaggan on Islay, Gairloch Wester Ross, and Castle Park Dunbar. This includes human enamel, dentine and bone isotopic data; and isotopic data from over 40 species of fauna as well as archaeobotanical material, and organic residues from pottery. This is the largest integrated dataset for mobility, diet and foodways from Scotland. By combining multi-isotope, multi-tissue and multi-species data at high-resolution on these nationally important sites, we have uncovered patterns related to possible dietary taboos and the “missing” dead, multi-scalar mobility and imported dietary practices, surprising culinary practices in the pots, and complex ecological relationships in coastal and island environments. The re-adoption of marine fish into medieval diets is not a straightforward process – even for islanders and coastal dwellers.

Evidence for Secondary Vessel Use? Investigating Subsistence Patterns in Late Neolithic, Britain, with a Multidisciplinary Organic Residue Approach

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Keywords: Organic residue analysis, Grooved ware, Secondary vessel use

Grooved Ware (GW) ceramic vessels are one of the key elements of Late Neolithic in Britain and Ireland (3200-2400 cal BCE), a period known for remarkable ceremonial complexes, often linked to large-scale feasting events. Situated in the Heart of Neolithic Orkney, the Ness of Brodgar produced one of the richest archaeological collections of its kind with over 40 discovered structures and the largest GW pottery assemblage in Britain. This provides an excellent opportunity to examine spatial and temporal variability in ceramic function across the site. Here, we report on one of the largest investigations of pottery use ($n=340$) from a single archaeological site. We deployed a multidisciplinary approach (lipid biomarkers, compound specific isotope analysis, Bayesian mixture modeling of compounds, proteomics, scanning electron microscope, technological aspects) to determine site dynamics and chronological variation.

Overall, pottery at the Ness of Brodgar yielded exceptional lipid preservation and was dominated by animal fats, mainly indicating the processing of dairy products. Some variation through time is evident suggesting intensification in ruminant carcass and dairy products towards the end of the sequence. Contrasting results were obtained from the different approaches, mainly isotopes and biomarkers, pointing to different phases of use over the life-history of a vessel. These results are perhaps linked to the seasonal cycles of activity at the Ness of Brodgar and show how methods with different levels of resolution can be combined to effectively enhance knowledge of complex culinary practices, demonstrating the potential for fine-nuanced resolution in future organic residue studies.

Trial by Fire, Testing the Taxonomic and Tissue Identifications of Proteomic Analysis with Experimental Foodcrust

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Keywords: Palaeoproteomics, Organic residue analysis, Ancient diet

Archaeological foodcrusts preserved on ceramic vessels provide crucial insights into ancient dietary practices. While lipid analysis has traditionally dominated this field, protein analysis is emerging as a powerful complementary technique, offering superior taxonomic resolution and the ability to differentiate components in mixed food residues. However, the reliability of proteomic approaches for archaeological foodcrusts remains poorly understood.

Due to variation in the protein content of different foodstuffs, the robustness of different proteins against degradation and the extent of reference proteome coverage, it is expected that some foodstuffs may be easier to identify, while others are more likely to be missed. To investigate the extent of these biases we performed a blind test using twenty experimentally produced foodcrusts with known contents. From these twenty samples proteins were extracted and analysed using several workflows. Our results clearly show that there are severe biases in the proteomic identification of complex dietary mixtures, including false positives and negatives. Only in a minority of cases the used foodstuffs could be identified and often no evidence for all components of a mixture was found. We present refined methodological strategies that minimize false positives while maximizing the retention of reliable protein identifications. This study demonstrates critical constraints in proteomic approaches to ancient food residue analysis and provides a practical framework for improving the reliability of future research in this field.

Lipid Residue Analysis and Compound-Specific Radiocarbon Dating to Characterise Vessel Use in Wales, Orkney and Ireland ca. 3600-2500 cal BC

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Keywords: Lipids, Radiocarbon dating, Neolithic

The Later Neolithic in Ireland, Orkney and Wales (ca. 3600 BC onwards) is a period characterised by societal changes visible in increasingly monumental megalithic funerary monuments and a change in settlement practice. Architectural and artefactual evidence suggests that communities in the study areas were connected by seaways, shared ideas and exchanged artefacts.

Studying organic residues in pottery and radiocarbon dating individual fatty acids informs us about various aspects of past diet and subsistence practices. It is possible to investigate whether diachronic change of vessel use took place, whether communities in the three study regions used the same pottery types at the same time for the same resources or whether past diet was governed by the local environment. Using this combined approach, a large lipid dataset from ca. 500 potsherds and 48 new radiocarbon dates directly associated with vessel use was obtained.

Despite the connectedness of these Irish Sea communities, several local trends could be observed. Local patterns indicated that food procurement differed between regions as well as between site types. Aquatic biomarkers in pottery from some Irish and Welsh sites suggested that the consumption of aquatic resources was more common in the British and Irish Neolithic than previously reported. Stable carbon isotope analysis of lipids also provided information on subsistence techniques such as foddering practices or land management.

Proteomics of Symbolic Objects from Eastern and Sub-Saharan Africa

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Keywords: Proteomics, African archaeology, Ostrich eggshell beads

Over the past decade, proteomics has revolutionized our understanding of ancient societies by unlocking secrets embedded in everything from dental calculus and skeletal remains to vessel residues. But one exciting frontier has remained largely unexplored—symbolic objects like beads. These small yet powerful artifacts, often overlooked, can hold invaluable biomolecular traces that reveal fascinating details about the people who crafted and wore them. In this study, we dive deep into residues accrued on archaeological shell beads, primarily made from ostrich eggshell, from Kenya, South Africa, and Malawi. Alongside ethnographic beads from South Africa, we uncover a trove of human-derived proteins from a variety of tissues, revealing intimate glimpses into the lives and activities of their wearers. Additionally, our analysis recovered a wealth of faunal proteins from domesticated livestock as well as wild terrestrial and aquatic species. These residues tell a compelling story of communities balancing hunting and foraging with emerging pastoral practices, unveiling complex, multi-faceted subsistence strategies. This study pushes the boundaries of what we thought possible, showing that archaeological beads can preserve a rich tapestry of daily life. We present a novel approach to shed light on past transitions in subsistence and culture, offering fresh insights with profound implications for the field of archaeology.

Evolution of human and non-human animals, plants, and microbes I

A Genetic Perspective on the Relationships between Ancient Papua New Guinea Highlanders and Modern Populations Worldwide

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Keywords: Ancient DNA, Population genetics, Papua New Guinea

Rapid advances in ancient DNA technologies have contributed to our understanding of human history, yet several regions of the world remain poorly covered, or not at all, due to the difficulties associated with recovering fossils and ancient biomolecules from tropical regions.

Here, we present an initial assessment of the genetic relationship between the earliest ancient humans from Papua New Guinea (PNG) so far, and modern populations. We extracted genetic data from 116 human bone fragments from three archaeological sites in New Guinea's East Highlands (Kiowa, Yuku, and Tsak Pumakos B). The samples were previously identified using palaeoproteomics (ZooMS) and were directly dated using radiocarbon.

Despite low endogenous DNA content, we obtained a 0.01-fold coverage genome from an ~8,200 year old individual from the site of Kiowa, which shows strong genetic affiliation to present-day Papuans, and evidence for Denisovan introgression. In addition, to enhance the resolution of our analyses, we applied in-solution target enrichment on 1.24 million known variable positions across the genome in order to compare 45 ancient Papuan individuals with other modern and ancient genomes.

By tracing changes in genetic ancestry over time, we elucidate the relationship between PNG ancient highlanders and modern populations represented in the Simons Genome Diversity Project and other datasets. This study provides the first direct genetic evidence about the contribution of Papuan ancient populations to the broader population history of Oceania, shedding light on an understudied yet key region in human evolution.

Evolutionary History of the Treponematoses: Ancient DNA Evidence for an American Origin of Syphilis, Yaws, and Bejel

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The origin and evolutionary history of the diseases associated with *Treponemal pallidum*, namely yaws, bejel, and syphilis, has been heavily debated. Most discussion centres on syphilis specifically, where evidence for the “Columbian” theory, which argues for a post-1493 introduction from the Americas, is weighed against “pre-Columbian” theories, which argue for its establishment in Europe in the Medieval period. While ancient DNA data have the potential to make meaningful contributions in addressing these controversies, molecular recovery of this pathogen in a known challenge. Here we present five *T. pallidum* genomes from contexts in the pre- (n=4) and peri-contact (n=1) Americas. These genomes represent sister lineages for all currently known sublineages of *T. pallidum*, which supports their common origin in the Americas. Molecular dating suggests their divergence from a most recent common ancestor within the last 9000 years. Together these data present the most convincing evidence thus far for an American origin of *T. pallidum*, consistent with the “Columbian” theory.

Ancient Genomic History and Oral Health in Pre-Columbian Coastal Populations of the Atacama Desert

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Keywords: Ancient DNA, Atacama Desert, Oral microbiomes

This study investigates the genetic history of early archaic Chinchorro and later agricultural populations along the coast of the Atacama Desert (northern Chile) and examines the impact of agriculture on the oral microbiome. We analyzed 125 teeth and 70 dental calculus samples from coastal and inland sites spanning 7,000 to 500 years BP, covering all cultural periods in the region. DNA was extracted, converted into single-stranded libraries, and sequenced.

From shotgun sequencing, we recovered 45 ancient mtDNA genomes with coverage ranging from 0.8X to 22X (Archaic, n=20; Formative, n=8; Intermediate, n=8; and Late, n=9), including samples from early Chinchorro sites such as Camarones-14 (7,000 BP) and Morro de Arica (5,500–3,600 BP). Mitochondrial DNA analyses reveal genetic links to modern indigenous populations in the southern cone. Currently capture of mtDNA genomes and whole genome is in progress to improve data recovery for reconstructing population history and genome-wide comparison with modern populations of South America. From our metagenomic analysis, our findings also indicate shifts in oral microbiome composition following the agricultural transition, with oral bacteria such as *Methanobrevibacter oralis*, *Pseudoramibacter alactolyticus* and *Tannerella forsythia* becoming more prevalent in agricultural populations. Overall, this study provides new insights into the cultural and biological diversity of past populations in the Atacama Desert and the emergence of local oral pathogens and dietary shifts over time.

Evolution of human and non-human animals, plants, and microbes II

Archaeogenomics of Maize Evolution in the South American Andes

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Keywords: Ancient DNA, Plant evolution, Maize domestication

Maize, a staple crop, evolved from the wild plant teosinte through human activities in Mexico approximately 9,000 years ago. Around 7,500 years ago, it was brought through Central America, and by 6,500 years ago, it had spread into South America.

While the timeline of maize's spread is well-documented, there remains debate about whether maize underwent complete domestication before reaching South America and how it transitioned from lowland Mexico to the high-altitude Andes.

To address this, we sequenced 137 archaeological genomes and 11 maize landrace genomes from the South American Andes. These genomes were analyzed alongside 600 previously *published Zea mays* genomes, with *Zea luxurians* and *Zea diploperennis* as outgroups. Additionally, we employed radiocarbon dating to determine the ages of the samples and used strontium isotope analysis to verify the geographical origins of ancient maize specimens.

Our findings provide insights into the evolution of maize between Andean cultures prior to subsequent waves of maize movement through South America and reveal an early diversification of lowland and highland genomes shedding light on how maize adapted to the high-altitude environments of the Andes.

Unveiling Equidae Evolution through a Rich Dental Proteome of an Arctic Pliocene (3.9 Ma) Hipparionine Horse

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Keywords: Palaeoproteomics, Equidae, Palaeontology

Ancient DNA analysis recalibrated several evolutionary events in the equid family tree, revealing the complex history of this group. However, the periods of greatest equid diversity are too remote to be compatible with aDNA recovery. Here, we use palaeoproteomic methods to recover protein sequences from a Pliocene (3.9 Ma) dental fragment of an hipparionin horse from the High Arctic Beaver Pond site in Canada. Despite the age of the fossil, we retrieved a remarkably well-preserved proteome from both dentine and dental enamel. Ultimately, we confidently identified at least 60 proteins represented by 14,184 peptides-spectrum matches, covering 7789 amino acids. STRING network analysis reveals three distinct tissue specific proteomes: enamel, dentine/bone, and blood plasma, each displaying differential degradation rates. Authenticity is supported by the conventional markers of ancient protein damage (high deamidation, shortened peptides). The extent of damage we observe is slight compared to that previously reported in Middle to Early Pleistocene mammalian dental proteomes from lower latitudes. A phylogenetic analysis of extant and extinct perissodactyls, using a tip-dating approach, identifies the High Arctic hipparionin as the earliest-diverging equid in the analysis. A Hipparionini-Equini split is identified in the Miocene, likely within the Middle Miocene, a time when Equidae diversification was high, particularly in North America. With a proteome sequence coverage at least 10-fold higher than those of previously described Middle–Early Pleistocene dental proteomes from lower latitudes, this study demonstrates the potential of palaeoproteomics to recover extensive proteomes even in deep-time, when preservation conditions are favourable for their survival.

Ancient Genomes Illuminate the Origins and Dynamic History of East Asian Cattle

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The evolutionary history of domesticated cattle in East Asia for the last 5,000 years remains largely obscure. Here, we investigate the origins and evolution of cattle genetic diversity in China by analyzing whole genome sequences of 166 ancient bovines spanning a 10,000-year period, encompassing now-extinct East Asian aurochs and domesticated cattle from key archaeological cultures. East Asian aurochs were distinct from other aurochs and harbored a basal taurine ancestry. The first domesticated cattle in the Yellow River region derived >10% of their ancestry from local aurochs with an uneven genome-wide distribution. Early cattle from Xinjiang were genetically distinct and partially contributed to the later northern Chinese cattle. Indicine admixture became widespread only in the Medieval period in northern China.

An Extinct Wildebeest Lineage Identified by Morphology and Ancient DNA of a 42,000-Year Old Molar from High-Altitude Ethiopia

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Keywords: Africa, Ancient DNA, Extinction

Over the last ~50,000 years, the majority of Earth's megafauna became extinct across the continents. Historically, Africa was thought to be an exception to the global pattern, with relatively few megafaunal extinctions during the late Quaternary¹. More recent work showed that the magnitude of African extinctions was considerably larger than appreciated, suggesting the exploration of new sites would lead to discovery of additional extinct taxa². We present the discovery of a new lineage of wildebeest (*Connochaetes*) based on palaeogenomic and morphological analysis of a 42,000-year old tooth from the high-altitude Middle Stone Age site of Fincha Habera, in the Bale Mountains of Ethiopia. Our analyses show the lineage diverged from extant wildebeest 790-240 thousand years ago, with subsequent limited gene flow. Ecological niche modelling suggests that the grassland habitat around Fincha Habera was unsuitable for either extant species, but we find no definitive evidence of high-altitude adaptation in the extinct lineage. Taken together, our findings suggest this extinct lineage, known from a single tooth (similar to the case of Denisovans), is a distinct group of wildebeest that may have been (i) hunted from the lowlands, (ii) obtained via exchange by the inhabitants of Fincha Habera, or (iii) resident on the high-altitude grasslands. Our study supports the idea that late Quaternary megafaunal extinctions in Africa are underestimated and through discovery of new fossil sites and application of state-of-the art techniques across disciplines, more cryptic extinctions will be revealed, improving our understanding of the evolutionary history of species in Africa.

References

1. Koch, P. L. & Barnosky, A. D. Late Quaternary Extinctions: State of the Debate. *Annu. Rev. Ecol. Evol. Syst.* 37, 215–250 (2006).
2. Faith, J. T. Late Pleistocene and Holocene mammal extinctions on continental Africa. *Earth-Sci. Rev.* 128, 105–121 (2014).

Paleogenomic Study Reveal the Origin and Expansion History of the Woolly Rhinoceros in Late Pleistocene

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Keywords: Woolly rhinoceros, Population history, Late Pleistocene

The woolly rhinoceros (*Coelodonta antiquitatis*) was a representative megafauna species dominating the northern Eurasia during the Late Pleistocene. However, our knowledge about its expansion across Eurasia and population history during the glacial cycles are still scarce, with the only available genomes limited to northeastern Siberia. We reconstructed 22 mitochondrial genomes and 14 nuclear genomes (0.5-10.6X) from fossil specimens across East Asia, Central Asia, and Eastern Europe, dated to 141-18 ka. Based on phylogenetic and population structure analyses, we revealed an East Asian origin of all the Late Pleistocene woolly rhinoceroses, with one East Asian individual basal to all other woolly rhinoceroses, and an early split between other East Asian individuals and northern Eurasians. A ~120-105-thousand-year-old individual from the Denisova Cave is basal to all non-East Asian individuals, suggesting Central Asia as the crossroad of the Late Pleistocene expansion of the woolly rhinoceros out of East Asia. Taken together, we propose that Late Pleistocene woolly rhinoceroses were originated from East Asia, a potential climatic refugium during the Penultimate Glacial Period (Marine Isotope Stage 6, ~191-130 ka). From the Eemian Interglacial Period (Marine Isotope Stage 5e, ~130-115 ka), the woolly rhinoceros migrated north to Central Asia, and eventually expanded across Europe and northeastern Siberia.

Evolution of human and non-human animals, plants, and microbes III

Uncovering pre-European Contact Leprosy in the Americas and its Enduring Persistence

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Keywords: Leprosy, *M. lepromatosis*, Americas

Leprosy is widely believed to have been brought to the Americas during European colonization, with the introduction of *Mycobacterium leprae*. However, the discovery of a second leprosy-causing bacterium, *Mycobacterium lepromatosis*, reported mainly in the Americas, raises questions about its emergence time and epidemiological history on the continent. To investigate this, we screened hundreds of histological biopsies from patients with suspected leprosy across the Americas, as well as wild animals like armadillos, for the presence of *M. leprae* and *M. lepromatosis*. We identified 36 *M. lepromatosis*-positive cases, and generated genome-wide data for 23 strains, increasing by seven-fold the number of available genomes from human-infecting strains of this species. Additionally, we screened archaeological skeletal remains for pre-contact evidence of *M. lepromatosis* in the continent, successfully detecting and recovering genomes from two sites: 1300-year-old remains from the west coast of Canada and 860-year-old remains from the east coast of Patagonia, Argentina. Our findings thus reveal a continent-wide distribution of the pathogen in pre-contact times. Molecular clock analyses also suggest that *M. lepromatosis* has been present in the Americas for millennia, with a rapid spread from North to South America during the late Holocene (1500–900 BP). We also identified two distinct *M. lepromatosis* clades infecting humans today, one of which dominates and appears to have expanded during colonial times. Together, our findings challenge previous assumptions about the history of leprosy in the Americas and provide valuable insights into the ancient and modern epidemiology of this disease.

2500 Years of Human Betaherpesvirus 6A and 6B Evolution

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Keywords: Viruses, HHV-6, Pathogens

Human betaherpesviruses 6A and 6B are double-stranded DNA viruses, specialised in infecting humans and are best known as the main causative pathogens of the common childhood infection “sixth disease”. Despite only being discovered in the 1980s, these viruses are speculated to have a much longer and more complex history within the human population than available modern data make clear. The viruses are carried by large fractions of the human population and can integrate into the human genome, leading to a wide range of clinical manifestations of varied severity. Here, we present the first nine full and two partial ancient genomes of HHV-6A and 6B, dating as far back as the Italian Iron Age (ca. 1100–600 BCE). We demonstrate that large fractions of the current HHV-6 diversity were already well established in the human population by the 14th century CE. Our data suggests that HHV-6B integrated into the human genome at the latest before the 1st-6th century CE, with two integrated clades being populated by ancient DNA genomes in our phylogeny, which further supports that they originated from likely much older ancient founder events. Additionally, we show that all known inherited chromosomally integrated (ici)HHV-6A clades were already represented in historical populations, confirming that HHV-6A no longer integrates into the human germline within populations of European ancestry and likely endogenized in early human history. Finally, our results demonstrate the unique suitability of archaeological remains and ancient DNA for the study of the evolution of integrated viruses.

Evolutionary History and Convergent Host Adaptation in Ancient *Salmonella* Enterica

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Keywords: *Salmonella enterica*, Host adaptation, Prehistoric

Salmonella enterica subsp. enterica is an extremely diverse bacterial pathogen: over 2600 different serovars have been described, many with a wide host range. A small number of serovars are adapted to infect specific hosts: of these, serovars Typhi and Paratyphi A, B and C cause human-specific systemic infections (typhoid and paratyphoid fever). Although Paratyphi C is one of the rarest human-specific serovars today, it has been shown to have been relatively widespread in the past: all published ancient salmonella genomes belong to or are ancestral to this lineage. Here, we present over 40 new ancient *Salmonella* genomes spanning Eurasia and dating between 5000 and 1000 years before present. This dense genomic dataset allows us to reconstruct the evolutionary history of this pathogen in unprecedented detail. This includes the identification of multiple extinct prehistoric lineages, which at one stage caused infections throughout Europe and Asia. Multiple lineage replacement events are observed throughout history and prehistory, and Bayesian phylogenetic analysis and molecular dating is used to date and identify host adaptation events within this lineage. Host-adapted sublineages (Paratyphi C, Choleraesuis and Typhisuis) continued to evolve host specificity independently from each other. We reconstruct signals of convergent host adaptation in the studied lineages and other host-adapted strains by analysing shared pseudogenes and recurrent gene gain and loss events. Enrichment analysis demonstrates a role for host interactions as a particular target of selection, and highlights the role of host-microbe interactions in pathogen evolution.

Genomic Footprints of Adaptive Responses to Past Epidemics in the Finnish Population

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Keywords: Natural selection, Population genomics, Infectious diseases

Infectious diseases are one of the main drivers of human evolution. Exposure to intense selective pressures such as epidemic outbursts of infectious diseases leaves signatures at the molecular level, particularly in genomic regions known to be involved in adaptive immune responses.

We study these signals in North Eastern Europe, particularly in the Finnish population, where we have information on infectious disease outbreaks and respective demographic impact (mortality). Historical infectious disease outbreaks such as smallpox, pertussis, and measles caused high infant mortality, providing a unique opportunity to robustly identify signatures of selection against these diseases. Although previous studies have identified genetic adaptations across different human populations, true local adaptation signals driven by these infectious diseases in the Finnish population may have remained undetected due to small sample sizes and the absence of ancient-DNA analyses.

Using an extensive collection of DNA, including ancient and contemporary samples, we test the hypothesis that immune-related regions of contemporary genomes show signs of selection. The study employs haplotype-based methods to detect the selection. Additionally, we use ancestral recombination graphs (ARG) to investigate allele frequency trajectories of alleles under selection and respective effective size changes to understand the impact of epidemics on genomic variation. Initial haplotype-based selection genome scans identified multiple loci from immune genes under selection. This project offers insights into understanding how infectious diseases have shaped genetic adaptation in the contemporary Finnish population.

Bronze Age *Yersinia Pestis* Genome from Sheep Sheds Light on Hosts and Evolution of a Prehistoric Plague Lineage

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Keywords: Zoonosis, Zooarchaeology, One Health

Most human pathogens are of zoonotic origin. Many emerged during prehistory, coinciding with domestication providing more opportunities for spillover into human populations. However, we lack direct DNA evidence linking animal and human infections during prehistory. Here we present a *Yersinia pestis* genome recovered from a 3rd millennium BCE domesticated sheep from the Eurasian Steppe belonging to the Late Neolithic Bronze Age (LNBA) lineage - a lineage until now exclusively identified in ancient humans from across Eurasia. Interestingly, the closest genetic relative to this genome is a human infection from the same cultural complex, implicating sheep in the overall transmission landscape of this exclusively pastoralist society. We show that this ancient lineage underwent ancestral gene decay paralleling extant lineages, but evolved under distinct selective pressures contributing to its lack of geographic differentiation. We collect evidence supporting a scenario where the LNBA lineage, unable to efficiently transmit via fleas, spread from an unidentified reservoir to humans, sheep, and likely other domesticates - increasing its known host range, and thus the human infection risk. Collectively, our results connect prehistoric livestock with infectious disease in humans and showcase the power of moving paleomicrobiology into the zooarcheological record.

Ancient DNA Reveals 4000 Years of Sheeppox Virus Evolution through Eurasia

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Animal domestication and mixed-species pastoralism likely influenced the health of domesticated animals by increasing the risks of disease transmission and cross-species pathogen exposure. Ancient DNA techniques have recently enabled the recovery of important animal viral genomes, such as Marek's disease virus in poultry and Equine herpesvirus 4 in horses. However, no ancient viruses have yet been retrieved from domestic ruminant remains. Here, we report the recovery of seven high-quality ancient sheeppox virus (SPPV) genomes, a disease-causing poxvirus causing high mortality and significant economic losses in herds today. Our SSPV genomes come from across Eurasia and span a temporal range of 4,000 years, from the Bronze Age to historical periods. Notably, this includes two genomes recovered from parchment, demonstrating the potential of using ancient animal skin as a source for reconstructing ancient animal poxviruses. Through pangenomic and molecular clock analyses, we provide insights into the recent evolutionary history of SPPV, and explore host adaptation of this pathogen over millennia.

Interaction between humans and non-human animals, plants, and microbes

Human Presence and Subsistence in the New Guinea Highlands over the Holocene

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Keywords: Changing diets, Holocene archaeology, New Guinea Highlands

New Guinea is a hugely relevant region for understanding ancient and modern hominin dispersals, admixture, and adaptation to changing sea levels and environments. The Pleistocene and early Holocene archaeological record is sparse and poorly understood especially in terms of organic remains, however, with improved biomolecular methods new fossils along with direct chronological and dietary interpretations can be made, particularly with Holocene material. This approach has been undertaken on the faunal bone assemblages from three New Guinea Highland sites – Yuku, Kiowa, and Nombe – with ZooMS, radiocarbon, and stable isotopic results presented in this paper. Over 2000 unidentifiable bone fragments were analysed using collagen peptide mass fingerprinting (ZooMS) and taxonomic identifications were possible for numerous faunal taxa along with human remains being identified from all 3 sites. Direct radiocarbon dates from both faunal and human remains paired with carbon and nitrogen stable isotope data reveal a complex pattern of shifting diets over time. These results are explored with reference to the changing montane-rainforests, first domestication of plants and introduction of commensal animals, as well as the movement of modern human groups and the introduction of Lapita people during the Holocene. Finally, we will present the exciting potential of biomolecular methods, on material from tropical environments with traditionally poor organic preservation, in answering key questions regarding human evolution and dispersal in Sahul and Remote Oceania.

Ancient Genomics Reveal Chinese Pig Domestication History Associated with Human Migration and Management

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Keywords: Paleogenomics, Domestic pig, Population history

As one of the earliest domesticated animals, the history of pig domestication has remained controversial, especially in East Eurasia. Here, to trace the origin and dispersal of East Eurasian domestic pigs, we generate 21 nuclear genomes and 23 mitogenomes from ancient pig/boar remains spanning from 5,800 BCE to 1,300 CE across China. By combining published Eurasian pig genomes, we demonstrate the continuous population history of pigs domesticated in northern China, which played a major role in shaping the genetic diversity and contributing to the unique black coat color of modern Chinese domestic pigs. The expansion of ancient pigs from northern China mirrors the spread of Yellow River millet farmers to the Yangtze River Basin and Southwest China, accompanied by genetic introgression from local wild boars. We also observe a loss of genetic diversity and an increase in inbreeding in northern pigs, potentially linked to captive breeding practices as early as 3,000 BCE. Our findings illuminate the domestication and dispersal history of pigs in China, which is closely associated with the human migration and societal development.

Lethal Plague Outbreaks in Lake Baikal Hunter–gatherers 5500 Years Ago

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Keywords: Microbes, Palaeodemography, Zoonosis

The rise of zoonotic diseases in prehistory is often associated with the Neolithic agricultural transition; with plague specifically linked to population declines in Late Neolithic Europe^{1,2}. Although plague is amongst the most devastating diseases in human history, early strains of *Yersinia pestis*, the causal agent of plague, lack virulence factors required for the bubonic form, and their severity remains unclear. Here, we describe strains of plague reported associated with early outbreaks among prehistoric hunter–gatherers in the Lake Baikal region in East Siberia, beginning from ~5600–5400 years ago (cal. BP). These outbreaks occur across four hunter–gatherer cemeteries, with a 39% detection rate for plague across all sites. By reconstructing kinship pedigrees, we show that small familial groups are affected, consistent with human-to-human spread of the disease, and the first outbreak occurred within a single generation. Intriguingly, the infections appear to have resulted in acute mortality events, especially among children. Zoonotic transmission is separately indicated by a *Brucella* infection in one of the children. Interestingly, we see differences in functional genomic variants in the prehistoric plague strains, including in the *ypm* superantigen known from *Y. pseudotuberculosis* today. The new strains diverge ancestrally to all known *Y. pestis* diversity and push back the *Y. pestis* divergence from *Y. pseudotuberculosis* by some 2000 years³. Our results show that plague outbreaks happen earlier than previously thought and were indeed lethal. The findings challenge the common notion that high population densities and lifestyle changes during the Neolithic transition were prerequisites for plague epidemics.

References

1. Rascovan, N. *et al.* Emergence and Spread of Basal Lineages of *Yersinia pestis* during the Neolithic Decline. *Cell* **176**, 295–305.e10 (2019).
2. Seersholm, F. V. *et al.* Repeated plague infections across six generations of Neolithic Farmers. *Nature* **632**, 114–121 (2024).
3. Susat, J. *et al.* A 5,000-year-old hunter-gatherer already plagued by *Yersinia pestis*. *Cell Rep.* **35**, 109278 (2021).

De-novo Assembly and Reconstruction of an Ancient *Streptococcus Pyogenes* Genome From a Pre-Columbian Mummy: Insights into the Evolution of a Human Adapted Pathogen

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Keywords: Pre-Columbian pathogen, *Streptococcus pyogenes*, Ancient prophage

Streptococcus pyogenes, also known as Group A Streptococcus (GAS), is a human adapted pathogen that can cause a range of clinical manifestations, including scarlet fever, skin infections, rheumatic fever, post-streptococcal glomerulonephritis and bloodstream infections. Recent studies have identified *S. pyogenes* as responsible of series of scarlet fever outbreaks along with the surge of invasive infections. Genomic studies have suggested that new virulence variants disrupt both innate and adaptive immune host responses, making *S. pyogenes* strains more transmissible and virulent over time, with impact on the first line of antibiotic treatment. Here, we subjected a tooth sample of a pre-Columbian Andean mummified individual linked to the Late Intermediate Period (1100 – 1450 AD), from the anthropological collection of The National Museum of Archaeology – MUNARQ in La Paz, Bolivia, to a shotgun metagenomics approach.

Initial analysis of the human mitogenome placed our individual within the Native American genetic diversity. Further *de-novo* assembly of the metagenome allowed us to reconstruct the complete genome of the pathogen *S. pyogenes*. Using phylogenetic comparative analysis enabled us to assign the ancient genome to its closest modern infective strains. The ancient strain carries a set of virulence factors indicating a pathogenicity similar to modern *S. pyogenes*. The presence of two intact prophage regions helped to place our ancient *S. pyogenes* strain in a broader context. Similar prophage predictions in modern closely related strains will help to understand its evolution and occurrence in different hosts and geographic regions.

Paleogenomic Insights into the History of Tuberculosis in South America Across Diverse Cultural Contexts

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Keywords: Tuberculosis, South America, Pre-Hispanic

Despite ample skeletal evidence of pre-Hispanic tuberculosis (TB) in northwestern and western regions of South America, the strain diversity and phylogeography of the *Mycobacterium tuberculosis* complex (MTBC) that circulated in the continent remains limited. This is partly due to challenges in recovering, authenticating, and analyzing MTBC DNA from archaeological remains. We provide new genomic-level evidence of a widespread distribution of TB in pre-contact times, reaching the Southern Cone. A multi-method metagenomic screening approach permitted the detection of MTBC DNA in multiple archaeological individuals from Argentina. Employing in-solution hybridization capture, we reconstructed a high-coverage (66x) MTBC genome from an individual dating to the end of the Middle Horizon (~1000 calBP) from the site of Lomitas de Saujil. Phylogenetic analysis positions this strain within *M. pinnipedii* subclade and closely related to all previously published genomes, extending the known range to Northwest Argentina. Using a novel approach for low-coverage data, we phylogenetically confirmed four additional cases in individuals from distinct sites including a hunter-gatherer, radiocarbon dated to ~1700 calBP. This provides the oldest DNA-

supported evidence of MTBC in South America, and the first detection in a hunter-gatherer population. Phylogenetic analysis indicates that, despite its antiquity, this strain is not basal to more recent pre-Hispanic cases, suggesting a dynamic host range and broader and deeper diversity of MTBC infecting ancient humans than previously recognized. This study extends the genomic identification of TB by 700 yBP and reveals TB in diverse ecological and cultural contexts, enhancing our understanding of ancient disease dynamics related to TB.

Methodological advances in biomolecular archaeology II

Composition Variations in Archaeological Human Bone Proteomes

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Keywords: Palaeoproteomics, Skeletal biology, Sampling selection strategies

The living skeleton is a complex unit formed through two different processes, endochondral and intramembranous ossification, and is largely composed of two different bone types, cortical and trabecular bone. These differences are generally not taken into account in palaeoproteomic research design, for example in relation to choosing bone sample locations, where the bone proteome is considered to be uniform across the skeleton. How the biology of the living bone influences proteomic composition variation and preservation within the human archaeological skeleton is largely unknown. Knowledge on bone sample locations containing larger proteomes during life, or preserving larger proteomes, would therefore enable evidence-based decision making processes for the study of archaeological specimens. Alternatively, some proteins of interest might only be recoverable from particular skeletal locations.

To resolve this, we designed a comparative study focusing on 10 healthy, adult human skeletons from the Netherlands. Proteomes were generated using shotgun proteomics from four skeletal locations: the parietal, rib, femur, and petrous bone. Cortical and trabecular bone proteomes were generated for the first three locations. Comparisons were made based on proteome size, composition, and deamidation degradation. We observe larger and more complex proteomes within cortical bone, especially in endochondral bone, compared to trabecular bone. The petrous bone stands out with the largest proteome. In degraded contexts cortical bone should be preferred, especially the petrous bone. If the petrous bone is not accessible, a combination of cortical and trabecular bone is recommended, as trabecular bone is found to contain few phylogenetically informative proteins not observed elsewhere.

Testing a Unified, Minimally-Invasive Protocol for the Analyses of Ancient Bone Remains

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Keywords: Radiocarbon dating, Palaeoproteomics, aDNA

The study of archaeological bone and teeth provides critical insights into past human and animal populations. However, the most widely used biomolecular methods, namely *radiocarbon dating*, *palaeoproteomics* and *ancient DNA* analyses, involve repeated destructive sampling, which poses ethical and preservation challenges.

In this talk, we discuss the development, both conceptual and analytical, of a unified, minimally-invasive and quasi non-destructive, workflow designed to optimize biomolecular data recovery while preserving the structural integrity and appearance of archaeological bones and teeth.

Our approach integrates traditional protocols in radiocarbon dating, palaeoproteomics (ZooMS), and ancient DNA analysis, unified under a first single-step extraction. The described protocol is designed to balance analytical precision with the conservation of valuable archaeological specimens, while acknowledging that non-destructive methods, while less “efficient”, help preserve cultural heritage remains and abide by ethical guidelines.

Through case studies spanning multiple types of remains (human bone, worked bone, teeth), we will demonstrate the protocol’s effectiveness in generating comprehensive data without compromising bone preservation. This standardized approach offers a practical framework for researchers seeking to balance analytical rigor with conservation priorities in bioarchaeological, palaeontological and forensic investigations.

Ultimately, refining minimally-invasive methodologies contributes to the broader effort of *sustainable archaeological research*.

Expanding the Isotopic Toolbox: Nitrogen Isotopes in Fossil Tooth Enamel and First Data of Early Hominins

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Keywords: Australopithecus ecology, Dietary reconstruction, Trophic behavior

Stable nitrogen isotope analysis ($\delta^{15}\text{N}$) is a vital tool for investigating dietary and trophic interactions in modern and ancient ecosystems. While $\delta^{15}\text{N}$ is typically measured in collagen or soft tissues, its application to paleontological contexts has been limited by the diagenetic loss of organic material, usually restricting studies to well-preserved specimens younger than 100,000 years. Tooth enamel, the densest mammalian tissue, offers exceptional preservation potential, but its low organic content has historically impeded $\delta^{15}\text{N}$ analysis.

We now measure $\delta^{15}\text{N}$ in mineral-bound organic matter in tooth enamel ($\delta^{15}\text{N}_{\text{enamel}}$), overcoming this limitation and enabling isotopic analysis of fossils far older than previously possible (i.e. millions of years) with minimal sample material (~5 mg). This development expands the isotopic toolbox for investigating ancient ecosystems and evolutionary trajectories.

Our studies demonstrates that $\delta^{15}\text{N}_{\text{enamel}}$ reliably reflects trophic-level enrichment, with a 3–4‰ increase per trophic step in the food-web, comparable to $\delta^{15}\text{N}$ from collagen, as shown with analyses of controlled feeding experiments¹ and modern mammals that lived in natural habitats².

Furthermore, isotopic data from Pliocene Sterkfontein Member 4 (South Africa) provide the first $\delta^{15}\text{N}_{\text{enamel}}$ measurements for *Australopithecus* and coexisting fauna³. Our findings reveal a variable but predominantly plant-based diet (largely C_3) for these early hominins. These results provide crucial insights into the ecological role of *Australopithecus*, emphasizing their reliance on plant-based diets.

The ability to measure $\delta^{15}\text{N}_{\text{enamel}}$ represents a transformative advancement for paleoecological research, offering a powerful tool to investigate the diets and adaptations of extinct species across deep time.

References

1 Leichliter, J. N. *et al.* Nitrogen isotopes in tooth enamel record diet and trophic level enrichment: results from a controlled feeding experiment. *Chemical Geology* **563**, 120047 (2021). <https://doi.org/10.1016/j.chemgeo.2020.120047>

- 2 Leichliter, J. N. *et al.* Tooth enamel nitrogen isotope composition records trophic position: a tool for reconstructing food webs. *Communications Biology* **6**, 373 (2023). <https://doi.org/10.1038/s42003-023-04744-y>
- 3 Lüdecke, T. *et al.* Australopithecus at Sterkfontein did not consume substantial mammalian meat. *Science* (in press). <https://doi.org/10.1126/science.adq7315>

Migration, mobility, and translocation III

Tracing Mediterranean Interconnection in South Iberia during Antiquity and Medieval Ages

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Keywords: Migration, Ancient DNA, Population admixture

Southern Iberia was a key hub for human mobility during Antiquity and Middle Ages, connecting the Mediterranean and bridging continents. Over 1,000 years, the region came under the control of different powers imbedded in five major historical periods: the Romans (2nd century BCE–5th century CE), Visigoths (476-711 CE), Byzantines (552-620/625 CE) and Andalusi (711-1492 CE), and the Christian Kingdoms (from 13th century CE). We analyzed 314 newly sampled individuals from 21 archaeological sites dating between the 2nd and 14th centuries CE. Our study explores the population changes driven by these major historical events. During the Roman period, Southeast Iberia became a key part of a Mediterranean-wide network. Genetic data show strong links with populations in North Africa and the Eastern Mediterranean, reflecting the region's role in trade, governance, and cultural exchange within the Roman Empire. This Mediterranean connectivity continued under Visigoth and Byzantine rule. Genetic data show a stronger North Africa–Southern Iberia connection during the Byzantine period, aligning with the Byzantine conquest of Spania launched from newly acquired African provinces. In 711 CE, the establishment of Al-Andalus brought cultural transformation and ties to the Muslim world. However, its impact on Mediterranean-wide mobility did not suppose an overall shift in the genetic landscape of Southern Iberia. Our findings highlight how successive political and military events reshaped Southern Iberia's population. Our study underscores the region's role as a crossroads of Mediterranean interconnection, at least until the Christian conquest and the massive population displacements, deportations, and annihilation it produced.

Challenging Traditional Migration Period Narratives with a Multi-Proxy Approach

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Keywords: Paired isotope-genetic analyses, Environmental models, Basel (Switzerland)

The 1st millennium CE in Basel (Switzerland) is characterised by diversity in the archaeological record and complex political, cultural, and economic interactions. The present study aims to investigate mobility patterns, subsistence strategies, and communication networks in the region at the periphery of the Late Roman Empire, which became the centre of Early Medieval crossroads between cultural entities. This objective is pursued through an in-depth analysis of ca. 150 burials dating to the 4th to the 8th centuries CE using an integrative approach encompassing AMS radiocarbon dating, archaeological, anthropological, isotopic, and genetic analyses, as well as environmental modelling. Preliminary results call into question the previously held view of large-scale migrations from the 4th/5th centuries onwards. Instead, the results indicate that regional mobility was prevalent – besides the presence of large-scale communication networks. This study underscores the pivotal role that bioarchaeological approaches play in unveiling hitherto unanticipated facets of social dynamics during the late antique and early medieval periods in *Basilia/Bazela*, thereby facilitating the reconstruction of individual life histories. The study further demonstrates the benefit of integrating multivariate models involving high-resolution climatic and environmental data to disentangle potential drivers of isotope variability at various scales.

Population Dynamics and Cultural Continuity in Post-Roman Central Europe

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Many modern Central European settlements originate from early medieval communities established on former Roman territories in the 5th century CE. Traditionally, this transition has been framed as a period of large-scale migrations and conflict, yet the demographic realities remain complex and understudied.

To explore these dynamics, we conducted an interdisciplinary study integrating historical, archaeological, isotopic, and genetic analyses. By examining 250 ancient genomes from southern Germany (5th–7th centuries CE) along the former Roman limes and comparing them with 1,200 modern genomes, we reconstructed patterns of mobility, genetic diversity, and social organization. Using population genetic diversity estimators, genomic summary statistics, and kinship analysis, we offer a refined perspective on demographic shifts, cultural continuity, and integration. Our findings reveal a dynamic interplay of migration, local mobility, and cultural assimilation. Mobility often mimicked large-scale migrations but reflected distinct social behaviors. Additionally, structured interactions between genetically diverse groups, characterized by non-random mating, played a key role in shaping the genetic landscape of the region.

The combined genetic and isotopic evidence aligns with historical and archaeological records while providing new insights into kinship structures and marriage practices. These findings enhance our understanding of the cultural and genetic legacies of this critical period and offer fresh perspectives on the demographic processes that shaped modern Central European populations.

Keszthely, Sirmium, Singidunum: Genetic Insights into the End of Roman Cities at the Limes.

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From the 1st to the 4th century, Pannonian provinces played a key role in the functioning and supply of the empire, with urban complexes reportedly counting thousands of individuals. During the 5th century, the Roman Empire administration progressively abandoned this area, possibly as a consequence of the growing instability of the region. Archaeological and historical sources report a continued usage of the infrastructures through and after the 5th century, and archaeological investigations suggest at least partial population continuity. Nonetheless, there is yet no systematic investigation of the genetic impact on urban populations at the turn of the 5th century.

Here, we present ~300 new autosomal genomes from former Roman cities in the region, dated between the 3rd and 7th century, complemented with a systematic strategy of isotope analysis to investigate diet and mobility. In concordance with previous literature, a wide genetic diversity in the area characterizes the population of the Roman period. Yet, the analysis of 5th century cemeteries reveals a disappearance of ancestries associated with the Eastern Mediterranean and African areas, shrinking the ancestral complexity to one that is comparable to that of “barbaric”-associated contexts in the area. Moreover, cemeteries dated to the 6th and 7th century present further homogeneous ancestry patterns: here, the analysis of biological relatedness and isotopes point to a recent settling in former urban complexes from (quasi-)local groups. This suggests sizable demographic changes at the fall of the Roman empire in this region and particularly in the contexts of former Roman cities.

Archaeogenetic Insights into 1st Millennium BCE Athenian Society

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Keywords: Phaleron cemetery, aDNA

The social developments in Athens during the 1st millennium BCE have profoundly influenced subsequent European history. Until now, our understanding of Athenian society from this period has been derived primarily from textual and archaeological sources. Recently, at Phaleron, Athens's principal port and a major center of the Mediterranean, excavations revealed the burial ground of more than 2,000 individuals, extending over an area of c. 10km². The site features diverse burial types, from simple pit and jar interments to so-called 'deviant' burials, some of which suggest violent deaths. This diversity offers a unique opportunity to integrate biomolecular methods with studies of population dynamics, social organization, and individual life histories during this transformative era.

We are presenting human genomic evidence from 110 individuals buried between the 7th and 5th centuries BCE at Phaleron. Our dataset reveals genetic continuity since the Late Bronze Age, but also the presence of individuals with distinct ancestries mostly from within the Mediterranean. Notably, the burial of an adult male with North African genetic ancestry, buried with classical Greek burial goods, and the double burial of a central Asian male with a male of local ancestry, raise the question of understanding and defining the Athenian citizen in the burial evidence. Finally, we observe a lower frequency of close-kin unions compared to the Bronze Age, indicating that such marital systems are not represented in the communities burying at Phaleron, and that written sources may be attributing older traditions to distinct social systems.

Genetic and Social Stratification in Medieval Estonia: Insights from Urban and Rural Burial Practices

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Keywords: Ancient DNA, Imputation, Medieval period

The 13th century in the Eastern Baltic was the period of crusades, which aimed to spread Christianity in the region of historical Livonia – the current territories of southern Estonia and northern Latvia. By 1227, a new ruling elite was established, mostly of German origin. These brought along new traditions and social stratification of the population. There was a change in burial practices: elite people were buried mostly inside the churches, while local people were buried in the churchyard or rural cemeteries.

Our study provides a unique insight into medieval Estonian society's social structure and migration dynamics. We collected and sequenced 61 Medieval individuals (1407-1640 CE) buried in the town cemeteries and churches in Tartu and Tallinn. We also included 135 individuals from rural sites to investigate the genetic origins of the medieval Estonian populations. The principal component analyses (PCA) reveals two distinct groups of the studied individuals: a more “local” one overlapping the modern Estonians and the “elite” one shifted towards modern Western European populations. The imputation of the genomes of 112 individuals allowed us to conduct genetic ancestry analysis for urban and rural populations. Our results suggest a clear distinction between these two groups, providing genetic evidence supporting the genetic and social stratification between the medieval Estonian elite of Western European ancestries, and local rural individuals.

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Environmental change, sustainability, and ancient ecosystems I

Ancient Beluga Genomes: Unraveling Long-Term Climate Impacts on Arctic Marine Mammals

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Key words: Ancient DNA, Beluga Whales, Arctic Climate Change

The global environment has changed dramatically over the past few decades, especially in the Arctic, and Arctic sea ice is predicted to decline by up to 94% by 2050. By losing their habitats, ice-associated marine mammals are highly vulnerable to changes in sea ice conditions. Historically, Arctic ecosystems have experienced dramatic shifts in sea levels and sea ice cover during the Late Quaternary. Yet, how climate change has affected Arctic marine mammals on a long-term scale is still not well understood. Although next-generation sequencing technologies have significantly advanced ancient DNA research, most work has focused on macrofossils from terrestrial species. As a result, relatively little is known about marine mammals, as they live and die at sea and thus have relatively limited fossil chronologies.

In this ongoing study, we employ whole-genome shotgun sequencing and mitogenome capture to recover genomic data from more than 50 ancient beluga whale (*Delphinapterus leucas*) specimens dating from the late Pleistocene to the early Holocene. By integrating their genomic data with isotopic analyses, we aim to investigate how past climate changes have influenced beluga whale populations, migrations, and adaptations. This work may provide valuable insights for understanding how global warming and diminishing Arctic sea ice might impact future marine mammal conservation efforts.

Phenotypic and Genotypic Diversity of Brown Trout During the Last Glacial Maximum

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Keywords: Brown trout (*Salmo trutta*), Intraspecific variation, Iberian glacial refugium

Brown trout (*Salmo trutta*) is a salmonid species of cultural significance in Europe, exhibiting remarkable intraspecific diversity. Resident (freshwater) and anadromous (migratory) ecotypes can coexist within the same population, with migratory behaviour being a threshold trait influenced by ecosystem carrying capacity and temperature. Migration provides a fitness advantage for trout due to increased dispersal ability and better adaptation to cold temperatures. Anadromous trout migrate seasonally and grow larger, making them a likely preferred target for hunter-gatherers (HG) during the Last Glacial Maximum (LGM).

To assess the LGM's impact on brown trout intraspecific variation in one of its key glacial refugia and provide insights into its evolutionary history, we analysed 269 Late Pleistocene salmonid vertebrae (~30-12 kyr BP) from 11 Cantabrian and French archaeological sites spanning pre-, intra-, and post-LGM phases.

On 207 vertebrae identified as brown trout using ZooMS and morphometric analysis, growth rate estimates were comparable to modern trout. Ancient mtDNA retrieved from 36 vertebrae enabled examining genetic diversity across time and space. $\delta^{13}\text{C}$ stable isotope analysis revealed both ecotypes, suggesting humans exploited anadromous trout alongside relatively large river-resident trout, which likely benefited from optimal glacial refuge temperatures. Anadromous trout presence in France, as far north as the Loire basin, supports post-LGM recolonization from the northern margins of the refugium. Ongoing phylogenetic analyses of modern and ancient brown trout mtDNA sequences aim to elucidate this aspect.

Overall, our findings highlight the resilience of LGM brown trout and the reliance of HG groups on river environments, enabling year-round subsistence strategies.

Arctic Faunal Phylogeographic History and Environment Reconstructed from 75 000 Year Old Bulk-Bone

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Keywords: Bulk-bone, Arctic, Mitogenome

The Last Glacial period in the Arctic is characterized by extreme climatic fluctuations, associated with glacial advances and retreats, changes in sea levels, and shifting sea ice extent. This resulted in major redistributions of flora and fauna that have left a lasting phylogeographic imprint still evident today. Nevertheless, the effects of these changes on pre-LGM biodiversity in the Arctic remain largely unresolved because of the the rareness of fossil remains caused by glacial erosion and flushing of glacial meltwater. The 75 000 year old bone-bearing layer in Arne Qvamgrotta, part of the Storsteinhola cave system (68.10° N 16.38° E) in Northern Norway, forms a unique exception. By combining comparative morphology and bulk-bone metabarcoding we identified 46 marine and terrestrial taxa, representing mammals, birds and fish. Subsequent shotgun sequencing and competitive mapping of bulk-bone allowed us to reconstruct almost complete mitogenomes from twelve species, including *Balaena mysticetus* (bowhead whale), *Rangifer tarandus* (reindeer) and *Haliaeetus albicilla* (sea eagle). This first Arctic MIS 5a assemblage reveals a non-analogous, coastal, cold-adapted, Arctic faunal community with taxa previously unknown from Fennoscandia. Moreover, all sequenced mitogenomes represent now extinct lineages, indicating significant faunal turnover in the Fennoscandian Arctic. Finally, mitogenome analyses refine inferred phylogeographic histories of a diverse group of species and contributes to our understanding of the underlying mechanisms of long-term biological responses to climate change.

Environmental change, sustainability, and ancient ecosystems II

Palaeoproteomic Investigations in Palaeolithic Siberia; the Case of Ust'-Karakol

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Keywords: ZooMS, Palaeolithic, Ust'-Karakol

The application of palaeoproteomics in archaeology and evolutionary anthropology has demonstrated the potential for biomolecular approaches to complement, and often resolve, osteoarchaeological and zooarchaeological research questions. Zooarchaeology by Mass Spectrometry (ZooMS) or collagen peptide mass fingerprinting, in particular, can assign robust taxonomic identifications to the level of family, genus and even species to highly fragmented unidentified skeletal remains. In this study, we examine a large assemblage of unidentified bone and teeth fragments excavated from Ust'-Karakol, an open-air site located in the Anuy river valley of the northwest Altai region, Siberia, Russia.

Using ZooMS, we analysed a portion (n=576) of the site's non-identifiable faunal assemblage to provide taxonomic identification and attempt the discovery of human remains. The results revealed diverse representation of fauna and the presence of two human fossils. In this paper, we compare our new results with the previously identified taxa from the neighbouring site of Denisova Cave, a key locus for Palaeolithic archaeology in the area, and place the human remains in a general context through direct radiocarbon dating. This comparative approach contributes to the unravelling of human-environment interactions during the Late Pleistocene in the Anuy river valley and provides new information for the study of Pleistocene ecosystems, palaeoecology and hominin subsistence strategies in key Altai region and Northern Eurasia in the past 50,000 years.

From Forests to Fire: Reconstructing Holocene Forest Ecosystems and Nordic Bronze Age Human Cave Activity at Solvikhola, Northern Norway

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Keywords: aDNA, Palaeoarchive, Archaeology

Caves offer exceptional preservation conditions for organics and archaeological remains, providing insights into biodiversity, ecosystem dynamics, and anthropogenic events over millennia. Solvikhola (67°12'46.2"N, Fauske, Nordland, Northern Norway), a cave excavated in 2023, revealed a remarkable palaeoarchive with a sedimentary deposit spanning the last 7600 cal. years. Ten distinct stratigraphic layers contained organic remains such as bones, shells, leaves, and charcoal, providing a unique opportunity to reconstruct past environments and prehistoric human activity in higher latitudes. Such long-term records are rudimentary and underexplored above the Arctic Circle, making Solvikhola an invaluable resource for understanding the region's distant past. Applying a multidisciplinary methodology including archaeology, ancient DNA (aDNA) bulk-bone metabarcoding (BBM), palynology, geology, and isotope analysis (¹⁴C-dating), we uncovered a hearth with a torch and a Bronze Age cultural layer. Moreover, we identified a diverse faunal sequence back to the Mesolithic. Osteological analysis identified 18 taxa across Amphibia, Aves, Mammalia, and Pisces representative of 24% of the bone fragments. Ancient DNA BBM considerably expanded taxonomic resolution, identifying more than 56 taxa from the remaining 76% of bone fragments. This dataset highlights a relatively stable forest ecosystem with small climatic fluctuations, hosting foxes, wolves, reindeer, small mammals (e.g., *Arvicolidae*, *Cricetidae*, *Leporidae*), and birds (e.g., *Strigidae*, *Hirundinidae*, *Phasianidae*) resembling the present-day ecosystem in Northern Norway. Notably, the discovery of archaeological remains dating to c. 2700 cal. BP offers evidence of early human activity at the cave site, contributing valuable data to the limited knowledge of human presence in this northern interior region.

Tales of Prestige Seekers and Mud Hunters: Dietary Variability Among Pre-Colonial Sambaquis Revealed through Stable Carbon, Nitrogen, and Sulfur Isotope Analysis

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Keywords: Stable sulphur, carbon and nitrogen isotopes, Human diets, Coastal adaptation, South America

The southern Atlantic Forest of Brazil preserves archaeological evidence of coastal populations that endured over extensive geographic areas during the Middle and Late Holocene. While it is well-established that these groups relied heavily on marine resources, the extent to which they adapted to the complexities of the coastal environment remains unclear. We measured the bulk collagen of 350 human individuals and 250 faunal remains for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ values. The isotopic distribution exhibits significant geographic variability, reflecting differences between mangrove and open sea ecosystems. A few individuals with extremely high $\delta^{34}\text{S}$, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values suggest prolonged consumption of pelagic species.

- a low-ranked yet high-risk resource compared to inshore fish, which provided the bulk of dietary protein for most individuals. These outliers are interpreted as evidence of “costly signaling”, associated with efforts to secure and maintain social prestige through political (leadership) or religious (shamanic) roles. Conversely, individuals with extremely low $\delta^{34}\text{S}$ values likely relied on resources from highly anoxic environments, such as mangrove sediments in the middle and upper estuary. Zooarchaeological evidence supports this interpretation, indicating that these individuals consumed benthic animals including molluscs and crustaceans from such habitats. Sulfur isotope analysis offers unprecedented insights into the social and subsistence organization of pre-colonial populations in Lowland South America. Our study reveals that the pursuit of high-risk resources to maintain social status dates back thousands of years in this region, and that some groups consumed significantly more molluscs and crabs than conventionally assumed.

Ungulate Ethology and Late Pleistocene Human Ecodynamics. A Multiproxy Analysis of Prey Species at El Mirón Cave (Cantabria, Spain).

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Keywords: Animal Migration, Ecological niches, Hunting season

To reconstruct the seasonal climate and environmental conditions when prehistoric humans lived and hunted animals after the Last Glacial Maximum, we study ungulate teeth from the Lower Magdalenian period (19.2–18.3 ka cal BP) discovered in El Mirón Cave, Cantabria, Spain. We measure the stable isotope composition of oxygen ($\delta^{18}\text{O}$ in enamel carbonate and phosphate bioapatite), carbon ($\delta^{13}\text{C}$, carbonate), and strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) through intra-tooth sequential sampling from ibex, horse, and red deer. Phytoliths from the dental calculus of the same teeth, along with samples from large bovids, were analysed to improve environmental data and understand animal dietary behaviour. The multi-isotopic results show that ibex exhibit the greatest intra-tooth (seasonal) variation, likely related to altitudinal ranging. Ibex also occupied different ecological niches and had distinct feeding behaviors compared to red deer and horses. Arboreal phytoliths were more common in the dental calculus of ibex than in large bovids and were absent in horses. Ibex consumption of flowering plants (Asteraceae) increased throughout the Lower Magdalenian human occupation of El Mirón, which may indicate the greater availability of these floras in cold environments that were becoming increasingly humid, possibly with more meltwater discharge. We discuss 1. how species-specific isotope and phytolith data reveal past hunters' environments, in which they intensively exploited ibex and red deer, and 2. the dynamic relationship of this mosaic landscape to past human behaviour, particularly during climatic transitions. This multiproxy analysis at El Mirón thus improves our understanding of how glacial-age animals and humans navigated past ecological dynamics.

Interaction between humans and non-human animals, plants, and microbes II

Plant Medicine, Stimulant and Cultural Keystone: Applying a Herbariomics Approach to Explore the Adoption of the Coca Plant by Humans

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Keywords: Coca, Domestication, Herbariomics

Native to the South American tropics, coca (*Erythroxylum coca* and *E. novogranatense*) is a crop plant with which humans have had a relationship for at least 8,000 years [1]. It is cultivated for its leaves which produce a multitude of alkaloids, famously including cocaine. This composite of alkaloids confers stimulatory, medicinal and nutritional properties and, combined with the entrenched ritual around leaf-chewing, coca retains an important place in the culture of millions of South Americans. Nonetheless, our botanical understanding of coca, e.g. in terms of its taxonomy and chemical ecology, remains patchy. This in turn hinders interpretation of its domestication process, with the two cultivated species separated by a pre-human timing of divergence [2] and the possibility of more than two origins [3]. Furthermore, the subtle domestication syndrome, typical of perennial tree crops and further underscored by the predominantly clonal propagation of coca, is non-trivial to characterise. Here, we present a herbariomics approach to delimiting diversity within the gene pool of coca and its wild relatives. To this end, we extracted genomic and biochemical data from ~200 *Erythroxylum* herbarium specimens to gain new insights into how *E. coca* and *E. novogranatense* adapted to human landscapes and were dispersed within them. Progressing research on the coca crop is timely, given the current discourse around legality of the coca leaf and sovereignty of Indigenous coca biodiversity in the context of coca monocultures and deforestation driven by the illegal cocaine market.

References

- [1] Dillehay *et al.* (2010) doi.org/10.1017/S0003598X00067004
- [2] Przelomska, Diaz *et al.* (2024) doi.org/10.1093/molbev/msae114
- [3] White *et al.* (2021) doi.org/10.1093/sysbio/syaa074

Once Upon a Time in the Americas: How 16th Century Epidemics Reshaped Indigenous Immunogenetic Diversity

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Infectious diseases are among the most important evolutionary forces, not only to humans but to many forms of life. Susceptibility foci for outbreaks, epidemics and pandemics can be discovered once we understand the roles interplayed by the host immune system and its genetic diversity, the pathogen arsenal, and the ecological scenario in which they meet. The study of past epidemiological events can be useful for understanding the way in which immunologically naïve individuals respond to newly introduced pathogens in specific environments.

Here, we present the story of one historic epidemiological event: the *Salmonella* epidemic in the 16th century Mexico. First, we explore the diversity of the pathogen *Salmonella enterica* Paratyphi C, proposed to be the causing agent of one of the *Cocoliztli* epidemics, events that took a toll on the Indigenous Americans from Mesoamerica at the beginning of the colonial period. The phylogenetic analyses of newly recovered *Salmonella* genomes from central Mexico together with previously reported ones from Teposcolula, Oaxaca, further confirm the epidemiological status of the disease. Then we travel to the Yucatan Peninsula, where we identified a shift in immune-related genetic markers, particularly concerning the human leukocyte antigen (HLA) complex in the genomes of the ancient inhabitants of Chichén Itzá, and the present-day Mayans from Tixcacaltuyub, which are genetic descendants of the Chichén Itzá inhabitants and are genetically comparable. That particular variant confers resistance to enteric fever, and it is one of the most frequent alleles among present-day Mexicans, both Indigenous and mixed-ancestry, illustrating an adaptive response to these introduced diseases.

With the incorporation of the newly generated genomes for *Salmonella enterica* Paratyphi C from central Mexico into the story, we provide further evidence that this strain may be the elusive pathogen that caused the first *Cocoliztli* in 1545 and left a fingerprint in the immunogenetic diversity of present-day (Meso)American populations.

Ancient Metagenomics Reveal Human-Microbial Interactions over Evolutionary Timescale

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The interaction with microbes has long been an indispensable piece in human history. Despite over 10,000 years history of utilizing and manipulating microorganisms to produce fermented food, little is known about the origin of the techniques and history of its development and spread. In addition to the scarcity of organic residues preserved over thousands of years, a major challenge is to obtain molecular evidence from the ancient remains. Here, with the great privilege of accessing several Bronze Age cheese samples (~ 3500-year-old) from the Xiaohe Cemetery (Xinjiang, China) and 700,000 newly designed probes targeting a *Lactobacillus* genome, we were able to interrogate the DNA information of probiotic bacteria that were regularly propagated and consumed by humans over thousands of years. We found that the Xiaohe population actively adopting techniques from western steppe populations and integrating them into their own culture. In addition, while kefir is a traditional fermented milk beverage that is still widely consumed today, the origin and spread of it is unclear. Through the reconstruction of the phylogeny of *Lactobacillus kefiranofaciens* – a key fermenter in kefir, we found there are two distinct spread routes in East Asia, with one primarily spread along the coastalline and the other spread within the mainland. We also identified dual-role gene clusters (i.e., benefit both the bacteria and their human hosts) gained by *Lactobacillus kefiranofaciens* through horizontal gene transfer over their thousand years of interactions with humans. Through retrieving the first ancient DNA study of dairy residue, our study reveals the history of dairy fermentation by a Bronze Age population as well as dynamic interactions between humans and microbes over thousands of years.

Describing Early Domestic Genetic Diversity using Ancient Sheep Genomes from Anatolia

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Sheep was amongst the earliest livestock species to be domesticated in the Fertile Crescent around 11.000-10.000 years ago. However, the precise geographic location and the number of founder subspecies of the wild ancestor, the Asiatic mouflon, remain contentious. Recent genomic studies suggested that the domestic form emerged either from several subspecies of mouflon or by a well-stratified mouflon population. However, the ancestral phylogeography of mouflon is still unknown, reflected by conflicting patterns of genetic and geographic divergence in mouflon subspecies of Anatolia and Cyprus.

To disentangle the history of early domestic sheep, we sequenced 43 high quality ancient genomes from various Pre-Pottery Neolithic (PPN) sites in southwest Asia, including Göbekli Tepe, an early megalithic site in Anatolia built by a community of foragers, and Aşıklı Höyük, a site reflecting the earliest stages of sheep management and domestication, with implications for sheep husbandry in western Anatolia and Europe. The pooling of these ancient genomes along publicly available (modern and ancient) genomes^{1,2} enables us to elucidate the genetic structure of pre-domestic and early domestic sheep populations in Neolithic southwest Asia. Besides showing a loss of diversity in the wild population, our results also hint at translocations of flocks and replacement by Eastern mouflon ancestry to form the modern Anatolian and Cyprian mouflon populations. We interpret these movements as the consequence of the Early Holocene process of deglaciation allowing colonization of new landscapes, but also acknowledge anthropogenic factors in the form of animal translocations, habitat deterioration, and domestic-mediated competitive exclusion of the wild form.

Manatees in the Margins: Population Fragmentation and Ecological Differentiation of African Manatees Revealed by Ancient DNA and Stable Isotopes

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Key words: aDNA, Tropical marine mammals, Historical ecology

Manatees remain chronically understudied, despite their cultural and economic significance and their vulnerability to extinction. African manatees (*Trichechus senegalensis*) inhabit diverse habitats from Mauritania to Angola, encompassing coastlines, rivers, estuaries, and lagoons, within biomes extending from tropical rainforests to arid deserts. Anthropogenic pressures have isolated some populations, potentially leading to significant genetic differentiation and diversity loss. However, due to the elusive nature of manatees, such genomic impacts remain underexplored. Here, we analyse aDNA and stable isotopes from historical African manatee specimens dated up-to 250 years BP, covering much of the current and historical range. Phylogenetic analyses of 29 mitogenomes reveal four geographically distinct clades, with evidence of deep-rooted divergence in the most northern clade (I) and relatively recent divergence in the most southern clade (IV). Lower genetic diversity is also observed at these range termini. Furthermore, low coverage, whole-genome nuclear data reveal fine-scale population structure and genomic diversity, while stable isotope analysis elucidates the distinct foraging behaviours of the southern clade (IV), which primarily forages in estuarine environments compared to our sampled northern manatees that forage more in riverine systems. Altogether these findings show that population structure has been maintained across both ecological and evolutionary timescales. Importantly, coupled with the known discontinuity of populations, our results indicate that African manatees are ecologically specialised making them likely to be vulnerable to environmental change. Manatees face a multitude of threats including pollution, climate change, illegal exploitation, and habitat destruction. Therefore, their specific ecological niches should be considered when informing conservation management actions.

Methodological advances in biomolecular archaeology III

Historical Records of Climate Preserved within Parchment Lipids

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Keywords: Lipids, Isotopes, Parchment

Parchment is an ancient material used for millennia to document litigation, art, and societal information. Formed from animal skins, its manufacture comprises repeated liming and scraping steps before drying under tension. Historical parchment serves as a valuable record of cultural heritage, but this also presents a considerable analytical challenge, with many studies limited to non-invasive techniques.

In this paper, we highlight the prospect of the molecular and isotope compositions of animal lipids from parchment as a time-sensitive record of its production and the animal's diet and environment. To be analysed isotopically, lipids must first be extracted from parchment using solvent. We report a novel, minimally invasive lipid extraction technique for this purpose.¹ By comparing the quantitative and qualitative compositions of lipids and validating our method in collaboration with The National Archives, UK, we demonstrate a viable means of sampling this untapped biological archive.²

Applying this method to historically significant membranes documenting UK Acts of Parliament, we present the first-ever $\delta^2\text{H}$ climate signal from parchment for 1814–1820 CE, providing evidence for the Year Without Summer.³ Using these proxy data and instrumental rainfall measurements, we developed a calibrated model which effectively captured extreme changes in precipitation.³ Assessment of the carbon isotopic composition of $\text{C}_{16:0}$ and $\text{C}_{18:0}$ has shed light on the Napoleonic Wars (1803–1815 CE) with evidence for changes in historical feeding practices.¹ These findings showcase historical parchments as unique palaeoenvironmental resources, paving the way for new knowledge of animal husbandry and human responses to climate change.

References

1. Johns, S. P. *et al.* Minimally Invasive Vacuum-Aided Extraction Technique for the Lipid Analysis of Historic Parchment. *Anal. Chem.* 96, 13811-13820 (2024).
2. Vermeulen, M. *et al.* Assessing the Effect of Minimally Invasive Lipid Extraction on Parchment Integrity by Artificial Ageing and Integrated Analytical Techniques. *PDST*, 230, 111076 (2024).
3. Johns, S. P. 'Historical Records of Climate Preserved Within Biomolecular Components of Calendrically-Dated Parchment Manuscripts. University of Bristol (2024).

Combined Time-Resolved Sr-O-C Isotopes and Proteomics Unravel the Ecology of Extinct Straight-Tusked Elephants at Neumark-Nord (~125 ka, Saxony-Anhalt, Germany)

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Keywords: Multi-isotope, Amelogenin, *Palaeoloxodon antiquus*

The nearshore sediments of the Last Interglacial site complex of Neumark-Nord (~125 ka) preserve multiple traces of Neanderthal activity within a lake-dotted landscape surrounded by a semi-open environment. Notably, skeletal remains of >70 straight-tusked elephant (*Palaeoloxodon antiquus*) individuals covered in anthropogenic cutmarks have been discovered. These findings, combined with a mortality profile skewed towards adult males, suggest selective Neanderthal hunting. Four elephant molars – two with non-local bulk enamel ⁸⁷Sr/⁸⁶Sr composition – were investigated to reconstruct intra-life mobility and ecological patterns of *P. antiquus*. For our spatially-resolved Sr-O-C isotope analysis we combined LA-MC-ICPMS to assess mobility with sequential intra-tooth $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ analysis by IRMS to identify seasonal variation in habitat use and diet. A specifically-developed Sr-isoscape of northeastern Germany was employed to track the provenance and movements of the four individuals. Enamel amelogenin analysis, performed by LC-MS/MS, identified all individuals as males. We found remarkably large intra-tooth ⁸⁷Sr/⁸⁶Sr variations that indicate (sub-)seasonal migration for all individuals during ~8 years of tooth mineralization. Interestingly, two males likely roamed near Neumark-Nord, while the other two roamed for years in more radiogenic areas, possibly near Palaeozoic mountain ranges up to 300 km away from the site they were eventually killed by Neanderthals. Distinct $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values suggest that the non-local elephants fed in more forested areas and drank from more ¹⁸O-depleted water sources than the local individuals. Overall, this multi-proxy approach provides insights at unprecedented temporal-resolution into the behavioural ecology and habitat use of *P. antiquus*, and underscores selective hunting strategies of Neanderthals.

Smaller, Faster, Better? Microfluidics & UHPLC for Ancient Amino Acids

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Keywords: Proteins, Biomineral, Lab-on-a-chip

The development of analytical technologies provides powerful new tools for biomolecular archaeology, particularly by increasing both sensitivity and resolution, vital for detecting the low biomolecule concentrations in fossil material. The types of complex matrices involved usually require bespoke method development, but this work can reap rewards not only in speed but also in data quality. Here we present the method development work we have done in the preparation and analysis of chiral amino acids in fossil samples, relevant for both amino acid dating and palaeoproteomics.

We developed a modular microfluidic system preparing tooth enamel samples for amino acid dating. The modular system comprised of three sequential glass microfluidic devices for: i) sample bleaching, ii) hydrolysable amino acid release, and iii) biphasic separation, before the relative concentrations and D/L values of key amino acids were then measured using reverse-phase high performance liquid chromatography (RP-HPLC). The microfluidic method provides consistent data across different genera and operators, with reduced sample mass and analysis time. This enables the analysis of smaller/more precious samples, and also shows the potential for some of the sample preparation to be carried out within the samples' country of origin, democratising the technique. We have also expanded the range of material analysed by "ultra" high pressure LC (rather than conventional RP-HPLC) from eggshell to foraminifera, bivalves, corals and tooth enamel, in most cases improving resolution, sample throughput, number of amino acids measured, and speed of the analysis. We present the trials, tribulations and triumphs of this approach.

Methodological advances in biomolecular archaeology IV

The Role of the Ceramic Fabric in the Preservation and Recovery of Proteins

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Keywords: Ceramic, Cooking, Extraction

While proteins have been successfully extracted and identified from a range of materials, their recovery from ceramic archaeological materials remains challenging. Targeting proteins trapped in calcified deposits has proven successful before. However, less is known about the mechanics of protein absorption into the ceramic matrix and the best strategies to extract them. This study uniquely investigates how ceramic mineral composition and structure, tempering and firing temperature influence protein absorption, extraction and identification. By systematically altering these variables, we built a range of ceramic briquettes. Then, in separate experiments, either raw milk or an anchovée preparation was cooked into the briquettes in controlled conditions. Finally, a guanidine hydrochloride extraction was used before the LC-MS/MS analysis. Overall, we were able to consistently identify more spectra and peptides on the high temperature fired than in the low fired briquettes. We hypothesize that proteins bind stronger to the rough surfaces of low-fired ceramics, making it hard to extract them, even though their higher porosity would mean that more proteins can penetrate and adsorb. Quartz-tempered ceramics, at both firing temperatures, furnished more identifications than any other clay, temper and temperature combination. Here, the alpha-beta quartz expansion, creates pores in the ceramic, increasing the available surface, while exposing itself for proteins to bind. Interestingly, by using open-search for the spectrum database querying, we identified several postranslational modifications, such as lactosylation in milk, that would be introduced in the cooking process.

Optimization of Dental Enamel Demineralization for Palaeoproteomics

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Keywords: Palaeoproteomics, Method development, Dental enamel

Proteins in archaeological and palaeontological dental enamel can preserve over very long time spans, even millions of years, and hold clues to our ancient past. Although the dental enamel proteome is small and highly conserved, it has been used to study phylogenetic relationships between extinct and extant taxa, and to assign genetic sex to individuals. However, prior to palaeoproteomic analysis, proteins need to be released from the dense and highly mineralized dental enamel matrix. There is currently no consensus on which demineralizing agent should be used, or at which concentration, to efficiently dissolve the mineral without damaging the endogenous proteins. Here, we compare dental enamel demineralization using FA (formic acid), HCl (hydrochloric acid) and TFA (trifluoroacetic acid) at three different concentrations (5%, 10% and 15%). We conduct the comparisons on a Holocene *Bos* specimen (St. Clemens Torv, Denmark), a Late Pleistocene *Bos* specimen (Callao Cave, the Philippines), and a Middle Pleistocene Rhinocerotidae specimen (Naxian Cave, China). We find that the total amount of extracted protein does not differ between acid types or concentrations, while the total intensity of identified proteins after mass spectrometry does depend on acid concentration, with higher concentrations leading to lower protein intensity. We find significant differences in post-translational modifications, peptide length and hydrophobicity, indicating that the choice of demineralization condition has a notable effect on the recovered proteome. It is thereby essential to choose the most appropriate dental enamel demineralization condition to ensure retrieval of the maximal amount of proteomic information from each destructive sampling.

Exploring an Integrative Approach: Metabolomics of Paleolithic Faunal Remains for Dietary Reconstruction and Radiocarbon Dating

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Keywords: Metabolomics, Paleolithic fauna, Diet reconstruction

Reindeer and horses are among the most common faunal remains at Eurasian Paleolithic sites. Unlike other mammals that went extinct during Marine Isotopic Stage 3, (e.g., cave bear, cave hyena), they survived despite abrupt climatic fluctuations. This raises questions about the factors influencing faunal extinction and survival (e.g., competition for resources, impact of climate, dietary flexibility) that geochemical analyses of bones can help address. To enhance these insights, we propose to integrate traditional approaches with metabolomics, to investigate the chemical integrity of bones organic fractions and fauna dietary flexibility.

Reindeer/horse bones (32) and collagen extracts (15) from the Portel-Ouest and Tournal caves (north Pyrenees, 50–15 ka BP) were analyzed by pyrolysis-gas-chromatography coupled with mass-spectrometry (Py-GC-MS) and data-processed by a specifically-dedicated metabolomics workflow. Stable isotope analyses ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and radiocarbon dating were also performed. Py-GC-MS enables a broad molecular characterization, detecting both native low molecular weight compounds and pyrolytic products of high weight molecules, while metabolomics greatly facilitates statistical treatment of complex datasets.

Our results highlight a dual significance: (1) it has efficiently identified endogenous biomarkers, such as protein derivatives, alongside exogenous compounds (potential contaminants). This constitutes a reliable screening tool to assess the chemical quality of bones/collagen extracts, that is crucial for isotopic measurements reliability. (2) It has explored the potential of Py-GC-MS to identify putative dietary biomarkers in ancient faunal remains.

Looking ahead, this methodology could become a preliminary step to optimize bones chemical treatments and could be routinely integrated into diachronic dietary reconstructions, complementing multi-isotopic studies.

The Potential of $\delta^{88}\text{Sr}$ – A Journey through Mass Spectrometry and Application

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Keywords: Strontium, MC-ICP-MS, Cremated remains

The novel $\delta^{88}\text{Sr}$ tool¹⁻³ has been applied to multiple sites across Europe to trace human diet. Few results have yet been published. Traditional dietary tracers such as C and N cannot be applied to cremated remains due to their volatile nature. Strontium, incorporated into the inorganic fraction of the bone, is thus an invaluable tool. Even the high temperatures reached during cremation do not affect the Sr isotopic ratios. As more remains are analyzed with this new tool, we gain deeper understanding but also more questions regarding the interpretation of this data. The abiding questions are how to use this new tracer and what are the next steps for $\delta^{88}\text{Sr}$? What are the key parameters when interpreting these new datasets? This presentation will guide you through some of the first datasets on cremated human remains. A trend of increasing $\delta^{88}\text{Sr}$ from Bronze Age to Roman occupation (e.g. Blicquy, Belgium) illustrates how we can track dietary shifts within a population over time. In parallel, we found statistically significant differences in the $\delta^{88}\text{Sr}$ values between individuals buried with different types of grave goods (e.g. Brinjeva Gora, Slovenia). By combining $^{87}\text{Sr}/^{86}\text{Sr}$, [Sr] and $\delta^{88}\text{Sr}$, with archaeological evidence and environmental data we gain key insights into human diet and mobility in the past. Finally, this presentation will discuss the use and application of $\delta^{88}\text{Sr}$ baselines.

References

1. Gerritzen, C. T., Goderis, S., James, H. F. & Christophe Snoeck. Optimizing Zr-doped MC-ICP-MS sample-standard bracketing to simultaneously determine $^{87}\text{Sr}/^{86}\text{Sr}$ and $\delta^{88}\text{Sr}$ for high sample-throughput. *Spectrochimica Acta Part B Atomic Spectroscopy* 217, 106955–106955 (2024).
2. Lewis, J., Pike, A. W. G., Coath, C. D. & Evershed, R. P. Strontium concentration, radiogenic ($^{87}\text{Sr}/^{86}\text{Sr}$) and stable ($\delta^{88}\text{Sr}$) strontium isotope systematics in a controlled feeding study. *STAR: Science & Technology of Archaeological Research* 3, 45–57 (2017).
3. Guiserix, D. et al. Simultaneous analysis of stable and radiogenic strontium isotopes in reference materials, plants and modern tooth enamel. *Chemical Geology* 606, 121000 (2022).

Methodological advances in biomolecular archaeology V

Archaic Ancestry Inference in Imputed Ancient Human Genomes

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Keywords: Archaic Introgression, Imputed Genomes, Adaptive Introgression

The study of interbreeding between Archaic Humans (Neanderthals and Denisovans) and Anatomically Modern Humans has reshaped our understanding of human origins and opened new research avenues into the impact of archaic introgression on human evolution. Some introgressed variants conferred an evolutionary advantage (adaptive introgression) in traits such as brain development, immunity, metabolism, pigmentation, and skeletal structure.

Despite advances in identifying and quantifying archaic introgression, most studies have focused on contemporary genomic data. Consequently, little is known about how archaic variation evolved within human populations after introgression. These variants continued to evolve, shaped by population-specific demographics and natural selection. While ancient DNA holds promise for addressing these questions, its poor quality has hindered progress. A promising approach is imputation, which enhances both the quality and depth of analysis in low-quality DNA.

Here, we assess the feasibility of detecting archaic introgression in imputed ancient genomes, addressing two key challenges. We evaluate imputation's accuracy in reconstructing introgressed fragments in ancient individuals and examine potential biases arising from using present-day reference panels.

Analysing twenty downsampled high-coverage ancient genomes, we assess three key aspects: the reliability of identifying archaic introgression, the quantification of introgressed fragments, and the accuracy of pinpointing introgressed haplotypes. While some common quality filters on imputed data were found to bias certain allele frequency results, imputation still provides a robust reconstruction of introgressed fragments, even surpassing that of the original high-coverage genomes. We confirm that imputation effectively reconstructs introgressed fragments, identifying both known archaic haplotypes and novel high-frequency segments from the past.

Analysing Networks to Uncover and Compare Genetic- and Cultural-Relatedness in Connected Ancient Cemeteries

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Keywords: Ancient DNA, IBD networks

Recent ancient DNA publications have begun to focus on every available individual from single cemeteries, or from several nearby cemeteries. Using measures of genetic relatedness, networks are formed by “linking” related individuals, and inferences are made by looking at the observable patterns, or from surface-level statistics in the networks. Here, we introduce the use of network regression and clustering methods to analyse these networks, allowing us to statistically test hypotheses about demographic and cultural observations.

Such questions may be, can we form “communities” or “clusters” of related individuals, and can these be understood as a mating network? Do similarities in unique burial items or customs correlate with individuals being closely genetically related? Are these patterns consistent, and even connected, across cemeteries, different sites or even different regions? Importantly, we explore these patterns while accounting for the many nuisance variables that could obscure these inferences, such as location, distance, or biased sampling. To illustrate the potential of this new approach, we introduce these methods in various prehistoric and historic contexts, showcasing how we can uncover patterns of similarity between genetically-related individuals.

References

Ringbauer, Harald, et al. "Accurate detection of identity-by-descent segments in human ancient DNA." *Nature Genetics* 56.1 (2024): 143-151.

Kolaczyk, Eric D., et al. "Statistical models for network graphs." *Statistical analysis of network data with R* (2014): 85-109.

Novel Avian Protein Sequences Improve Resolution of Palaeoproteomic Approaches to Taxonomic Identification and Reveal Widespread Intraspecies Variability

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Keywords: Palaeoproteomics, Taxonomic identification, Methods

Large curated protein databases are essential for confident palaeoproteomic applications to taxonomic identification, especially for taxa with high species diversity. We present a new pipeline to annotate protein sequences from genomic data allowing us to leverage rapid advancements in avian genomics. We annotated bone and eggshell protein sequences for 112 Anatidae (ducks, geese and swans) species, a biologically complex group of birds that are central to many archaeological and ecological questions. This extensive dataset based on 160 genomes allows us to address a fundamental issue in using ancient proteins for reconstructing evolutionary relations as well as for taxonomic identification: the extent of intra-taxon and inter-taxon variability of amino acid sequences.

We found evidence for widespread intraspecies variation in protein sequences, challenging the assumption that protein sequences vary only at the species level or above. Moreover, we present a phylogenetic tree from 13 proteins that aligns with mtDNA-based phylogenies, supporting the use of proteins for examining evolutionary relationships when multiple proteins are considered. However, our phylogenetic trees based on single proteins vary greatly in topology, underscoring the need for caution when using fragmented proteins for identifying taxonomic and evolutionary relationships.

With the comprehensive taxonomic coverage of Anatidae proteins, clear taxonomic patterns emerge that enable reliable identification of bone and eggshell. We demonstrate this application to archaeological material from Teotihuacan, Mexico, and Shubayqa, Jordan. We highlight that extensive curated protein datasets accompanied by rigorous standards for assessing single amino acid polymorphisms as taxonomic biomarkers are fundamental for correct taxonomic identification.

Refining Deamidation Estimation in MALDI-TOF Data: development and application of the MALDI Deamidation Score (MDS)

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Key words: Deamidation, MALDI-TOF data, Denisova cave

Estimating deamidation from MALDI-TOF MS spectra of bones has most frequently been achieved using the q2e method (Wilson et al., 2012) due to its high-throughput capacity and ease of use. Despite this accessibility, q2e is only capable of estimating deamidation at the peptide level on a fixed, non-modifiable peptide list and does not generate a sample-wide summary. The introduction of the Parchment Glutamine Index (PQI) (Nair et al. 2023) presented an alternative method for deamidation estimation. Initially designed for a large ZooMS dataset of parchment, it utilises weighted least squares and a linear mixed-effects model (LMM) to generate deamidation estimation on both the peptide and sample level. To address the limitations of q2e and expand the applicability of PQI to a wider range of archaeological tissues and MALDI-derived data (such as ZooMS data on bone collagen), we developed the MALDI Deamidation Score (MDS), an iteration of the PQI method optimized for handling large-scale datasets. Compared to PQI, MDS is more streamlined for the analysis of multi-species data with customisable peptide lists, offering a dramatically decreased processing time while being able to normalise the peak intensity for higher accuracy. Through a case study on the published Denisova Cave ZooMS assemblage (Brown et al., 2021), we demonstrate that different peptides exhibit varying deamidation patterns over time, making the use of a single peptide to represent overall deamidation potentially biased. Such information is invaluable for investigating key questions such as site formation processes, especially when contextualized with other lines of archaeological evidence.

References

- Brown, S., Kozlikin, M., Shunkov, M., Derevianko, A., Higham, T., Douka, K., & Richter, K. K. (2021). Examining collagen preservation through glutamine deamidation at Denisova Cave. *Journal of Archaeological Science*, 133, 105454.
- Nair, B., Palomo, I. R., Markussen, B., Wiuf, C., Fiddymment, S., & Collins, M. J. (2023). Parchment Glutamine Index (PQI): A novel method to estimate glutamine deamidation levels in parchment collagen obtained from low-quality MALDI-TOF data. *Peer Community Journal*, 3(e10). <https://doi.org/10.24072/pcjournal.230>
- Wilson, J., van Doorn, N. L., & Collins, M. J. (2012). Assessing the extent of bone degradation using glutamine deamidation in collagen. *Analytical Chemistry*, 84(21), 9041–9048.

Ancient RNA Expression Profiles from the Extinct Woolly Mammoth

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The analysis of ancient DNA has recently gained considerable momentum, allowing the study of extinct and extant organisms that lived up to 2 million years ago¹. This has enabled the reconstruction of genomes and historical ancestry of multiple extinct species, as well as revealing the complex nature of the bygone ecosystems where they once thrived. However, current DNA sequencing techniques cannot alone provide information about tissue identity, gene expression dynamics or transcriptional regulation – all of which is encoded in the RNA fraction, and critical to fully understanding the biology of these now lost species. Here we report RNA expression profiles from 10 woolly mammoths dating to the Late Pleistocene. One of these, dated to ~39,000 YBP, yielded sufficient detail to recover tissue-specific biological functions essential for skeletal muscle and skin metabolism. Our findings represent the oldest ancient RNA sequences recorded to date, enabling the reconstruction of transcriptional signatures in the extinct woolly mammoth. With our results, we showcase the potential to study ancient RNA molecules decoding preserved transcriptomes from deep time, pushing the field beyond the study of ancient DNA and proteins, and anticipating a new era of integrative palaeo-studies combining genomics, proteomics and transcriptomics.

Migration, mobility, and translocation IV

A Comparison of Genetically Visible Mobility Patterns in Southwest Asia and East Asia through the Holocene

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Keywords: Regional comparison, Modes of mobility, Sex-biased mobility

Here we compare mobility patterns observable from paleogenomic data in two broad regions of Asia which both saw the emergence of food production, metallurgy, long distance transport technologies, and institutionalized inequality.

We use various population genomic signatures from published data to explore various modes of mobility including migration (mass movement), background mobility (continuous low-level movement), as well as sex-biased mobility patterns.

In Southwest Asia and its neighbouring regions, represented by c.1300 human paleogenomes, we find relatively ubiquitous signals of interregional movement and admixture through the Holocene: continuous increase in genetic diversity, a trend towards longer-distance movement over time and a trend towards increasing male-bias in admixture (patterns reported in our earlier work¹).

Patterns we observe in Holocene East Asia, represented by c.1000 paleogenomes, appear less uniform. In this region, ancestry profiles as well as diversity levels tend to show more dramatic shifts than those observed in Southwest Asia. We also do not find a temporal trend in sex-biased in admixture in the latter region.

Although various explanations may be possible for the observed differences among regions, we speculate one reason might be migration (mass movement) having a larger contribution to observed genetic changes than background mobility in East Asia than in Southwest Asia. Such differences, in turn, could be attributable to historical contingency, differences in social/political organisation, as well as the predominance of land versus marine transport.

Reference

¹ Koptekin, D. et al. Spatial and temporal heterogeneity in human mobility patterns in Holocene Southwest Asia and the East Mediterranean. *Current Biology* 33, 41-57 (2023).

Origins and Decline of a Farming Population in the Southern Andes

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Keywords: Agricultural transition, Isotopes, Ancient human and pathogen genomes

The transition to agriculture was one of the most transformative processes in human history, profoundly reshaping societies across regions and time. Despite the significant insights archaeogenetic research has provided into the agricultural transition, our understanding remains heavily skewed toward certain continents. Here, we investigate the unique history of agriculture in the Southern Andes, focusing on the Uspallata Valley (UV), in modern-day Argentina. By combining genomic, isotopic, and archaeological evidence, we reconstructed the demographic, social, and ecological dynamics of hunter-gatherer and farming societies in the region over 2,000 years. Our results reveal genetic continuity between local hunter-gatherers and farmers in the region, demonstrating that agriculture in the UV was adopted through cultural transmission rather than migration. Intensive maize farming, which peaked between 850 and 600 BP, coincided with social and ecological challenges, including demographic decline, malnutrition, and the presence of infectious diseases like tuberculosis. These crises triggered trans-generational migrations of farming families from nearby regions, as evidenced by strontium isotopes and kinship networks, which also suggest a matrilineal social organization. Furthermore, genome analyses revealed that UV and nearby populations carried a distinct genetic component that persists to present-day, named Central Western South American (CWSA), indicating their deep divergence and unique population history compared to other South American groups. Our work counted with the involvement of Indigenous Huarpe communities, whose oral histories enriched the interpretation of these findings. This work contributes to global discussions on agricultural transitions by providing insights into the ecological and social pressures that shaped Andean farming societies.

Genetic Relationships of Ancient Caribbean Individuals from the Dominican Republic

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Keywords: Caribbean, Relatedness, Archaeogenetics

In the Caribbean, the period spanning from 2800 to 400 BP saw the emergence and increase in pottery production, marking a time of cultural exchange and diversification. While previous research has effectively described the general settlement patterns in the islands, there are still debates on the details of population connections among Caribbean archaeologists and geneticists.

This study contributes to the debate by enhancing our understanding of the pre-European Caribbean, particularly in the Dominican Republic. We report genome-wide data for 29 ancient individuals from archaeological sites in the northern Dominican Republic. Two sites, El Flaco and El Carril, are geographically close to each other and were occupied contemporaneously from approximately 750 to 500 years before present (BP). The Archaeological context suggests close interactions and exchanges with sites further away.

Focused on genomics, but integrating isotopic data, AMS-dating, and the cultural context, this study includes data from published nearby and distant sites to explore potential genetic relationships between them. By employing established methods to infer genetic relatedness, such as tracts that are identical by descent (IBD), this research examines the connections within the archaeological sites. We put a more diligent focus on the dating of the various connections to add detail to the understanding of changes in mobility and demographic patterns over the period spanning from 2800 to 400 BP in the Caribbean.

Unearthing Historical Migrations and Individual Identities via Identical-By-Descent (IBD) Connections to 17th Century St. Mary's City, Maryland

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Keywords: Identity-by-descent, Migration, Re-identification

Immigration has profoundly shaped the demography of the United States throughout its history, yet the colonial-era founder populations who were among the first European settlers in the region have left a genetic legacy that endures to the present. In this study, we demonstrate that identical-by-descent (IBD) segments of DNA can be used to directly trace the migrations of these colonial-era populations and their descendants and even to recover the identity of otherwise nameless individuals. We analyzed the genomes of 48 individuals from 17th century St. Mary's City, Maryland—the first permanent English settlement in the colony—and identified over 1.3 million genetic relatives among approximately 11.5 million consenting research participants in 23andMe, Inc.'s genetic database. Our analysis revealed significant genetic sharing with participants from Midwest England and Wales, pinpointing likely places of origin of many of the colonial city's earliest inhabitants. Additionally, we observed strong genetic connections among participants from Kentucky, reflecting a well-documented westward migration that began during the late 18th century. By integrating genealogical information from 23andMe research participants who share the closest genetic connections to the St. Mary's individuals, we propose possible identities for otherwise nameless individuals buried at St. Mary's Brick Chapel. In this study we provide a powerful framework for reconstructing historical migrations, localizing biogeographic origins, and restoring the identity of unknown historical individuals, by combining genetic and genealogical approaches

POSTER PRESENTATION ABSTRACTS

27 AUGUST 2025

Ancient biomolecules and museums

Extracting Microbial DNA from Waterlogged Archaeological Wood

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Keywords: DNA extraction, Microbial community, Waterlogged archaeological wood

The quality of microbial DNA extracted from waterlogged archaeological wood is critical for generating high-quality sequencing data for microbial community analysis. Factors influencing microbial community composition includes the DNA extraction method, which directly impacts downstream sequencing results. This study evaluates the efficacy of three commercially available DNA extraction kits - Bead-Beat Micro AX Gravity, DNeasy PowerSoil Pro and MagAttract PowerSoil Pro DNA - using samples of waterlogged archaeological wood from a Neolithic fish-trap system dating to c. 4300-2800 CE preserved under anoxic conditions. The Bead-beat kit yielded the highest DNA quantity, as measured by Qubit fluorometry. Samples were sequenced using Illumina 16S amplicon sequencing of the V3-V4 region to explore the microbial composition. Microbial community analysis identified bacteria such as *Marinimicrobia* and *Desulfobacterota*, which are commonly associated with marine and low-oxygen environments, indicating the successful recovery of environment-specific microbes. The most abundant bacteria, *Dehalococcoidia*, are consistent with prior findings in wooden shipwrecks or nearby sediments (Hampel et al. 2022).

Comparative analysis of microbial community profiles revealed minor differences between the extraction methods, with the Bead-Beat kit recovering the highest number of amplicon sequence variants (ASVs). Additionally, the sample location within the wooden pole (splintwood vs. heartwood) influenced microbial composition, with consistent patterns across all three kits. This study highlights the importance of selecting an appropriate DNA extraction method to optimize microbial community profiling in waterlogged archaeological wood. These findings contribute to methodological standardization and provide valuable insights for advancing biomolecular archaeology and the preservation of cultural heritage.

Reference

1. Hampel, J. J., R. D. Moseley, R. L. Mugge, A. Ray, M. Damour, D. Jones and L. J. Hamdan (2022). "Deep-sea wooden shipwrecks influence sediment microbiome diversity." *Limnology and Oceanography* 67: 482-497. doi: 10.1002/lno.12008

A Collaborative Approach to (Biomolecular) Archaeological Research: The Tromsø University Museum Framework

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Keywords: Collaborative framework, Interdisciplinary dialogue, Research museum

As a university museum in Norway, The Arctic University Museum of Norway provides a multi-disciplinary institutional framework where diverse stakeholders in archaeological research—field archaeologists, curators, conservators researchers, and biomolecular specialists—are integrated within a single institution. This collaborative structure supports a holistic approach to archaeological studies, combining theory, methods, and reasoning from a wide range of fields such as art history, history, anthropology, ethnology, chemistry, etc. Among the newer fields incorporated into this multidisciplinary approach are ancient biomolecules, whose analysis requires careful methodological and ethical considerations.

By working together, we tackle key challenges in sampling archaeological materials, such as ensuring the representativeness of the archaeological record, evaluating preservation states, and selecting appropriate methodologies. At Tromsø University Museum, these decisions are guided by open interdisciplinary dialogue, ensuring sampling strategies balance scientific advancement with ethical responsibility towards cultural heritage.

This model allows us to critically explore the potential of museum collections as invaluable repositories for biomolecular research. In line with our national responsibility for cultural heritage management to ensure open access to archives, including metadata, we strive to ensure that the data generated is stored in standardised formats that facilitate accessibility and readability for future research. This practice guarantees long-term documentation of the analyses and enhances future research opportunities.

This example demonstrates how a collaborative, interdisciplinary environment can both advance biomolecular research and protect cultural heritage.

ZooMS and Isotopic Analysis of El Hammar and El Hattab II Caves

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The identification of fossil taxa is the main goal of paleontology. Knowing the composition of communities is fundamental to characterizing past ecosystems and understanding changes in environment and climate. However, taxonomic identification of animal remains is not always straightforward due to the high degree of fragmentation that bones tend to present.

Recently, a technique of molecular biology (proteomics) has been developed for the identification of bone fragments, through the collagen peptide fingerprinting by mass spectrometry (ZooMS). This requires the collagen to be removed from the bones and that this collagen maintains a good degree of preservation. Breaking the collagen molecule between specific amino acids with trypsin, the obtained set of peptides can be identified by MALDI-TOF (Matrix-Assisted Laser desorption/ionization, time of flight). Some of them are characteristic of a certain taxon, and their presence or absence will allow us to differentiate the taxon from which they come, working as true markers.

In this work we applied those techniques to animal bones remains from the cave sites of el Hammar and Hattab II, both located in northern Morocco during End-Pleistocene and Holocene.

In those two sites, the bone sample is usually fragmented and therefore taxonomic identification was only possible in a low percentage of the remains. However, the results obtained allowed us to broaden the faunal spectrum and the number of remains identified, including ursids, felines and several ungulates. Not only that, but also, we make an isotopic analysis for our samples to reconstruct the ecology of our species: the collagen, and then analyzing the carbon and nitrogen isotopic ratio. In addition to reporting on the type of diet of the organisms, they reflect environmental variables such as the degree of tree cover, temperature, humidity of the environment or the altitude and orographic conditions of the area where they lived.

Unprovenanced bronze ritual vessels from museums reveal contents through bottom-up proteomics

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Keywords: Bronze vessels, Proteomics, Early China

Unprovenanced ancient Chinese ritual bronzes have provided crucial data for the understanding early dynastic Chinese ritual practices but remain underutilized in biomolecular research. Textual evidence indicates that such vessels were used by the elites to offer food and drink to their ancestors to maintain the relationship between the living and the dead, obtain favour and guidance on personal and stately matters. Thus understanding vessel usage is crucial for reconstructing ancient ritual practices and the social status of specific foodstuff. We analysed corrosion from eight bronze vessels from the late Shang (1250–1046 BCE) and early Western Zhou (1046–977 BCE) periods held by the Museum Rietberg, Zurich, using bottom up proteomic analysis (LC-MS/MS). Despite the low protein recovery and the high level of keratin contamination, we were able to detect protein matches for ginger (*Zingiber officinale*) and *Monascus purpureus* (a fermentation agent) in a *hu* wine vessel. These findings provide the first biomolecular evidence for the storage of fermented grain beverages in a *hu* vessel and reveal the sensory experience of late Shang worship, where foodstuff was heated to create odors that rose to the ancestors. The ingredients imply continuity in the fermentation practices relating to ritual food culture from the Neolithic to the Dynastic period, since archaeobotanical research has found microscopic remains of *Zingiber* and *Monascus* in Neolithic ceramic vessels. Our analysis demonstrate that unprovenanced bronze museum artefacts may still hold the key to ancient practices.

Genomic insights into *Hippopotamus amphibius* through museomics

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Keywords: Museomics; Population genomics; Biodiversity

Ancient DNA studies and museomics are reshaping the value of museum collections, revealing their potential to bridge cultural heritage and genomics. By extracting genetic information from preserved specimens, these approaches facilitate significant advancements, including the reconstruction of phylogenetic relationships, the mapping of genetic erosion, and the monitoring of species biodiversity. This study focuses on *Hippopotamus amphibius*, a species which is still underrepresented in genomic research. Five subspecies have been proposed based on morphology, but their validity remains controversial due to limited molecular characterization, which has been confined to partial fragments of the mitochondrial DNA and few nuclear markers. To address this issue, we sequenced the genomes of historical museum specimens from the last few centuries and integrated them with data from contemporary individuals. We applied population genetics methods to: (i) comprehensively characterize the genomic history of *H. amphibius*, (ii) clarify intraspecific diversity and subspecies classification, (iii) explore the demographic history of the species, including the potential impact of human activities since the XX century, and (iv) investigate the evolutionary history of the *Hippopotamidae* family, by also comparing it with *Choeropsis liberiensis*, for which a few specimens were also sequenced. Our findings highlight the role of museum collections as significant archives of biodiversity and their evolution over time. By integrating these findings into exhibits and educational programs, we aim to foster public engagement with conservation and biodiversity and emphasize the importance of genomic data in enhancing the invaluable natural heritage preserved in museums.

Tracing time's footprints: exploring feeding ecology and historical changes of Mediterranean common dolphin (*Delphinus delphis*) over two centuries

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Keywords: stable isotopes, ecological history, *Delphinus delphis*

The Mediterranean population of the common dolphin (*Delphinus delphis*) presents an enigmatic natural history in the last centuries. Once widely abundant in the 19th and early 20th, the species faced a significant decline across most of the basin by the late 1960s. The causes and mechanisms behind this decline remain poorly understood. The IUCN classifies the Inner Mediterranean population of common dolphins as Endangered. Here, we analyzed stable isotopes of nitrogen and carbon ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) on skeletal remains collected in Mediterranean natural history museums to investigate spatio-temporal trophic shifts and niche partitioning of the Mediterranean common dolphin over the past 200 years. Comparisons with contemporary populations of *Stenella coeruleoalba* and *Tursiops truncatus* which co-occur in the Mediterranean, revealed a marked change in the common dolphin's ecological history. We found that historically common dolphins occupied a well-defined isotopic niche separate from other species, while the contemporary populations shifted to overlap those of the striped dolphin. We cannot exclude that this significant shift could be driven by habitat and trophic competition with human activities as prolonged and intense fishery stock exploitation has occurred in the basin. However, it appears that a key factor in the common dolphin's decline was competition with the widespread striped dolphin, which has effectively "squeezed" the common dolphin's habitat. This is the first long-term evidence of an ecological and/or trophic shift of the Mediterranean common dolphin, which could be the starting point for understanding the causes and impacts of the species' near-collapse.

Genetic Check-Mate: Unlocking the Secrets of the Sandomierz Chessmen

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Keywords: Medieval chess, Ancient DNA, mtDNA

Sandomierz chessmen are among the most valuable relics in the collection of the Sandomierz Castle Museum (Poland). This set is the only nearly complete collection of early medieval chess pieces discovered in Central Europe, with only three pawns missing. Made from bone, these pieces are also among the smallest medieval chess pieces, with a maximum height of only 25 mm. All pieces were discovered during archaeological excavations in 1962, under the floor of a medieval building near a Dominican monastery. Owing to uncertainties in the chronology of the archaeological context and available archaeozoological evidence, various hypotheses have been proposed regarding the origin of the bones used to craft chessmen. These hypotheses range from that of marine species to that of exotic terrestrial animals. To identify the animal species from which the Sandomierz chessmen were carved, we conducted genetic analysis of the three pawns using targeted mitochondrial DNA enrichment and high-throughput sequencing. The chessmen were divided into two sets based on their ornaments. For the analysis, we selected one pawn from each set. A third pawn, thought to be a replacement for a missing piece, was considered to have been produced later than the entire set. Mitochondrial DNA analysis revealed that each pawn was made from the bones of a different animal: one from a domestic horse (*Equus caballus*), another from a cow (*Bos taurus*), and the third from a red deer (*Cervus elaphus*). However, it was not possible to determine the precise geographical origins of these animals.

A Glimpse into the Prehistoric Qatari Society

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Keywords: Archaeology, Stable isotopes, Ancient DNA, Evolution

Background. The Arabian Peninsula holds a wealth of historical information. Yet, the prehistoric societies and population movements of Middle Easterners are only marginally understood.

Methods. Here we have excavated burials within the Qatari Peninsula and applied a multidisciplinary approach of archaeological, morphological and molecular analyses to expand our current knowledge of prehistorical Qatari societies during the Neolithic to the Late Iron Age. By also combining samples retrieved from previous excavation campaigns, a total of 41 cairns and 64 skeletons were analyzed. The remains retrieved from 64 individuals underwent a detailed morphological assessment. Seventy-five samples were submitted for bioapatite dating and Sr, C and O stable isotope analyses. A total of 20 petrous bone/tooth samples were processed for ancient DNA analysis.

Results. Our samples cover a transect of time spanning from the Neolithic to the Late Pre-Islamic period (6.5-1.5 kya). Several individuals were found with musculoskeletal stress markers and dental pathologies, suggesting that the individuals studied might have used the teeth for daily activities. Periostitis and Schmörl's nodes found in the skeletal remains could suggest the presence of metabolic diseases, and degenerative pathologies.

All the human and faunal samples analyzed displayed $^{87}\text{Sr}/^{86}\text{Sr}$ values within the local Sr range. Overall, the stable isotope dietary results suggested a diet with high amount of input C4/CAM plants. Shotgun aDNA sequencing and capture experiments on the studied skeletal elements showed little to no aDNA preservation. The aDNA sequencing data is still being evaluated.

Conclusion. This project provides preliminary insights on the prehistorical Qatari society.

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Embodied Identities in Life and Death

Early Colonial Interactions and Indigenous Enslavement in the Spanish Colonized Caribbean Through an Archaeogenomic Lens

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This study investigates the El Chorro de Maíta site in Cuba, one of the largest indigenous Caribbean cemeteries, dating to the peri-colonial era. This period was characterized by the European invasion, displacing indigenous societies through systems of forced labor, such as the *encomienda*. Concurrently, the Caribbean became a significant trading hub among the Trans-Atlantic slave routes.

Excavations at El Chorro de Maíta, ongoing since the 1980s, have yielded over 150 individuals. Combined mortuary, osteological and multiple isotope analysis have identified individuals of local origin, and non-local individuals, some from other places in the Caribbean, extending to possibly the Yucatán Peninsula in Mexico. This aligns with historical evidence of forced relocation of indigenous groups within the Caribbean and Central American regions due to slavery. Additionally, remains of an individual of “European” ancestry and associated material culture, as well as an individual of “African” descent were identified in the site. The diversity observed at this site exemplifies the complex cultural and genetic exchanges that occurred during the European colonial period in the Americas.

Of the teeth recovered from the site, we successfully obtained the genomes of 32 individuals, and were able to conduct population genomics analysis. Our results have so far corroborated the ancestral assignments based on archaeological, osteological and isotopic evidence.

The genetic data is integrated with bioarchaeological data and grave good analysis to unravel the complex population interactions at the height of colonialism's impact in Central and Southern America. Adding a genetic characterization of the individuals will help produce a more detailed reconstruction of the type of society that existed at El Chorro, as well as the circumstances surrounding each individual.

Embodied Identities in Megalithic Funerary Spaces: Revisiting the Aveyron Dolmens through Archaeo-Anthropology and Palaeogenomics

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Keywords: Megalithism, Funerary practices, Museum collections

The LINK project explores the functioning of human communities between the Atlantic and Mediterranean seaboard during the Neolithic and Bronze Age, using an approach combining archaeological, anthropological and palaeogenomic studies. This work not only sheds light on burial practices and population dynamics but also demonstrates the scientific potential of re-evaluating underutilized and poorly documented museum collections.

With more than 800 dolmens, the Aveyron region in France has one of the highest geographical concentrations of megalithic monuments in Europe. This density makes the region an ideal laboratory for studying the potential interactions among contemporary funerary monuments. However, these archaeological and anthropological collections, originating from early excavations, suffer from incomplete documentation and out-dated study. In collaboration with curators from the Musée Fenaille (Rodez, Aveyron), we reassessed osteological remains from twelve dolmens and two sepulchral caves. Genomic data from 80 individuals revealed remarkable DNA preservation, despite the collections' complex histories. Archaeoanthropological studies highlighted different uses of these monuments, notably linking burial space management and the number of individuals deposited. Palaeogenomic results further clarified funerary practices, occupation dynamics and highlighted links between sites giving unprecedented insights into the social organization of these communities.

By bridging funerary archaeo-anthropology and palaeogenomics, this research revitalizes overlooked museum collections, reaffirming their scientific and cultural significance. Beyond the production of new knowledge, it underscores the essential role of curatorial collaborations in preserving and reinterpreting these assemblages, fostering new connections between past identities, heritage, and contemporary audiences.

Medieval Work and Disability as Embodied Experience: The Case of ELS215 from Bone to Biomolecule

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Keywords: Isotopes, Biomolecules, Calculus

This paper will present an extended osteobiography of a female individual (burial designation ELS215) buried in the medieval monastic cemetery of Elstow Abbey (Bedfordshire, UK). We present evidence from osteological analysis, dental calculus analysis, proteomics, and stable isotope analysis to tell an integrated story of a medieval woman who both performed manual work within her community and experienced extreme physical impairment from advanced Pott's Disease (tuberculosis of the spine). We offer evidence suggesting the type of work in which this individual engaged, as well as her diet, oral health, and possible ingestion of medicinal materials. We also demonstrate the use of forensic facial and posture estimation and graphic art to share ELS215's story with a broader public. This case study is part of the AHRC-funded project Word of Mouth: Embodied Stories of Premodern Women at Work. The Word of Mouth project seeks to recover, contextualise, and synthesise women's stories, providing an unprecedentedly fine-grained picture of women's work and occupational health in monastic contexts. Moving between archive and laboratory, we integrate historical research with archaeological science to transform the way we see women and disabled individuals in the European past.

Cloistered lives: the case of S. Maria della Stella, Saluzzo (Italy)

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Keywords: Monastic women, proteomics, stable isotopes

As part of the project “Word of Mouth: Embodied Stories of Premodern Women at Work” funded by the UKRI Arts and Humanities Research Council, we have undertaken a comprehensive analysis of 48 human skeletal remains to explore the daily life of a post-medieval Cistercian nuns’ community in Saluzzo (Piedmont, Italy). The existence of historical documents related to this community makes the S. Maria della Stella assemblage exceptional, providing a rare glimpse into the lifestyle of a cloistered religious order as well as their 18th-century dietary habits. The micromorphological analysis of “micro-débris” of diverse origin (animal, plant, mineral) and lipid profiling of dental calculus confirmed the consumption of maize and other starch-based foods, while milk proteins were detected in one individual. Stable isotope analyses were conducted to assess the dietary habits of the individuals and were combined with osteology, which documented the impact of work and religious activities on the monastic women’s skeletons, including the presence of pathological markers signalling kneeling. The data were interpreted in the light of potential differences in status within the S. Maria della Stella Monastery, shedding light on the cloistered lives of the nuns and demonstrating the fundamental need for integrating bioarchaeological findings and historical documentation.

A Genomic Transect through the Later Prehistory of Luxembourg

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Luxembourg is well-known in ancient DNA research for Loschbour, the high-coverage genome that served as reference for Western Hunter-Gatherer populations (Lazaridis et al. 2014). Bordered by the Moselle River, a tributary of the Rhine, Luxembourg provides a regional snapshot of population changes observed at continental level. A project initiated in 2021 with the support of the Institut National de Recherches Archéologiques (INRA) aimed to find out more about the populations that occupied the territory of the Grand Duchy of Luxembourg during the Neolithic, Bronze and Iron Age (Brami, Zedda et al. 2023). In total, 18 new shotgun-sequenced genomes (including two to >10x) were produced from human bones and teeth of 13 archaeological sites, dating from the 5th to the 1st millennium BC. As anticipated, the new genomes reflect major temporal shifts in population structure, with the arrival of "Neolithic" and "Steppe" ancestries. Interestingly, the diversity in regional burial practices at any given time does not correspond to a similar diversity in genetic ancestry. For instance, the ancestry profiles of those buried in collective graves under rockshelters and individual flat graves during the 3rd millennium BC are virtually identical. We also report on the analysis of IBD sharing patterns and pathogens.

Exploring the Link Between Human Mobility Changes and Spatiotemporal Variations in Burial Rite Practices

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Describing and interpreting cultural history and transmission has been a central focus of archaeology for over a century. New information coming from ancient DNA has revealed the fundamental role of demography in shaping both genomic and cultural change. Yet, the effects of demographic processes on the spatial and temporal distributions of cultural and genomic patterns are still poorly understood. Here, we examine the relations between demography and space-time patterns of genomic variation and cultural traditions, by testing whether shifts in genomic patterns correspond to spatiotemporal changes in burial rites. Our approach uses data accessed from the Big Interdisciplinary Archaeological Database (BIAD), linking mortuary information from approximately 4,200 individuals with genetic ancestry and mobility data inferred from over 1,300 human genomes, spanning vast parts of Western Eurasia over the last 10,000 years. By integrating and modeling these diverse datasets, we aim to provide a comprehensive understanding of how human mobility and demographic change intersect with cultural traditions, offering new insights into the dynamics behind culture history, and the extent to which genes and culture are transmitted in parallel.

Archaeogenetics in the Traisen Valley: Mobility and Social Structure during the Iron Age in northeast Austria

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Spanning from eastern France to the northern Balkans, the Early La Tène culture of the central European Iron Age is associated with high levels of artistic crafts, as well as intensification of long-distance trade relationships with groups from Scandinavia to the Mediterranean. In this project, we analysed ancient DNA from 235 individuals buried at seven sites within the Traisen river valley in northeastern Austria, a core region of the Early La Tène period, to understand the expression of community structure and cross-regional connections within a microregion. Our results showed high levels of genetic relatedness where closely related individuals were buried in physical proximity within the same burial grounds, while distant IBD relationships also spanned across the valley. The majority of burials shared a similar genetic profile most comparable with Iron Age individuals from Germany and France. However, ca. 15% were genetically more similar to contemporaries from northern and southern Europe. About half of these individuals with non-local ancestry were also part of reconstructed pedigrees, where the level of genetic dissimilarity decreases from older to younger generations. Finally, we combined the genetic and osteological results with categorised grave inventories and could identify a set of deposited objects associated with genetic sex, as well as a general trend of lower numbers of object types being present in the graves of individuals with non-local genetic profiles. Our project illustrates the value of small-scale studies utilising multidisciplinary approaches to gain insights into past behaviour and identities.

Unwrapping Past Embalming Practices through the Multidisciplinary Analysis of Egyptian Mummified Heads

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Keywords: Embalming, Lipids, Radiocarbon dating

Excavations in Egypt in the early 1900's by the Italian Archaeological Mission yielded a wealth of anthropological remains, collectively referred to as the "Marro collection." This collection includes a large corpus of mummified heads that for over 100 years have never undergone a systematic study that also includes their chronological contextualization. The current research sheds light on this collection through the application of radiocarbon dating and lipid residue analysis. Here we present new AMS radiocarbon dates for 23 samples of wrapping material and hair, which show that the majority of the studied remains date to 1000–50 BCE, including the Third intermediate, Persian, and Ptolemaic periods. Four heads stand out as unique, dating much earlier to the Predynastic period and the Old and Middle Kingdoms. Gas Chromatography-Mass Spectrometry was then applied to characterize the embalming practices and identify whether there was diachronic variation. The biomarker approach allowed the identification of conifer and *Pistacia* resin, beeswax, castor oil, bitumen, and potentially animal fat, often in diverse combinations. This suggests that while preserving the physical body of the deceased was essential to ensure the soul transitioned to the afterlife, there was some flexibility in the substances that could be used to treat the head and bandages, rather than one consistent recipe.

Romans Versus Barbarians: a 3-5th Century Genomic Study from the Carpathian Basin

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Keywords: Genomic diversity, Differences, eastern and western side of the Danube

The Carpathian Basin was an interaction zone between the Barbarians and the Romans during the 1-5th centuries CE. This paper summarises new genomic datasets from the Roman period Carpathian Basin. Pannonia gave home to various Romanised people West and South of the Danube here, while the Sarmatian tribes of Iranian origin occupied the Great Hungarian Plain, east of the Danube in the 1st century CE, and persisted there until the Hunnic period.

In the present study, we analyzed 50 samples from the 3rd–5th centuries from the Pest Plain, located east of Pannonia as part of the project NKFI K142304. We carried out detailed statistical analyses and explored kinship networks. Our findings indicate that the genetic composition of the 3rd–4th century Sarmatian individuals is primarily homogenous, with similarities to Central European populations. However, the genetic makeup of the 5th-century individuals reveals elements of both East Eurasian and Near Eastern/Caucasian origins, likely introduced through new eastern population influxes during the Hunnic period. On the western side of the Danube, after analysing approximately 100 Roman-period individuals in the framework of the MTA-BTK Momentum Bioarchaeology Research Programme, we observe a significant variety of ancestries, in stark contrast to the more homogeneous 3rd–4th century Sarmatian communities. With this paper we present our initial investigation into intercultural and interethnic genetic connections among contemporaneous communities on the edges of the Roman world.

Ancient DNA exposes the presence of *Yersinia pestis* in Copper Age Italy

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Keywords: aDNA, *Y.pestis*, Coinfection

The infection of humans by the causal agent of Plague, *Yersinia pestis*, has been attested as far back as 5500 BP in Eastern Siberia¹. Although the specific etiology of the disease caused by these Stone Age strains remains unclear, the bacterium swiftly spread westwards towards Europe, reaching the continent during the Neolithic.

In this study, we analysed 9 genomic samples originating from 6 different human individuals modelled to date to 5025 BP (cal.) from the site of Grotta della Spinosa, in Tuscany, Central Italy. Metagenomic screening of these samples reveals one individual (GSP013) to be co-infected by *Y. pestis*, *Hepatitis B virus (HBV)* and *Human parvovirus B19*. At least two further individuals were infected with *HBV*, indicating its wider presence within the community.

The phylogenetic placement of *Y. pestis* in GSP013 shows that this strain is closely related to the earliest LNBA Caucasus strains of the bacterium, basal to later European diversity. This represents the earliest evidence of *Y. pestis* in the Italian peninsula (and Southern Europe more widely) to date, predating previously discovered strains by ~300 years. This contributes to our understanding of *Y. pestis* transmission in prehistoric Europe and offers insights into disease dynamics in Italian communities during the 3rd millennium BCE.

References

1. Macleod, R. et al. Lethal Plague Outbreaks in Lake Baikal Hunter–gatherers 5500 Years Ago. *bioRxiv* (2024).

Bringing up Baby: Evaluating the Physiology/Diet Interplay on Isotope Composition

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Keywords: Isotopes, Infant, Nutrition

Food is central to a person's life and diet in early life has especially important repercussions on the health, development and survival of the child. Incremental analysis of nitrogen and carbon stable isotope ratios in dentine from human remains has become an essential tool to help archaeologists answer questions such as how long babies were exclusively breastfed for or at what age they were weaned from the breast. However, there are uncertainties around the influence of metabolic factors like stress and growth on isotope values in early life. The metabolic effect has implications when interpreting isotopic data in relation to infant feeding, but also present the potential to provide information on nutrition and health during this critical life period. This paper uses stable light isotope longitudinal data from modern babies with known feeding, health and weight histories to explore how infant physiology and diet interact to shape the isotope composition of protein tissues and how such new understandings of the physiological effect affect the interpretation of archaeological isotopic data.

Ancestry and Kinship in a Romano-British Cemetery at Poundbury Camp, Dorset (UK)

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Keywords: Romano-British, Kinship, Ancestry

The extent to which the local population of Britain integrated with individuals associated with the Roman occupation (43-410 CE) remains a topic of debate. Written sources highlight the migration of peoples from across the Roman Empire to Britain, but there have been limited genetic studies focusing on within-life migration of Roman era individuals. Kinship in Romano-British burials has been mainly assumed from archaeological burial context, and is rarely explored in depth.

This research focuses on investigating the ancestry and kinship of individuals selected from a Romano-British cemetery population (Poundbury Camp, Dorset, UK), to build a greater understanding of society in the later Roman period.

Poundbury Camp cemetery served the regional centre of *Dunovaria*, the location of modern day Dorchester in Dorset, UK (4th- early 5th centuries CE). The cemetery is notable for its organised layout and the use of mausolea, which has resulted in family connections and social hierarchies to be postulated. Here, aDNA techniques are applied to individuals from the site to understand any social or ancestral basis to the organisation of the cemetery. Individuals represented by a range of burial practices were selected including those from east-west and north-south burial orientations (potentially related to Christian vs pagan beliefs), individuals from inferred elite and standard burial contexts, and individuals previously estimated to be from familial groups. This allows investigation into kinship and ancestry to assess differences between groups based on their archaeological contexts.

Insights into Neolithization in Upper Mesopotamia: Çayönü Tepesi

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Keywords: Archaeogenomics, PPN, Upper Mesopotamia

Upper Mesopotamia was one of the key regions in the Neolithization process. Çayönü Tepesi, in particular, holds significant importance due to its continuous occupation from the beginning to the end of the Pre-Pottery Neolithic period. This uninterrupted timeline provides an opportunity to study cultural developments and transitions at this early Neolithic site, from changing architectural trends to the management and domestication of animals. We have generated 50 ancient shotgun genomes from Çayönü Tepesi, spanning the Pre-Pottery Neolithic (approximately 8500 – 7500 BCE) and compared these with data from settlements in Upper Mesopotamia and neighbouring regions. By analyzing the changing genetic profiles, genealogical relationships, postmarital residence practices, and consanguinity patterns among individuals buried at Çayönü Tepesi in both residential and communal contexts, we aim to understand the social and demographic dynamics of this site and its connections with neighbouring regions during this transformative period.

Detecting Osteoporosis in Past Populations - A Bioarchaeological Approach

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Keywords: Osteoporosis, Proteomics, Lipids

Osteoporosis is a metabolic bone disease characterised by reduced bone mass and skeletal fragility. This pathology has a complex causative nature, therefore investigating the prevalence and aetiology of osteoporosis in past societies offers a broad set of insights about the life conditions of pre-modern individuals. Even though multiple methodologies have been applied in bioarchaeological and palaeopathological studies of osteoporosis, there are still considerable limitations in definitive diagnosis of this disorder in ancient skeletons. Here, we explored the potential of shotgun proteomics and targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of steroids to investigate the occurrence of osteoporosis in a mixed-age group of female monastic women who lived in the 18th century in Piedmont, Italy. Proteomic analysis included development of a novel protein extraction method, aimed to reduce the abundant signal from collagen, thereby enhancing the detection of non-collagenous proteins (NCPs). This optimised protocol was used in a comparative tandem mass tag (TMT)-based quantitative proteomic experiment between osteoporotic and control groups in modern and ancient sample pools. Targeted LC-MS/MS analysis of steroids was applied for the first time to human bone tissue, demonstrating the potential of this technology to detect and quantify steroid molecules in modern and archaeological samples.

Slices of Teeth, Slices of Life: Serial Stable Isotopes and Palaeoproteomics for the Study of Ancient Mesoamerican Populations

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Keywords: Dietary practices, Weaning age, Amelogenin

Ancient Mesoamerican human remains have been used for some of the earliest stable isotope analyses. These pioneer works allowed the identification of a strong reliance on maize and C₄-derived products, as well as an overall limited meat consumption. Yet, recent developments in biomolecular archaeology have unlocked the possibility of accessing many more details of the life of deceased individuals that have not been applied to their full potential to Mesoamerica, yet. In this study, we used carbon and nitrogen dentine serial stable isotopes on thin slices of teeth to identify the evolution of dietary practices, including weaning patterns. We combined this analysis to the palaeoproteomics of enamel for amelogenin-based sex identification. Our approach was first applied to five individuals from two sites in Western Mexico (northern Michoacan and southern Guanajuato) to better understand the diversity of weaning and feeding practices in interrelated cultural groups. These results are further combined with the analysis of bone collagen and bioapatite stable isotopes (C, N, O) from these individuals as well as 50 others from the same region, along with animals and plants. With this approach, we gain a deeper understanding of the territory exploited by each of the groups and how they changed through time, but also how the recruitment of the population into the different funerary structures may have played a role in this differentiation.

Solving the Mystery of M141: A Multi-Disciplinary Re-Assessment of Commingled Human Remains Outside the City Walls of Romano-Byzantine Ibida (3rd-5th C. CE) (Romania)

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Keywords: Isotopes, Commingled, Mobility

Commingled assemblages of human remains present the most complex and often neglected deposits in biomolecular research. This study employs a multi-disciplinary approach (*chaîne opératoire*, archaeoethanatology, osteology, isotopes, proteomics), including the first multi-isotopic ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$, $^{86}\text{Sr}/^{87}\text{Sr}$, $\delta^{18}\text{O}$, Pb) study of any Roman period (1st-7th c CE) site from this region, to re-examine a suspected ‘massacre of outsiders’ in a commingled deposit known as M141 (MNI=37, n=20) discovered at the Romano-Byzantine *Limes* fortress Ibida (Romania). Remains from M141 are analysed in the context of a wider contemporaneous necropolis (n=71) including a high-status mausoleum (n=12).

Analysis of the human remains reveals that M141 is a secondary deposit, comprising adults and non-adults, both sexes and minimal perimortem trauma. Intriguingly, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from bone collagen and bulk dentine identify distinct dietary histories, with many of the individuals from M141 consuming C_4 foods, likely millet, in contrast to the C_3 -dominant diet of the contemporaneous high-status individuals and the necropolis population. $\delta^{34}\text{S}$, $^{86}\text{Sr}/^{87}\text{Sr}$ and $\delta^{18}\text{O}$ analysis of tooth enamel identify potential non-local individuals within both M141 and the necropolis. Pb isotope concentrations from tooth enamel are variable, but the high-status group stands out for possessing consistent Pb ppm values >0.5, suggesting long-term Roman cultural influence.

Altogether, multi-proxy analysis indicates that the M141 group were not victims of a massacre. Isotopic analysis of the differing burial contexts at Ibida has unmasked the diversity exhibited at this site, underscoring the underappreciated role of commingled deposits in reconstructing complex human experiences.

Redressing the Balance: Exploring Personhood in Roman-period York Using a Multi-isotope Approach

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Keywords: Personhood, Stable isotopes, Demography

York was a significant provincial capital strategically placed on the northernmost fringe of the Roman Empire between the 1st to 5th centuries AD. Excavations across York have revealed a substantial and complex Roman-period funerary landscape of over 1,000 known burials, including contemporaneous cremation and inhumation practices and extraordinary funerary behaviour (eg: decapitation and gypsum burials). While previous research on York's Roman-period population over 10 years ago successfully employed isotopic, osteological and aDNA analysis, these studies were focused on a subset of the Roman population from four cemeteries, resulting in a sampling bias towards males. This dataset provided an emerging image of a dynamic and diverse society which remained deeply connected to the rest of the Roman Empire despite its location on the fringes.

This research project analyses a further 193 burials from 14 cemeteries, to explore a representative view of life in Roman-period York. Through a multi-isotopic (C, N, O, Sr, S, Pb) approach across the known funerary landscape, an objective picture of population diversity is sought to explore how diet (n=193) and mobility (n=60) varied at an intra- and inter-individual and population scale, with preliminary results already indicating the potential for greater variability in female diet in the city. The incorporation of interdisciplinary evidence from funerary contexts with the osteological and biomolecular data provides the necessary contextual dimension to explore all social groups, answering questions relating to personhood, mobility and social behaviours and highlighting new insights into the lived experiences of the people of Roman-period York.

Archaeogenetic Evidence of a Late Bronze Age Population Crash in the Cantabrian Region (Northern Iberia)

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Keywords: Archaeogenetics, Population bottleneck, Iberia Late Bronze Age

The Late Bronze Age period (1200-900 BCE) in Northern Iberia is characterized by a scarcity of human remains. One exception is El Espinoso cave (Ribadedeva, Spain), located on the central coast of the Cantabrian region and containing a collective burial dated to 1235-1099 cal. BCE. To provide a window into the life and death of the people buried at El Espinoso, we generated genome-wide data from 39 individuals. Our results demonstrate that all were part of a close kin group organized around eight nuclear families with a predominance of sibling relationships, and that whoever placed the bodies must have been aware of these familial relationships, as members of the same nuclear families were closer together in the cave. The same mitochondrial lineage was present in 83% of the individuals and in all families, suggesting a social organization with a matrilineal bias. This group displays one of the strongest signals of endogamy in the whole archaeogenetic record, with evidence of both sustained low population sizes and recent close kin unions in all analyzed individuals, including a case of a woman reproducing with her paternal uncle. Based on distant family links with an individual who died ~150 years earlier from flea-adapted plague at El Sotillo (180 kilometers to the southwest), we postulate that El Espinoso community represents the final stages of a plague-related population collapse in Northern Iberia during the last centuries of the 2nd millennium BCE.

Ritual Murder of Women: Biomolecular Insights into the Middle Neolithic Case of Saint-Paul-Trois-Châteaux (Rhône Valley, France)

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Keywords: Sacrifice, Neolithic, Women

The identification of ritual murder cases in the archaeological record and the characterization of human sacrifice in past societies remain subjects of ongoing debate. In the context of the European Neolithic, several hypotheses have been proposed to interpret ritual killing, including (i) retainer sacrifice, in which individuals are killed to accompany someone of higher social status in death, and (ii) agrarian rituals, particularly those involving women and symbols of agriculture. At the site of Saint-Paul-Trois-Châteaux (Middle Neolithic, Rhône Valley, France), the atypical burial positioning of two women, within a structure aligned with the solstices, along with their association with deliberately broken grindstones, suggest a case of ritual murder. Extensive archeo-anthropological analyses indicate a ritualized form of homicidal ligature strangulation. Given the complexity of interpreting this archaeological evidence, an interdisciplinary approach incorporating biomolecular data is essential. To this end, we conducted genome-wide analyses of the two sacrificed women, a third woman buried in the same pit, and other individuals from the site. Despite the challenges posed by limited molecular preservation, our findings contribute to a deeper understanding of the transcultural phenomenon of ritual murder that influenced Early and Middle Neolithic sites across central and southern Europe.

Genetic Histories of Kinship and Ancestry in Roman Britain

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Keywords: aDNA, Roman, Britain

The genetic impact of the Roman Empire in Britain has been hypothesised to be low, especially in rural regions, with the bulk of ancestry likely deriving from the preceding Iron Age. Nevertheless, several isotope studies, as well as the identification of ancestry outliers, including in rural contexts, suggest a potential heterogeneous picture.

Here we focus on a small Late Roman rural cemetery (4th century CE) in Childrey Warren, Oxfordshire (England). The demographic profile ranged from neonates to older adults, and included one decapitated individual with the skull below his feet. We conducted an analysis of genetic relatedness and ancestry on eight individuals (average coverage: 0.9-1.9x). We identified two family clusters involving six closely-related individuals, one of which possibly spanning four generations. Two additional individuals shared a total of >40 cM in IBD (identity-by-descent) with several elements from one of the family clusters. Runs of homozygosity (ROHs) were rarely present and always <8 cM, indicating outbreeding. In line with this, the majority of the individuals carried ancestry broadly consistent with that found in Iron Age Britain, except for one adult male whose ancestry points to contribution consistent with continental sources with increased ‘Early European Farmer’-related ancestry.

The genetic results point to a small community burying their relatives in this small cemetery, but nevertheless in contact with other communities elsewhere. By combining aDNA with osteology and stratigraphy, interdisciplinary studies such as this one provide insights on different aspects of life and death during the Late Roman period in Britain.

Applying Ancient DNA Techniques to Formaldehyde-Fixed Samples: Reexamination of Landmark Case Auguste Deter

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Keywords: aDNA techniques, Alzheimer's disease, Historical fixed samples

Historical tissue samples from museums or preserved collections, treated with fixatives, serve as time capsules that present opportunities for re-examining past genetic, morphological, and ecological data, thus offering insights into evolution, conservation biology, medicine, and forensic science. DNA contained in historical fixed samples exhibits damage similar to that observed in ancient DNA (aDNA), including low quantity, short fragment size, and cross-linking between DNA and proteins, limiting its utility for genetic research. By applying aDNA techniques, we successfully retrieved DNA from fixed brain slides of Auguste Deter, the landmark case studied by Alois Alzheimer that contributed to the characterization of the disease, which was later named after him. We extracted DNA from slides handled and fixed at the Munich University Hospital for Psychiatry in 1906. Using single-stranded Next Generation Sequencing library preparation we isolated sufficient DNA (median fragment size around 28 bases) from approximately 0.5mg of input material to reconstruct the genome of this individual to about 8x coverage. Through both shotgun and targeted sequencing, we generated genome-wide nuclear data confirming that the DNA is from a Western European female. Currently we are analyzing potential causal single nucleotide variants (SNVs) in genes associated with early-onset Alzheimer's disease, to resolve conflicting statements about a possible genetic basis. Our study serves as a historical benchmark for understanding how the disease has manifested over time. The methodologies developed in this project provide a framework for analyzing other rare and medically preserved samples, paving the way for new insights into historical and pathological conditions.

Understanding Mediaeval Wallachian Diet, Health and Mobility through a Stable Isotopes and Paleopathology Analysis

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Keywords: Stable isotopes, Biological anthropology, Paleopathology.

This study explores dietary patterns, health outcomes, and mobility in Mediaeval Wallachia through stable isotope analyses, focusing on skeletal remains from the largest mediaeval cemetery in the region, located in Buftea, Romania. Spanning the 14th to 17th centuries, the site contains 10 double burials, one of which also features a reburial, offering a unique perspective on the social and biological aspects of this population. Using carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$), sulphur ($\delta^{34}\text{S}$) and oxygen ($\delta^{18}\text{O}$) isotope analyses, the research reconstructs paleodiets, examines nutritional stress and identifies mobility patterns among 21 individuals, comprising both adults and subadults. As reliance solely on traditional anthropological methodologies proves insufficient, the stable isotope perspective is crucial in assessing the influence of dietary variables on individual and populational health. The pathological analysis revealed a high prevalence of mortality among subadults, as well as non-specific infectious disease markers, including periostitis and osteomyelitis, likely exacerbated by nutritional deficiencies and potential epidemic events. The isotopic results indicate a mixed diet consisting of terrestrial C_3 and C_4 plants, animal proteins, and freshwater resources, with distinct dietary differences observed based on age and sex. Additionally, isotopic variations suggest mobility patterns, with some individuals likely originating from outside the immediate region, while the majority of the sample exhibits more homogenous local origin and mobility signatures. By highlighting the interplay between diet, disease, and social structure, the research contributes to broader discussions on human adaptation and resilience in historical populations, underscoring the critical role of stable isotope analyses in paleodietary studies.

Genomic Signs of Community Organisations and Social Changes in the Early Copper Age of the Eastern Carpathian Basin

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Keywords: Archaeogenomics, Copper Age, Carpathian Basin, Identity by descent, Kinship

This paper presents a genetic analysis of Late Neolithic (LN) and Early Copper Age (ECA) populations in the Carpathian Basin, utilizing 203 newly sequenced genomes from 4800-3900 BCE. By employing well-established population genomic and identity by descent (IBD) segment-sharing network genetic dynamics of the era and region.

Our findings indicate that complex changes from LN to ECA periods on the Great Hungarian Plain were primarily driven by internal social reorganizations rather than new immigrant arrivals, despite notable changes in settlement patterns, burial practices, and material culture. During the ECA, the genetic composition of populations in the Polgár area remained stable and relatively closed. Evidence from the extensively analyzed ECA Tiszapolgár-Basatanya cemetery highlights intricate kinship systems, including consanguineous relationships, suggesting a closed, patrilineal community with first-cousin marriages and a restricted effective population size. Estimates of effective population sizes of this community hint at a drastic community reorganization, with further examples from the ECA Carpathian Basin. Although this is a process yet to be fully understood, theories explaining this phenomenon are discussed.

Furthermore, we report that increased genetic diversity in the contemporaneous eastern sites of the Great Hungarian Plain, such as Urziceni-Vamă, indicates connections with Eastern/Southeastern Europe. This cemetery exhibited a more open community organization with frequently detected sibling relationships but limited consanguinity and a more diverse population, suggesting different social structures compared to those observed in the Polgár microregion.

These findings caution against overgeneralizations based on single-site genetic analyses and highlight the diverse social organization and biological relatedness within the Carpathian Basin during the ECA periods.

Out of the Mouth of Babes: Dental Enamel Sex Estimation and Oral Pathology in Sarmatian Children on the Great Hungarian Plain

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Keywords: Paleoproteomics, Bioarchaeology, Roman Period

The study of childhood health in the past has recently experienced an increased interest in the science community, although the emphasis has still very much been on the Western sphere and on the Roman Empire. Hoping to rectify this trend in childhood paleopathology, the current investigation shifts the lens of interrogation from the Roman Empire to its edges, and from gentes and provincials to the ‘barbarians’ outside their borders, to get a better understanding of health and disease in infants and children from communities east of the Roman *limes*. Non-adult individuals with age-at-death of ~1-12 years-old from 10 sites on the Great Hungarian Plain have been selected for macroscopic documentation of oral pathologies and childhood stress indicators: dental calculus, dental caries, and linear enamel hypoplasia have been identified visually and by probing based on well-established criteria and scored as present or absent on both deciduous and permanent teeth. Working with archaeological skeletal remains of pre-pubertal individuals makes osteological sex estimations based on morphological techniques very unreliable and problematic; therefore, we used a streamlined mass spectrometry-based workflow for the estimation of sex using targeted amelogenin peptides from dental enamel by employing a minimally invasive sampling strategy via acid etching and high-resolution liquid chromatography coupled to mass spectrometry using parallel reaction monitoring assay. One of the main objectives of this project is to investigate whether there were any cultural and biological differences between male and female children in Sarmatian communities; this is accomplished by integrating methods from bioarchaeology and paleoproteomics.

Archaeogenetic Insights into a Megalithic Burial Monument in Southern France

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Keywords: Population genetics, Kinship, Archaeology

Megalithic monuments are iconic features of the European prehistoric landscape and are associated with Neolithic farming communities along the Atlantic Façade, the North Sea, the Baltic Sea and the Mediterranean. France alone is home to over 4,500 dolmen structures, yet their precise function and significance remain subjects of ongoing debate. In this seminar I will focus on the site ‘Dolmen de l’Ubac’ (Goult, Vaucluse, France). This late Neolithic burial monument contains primary deposits and disarticulated remains of approximately 40 individuals, 22 of whom were genetically analysed. These remains show no distinct archaeological patterns of gender or social status and were linked to four distinct phases of use. By integrating archaeological, anthropobiological, and genetic evidence, this research investigates the burial practices and social structures associated with this site. Key questions include the biological characteristics of the buried individuals, potential kinship patterns, and whether selection criteria based on age, gender, or status are evident. This monument stands out as one of the few dolmen sites in Southern France with access to well-preserved burials, offering valuable insights into late Neolithic burial practices in the region.

Untangling identity in the Danish Viking Age Through Proteomics Analyses of Fur Potentially Used as Power Dressing

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Keywords: Proteomics, Viking Age, Fur

Extensive international trade and exchange of goods is one of the major characteristics of the Viking Age, the final era of the Scandinavian Late Iron Age (~800-1050 C.E.) Fur is often mentioned as a key commodity and an important marker of elite status. Written records describe how species such as arctic fox, beaver, marten, ermine, and sable had a particular economic value and signalling power in dress. Fur rapidly degrades, but has been preserved under favourable preservation conditions such as in deep chamber graves of the Viking Age elite and in connection to metal objects. Species identification of those present an opportunity to discover the use of non-local animals and speculate about the visual appearance of fur in the dress and its role in mortuary practices, visually reflecting the deceased individual's identity. Microscopy and DNA analysis have been used for species identification, but are challenged by harsh archaeological environments, whereas proteomics has shown to be successful.

Here, we identify fur samples from a rich, female Viking Age grave dating to around 1000 AD, Hørning, based on proteomics methods. Proteomics results along with the human remains of the graves and the other grave goods including textiles can help to shed light on the Viking Age individuals' affordability, identity in life as well as the practices surrounding her death. We finally compare the grave to two other rich female graves with a similar burial style and discuss the value of fur as a visual marker of status in Viking Age Denmark.

The Origin of Social Inequalities in Northern Italy: Clues from Ancient Genomes

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Keywords: Relatedness, Ancient genomes, Burials

Uncovering the dynamics promoting the onset and perpetration of social inequalities in prehistoric societies is a major challenge. We now have the opportunity to exploit archaeogenomic data to describe, with unprecedented resolution, past population structure and processes and to shed light on sociocultural dynamics (e.g., the lineage-based transmission of social status and wealth) which are the basis of the onset of inequalities. Two major population dynamics within the last 10,000 years impacted the genomic composition of Europeans: the Neolithic expansion and the Bronze Age migration from the Steppe; despite the importance of these events, our genetic understanding is mainly built upon pan-European sampling strategies, resulting in limited knowledge about the impact of these migrations at the level of single societies. In this project, we propose a high-resolution multidisciplinary study of three burial sites in Northeast Italy from the Neolithic, Eneolithic, and Bronze Age periods to infer the social and genetic structure and their possible change in this time transect. We will sequence whole genomes of 110 individuals to provide a detailed reconstruction of biological relatedness that, combined with a deep resolution chronology, the fine assessment of grave goods and burial practice, individual mobility and dietary resources access provided by stable isotopes analysis, will give us information about the presence of inequality between the members of each burial site. The results of this study will help us to shed light on the onset of social inequality in Northern Italy, and on the cultural and biological mechanisms that promoted its development.

Female lineages and changing mortuary practices in Neolithic Çatalhöyük: Insights from genomic and archaeological data

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Keywords: Neolithic Çatalhöyük, kinship relations, female lineages

The Neolithic site of Çatalhöyük, located in the Konya Plain of Anatolia, is well known for its large size, prolonged occupation, and abundant traces of complex symbolism. We co-analyzed whole-genome and archaeological data from burials spanning the early (7100–6700 BC), middle (6700–6500 BC), and late (6500–6300 BC) periods of Çatalhöyük East Mound to investigate house-based social organization and gendered social relations. In the early period, individuals were often buried with close relatives, but this pattern declined during the middle and late periods. Instead, children from multiple families were buried in the same building and shared similar diets as indicated by strontium and oxygen isotope analysis. Furthermore, burials within the same buildings were more often related through maternal rather than paternal lineages and grave goods were significantly more common in the burials of subadult females compared to male subadults. Our findings illustrate how kinship relationships evolved through time, while certain mortuary practices emphasizing female lineages may have continued to shape the house-based social structure of Neolithic Çatalhöyük. This pattern of social dynamics persisted even amidst changing kinship practices.

Genetic and Social Complexities of Late Roman Emona, Slovenia

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Keywords: Ancient DNA, Urban Roman Burial Sites, Late Antiquity

This study explores the genetic diversity, ancestry, and social structure of Emona, a Roman colony located in modern Ljubljana, Slovenia, during its peak and decline from 250–450 CE. Using ancient DNA methods and bioinformatics, combined with rich archaeological data, this research aims to investigate the complex interplay of migration, integration, and cultural practices in this urban settlement.

Situated at a strategic crossroads of the Roman Empire, Emona was home to a diverse population. Preliminary ancestry proximal models show localized influences from the Balkan peninsula and Eastern Mediterranean. Preliminary genomic analyses suggest significant genetic diversity and reveal patterns of sex-biased admixture, reflecting the colony's role as a military and trade hub. Differences in burial contexts further indicate potential links between ancestry, cultural practices, and social status, with some evidence of family groupings in graveyards suggesting a tradition of familial interment.

The study also examines population continuity during Emona's last 300 years, a period marked by economic fluctuations and sociopolitical turmoil. Radiocarbon dating and genetic drift analyses provide insights into Emona's population (dis)continuity despite these challenges.

This research uniquely integrates genetics and archaeology to provide a high-resolution snapshot of urban life in the late Roman Empire. By focusing on a single site with extensive historical records, the project sheds light on broader patterns of migration, cultural exchange, and social organization, contributing to a deeper understanding of ancient Mediterranean societies.

Evolution of human and non-human animals, plants, and microbes

Detecting Evolutionary and Functional Signatures through Archaic and Ancient Polymorphic Transposable Elements Under Selection in Modern Humans

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Keywords: Evolution, Introgression, Selection

Previously considered “junk DNA”, transposable elements (TEs) have been re-evaluated as genetic components with relevant impact on gene function, and as useful genetic markers of human evolution. The present study aims at highlighting functional and evolutionary signatures in modern populations by leveraging on gene-associated polymorphic TEs previously detected in ancient and archaic humans. To achieve so, a panel of eight ancient and archaic whole genome sequences have been preliminarily analysed with the MELT and TypeTE software tools, to detect reference and non-reference TEs belonging to the Alu, LINE and SVA families. After quality control, the remaining TEs were searched in 20 non-admixed populations of the 1000 Genome Project dataset, retaining for further analysis only those with a frequency of at least 0.05 in at least one modern population. Gene-associated TEs were detected and haplotypes within 5Mb around these TEs were further scanned for local signatures of positive selection (rEHH) and adaptive introgression (VolcanoFinder) in each population. Their emergence in the human lineage was also dated exhaustively by applying the GEVA software. Gene network analysis, functional annotation and overrepresentation were performed with STRINGdb, DAVID and PANTHER. Results show a marked influence of archaic and ancient polymorphic TEs on functional pathways and disease-related phenotypes in modern human populations, particularly those associated with immune response, brain connectivity and development, mental and behavioural disorders, anthropometric measurements and cardio-pulmonary functionality. These signals were sometimes limited to specific macro-areas, while in other cases they showcased recognizable geographical clines reproducing known human migration routes.

Mycobacterium Leprae Genomes from Central Europe Provide Clues into the Past Diversity of the Leprosy Bacterium

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Keywords: Ancient DNA, Leprosy, Human pathogen

Leprosy is one of the oldest recorded diseases in human history, caused by the bacterial species *Mycobacterium leprae* and *Mycobacterium lepromatosis*. It affects the peripheral nerves, respiratory system, skin and eyes, resulting in skeletal involvement and limbs and facial deformation. These characteristic skeletal lesions in hand, feet and facial bones have been used by palaeopathologists to identify this disease in the past. Despite its long association with humans, little is known about the disease's origins and whether other species have played a role in human cases.

To date, 49 ancient *M. leprae* genomes have been recovered from individuals from Europe (n=48) with the exception of one case from Northern Africa. The published ancient dataset is biased towards the High and late Middle ages, with only 9 genomes predating 1100 years ago, thus limiting the knowledge of the past diversity of this bacterium.

Here, we present the 8 oldest *M. leprae* genomes dating to 100 BCE-900 CE from Central Europe. We identified three different *M. leprae* genotypes in our dataset. Two Austrian genomes that date to 2000 years ago form a clade with the oldest known genome from Northern Africa, representing the earliest finding to date of the 3L genotype in Europe. Together with previously published genomes, this dataset shows that by the Early Middle Ages a high diversity of *M. leprae* was already present in Europe. We will discuss the implications of the older diversity than previously expected, and provide preliminary results from functional comparisons between new ancient strains.

References

1. Winter, A., Henderiks, J., Beaufort, L., Rickaby, R. E. M. & Brown, C. W. Poleward expansion of the coccolithophore *Emiliania huxleyi*. *J. Plankton Res.* **36**, 316–325 (2014).
2. Jakobsson, M. *et al.* Pleistocene stratigraphy and paleoenvironmental variation from Lomonosov Ridge sediments, central Arctic Ocean. *Glob. Planet. Change* **31**, 1–22 (2001).

3. Razmjooei, M. J. *et al.* Revision of the Quaternary calcareous nannofossil biochronology of Arctic Ocean sediments. *Quat. Sci. Rev.* **321**, 108382 (2023).

Searching ancient genomes for footprints of human self-domestication hypothesis

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Keywords: Self-domestication hypothesis, aggression, hominid evolution

Modern human civilization is a product of millions of years of gradual evolution punctuated by major transitions in terms of life-style, technological breakthroughs or societal awakening. Some of the most relevant characteristics relate to more complex forms of cooperation, increased self-control, and cumulative culture. The theory of self-domestication hypothesizes that approximately 300 000 years ago, *Homo sapiens* started to experience a process of selection for overall greater docility and lesser aggression, a process that we expect to have left footprints in genes nowadays known to be associated with docility or aggressive-like behaviors. Thus, in order to explore evidence at molecular level for the self-domestication hypothesis, we profiled the evolution of genetic diversity in aggression-related genes across hominin history. Genetic variation linked to aggressive behaviour in modern humans is nowadays well-established and has been found to aggregate in key genes or genomic regions. Here, we present results of the first stage of the study. By building a pseudo-reference genome constituted solely by the abovementioned genomic regions, mining the European Nucleotide Archive for DNA sequences of Neandertals (n = 33) and anatomically modern humans (n = 133), and applying stringent filtering to call structural variants (SNPs), we were able to reconstruct evolutionary trajectories and inspect for signatures of selection. To contextualize our findings to other extant hominid lineages, we utilized chimpanzee and bonobo sequences, species with well-studied behaviour differences in aggression, and ample availability of genomic sequences for the same sets of genes.

Invisible Diseases of Pastoralists: A Complete Genome of *Erysipelothrix Rhusiopathiae* Recovered from Early Medieval Human Remains in the North Caucasus

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Keywords: *Erysipelothrix rhusiopathiae*, Early Medieval, Zoonotic pathogen

Covarious interactions between humans and domestic animals are of utmost significance for palaeoecological reconstructions. One way to study these interactions is to monitor pathogens shared by humans and animals. Given that traces of transmitted infections are not always visible in bone tissue and morphological manifestations of the bone tissue, when present, may be similar for the different infections, genomic analysis is an effective way to reveal the pathogen burden in a population.

Bacterium *Erysipelothrix rhusiopathiae* affects both humans and animals, especially domestic pigs, causing a disease known as erysipeloid. The disease has three clinical forms: cutaneous, generalized and septic and can be fatal, especially in the presence of comorbidities. In this study, complete genome of *E. rhusiopathiae* was reconstructed from DNA recovered from tooth pulp of a 1,400-year-old human skeleton from an early medieval Alan burial. The ancient genome is phylogenetically closest to those of modern *E. rhusiopathiae* isolated from wild boars and domestic pigs in Western Europe.

Paleoanthropological examination of the skeleton revealed signs of severe infection of unclear origin in the spine, ribs and sternum that are consistent with bone tuberculosis. However, no trace of mycobacterial DNA were recovered. In contrast, reads of ancient *E. rhusiopathiae* DNA were highly abundant. It is thus possible that the *E. rhusiopathiae* infection, alone, or exacerbated by an additional unidentified disease, was the cause of observed abnormalities.

The Upper Palaeolithic Roots of European Mesolithic Hunter-Gatherers

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Keywords: Demographic modeling, Hunter-gatherers, Western-Eurasia

In recent years, numerous published genomes have fundamentally expanded our understanding of the population history of western Eurasia. However, due to the temporal distribution of the available data, which predominantly date to the Neolithic and later times, our knowledge of earlier periods in human history remains limited. Recent aDNA studies have been able to characterize the complex spatio-temporal relationships of Eurasian hunter-gatherers. However, precise demographic models, particularly those enabling comparative analyses and quantitative parameter inference are still lacking.

To fill this knowledge gap, we compiled a dataset consisting of high-resolution genomes bridging over 30kya from the Upper Palaeolithic onwards. To increase data-resolution in key geographic and temporal aspects, we produced high coverage genomes (>10X) of one Palaeolithic and three Mesolithic hunter-gatherers from Germany in addition to two hunter-gatherers from the Eastern Eurasian steppe region in Russia.

Coalescent-based demographic inferences with fastsimcoal2 show that lineages associated with western Eurasian hunter-gatherers of the Upper Palaeolithic comprise a substantial part of the ancestry of both eastern (EHG) and western (WHG) European hunter-gatherers. Our results further suggest that WHGs and EHG separated only after the Last Glacial Maximum and did not replace earlier HG populations, as previously reported, but partially descended from them.

Paleogenetic Analysis of the Saint-Memmie (Marne, France) Hypogeum from the Late Neolithic

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Hypogea are subterranean tombs that can contain the remains of dozens of individuals. During the Late Neolithic, there was a flourishing of hypogea in the Paris Basin (France). Most of them are located in the Marne region, along a cuesta between the cities of Épernay and Sézanne. Famous examples include Les Mournouards, excavated by Leroi-Gourhan during the 1960s, and Mont-Aimé.

In 2019, a rescue excavation in the city of Saint-Memmie uncovered a new hypogeum on the banks of the Marne River. This site contained the remains of 55 individuals dating back to 3,000 BCE. Paleogenomic analyses were conducted on 52 of these individuals, enabling the recovery of ancient genomes with up to 1X coverage.

Biological relatedness was identified among these individuals, revealing close (1st- to 2nd-degree) and more distant familial links, and their role in the structuring of the tomb. They were also compared to contemporaneous samples from Mont-Aimé and Les Mournouards to better understand genetic structuring at the regional level.

Comparisons between these individuals and other Neolithic and Bronze Age samples show that they belonged to European Farmer groups, with varying degrees of ancestry from Western European Hunter-Gatherers but none from Steppe populations. This suggests that the Steppe ancestry, identified in the slightly later group from Les Bréviandes, arrived in the Paris Basin after 3,000 BCE.

Limits and Best Practices for Local Ancestry Inference in Imputed Ancient Genomes

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Keywords: Ancient DNA, Local ancestry inference, Admixture dating

Recent advances in imputation of ancient genomes have created the opportunity to apply state-of-the-art methods of statistical genetics to ancient genomes of different species to study various aspects of their evolutionary histories across large timescales. One popular class of such methods, known as local ancestry inference (LAI), aims to “paint” an individual’s genome as a mosaic of segments inherited from ancestral source populations. Despite their great potential to reveal patterns of genetic ancestry across the genome, most LAI methods have been designed to study relatively recent admixture events specifically in human history, using large panels of present-day genomes. However, it is currently unknown how reliable LAI is in aDNA datasets, which are characterized by scarce geographic and temporal sampling, various degrees of divergence between ancient source proxies and admixing populations, and their complex demographic histories. In this study, we evaluated the performance of four LAI methods, RFMix, FLARE, MOSAIC and simpLAI, together with a popular admixture dating software DATES, under different demographic scenarios, various temporal sampling schemes and sample sizes, as well as admixture dates. We quantified their accuracy as a function of these parameters, and tested to what extent does inclusion of source proxies pre- or postdating the admixture event or grouping putative sources from different time points, affect the inferences. Our study establishes the limits of LAI in ancient genomes and provides general guidelines for LAI in future studies, which we demonstrate by a proof-of-concept LAI in a set of Steppe Bronze Age ancient human genomes.

Analysis of a 5,500-year-old *Treponema pallidum* Genome from Sabana de Bogotá, Colombia

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The origin, diversification, and spread of treponemal diseases have remained one of the most contentiously debated topics in the history of infectious diseases. Despite the growing body of genomic data, the emergence and evolution of the causative pathogens, *Treponema pallidum* subsp., remain unclear. Paleogenomics is an emerging field which allows to explore the evolutionary history of human-associated pathogens. By recovering ancient DNA data from a hunter-gatherer at the archaeological site of Tequendama I (Colombia), we reconstructed a *Treponema pallidum* genome at a ~1.7X mean genome coverage, radiocarbon-dated to ~5,500 yBP. Our analyses reveal that this genome is basal to all known *T. pallidum* subspecies, being also very different from previously reported ancient *T. pallidum* genomes. It represents a putatively extinct sister clade that diverged before the most recent common ancestor of all known modern and ancient strains. This ancient genome displays a gene content similar to modern genomes, with all genes associated with virulence being covered for at least ~50% of their sequence. These observations indicate that treponematosi has a long evolutionary history in South America which possessed a previously unknown diversity of treponemal strains during the middle Holocene. Our findings support previous paleopathological research in the region, demonstrating that an ancestral form of *T. pallidum* was infecting people in South America as early as ~5,500 yBP, during a time of transition to agriculture and population expansions. Our results provide the earliest genomic evidence of a treponemal infection in humans, shedding light on the earlier stages of *T. pallidum* evolution.

Paleogenomic reconstruction of pathogens from the 19th-century in Tlatelolco, Mexico

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Keywords: pathogens, paleogenomics, Mexico

The Conquest and colonization of the Spanish Empire brought significant ecological disruptions to Mexico, leading to new infectious diseases. As a result, diseases like typhus, smallpox, measles, diphtheria, and cholera later impacted the social and biological conditions in 19th-century Mexico City. At the archaeological site of Tlatelolco, an ancient Mexica city and the last stronghold of resistance during the Conquest, a burial pattern in the Great Platform resembled a mass grave, likely due to an epidemic. To understand the infectious agents of this epidemic, teeth samples from twenty individuals at the site were analyzed using shotgun sequencing and Kraken2 to identify pathogenic species. The bacterial pathogens identified include the oral red-complex bacteria—*Tannerella forsythia*, *Porphyromonas gingivalis*, and *Treponema denticola*—associated with periodontal disease in one individual. Additionally, we detected Human Parvovirus B19 (B19V) in three individuals. By using high-depth sequencing, we reconstructed a genome of *T. forsythia* at approximately 10X coverage and compared it to previously reported ancient genomes. Finally, we plan to use B19V capture-enrichment to increase the coverage of this viral pathogen. Integrating paleogenomic, bioarchaeological, and historical evidence will provide insights into the primary epidemic outbreaks of the 19th century in Mexico.

Characterization of Late Pleistocene European Small Hamsters (Subfamily Cricetinae) Based on aDNA Analysis

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Keywords: Cricetinae, Late Pleistocene

It is assumed that during the Pleistocene steppe, taxa now found in Central Asia may have expanded into Europe during glaciations when open environments were present. We generated the mitochondrial genomes of 34 Late Pleistocene and Holocene remains of small hamsters (subfamily Cricetinae) from Central Europe, the Balkans and Anatolia to check if this rule holds true in the case of this group.

Late Pleistocene European small cricetids are usually assigned to grey dwarf hamster (*Nothocricetulus migratorius*), an extant species inhabiting Western and Central Asia and Eastern Europe. Instead, among the 16 samples from Late Pleistocene Central and Western Europe, the only species found was the Dzhungarian Hamster (*Phodopus sungorus*) currently occurring in northern Kazakhstan and southern Russia. Our phylogenetic reconstruction revealed one major lineage composed of all modern and some Late Pleistocene samples which coalesce around 25 thousand years ago, while older samples were located outside this clade. Such a topology suggests multiple expansions of the Dzhungarian Hamster to Europe, with the most recent one occurring after the Last Glacial Maximum.

In the Balkans, we found that the Late Pleistocene grey dwarf hamsters formed a distinct clade sister to the lineage formed by population from the Qurama Mountains in Uzbekistan, and not related with modern samples from Southeastern Europe. The Anatolian Holocene samples clustered with a modern population of species from the same region.

Our results indicate that steppe species, respond to past climatic and environmental changes in an individualistic manner, and generalizations about their evolutionary histories are unwarranted.

Phylogenetic and Functional Insights from Ancient Oral Archaea Across Time and Space

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Keywords: Microbiome, Dental calculus, Metagenomics

Although oral bacterial diversity is increasingly studied, commensal archaea, such as *Methanobrevibacter*, remain underexplored. Advances in *de novo* metagenome assembly have revolutionized the study of ancient microbes, enabling detailed investigations into the evolutionary history of individual microbial species. Specifically, assemblies from ancient dental calculus offer a unique opportunity to investigate commensal oral microbes in greater detail. In this study, we report the discovery that the archaeal genus *Methanomethylophilus*, previously unreported in the mouth, is prevalent across our samples, and we reconstruct 54 metagenomically assembled genomes (MAGs) belonging to two species of this genus in humans. Our analysis of these MAGs reveals patterns of geographical clustering, shedding light on microbial biogeography and its potential to track human migration. Additionally, we analyzed 228 MAGs of *Methanobrevibacter* from ancient dental calculus and plaque samples spanning Neanderthals to modern humans across five continents. We confirm earlier reports that ancient DNA previously identified as *Methanobrevibacter oralis* likely originates from a group of three unnamed species within this genus. By resolving the phylogeny of this genus, we identify a fourth species cluster, expanding the previously known diversity. Our analysis of *Methanobrevibacter* genomes uncovered genes associated with resistance to antimicrobial compounds including metronidazole, a drug commonly used in modern dental procedures, but only in ancient and non-industrial genomes. Its absence in modern industrial populations suggests shifts in selective pressures over time. Overall, this research underscores the value of commensal oral archaea as tracers of human movement and microbial evolution, illuminating the co-evolution of humans and their microbiota.

Ancient wolf population dynamics and ancestry of dogs in Southern Europe

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Keywords: paleogenomics, mitochondrial DNA, canids

Wolves have walked alongside humans for millennia, first as competitors for territories and prey and then, following the process of domestication, as their most faithful friends. However, the geographic and temporal origins of dog domestication remain controversial. In Europe, especially in the South, almost no data are available on the genetic diversity of wolves prior to their domestication and on early dog DNA variability.

Using mitochondrial genomes from ancient wolf and dog remains from Italy, we investigated two fundamental aspects of their evolutionary narrative: (1) the genetic diversity and structure of the ancient wolf populations and (2) the spatiotemporal dynamics of mitochondrial haplogroups in early dogs. Through shotgun approach and mitochondrial capture, we obtained complete mitogenomes of 39 samples, of which 22 wolves and 17 dogs ranging in age from ~40 to 3 thousand years ago and in depths from 1.1 to 869.9-fold coverage (mean 108.9 x).

The results provided an overview of the temporal patterns of the Italian wolf population dynamics, revealing a high variability and a pronounced connection with Eurasian coeval samples.

Dynamics of dog populations highlighted the occurrence of the main dog haplogroups (A, B, C, D), with a greater presence of haplogroup A, followed by C and D.

These results will surely contribute to clarifying the population changes and current morphological and genetic uniqueness of the Italian wolf population while shedding light on local domestication dynamics.

Coprolite Insights: Ancient Homo GI Microbiome

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Keywords: Metagenomics, Paleofeces, Coevolution

Recent research suggests that 'core' taxa of the gastrointestinal microbiome have been evolving alongside (and inside) us dating back to our early *Homo* ancestors, prior to the split of contemporary human and Neandertal lineages, and long before the advent of industrialized food systems and agriculture. This study performs metagenomic analysis on ancient shotgun sequence data extracted from fossilized paleofeces (n=3) recovered from the paleolithic cave site Lapa do Picareiro in central Portugal. The coprolites, suspected to be Neandertal in origin, were recovered in sediment layers dating to 47-49kya. Ancient DNA sequences were classified with MetaPlan4 and compared to other publicly available sequence data of *Homo* coprolites. Our results support the idea of core gastrointestinal microbiome taxa originating in early *Homo* through the similarities in taxa between the Picareiro coprolites, other paleofeces and contemporary hunter gathering communities. Specifically, we identified 15 of 24 taxonomic families strongly associated with the hominin gut and include genera like *Blautia* and *Coproccoccus* that are still key members of the human gut today. These similarities highlight the role of dietary fibre consumption in combination with evolutionary relationships in establishing the intestinal microbiome of an organism. The characterization of an ancestral *Homo* gastrointestinal microbiome is particularly relevant in the context of decreasing gastrointestinal diversity associated with consumption of industrialized diets characterized by low fibre and high carbohydrate intake.

Genetic Identification of Cetacean Remains from the Archaeological Site of Grotta Dell'uzzo (North West Sicily, Italy)

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Keywords: Ancient DNA, Cetaceans, Climate changes

The archaeological site of Grotta dell'Uzzo (North West Sicily, Italy) is known for the discovery of a relatively large cetacean assemblage dating back to the Mesolithic-Neolithic transition. The accumulation of cetacean bones has been related to opportunistic exploitation of stranded individuals by hunter-gatherers during a period encompassing the 8,200 yrs cal BP event. An abrupt decrease in global temperature occurred during this period with profound changes in ocean circulation and sea level, as a consequence, the coastal area near the cave probably took on the features of a mass strandings hot spot. In order to clarify the impact of past climate changes on cetacean diversity in the Mediterranean Sea, the first step is the correct identification of bone remains from archaeological sites. Cetacean bones from Grotta dell'Uzzo are mostly fragmented and difficult to identify morphologically, so ancient DNA analysis was performed to correctly assign them to species. DNA was extracted from bone powder or small pieces and, two mitochondrial DNA fragments were amplified and sequenced. The obtained sequences were assigned to species after comparison with those available in GenBank. Our results highlight the presence in the cetacean assemblage of Grotta dell'Uzzo of bones belonging to both Mysticetes and Odontocetes, also revealing cases of morphological misidentification of remains. A correct taxonomic identification of cetacean bones is essential to highlight variation in distribution or extinctions in response to past climate changes, which may provide us with useful information to predict the impact of ongoing changes on cetacean populations.

Assembly of Ancient and Modern Oral Metagenomes Reveals New Insights into Red-Complex Bacteria

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Keywords: Ancient metagenomics, *De novo* assembly, Oral microbiome.

Assembling historic and modern oral bacterial genomes can shed light on bacterial evolution. *Tannerella* and *Porphyromonas* are bacterial genera linked to periodontal disease, an inflammatory condition that can lead to tooth loss. Understanding their evolution may reveal insights into their role in oral disease development. We performed genomic and phylogenetic analyses on a global set of isolates and metagenome-assembled genomes of the genera *Tannerella* (n=238) and *Porphyromonas* (n=505), including from published ancient dental calculus samples (up to 14,800 years old) and modern saliva, tongue, dental plaque, and dental calculus samples from present-day living populations. We identify a new, previously misidentified *Tannerella* species in modern and ancient humans that differs from *T. forsythia* in gene content, including virulence factors. We reveal a distinct clade of salivary *T. forsythia* strains in Oceania. Within *Porphyromonas pasteri*, we observe a distinct phylogenetic grouping based on the oral site from which the sample was collected, whereas no such pattern is found in our *P. gingivalis* phylogeny. A phylogenetic analysis of the *P. gingivalis fimA* gene coding for the major fimbrial protein reveals the genes cluster by genotypes defined in the literature, and that no ancient genes are found in genotypes I and Ib. Finally, we find the Oceanian and Asian *Tannerella* and *Porphyromonas* genomes to be enriched in pseudogenes compared to other genomes. Using a *de novo* assembly approach improves the diversity of oral genera found in reference databases and enhances our ability to study the evolutionary history of these taxa.

Dynamics of Resistance and Susceptibility to Pathogenic *Yersinia* Species in Domestic and Wild Taxa

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Keywords: Domestication, Disease, Plague

The agricultural transition is associated with an increase in infectious disease due to wider zoonotic opportunity. The pathogen *Yersinia pestis*, the agent of plague, emerged during this period, the emergence of which has been linked to domestication processes and human subsistence change (Andrades Valtueña *et al.* 2022). Most domestic mammals are resistant to plague, which has been linked to evolutionary processes relating to their domestication (Barbieri *et al.* 2020). However, closely related wild mammals also appear to be resistant. Using an integrated literature review and bioinformatic approach I demonstrate that there is limited association between the domestic status of taxa and resistance to pathogenic *Yersinia*, including *Y. pestis*, indicating a limited role of pathogen-mediated evolution for resistance as part of the domestication process, beyond that observed in primary *Y. pestis* sylvatic reservoirs.

To test for a genotypic basis for resistance to plague I screened taxa for the presence of FPR1, a protein proposed to act as a *Y. pestis* binding receptor (Osei-Owusu *et al.*, 2019). Correlation between FPR1 presence in mammals and susceptibility to *Yersinia* suggests a genotypic basis for resistance, though FPR1 absence does not predict *Y. pestis* resistance. The presence or absence of FPR1 occurs at the superorder taxonomic level, as do patterns of resistance and susceptibility to disease. Using modern data to elucidate past pathogen dynamics I call for a renewed investigation into accepted assumptions regarding the wild or domestic status of taxa and their role in pathogen evolution.

References

- Andrades Valtueña, A. *et al.* (2022) Stone Age *Yersinia pestis* genomes shed light on the early evolution, diversity, and ecology of plague. *Proceedings of the National Academy of Sciences*, 119(17), p. e2116722119.
- Barbieri, R. *et al.* (2020) *Yersinia pestis*: the Natural History of Plague. *Clinical Microbiology Reviews*, 34(1), p. 10.1128/cmr.00044-19.
- Osei-Owusu, P. *et al.* (2019) FPR1 is the plague receptor on host immune cells, *Nature*, 574(7776), pp. 57–62.

Mineralised Tissue Formations as an Alternative Source for Human and Pathogen DNA

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Keywords: Mineralized tissue formations, Ancient DNA, Pathogens

While still a rather rare finding in archaeological excavations, mineralized tissue formations (calculi), such as bladder-, kidney- or gallstones, were likely not uncommon in past human populations. Often overlooked or mistaken as stones, they can, however, be a valuable source for both host and pathogen DNA. Calculi have diverse underlying causes; they can be linked to hydration and diet, metabolic diseases, tumors and metastases, as well as chronic and infectious diseases. Especially the latter two are of great interest for pathogen research, as e.g. bacteria become enclosed in the course of formation. However, as the archaeological record is sparse, not many objects have been studied yet regarding ancient host and pathogen DNA.

The “Pathologisch-anatomische Sammlung im ‘Narrenturm’, Naturhistorisches Museum Wien (PASiN-NHM)” houses a large collection of calculi comprised of contributions by various people, such as Leopold von Dittel, a surgeon and urologist in Vienna during the 19th century.

Here, we present genetic data from a pilot sample set of calculi from this collection, including gall, bladder and kidney stones. While exploring different sampling strategies to best preserve stone formations, we assess overall DNA preservation of both host and microbial DNA and examine the species composition across different sample types. Our results show an overall excellent DNA preservation. Regarding species composition, the calculi are very heterogeneous, even differing greatly within the same type of sample. From one of these calculi, a presumed uterine myoma from a medieval site in Austria, we were able to recover a high coverage *Mycobacterium tuberculosis* genome.

Studying Evolution Through the De Novo Reconstruction of Microbial Diversity from Ancient DNA

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Keywords: Ancient DNA, Microbes, Simulations

Ancient DNA metagenomics provides a unique opportunity to directly observe past microbial diversity as a snapshot in time and study its evolution. Until now the analysis of metagenomic data of ancient samples has mainly been conducted by reference-based mapping methods that cannot capture previously unobserved microbial diversity. However, researchers have started to adapt novel strategies that do not require the presence of a reference genome, such as de novo assembly. Ancient DNA presents some unique challenges, and most researchers have so far applied strategies implemented for modern DNA samples to de novo assemble their ancient DNA data without evaluating how accurate these are. In this study, we simulated a metagenomic sequencing dataset that is similar to ancient dental calculus, a commonly studied human-associated microbiome sample that is well represented in the archaeological record. We evaluated different de novo assembly software and could show that DNA molecule length is the strongest predictor for the success of de novo assembly. In contrast, ancient DNA damage is of less importance. Increasing the sequencing depth can help to overcome the effect of shorter DNA molecules and enables the reconstruction of DNA sequences similar in length to the ones derived from modern DNA. Applying this knowledge to a set of deeply sequenced human palaeofaeces samples, we were able to successfully reconstruct previously undescribed microbial diversity in the human gut microbiome.

Highly Accurate and Efficient Diagnostics of Tuberculosis in Ancient Human Remains

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Keywords: *Mycobacterium tuberculosis*, Ancient pathogen, Phylogenetic lineages

Tuberculosis is an infectious disease that has accompanied mankind throughout much of its history. Until recently, morphoscopic techniques have been the main way of studying the prevalence of tuberculosis in past. The development of genetic (especially genomic) technologies has allowed the study of ancient bacterial genomes from skeletal remains, shedding light on the evolution of extant pathogens. We analyzed a series of skeletons from a Kozino village cemetery (Moscow Region) dating from the Late Medieval to Modern periods. Based on morphological criteria, a quantitative index that indicated the probability of tuberculosis was derived. In parallel, we performed genomic screening of material from skeletal remains and carried out identification of phylogenetic lineages of ancient *Mycobacterium tuberculosis* using existing SNP. The results of our analysis reveal a spread of *M. tuberculosis* lineage 4 (L4) in Eastern Europe during the Late Medieval.

A Legacy of Genetic Entanglement with Wolves Shapes Modern Dogs

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Keywords: Dogs, Wolves, Hybridization

Dogs evolved from gray wolves under human influences during the late Pleistocene and have been ubiquitous in human societies ever since. Previous research has suggested some dog-to-wolf gene flow especially in regions with declining wolf populations, and rare instances of wolf-to-dog introgression associated with adaptive advantages such as high-altitude survival. However, any widespread gene flow has fallen below thresholds of detection of previous genome-wide statistical assessments. To reexamine evidence of dog-wolf gene flow, we analyze 2,693 published dog and wolf genomes and combine highly sensitive local ancestry inference and phylogenomic analyses of nuclear genes, mitochondrial genomes, and Y-chromosome sequences. Although dogs and wolves segregate strongly at the nuclear level, no individual nuclear gene tree supports dog monophyly, indicating a more complex scenario. Uniparental markers—mitochondrial genomes and Y-chromosomes—show mixed and interleaved dog and wolf clades with strong support, and highly incongruent phylogenetic topologies. Using local ancestry inference, we find 64.1% of modern breed dogs carry wolf ancestry covering around 0.14% of their nuclear genome from admixture that occurred nearly a thousand generations ago. Among free-living village dogs (n=280), 100% of analyzed genomes carry wolf ancestry. We find that wolf ancestry correlates with functional traits including size, breed category, and personality characteristics. In village dogs, wolf ancestry is enriched at olfactory receptor genes, suggesting adaptive introgression for sensory acuity, allowing these dogs to survive in more challenging environments. In total, dog-wolf admixture has likely been an important factor in shaping the evolution of modern dogs.

The 3,300-year-old *Yersinia pestis* Genome of an Ancient Egyptian Mummified Individual

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Keywords: Plague, Mummy, Museo Egizio

Yersinia pestis, the bacterium responsible for plague, has caused three major pandemics throughout recorded history. Paleogenetic studies of human skeletons have also traced the presence of *Y. pestis* back to prehistoric times, as early as the Neolithic period, though these discoveries have been geographically confined to Eurasia.

In this study, we present evidence of *Y. pestis* DNA in an ancient Egyptian mummy, an adult male from the Museo Egizio collection in Turin, Italy. The individual, anthropogenically mummified, has been radiocarbon-dated to the transitional period between the end of the Second Intermediate Period and the beginning of the New Kingdom (3290 ± 45 years BP; 1686–1449 cal. BC). While the mummified individual's exact provenance within Egypt remains uncertain, analyses of bone tissue and intestinal content using shotgun metagenomics revealed the presence of *Y. pestis* DNA in both samples. This might suggest a broad tissue tropism of the pathogen during an advanced stage of infection.

Subsequent targeted enrichment methods yielded low-coverage genome-wide data for both the human host and the *Y. pestis* pathogen. This marks the first protohistoric *Y. pestis* genome discovered outside Eurasia, providing molecular evidence of plague in ancient Egypt. Nevertheless, the prevalence of the disease during this time remains unclear. Further genomic analysis aims to position this ancient strain within the known diversity of *Y. pestis*, examine virulence-associated genes, and explore potential modes of transmission and pathology.

Evolutionary Dynamics of Extinct and Extant Moose in North America

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Keywords: Hybridization, Introgression, Migration

The Eurasian moose, ancestor of *Cervalces* sp. (*C. scotti* and *C. latifrons*), entered North America via the Bering Land Bridge around 800 thousand years ago (ka)¹ and moved southwards during periods of glacial retreat, eventually inhabiting all of boreal North America. The modern moose (*Alces alces*) entered North America just before the submersion of the Bering Land Bridge (14-10.5 ka) and quickly spread southwards as the ice sheets retreated². *A. alces* migration closely mirrors that of human dispersal into North America, so these dynamics can serve as a proxy to better understand human migration. *Cervalces* sp. went extinct in North America ~10 ka, likely due to competition from *A. alces* and climate change at the end of the Pleistocene. While there is some debate over whether *A. alces* encountered *C. scotti* to the south of the ice sheets prior to *C. scotti*'s extinction, *A. alces* would have encountered northern *C. latifrons* populations in Yukon/Alaska before dispersing southwards. Hybridization commonly follows range expansions due to shifts in environment, when a reproductive barrier such as physical isolation is removed³. Thus, it is possible that upon contact between *A. alces* and *Cervalces* sp., the two species hybridized. To test this, we have generated ancient genomic data from multiple *Cervalces* sp. samples from Yukon, Canada, and have compared these to 23 modern *A. alces* genomes. The results provide novel insights into the movements and evolution of megafauna in North America in relation to past climatic changes at the end of the Pleistocene.

References

1. Churcher, C. S. & Pinsof, J. D. Variation in the antlers of North American *Cervalces* (Mammalia; Cervidae): Review of new and previously recorded specimens. *J. Vert. Paleontol.* 7, 373–397 (1988).
2. Hundertmark, K., Hundertmark, K., Bowyer, R., Shields, G. & Schwartz, C. C. Mitochondrial phylogeography of moose (*Alces alces*) in North America. *J. Mammal.* 84, 718–728 (2003).
3. Chunco, A. J. Hybridization in a warmer world. *Ecol. Evol.* 4, 2019–2031 (2014).

Genomic Evidence of *Yersinia pestis* from Iron Age Romania

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Keywords: *Yersinia pestis*, Plague

Yersinia pestis, the aetiological agent of plague, has been identified as the cause of three historical pandemics, the earliest of which began in the 6th century CE. Yet, ancient DNA evidence indicates that *Y. pestis* was present in Eurasia as far back as the Neolithic and persisted through the Bronze and Iron Ages. Most of these early variants lack key genomic adaptations associated with flea-borne transmission and belong to deeply diverging genomic lineages that are today extinct. While it is unclear whether these variants could cause widespread disease at an epidemic level, there is growing evidence of localized outbreaks in some prehistoric communities. Here, we identified *Y. pestis* in individuals from two Iron Age (10th – 7th centuries BCE) archaeological sites in Romania using non-targeted metagenomic screening. A subsequent whole-genome targeted enrichment approach improved *Y. pestis* coverage among positive genomic libraries, enabling us to explore the phylogenetic context of these genomes within ancient plague lineages. Additionally, we analyse novel insertions and deletions for possible impacts on the pathogen’s virulence and transmission. These genomic results, examined alongside archaeological and paleodemographic data retrieved from both sites, provide deeper insight into how *Y. pestis* interacted with its human and ecological environment during the Iron Age and its potential impact on ancient communities.

Population History of Pre-Colonial Maritime Societies of São Paulo State (Brazil): An Archeogenetic Analysis of the Piaçaguera Shellmound

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Keywords: Shellmound, Archeogenetics, South America

Sambaquis are the intentional, century-long accumulation of seashells from molluscs and marine fauna and are found intermittently along the Brazilian coasts and rivers. These constructions mark human presence in the coastal regions during the Holocene, between 8,000 and 1,000 years ago (kaBP - thousand years before the present). The population history of sambaqui-building groups has been extensively investigated through cultural and bio-anthropological markers and, more recently, through studies involving ancient DNA. So far, molecular analyses have included sambaquis from Santa Catarina, Paraná, Rio de Janeiro, and Espírito Santo, without the inclusion of coastal sambaquis from the state of São Paulo. Therefore, questions about the origin and dispersion of Sambaqui groups on the southernmost east coast still remain under debate. The current project has retrieved nuclear and mitochondrial data from 20 individuals excavated from the Piaçaguera sambaqui, São Paulo. All individuals exhibited a high percentage of endogenous DNA, ranging from 35% to 75%, and a C>T substitution rate exceeding 50%, which strongly indicates the ancient nature of the samples. Preliminary results suggest that the population from the Piaçaguera coastal shellmound is more closely related to nearby riverine shellmounds than to more distant coastal shellmounds. Further analysis will focus on elucidating the local population dynamics of the sambaquis between coastal and riverine regions of São Paulo.

Palaeoproteomic Analyses of Bone Fragments from Palaeolithic Crimea Reveals Two New Hominins

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Keywords: Palaeolithic Archaeology, Palaeoproteomics, Ancient DNA

The Crimean Peninsula contains significant Palaeolithic sites, including Kabazi II, Kabazi V, Siuren I, Starosel'e, and Chokurcha I. The region has been considered a potential refugia for Neanderthals before their replacement by *Homo sapiens*. This hypothesis is supported by lithic analysis and radiocarbon dating.

In this study, we used collagen peptide fingerprinting, Zooarchaeology by Mass Spectrometry (ZooMS), to identify morphologically unidentifiable bones to genus or species level and screen for potential human remains, which are rare in Palaeolithic contexts.

We analysed 628 bone fragments with ZooMS, finding that 82.3% had sufficient collagen preservation for taxonomic identification. Our results suggest Palaeolithic humans primarily hunted horses (Equidae) at Kabazi II, Kabazi V, and Starosel'e, while other sites revealed evidence of deer, mammoth, and other large ungulates, offering insights into the region's paleoenvironment and subsistence strategies.

Importantly, three bone fragments displayed collagen fingerprints matching Hominidae: two from Starosel'e and one from Siuren. Radiocarbon dating of the Starosel'e bones revealed them to be 44–46,000 and 42–44,000 years old, close to the transition between the disappearance of Neanderthals and early dispersal of *Homo sapiens*. We obtained ancient DNA analysis from the younger bone, finding very low endogenous content (<0.1%). However, we could recover a 1-fold coverage of mitochondrial genome falling on the Neanderthal lineage.

The results demonstrate that ZooMS is a powerful tool for identifying previously unrecognised faunal remains, helping reconstruct paleoenvironmental, and potentially new hominin remains that can be directly dated, genetically sequenced, and analysed for dietary isotopes.

Preliminary Exploration of Genetic Data of an Iron Age Skull from Latvia with Multiple Lytic Bone Lesions

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Keywords: Lytic lesions in skull, Differential diagnosis, aDNA

Different types of bone lesions possibly attributable to tumours have been previously identified in archaeological human remains from all periods of time including prehistory, but our knowledge about genetic variability associated with them is limited.

In this study we analysed a skull from a 10th century CE burial from the Čunkāni - Dreņģeri cemetery in Latvia. The burial was severely disturbed, with only a fragmentary skull, mandible, and the first cervical vertebra preserved. Analysis was done macroscopically and using computer tomography. The morphology of the bones indicate that the individual was a 40 to 50 years old female.

Non-traumatic lesions were observed in the skull vault, in the form of sharply defined holes from 5 to 25 mm in diameter, which penetrated both tables of the skull. One of the possible causes of the pathology could be multiple myeloma. Identification of myeloma in archaeological human remains is complicated by the similarity between bone lesions of myeloma and some types of metastatic carcinoma. Another possible causative agent for the severe bone lesions could be an infection with *Mycobacterium tuberculosis*.

Bone powder was drilled from petrous bone and four other sites in the vicinity of bone lesions. Obtained sequences were aligned to human genome reference to search for variants also found in oncological databases. Additionally, metagenomic analysis was performed.

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Oral Bacterial Genome Evolution During the Neolithic Transition

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Keywords: Neolithic transition, Population genetics, *De-novo* assembly

The interest in ancient metagenomics is increasing with over 1.700 ancient microbial genomes, partial genomes, and metagenomes characterized. However, most studies focused on aligning sequences to modern reference genomes, limiting discoveries to known taxa and introducing a reference bias, since there are genetic differences between ancient and modern genomes. A way to overcome this issue would be to perform a *de novo* assembly of metagenomes, resulting in a set of genomes of all the species that are present in the sample. We evaluated multiple *de novo* assembly pipelines using simulated ancient metagenomes and identified the optimal pipeline for reconstructing ancient metagenome-assembled genomes.

We will apply this approach to metagenomic data from ancient human dental calculus covering the Neolithic transition, one of the main cultural and demographic events in prehistoric Europe triggered important changes in the evolution of both humans and their oral microbiome.

We collected published shotgun metagenomic data from samples of different geographical areas in Europe ranging from the Palaeolithic to the Bronze Age, as well as newly sequenced samples from Italy. We focus on reconstructing the ancient genomes of, *Olsenella* sp. oral taxon 807 (OT807) and *Anaerolineaceae* oral taxon 439 (OT439), two bacterial species found a higher abundance the oral microbiome of Neolithic farmers. Through genomes reconstruction, we will explore more in depth the genetic variability of these species and perform functional annotation to identify genes that may have been lost/gained and can have an impact in the metabolization of food/medicines.

Genetic Research into Pre-Colonial Dogs from the Caribbean Region Using Ancient DNA

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Humans and dogs have long, intertwined histories, sharing common lifeways. Their close relationships are attested in the archaeological record, i.e., through human-dog burials, shared migration and dietary patterns, and a prominent role of dogs in the cultural fabric of human history, art, and symbolism.

In the Americas, a wide variety of Indigenous dog breeds exhibit a large genetic variation, however the degree of the genetic replacement with dog populations after European colonial invasion is still debated. Ancient DNA from pre-colonial contexts reveals a set of mitochondrial DNA sequences unique to the Americas, with haplogroups that are absent in European dogs. However, to fully understand the extent of intermixing or replacement, it is essential to conduct analyses at the genome-wide scale, which is focus is the main focus of this project. We have generated genome wide data from canine remains from several Caribbean islands of the Lesser and Greater Antilles – spanning a time period from 2350–400BP – which, in the majority of the cases, were buried in habitation sites – often together with humans.

With this analysis, we are hoping to provide a more nuanced overview of the genetic composition of Indigenous dog breeds from the Caribbean, and relate them to other published individuals from ancient and present-day populations. Dogs move in close proximity and/or in association with humans; therefore, comparing dog and human population histories in the Caribbean will also contribute to human genetic histories.

Tracing *Gephyrocapsa Huxleyi* in Arctic Ocean Sediments through Time and Space

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Keywords: Arctic Ocean, *Gephyrocapsa huxleyi*, sedaDNA

Coccolithophores are calcifying phytoplankton constituting the base of the food-web in the ocean. Their growth in the surface layer, followed by their sinking to the seafloor upon death, influences the carbon flux between the ocean, and the atmosphere. They therefore play an important role in the marine carbon cycle. In situ, and satellite observations show that the widespread, and abundant coccolithophore *Gephyrocapsa huxleyi* is expanding into polar waters. Changes in its global distribution, and abundance relative to non-calcifying phytoplankton are likely to affect the marine carbon cycle, and subsequent feedbacks in the climate system¹.

G. huxleyi's first appearance in the Arctic Ocean (AO), believed to be during the last interglacial period (MIS 5, 71-130 ka), is a key biostratigraphic datum in AO sediments². However, identifying nanofossils is challenging because of low abundances and poor preservation due to unfavourable taphonomic conditions. This has led to ambiguous biostratigraphic age estimates, a great concern given their importance to palaeoceanographic research in the AO³. The first appearance, and the factors driving the diversification, and distribution of *G. huxleyi* in the Arctic Ocean (AO), as well as its relationship to the marine carbon cycle, remain to be assessed.

To better understand the distribution of *G. Huxleyi* in the AO, we analyse sedaDNA from 10 cores from the AO using a shotgun approach. Integrating metagenomics with fossil, and climate proxy data, we also aim to more confidently place the first appearance of *G. huxleyi* in the biostratigraphic framework of Quaternary AO sediments.

References

1. Winter, A., Henderiks, J., Beaufort, L., Rickaby, R. E. M. & Brown, C. W. Poleward expansion of the coccolithophore *Emiliana huxleyi*. J. Plankton Res. 36, 316–325 (2014).
2. Jakobsson, M. et al. Pleistocene stratigraphy and paleoenvironmental variation from Lomonosov Ridge sediments, central Arctic Ocean. Glob. Planet. Change 31, 1–22 (2001).
3. Razmjooei, M. J. et al. Revision of the Quaternary calcareous nanofossil biochronology of Arctic Ocean sediments. Quat. Sci. Rev. 321, 108382 (2023).

Mycobacterium Tuberculosis Diversity in the Post-European Contact Americas

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Keywords: Tuberculosis, Americas

Today, tuberculosis (TB) incidence in the U.S. is among the lowest globally, however, it remains the leading cause of death due to a single infectious agent worldwide. TB is caused by strains belonging to the *Mycobacterium tuberculosis* complex (MTBC). Ancient DNA research has revealed that nearly 1,000 years ago, pinnipeds introduced *Mycobacterium pinnipedii* to coastal South American populations. These *M. pinnipedii* strains spread inland via human and/or animal hosts but were later replaced by more virulent *Mycobacterium tuberculosis* strains introduced after European contact. This study investigated MTBC strain diversity and replacement dynamics in the post-European contact Americas. Skeletal remains of 43 individuals with TB lesions from archaeological sites in the U.S., Mexico, and Canada were screened for MTBC DNA. Four samples yielded authenticated ancient MTBC DNA, with all four strains belonging to the European-origin *M. tuberculosis* Lineage 4. A partial genome recovered from an individual buried in the Highland Park cemetery, New York (1826–1863 CE), and a near-complete genome recovered from an individual from Tlatelolco, Mexico City (1685–1928 CE), were identified as sub-lineage 4.10, a globally distributed generalist sub-lineage. Within 4.10, the Highland Park strain belongs to a further sub-lineage 4.9 which comprises the *M. tuberculosis* reference strain, H37Rv, and was prevalent in Great Britain in the 18th-19th centuries, whereas the Tlatelolco strain belongs to sub-lineage 4.8 which is commonly found in Latin America today. Overall, this study highlights the complex dynamics of TB transmission in the Americas during the post-contact period.

Characterization of the Ancient Human Oral Microbiota Recovered from Teeth

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Keywords: Ancient oral microbiota, Comparative metagenomics, Compositional data analysis

Metagenomic sequencing of ancient DNA extracted from dental calculus has yielded important insights into the composition and functional diversity of the human oral microbiota in the past. Ancient human teeth have been sampled widely for studies of human host DNA, however their characteristics as potential sources for ancient human oral microbiota have yet to be determined.

Here, we present a comprehensive characterization of the microbiota retrieved from 1,313 ancient humans ranging from the Upper Paleolithic to historic times. Analyzing species abundance profiles derived against a modified version of Genome Taxonomy DataBase (GTDB), we demonstrate that oral microbiota are frequently recovered from ancient human teeth samples. We further characterize the composition of the teeth microbiota of 217 high abundance samples against large-scale reference dataset including publicly available modern and ancient oral microbiomes.

Our study demonstrates that ancient metagenomic data extracted from ancient human teeth can provide a hitherto under-exploited resource to study human oral microbiome evolution.

Deciphering the Genomic History of the Rendena Valley People

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Keywords: Population genomics, Mitochondrial and nuclear genomes, Italy

Genomic studies have greatly increased our knowledge of the genetic history of human populations. Present-day Italians (i) harbour a higher genetic diversity compared to other European populations, an effect of various genetic contributions since the Upper Paleolithic, and (ii) cluster into four major groups: Sardinia, Northern, Central and Southern Italy. This diversity is being further explored at the microgeographic level. In this study, we analyzed the population genomics of a community from the Rendena Valley, in the Italian Region of Trentino-Alto Adige/Südtirol. A total of 125 individuals having ancestors living in this valley ('Rendeneri') were sampled. First, we assessed their mitochondrial DNA variation by sequencing complete mitogenomes from 95 Rendeneri maternally unrelated and with the grandmother from Val Rendena. Haplotype and haplogroup distributions show high internal variability and an unusually high frequency of haplogroup U5a (18%), the highest detected so far in Italy. Age estimates of the main subclades U5a1a1 and U5a2c suggest Bronze Age connections with central-eastern Europeans and populations from central Asia, including some nomadic groups (Yamnaya) from the Pontic-Caspian steppes. To further investigate this hypothesis, 48 individuals were selected for genome-wide genotyping with the Human Origin SNP chip. Exploratory analyses of allele frequencies provided new insights into the genomic makeup of the valley. They not only confirm the high level of variation in the Rendereni, but also highlight their peculiar placement relative to the genomic cline previously reported for the Italian Peninsula, thus raising additional questions about past demographic events.

Investigating Past Human Mobility and Interactions in the Southern Cone of the Americas using Phylogenomics of Ancient Commensal Microbes



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Key words: Oral Microbiome, Paleogenomics, Demography

Dental calculus, the calcified biofilm that accumulates on teeth's surfaces during an individual's life, preserves an exceptional archaeogenetic record of hundreds of microbial species that are part of the human oral microbiome. However, most of what we know about oral microbiomes is based on modern samples, and little is known about how these human-microbe associations were established over large spatiotemporal scales. Previous metagenomics works –on modern and ancient samples– have found relatively low differences in the oral microbiome composition of populations from different periods and continents. Thus, we hypothesised that if oral microbial species are maintained within human populations over long periods, their genomes could reflect human population movements through time. To test this hypothesis, we sequenced and analysed ancient dental calculus samples from multiple individuals from the Southern Cone of the Americas, a region with a rich archaeological record, but underexplored using paleogenomic approaches. Phylogenomic methods were used to reconstruct the relationship between strains of oral microbial species

recovered from individuals across multiple spatiotemporal contexts. When combined with archaeological data, we revealed several species with strong spatiotemporal associations with their host populations. Together, our results bring not only unprecedented information about the oral microbial diversity associated with indigenous populations of South America but also novel insight into the long-term association between humans and their commensal microbes. We propose that this type of data and the implemented analytical approaches could be also used as a novel and complementary approach to investigate the mobility and interactions of ancient human populations.

Palaeogenomic Study of the Extinct *Bubalus mephistopheles* Shed Light on the Evolutionary History of the *Bubalus* Genus and Domestic Water Buffaloes

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Keywords: *Bubalus mephistopheles*, Water buffalo, Ancient DNA

The water buffaloes (*Bubalus spp.*), once widespread across Eurasia in Pleistocene, are now mostly restricted to tropical South and Southeast Asia. The extinct *B. mephistopheles* was the last water buffalo species found in East Asia during the Holocene. Its close relationship with human makes it once suggested to be the wild ancestor of the swamp buffalo. However, this species disappeared from around 3,000 years ago, with its taxonomy status, genetic relationship with other buffalo species especially the domestic water buffalo, remain largely unclear. To address these questions, we generated 30 mitochondrial genomes and six nuclear genomes of *B. mephistopheles*, together with one Late Pleistocene *Bubalus wangsajoki*. By integrating with modern water buffalo genomes, we revealed the taxonomic relationship of *B. mephistopheles* with other *Bubalus* species, and suggested that it was conspecific with the *B. wangsajoki* which was found in Pleistocene Northeast China. We further detected gene flow between *B. mephistopheles* and modern swamp buffaloes, indicating that the genetic diversity of domestic water buffaloes were also shaped by post-domestication introgressions from wild species, as described in domestic cattle. This study provides new molecular insights for understanding the evolution of the *Bubalus* genus, and the dispersal of swamp buffaloes.

Reconstructing the evolutionary history of Parvovirus B19

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Keywords: Parvovirus B19, ancient DNA, paleo virology

Human parvovirus B19 (B19V) is a widespread, significant human pathogen. Erythroparvovirus B19V poses a substantial epidemiological concern for children and pregnant and immunocompromised individuals. Despite its mild, self-limiting nature in immunocompetent individuals, B19V can cause severe complications in susceptible groups. B19V has a small, single-stranded DNA genome (~6,000 bp), which is classified into three main genotypes with 5-10% sequence divergence. These genotypes exhibit regional and temporal patterns of occurrence. Following primary infection, B19V can establish lifelong persistence in various tissues, including the bone marrow.

Advances in ancient genomics enabled the retrieval of genome-wide data from human bacterial and viral pathogens. Signatures of B19V have previously been detected in individuals spanning from the Neolithic to the modern period across Eurasia and the Americas. Applying a metagenomic approach that integrates taxonomic assignment we screened a large collection of shotgun sequencing data from ancient individuals. We combated the high sequence divergence within the B19V by implementing a multireference-based mapping strategy, when recovering ancient genomes from individual reads.

We detected a B19V signal in 1892 individuals, spanning over ~9000 years. After applying filtering and authentication steps, we performed whole-genome B19V DNA capture for 146 positive samples to enrich the pathogen DNA. We aim to evaluate B19V DNA preservation and transmission patterns in various archaeological contexts.

Our study demonstrates the feasibility of detecting B19V infections in ancient individuals. Implementing diverse methodologies to detect and recover genomes of ancient B19V infections represents a crucial step in investigating the evolutionary trajectory and historical significance of this virus.

Building Ovicaprine Bridges: Combined Palaeogenomic and Palaeoproteomic Analysis for the Study of Caprinae Early Domestication

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The advent of domestic species marks a turning point in our (pre)history. With the cultivation and herding of domesticated plants and animals, humans became less reliant on what they could gather and hunt, shifting societies and their economies to controlled food production. Starting around 10,000 years ago, the domestication of sheep (*Ovis aries*) and goats (*Capra hircus*) is now understood as a geographically complex and gradual process. The management and control over reproduction of populations of the wild representatives *Ovis orientalis* and *Capra aegagrus* consequently led, after many generations of breeding in the anthropogenic environment, to the domestic forms of caprines currently herded. It is thus crucial for understanding these very first steps of animal domestication to specifically characterise archaeological faunal assemblages dating to this transition towards more intensive livestock management.

The present paper aims at inferring the nature of caprine domestication in the Zagros Mountains using biomolecular methods. Genetic analysis showed limited success, but we nevertheless report the first ancient *Capra* specimens from Shanidar Cave and Tepe Guran, widening our temporal understanding of the local wild and managed goat populations.

Some of the sampled specimens failed aDNA recovery and were thus sub-sampled for ancient protein extraction and characterisation through Zooarchaeology by Mass Spectrometry (ZooMS). We therefore report the first use of a dual biomolecular approach on faunal assemblages from southwest Asia, by combining ancient DNA and ancient protein analyses, shedding new light on the initial steps of Caprinae domestication.

Bioarchaeological Perspectives on Horse-Human Relationships in Bronze and Iron Age Britain

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Keywords: Bioarchaeology, Human-animal relationships, Mobility

Horses in Bronze and Iron Age Britain (2500 BCE to 43 CE) played an increasingly significant role in human society, yet important questions remain about their relationship with people and their presence in the landscapes during this period. Key uncertainties include whether the native horse population is extinct by the time domestic horses arrive in Britain, the timing of the domestic horse introduction, and whether these animals were closely managed or allowed to roam freely once introduced. The UKRI-funded 'Rewilding' later prehistory project is employing a multi-stranded approach, integrating bioarchaeological, zooarchaeological, and material culture analyses to address these questions.

Despite several discoveries of horse remains initially identified as being from much earlier contexts, the earliest radiocarbon date from the Holocene currently lies between 1423 and 1131 cal BCE. To refine this timeline, we have generated fifty-two new radiocarbon dates which allow us to significantly push back the earliest confirmed arrival of domestic horses into Britain. New dates, together with aDNA analysis for fifty-eight individuals, are also shedding light on the fate of native wild horses in Britain.

To approach the mobility, management, and trade of domesticated horses, we have coupled genetic analysis with laser-ablation strontium isotope analysis on material from sixty-four individuals. Our assessment of isotopic patterns of landscape mobility, genetic population structure and relatedness in horse co-burials test current theories that consider the absence of juvenile horses in Bronze and Iron Age assemblages as evidence that horse populations roamed freely with selected individuals captured, broken-in, and trained.

Identification of Ancient Equids with Shotgun Proteomics. Revisiting Biomarkers and Biomolecular Aging

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Keywords: Equids, Paleoproteomics, Degradation

The taming and breeding of Equids emerged with an intriguing delay compared to the livestock of the Early Neolithic package (sheep, goat, cattle, pig), but then spread rapidly as it led to novel capabilities for the exploitation of natural resources and for intercultural interactions over increasingly larger territories. The eventual arrival of the modern horse (*Equus caballus*) and donkey (*Equus asinus*) and their eventual confluent husbandry in the Iberian Peninsula provides a paradigmatic framework to understand the rate and fate of the dissemination and management of equids in the Western Mediterranean. The rationalization of the equid archaeological record from taxonomical parameters has been traditionally jeopardized by the overlapping morphological features of the skeletons of domestic horses and donkeys, taking into account intra-species variability. The present study aims at the taxonomic identification by shotgun proteomics (nLC-MS/MS) of forty-five samples (with 3 biological replicate each) collected from twenty archaeological sites in Southern Iberia. The study covers specimens from the southern Iberian Peninsula, ranging from the Upper Paleolithic to the Middle and Modern Ages. The aim of the investigation is twofold. On the one hand, strategies are devised to identify *Equus caballus*, *Equus asinus* and their hybrids over time. This is challenging due to the limited variability of collagen within the equid species, for which we have five potential amino acid markers, whose reliability will be assessed with our proteomic data and associated genomic information. On the other hand, we take advantage of the broad chronological range scrutinized in our study to lay out an assessment of protein degradation from different types of chemical alterations. The correlation between biomolecular damage with specimen ages and environmental conditions of the archaeological sites will be discussed.

Tracing early hop use through stable isotope analysis

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Keywords: hops, isotopes, brewing

The use of hops (*Humulus lupulus*) in beer production goes back c. 1,500 years in Sweden, but it is largely unknown what type of hops were used for brewing. Archaeologically, the small hop fruits inside the hop cones, are preserved either in desiccated state or under waterlogged conditions. In this pilot study, we examine wild and cultivated modern hops, as well as medieval archaeological hop remains, with the aim of developing a methodology for distinguishing wild, locally cultivated, and imported hops through the analysis of stable carbon, nitrogen, and sulphur isotopes of hop fruits.

Eliminating the Gap: New Radiocarbon Dates and aDNA Identification Suggest Chronological Overlap between Humans and the Extinct Mallorcan Dwarf-Goat

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Keywords: aDNA Authentication, Chronology, Human-Caused Extinction

Island ecosystems are known to be particularly sensitive to disruption caused by the introduction of humans, with many documented extinctions of island fauna occurring soon after the arrival of humans. In the case of the endemic dwarf goat of the Balearic Islands, *Myotragus balearicus*, the timing of the arrival of humans in relation to the extinction of *Myotragus* has been a subject of much debate. To secure a new chronology of the extinction of *Myotragus* and the settlement of Mallorca, we apply ancient DNA techniques to authenticate the morphological identifications of faunal remains that have been directly radiocarbon dated. These faunal remains have directly overlapping radiocarbon dates and were morphologically identified as *Myotragus* (4413–4158 years calBP) and an introduced ovicaprid domesticate (4413–4149 years calBP), which serves as a proxy for human settlement. These remains push forward the most recent date ever obtained for *Myotragus* by approximately 229 years and provide compelling evidence for the cohabitation of humans and *Myotragus* on Mallorca. Using a series of laboratory and analytic protocols to optimize the recovery and interpretation of endogenous aDNA, we were able to generate sufficient molecular data to not only confirm the morphological identifications of both specimens (*Myotragus balearicus* and domestic goat, *Capra hircus*) but also to generate 2 partial mitochondrial genomes for *M. balearicus*. While the exact causes of the dwarf-goat's extinction are still uncertain, our results present strong support for the existing hypothesis that the arrival of humans directly contributed to the extinction of *Myotragus*.

Reconstruction of Oral Environments of Ancient Hunter-Gathers in Japan Using Palaeogenomics and Palaeoproteomics

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Keywords: Dental calculus, Ancient oral microbiome, Hunter-gatherers

The Jomon period (ca. 16,500–2,300 years ago) marked a hunter-gatherer period in Japan, during which populations on Honshu, a mid-latitude region, developed diverse diets and lifestyles. The populations of the eastern and western parts of Honshu showed different life strategies and eating habits and had different oral conditions. These differences point to the significant influence of diet and lifestyle on oral microbiomes, yet the molecular mechanisms driving these regional variations remain unclear. To uncover the molecular basis of these disparities, we analysed dental calculus from 10 individuals (ca. 5000–2300 years BP) from two different Jomon sites from both the eastern and western parts of Honshu. Mass spectrometry-based proteomics was applied to eight samples. Protein preservation was confirmed with OSSD, and all eight samples met stringent criteria. Furthermore, applying shotgun DNA sequencing to the same sample set, we checked contamination with decOM and found that all individuals harboured ancient bacterial genomes, with 50–80% derived from ancient oral microbes. Based on these data, regional differences in microbial compositions and interaction between host and oral microbes were assessed. We observed differences in the abundance of specific bacterial species between individuals from eastern and western regions, suggesting that distinct subsistence strategies influenced oral microbiome composition. These findings highlight the influence of ancient lifestyles and environmental adaptations on oral health and microbiome evolution. By integrating molecular and archaeological evidence, this study provides a deeper understanding of how regional differences shaped the oral microbiomes of Japanese hunter-gatherers.

Raw Material Selection Strategies in Leather Production: Equus Sp. and Cultural Dissonance in Copenhagen's Medieval Period and Renaissance

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Keywords: Leather artefacts, ZooMS, Horse

The exploitation of horses throughout the Scandinavian Medieval Period and Renaissance reveals a duality. On one hand, horses were primarily accessible to the elite and although substantiated evidence is lacking, it is generally believed that horse meat was not consumed during the medieval period - possibly due to a Christian prohibition against an old pagan custom. On the other hand, stigma surrounds the handling of horse hides. Medieval skinnners working with horse hides have been described as “dishonest”, while Stockholmers were specifically forbidden from using horsehide in shoemaking. Meanwhile, the archaeological record narrates a gap between social norms and actual behaviour. Medieval horse bones were found in refuse layers in the butcher's district of Copenhagen suggesting consumption of horses. Such cultural dissonance is further evident in this study, where around 4,000 samples, originating from approx. 1,000 highly modified leather objects have been taxonomically identified via the proteomic approach ZooArchaeology by Mass Spectrometry (ZooMS) from Medieval and Renaissance Copenhagen and Odense, Denmark, revealing a preference for cattle and sheep. However, a surprisingly high number of *Equus* sp. samples were also present. The large quantity of *Equus* sp. found in leather objects contrasts with the relatively small number of horse bones identified in a nearby excavation. This discrepancy highlights the complexity of horses as a raw material in Copenhagen's historical period, suggesting that different body parts were integrated into distinct production systems.

Genomic Evidence for the Dispersal of Early Domestic Sheep into Neolithic Central Asia

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Keywords: Domestication, Ancient DNA, Sheep

The domestication of sheep and their dispersal into Central Asia has transformed lifeways in the region. Recent research discovered the early presence of domestic sheep at Obishir V in southern Kyrgyzstan, and proposed an agropastoral expansion to the Eurasian interior by ca. 6000 BCE, almost 3,000 years earlier than expected. Despite these findings, much regarding the origins of livestock in montane Central Asia remains unresolved, such as how domesticated sheep dispersed into Central Asia and whether Neolithic sheep populations at other Central Asian sites, such as Jeytun in Turkmenistan (ca. 6500 BCE), might have followed similar or different dispersal and management trajectories. These unresolved questions on the early history of Central Asian domestic sheep also stem from a lack of deeply-sequenced sheep genomes from the region. Here, we explore the results of our analyses of ancient genomes from Neolithic Obishir V and Jeytun, alongside published domestic and wild sheep genomes from Neolithic Iran, Anatolia, and Europe. We find that hybridization with wild sheep may have played a role in the establishment of some Neolithic pastoralist systems in Central Asia. Overall, our results illuminate the complex nature of the dispersal and management of sheep in Neolithic Central Asia.

Strength in Numbers: DNA Preservation, Sex Ratios, and Species Identifications in >1,300 Ancient Canids

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Keywords: Canids, aDNA, Domestication

The Skoglund Ancient Genomics Laboratory is currently undertaking a large-scale ancient DNA project to piece together the unique relationship between humans, wolves, and dogs in the past. We are examining this from several different perspectives: exploring the genetic history of Ice Age wolves; tracing domestication from wolves into dogs; and reconstructing how dogs adapted to changing human environments. The project is based on close collaborations and data sharing with zooarchaeological researchers, where initial results are made immediately available for inclusion in external studies and reports.

Here, we have used initial aDNA results from over 1,300 canid specimens across a large spatial and temporal range to investigate broad trends in the data. First, skeletal element preservation confirmed high endogenous preservation in ossicles and petrous bones, and produced new evidence for improved DNA yield in cementum over dentine samples. Second, a new pipeline for genetic sex estimations has revealed a bias in male remains in the archaeological record, mirroring those in other mammalian fossils (Gower *et al.* 2019) and some modern wolf populations (Packard & Mech 1980). Third, we will be implementing a species identification pipeline to distinguish dog, wolf, and other canid samples, with the potential for identifying unique specimens in unexpected archaeological contexts. Overall, this presentation outlines some of our newest contributions to understanding human-canid relationships, and how we plan to continue addressing questions of this nature in future studies.

Divine Creatures: New Molecular Research on the Egyptian Animal Mummies at the British Museum

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Keywords: Animal mummies, Molecular analysis, Museum collections

The British Museum curates one of the largest animal mummy collections outside Egypt. The collection comprises some 500 mummies, including cats, crocodiles, ibises, snakes and fish. Despite its range, the collection has received scant research attention and is a priority for improved storage and conservation. The Museum has now embarked on an ambitious new project bringing together curatorial, scientific and conservation expertise in the first systematic study of this material. Molecular analysis of the embalming materials is an important component of the multi-analytical scientific programme, alongside X-ray imaging and radiocarbon dating.

Animal mummies are a source of fascination for Museum visitors, providing unique insights into ancient Egypt. Believed to represent miniature versions of their gods (e.g. cat-goddess Bastet), these offerings were thought to establish a connection with the divine. The scale of production had significant cultural and social ramifications. Millions of animals were bred, with impacts on the local and national economies, and probably also on the local fauna.

This paper will focus on the molecular research, which aims to identify the mummification materials and techniques employed, to evaluate variation across species, production centres and time, and to compare with human mummification practices. Overall, the project aims to advance understanding of the evolution of animal mummification in ancient Egypt, its ecological and economic impacts, and its social and cultural significance. The research aligns with a new exhibition on animal mummies taking place at the Museum in 2026 and will inform future permanent displays.

Dietary Strategies in Medieval Urban Cat Populations through Multi-Proxy Stable Isotope Analyses of Bone Collagen

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Keywords: Isotopic ecology, Zooarchaeology, Anthropogenic ecosystem

The well-established principles of carbon and nitrogen isotope pools and fluxes in natural ecosystems make the stable isotope analysis of animal tissues an extremely useful tool in studying dietary preferences, food web dynamics and reconstruction of past ecology. However, in anthropogenic environments, the natural processes are disturbed; in particular, non-local dietary items can be imported, which makes interpreting past animal ecology very challenging. In this paper, we use a domestic cat as an example of species adapted to live in urbanized environment. Despite being domesticated and obligate carnivore, the cat is flexible in its dietary choices. It can easily switch its subsistence strategies between benefiting from human-provided food, opportunistically exploiting synanthropic environment, and raiding the wilderness. A recently published paper¹ revealed high isotopic variability in medieval urban cats, likely representing the availability of many variable food sources or subsistence types. To address these problems, we selected a collection of cat bones from a late medieval town of Gdańsk in northern Poland, an example of a domestic cat population living in one locality. We measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of individual amino acids and $\delta^{34}\text{S}$ values in collagen, in addition to previously reported bulk carbon and nitrogen stable isotope data from the same samples. Using this combined approach we discriminated between marine and terrestrial resources and identified several isotopic groups of cats representing different feeding strategies within the Gdańsk town. Our data reveal that the dietary sources of several cats in Gdansk were based on imported crops (non-local foods).

Reference

1. Krajcarz, M. et al. Stable isotopes unveil one millennium of domestic cat paleoecology in Europe. *Sci. Rep.* 12, 12775 (2022).

Pathological Dental Calculus?: Exploring the Oral Microbiome in Individuals with Leprosy

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Keywords: Ancient DNA, Oral microbiome, Leprosy

This pilot study examines the role of how leprosy (Hansen's disease) and *Mycobacterium leprae* can affect the formation of dental calculus and the composition of the oral microbiome. Three individuals from the medieval leprosarium of St Mary Magdalen's, Winchester, United Kingdom have both skeletal lesions highly consistent with leprosy, as well as unusual dental calculus. These three individuals have gross unilateral dental calculus of an atypical morphology. This dental calculus formation is suggestive of impaired masticatory function, trismus, and/or paralysis.

Leprosy can cause extensive damage to the mouth and nasal areas, and can also affect the facial and trigeminal nerves possibly leading to facial paralysis. We use shotgun metagenomics to investigate the make-up of the oral microbiome from dental calculus on the affected and unaffected sides, to compare microbial composition, bacterial metabolism, and possible functional differences.

Exploring the Impact of Anthropogenic Environmental Factors on Non-Human Mammalian Oral Health: A Study of Dental Calculus from Smithsonian Institution Collections

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Anthropogenic impacts on mammalian oral health can be studied via the detailed contextualization of ancient and historic dental calculus. This study examines the microbiomes generated from dental calculus sub-samples of 63 non-human mammalian individuals across 16 different species housed at the Smithsonian Institution National Museum of Natural History (SI-NMNH). To contextualize microbial variation within this dataset, we utilized qualitative accession data along with quantitative data to identify possibly meaningful points of comparison along standard and non-standard analytical dichotomies. These include captive v. wild, foregut v. hindgut, year of death, anthrome type and geographic location. We utilize a pipeline designed for the exploration of non-human oral microbiome datasets which are severely underrepresented in reference databases. Finally, by integrating museum accession records with oral microbial profile data we reiterate the benefit of utilizing qualitative museum accession data within historic oral microbiome studies.

Tracing the Emergence of Millet Agriculture in Northern China: Radiocarbon Chronology and Stable Isotope Insights from the Xinglong Site

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Keywords: Early millet agriculture, Radiocarbon dating, Stable isotope analysis

The transition from foraging to farming marks a fundamental shift in human history, yet the precise timing, scale, and ecological impact of early agriculture in northern China remain unclear. This study integrates radiocarbon dating and stable isotope analysis with published archaeobotanical evidence to investigate the origins and development of early rain-fed millet farming at the Xinglong site, associated with the Yumin culture.

Previously published archaeobotanical studies identified charred broomcorn and foxtail millet seeds, with broomcorn millet increasing over time. Direct radiocarbon dating of charred broomcorn millet seeds confirms early cultivation at Xinglong.

To refine the chronological framework, we conducted new radiocarbon dating on key archaeological features, revealing a long-term occupation sequence (~11,500–10,500 cal BP and ~8,800–7,000 cal BP), spanning the transition from foraging to early millet agriculture. Stable isotope analysis of human remains indicates a progressive dietary shift toward millet dependence, while animal remains suggest subtle ecological changes linked to early cultivation. These findings highlight the role of millet farming in shaping subsistence strategies and human-environment interactions.

By integrating a refined radiocarbon chronology, stable isotope analysis, and archaeobotanical findings, this study provides new insights into the emergence and development of early agriculture at Xinglong. The results contribute to broader discussions on Neolithic transitions, millet-based subsistence, and the dynamic interplay between emerging agricultural practices and environmental change in northern China. These findings emphasize the value of biomolecular archaeology in reconstructing early farming societies.

Evaluating Agricultural Practices in the Coast and Valleys of the Atacama Desert (1000 BC – AD 1450): An Approach to Crop Management using Carbon, Nitrogen and Strontium Isotopes



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Keywords: Isotopic variation, Fertilizers, Crops

Despite its extreme aridity, agriculture was key for pre-Colombian human subsistence in the Atacama Desert (Chile). Irrigation combined with different types of fertilisers supported intensive crop production, particularly in the Tarapacá region (1000 BC-1450 AD). Although well described from inland Tarapacá, little information is known for sites on the Pacific coast. Coastal agriculture was assumed impossible due to the harsh desert conditions, and crops found in coastal sites were assumed to be derived from inland valleys or oases. Here we examine agricultural practices in the coast and interior of Tarapacá, considering the use of fertilisers and geographic origin of the crops. Due to the excellent preservation that characterises the Atacama, stable carbon and nitrogen analysis were carried out on a total 315 desiccated coastal and inland plants. Strontium isotopes were analysed for 46 crops from coastal and interior sites. Results show that crops from both geographical zones were fertilised (camelid dung or seabird guano). Inland crops show local origin with limited exceptions. Coastal crops do not show $^{87}\text{Sr}/^{86}\text{Sr}$ values indicative of either local coastal habitats or those expected from sites in the interior. However, their values suggest these crops likely originated from the coastal valleys of the area now found in the extreme north of Chile and/or southern Peru. These results require new thinking and discussion on the exchange of crops in the region, where people not necessarily traded plants east to west but also in a north-south direction.

Biomolecular Analysis of Pleistocene Canids Indicates Widespread Connectivity and Close Human-Dog Association Across Western Eurasia

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Wolves (*Canis lupus*) were the first non-human species to enter into the anthropic niche, ultimately giving rise to domestic dogs (*Canis domesticus*). This process is thought to have occurred during the Late Pleistocene, although the exact timing and location is unknown. This is largely due to the absence of genetically definitive dogs dating to this period, with the majority of putative dogs identified using morphometric analysis [1,2]. Dogs were widespread by the Mesolithic however, and deeply integrated into human culture, from being exploited as traction animals in the Arctic through to sharing diet and funerary behaviours with humans. The Late Pleistocene therefore represents a key transition in human-dog coevolutionary histories.

Here, we analysed several canids from Late Pleistocene deposits across West Eurasia using combined archaeological and biomolecular data to identify dogs from the Late Pleistocene and investigate the role they played in Palaeolithic human cultures. We identified the earliest known dogs, which were excavated from Palaeolithic contexts in the UK (15kya; Gough's Cave) and Turkey (16kya; Pınarbaşı). These Palaeolithic dogs were associated with geographically, culturally and genetically distinct human populations, but were highly genetically similar. Isotope analysis revealed a shared diet of dog and humans at each site, alongside a shared post-mortem treatment of both dogs and humans. These results not only indicate rapid dispersal of a single dog population across West Eurasia during the Late Pleistocene, but close interaction both in-life and death between dogs and humans regardless of the behaviour, culture and ecology of the associated human population.

References

1. Janssens L, Giemsch L, Schmitz R, Street M, Van Dongen S, Crombé P. 2018 A new look at an old dog: Bonn-Oberkassel reconsidered. *J Archaeol Sci* 92, 126–138.
2. Germonpré M, Sablin M V., Stevens RE, Hedges REM, Hofreiter M, Stiller M, Després VR. 2009 Fossil dogs and wolves from Palaeolithic sites in Belgium, the Ukraine and Russia: osteometry, ancient DNA and stable isotopes. *J Archaeol Sci* 36, 473–490.

Antibiotic Resistance and Microbial Communities in the Environment of an 18th-century Slaughterhouse

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Keywords: Antibiotic resistance, Microbiomes, aeDNA

Microbes, the first living organisms on Earth, have evolved to adapt to various environments. The microbial diversity in the human gut microbiome is crucial for health, and similarly, microbial diversity in agricultural environments is vital for soil health and interconnected health between humans, animals, and the environment (One Health). Although antibiotics were discovered in the early 1900s, microbes have produced antibiotics long before, and antibiotic resistance is ancient. However, the role of antibiotic-producing bacteria (APB) in resistance development is unclear. While the use of antibiotics causes a strong selective pressure for antibiotic resistance, also other anthropogenic activities are speeding up its spread. Manure and garbage piles in agricultural environments are habitats where both antibiotic producers and pathogens thrive, providing an opportunity to study the role of producers in the emergence of antibiotic resistance.

This study explores the evolution of antibiotic resistance through agricultural history by investigating microbial communities, antibiotic resistance genes (ARGs), and potential APB from an 18th-century slaughterhouse in Turku, Finland. Twelve samples from archaeological excavations were analyzed using metagenomic sequencing. Additionally, dormant bacteria were cultivated and their genomes sequenced.

From the metagenomes, 60 ARGs were detected. Nine gram-positive bacteria were isolated and identified: *Arthrobacter* sp., *Paeniglutamicibacter quisquiliarum*, *Paeniglutamicibacter sulfureus*, *Streptomyces* sp., *Microbacterium murale*, *Bacillus licheniformis*, *Peribacillus frigoritolerans*, *Rhodococcus qingshengii*, and *Streptomyces clavifer*. Here, we present our current and developing results, which will elaborate our understanding of the impact of agriculture on the emergence of antibiotic resistance.

Exploring spatiotemporal variation in pathogen detection rates using deep sequencing data

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Keywords: metagenomics, ancient DNA, pathogens

Over the past decade, advances in the identification, enrichment, and genomic analysis of ancient DNA from pathogenic microorganisms have yielded major insights regarding the burden of specific infectious microbes in past human populations.¹ Moreover, recent studies leveraging dramatic increases in the breadth and depth of available ancient DNA datasets have begun to explore both spatiotemporal variation in pathogen preservation as well as possible sociocultural drivers of this variation.² Building on these efforts, we implemented a high-throughput pathogen screening pipeline optimized to detect more than 600 pathogenic bacterial, viral, and eukaryotic taxa in human skeletal remains. Using this approach, we tested for the presence of pathogens in more than 20,000 ancient individuals sequenced as part previous studies of ancient human population genetics. The dataset is highly diverse, spanning c. 34,000 years of human history and including individuals recovered from the territory of more than 100 modern countries. Analysis of this rich resource promises to yield new insights regarding the spatiotemporal distribution of pathogens known to have impacted past human populations, opening new opportunities to explore the drivers of changes in the burden of infectious disease through time.

References

1. Spyrou, M.A., Bos, K.I., Herbig, A., Krause, J. Ancient pathogen genomics as an emerging tool for infectious disease research. *Nat. Rev. Genet.* **20**, 323-340 (2019).
2. Sikora, M. *et al.* The landscape of ancient human pathogens in Eurasia from the Stone Age to historical times. *bioRxiv* (2023).

Exploring Human-Parasite Interactions: A Palaeoparasitological Study of Human Coprolites from the Aragón-1 site, Atacama Desert (3300 BCE – 530 CE)

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Keywords: Palaeoparasitology, Northern Chile, Coprolites

Palaeoparasitological studies provide valuable insights into the presence of parasites in human groups and their relationship with diverse lifestyles, health conditions and cultural practices in past populations. The transition from the Late Archaic Period (3050 - 1750 BCE) to the Formative Period (1000 BCE - 900 CE) in the Tarapacá region, Chile, is marked by significant changes in human lifestyles, including a shift from primarily hunter-gatherer practices to more sedentary pattern, such as the adoption of agriculture and pottery. We recovered and analyzed twenty-two human coprolite samples from the Aragón-1 archaeological site (3300 BCE to 530 CE) located in the Pampa del Tamarugal, Atacama Desert, with the purpose of evaluating human-parasite interactions and understand lifestyle changes during these periods. We found *Dibothriocephalus/Adenocephalus* sp. eggs, indicating parasite infection through the consumption of undercooked fish during both the Late Archaic and Formative periods. Our analysis revealed a close ecological interaction between this parasitic genus and the humans who inhabited Aragón-1. The presence of this parasite decreases significantly towards the later period, a phenomenon that could be explained by cultural and/or environmental factors. Culinary practices, care and prevention, and El Niño y La Niña phenomenon could be influencing this human-parasite dynamics. This study contributes to a deeper understanding of the cultural practices of the Aragón-1 individuals, highlighting dietary habits based on marine resources and mobility between the inland and coastal areas of the Pacific Ocean. It also deepens our understanding on the relationship between humans and this parasite.

Paleogenomic Insights into the Dispersal of Domestic Cats into Europe and Selection Patterns Over Time

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Keywords: Domestic cat, Dispersal, Selection

Zooarchaeological and genetic evidence from the last two decades demonstrated that domestic cats originated from the North African and Near Eastern wildcat, *Felis lybica lybica*. The commensal relationship between humans and cats started about 11 thousand years ago in the Neolithic Levant. Recent paleogenomic evidence showed that cats were introduced to Europe several millennia later, in the Roman era. Yet, archaeozoological and ancient mitochondrial DNA data from northwest Europe suggest that domestic cats were already present in this region in the 1st millennium BCE, in Iron Age settlements. Until now, only three cats from Europe dated to this period have been analysed at the nuclear level, thus leaving uncertainty on the times and circumstances of the human mediated dispersal of domestic cats into Europe.

To address that, we analysed the DNA of 30 cat remains dated from the Bronze Age to the Roman era from northern and western Europe. We built double-stranded genomic libraries and generated low-coverage genome-wide data via shotgun sequencing. The temporal transect of genomic variation that we reconstructed made it possible to refine the timing of the introduction of the domestic cat to Europe. In addition, to investigate patterns of selection in the history of cat domestication, we show here the preliminary results of the analysis of cat phenotypic variants across time and place, with a particular focus on the sex-linked orange coat colour.

Ancient DNA from the Tiny Mediterranean Archipelago of Columbretes: A Deadly Crossroads of Extinct Snakes and Pirates

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Keywords: Ancient DNA, Snakes, Islands

The Columbretes Islands, a small volcanic archipelago, lie 50 km off Spain's Mediterranean coast. Last connected to the mainland at the end of the Last Ice Age, they were known to ancient Greek and Roman sailors as **Ophiusa** and **Colubraria**, names reflecting their once-abundant snake population. Despite this, Barbary pirates used the islands as a base for raids on the Iberian coast during the Middles Ages.

The snakes are now extinct, following an eradication in the 19th century, that involved burning the whole island, to facilitate construction of a lighthouse. The identity and origins of the snakes remained a subject of debate ever since.

To clarify this, we analyzed ancient DNA from the main island few available remains, including a 3,000-year-old fossil assemblage excavated in 2005. The collection, mostly reptile and bird bones, also contained mice. We genetically identified the island's extinct snake as *Vipera latastei*, alongside previously unrecorded paleofauna, such as *Chalcides bedriagai*. The mice were identified as *Mus musculus*, rather than *Mus spretus*.

Additionally, we recovered DNA from the island's only known archaeological remains, a human burial of uncertain age (undergoing work), discovered in 1990 after a cliff cave collapse, whose genome appears as a mixture of various Mediterranean ancestries.

Stable Isotope Insights into Horse Management and Human-Horse Relationships in Bronze Age China (ca. 1300-770 BCE)

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Keywords: Stable Isotope, Horse, Western Zhou Dynasty

Archaeological and documentary evidence from Shang and Zhou periods indicates that the acquisition and management of horses were significant issues within early dynasties. However, the limited nature of such materials restricts detailed understanding. Stable isotope analysis provides insights into the diets and origin of these horses, which is important for understanding the role of horses and their interaction with human society. Our study focuses on key Western Zhou sites: Zhouyuan (the capital region and a major center for horse consumption), Yaoheyuan (a newly discovered site on the northwestern frontier), and Liulihe (on the northern boundary of the Zhou's eastern land). By integrating multi-isotope data (C, N, Sr, O) of bones and enamel samples with radiocarbon dating, we discuss the methods and temporal changes in horse acquisition and management at these sites. We also compare these findings with data from other regions, examining similarities and differences in horse management practices across the Western Zhou and surrounding steppe regions (west and north), as well as the source of horses and potential horse exchange networks. Preliminary data from Late Shang sites may present a different narrative, which we are also investigating.

All your Eggs in a Network: the Case of Çatalhöyük

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Keywords: Eggshell, Proteomics, Socio-ecological networks

Çatalhöyük has provided some of the most intriguing findings related to complex human-avian interactions. Birds were exploited chiefly for symbolic purposes as attested by osteological markers of feather removal, intentional deposition in burials and use of bird bones to make musical instruments. The role of the crane (*Grus grus*), which is also depicted at the site, has become especially famous due to Russell and Mc Gowan's¹ hypothesis of a "Crane Dance" being reenacted at Çatalhöyük. Previous proteomics studies² targeting eggshell remains from the site revealed the presence of a range of Anseriformes species as well as Gruiformes, but could not resolve the taxonomy further. Furthermore, this study highlighted that eggs seemed to straddle the world of the living and the world of the dead, with some fragments recovered from burials and others discarded in middens. Here we re-examine this data in the light of major advances in reference databases used to discriminate between bird taxa. We also use available contextual data and network analysis to better understand the role(s) of eggs at the site, attempting to glimpse the complex multi-species relationships that were taking place at Çatalhöyük at the dawn of Neolithic lifeways.

References

1. Russell, N. & Mc Gowan, K. J. Dance of the Cranes: Crane symbolism at Çatalhöyük and beyond. *Antiquity* 77, 445-455 (2003).
2. Demarchi, B., Presslee, S., Sakalauskaite, J., Fischer, R. & Best, J. The role of birds at Çatalhöyük revealed by the analysis of eggshell. *Quat. Int.* 543, 50–60 (2020).

The Genomic Make-Up of Ancient Wild and Domestic Cats from the Levant and North Africa

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Keywords: Cat domestication, Paleogenomics, *Felis lybica lybica*

The domestic cat (*Felis catus*) descends from the North African and Near Eastern wildcat subspecies *Felis lybica lybica*. Its close association with humans facilitated its global spread, making it one of the most successful mammal species. Recent archaeogenomic and zooarchaeological studies indicate that domestic cats were introduced to Europe within the last 2,000 years, much later than previously assumed. However, the scarcity of genomic data from the Near and Middle East and North Africa leaves key questions unresolved, particularly regarding the origins of domestic cats and the contributions of the two proposed centres of domestication, the Neolithic Levant and Pharaonic Egypt.

To fill this gap of knowledge, we generated 60 low-coverage (0.1- to 0.6-fold) genomes from cat remains from North Africa, the Near and Middle East, and the Iberian and Italian peninsulas dated from the 7th millennium BCE to the 15th century CE, along with 23 low- to high-coverage (0.4- to 15-fold) genomes of present-day and museum wildcats from the Levant and central and northern Africa. Our findings suggest that North Africa was the original centre of cat domestication. As domestic cats spread to the Levant and the Middle East, they most likely acquired Levantine genetic ancestry through admixture with local *F. l. lybica* populations. This work lay the foundations for further disentangling the origins of domestic cats and tracing their expansion across Southwest Asia.

Past Human Activities Affected Microbial Communities in Ancient Archaeological Sediments from the Alverson Site, Uzbekistan

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Keywords: SedaDNA, Human activity, Microbiome

Sedimentary ancient DNA (sedaDNA) from archaeological sites holds a large potential for reconstructing past human-environment interactions, yet it remains poorly understood due to limited studies. Here, we analyze sedaDNA from 26 sediment samples collected across three layers (Sectors 2, 3, and 9) at the Dalverzin site, a Bronze-Iron Age open-air settlement dated to 1500–800 cal BC in the Fergana Valley of Uzbekistan, to explore both plant and microbial communities influenced by ancient human activity. DNA sequencing and taxonomic profiling reveal distinct environmental signatures. Sector 2 showed a dominance of *Phragmites*, suggesting a wetland habitat, alongside native and human-utilized taxa like *Glycyrrhiza*, *Arundo*, *Equisetum*, *Hippophae*, and *Salix*. Our microbial analyses, thermophilic bacteria - including *Planifilum*, *Thermobifida*, and *Thermomonospora* - were abundant in the Sector 9 layer but absent in the others. Given that Sector 9 was a residential area, we hypothesize that human activities sustained elevated temperatures (more than 40°C), fostering a unique thermophilic microbiome. This study provides new insights into potential human influences on ancient microbiomes, offering new perspectives on the intersection between human habitation and soil microbial dynamics.

Genomic and isotopic insights into cat dispersal and diet from Viking to medieval northern Germany

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Keywords: biomolecular archaeology, cat-human relationship, northern Europe

Bioarchaeological studies of ancient wild and domestic animals provide critical insights into animal translocation and human-animal relationships over time. Domestic cats (*Felis catus*), in particular, have a long history of association with humans as companions and pest controllers. This makes them valuable bio-proxies for reconstructing human movements via sea and land routes, as well as dietary shifts in the anthropogenic niche.

In this study, we performed genomic and stable isotope analyses on over 90 cat remains from two major trade hubs in northern Germany: the Viking settlement of Haithabu (AD 800 - 1050) and its medieval successor, the town of Schleswig (AD 1050 - 1250). Ancient DNA analysis showed no clear geographical pattern in mitochondrial DNA ancestries from the two sites, all of which belong to clade IV of *F. s. lybica* and are assigned to haplogroups A, C, and D. This likely reflects the widespread dispersal of domestic cats in Europe starting from the Roman era. Nuclear DNA analysis revealed limited gene flow from European wildcats, consistent with previous findings on ancient domestic cats from other regions in Europe. Stable carbon ($\delta^{13}\text{C}$ $\mu = -19.1 \pm 0.7\text{‰}$) and nitrogen ($\delta^{15}\text{N}$ $\mu = 10.5 \pm 1.1\text{‰}$) isotope ratio analysis of cats from both sites indicates that most cats had mixed diets involving some marine foods supplied by or scavenged from humans.

This multidisciplinary approach enables a comprehensive reconstruction of human-mediated cat dispersals and their dietary patterns during pivotal socio-economic transformation.

Expanding Dietary and Ecological Insights of Archaeofauna at Aqaba Castle, Jordan, Using Non-Exchangeable Hydrogen Isotope Ratio ($\delta^2\text{H}$) Analysis

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Keywords: Non-exchangeable hydrogen isotope ratios, Middle Eastern archaeology, Animal diets

Non-exchangeable hydrogen isotope ratios ($\delta^2\text{H}$) are an underused proxy for studying past human-animal relationships, offering insights into diets, water intake, and ecological ranges. This study examines 218 bone collagen samples for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^2\text{H}$ values of wild and domestic terrestrial species, and marine fish from Ottoman-period Aqaba Castle, Jordan (16th–19th century CE). In this hyperarid region, high $\delta^{15}\text{N}$ values reflect the elevated $\delta^{15}\text{N}$ baseline of plants, causing overlaps in taxa with varying trophic levels. $\delta^2\text{H}$ helps mitigate this by acting as an additional trophic level indicator. Domesticated herbivores mostly possess $\delta^2\text{H}$ values close to that of predicted local rainfall, while hypercarnivorous cats (*Felis catus*) exhibit the highest $\delta^2\text{H}$ values, averaging +42.4‰ above herbivores. Fish also show high $\delta^2\text{H}$ values, correlated with trophic level ($r = 0.84$, $R^2 = 0.70$) and estimated body size ($r = 0.61$, $R^2 = 0.37$). Cattle and chickens, have the lowest $\delta^2\text{H}$ values of all terrestrial species, likely due to their significant water requirements and the consumption of ^2H -depleted aquifer water. Mapping wild gazelle (*Gazella* sp.) and chukar (*Alectoris chukar*) $\delta^2\text{H}$ values on rainwater $\delta^2\text{H}$ isoscapes indicates limited home ranges in the surrounding hyperarid highlands, in line with their expected behaviours. We assess the utility of $\delta^2\text{H}$ for determining fish consumption using Bayesian dietary models for cats and hyenas (*Hyaena hyaena*), revealing higher fish consumption by cats when $\delta^2\text{H}$ is included alongside $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. This study highlights the value for $\delta^2\text{H}$ values to enhance interpretations of animal diets and behaviours in archaeological contexts.

Optimising Sheep Reproductive Strategies in Roman Iberia: The Case Study of the Villa of Els Tolegassos (Catalonia).

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Keywords: Stable oxygen isotope, Seasonal reproductive patterns, Early Roman period

The Roman conquest of the Iberian Peninsula entailed a profound process of transformations in the sociopolitical and socioeconomic organisation of the local communities. These also implied changes in livestock farming practiced over centuries, with optimisation, selection, and specialisation being the desired criteria, in order to satisfy the new colonial system. Sheep are one of the animals for which both zooarchaeology and written sources suggest exploitation mainly focused on the intensification of wool and milk production after Romanization.

This study aims to provide innovative data on the reproductive patterns of sheep in the northeast of the Iberian Peninsula during the early Roman period and to compare them with immediately previous data from the same area, as a way to start to characterise this specialised Roman livestock farming.

To investigate the season and seasonality of births and the duration of the lambing period, sequential oxygen isotope ($\delta^{18}\text{O}$) analyses were carried out on the enamel of eight lower second molars of sheep from the small villa of Tolegassos (Alt Empordà). Preliminary results reveal a selection of a single birth season between late winter and mid-spring. These very homogeneous results differ from those obtained at Mas Castellar de Pontós, a Late Iron Age rural settlement located 17 km northwest of Tolegassos, where two different birth seasons were detected. These data suggest the optimisation of sheep reproductive control patterns linked to a specific production system in this rural Roman villa.

Metagenomic and Proteomic Studies of Stone Age Anthropogenic Birch Tar Samples

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Keywords: Biomolecules, Human oral microbiome, Stone Age

Birch tar, a widely used Stone Age adhesive, has emerged as a unique and valuable source of ancient biomolecules. Birch tar was produced through the dry distillation of birch bark (*Betula* sp.) and was often processed by chewing. Remarkably, birch tar chunks surviving from thousands of years ago have been shown to preserve ancient human and oral microbiome DNA (aDNA), addressing the scarcity of human remains in Baltic regions for the Prehistoric period.

Our research project focuses on analyzing Stone Age birch tar samples (2120 BC to 9340±50 BC) from Estonia, Latvia, and Lithuania.

The purpose of the project is to investigate the oral microbiome, the immune responses and diet of ancient Baltic populations. Using metagenomics, we aim to explore the diversity, prevalence, and evolutionary trends of the human oral microbiome (HOM), shedding light on the lifestyle of hunter-gatherer communities. Then, palaeoproteomics analyses will identify peptides linked to oral microbial species and human immunity, with a particular focus on RNA viruses, whose prevalence in prehistoric times remains largely unknown. Finally, dietary analyses will further unravel specific details about food consumption and seasonal dietary habits of Baltic hunter-gatherers. By analyzing the birch tar samples, this research could significantly deepen our understanding of prehistoric health conditions, human-pathogen co-evolution and immunity adaptation in Baltic countries.

We have generated preliminary results from eight samples from different Estonian's sites (9210 - 9340±50 BC) showing the source and abundance of some microbial species, providing a first insight on health conditions of these communities.

The Sex(y)-Ing Part of Enamel: Estimating Sex Ratios in Hunted Ungulates from the Late Pleistocene

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Keywords: Palaeoproteomics, Zooarchaeology, Dental enamel sexing

The precise documentation of the sex composition of hunted faunal assemblages is crucial for a broader understanding of hominin hunting strategies. Traditionally, animal sex determination has been complicated due to the high fragmentation rate of Pleistocene and Holocene faunal assemblages, which prevents assessing skeletal dimorphism between males and females. The characterisation of amelogenin X and Y proteins allows access to genetic sex-related information in humans, and is proving applicable to (some) other mammalian species.

Here we explore the characterisation of X and Y amelogenin sequences of the main hunted and herded ungulate families and subfamilies to be found in the archaeological record: Cervidae, Equidae, Suidae, Bovinae and Caprinae. For each family, we studied approximately 12 modern specimens with known genetic sex, in roughly equal male:female ratios. Extractions were conducted using an acid demineralization approach without subsequent protease digestion, followed by LC-MS/MS analysis. Except for Suidae, which, for biological reasons, does not present any variation between the X and Y proteins, most of the reference specimens were confidently assigned to the correct sex. We then applied sex determination using enamel proteins to *Cervus elaphus*, *Equus ferus* and *Bos/Bison* specimens from the MIS5-3 site of Axlør (northern Iberia), which has evidence of Neanderthal occupations.

We show that amelogenin X and Y are great targets to perform sexing of ungulate dental enamel and that such information can be routinely obtained from Late Pleistocene specimens. Applications of this approach can thus enhance our comprehension of hunting strategies (if any) or early herding management.

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Authentication and reliability of ancient molecular datasets

Improving Data Archiving Practices in Ancient Genomics

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The long-term value of ancient genomic data will only be fully realised if it is appropriately archived in public databases. I surveyed publicly archived data from 42 recent ancient genomics studies, and found widespread shortcomings. Half of the studies archived incomplete datasets, preventing accurate replication and representing a loss of potentially useful data from thousands of remains. Inappropriate filtering and poor metadata reporting further complicates reuse of data by other researchers. I will discuss these issues, and make six practical recommendations for producers of ancient genomic data: (1) archive all sequencing reads, not just those that could be aligned to a reference genome, (2) archive read alignments too, but as secondary analysis files, (3) provide correct experiment metadata on samples, libraries and sequencing runs, (4) provide informative sample metadata, (5) archive data from low-coverage and negative experiments, and (6) document archiving choices in papers, and peer review these. As datasets continue to grow, I argue that ancient genomics as a field cannot afford to continue to neglect data archiving.

Deinotherium Teeth Samples Reveal Enamel Protein Sequence Preservation in the Iberian Peninsula Across the Miocene

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Keywords: *Deinotherium*, Paleoproteomics, Enamelome

The extinct genus *Deinotherium* belongs to an ancient proboscidean lineage, characterised by the presence of backward-growing mandibular tusks (lower incisors). This taxa was widespread throughout Eurasia and Africa during the Middle and Late Miocene and survived in Africa until the Early Pleistocene. By LC-MS/MS, we have analysed the protein content of seven *Deinotherium* cheek tooth enamel samples from two Miocene sites of the Vallès-Penedès Basin (NE Iberian Peninsula): Abocador de Can Mata (various localities that span between 12.4 and 11.6 Ma), corresponding to *Deinotherium levius*; and Can Llobateres 1 (9.8 Ma), corresponding to *Deinotherium giganteum*. Our results suggest the presence of enamel protein sequences in all the analysed samples, with being AMELX the most abundant one. New bioinformatic approaches have also been applied to increase the coverage and resolution of identified peptides, searching for lineage-specific variants. This data pushes the protein sequence preservation record in the Iberian Peninsula to more than 12 Ma, increasing the knowledge of peptide sequence recovery in Miocene samples.

Bias in the Recovery of Human aDNA from Sediments and Bones Using TWIST Genome-Wide In-Solution Target Enrichment

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The scarcity of fossils limits our understanding of the genomic characteristics of Pleistocene humans, particularly the timing of specific genetic admixtures. These constraints hinder efforts to reconstruct genetic and cultural associations in past societies. Iberia served as one of the main refugia for both humans and animals during the Last Ice Age (30,000–19,000 years BP). Recent sedaDNA analyses from El Mirón Cave (Cantabria, Spain) have demonstrated continuous human occupation and the survival of carnivore species in the region throughout the glacial period. Moreover, mitochondrial sedaDNA evidence suggests population continuity during the Solutrean (24,000–21,000 years BP). By preparing hundreds of libraries, we recovered over 50,000 nuclear SNPs from a single sedaDNA sample from a Solutrean layer in El Mirón, complemented by newly generated bone genomic data from Isturitz, El Mirón, and Chufín. This dataset bridges a significant gap of several thousand years during which human remains and consequently, genomic data are scarce. It provides strong evidence for the genetic continuity of human groups in this region from the Aurignacian to the end of the Solutrean. Our analyses implemented both shotgun and capture approaches, and identified notable biases in the TWIST capture method when applied to heavily degraded samples typical of most Pleistocene materials. These biases manifest as differential edit distances and longer read lengths. These findings underscore the critical need for selecting appropriate strategies to recover genomic material from Pleistocene sediments and bones, which often exhibit very low endogenous DNA content.

Protein Preservation Through Time and Space - Comparative Analysis Between Study Type and Archaeological Context

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Keywords: Paleoproteomics, Meta-analysis, Skeletal proteome

As paleoproteomics continues to advance, an increasing number of datasets from archaeological materials, including human remains, are available. However, these are still relatively few, and no comparison between ancient proteomic datasets exists. In our paper, we compare five paleoproteomic datasets, comprising four from archaeological and one from a forensic context. The datasets represent a range of geographical locations and time periods, spanning from the 2nd century CE to modern times, and include one previously unpublished dataset of three individuals from late 18th century Finland.

Variations in wet lab methods and advancements in mass spectrometry over the past 15 years of paleoproteomic studies have made cross-study comparisons difficult. Furthermore, limited knowledge about how time and taphonomic factors influence protein preservation in skeletal remains requires further research. Therefore, meta-analyses combining previously published data with newly generated datasets offer a valuable yet underutilized opportunity to deepen our understanding of ancient proteins. Our study demonstrates that historical burial crypts offer excellent conditions for protein preservation, in that certain immune-related proteins are exceptionally well preserved in our soft tissue samples.

We show that comparing multiple datasets can reveal patterns of preservation as well as shed light on how temporal and taphonomic factors influence the recoverability of different proteins. We also aim to open discussion about the research potential of existing data and encourage discussion about data availability and long-term storage as necessary steps for conducting future meta-analyses of proteomic data.

Clear as Milk: Challenges in Authenticating Proteins Identified in Ancient Pottery

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Ancient peptide and protein authentication is one of the most critical challenges facing palaeoproteomics. Though several methods for authentication have been proposed (e.g. glutamine deamidation rates), the efficiency and reliability of these techniques are variable and often dependent on total protein recovery rates. Increased discussions of protein authentication efforts are thus necessary to further standardize and improve the proteomic method as it is applied to archaeological material. Here, we present a case study in which shotgun proteomics identified Bovinae beta-lactoglobulin, alpha-S1-casein, and alpha-S2-casein in a Late Moche cooking pot from Huaca Colorada in Peru. The detection of bovine proteins in this region and time period is highly unlikely, necessitating a rigorous evaluation of the results. We outline our laboratory contamination controls and bioinformatic approaches for assessing the origin of the milk peptides, and discuss challenges in addressing our research question: How old's the milk? Our findings underscore broader challenges in the field, particularly when conventional authentication tests yield inconclusive results. This raises a critical issue for the proteomics community: In the absence of definitive authentication methods, can we fully trust our data? To advance the field, we emphasize the importance of reporting negative results and failed attempts, which are often underrepresented in the literature. By fostering transparency and collaboration, we can move closer to establishing standardized protocols for protein extraction, analysis, and authentication in palaeoproteomics.

Benchmarking Workflows for Ancient DNA Analysis of Polyploid Crop Species

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Keywords: Ancient DNA, Crops, Benchmarking

Recent advances in ancient DNA analysis have enabled researchers to reconstruct the histories of both extinct and extant species, providing deeper insights into past ecosystems and evolutionary processes. However, the highly fragmented and degraded nature of aDNA, characterised by deamination and other forms of damage, requires specialized bioinformatic tools and pipelines tailored to these unique challenges. While such tools have been extensively applied to human and animal genomes, their performance and suitability for polyploid plant species remain largely unexplored. In this study, we evaluate the effectiveness of existing aDNA pipelines on large, complex plant genomes, focusing on major crop species like wheat and maize. We assess the utility of these packages using shotgun sequencing and target enrichment data and compare bioinformatic tools in terms of ease of use, computational resource requirements, runtime, and accuracy. In particular, we examine the impact of ploidy using diploid and allopolyploid taxa. By identifying the most efficient pipelines for these crops, our findings aim to ensure reproducibility and high-quality results for downstream analyses of species with complex genomes.

Evaluation of Dyes and Protein Survival in Charred Textiles: The Case of Pompeii and Greek Area Samples

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Keywords: Protein degradation, Charred textiles, Unique workflow

Archaeological textiles are invaluable artifacts from ancient society, cherished for their significant historical and cultural importance; in some cases, they represent a key to understand major events faced by a civilization, such as in the Pompeii and Vesuvian area. Such relics, because of their fragile preservation and degradation, require the use of highly sensitive analytical techniques for thorough examination.

For this reason, the PARCA project aimed at developing a new protocol for the simultaneous investigation of dyes and keratins (IFs) (and keratins associated proteins (KAP)) components of wool-based materials. Several tests were performed, comprehensive of different natural dyes and two protocols, the TCEP/CAA method and modified urea method, proved to be effective in pursuing the scope of the project, reducing number of samples required but maximizing the information obtained from each sample, aimed at a comprehensive approach¹.

The defined protocols were applied to mock-ups thermally aged up to 300°C to evaluate the performance of the extractions and the survival threshold of the dyes and proteins, showing characteristic trends especially between 200 and 250°C. This was correlated with FTIR and SEM data for non-invasive evaluation as well. These data are now compared with the results of the analysis of textiles from Pompeii and Greece. The results will be presented during the conference.

Peptides and dyes were separated on a Vanquish core/Vanquish Neo and detected on an Orbitrap Exploris 240. Peptide data were analyzed with PEAKS 12, (database sequences from NCBI and de novo identification); dyes were analyzed through Qualbrowser.

Reference

Serafini, I., Favero, G., Curini, R., Kavich, G. M., Cleland, T. P. Development of a Combined Protein and Dye Extraction Approach for the Analysis of Keratin-Based Textiles, *J. Proteome Res.* 23, 3890–3903, (2024).

Changepoint: Reference-free Authentication of Ancient DNA

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Keywords: Authentication, aDNA, Changepoint

A persistent challenge in paleomicrobiomics is finding a robust method to authenticate complex mixtures of ancient DNA (aDNA). Current bioinformatics pipelines involve a two-phase assessment of mapping individual species genomes, followed by aDNA authentication by comparison to a known-reference genomes (i.e. Mapdamage2.0), limiting our ability to authenticate unmapped sequences. This is further complicated by the positive bias towards detecting species that are more resilient to taphonomy or have abundant reference genomes. To alleviate the difficulty of aDNA authentication, we propose a method to authenticate aDNA without reference genomes. Here, we develop Changepoint, a new bioinformatics tool that quantifies terminal cytosine residues in ancient metagenomic data sets and a likelihood ratio test to identify the presence of aDNA based on the comparison of best fit between two models: expected DNA decay vs no decay. Using simulated aDNA data generated from gargammel, damage evaluation was not influenced by endogenous content when at least 100K sequences were available. We further benchmarked ChangePoint against other commonly used aDNA authentication methods (i.e., Mapdamage2.0 and PyDamage) using published ancient oral microbiome and raw paleofeces data. Contamination with modern DNA was detected using Changepoint across published data. Changepoint represents a novel, reference-free tool that allows users to reliably assess the aDNA authenticity of an entire metagenome without the need for reference genomes.

Diet and foodways

Four-lugged pots and the dairying revolution in northwestern Anatolia: lipid residues from Barcın Höyük (6600-6000)

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Keywords: Dairy, Organic residue analysis, Anatolia

Research has identified Northwestern Anatolia as a key region for the development of dairying in the seventh millennium BCE, yet little is known about how this practice evolved there. This presentation will focus on Barcın Höyük, a site located in Bursa's Yenişehir Valley, which ranges chronologically from 6600 BCE, when the first evidence of settled life appears in the Marmara Region, to 6000 BCE, when Neolithic habitation at the site ceases. Applying organic residue analyses to sherds diagnostic by vessel category and type, this paper aims at identifying which ones may have been primarily used to store, process, or consume dairy products. The lipid residue data discussed in this presentation will derive from 143 isotopic results subsampled from 173 organic residues obtained from 805 Neolithic potsherds, which suggest the existence of some degree of specialisation, as bowls and four-lugged pots may have been the preferred containers for processing milk. The discovery of abundant dairy residues even among the earliest ceramics indicates that the pioneer farmers arrived in the region already with the knowhow to prepare these foodstuffs and would develop new foodways that would help introduce dairy into the European continent. In fact, these skills and the reliance on secondary products may have given them one of the necessary tools to successfully venture into the unfarmed lands of Northwest Anatolia in the first place.

The Role of Fish in the Diet of Pre-Columbian Mound Builders of the Patos-Merín Lagoon System Through Stable Isotope Analysis

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Keywords: South America, Coastal wetlands, Bone collagen, Paleodiet

The study of human diet through stable isotope analysis is well-established in South American archaeology, offering robust insights into the economic and social practices of pre-Columbian groups. However, certain regions, such as the Patos-Merín Lagoon basin (southern Brazil and eastern Uruguay), remain understudied, with gaps in faunal isotopic data limiting a broader understanding of archaeological patterns. This region contains thousands of earthen mounds constructed by Indigenous groups between approximately 5000 and 250 years ago. Zooarchaeological studies have revealed a diverse range of exploited resources, with fish present in nearly all analyzed archaeological assemblages. Nevertheless, their contribution to the human diet remains a subject of debate due to the lack of isotopic baselines. Here we present the results of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis of fish remains recovered from archaeological earthen mounds in the region. Fish bone collagen exhibits significant isotopic variability, which must be considered when interpreting human stable isotope data and calibrating radiocarbon dates. Our results provide a reference for studying pre-Columbian coastal wetland adaptations and contribute to marine biodiversity conservation in habitats that are increasingly vulnerable to human activities and climate change.

Evolving Lipid Evidence: Decoding Contributions of Aquatic Fat in Early Pottery of the Southeastern United States

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Keywords: Earliest North American pottery, Lipid residue analysis, Late Archaic shell rings

New biomolecular data from lipid residue analysis conducted on one of the oldest potteries in North America now allows for a better understanding of Late Archaic (5000-3000 cal. BP) foodways in the southeastern United States. These pots have been uncovered on coastal sites referred to as shell rings, which represent the first year-round villages and large-scale feasting sites. Molecular and isotopic analyses were conducted on 60 pottery sherds and 20 baked clay objects (BCO) from St. Catherines and McQueen, two well-known shell rings located on St. Catherines Island (Georgia, USA), and dating respectively from 4300 to 4100, and 4100 to 3900 cal. BP. BCO are low-fired clay nodules that are thought to have been used in an indirect cooking method, although this hypothesis had never been tested by lipid residue analysis before. Results indicate a ubiquitous contribution of aquatic origin alongside plant and terrestrial animal-derived lipids in residues from pottery and BCO samples, validating previously untested hypothesis. Fatty acid ratios and trace amounts of aquatic biomarkers, however, led to erroneous initial interpretations and highlight the necessity of designing a multi-steps analytical protocol when conducting lipid analysis of ancient ceramics. Single-compound isotopic values are coherent with a mixing of marine aquatic resources and plant oils and/or animal fats. In addition, decorated sherds more often yielded lipid profiles indicating terrestrial animal fat contributions than undecorated sherds. Venison has frequently been described as an important feasting resource, suggesting that these pots were used during special gatherings.

Examining childhood diet in Iron Age Orkney using compound specific isotope analysis

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Keywords: Iron Age diet, Fish, Orkney

The role and significance of marine resources to human diet in Iron Age Orkney is insufficiently understood, with bulk carbon and nitrogen isotope analysis suggesting that marine consumption was, at most, minimal. Compound specific isotope analysis of amino acids (CSIA-AA) offers greater dietary precision and presents an exciting new approach to investigate Orcadian diets. Here, we apply this technique to a range of marine and terrestrial faunal samples and teeth from the remains of two middle Iron Age humans from The Cairns. Multivariate analysis and Bayesian modelling reveal clear dietary differences between the two human individuals during their early lives, with one showing strong evidence of marine resource consumption over terrestrial animals such as cattle. These findings demonstrate that the consumption of marine resources was not avoided at The Cairns during the middle Iron Age, opening up the possibility of a wider practice of marine resource exploitation throughout the Orkney Islands during this period.

Exploring the Evolution of Atlantic Fish Trade in Western Mediterranean through Stable Isotope Analysis of Medieval and Early Modern Fish Remains

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Spain hosts one of the largest fishing industries in the EU, yet the origins and historical development of this commercial endeavour remain subjects to debate. Exported salted fish was a relevant commodity in the Iberian Peninsula from the medieval period onwards, supplementing the local fisheries. Atlantic species such as the European hake (*Merluccius merluccius*), European pilchard (*Sardina pilchardus*), and Conger eel (*Conger conger*), were primarily traded by Galician merchants from the 14th century onwards. Determining the provenience of these products, however, remains challenging due to these species exhibiting populations in both the NE Atlantic and Mediterranean. The rise of cod (*Gadus morhua*) trade during the second half of the 16th century, closely tied to the *aguardiente* (brandy) trade - exchanged through the same commercial routes marked a significant shift. It has been suggested that the beginning of this cod trade is linked to the exploitation of the Newfoundland stocks, making it essential to determine the geographic origin of traded cod remains to understand the evolution of fish trade patterns in the western Mediterranean.

Here we use $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses on bulk collagen extracted from European hake, Conger eel, and cod from archaeological sites in NE Iberia to trace the geographical origin of these fish, comparing the results with the isotopic signatures of modern reference specimens. Our results provide new insights on the scope and reach of medieval and early modern fish trade networks, offering a clearer picture of the contribution that Atlantic fisheries had to the western Mediterranean fish trade over time.

Lifting the Lid on the Hebridean Neolithic: Using Organic Residue Analysis to Reconstruct Foodways in the Hebridean Social Landscape

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Keywords: Neolithic, Ceramics, Organic Residue Analysis

This contribution will present key results of my doctoral project which focuses on the analysis of dietary practices and the subsequent interpretation of foodways and the related societal frameworks of the early farming communities of the Early-Middle Neolithic of the Outer Hebrides, Scotland c. 3700-3200 BCE. The project utilises cutting-edge organic residue analysis of Neolithic Hebridean pottery lipids from islets, domestic sites and tombs – both GCMS analysis to scan for specific trace biomarkers, and stable isotope analysis to determine the origins of animal fats and subsequently to determine what foodstuffs were cooked and eaten in these vessels.

Hebridean crannogs are artificial islets set within lochs. Underwater surveys have recovered large quantities of Neolithic ceramics from the loch-beds surrounding some of these islets. It has been proposed that these islet sites were important centres for the formation of community identity via ‘commensal activities’ such as feasting (Copper and Armit 2018). Therefore, by re-constructing the dietary patterns of these sites, the role of food in these commensal dynamics can be explored.

My work extends previous analyses of pottery residues from four Hebridean islets, bringing in additional crannog sites for comparison and increasing the representation of key forms of pottery to test hypothesised differences in use. I have compared these results with those from both domestic and tomb contexts and have determined a difference in dietary patterns between these different types of sites. This also means that there was likely a difference in both the activities and the social meaning of these sites.

Reference

Copper, M. and Armit, I. 2018. A Conservative Party? Pots and People in the Hebridean Neolithic. *Proceedings of the Prehistoric Society* (84): 257-275

The Early Spread of Rice (*Oryza sativa*) in Iron Age Central Asia: New Paleoproteomic Evidence from the Mongolian Steppe (c. 250 BC-150 AD)

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Keywords: Archaeobotany, Proto-globalization, Paleoproteomics

Rice (*Oryza sativa*) is an ancient cereal crop of global cultural and economic importance. It has been exploited as a wild food resource for more than ten millennia and as a domesticated food resource for at least 7000 years (Fuller 2011). Nevertheless, the spatiotemporal, social, and climatic dimensions of rice's dispersal from its East Asian core zone remain unclear. Using liquid chromatography tandem mass spectrometry (LC-MS/MS) we report the first successful paleoproteomic identification of rice in human dental calculus. Results derive from a multiregional set of Late Iron Age (c. 250 BC-150 AD) individuals from the Mongolian steppe who occupied areas as diverse as the boreal forest zone and the Gobi desert. These results provide the earliest evidence for rice presence on the Mongolian steppe and the earliest direct evidence for its dispersal into the Central Asian geocultural zone more generally. In this paper, we explore the potential cultural, geopolitical, and climatic factors behind its regional dispersal. We further contextualize and evaluate our findings through their consideration within an emerging body of regional archaeobotanical and biomolecular data, which suggest a process of Iron Age agricultural intensification and circulation that refutes Mongolia's longstanding perception as an outlier to supraregional prehistoric agricultural developments. Finally, we consider these data within larger frameworks of archaeological and ancient textual evidence for the growth of Eurasian inter-empire networks around the turn of the first millennium AD. We ultimately recognize a pattern of intensified subsistence and culinary transformation on the steppe during this time.

Reference

Fuller, D.Q. Pathways to Asian Civilizations: Tracing the Origins and Spread of Rice and Rice Cultures. *Rice* **4**, 78–92 <https://doi.org/10.1007/s12284-011-9078-7> (2011).

What's on the Menu? Feasting on the Frontier Steppe of Medieval Mongolia

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Keywords: Millet, Steppe diet, Frontier garrison, Medieval Mongolia, North China

This research represents the first comprehensive analysis of pottery from the Medieval period in China and Mongolia, with a particular focus on ceramics associated with the Medieval Wall System (MWS). Utilizing advanced analytical techniques—including 3D scanning, petrography, and organic residue analysis—it investigates the economic foundations, dietary practices, and social dynamics of the communities that constructed and inhabited the MWS.

The study identifies domesticated plants, particularly various types of millet, as a critical food source on the steppe, suggesting millet's role as a dietary staple for these populations. This is complemented by cultivated grains, domesticated animals, and wild resources obtained through hunting and fishing. Notably, the findings present the first archaeological evidence of foxtail millet in Mongolia and indicate the possibility of millet cultivation on the Mongolian steppe.

In addition to shedding light on dietary practices, the study provides valuable insights into ceramic technology, vessel usage, and trade in the medieval frontier. It highlights the economic and social intricacies of garrison communities at both local and regional levels, contributing to a deeper understanding of the archaeology and history of Mongolia and North China during this period.

Adjusting Carbon Isotope Threshold Values for Tracing C₄ Plant Consumers Across Europe

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Keywords: Millet, Paleodiet, Ecological diversity

Millet is highly nutritious and drought-resistant crop which was an essential source of food in past agricultural systems across the world. Since their first introduction in Europe during the Bronze Age, the intensity and geographic spread of millet consumption across Europe varied over time and between regions. Being a C₄ crop, millet use in Europe as a dietary input is well attested through stable isotopic analysis of human and animal tissues. To identify levels of C₄ plant consumption, the commonly used carbon isotopic threshold value ($\delta^{13}\text{C}$) in bone collagen is $-18.00\text{‰}^{1,2}$. However, C₃ plant isotopic composition varies depending on environmental conditions, which are in turn reflected throughout the food chain, whereas C₄ plant $\delta^{13}\text{C}$ values remain largely stable. The C₃ plants in northeastern Europe show considerably lower $\delta^{13}\text{C}$ values compared to other regions, meaning that a constant threshold cannot be meaningfully applied to all regions equally; rather, it is necessary to adjust the detection baseline as a function of the environment.

The goal of this study is to adjust the baseline $\delta^{13}\text{C}$ threshold value for the detection of millet consumption based on environment, thereby enabling a more accurate methodology for identification of millet-eaters within various ecosystems. We achieve this goal by collating published $\delta^{13}\text{C}$ values of archaeological charred grains from C₃ crops, and by integrating multivariate environmental models. We consider the influence of diverse environmental settings across Europe in C₃ plants isotope variability, which represents the baseline for paleodietary and ecological studies.

References

Pearson, J. A. et al. New light on early caprine herding strategies from isotope analysis: a case study from Neolithic Anatolia. *Journal of Archaeological Science* 34, 2170–2179; 10.1016/j.jas.2007.09.001 (2007).

Lightfoot, E., Liu, X. & Jones, M. K. Why move starchy cereals? A review of the isotopic evidence for prehistoric millet consumption across Eurasia. *World Archaeology* 45, 574–623; 10.1080/00438243.2013.852070 (2013).

Archaeogenomic Investigation of Stratified Domestication of Maize in South America using Brazilian Archaeological Maize

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Maize was first domesticated in the lowlands of what is now Mexico at approximately 9000 years ago from the wild Balsas teosinte (*Z. mays* ssp. *Parviglumis*). Maize was later quickly transported into South America around 6500-6300 years ago. A stratified model of South American suggests maize left Mexico in a semi-domesticated state and underwent full domestication via a secondary domestication center in the Northwest Amazon (Kistler, et al. Science 2018).

From the Peruaçu Caves National Park, Minas Gerais, Brazil, fourteen archaeological maize specimens (1010-570 BP) have been identified as holding unique teosinte-like morphological characters not yet found elsewhere in South America (Costa, et al. Sci. Adv. 2024). This project will generate and analyze genomes from several of these teosinte-like maize from Peruaçu along with other more typical-looking maize from Peruaçu and other Brazilian archaeological sites relatively contemporary to each other. The project will study which domestication genes can be identified from individual specimens and how do their genomes relate to other archaeological and modern maize. From this, the project will further explain the local phenomenon of “primitive-type” Peruaçu maize, as well as the broader history of South American maize domestication.

Early Iron Age Foodways: Revisiting South African Faunal Assemblages Using ZooMS

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Keywords: South Africa, Zooarchaeology, Collagen fingerprinting

The introduction of agropastoralism in northeastern South Africa coincides with the arrival of the Bantu-speaking people in the first millennium CE and is known as the African Iron Age. Faunal remains enable us to reconstruct the shift in human-animal relationships and past foodways at this key transition. Unfortunately, in many northeastern Early Iron Age sites, the fragmentation and paucity of specimens have hampered a comprehensive understanding of pastoralism's spread. The fundamental issue of taxonomic identification of fragmented specimens is the first major hurdle, as morphology-based zooarchaeological studies can only achieve broad classification, potentially leading to misinterpretations in the composition of the faunal assemblage and thus the subsistence patterns of Early Farming Communities. In this study, we employed Zooarchaeology by Mass spectrometry (ZooMS) to identify 68 bone fragments from five Early Iron Age sites (Lydenburg Heads, Riverside, Doornkop, Langdraai and Lebalelo). 60% of the analysed specimens yielded good-quality MALDI-TOF spectra, allowing taxonomic identification. The presence of wild and domestic species (*Bos taurus* and *Ovis aries*) indicates the persistence of hunting during the introduction of agropastoral practices. Furthermore, the lack of browsing species suggests a selection towards grazers as a meat source, despite settlement preferences in riverside and wooded environments during the Early Iron Age. The identification of a canid specimen at the Langdraai site highlights the necessity for further proteomic research, which could enable differentiation between wild and domestic canid species. This research sheds new light and emphasizes on the potential of biomolecular approaches in understanding Early Farming Communities subsistence strategies.

Identifying animal management practices at Treby Borg using stable isotopes

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Keywords: Animal management, Isotopes

The Iron Age ringforts are one of the most prominent archaeological features on the island of Öland, off the Swedish east coast. Apart from Eketorp borg, only eight of the sixteen ringforts which are still visible have been excavated, at best to a very partial extent. Therefore, questions about their chronology, relation to the surroundings and societal role(s) remain [1]. Existing hypotheses include defensive, cult, political or trade functions. So far, artefactual evidence retrieved from some of the ringforts as well as burial grounds suggests that the island had a pivotal role in the flow of good and people in the Baltic region. The faunal remains retrieved from the ringforts represent a key resource for a better understanding of the economy during their use. In particular, comparison of feeding practices and animal mobility – as revealed through isotope analyses – with sites of different nature (e.g., burial grounds, sacrificial sites such as Skedemosse) provide the opportunity to assess the potential higher status and/or wide-ranging connectivities of the ringforts. The island being a geographically constrained area, it has been proposed that cattle were imported from the Swedish mainland.

Reference

1. Lidén, K. *et al.* New Research Programme: Crisis, Conflict and Climate: Societal Change in Scandinavia 300–700 CE. *Current Swedish Archaeology* **31**, 213–218 (2024).

Flatfish in Focus: Developing a ZooMS Reference Database to Identify Archaeological Pacific Flatfish

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Keywords: ZooMS, Indigenous fisheries, Flatfish

Archaeological fish remains provide detailed information on paleoenvironmental conditions, marine anthropogenic impacts, and human-animal relationships in the past. Fish bones, however, are challenging to identify to species due to their morphology, a need for accessible and comprehensive comparative collections, and destructive taphonomic forces creating fragmentary assemblages. On the Pacific Northwest Coast of North America, archaeologists have focused on salmon and herring, two archaeologically abundant cultural and ecological keystone species, leading to other fish taxa being understudied. To shine a light on one such underrepresented taxon, we are constructing a ZooMS reference database to identify archaeological Pacific flatfish, representing species like halibut, flounders, and soles. The database presents unique biomarkers, confirmed using LC-MS/MS, for 26 flatfish species endemic to the Northwest Coast, enabling the reliable identification of morphologically indistinct or fragmentary Pacific flatfish to the species level. In collaboration with xʷməθkʷəy̍əm (Musqueam) First Nation, we demonstrate the efficacy of this database through the analysis of flatfish elements from several archaeological sites in traditional xʷməθkʷəy̍əm territory. The ZooMS analysis of flatfish remains across several archaeological contexts will provide insight into the diversity of flatfish caught and consumed by xʷməθkʷəy̍əm ancestors, as well as aiding the reconstruction of marine ecosystems prior to the advent and ongoing process of colonialism.

Dietary Practices of a Rural Society in Western Germany Under Rome: Lipid Analysis of Pottery Covering Major Historical Transitions (1st to 4th Century CE)

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Keywords: Organic residue analysis, Roman Germany, Diachronic study

Organic residue analysis of archaeological pottery allows us to study the economies (subsistence, trade), social structure, and ritual practices of past populations, as well as observe the development of these aspects through time. In our study, we analyzed 142 pottery sherds from two settlements (a *vicus* and a *villa rustica*) near Hermeskeil (co. Trier-Saarburg) in Western Germany, populated by the Treveri people. These sites cover the timeframe from the onset of Roman rule in the area (Late La Tène/Early Imperial) to the economic decline caused by political unrest and frequent Germanic raids (mid-3rd to mid-4th century CE). The sherds were subjected to multiple (2–3 ×) sampling, sequential extractions (solvent followed by acidified methanol extraction), and gas chromatographic analysis. Up to 93 % of the vessels contained significant amounts of lipids ($> 5 \mu\text{g g}_{\text{pottery}}^{-1}$). Multiple sampling was shown to have helped to achieve that number. The vast majority of extracts bore signatures associated with animal fats, while plant biomarkers were exceedingly rare. Principal component analysis indicated a relatively monotonous diet. In addition, thermal decomposition products were detected in high abundance at one of the sites. Some of these compounds have not been previously reported. The aim of this project is to track dietary practices in a geographically limited context during a historically significant era that saw a change in power, a period of prosperity and integration into an empire-wide trade network, the Crisis of the Third Century, and finally economic collapse and displacement.

Lipids, Lapita, and Lore

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Keywords: Pottery, Residue, Vanuatu

Pottery was introduced in Remote Oceania around 3,000 years ago by communities known as Lapita peoples who settled on large island groups from New Guinea to Tonga and Samoa. Despite being the most frequent artefact left by the past societies who inhabited the 4,000 km chain of Oceanic islands, little is known about how the pottery vessels were used, particularly the foods and products that were cooked and stored in them.

We are presenting here the results from the analysis of lipids preserved in archaeological pottery from the Lapita cemetery site of Teouma (2870–2920 BP), in Vanuatu, Southwest Pacific. We compare pots of different forms and decorative styles to assess potential variations in use.

Lapita pottery is often viewed as carrying ideological meaning, particularly through its distinctive dentate-stamped decorations. By analysing the residues in highly decorated pots associated with burials and comparing them to those in undecorated pots, we provide direct evidence for this hypothesis. To refine our interpretation, we compare these results with experimental cooking vessels.

This pilot study lays the groundwork for a new 3-year project integrating lipid analysis with traditional knowledge from Vanuatu communities, among the few in the Pacific where traditional pottery manufacture has continued into the 21st century.

Characterization of the Fish Products Lipidic Signatures in Ethnographic Pots for a Better Interpretation of Archaeological Remains

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Keywords: Lipids, Fish products, Ethno-archaeology

There are still open questions regarding the lack of biomolecular evidence of aquatic products in European Neolithic ceramics, despite the availability of these resources (Craig *et al.*, 2013). It is still unclear whether this reflects ancient foodways (no consumption or not in pottery), or whether their chemical composition is highly conducive to natural degradation, leading to difficulties of detection.

Ceramic vessels from the Diola Kassa in Lower Casamance (Senegal) were studied: 6 ethnographic vessels used for fish-based recipes, and 8 ethno-experimental vessels in which fish was experimentally processed several times. To assess the interpretative potential and limitations of this ethno-archaeological method, we also studied an archaeological dumping site, “La Poubelle des Mamans”, located in Lower Casamance (Debels *et al.*, 2024) and vessels buried during 1 and 2 years.

These vessels have been chemically investigated using suitable protocols to detect aquatic products (Cramp & Evershed, 2014). Lipids were recovered with acidified methanol extractions, GC-MS and isotopic analyses were performed.

We have observed that, despite repetitive use, none of the recipients showed all the biomarkers of aquatic resources listed in the literature. There is a wide variability in the molecular composition of the vessels with no discernible trend according to the duration of use or extraction yield.

These results will help the interpretation of archaeological lipid extracts. They provide clues to assess the extent to which fish-based diets can be inferred from the lipids absorbed in pottery and objectify the lost information. By providing interpretative keys to study aquatic products consumption, we attempt to understand the relationship between past societies and their environment.

Food in Medieval Northern Italy: Paleoproteomic Evidence from a Rural Village

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Keywords: Food, Paleoproteomics, Middle Ages

The study of food in past societies presents itself as an increasingly dynamic and innovative research field, combining archaeological methodologies with those of disciplines such as physics and chemistry. The fruitful encounter of this knowledge makes it possible to enrich our understanding of past habits with new data. Food offers a unique perspective for examining everyday practices and social behaviour.

In this study, with a paleoproteomic approach, we analysed encrusted food remains attached to “Pietra ollare” (soapstone) vessels from the medieval village of Nogara, near Verona, in Northern Italy. The fragments were recovered from layers inside and around two houses dated between the 9th and the 11th centuries.

Thanks to our protocol, we successfully extracted and identified numerous animal proteins preserved inside the charred material. These data give us new information on the food habits of the inhabitants of the time and enrich our previous knowledge on the site based on archaeobotany and archaeozoology.

Although preliminary, our results contribute to a deeper understanding of the dietary practices in Nogara, offering a glimpse into the everyday life of a rural settlement during the Early Middle Ages in Northern Italy.

Resilience and Adaptation: Isotopic Reconstruction of the Impact of Climate Change and Human Interventions on the Nile River, Plant Biodiversity and Ancient Egyptian Foodways

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Keywords: Ancient Egyptian diet, Isotope ecology, Water Justice

This project reconstructs the impact of climatic changes and human interventions—such as changes in agricultural practices and damming—on the Nile River and the associated plant and food biodiversity, both in the past and present. Archaeological, ethnographic, and modern botanical samples are compared and analyzed using stable isotopes to reconstruct ancient water availability, soil fertility, and changes in relative humidity. Ancient desiccated botanical materials buried as food offerings from three different sites in Egypt (Nag ed-Deir, Deir el-Ballas, and Thebes) are compared to ethnographic and modern samples of the same species, which were measured isotopically to understand botanical adaptation to these climatic changes, including ancient water oxygen isotopes. Clumped isotopes on freshwater shells were also analyzed to reconstruct the ancient temperature of the Nile River.

Given the profound influence of the Nile River on ancient Egypt, both physically and culturally, this project explores the archaeology of the Nile River and the water-plant relationship, along with traditional ecological knowledge, to understand the social history of food in ancient Egypt and community resilience against climatic changes. One significant result is that, despite plant samples showing episodes of water-stressed conditions, ancient community resilience was expressed through the intensification of heat-tolerant species in diet and animal fodder.

This project is an ongoing effort to use deep-time archaeological and ethnographic data to help modern communities along the Nile build resilience against climate change and its impact on their food and water resources. The research also serves as advocacy for water justice along the Nile.

Integrating Early Neolithic Dietary Data and Kinship Practices at Hazleton North

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Keywords: Stable isotopes, Diet, Neolithic

The Hazleton North long cairn is an Early Neolithic mortuary monument in Gloucestershire, England, dating to the 37th century BC. Recent aDNA analysis has revealed that the tomb was primarily used by five generations of individuals connected by paternal descent, the first generation of which was comprised of one male and four female individuals whom he reproduced with (Fowler et al., 2021). While paternal descent largely influenced inclusion within the tomb, descent from the first-generation females affected which of the two chambered areas a person's remains were placed within: the descendants of two of these women were placed within the southern chambers, and the majority of descendants from the other two within the northern chambers. Other individuals were also present who were not biologically related to the main patriline. Here, we present new stable carbon and nitrogen isotope results from incremental dentine for the Hazleton North individuals to examine dietary practices during early life through to early adulthood at a high temporal resolution. We will also integrate the isotopic data with the aDNA results to investigate whether dietary practices within the group were influenced by biological relatedness, including between different sub-lineages, between individuals from different sides of the tomb, across different generations, and between individuals who were biologically related to the main patriline and those who were not. Combining the isotopic and genetic data will offer a more complete view of Early Neolithic lifeways at Hazleton North than previously possible by considering how dietary and kinship practices may have intersected.

Reference

Fowler, C., Olalde, I., Cummings, V. *et al.* A high-resolution picture of kinship practices in an Early Neolithic tomb. *Nature* 601, 584–587 (2021). [https://doi.org/10.1038/s41586-021-04241-](https://doi.org/10.1038/s41586-021-04241-4)

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Towards a Culinary Archaeology of Cacao-Based Recipes in El Salvador and Isla De Sacrificios, Mexico

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Keywords: Organic residue analysis, Theobromine, Chocolate

Several societies in Mesoamerica and Central America were shaped by the production and consumption of cacao for over two thousand years. Cacao was a divine food at the heart of ritual life, used as money and in culturally significant preparations. Organic residue analysis studies have successfully identified theobromine, the cacao biomarker, in vessels from Guatemala, Honduras, Belize, Mexico and the US, among others. However, the role of Central America in the adoption of chocolate beverages and the diversity of cacao-based drinks and foods remain little investigated. Using LC-MS and GC-MS, we are analysing for the first time vessels from archaeological contexts in El Salvador, the birthplace of the chocolate recipe. The study also includes vessels from the British Museum collection, notably from Isla de Sacrificios, an overlooked Postclassic pilgrimage site off the coast of Veracruz, Mexico. This will improve our understanding of the BM collection and contribute to developing engaging narratives for the collection on display.

The analysis has a methodological angle, offering a comparison of different extraction and analysis methods to retrieve compounds associated with the preparation of chocolate drinks and cacao-based foods. It also goes beyond the presence/absence of cacao, by looking at other ingredients potentially added to the preparation. From that perspective, we aim to reconstruct the culinary complexity involved in the use of cacao, the chocolate preparation and consumption over time by comparing our results with archaeological, ethnographic and historical sources emphasizing the importance and diversity of uses of cacao for Mesoamerican and Central American foodways.

Dairy, Fruit, and Beyond: Consumption Practices and Culinary Activities in the Third-Millennium South Caucasus

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Keywords: Consumption practices, Ruminant fat cooking, Southern Caucasus Bronze Age

In the Southern Caucasus, the still enigmatic disruption of the long-lived Kura-Araxes socio-cultural system and the abrupt emergence of Early Kurgan communities marks a profound societal change, influencing settlement patterns, ways of life, funerary traditions and many elements of the material culture, particularly Pottery traditions. This paper explores these changes through a diachronic investigation of consumption practices and culinary activities at the third-millennium settlement of Qaraçınar (Azerbaijan). Recent research conducted within the framework of the KUR(A)GAN project has expanded our understanding of the dietary habits of the Kura-Araxes and Early Kurgan communities through the analysis of organic residues preserved in ceramic vessels, providing valuable insights into their culinary practices. Among the diverse plant and animal resources identified in the Qaraçınar pottery, this study highlights the prominent roles of dairy and fruit products in Early and Middle Bronze Age consumption and culinary traditions. The paper also provides new insights into molecular markers associated with the thermal processing of ruminant fats, offering a refined perspective on potential food preparation techniques within these communities. Furthermore, the study investigates the functional roles and usage patterns of Kura-Araxes and Early Kurgan ceramic vessels, contributing to a deeper comprehension of the cultural and societal transformations during this period.

Protein Preservation Mechanisms in Cooked Pottery and Optimization of Protein Extraction Methods

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Keywords: Cooking experiments, Protein survival, SP3

Since the emergence of pottery in the Late Paleolithic period, it has been crucial in the daily lives and production activities of ancient peoples. The proteomic analysis of adsorbed organic residues in pottery provides valuable insights into ancient plant and animal use, culinary practices, and pottery functions. However, this research area remains under-explored and challenging due to limited understanding of protein preservation and the lack of effective extraction methods. This study aimed to explore protein preservation in pottery by simulating cooking experiments and optimizing extraction methods. Milk was cooked in replica pottery and the slices were used for further analysis. Using laser scanning confocal microscopy, we observed a distinct protein distribution pattern on modern pottery slices, revealing a concentration of casein on the inner wall, penetrating up to 2 mm. Stratified sampling of pottery slices confirmed that milk proteins primarily resided within the inner wall surface layer, spanning approximately 3 mm, guiding future sampling strategies for residual protein analysis in pottery. Furthermore, we compared three extraction methods—FASP, SP3, and iST—using pottery powder incubated with whey protein/casein and pottery shards cooked with milk. LC-MS/MS mass spectrometry analysis indicated SP3 was superior, showing higher efficiency and stability. Our findings also highlighted variations in protein binding capability to pottery powder, impacting their survival rates. Additionally, differences in enzymatic cleavage introduce further complexities in the analytical process. Finally, Sp3 was conducted on some ancient pottery shards and caution must be exercised in interpreting residue analysis results.

Raw Fish, Maize and Quinoa? Evaluating Long-term Culinary Practices in the Atacama Desert (450 BC-AD 1450) Through Lipid Residue Analysis of Pottery

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Keywords: Lipids, Pottery, Atacama Desert

Ancient pottery from domestic contexts in the Atacama Desert is generally assumed to be used for cooking practices based on their exposure to fire as observed through macroscopic analysis. To assess this proposal, here we use organic residue analysis (ORA) to evaluate culinary practices of the ancient human groups that inhabited northern Chile. We analysed pottery fragments from the coast, valley and precordillera of the Tarapacá region, northern Chile dating from the transition to agriculture to the establishment of control of the Inca Empire. Exceptionally, we were able to produce a local, ancient fatty acid carbon isotopic baseline for archaeological crops, fish, rodents and camelids, thanks to the excellent preservation that characterises the Atacama Desert. The absence of heating biomarkers suggests that pottery was mainly used for storing, processing and serving commodities. Marine products were mainly consumed at the coast and valley, complemented with C3 plant resources. Pre-inca sites in the precordillera show a reliance on C4 plants, changing drastically towards the Inca presence, as local populations shifted to C3 resources, which we believed was influenced by the empire. We conclude that the use of pottery was highly diverse through space and time, with uses going beyond culinary practices, such as ritual burning of plant materials and possible medicine uses. These findings show that ORA on pottery can also provide insights into socio-political processes and changes experienced by local populations.

From Hearth to Castle: Dietary Insights and Social Dynamics Between Botolphs and Bramber Castle in Sussex, England (950-1300 AD)

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Keywords: Dietary reconstruction, Norman Conquest, Sussex

This study explores the dynamics between rural and elite settlements in England from 950-1300 AD, focusing on Botolphs, a rural site on the banks of the River Adur, and Bramber Castle, a nearby elite stronghold built following the Norman Conquest. To achieve this, complete pottery assemblages from both sites were systematically categorised and documented, ensuring a robust and representative sample selection. Selected cooking pot fragments were then analysed using Gas Chromatography (GC), Gas Chromatography-Mass Spectrometry (GC-MS), and Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS), to reconstruct dietary patterns.

While the castle is traditionally viewed as a noble residence, the castle's occupation dynamics, including potential roles as a military outpost manned by guards, will be examined. Simultaneously, the research will emphasise how rural life at Botolphs was shaped by, and responded to, regional economic and social forces, including those emanating from Bramber Castle. Therefore, this analysis aims to illuminate not only culinary practices but also the broader social dynamics that defined these contrasting communities. The project particularly emphasises understanding the living experiences and social responses of the elites at Bramber Castle and the historically under-represented rural populations at Botolphs. Ultimately, the study aims to enrich our understanding of the social fabric during the Norman Conquest and highlight how rural and elite communities responded to significant socio-economic changes. This nuanced approach not only illuminates the complex dynamics of this pivotal period but also contributes to a larger project investigating continuity and change in the living experiences of Adur Valley communities.

Dietary Variability and Inequality at Trinity Burial Ground, Kingston-Upon-Hull: Results from Incremental Dentine Isotope Analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$)

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Keywords: Incremental dentine isotope analysis, Inequality, Industrial-era diet

Understanding individual dietary variations can provide valuable insights into the sociocultural and ecological conditions of past societies, as food production and consumption are shaped by environmental, technological and cultural processes. While diets of the industrial-era Britain are often studied through documentary evidence (e.g., economic texts, cookbooks, surveys), these sources disproportionately represent urban factory workers, rural laborers and upper-class individuals, neglecting individual variabilities and regional patterns influenced by local economies and environments. This study addresses these gaps by examining dietary patterns in a maritime, mercantile-focused community at Trinity Burial Ground, Kingston-Upon-Hull, England (1785–1861), using stable isotope analyses ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of incrementally sectioned tooth dentine collagen. Dietary histories of 18 individuals, spanning infancy to early adulthood were reconstructed from 31 teeth. Results indicate that the population primarily consumed protein from terrestrial C_3 ecosystems, supplemented by freshwater and marine proteins, reflecting Hull's location at the intersection of the Humber Estuary and the North Sea. Notably, socioeconomic status (SES) influenced dietary quality, with higher SES individuals showing greater and more stable access to meat, as indicated by $\delta^{15}\text{N}$ profiles. While males consumed slightly more marine foods, females exhibited slightly higher $\delta^{15}\text{N}$ values during childhood (3–10 years), potentially reflecting dietary or stress-related physiological differences. Some individuals showed notable dietary shifts, likely tied to migration or changes in social identities (e.g., age, familial roles, occupations). This study highlights the advantage of incremental dentine analyses in capturing individual dietary nuances and complements documentary evidence on industrial-era diet by providing insights into understudied maritime populations.

Contents of Early Vessels in the Eastern Gulf: Organic Residue Analysis of Sixth-Fifth Millennium BC Imported Mesopotamian Vessels in South-Eastern Arabia

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Keywords: Southeastern Arabia, 'Ubaid pottery, Lipid residues

The earliest known pottery vessels in south-eastern Arabia are Mesopotamian in origin, known as 'Ubaid or black-on-buff pottery, and date from the late sixth to the mid-fifth millennium BC. At this time, aceramic Middle Neolithic Arabian populations were mostly mobile; engaging in hunting, gathering, fishing and herding. They were also navigating around the Persian Gulf coast, exchanging products such as softstone, obsidian, haematite, livestock and pottery, with 'Ubaid pottery either being exchanged directly or down-the-line with Mesopotamian communities. It is also hypothesised that 'Ubaid pottery and/or the products within them carried connotations of prestige in Middle Neolithic Arabian communities, and were used in specific contexts to display wealth and/or status while serving food. As the possession and exchange of pottery was possibly a "profoundly symbolic and political act", 'Ubaid pottery may have been linked to new means of wealth accumulation and display, redistributed in acts of ceremonial gift-giving or exchange at communal or feasting events. This poster will present the results of organic residue analysis of 'Ubaid vessels from two coastal sites in south-eastern Arabia, Akab and Umm al-Quwain 2, to discuss the contents detected within 'Ubaid pottery and raise questions about 'what pottery was good for' for aceramic Middle Neolithic coastal Arabian communities. As indigenous pottery production in southeastern Arabia only began a millennia later in the third millennium BC, the results provide unique insight into the symbolic role of ceramics for a mobile population.

What Lies Behind Li Tripods? Nuancing the Shang-Zhou Transition through a Multifaceted Approach to Ceramics

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Keywords: Lipid Residues, Ceramic Analysis, Bronze Age China

Craft traditions and culinary practices are important proxies for investigating past social identities and generating insights into culture contact, migration, and colonialism. This research focuses on identifying changes and continuities in community-specific production and uses of *li* tripod cooking vessels before, during, and after the Zhou Dynasty expansion into what is now the Shandong Province in eastern China. Rather than deriving ethnic identity from ceramic form and style, we concentrate on how these vessels were created and utilized by combining ceramic petrography, use-alteration inspection, and the molecular and isotopic characterization of lipids residues. The combination of these methods provides insight into social practices via a single ubiquitous artifact: *li* cooking vessels. Petrographic analysis shed light on how and where these vessels were produced, while lipid and use-alteration analysis reveal how they were used and what foods were cooked in them. This multifaceted approach to ceramic analysis was designed to provide a nuanced portrait of the Western Zhou transition, traditionally viewed as a homogeneous process of conquest, colonialization and acculturation. In this presentation, we discuss data from 105 vessels uncovered at three distinct sites: Yangxinliwu (Shang period), Gaoqingcaopo (Shang-Zhou transition), and Kanjiazhai (Zhou period). The comparison of lipid and isotopic profiles, production and import patterns, and preferred preparation methods (dry/wet) at each of these sites allow us to situate communities along a continuum where similar social aspects gleaned from ceramic analysis suggest conquest, assimilation, and absorption, while total difference reflects local variability and resistance to foreign acculturation.

Stable Isotope Analysis of Amino Acids: Comparison of GC-c-IRMS and LC-MS Results

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Keywords: CSIA, GC-C-IRMS, LC-MS

For over 30 years, compound-specific amino acid analysis has been used to investigate past diets. Unlike bulk stable isotope analysis, this method has not become a regular tool, likely due to its complexity and cost. However, it potentially offers more detailed insights into trophic levels and resource use.

To address complex questions about past subsistence practices, it is essential to understand how to analyse carbon stable isotope values from amino acids. This can be done using either gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) or liquid chromatography-isotope ratio mass spectrometry (LC-MS).

In this paper, we will compare the benefits and limitations of these two methods through a case study from the 3rd millennium BCE in the Eastern Baltic. We will examine the entire 'life cycle' of both methods to highlight their advantages and disadvantages. Our goal is to make these promising techniques more understandable and accessible to researchers, moving towards a more inclusive field of biomolecular archaeology. Understanding these methods is crucial for applying them to questions about the diets of past populations.

5 A Day: The Role of Plant Substances in the Ancient Diet of Corsica during Protohistory

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Keywords: Lipids, Plants products, Corsican protohistory

The Mediterranean area is known for its poor preservation of organic matter, particularly plant products, which are under-represented due to the predominance of lipids in organic residue analyses and the low lipid content of plants. However, recent analyses of organic residues have revealed excellent preservation of organic molecules in Corsican pottery (Drieu *et al.*, 2018; Rageot *et al.*, 2016), offering new research perspectives on foodways in the Mediterranean.

In this work, we focused on the identification of plant substances using an innovative method (Garnier and Valamoti, 2016) that allows, from a single sample, the detection of lipids (including triacylglycerols) and short-chain acids present in plants via GC-MS and LC ESI-MS/MS methods. Fifty samples from three protohistoric sites were analysed.

The results show that organic compounds were well preserved, depending on the archaeological sites, with a wide range of molecules identified, from triacylglycerols to short-chain acids. These data highlight the diversity of plant substances used in culinary practices during the Bronze and Iron Ages (grapes, vegetable oils and waxes). LC ESI-MS/MS analyses allowed us to determine the origin of the oils (olive). Other plant substances identified include birch bark tar and conifer resins and tars, which appear to have been used repair ceramics.

This new approach expands the range of molecules studied, including a more detailed study of triacylglycerols, provide new insight into the consumption of plant products and the early domestication of plants such as olives and vines on the island.

A Comparison of Organic Residue Analysis on Pottery Vessels from Layer 7 Aşağı Pınar with Results from Other Layers

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Keywords: Lipids, Pottery, Dairying

Aşağı pınar (6.200- 4.900 BC) has been part of the earliest discussions on organic residue analyses on pottery from Thrace in Anatolia (Evershed et al. 2008; Özbal et al. 2014). The results suggest that the inhabitants relied on dairy products. Nonetheless, given the lack of chronological refinement it has not been possible to ascertain whether the said practices were adopted already by the earliest site dwellers, or they were embraced by occupants from later levels. To solve this question and obtain a comprehensive database exploring pottery use, sherds from distinct vessel types from Layer 7 were screened for lipid and analysed to characterise preserved food remains. Additionally, sherds from layers 2 to 6 were studied to diachronically explore pottery use and assess traits exclusive to Layer 7.

All in all, from six different layers, 169 sherds were studied following TLE and acidified methanol extractions and analyses through GC-FID, GC-MS and GC-IRMS.

The analysis of lipids yielded fats including dairy, ruminant and non-ruminant animal fats, as well as terrestrial and marine plants and beeswax. The aquatic plants could indicate a broadening of the diet of the Neolithic Thrace. Preliminary results indicate that dairying would have been a strong practice at the site from Layer 6 onwards, but not in Layer 7. This hence implies that dairying was present at the site beginning in the first half of the 6th millennium BC. Results show that the transformation and consumption of plant and animal products from terrestrial and aquatic environments was practiced indistinctively across different ware types.

Evidence for Intensified Dairying at the Corded-Ware Cultural Horizon in Central and Northern Europe Using Lipid Residue Analysis and Zooarchaeology

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Keywords: Corded Ware, Dairying, Lipid residue analysis

The appearance of the Corded Ware Culture across many areas of Central and Northern Europe during the 3rd millennium BC was a major period of cultural transition, which has been linked to a series of human migrations from the Pontic-Caspian steppe region. Some previous zooarchaeological work has indicated that this also may have been a time of economic and agricultural change, with intensification of cattle husbandry. It is not known, however, how widespread this shift was, its exact nature or whether or not it is linked to the arrival of new human groups into the region. This paper will present extensive lipid residue analysis on pot and foodcrust samples to examine the use of pottery across the transition to Corded Ware in two regions: The Netherlands and Switzerland. This work shows an increase in dairy residues in Corded Ware pottery in both regions. The results will be presented alongside zooarchaeological work investigating evidence for changes in animal husbandry at this time.

Dryland Agriculture in Early Neolithic Northern China: Organic Residue Evidence from the Yumin Culture

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Keywords: Organic Residue Analysis, Millet Agriculture, Xinglong Site

The Yumin Culture, recently discovered in the Bashang region at the junction of Hebei and Inner Mongolia, represents an Early to Middle Neolithic archaeological tradition dating back 8,500–7,000 years. The region’s unique geographical setting provides a valuable context for exploring early subsistence strategies. Recent excavations at the Xinglong site in Kangbao County, Hebei, have yielded abundant materials for studying subsistence practices in northern China during the Early Neolithic. This study employs organic residue analysis of pottery samples from the Xinglong site to investigate the subsistence strategies of its inhabitants, with a particular focus on millet agriculture. By analyzing organic residues of pottery, the research explores how this community adapted to its environment through a combination of agriculture and foraging.

The findings reveal the diversity and sustainability of its subsistence system, highlighting the pivotal role of millet agriculture in supporting long-term settlement. This research also contributes to broader discussions on the socio-economic impacts and ecological sustainability of early agricultural societies during the Early Neolithic.

Shell Middens As sedaDNA Archives: Expanding Perspectives on Mesolithic and Neolithic Dietary Practices in the North Sea Region

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Keywords: Shell middens, Sedimentary ancient DNA, North Sea

Shell middens are anthropogenic accumulations of shells and other ‘kitchen waste’ that provide insights into past subsistence strategies, particularly in relation to marine resource consumption, and associated cultural practices. The archaeological evidence from coastal shell middens in the North Sea region indicates that Late Mesolithic hunter-gatherer-fisher communities (~5,400-4,000 yr BC) intensively exploited marine resources. This was followed by a dietary shift into terrestrial foods, coinciding with the arrival of farmers to the region and the start of the Neolithic (~3,900 yr BC). Ancient environmental genomics has demonstrated its valuable contribution to the understanding of archaeological contexts, however, its application to shell middens remains largely unexplored. In this study, we investigate two shell-midden deposits from the Jutland Peninsula (Denmark) – Vestre Strandallé and Sindholt – spanning the Late Mesolithic and Early to Middle Neolithic periods. We conducted sedimentary ancient DNA (sedaDNA) analysis on 41 samples, complemented by radiocarbon dating (C14), to identify genetic traces of plants and animals that could offer additional insights into dietary patterns and integrate previous zooarchaeological research. Serving as a proof of concept, this work examines the preservation of ancient environmental DNA in shell midden contexts and its potential to expand our knowledge of dietary practices and human-environment interactions during the transition from foraging to early farming.

Dairying Sustained Seasonal Transhumance in the Bronze Age Western Tianshan Mountains

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Keywords: Ancient proteins, Milk, Adunqiaolu

The Adunqiaolu site and cemetery, located in the Bortala Valley of the Western Tianshan region in Xinjiang, lie at the crossroads of ancient trans-Eurasian exchanges. This site provides crucial evidence for the seasonal transhumant pastoralism that characterized subsistence strategies during the second millennium BCE. In this study, proteomic analysis was conducted on dental calculus from individuals at the Adunqiaolu site. The findings reveal the extensive use of dairy products derived from cattle, sheep and goat by late Bronze Age pastoralists. This dietary reliance on dairy products underscores the key role of diversified secondary product exploitation in sustaining transhumant pastoralism during the Bronze Age, paralleling the economic strategies of modern pastoral communities in Xinjiang. These results fill a significant gap in our understanding of the intensification of secondary product use in the Western Tianshan region.

Environmental change, sustainability, and ancient ecosystems

Ancient DNA Preserved in Archaeological Sediments from Stone Age Mongolia

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Keywords: Sedimentary ancient DNA, Eastern Eurasia, Upper Palaeolithic

Little is known about the early modern humans who colonized Eastern Eurasia and the local archaic human groups, such as Neandertals and Denisovans, with whom they potentially interacted during the Middle-to-Upper Paleolithic transition. The scarcity of skeletal remains from this period and region poses a barrier to morphological analyses for classifying humans as modern or archaic, as well as for extracting ancient DNA (aDNA). To overcome this, we utilized archaeological sediment as a source of aDNA independent of skeletal remains. We collected several hundred sediment samples from four archaeological sites with evidence of human occupation spanning the Middle to Upper Paleolithic in Mongolia: Kharganyyn Gol 5, Tolbor-4, Tolbor-21, and Tsagaan Agui cave. These samples were screened for aDNA preservation through shotgun and targeted sequencing of ancient mitochondrial DNA from the most abundant mammalian families found in Eurasia during the Stone Age. We found evidence of aDNA preservation at all sites, demonstrating the potential of the approach to isolate ancient human DNA from different time periods in the region. Initial results using targeted sequencing of human DNA provide the first insights into the ancestry of the early modern humans who occupied the region during the Initial Upper Paleolithic. Our results show the potential of ancient environmental DNA approaches and higher-density sampling of sediment in archaeological sites to reconstruct the past, offering valuable insights into the ancestry of early modern humans who roamed Eastern Eurasia during the Upper Paleolithic.

Uncovering the Exploitation of Animal Resources in Early Medieval Pohansko Using ZooMS Analysis

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Keywords: ZooMS, Early Medieval Europe, Species identification

Pohansko, located near Břeclav in the Czech Republic, was a significant center of the Early Medieval Great Moravian Empire. Excavated since 1958, this archaeological site has provided invaluable insights into life in Early Medieval Europe. Pohansko is divided into three main areas: the central settlement, the southern suburbium, and the northeastern suburbium (Pohansko severovýchodní předhradí, PSP).

This study focuses on the exploitation of animal resources in the northeastern suburbium through Zooarchaeology by Mass Spectrometry (ZooMS) analysis of faunal remains. The analyzed remains, excavated from post holes in the northeastern suburbium between 2018 and the present, included small, highly fragmented bones that were unidentifiable through traditional zooarchaeological methods. These fragments were analyzed using the ZooMS method to identify the species.

The results correspond to previous archaeozoological studies, revealing a predominance of domesticated species, with cattle dominating the assemblages, alongside remains of goat and pig. Remains of wild animals like deer, wild boar, and notably, beaver were also identified. The presence of deer and wild boar suggests that hunting supplemented food resources. The beaver remains, many of which exhibit cut marks, indicate their exploitation for fur and meat.

These findings shed light on the dietary practices and resource management strategies in the northeastern suburbium, emphasizing a heavy reliance on domesticated animals while integrating wild species into subsistence and craft economies. This research validates earlier findings and highlights the utility of biomolecular methods, particularly ZooMS, in reconstructing past ecosystems and understanding the socio-economic dynamics of Early Medieval communities.

References

1. Dreslerová, G. (2023). Pohansko u Břeclavi: sociální a ekonomická stratifikace velkomoravského centra na základě archeozoologických analýz (Vol. 529). Masarykova univerzita.
2. Dresler, P., Dreslerová, G., Doláková, N., Kočár, P., & Kočárová, R. (2022). Beaver as proof of the change of natural environment and economy of the first half of the 10th century AD. *Archaeologia Austriaca*, 106, 117-136.

3. Macháček, J., Přichystalová, R., Dresler, P., & Sládek, V. (2017). Břeclav–Pohansko VII.: Kostelní pohřebiště na Severovýchodním předhradí (Vol. 455). Masarykova univerzita.

A Sequence of Southern Sweden: Sedimentary Ancient DNA Analysis of Environmental Change on the Kullen Peninsula

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Keywords: Ancient sedimentary DNA, Deglaciation, Marine-terrestrial transition

The Kullen Peninsula in the northwestern corner of Skåne county in southern Sweden was amongst the first land areas in Sweden to become ice free following the most recent deglaciation. Previous investigations of the small lake of Kullatorpssjön, situated on the peninsula, demonstrate that sedimentation began 17,000-18,000 cal. BP into what was then a brackish lagoon before isostatic rebound resulted in the isolation of the lake from the neighbouring Kattegat Sea 1000-1500 years later. Given the unique geographical position of Kullatorpssjön in southern Sweden and its dynamic palaeoecological history from a glaciated to marine to terrestrial environment, it is of great interest to further elucidate the timing and composition of postglacial colonising floral and faunal communities, as well as the timing of early humans, on the peninsula. Here, we use sedimentary ancient DNA (sedaDNA) shotgun metagenomic and metabarcoding approaches, applied to ~80 samples from a 4-meter core collected from Kullatorpssjön in 2024, to investigate the critical shifts in the coastal environment of the Kullen peninsula ca. 18,000-10,000 cal. BP.

Stable oxygen isotope composition ($\delta^{18}\text{O}$) of freshwater apple snails (*Pomacea*) as a proxy for reconstructing seasonality, subsistence strategies, and settlement patterns of Amazonian pre-Columbian foragers

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The Bolivian Llanos de Moxos hosts an unparalleled archaeological record, consisting of thousands of sites known as forest islands, formed by the first inhabitants of Amazonia during the Early and Middle Holocene. Although our knowledge on the pre-Columbian hunter-gatherer populations in the Amazon has notably increased in recent decades, information on their social and economic organisation remains scarce. Excavations conducted so far on the forest islands have shown that several archaeological assemblages were formed as shell middens, containing numerous freshwater snails from the *Pomacea* genus. Determining the seasonality of mollusc collection could have significant implications for shedding light on human behavioural strategies, particularly those related to subsistence and mobility. To achieve this goal, we are conducting stable oxygen isotope ($\delta^{18}\text{O}$) analyses of *Pomacea* shells recovered from the forest islands. $\delta^{18}\text{O}$ values can serve as indicators of changes in seasonal rainfall if aquatic water temperatures remain stable, enabling us to establish the period of the year when they were collected by humans. However, before analysing ancient shells of a given species, the proxy must first be calibrated using modern representatives. Here we show that $\delta^{18}\text{O}$ values of modern *Pomacea* shells can be used as an effective climate proxy in this region. Our preliminary results demonstrate that this species accurately record variations in seasonal rainfall throughout the year, allowing us to determine the season when they were harvested by pre-Columbian populations. Additionally, we undertook stable oxygen isotope analyses of archaeological *Pomacea* shells recovered from Los Chuchíos and Isla Encantada sites, and we present the preliminary results of this work.

Cultural and Economic Responses in Prehistoric Baltic Coastal Societies during the 8200 cal BP Cold Event

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How did prehistoric societies respond—both culturally and economically—to environmental change? This project explores how humans used culture to cope with sudden environmental shifts and identifies different patterns of human behaviour in prehistoric coastal societies of the Baltic Sea region. One of the key global environmental events studied is the 8200 cal BP cold event in the Mesolithic, which has been linked to cultural changes worldwide but remains largely unexplored in the Baltic Sea region.

By integrating archaeology, bioarchaeology, marine mammal ecology, and environmental studies, we assess the impact of environmental change on human coastal societies and marine ecosystems. To date, 233 bones from 13 species—including humans, terrestrial mammals (aurochs, red deer, elk, roe deer, wild boar, bear, wolf, and dog), and marine mammals (grey seal, harp seal, ringed seal, and harbor porpoise)—have been analyzed from Middle Mesolithic sites. These sites, chronologically linked to the 8.2 ka event, are associated with the hunter-fisher-gatherer Kongemose Culture (c. 8500–7400 cal BP) in southern Sweden and related cultures in east-central Sweden.

Stable isotope analysis (oxygen, carbon, and nitrogen) has been conducted to reconstruct paleoclimate and diet, revealing a significant decline in oxygen isotope values from 7000 to 6000 BC, likely corresponding to the global cooling of the 8.2 ka event.

This paper presents preliminary findings from this multidisciplinary study, demonstrating the interplay between climate change, human adaptation, and marine mammal ecology in prehistoric coastal societies.

Pottery Residue Analysis Revealed Dairying Sustained Prehistoric Southern Tibetans in Adapting the Worsening Climate After 3,000 BP

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Keywords: Pottery residue, Dairying, Prehistoric Tibetans

The Tibetan Plateau (TP), which is the roof of the world and is more than 4000 m above sea level (masl) on average, is one of the harshest environments characterized by perennial cold temperatures, resource shortages and hypoxia. To face these challenges, Tibetans adapt to extreme environments genetically and culturally. Since the late Holocene, Tibetans have adopted pastoralism based on sheep and/or yak and still heavily rely on it at present. Dairy products, which are important ingredients of the daily diets of Tibetans living in environments with low biomass productivity, can provide calories and nutrients without slaughtering animals. Direct evidence for the consumption of milk is rare in TP and later archaeological records. Integrated archaeological and molecular evidence can provide a deeper understanding of broader dietary patterns. Here, we present direct chemical evidence for early dairying practices within the TP through lipid residue analysis of archaeological pottery sampled from prehistoric Tibetan archaeological sites. The analysis results provide direct evidence of dairy consumption and indicate that dairying played a key role in sustaining prehistoric southern Tibetans adapting to the worsening climate after 3,000 BP.

Palaeoenvironmental Reconstruction of Bouldnor Cliff, a Submerged Mesolithic Forest of Cultural Significance

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Keywords: sedaDNA, Palaeoenvironmental reconstruction

Submerged costal sites have been shown to hold vital information relating to our history that predates the Neolithic period in Northwestern Europe ¹. One such submerged costal site is Bouldnor Cliff, the first submarine Mesolithic site to be discovered in the UK, located off the coast of the Isle of Wight². The site has risen in prominence over the past decade, becoming a key site culturally linking the UK to mainland Europe, although it is rapidly being lost to costal erosion. To date only a small area has been studied, providing a glimpse into the Mesolithic period, suggesting strong cultural connections with the European mainland. We therefore aim to reconstruct the palaeoenvironment of the Solent area, encompassing Bouldnor Cliff, from the late Pleistocene to the early Holocene utilizing sedimentary DNA, palynology, sedimentology and archaeology. We hypothesise that the Solent was part of a wider area of Southern Britain that provided glacial refuge in the North, and that Bouldnor Cliff was a key cultural connection to the South.

Rewilding Humans: Evaluating The Dietary Changes Recorded in the Hair of Foragers

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Keywords: Diet, Isotopes, Hair

What did people eat in the past? How and where did they procure and process their food? What were the impacts of changing resources on their nutrition and health? These are all big questions in archaeology where the ultimate aim is to understand the lives of our ancestors. Bioarchaeology has huge potential for us to explore how, through time, humans have adapted their pre-ultra-processed diets in response to environmental, economic, technological, and political transitions. The problem archaeologists face is that dietary evidence is fragmentary: much has been lost over time and is limited to what people left in their rubbish pits and the few human remains we can test for nutritional evidence. This means that we miss any clues about foodstuffs eaten raw, away from home, or which do not survive being buried. This preliminary study utilises stable isotope analyses of incremental hair samples from modern ‘hunter-gatherers’ who consumed a wild-only diet for 3 months. Working with members of the Wildbiome project, this study explores how a wild-only diet is reflected in the dietary isotopes of human consumers, and how a change from a modern to a wild-only diet can potentially impact the health of the participants. Given the current world challenges around food insecurity, it is timely to explore the potential positive impacts of a wild-food diet.

Towards more Sustainable Analysis of Archaeological Dental Calculus

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Key words: Sustainability, Diet, Dental calculus

From the 1990s, the investigation of incorporated material into dental calculus (mineralised plaque) has become an important source of direct dietary information of past people. While this started with the examination of microscopic remains, the last decade has shown a wide increase in the use of different biomolecular methods to characterise consumed material. The different sources of biomolecular and microscopic information are complementary and necessary to get a more holistic idea of ancient diets. However, current protocols often involve different extraction methods on separate samples of calculus, despite co-extractions being more sustainable and comparable.

Here we present our journey towards more sustainable analysis of archaeological dental calculus, not only applicable to the more judicious use of material but also what the results can provide for modern sustainable food use. This is done through developing a co-extraction protocol that allows for lipids, proteins, and microremains to be extracted from the same sample. This approach considered the impact of previous demineralisation to lipid retrieval and potential changes in protein damage. The final tests were done on calculus from a medieval individual from modern-day Italy, in triplicate, with two different lipid extraction techniques. Finally, we apply this technique to samples from Neolithic Ireland (Carrowkeel, Dowth Hall). The transition to the Neolithic and the food producing way of life is commonly understood to be amongst the most fundamental changes in prehistory, and understanding this change is important to contextualise current foodways and the future of sustainable practices.

Genetic History of Bacteria in Human-Impacted Environments

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Keywords: aDNA, Microbiomes, Ecosystems

We live in the Anthropocene where our habitat is in a constant flux as climate is changing radically. Although temperatures have fluctuated between cold and warm periods throughout the history of our planet, it is clear that we have caused the shift from Holocene to Anthropocene with our actions. Human activities have weakened many of the ecosystem services provided by microbes, including biogeochemical cycles. In addition to anthropogenic activities, genetic evolution of microbes has been shown to cause changes in the ecological cycles of elements. Also, changes in land use has been shown to trigger epidemics or even pandemics.

To understand the problems that we will face in the future, we need a better understanding on the chain of events that lead to alterations in microbiomes and their activities which are harmful to us. My research focuses on three key questions: (1) How have changes in climatic conditions affected microbiomes and their functions? (2) How have human-induced environmental changes influenced the diversity and functions of microbiomes, and how do these interact? (3) What is the significance of microbial diversity in microbial diseases/resistance and their spread from prehistoric to modern times?

To explore these questions, samples have been collected from archaeological excavations and peat bogs from Southern to Northern Finland, covering from before human influence to the present day. Total environmental DNA from the samples is extracted and shotgun sequenced. The sequencing data will be analyzed with bioinformatic tools to uncover past and present microbiome dynamics.

Microbial Seed Banks – Another Window to the Past

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Keywords: Microbial spores, Bacterial isolates, Genetic evolution

Recent advances in DNA techniques have substantially increased our understanding on ancient pathogens¹ and brought to life entire ecosystems of the past². These amazing works have been commonly carried out by applying metagenomics on archaeological specimens or samples from deep sediment layers. However, there might be more windows to the past. Dormant forms of microbes, also known as “microbial seed banks”, can persist extremely long periods of time in the right conditions³. My research group isolates spore forming bacteria from archaeological samples we have obtained aseptically from excavations. We have recovered bacteria belonging to *Streptomyces*, *Bacillus* and *Peribacillus* from their dormant forms. We use these isolates and their genomes to study the evolution of antibiotic resistance, because bacteria in these genera are known to be antibiotic producers. Both, antibiotic resistance and antibiotic biosynthesis are ancient. Over billions of years, microbes have interacted with each other by synthesizing bioactive molecules that weaken other microbes and by developing genetically encoded resistance mechanisms towards these molecules. Thus, in addition to antibiotic resistance, the ancient antibiotic producers recovered from dormant forms can be used to study the ecology and evolution of antibiotic biosynthesis. The two examples above show that despite the window to the past offered by microbial seed banks is narrow compared to metagenomic approaches, it can be utilized to answer many questions. In addition to the two examples, genomes of microbial seed banks could offer valuable insights on genetic adaptation of bacteria in response to transition from the Holocene to the Anthropocene.

References

- (1) Spyrou, M.A., Bos, K.I., Herbig, A. *et al.* Ancient pathogen genomics as an emerging tool for infectious disease research. *Nat Rev Genet* 20, 323–340 (2019). <https://doi.org/10.1038/s41576-019-0119-1>
- (2) Kjær, K.H., Winther Pedersen, M., De Sanctis, B. *et al.* A 2-million-year-old ecosystem in Greenland uncovered by environmental DNA. *Nature* 612, 283–291 (2022). <https://doi.org/10.1038/s41586-022-05453-y>
- (3) Lennon, J., Jones, S. Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nat Rev Microbiol* 9, 119–130 (2011). <https://doi.org/10.1038/nrmicro2504>

Hydrogen isotopes in Pleistocene fauna and environmental change

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Keywords: Hydrogen isotopes, Bone collagen

We investigate stable hydrogen isotope ratios ($\delta^2\text{H}$) of collagen of Pleistocene horses from sites in Britain and Belgium. Isotopic variations over time are noted, particularly a $\delta^2\text{H}$ excursion at ~15,000 BP which slightly precedes a later shift in $\delta^{34}\text{S}$ and $\delta^{15}\text{N}$. At the same timepoint, there is a significant difference between the UK and Belgian horse collagen $\delta^2\text{H}$ values, indicating a geospatial component to hydrogen isotope variation, as expected. While further work is needed, we highlight the potential of bone collagen $\delta^2\text{H}$ across long time sequences for palaeoenvironmental reconstruction.

Localized Management of Novel Crops in the Mountains of Central Asia during the Bronze and Iron Ages

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Keywords: Plant stable isotopes, Cultivation strategies, Agropastoralism

The mountains of Central Asia were critical pathways for the translocation of domesticated crops and animals and potentially their associated technologies, labor strategies, and social institutions. This translocation, often referred to as the Trans Eurasian Exchange or Prehistoric Food Globalization, spread Southwest Asian domesticates such as wheat, barley, peas, sheep/goat, pig, and cattle along with the Central Asian steppe domesticated horse, westward into Europe and eastward to South Asia and East Asia. A parallel movement of East Asian domesticates, such as broomcorn and foxtail millet, rice, and pig travelled south and west. Archaeological evidence attests to the routes and chronologies of this process that began prior to 5000 BCE and gained momentum during the 3rd-2nd millennium BCE. However, how local communities incorporated these novel crops and animals have only recently begun to be investigated, revealing complex systems of adoption and the strength of deep-seated culinary institutions and management systems. In this poster, we present results of plant stable isotope and macrobotanical analyses of key sites in the mountainous areas of Central Asia to investigate whether crops and their cultivation strategies travelled as a package across Eurasia, or if they disarticulated and were localized to fit the needs of each new community. The agropastoral communities at these sites, located in eastern Kazakhstan and Kyrgyzstan and western and central Tibet, incorporated plant cultivation in unique ways, localizing agricultural labor investment strategies into their own environmental and social systems.

ZooMS Identifies Fragmented Fauna from MIS 2 to 1 at Boomplaas Cave

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Keywords: Boomplaas Cave, Zooarchaeology by Mass Spectrometry

Intense fragmentation of faunal remains from archaeological sites is ubiquitous. In many cases, faunal assemblages are dominated by morphologically unidentifiable remains. For example, the vast majority of the large mammal remains from Boomplaas Cave could not be identified beyond the mammal size class using traditional morphological methods ¹. If they could be identified, these remains could potentially provide additional insight into human-animal relationships, dietary choices, and how they changed through time. Zooarchaeology by Mass Spectrometry (ZooMS) may enable taxonomic identifications when faunal remains cannot be identified using morphology ². ZooMS, however, is a destructive technique that is dependent on collagen preservation. Thus, Near-infrared Spectrometry was employed to estimate collagen yields before utilising ZooMS.

Palaeoenvironment and/or demographic change is expected to result in faunal turnover at Boomplaas Cave during MIS 2 to 1 as seen in Faith's (2013) identifiable faunal sample. Non-identifiable bones, or those that cannot be identified beyond bovid size class were analysed here. Differences between the morphological and ZooMS identified fauna are expected and have been documented at other archaeological sites ³. Preliminary findings will be presented, providing insights into sample selection, methodology, and subsistence behaviour at Boomplaas Cave during MIS 2 to 1.

References

1. Faith, J. T. Taphonomic and paleoecological change in the large mammal sequence from Boomplaas Cave, Western Cape, South Africa. *Journal of Human Evolution* **65**, 715–730 (2013).
2. Buckley, M., Collins, M. J., Thomas-Oates, J. & Wilson, J. C. Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry: Species identification of bone collagen using MALDI-TOF-MS. *Rapid Commun. Mass Spectrom.* **23**, 3843–3854 (2009).
3. Sinet-Mathiot, V. *et al.* Identifying the unidentified fauna enhances insights into hominin subsistence strategies during the Middle to Upper Palaeolithic transition. *Archaeol Anthropol Sci* **15**, 139 (2023).

Metagenomic Analysis of sedaDNA from Palaeolithic Cave Sites in Serbia

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Keywords: sedaDNA, Caves, Paleolithic

Palaeolithic research in Serbia has considerably advanced within the last 20 years, with the discovery and excavation of a growing number of Pleistocene cave sites. These sites provide archaeological contexts to study human occupations and their interactions with the surrounding ecosystems. As natural archives, caves preserve diverse biological sources which are integrated within the sediment and can be used to capture shifts in species interactions and ecosystem responses. The stable microclimate of caves is thought to be beneficial for the preservation of ancient sedimentary DNA (sedaDNA). Because of its highly complex composition, sedaDNA allows for the analysis of past biodiversity through biomolecular and metagenomic approaches.

Here, sedaDNA was extracted from Serbian caves spanning from the Lower to the Upper Paleolithic. Shotgun sequencing and bioinformatics pipelines based on mitochondrial DNA were implemented. The findings were authenticated using various classification pipelines such as MALT, aMeta and quicksand to confirm aDNA preservation and its reliable assignment. We reveal the presence of mammalian taxa that generally agree with the archaeozoological data and associated archaeological contexts, demonstrating the potential of Serbian caves for the study of ancient biodiversity. By integrating shotgun sequencing and bioinformatic workflows with optimized parameters, this research reconstructs past faunal diversity and provides insight into ecological dynamics and species interactions over time. In particular, we uncover ecological patterns which raise questions about the plasticity of cave ecosystems and the resilience of species to environmental fluctuations and human activities.

Microbial Dynamics of Ancient Peatlands: Can this Help Understand Current Rapid Changes?

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Keywords: Peatlands, Metagenomics, Roman

Peatlands are waterlogged, anaerobic environments and provide long-term carbon storage. Peatlands are vulnerable environments and susceptible to climate change compared to other ecosystems. Climatic changes can cause drying of the peatlands, releasing greenhouse gases and subsequently changing the microbial environment. In this poster, we focus on peat cores taken from a Roman fort site, Magna, in Northumberland, UK. Cores, taken from one recently dried location (Core A (4-56 cm)) and one waterlogged (Core B (4-128 cm)) location were radiocarbon dated. The microbial community was characterised using 16S rRNA metabarcoding and compared with chemical data to explore variation within the microbial community network.

Preliminary results of 16S rRNA metabarcoding showed that across all samples there was high robustness of the microbial community network. The network was most complex in shallow (0-20 cm) and deep layers (80-120 cm), where chemical elements Fe, Mn and P showed higher concentrations, compared with middle layers (20-80 cm) where the accumulation of Cd and Pb reduced community complexity, reflecting the negative effects of heavy metal pollution. Between the cores microbial diversity was highest overall in waterlogged core B with diversity of methanogens responsible for creation of methane highest in dried core A. There were also shifts in *Desulfobacteriota* and *Sideoxydans* which could have consequences for preservation.

Study of microbial dynamics within peatland could allow understanding of nitrogen and carbon dynamics. This can help inform management decisions around protection of archaeological land and timeliness of intervention in peatland restoration.

Historical Ecology of Elasmobranchs in the Early Modern Period from Proteomics and Marine Shagreen

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Keywords: Proteomics, Elasmobranchs, Marine historical ecology

The start of our most profound negative impacts on marine ecosystems coincided with the improved fishing technology, colonial expansion, and more efficient trade routes of the early modern period. Overlapping this time period was a peak in the demand for marine shagreen in northwestern Europe between the late 17th and early 19th centuries. Marine shagreen is a luxurious, storied leather made from the skins of elasmobranchs (sharks, rays, and skates) which was incorporated in a range of objects from cases for valuable objects to sword handles. Once elasmobranch skins have been turned into shagreen, more precise taxonomic identification than shark or ray is not possible. Preliminary review of historical sources indicates at least 17 species, spanning the Atlantic Ocean to the Indo-Pacific, were targeted for creating this material. Applying a multimethodological approach, encompassing proteomics, to studying this material, provides us with an opportunity to refine our knowledge of which species and seas were exploited to meet the demand for marine shagreen. Today, one third of all elasmobranch species are classified as being threatened with extinction by the IUCN. A key challenge to conservation efforts is the lack of historical data, long term perspectives, and ecological baselines. Studying these historic objects offers valuable insight into reconstructing the exploitation history and past biogeography of elasmobranchs. This poster will present pilot data from applying proteomics to marine shagreen, introduce a newly formed palaeoproteomics and elasmobranchs working group, and discuss the advantages of working closely with museums and conservators.

Stable Carbon Isotopes of Lost Crops of Eastern North America

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Keywords: Agriculture, Carbon, Experimental

Stable carbon isotope research is a widely available tool for studying past agricultural strategies of the Eurasian continent and its crops. In Eastern North America (ENA), stable carbon isotope studies of agriculture have been limited as researchers tend to focus upon maize (*Zea mays*), a C₄ crop. However, there is a suite of C₃ crops that were domesticated in this region, sometimes referred to as lost crops. They include goosefoot (*Chenopodium berlandieri*), erect knotweed (*Polygonum erectum*), sumpweed (*Iva annua*), maygrass (*Phalaris caroliniana*), and little barley (*Hordeum pusillum*). The lost crops were domesticated starting c. 4400 B.P. then put into agriculture over the consequent millennia, fuelling urbanization at Cahokia, the largest precontact North American settlement. They then fell out of use following European contact and Indigenous removal. The free-living descendants of the lost crops are still on the landscape and have been collected through efforts of Washington University's Paleoethnobotany Lab members. We plan to conduct growth experiments with these plants to identify their baseline $\delta^{13}\text{C}$ under varying water treatments. We will then test archaeological specimens to identify agricultural strategies of the past and how they changed through time at Cahokia and the surrounding settlements. This will be the first isotopic study of this nature in ENA. This work has far-reaching implications including identifying agriculture practices at other settlements in ENA, comparison with similar taxa in other regions of the world, and agricultural reinvigoration of a biodiverse suit of crops.

Sedimentary ancient DNA from the Middle Palaeolithic Cave Site Ararat-1, Armenia

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Keywords: sedaDNA, Transcaucasus, MIS3

Marine Isotope Stage 3 (ca. 60-27 Ka) witnessed rapid global changes in population dynamics including the possible turnover of sub-populations of Neanderthals and Modern Humans.

Southwestern Asia was a melting pot during this time with multiple hominin species present in the region possibly co-occurring with the cultural transition from the Middle to Upper Palaeolithic, and rapid environmental change and ecological fluctuations. Spatial effects of these climatic oscillations on hominin populations and terrestrial mammalian communities are however essentially unknown, especially in the Armenian highlands/Southern Caucasus region.

Ararat-1 is an Armenian Middle Palaeolithic cave site (35-52 Ka) with lithic remains and anthropogenic ashes. In this project, we harness sedaDNA to further investigate presence of early humans/ other mammals and to genetically validate microscopic observations of preserved organic material from the resin impregnated blocks.

Samples included bulk sediment and resin-impregnated blocks to generate shotgun libraries and mammal mtDNA capture. Mammal DNA was more abundant in the resin block samples, and more mammalian signal was found in the block deeper in the cave. Identified taxa included Hominids, Hyaenids, Felids, Bovids and Ochotonids. Hyaenas shed DNA primarily at the back of the cave and lions throughout the site. Results suggest that hominin use was ephemeral and concentrated at the back of the cave, alternating with large carnivores.

Migration, mobility, and translocation

Genomic Insights into an Irish Hunter-Gatherer Community

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Keywords: Demography, Kinship, Migration

Continuous human occupation of Ireland began around ten thousand years ago, when hunter-gatherer populations from Europe made their way to Irish shores. Despite being the sole human settlers on the island for 40% of its occupation, extremely little is known about these people. Only two genomes of individuals from this Irish Mesolithic have been sequenced, both from near the end of this era. Here, we present whole genome sequences for four novel Irish Mesolithic individuals from Killuragh Cave in the south of the island, increasing the total Irish dataset from two to six genomes and containing likely the oldest Irish genome ever sequenced. The individuals buried in Killuragh share highly inflated levels of identical-by-decent (IBD) segments with one another, relative to an Irish Mesolithic genome from Sramore Cave in the north, making this the first Mesolithic kin group genetically characterised in Ireland or Britain. No evidence of strict patrilocality or matrilocality is observed, as both Y chromosome and mtDNA diversity are high. In the European context, IBD data show greatest connection with Western Hunter-Gatherer individuals of North-Western and Northern Europe, rather than Atlantic coastal regions, suggesting a continental, and not Atlantic, initial colonisation of Ireland. Using simulations, we show that the island population these individuals belonged to was small, with effective population size estimated at 332 (95% C.I. 266-421), and underwent a period of extreme and extended isolation, resulting in some of the greatest degrees of Runs-of-Homozygosity (RoH) and shared allelic drift identified in any pre-Neolithic population.

Investigation of Admixture Between Neanderthal, Denisova and Central and Southeast Asian Populations

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Keywords: Archaic admixture, Migration, Population genetic

A decade ago, the sequencing of Neanderthal and Denisovan genomes revealed that our species, upon leaving Africa and migrating through Eurasia, interbred with these archaic hominins. This discovery spurred extensive research on archaic admixture in European, East Asian, and Oceanian populations, which show 1%–3% Neanderthal ancestry. East Asian and Oceanian populations also harbor Denisovan ancestry, with East Asians inheriting less than 1% and Oceanians up to 4%. However, the extent of archaic admixture in other Asian populations remains largely unexplored.

In this study, we investigate evidence of admixture between Neanderthals, Denisovans, and the ancestors of present-day Central, and continental Southeast Asian populations. Central Asian populations inherited approximately 2.5% of their genomes from Neanderthals, while Southeast Asians inherited around 2.7%. This ancestry results from a single event involving a Neanderthal population genetically close to the Vindija Neanderthal.

In contrast, Denisovan ancestry in these populations is below 1% and appears to result from multiple admixture events with distinct Denisovan populations, whose genetic distances from the Altai Denisovan genome vary. This suggests that, as they migrated across Asia, the ancestors of Asian populations encountered different Denisovan groups.

San Marcial De Rubicón: Paleogenomic Analysis of the First European City in the Canary Islands

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Keywords: Paleogenomics, European colonization, Slavery

San Marcial de Rubicón (SMR) is known as the first European overseas city funded within the Age of Discovery (15th – 17th centuries). SMR was located on the island of Lanzarote (Canary Islands) and it was founded in 1402 by the French explorer Jean de Betencourt. In this study, we analysed human remains from this historic site, including two individuals excavated within the city limits and two in the vicinity, with the aim of shedding light on the impact of the European colonization on indigenous communities.

The two individuals from SMR are male and their mitochondrial DNA (H3 and U5) and Y chromosome (J-M304 and R-M269) lineages are common in European populations. However, the other two individuals are female and have mitochondrial DNA lineages associated with North Africa, particularly haplogroup U6. Principal components analysis confirms that the two male individuals are similar to modern European populations, while the two females are closer to the Canarian indigenous population and present-day North Africans. These findings may represent the early stages of admixture and acculturation between the indigenous population (mostly women) and European society (composed primarily of men). However, the results of unsupervised clustering analyses show that SMR individuals, compared to the indigenous population of Lanzarote, have an excess of components associated with the Middle East and sub-Saharan Africa, which is more prevalent in present-day North Africans (due to Arab expansion and trans-Saharan migrations). Therefore, these individuals could potentially be associated with the North African slave trade.

Mobility and Social Structure in Late Antique Italy at the Edge of Longobard and Byzantine Territories

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Keywords: Ancient DNA, Mobility, Late Antiquity

From the 5th to the 8th century CE, a period historically known as Late Antiquity, intense socio-economic changes, political instability, and massive migration events reshaped the former Western Roman Empire, especially along the Italian Peninsula.

To assess the genetic and social influences of these events on the Italian population, we aim to conduct archaeogenetics and mobility analysis on 44 individuals from three Late Antiquity funerary areas in the modern Marche region of central Italy: Fossombrone (27 individuals), Castel Trosino (10 individuals) and a re-analysis of Pesaro (7 individuals), which has already been genetically sequenced¹.

All selected sites played a strategic role in Late Antiquity as centres of both peaceful and conflict-driven interactions among Romans/Byzantines, Goths, and Longobards. Fossombrone, located along the *Via Flaminia*, was part of the Byzantine Duchy of the Pentapolis until the Longobard conquest. Castel Trosino within the Duchy of Spoleto (*Longobardia Minor*), stood at the boundary between Longobard and Byzantine political entities. Pesaro, like Fossombrone, was one of the main centers of the Exarchate of Ravenna, under the formal rule of the Eastern Roman (Byzantine) Empire.

Preliminary findings from our ancient samples seem to suggest a complex interaction scenario, characterized by significant genetic variability and, in some individuals, a genetic shift toward Eastern or Germanic components.

Historical evidence and preliminary genetics data makes our sites excellent case-studies for exploring population dynamics and individual-level stories through genetic and molecular anthropology approach.

Reference

1. Ravasini, F. et al. The genomic portrait of the Picene Culture: New insights into the Italic Iron Age and the legacy of the Roman expansion in central Italy (2024). doi:10.1101/2024.03.18.585512

The Last Supper on a Phoenician Ship: Organic residue analysis of Archaic cooking pots and transport amphorae from the Xlendi wreck, Malta

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The on-going excavations of the Phoenician shipwreck at Xlendi Bay in Gozo (Malta) continue to reveal new insights into trade, subsistence, and seafaring in the Iron Age Mediterranean. Dating to around 650 BCE, this shipwreck and the cargo it contains represents direct evidence for trade between Malta, Sicily, and North Africa. In addition to the numerous ceramic transport containers, five used cooking pots were recovered from the wreck. The scorch marks present on the bottom of these pots, and the well-preserved carbonised food crust on their interior suggest that these cooking pots were used immediately prior to the sinking of the ship, thus constituting the ‘last supper’ these sailors had prior to the shipwrecking event. Few Phoenician shipwrecks have been thoroughly excavated, and little is known about the daily subsistence activities of mariners in the 7th century BCE. This paper presents results of organic residue analysis by GC-MS and GC-C-IRMS of ceramic cooking pots and transport amphorae recovered from the Xlendi shipwreck and discusses the wide variety of molecules detected. We explore what this new information can tell us about life on board ships in the early Iron Age, and how this can help us understand subsistence strategies during maritime trade voyages. We further examine how food items in the cooking pots relates to the lipids detected in the cargo of amphorae discovered on the ship. This collaborative study sheds light on long-distance maritime trade, translocation, diet, and mobility in the Iron Age.

A genetic exploration of Bronze Age connectivity in northwestern Europe

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Keywords: Demography, Migration, IBD

The initiation of the Bronze Age in western Europe was a period marked by large-scale demographic changes. The era was defined by an influx of ancestry related to steppe pastoralist populations, the movement of the Yamnaya from the steppe leading to a reduction in Early European Neolithic farmer (EEF) ancestry. Britain witnessed an approximate 90% population replacement during this time, associated with the spread of Bell Beaker Culture. The Bronze and Iron Age populations of Britain have been well characterised, with later continental migrations to the south of the island identified, providing candidate windows for Celtic Language entry. However, little is known about the population of the neighbouring island of Ireland, with only three genomes thus far published, all from the northeast. Here we present a new collection of Irish Bronze and Iron Age genomes, exploring patterns of British and continental influence in Ireland through the period. We quantify EEF ancestry through time and space in Ireland, which contrasts with British trajectories. We further use chromopainting to quantify the ancestry contributions from the preceding Neolithic of the islands, as well as continental populations, to British and Irish Bronze Age individuals. We further characterise patterns of mobility within and between archaeological sites across the islands using IBD and explore sex-biases in burial.

(All collaborators will be acknowledged)

Reconstruction of the Ancestry and Genetic Structure of Ancient Alpine Groups in the Iceman's Territory Over ~5000 Years (From Late Mesolithic to Middle Bronze-Age)

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Keywords: Ancient DNA, Trentino Alto Adige, Prehistoric Alps

The Eastern Italian Alps (EIA, Trentino-Alto Adige region) played a crucial bridging role between Mediterranean and Northern Alpine populations since Prehistory. However, few prehistoric individuals from that region have been genomically analysed so far. Among them, the Iceman (Copper Age, 3368-3108 BC) showed a relatively high Anatolian-Neolithic-related ancestry and low hunter-gatherers (HGs)-related ancestry.

To investigate the extent of major demographic events in the EIA and how the genomic structure of Alpine groups varied over time, we analysed 47 individuals dated from the Late Mesolithic (6,380-6,107 BC) to Middle Bronze Age (1,601-1,295 BC).

The Mesolithic individual shows high genomic affinity with Western Hunter-Gatherers (HGs), also with genomic contribution (~16%) from Eastern HGs and reveals genetic admixture between these two HGs groups occurred from ~13,7 to 8,3 BC. The genomes of Middle Neolithic Alpine individuals show a substantial shift in ancestry (from at least ~4,600-4,400 BC) with ~87% of component related to Anatolian farmers with a further limited contribution from HGs. Most individuals from the Neolithic onwards have similar genomic structure resembling that of the Iceman, supporting genetic continuity. Few individuals carried different ancestries, such as the Steppe-related ones appearing ~2400 BC. The study also suggests local and non-local admixture events between HGs and Neolithic farmers from this Alpine area.

In this contribution, the results of the comparison of maternal and paternal lineages and kinships found in specific funerary contexts will also be presented, and the genetic results on the Iceman placed in a local context.

Paleogenetic Diversity in Coastal Populations of the Far North Region in Chile

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Keywords: Paleogenetics, South America, Chinchorro

Along the coast of the Atacama Desert, the northernmost region of Chile, the Chinchorro culture was developed between 9,000 and 3,400 years BP. The Chinchorro people were adapted to a coastal environment, and one of their most striking features was their artificial mummification technique. Their lifestyle, mortuary practices, mobility patterns, diet, and social practices have been extensively studied, contributing to our understanding of early human occupations in this region. Meanwhile, their genetic diversity has only been studied by analyzing major mitochondrial haplogroups and a single low-depth genome. This research aims to characterize and describe the genetic diversity of early human populations associated with Chinchorro, evaluating the relationship through time and with the surrounding interior valleys. We sequenced a total of 80 samples from the Archaic to the Late Period (Inka) to evaluate the percentage of human DNA, quality, and authenticity of the genetic material. From this first screening, the highest percentage of endogenous DNA was 2.5%, and we recovered 34 mitogenomes. A total of 13 individuals were associated with the Chinchorro culture, and the predominant lineage (N=10) is A2+(64). Meanwhile, we see an increase in B2 and C1 lineages in the following periods, particularly the former reaching almost 70% in the late periods (Middle Horizon onward). This change in mitochondrial diversity may suggest a population replacement associated with migrations from the highlands. Further research is being conducted to increase the number of mitogenomes through enrichment protocols and generate whole genomes from representatives of each period.

Looking for Champlain's Cows: Cattle Origins, Movement, and Management in Colonial New France

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Keywords: Cattle, Isotopes, Ancient DNA

The colonization of the Americas brought European domestic species to the Western Hemisphere, fundamentally altering local ecosystems and reshaping the socio-economic structures of post-Columbian societies. Among these species, cattle (*Bos taurus*) emerged as a foundational actor of colonial economies and environmental transformations, particularly in the Lower St. Lawrence Valley, where their introduction facilitated the expansion of agricultural frontiers for European settlers. This study examines the origins, mobility, and management of colonial cattle in New France through the combined application of zooarchaeological, archaeogenetic, and isotopic methodologies.

This research is guided by three principal objectives: (1) tracing the geographical origins of colonial cattle using ancient DNA analysis, (2) analyzing patterns of movement and exchange of livestock within colonial North America through isotopic and genetic evidence, and (3) reconstructing historical husbandry practices via demographic and isotopic analyses of cattle remains. Drawing on faunal collections from 25 archaeological sites across Quebec, this multidisciplinary approach elucidates the integral role of cattle in shaping colonial economies and their integration into diverse cultural frameworks, including their interactions with Indigenous communities.

By synthesizing historical, genetic, and isotopic datasets, this study contributes fresh insights into the introduction and adaptation of cattle in North America. The outcomes not only deepen our understanding of colonial dynamics but also support the conservation of heritage breeds, such as the Canadienne cow, which highlights Quebec's agricultural and cultural heritage.

Complete Mitochondrial Genomes Provide Evidence on the Geographical Origin and the Temporal Evolution of Ancient Goats from Canary Islands

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Keywords: Ancient DNA, Fauna, Canary Islands

Archaeological and linguistic evidence have confirmed the North African origin of the Canarian indigenous people. Additionally, radiocarbon data has shown that, excluding the temporal Roman purple dye workshop exploited in the islet of Lobos around the 2nd century BC, the permanent settlement of the archipelago occurred by the 2nd century CE and lasted until the European colonization in the 15th century.

An alternative way of obtaining information on the geographic origin of populations is by analyzing their domestic species. One of the most abundant domesticates in the indigenous archaeological records are goats, providing a great opportunity to explore its origin and temporal evolution.

In this study, we apply next-generation sequencing to the recovery of whole mtDNA genomes of ancient goats from the Canaries (n=75), including animals associated to the Roman workshop, the indigenous occupation of archipelago and the colonial society.

Phylogenetic analysis of the Canarian ancient goat mitogenomes indicates they belong to two different clades: A2a and the newly defined A8. Both have been observed in present-day goats from the Middle East and North Africa, confirming previous results. Regarding the Roman goats, individuals from Lobos belong to the same clades as the indigenous ones. The sharing of specific haplotypes by Roman and indigenous goats from the islands closer to the African continent suggests that both settlements might have overlapped, leading to the movement of goats from the neighboring islands to Lobos. All historical samples fall within A8, pointing to the continuing use of indigenous herds up to the 18th century.

A Glance at Mitochondrial Haplogroup Change Through Time in Lithuania

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Keywords: Iron age, mtDNA, Lithuania

The present study is a follow-up of previous mtDNA studies on the modern Lithuanian¹ and the Stone Age² populations. Our objective is to address the existing knowledge gap concerning the structure and variation of haplogroups over time in the region.

We have analysed individual remains' DNA from Roman (N=28), Migration (N=49), Viking (N=24), and modern (N=145) periods from Eastern, Central, and Western regions of Lithuania. Ancient DNA was processed as described in Witas et al.³. Modern DNA was extracted from venous blood using phenol-chloroform extraction or automated paramagnetic particle purification (Freedom EVO® Nucleic Acid Purification Workstation) methods. Complete modern mtDNA genome sequences were derived after in-solution capture enrichment with an average of 352-fold coverage depth (Illumina GAII platform). Haplogroups were classified using Haplogrep v2 software.

The analysis yielded a total of 13 haplogroups (A, B, H, HV, I, J, K, L, N, T, U, V, W) across historical periods. Haplogroups H and U were consistent throughout the Iron Age and modern period. In contrast, during the Roman and Viking periods, the detection of Haplogroup L was noted.

The study revealed both qualitative and quantitative changes of mitochondrial haplogroup structure over time in the region. Haplogroup H continuously remained as a major defining haplogroup within the population. L haplogroup, which is associated with African ancestry and is found at higher frequencies in southern Europe, is not present in the modern sample group, suggesting the possibility that it may have disappeared from the gene pool of the Lithuanian population.

References

1. Kasperavičiūtė D., Kučinskas V. and Stoneking M. Y chromosome and Mitochondrial DNA Variation in Lithuanians. *Ann Hum Genet* **68**, 438–52 (2004).
2. Jankauskas, R. Ancient Mitochondrial DNA from Stone Age Lithuania and the Possible Origins of the First Inhabitants. *Archaeologia Baltica* **13**, 32–36 (2010).
3. Witas, H. W. et al. Hunting for the LCT-13910*T Allele between the Middle Neolithic and the Middle Ages Suggests Its Absence in Dairying LBK People Entering the Kuyavia Region in the 8th Millennium BP. *PLOS ONE* **10**, e0122384 (2015).

An Archaeogenetic Perspective on the Impact of European Colonization on the Formation of Mendoza City, Argentina

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Keywords: Ancient DNA, European colonization, Southern Cone

The colonization of the Americas by Europeans represented an unprecedented collapse of civilizations that deeply transformed Native American populations at multiple levels. However, its impact on the Southern Cone remains understudied. The city of Mendoza, founded in 1561 on the eastern slope of the Andes, became one of the southernmost stable Spanish settlements in the Americas. While initially small in size, Mendoza became a key commercial hub between the Pacific (through Chile) and the Atlantic (through Buenos Aires) throughout the colonial period and illustrates the complex population processes influenced by European colonization. Archaeological and anthropological studies have described a dynamic social organization classified into three major categories and their derivatives: Europeans and their descendants, local and non-local indigenous populations, and African enslaved individuals and their descendants. This social stratification reflects the broader patterns of colonization in the Southern Cone, where processes of admixture and cultural exchange reshaped the demographic landscape. By integrating paleogenomic and archaeological approaches, we studied 11 individuals from three different catholic cemeteries: San Francisco, La Caridad and La Merced. We found evidence of heterogeneous genetic affinities of Mendoza's inhabitants, tracing their genetic ancestries back to European, indigenous and African populations, as well as admixture between them. This genetic heterogeneity is reflected in burial practices, with a link between burial location and social class, though exceptions suggest complex social dynamics beyond ancestry alone. These findings align with the genetic composition of modern-day Argentinean populations, illustrating how present-day genetic diversity in the Americas was largely influenced by European colonization.

Sedimentary Ancient DNA (sedaDNA) from Borderlands: Understanding the Lower Germanic Limes Dynamics Through Biodiversity Reconstruction

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Keywords: Roman Limes, sedaDNA, Metagenomics

Sedimentary ancient DNA (sedaDNA) is a promising tool for reconstructing past biodiversity and past human-environment relationships within the archaeological context. Applied to borderland studies, sedaDNA can reveal the complexity of human interactions that are often overlooked. The Lower Germanic Limes (so-called "limes") consisted of the border of the Roman Empire, extending from the Rhine Delta to Remagen. Usually portrayed as a military phenomenon, the limes goes beyond its militarized scope, it also served as a contact and exchange zone for different cultures. To better represent the limes' complex dynamics, this study uses the biological information recovered from sedaDNA to understand consumption, mobility patterns, and landscape use across the limes' borderlands. We have accessed several archaeological sites from the Late Iron Age to the Roman period (200 BC - 300 AD) across the Netherlands and Belgium. We have generated metagenomic data from stored pollen trays and/or in-site samples from specific contexts, e.g., latrines, ditches, cesspits, and wells. We have obtained robust taxonomic profiles at multiple levels, including at species. The preliminary results indicate the presence of human-associated taxa, including domesticated animals, cultivated plants, introduced species, and organisms linked to human-induced environmental changes. In addition to corroborating previous findings from standard bioarchaeology methods, our study revealed new taxa at certain sites. These initial results demonstrate how sedaDNA can illuminate the complex dynamics of the limes, challenging the traditional view of it as merely a militarized borderland.

Shaping Identity: Exploring Artificial Cranial Modification and Patterns of Mobility in Late Antiquity to Early Medieval Europe

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The practice of Artificial Cranial Modification (ACD) is the permanent modification of an individual's skull, achieved through the use of compressive devices (such as wraps, hats or bandages) in early childhood¹. This process produces an elongated cranial shape and has been practiced across various cultures worldwide throughout history, serving aesthetic purposes and as a marker of group identity or social distinction. In Europe, evidence for ACD first appears in the archaeological record during the 2nd century, becoming more prevalent between the 4th and 6th centuries. It is debated whether the practice was linked to migrating groups, as its spatiotemporal distribution suggests, or if it originated in the East and spread independently as a cultural trend.

During the Late Antiquity/Early Medieval transition, the frequency of ACD was exceedingly rare in 5th –6th century Central Europe but increased significantly further East. In Eastern Europe ACD is practiced in males and females alike, while in Central Europe it's found almost exclusively in female skeletons². Additionally, higher female genetic diversity was observed in several sites from the region, suggesting the practice of female exogamy², even though the archaeological and historical sources do not rule out larger population movements³. Our study aims to conduct a comprehensive investigation of this phenomenon by analyzing newly sequenced and previously published ancient genomes from key sites, such as the 4th-century Roman city of Viminacium (Serbia) and the early Medieval site of Weilheim (southern Germany), to explore the complex patterns of cultural practices and mobility in this period.

References

1. Veeramah, Krishna R., et al. "Population genomic analysis of elongated skulls reveals extensive female-biased immigration in Early Medieval Bavaria." *Proceedings of the National Academy of Sciences* 115.13 (2018): 3494-3499.
2. Hakenbeck, Susanne. "'Hunnic' modified skulls: physical appearance, identity and the transformative nature of migrations." (2009): 64-80.
3. Velte, Maren, et al. "Between Raetia Secunda and the dutchy of Bavaria: Exploring patterns of human movement and diet." *Plos one* 18.4 (2023): e0283243.

Genomic Composition of Migration Period Horses in the Carpathian Basin

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Keywords: AncientDNA, Domestication, Horse

Previous studies have successfully recovered general trends in the horse domestication process. These revealed that the ancestors of all domesticated lineages originated from a 2nd millennium BCE radiation from the western steppes, and a population turnover around the beginning of the second millennium CE leading to the foundation of the majority of modern breeds. However, little is known about details and regional variation of these processes, especially in Central Europe during the early medieval period. To fill this gap, we shotgun sequenced more than 130 horse genomes from the territory of modern day Hungary, to an average of ~1x fold genomic coverage, mostly belonging to the 4th to 8th centuries CE. We use both allele-frequency-based and IBD methods to find answers for breeding customs, commerce and general genomic composition of the prevailing populations of the region. Moreover, we aimed to co-analyse the dataset with human ancient DNA data (i.e. the horse and its rider), and to compare the results with morphological ones and historical records in a multidisciplinary manner.

The project is embedded within the ERC HistoGenes Synergy Grant.

Population Shifts in the 5th & 6th Centuries CE in Central Europe. Integrating, Historical, Archaeological and Archaeogenomic Evidences from Whole Cemeteries



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Keywords: Archaeogenomics, Mobility, Migration Period

The 5th-6th centuries marked a period of profound transformation in Europe from the political dismantlement of the Western Roman Empire to the establishment of fragmented Barbarian Kingdoms. During the 5th century, various populations settled the border provinces of the former empire. The Carpathian Basin and surrounding area had a central role during these centuries. Initially, it was the center of the Hun Empire, later succeeded by the kingdoms of the Gepids in the east and Ostrogoths in the west. Around the beginning of the 6th century a new political force appeared in the region associated with the Longobards, whose origin is a matter of intense academic debate. The accounts from written sources coincides with the emergence of new burial communities spreading between present-day Czech Republic and the western part of the Carpathian Basin (the former Pannonia). The Longobards ruled these regions until 568 CE when historical sources describe their migration to Italy. Previous archaeogenomic studies have revealed the presence of a highly heterogeneous population in this region. Individuals with northern and southern European ancestries are found buried in the same cemeteries starting from the 5th century and later during the 6th century. In this study, we performed an exhaustive sampling for archaeogenomic analyses of 15 cemeteries from the 5th and 6th centuries located in the hypothesized core of the Lombard Kingdom. Our analyses allowed us to investigate at a finer scale the demographic shifts associated with the political and cultural transformations occurred during the Migration Period in the region.

Genomic Testing of Hypotheses on the Origins of the Grecìa Salentina Population and Language

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Keywords: Human population genetics, Gene and language phylogeny, Greek linguistic enclave

The origins of the Grecìa Salentina (GS) population, a Greek-speaking enclave in Southern Italy comprising 55,000 individuals across eleven communes, remain a topic of significant debate, with hypotheses pointing to either the *Magna Graecia* period (8th–5th century BCE) or the Byzantine era (early Middle Ages).

We performed whole-genome sequencing of 27 present-day consenting GS inhabitants. The results were compared with a subset of the large public dataset of ancient and modern individuals in the Allen Ancient DNA Resource. A total of ~7,000 European and Middle Eastern samples from the Paleolithic to the Middle Age were selected, with a particular focus on Italian, Greek and Anatolian data from the historical period critical to discriminate between alternative hypotheses on the formation of the GS community.

Principal Component Analysis (PCA), maximum likelihood genetic clustering methods such as Admixture, and F-statistics were employed to identify ancestry patterns.

As one of the last opportunities to study this community before its linguistic heritage disappears, this research provides critical insights into the history of human migration and cultural transmission in the Mediterranean. By integrating genetics, linguistics, and anthropology it addresses long-standing questions about the origins and legacy of this unique enclave.

Work approved by Comitato etico ASL Lecce, and Unione dei Comuni della Grecìa Salentina. Supported by PRIN2022 PC2TSX.

Palaeoproteomic Investigation on the Arrival of Sheep in Southern Africa

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Keywords: Ancient proteins, Pastoralism, South Africa

We employed palaeoproteomics to confirm species identification of two sheep bones discovered in the Later Stone Age levels of Blombos Cave, South Africa. Initially attributed based on their morphological traits, these specimens are identified as part of a sheep mandible and a calcaneum. Direct AMS ¹⁴C dating revealed their ages to be 1960 ± 50 cal. BP and 1880 ± 55 cal. BP respectively. Our findings verify the initial identification as sheep bones through identification of diagnostic collagen proteins and provide additional evidence regarding the theories relating to the route via which pastoralism was introduced to the southernmost Cape. These results offer valuable insights into the development of herding practices in southern Africa and the interactions between past human populations and their environment.

Between the Devil and the Deep Blue Sea: Sulfur Isotope Analysis of Geographical and Socioeconomic Mobility in 18th and 19th-Century Kingston-Upon-Hull, England

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Keywords: Sulphur isotopes, Industrialisation, Immigrant identities

Stable sulphur isotopes enable archaeologists to reconstruct mobility patterns, often utilised to estimate proximity to coastal and/or wetland regions. Marginal fractionation occurs when animals metabolise and incorporate sulphur throughout a food chain, allowing for direct evidence of environmental provenance with often substantial differences between terrestrial and marine origins. However, anthropogenic complications occur when analysing sulphur isotopes, as industrial pollution likely alters the biogenic values and thus hinders accurate interpretations. To better understand the shifts in sulphur isotopes in conjunction with industrialisation, this novel study explores a population of 119 individuals from the port-city Kingston-Upon-Hull, England during the Industrial Revolution. The sulphur isotope data from the rib collagen of the Trinity Burial Ground (c. 1785-1861) assemblage coupled with burial typology analysis allows for a holistic reconstruction of possible urban-rural mobility, class differences, and (non-)local identities. The findings reveal the probable local range for the population, established by a hypothesis that the intensification of sulphuric pollution exposure leads to site-level focusing of sulphur isotope values. The results further identify 24 non-locals, with significantly distinct patterns for males and females perhaps due to gendered occupational differences as maritime and domestic labourers, respectively. In addition, assuming earth cut burials relate to lower classes and tombs relate to upper classes, only select individuals within the local group may be associated with higher socioeconomic status—exemplifying constrained social mobility for non-locals. The study contributes to the nuanced investigation of identity politics and migration patterns with an original application of sulphur isotopes in industrial bioarchaeology.

Stone Age in The Baltic Region: Revealing the History of First Settlers in Estonia

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Keywords: Ancient DNA, Baltic, Stone age

This study provides valuable insights into the origins and migration patterns of the first settlers in Estonia and contributes to our understanding of the social, cultural, and genetic dynamics of prehistoric societies in the Baltic region. We focus on the analysis of ancient human DNA extracted from birch tar and teeth samples to explore the genetic composition of the first settlers of Estonia. The samples, dating from the early Mesolithic to the early Neolithic period (approximately 8700 BCE to 4000 BCE), capture the transition from the pre-pottery period to the Comb Ware culture.

Our research includes a total of 14 samples from seven archaeological sites in Estonia, including the most ancient one (8751–8433 calBCE) in the Eastern Baltic region. The findings reveal that Estonia's early Mesolithic settlers were genetically closer to eastern hunter-gatherers, while individuals from the late Mesolithic and early Neolithic periods show stronger genetic affinities with western hunter-gatherer populations. This genetic shift reflects the broader migration patterns and interactions between eastern and western hunter-gatherer groups across northern Europe.

Additionally, the successful extraction of ancient DNA from birch tar highlights its potential as an alternative source for genetic material, particularly in regions where traditional archaeological remains are scarce.

Refined Insights into Bronze Age Upper Mesopotamian societies from ancient DNA: A Study from Tell Chuera

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Keywords: Ancient DNA, Bronze Age, Human mobility

The Early Bronze Age (EBA, 3100-2000 BCE) in the Near East marked milestones in human civilization, including the advent of writing and the earliest cities and states. Considerable urban growth began around 2700 BCE in Upper Mesopotamia, with central sites accruing political influence over surrounding towns and villages. Despite a suggested major drought event leading to site abandonment by the late third millennium BC, archaeological and historical records indicate further "globalization" during the subsequent Middle and Late Bronze Ages (2000-1200 BCE). However, due to the scarcity of published ancient DNA data from this area, the effects of these profound societal shifts on human mobility and demographics remain unknown.

In this study, we generated genome-wide SNP data with exceptionally high quality from 27 individuals excavated from Tell Chuera, a prominent tell site in northern Syria. Initially populated at the outset of the EBA, Tell Chuera was inhabited as a large urban settlement until around 2200 BCE. After approximately 800 years of abandonment, the site was resettled in the mid-2nd millennium BCE and persisted until the 12th century BCE. Besides illuminating the genetic landscape of this period and region, these samples present a unique chance to study genetic and demographic patterns, tracing changes from an influential city-state during the EBA to a minor city of the Middle-Assyrian empire.

A High-Coverage Brown Bear Mitogenome from Early Holocene Sediment in Northern Sweden

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Keywords: sedaDNA, Brown bear, Mitogenome

During the early Holocene, plants and animals migrated into Scandinavia as the Fennoscandian ice sheet gradually retreated. Bi-directional colonisation events have been suggested for several species, including humans, brown bears, toads, shrews, and field voles. In brown bears, two different populations, represented by different mitochondrial clades, were involved in the colonisation: those carrying clade 1a took the southern route through Denmark and brown bears carrying clade 3a arrived via the northern route through Finland. These two groups formed a contact zone located around central Sweden. Based on mitochondrial DNA, it has been suggested that this structure was upheld by female philopatry throughout the Holocene, including a recent (~100 years ago) hunting-induced population bottleneck. Ancient DNA preserved in lake sediments across timescales of millennia can provide valuable insights to the colonisation routes of both plants and animals as a complement to osteological and other palaeolimnological studies. Here, we present a high-coverage ancient brown bear mitogenome extracted from a lake sediment layer from northern Sweden dated to 9.6 cal. ka BP, which is close in time to when the area became deglaciated. This complete mitogenome represents the oldest known brown bear DNA from northern Sweden. Our analyses suggest that this mitochondrial genome originates from a single individual belonging to an unexpected clade given the present-day distributions of brown bears.

Ancient Southeastern Anatolian Sheep: Genomic Evidence of Eastern Affinities

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Keywords: Archaeogenomics, Sheep, Southeastern Anatolia

Southeastern Anatolia occupies a central place in the Fertile Crescent—an area widely recognized as pivotal in the earliest stages of animal domestication. Despite its significance, ancient sheep genomic data from this region remain sparse. Here, we present eight new paleogenomes (0.03X-0.72X coverage) obtained from Early Bronze Age to the Hellenistic period Southeastern Anatolian excavation sites named as Karahöyük (n=3), Kuriki Höyük (n=1), Tatıka Höyük (n=1), Oylum Höyük (n=1), Tatarlı Höyük (n=1), and Yenice Höyük (n=1). Population genetic analyses of these new sheep paleogenomes with published datasets provide key insights into sheep population demography. Both PCA and D-statistics analyses indicate that southeastern Anatolian sheep genomes dating from the 3rd-1st millennia BCE, show closer affinities to Iranian Neolithic sheep than the earlier Anatolian Neolithic sheep from western and central Anatolia. This suggests remoulding of sheep gene pool via significant contribution from eastern lineages. Although the exact timing of this influx remains uncertain, our findings highlight the dynamic and evolving population structure of sheep in this crossroads region.

Genetic Characterisation of Individuals from Viking Age Southern Latvia

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Keywords: aDNA, Viking Age, Latvia

The movement of past populations around the Baltic Sea is a hot topic of historical research. The Viking age was a time of intense trade, military and cultural interactions across the Baltic Sea basin. The long, accessible coastline and navigable waterways in the territory of modern day Latvia have historically made this region accessible for intercultural contacts.

In total, samples from 25 individuals from four burial sites from ancient Semigallian territory were processed and data from 14 of those could be used for further analysis. Of the 11 identified mitochondrial haplogroups three (T2b, U5b1e1 and H5a1a) coincided between different burial sites located within a 13 km radius, indicating possible local maternal ancestry lines spanning up to five centuries. From 14 individuals eight were female and six male, all of which were from N Y-chromosomal haplogroup branch, characteristic to Eastern Baltic populations. The grave inventory of one of the individuals - a child analysed in this study - contained a three-winged brooch originating from Gotland, raising the question about the possible Scandinavian ancestry of this child.

Kinship analysis of the individuals does not indicate first or second degree relatedness, and run of homozygosity analysis indicates a larger reproductive population. Together with Admixture results showing a limited affinity with Viking Age inhabitants of Gotland, Sweden, our findings indicate the Eastern Baltic population being contiguous with other populations around the Baltic Sea.

Out-of-Anatolia Migrations during the Neolithization of Western Eurasia

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Keywords: Ancient DNA, Neolithic transition, Western Anatolia

Western Anatolia was a key centre in the Neolithization of Western Eurasia, serving as a bridge for spreading farming and social innovations from the Fertile Crescent into Europe. Using 30 new palaeogenomes from Anatolia (c. 8000–6000 BCE), we trace the genetic and cultural dynamics of Neolithization. Our analysis reveals genetic continuity from late Upper Pleistocene hunter-gatherers in the region, together with significant admixture with incoming populations from Central Anatolia. This admixture contributed to the formation of populations that later played a role in the Neolithic expansion into Southeast Europe. We further co-analyze genetic and cultural similarities across Southwest Asia and the Aegean villages using 58 material cultural traits. While material culture exhibits notable diversity among Aegean Early Neolithic communities, this diversity does not correlate with genetic differences, highlighting a decoupling of cultural and genetic dynamics. The absence of a correlation between genetic and cultural distances suggests that sociocultural affinity patterns may have changed at a different speed and following different processes when compared to the genetic affinities captured by ancient DNA data.

The Genetic History of the Livs, a Finnic-Speaking Community in the Late Iron Age and Early Medieval Eastern Baltics

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Keywords: The Livs, Human ancient DNA, Demography

During the Late Iron Age and early medieval period, various ethnic groups co-existed in the Eastern Baltic region, influencing each other due to shared territory, cultural exchange and trade. Among them were the Livs, a people who inhabited the eastern coast of the Baltic Sea and spoke Livic, a language belonging to the Finno-Ugric branch of the Uralic family. Understanding origin of the Livs and their interactions with neighbouring groups provides valuable insight in formation of Finnic- and Baltic-speaking communities in the region.

In this study, we investigate the population structure of the Livs and their place among medieval/early modern and contemporary populations from the eastern Baltic region using ancient DNA (aDNA). We generated whole-genome sequences from individuals from burial sites of Livic (n=40) and mixed (Baltic, Finnic) contexts (n=16) dating to 10th to early 13th century AD from what is now Latvia.

Genetic analysis revealed that the studied individuals share a close affinity with modern populations from Eastern Europe, while Livic individuals exhibit notable genetic diversity. Analysis of identical-by-descent genomic segments shared both among ancient individuals and between ancient and modern populations suggests that the Livs were genetically closer to other ancient individuals from Latvia than to those from Estonia. However, an exception was observed in individuals from the Siksälä site in southeastern Estonia, who displayed the opposite pattern.

Unveiling the Genetic Ancestry of Extinct Lion Populations

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Keywords: Population genomics, Genomic diversity, Museonomics

Lions (*Panthera leo*) were historically one of the most widespread large carnivores on the planet, ranging from the southern tip of Africa through the Middle East and Asia. Centuries of persecution and habitat destruction have led to lions occupying only 10% of their native range, leaving them restricted to a subset of African countries and a small population in India's Gir Forest.

Understanding how this dramatic decline has affected lion population structure and genetic diversity is critical for their conservation and management, as contemporary efforts rely heavily on captive breeding programs and translocations, and rewilding efforts have been adopted or proposed in a number of landscapes. Local extinctions and intentionally formed metapopulations (through mixing of distinct populations) complicate the ability to infer the historic population structure and demographic history of lions. Here, we generated and analysed genome-wide data from over 700 modern and historical lions to assess how persecution, translocation, and habitat fragmentation have shaped genetic diversity and local extinction. Our results provide insight into the pre-colonial dispersal capabilities of this large and iconic predator and contextualize patterns of modern population structure, allowing us to make more precise recommendations on how to preserve and restore genetic diversity of this iconic large predator.

Landscape and Topography in Mobility Analysis: A Combined Use of $^{87}\text{Sr}/^{86}\text{Sr}$ and GIS Modelling

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Keywords: Mobility, Strontium, Landscape

Strontium isotope analysis ($^{87}\text{Sr}/^{86}\text{Sr}$) is an established method to reconstruct mobility in archaeological populations. It often relies on comparisons between human values and a baseline for an area defined as “local” for an archaeological site. The extent of this definition can vary depending on site and researcher. Defining local using travel time from a site is one suggestion, but this requires an assessment of how the surrounding landscape might impact the distances covered within a given time. Introducing topography as an explanatory variable in the definition of local helps define the extent of territory accessible from a site.

This study looks into the mobility patterns of individuals buried at Kaptol; an Iron Age (8th - 6th c. BC) site of regional importance located on the southern slopes of Papuk mountain, Northern Croatia. Kaptol is located at a strategic location, close to important communication routes, such as the Sava river. This connectivity is displayed in material recovered from the tumuli of the two necropolises, Čemernica and Gradca, revealing links to the Eastern Alps, the Southern Pannonia, and the Central Danubian and Balkan regions. $^{87}\text{Sr}/^{86}\text{Sr}$ analysis was conducted on the cremated remains, and a first evaluation of bioavailable $^{87}\text{Sr}/^{86}\text{Sr}$ of the Papuk geological-region is provided through the analysis of collected plants. Landscape use and human-environment interactions are explored with the combined use of $^{87}\text{Sr}/^{86}\text{Sr}$ and Least-Cost Site Catchment around Kaptol, to understand the impact of the landscape and topography on human mobility.

Archaeogenomic Perspectives on the Greek Colonisation of Southern Italy

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Keywords: Ancient DNA, Greek colonisation, Migration

The Greek colonisation of southern Italy represents a pivotal chapter in Mediterranean history. However, the nature of this colonisation and its impact on indigenous communities remain subjects of debate. To investigate these dynamics, we generated genome-wide data from 44 individuals across four sites, encompassing both indigenous settlements and Greek colonies immediately prior and after Greek colonisation (9th–8th centuries BC). Ancient DNA was extracted from petrous portions of temporal bones or teeth, with data produced using double-stranded libraries and targeted enrichment via the Twist 1.4M human ancient DNA panel. The dataset comprised 27 females and 17 males, for whom mitochondrial DNA and Y chromosome haplogroups were determined. Among the 17 males, only 11 had sufficient Y chromosome DNA for haplogroup determination, and of these, only four were associated with Greek colonies. Although the number of Y chromosome haplogroups linked to Greek colonies is low, it is noteworthy that haplogroup E-V13, previously hypothesized to be associated with Greek colonisation, was absent. Genome-wide analyses will provide further insights into the origins of the Greek colonists and admixture patterns with local indigenous populations. These findings contribute to a deeper understanding of the complex interactions that characterized one of the most significant episodes of cultural and demographic transformation in the ancient Mediterranean.

Whole-genome Ancestry of an Old Kingdom Egyptian

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Ancient Egyptian society flourished for millennia, reaching its peak during the Dynastic period (~3,150-30 BCE). However, due to poor DNA preservation, questions about regional interconnectivity through time have not been addressed as whole genome sequencing has not been possible to date. Here, we sequenced a 2X coverage whole genome from an adult male excavated at Nuwayrat (Nuerat, نويرات). Radiocarbon-dated to 2,855-2,570 cal BCE, he lived a few centuries after Egyptian unification, bridging the Early Dynastic and Old Kingdom periods. The body was interred in a ceramic pot within a rock-cut tomb, potentially contributing to the DNA preservation. Most of his genome is best represented by North African Neolithic ancestry, among currently available sources. Yet, ~20% of his genetic ancestry can be traced to genomes representing the eastern Fertile Crescent, including Mesopotamia and surrounding regions. This genetic affinity is similar to ancestry appearing also in Anatolia and the Levant during the Neolithic and Bronze Age. Although more genomes are needed to fully understand the genomic diversity of early Egyptians, our results indicate that contacts between Egypt and the eastern Fertile Crescent were not limited to objects and imagery (e.g. domesticated animals and plants, writing systems), but also encompassed human migration.

Tracing Population Continuity and Genetic Relationship in Central Italy: Genomic and Isotopic Data

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Keywords: ancient DNA, mobility, genetic ancestry

During the Middle Ages, the region of Lazio, was a strategic crossroad of different cultures. This study focuses on two central Italian populations: Santa Severa (7th–15th century CE), a key Mediterranean trade hub, and Allumiere-La Bianca (15th–16th century CE), among Italy’s first alum mining settlements. Alum was a critical resource in Medieval trade between western Europe and the Middle East.

To investigate genetic variability and individual mobility, we applied genomic and strontium (Sr) isotope analysis. Sr isotopes from tooth enamel reflect an individual’s childhood geological environment, thus informing on geographical origins. Here, we aim to reconstruct individual mobility patterns of both human communities by combining strontium isotope analysis, paleogenomics and archaeological evidence.

Furthermore, low-coverage genomes were obtained from 40 individuals: Allumiere-La Bianca (n=13) and Santa Severa (n=27). Additionally, six Etruscan (6th–3rd century BCE) and five Roman (3rd century BCE–5th century CE) genomes from Santa Severa were analysed to assess genetic continuity through time.

Etruscan and Roman genomes from Santa Severa fall in the genetic variability of Etruscans and Romans already published, evidencing genetic discontinuity in Santa Severa between Etruscan and Medieval periods. Genetic differences have also been highlighted between the Early Medieval population of Santa Severa and the Late Medieval population of Allumiere-La Bianca. Among the Medieval individuals analysed genetically, we pointed out several outliers with non-local strontium isotopic values, suggesting origins outside the region.

Future work will focus on increasing genome coverage ($\geq 0.5X$) to enable high-resolution analyses of ancestry and population structure through imputation.

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Genetic Data from High-ranking Middle-Late Avar Period Communities Reveal Multiple Origins

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Keywords: Avar, Population genetics, Ancient DNA

The Avar Khaganate ruled for two and a half centuries in the Early Medieval Carpathian Basin, with its elite coming from the East Asian region. Here, we present 317 ancient human individuals from four Avar period burial sites, located in Slovakia and Hungary. Genome-wide SNPs were captured, then were enriched and sequenced. Two of these sites were used by upper-ranking groups in the Middle and Late Avar periods (late 7th-8th centuries CE). The genomic ancestries from two elite communities in these sites indicate different origins, where the first community has links to East Asia, and the other likely originates from around the Eastern European area. In Kiskundorozsma Kettőshatár-II, we present a group likely from the ‘highest elite’ strata of the society, and observe a lack of kinship links with the other communities buried in immediate vicinities. In Komárno-Lodenice, there is another high-ranking warrior group subordinate to the highest elite, who show possible admixture with groups of East Asian ancestry. These sites were robbed within decades after the burials of the individuals, which can hint at the fate of the falling Avar Khaganate’s elite. Constructed pedigrees shed light on several social patterns such as patrilocality and female exogamy, and genetic events that replaced certain groups with others.

Exploring Consumption and Environmental Patterns During the Roman Period in Harelbeke, Belgium, via sedaDNA Analysis

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Keywords: sedaDNA, Roman period, Archeogenomics

Recent studies show that sedaDNA methods could provide valuable information about the biodiversity of past environments. This study explores the use of sedaDNA methods to investigate the change in past consumption habits and their implications on the northwestern border zones of the Roman Empire (the Lower Germanic Limes). We obtained ancient DNA from latrine contents from two different Roman phases that gave an insight into the biodiversity of the region's past environment, consumption patterns, and increasing Roman influence in the local culture through the variety of domestic animals and plants. Our study shows an increase in the abundance of Roman-associated taxa, which implies the effect of changing trade relationships in the local area. Furthermore, while our findings were supported by previous archaeozoological and archaeobotanical research, they also demonstrated the complementary nature of sedaDNA studies with other archaeological methods for investigating historical periods. Therefore, this study highlights the potential of sedaDNA methods for improving our knowledge of the past and contributes to our understanding of ancient societies and their interactions with the environment. Moreover, the future sedaDNA research in the Limes area has the potential to provide a more comprehensive understanding of consumption habits and trade routes in the borderlands of the Roman Empire.

The Green Sahara Genomic Landscape: Tracing Ancestries across North Africa and Sahel through Joint Analysis of Modern and Ancient Genomes

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Keywords: Africa, Evolutionary genetics, Population genomics

During its last humid phase (12,000–5,000 ya), the Sahara was fertile and widely populated, allowing coexistence and interactions among various inhabitant groups. The subsequent desertification created the largest geographic barrier in Africa, driving the evolutionary divergence of North Africa and the Sahelian belt. In order to characterize at a higher resolution possible genetic signals related to the last Green Sahara and the trajectories of subsequent migrations, we sequenced 118 high-coverage (30x) human modern genomes from five North African and seven Sahelian countries, integrating them with 43 previously analysed samples (D'Atanasio et al. 2023) and >2,400 modern and ancient genomes from literature.

The analysis of this comprehensive dataset highlights significant genetic heterogeneity, with notable cases of stratification, such as in Sudan - where genetic signals link this region to both North Africa and Sahel - and the presence of a relevant sub-Saharan ancestry in some culturally Berber populations, like nomadic Tuareg and Egyptian Siwa, that could point to possible early events. Crucially, the inclusion of ancient genomes has revealed that early ancestries are differentially distributed not only between northern and southern regions of the Sahara but also longitudinally following distinct patterns along the Sahel.

Further analyses aim to distinguish between older and more recent admixture events and to date them. In particular, >150 ancient African samples, spanning from the Neolithic to the Early Christian period, are essential for uncovering genetic signals across an extensive temporal range and shedding light into the historical trajectories and evolutionary dynamics around the present-day Sahara.

Reference

D'Atanasio, E. et al. The genomic echoes of the last Green Sahara on the Fulani and Sahelian people. *Current Biology* 33(24), 5495-5504. (2023)

Paleogenetic Analyses of Prehistoric Caves in Belgium's Meuse Valley Reveal Distinct Patterns of Local Population Dynamics

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Keywords: ancient DNA, Mesolithic-Neolithic transition, Belgium

Southern Belgium, and more specifically the valley of the middle Meuse, is renowned for its rich archaeological record associated with prehistoric periods. Numerous human remains, dating from the Middle Palaeolithic to the Bronze Age, have been discovered in over 400 caves, providing unique fossil assemblages despite a significant hiatus between the Middle Mesolithic and the Middle Neolithic.

Here, we present the first results of a paleogenetic analysis conducted on remains from 14 collective cave deposits, initially targeted for their Mesolithic attribution within the frame of the ROAM project: Migration, diet, and health of the first permanent settlers of Belgium: inter- and multi-disciplinary perspectives. Most of these remains originate from early excavations, some dating back to the early 19th century and rarely accompanied by precise documentation. Therefore, a systematic radiocarbon dating campaign was undertaken to better understand the internal chronology of the burials.

The results demonstrate that several cave burials previously identified as exclusively Mesolithic are mixtures of depositions spanning multiple periods. Focusing on the Mesolithic and Neolithic periods, we provide new insights into demographic movements, previously unknown from a biological perspective in the region. While the Mesolithic groups align with known patterns in Western Europe at the time, all Late Neolithic individuals exhibit a strikingly different profile, characterized by an unexpectedly high hunter-gatherer inherited component. This is similar to findings from a few sites in nearby regions, such as Blätterhöhle¹ or Niedertiefenbach² in Germany. In Belgium, however, the archaeological evidence complements the genetic findings, highlighting unique local dynamics of acculturation³.

References

1. Lipson, M. et al. Parallel palaeogenomic transects reveal complex genetic history of early European farmers. *Nature*, **551**, 7680, 368-372 (2017).
2. Immel, A. et al. Genome-wide study of a Neolithic Wartberg grave community reveals distinct HLA variation and hunter-gatherer ancestry. *Commun Biol*, **4**, 113 (2021).

3. Messiaen, L. The Neolithization Process in the Lower-Scheldt Basin (Belgium, mid-6th to mid-4th Millennium cal BC) from a Lithic Technological Perspective. *Lithic Technology*, **48**, 2, 168–193 (2022).

A Genetic Bridge over the Gulf of Finland: Tracing the Origin of Genetic Connectedness Between Finns and Estonians

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Keywords: ancient DNA, human population genetics, migration

The Finnish population is a unique example of a genetic isolate affected by a recent founder event. Previous studies have suggested that the ancestors of Finnic-speaking Finns and Estonians reached the circum-Baltic region by the 1st millennium BCE. However, high linguistic similarity points to a more recent split of their languages. We set out to study genetic connectedness between Finns and Estonians directly.

In a study published in 2021¹, we searched for long shared allele intervals (LSAIs; similar to identity-by-descent segments) in unphased data for >143,000 present-day Estonians, 99 Finns, and 14 imputed ancient genomes from Estonia. We found unexpectedly high levels of individual connectedness between Estonians and Finns for the last eight centuries, contrasting their clear differentiation by allele frequencies. High levels of segment sharing between Estonians and Finns predate the demographic expansion and the last phase of the settlement process of Finland.

Here, to shed more light on the origins of the connectedness between Estonians and Finns, we extracted and sequenced ancient DNA from 16 additional prehistoric individuals from Estonia dating to 1,400 calBCE to 700 calCE, including the first genomes from 300–700 calCE. We show that already the 300–700 calCE individuals had higher levels of individual connectedness with modern Estonians and Finns than individuals from earlier time periods, including preceding individuals from 600–100 cal BCE. However, the 300–700 calCE individuals do not yet show region-specific connectedness within Estonia to the extent that medieval and modern (since 1200 CE) Estonian individuals do.

Reference

1. Kivisild, T. et al. Patterns of genetic connectedness between modern and medieval Estonian genomes reveal the origins of a major ancestry component of the Finnish population. *Am. J. Hum. Genet.* 108, 1792–1806 (2021).

Ancient genomes reveal demographic trajectories during the Classic Maya period

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Keywords: Maya civilisation; Admixture; Population collapse

Copán was a major capital at the southeasternmost extreme of the Classic Maya civilisation, serving as a crossroads connecting Central and South America. In 426/427 CE, the city witnessed the establishment of a royal dynasty, which endured for approximately 400 years. Despite extensive historical and archaeological records, there remains limited information regarding the genetic profile of people who resided in Copán. Here, we present genomes from seven Classic Copán individuals, including a potential member of the ruling dynasty and an accompanying sacrificial burial. Our analysis shows that the population associated with the Classic Copán culture forms a genetic cluster with the Late Archaic population from Belize, as well as with the Terminal Classic Maya from Chichén Itzá, the Colonial Maya from Campeche, and present-day Maya, all from Mexico. Using Late Archaic Belize as a source of local ancestry, we identify an influx of ancestry associated with highland Mexican populations from the Early-to-Middle Classic period onward, underscoring the role of gene flow in the formation of the Classic Maya state. Estimates of effective population size suggest a decline at the end of the Classic period, when Maya civilisation experienced widespread destabilisation and collapse.

Regional Genetic Differences of the Carpathian Basin Population in the 10th Century AD

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Keywords: Carpathian Basin, Medieval, Genomics

The population composition of the Carpathian Basin was shaped by diverse genetic influences. The arrival of the Hungarian conquerors marked the 10th century as a new chapter in the region's genetic and cultural history.

We analyzed genome-wide data from over 220 individuals (10th c. AD), combining previously published datasets of more than 150 individuals with nearly 70 new samples. The burial sites' extensive spatial distribution enables us to analyze the 10th-century population both on a macro- and microregional level. Our multidisciplinary approach integrates identical-by-descent (IBD) and kinship analysis, population genetics and isotopic data.

Our results indicate that the incoming population constituted approximately 10% of the total inhabitants. Unlike the Avars, genetic data suggest that Hungarians actively mixed with the local population from the beginning. While we found no evidence of large families, numerous individuals had extensive genetic connections.

IBD analysis of samples from the Carpathian Basin reveals that before and during the early 10th century, the Danube acted as a significant boundary, with the newly arrived Hungarians predominantly localized in the Great Hungarian Plain. However, from the mid-10th century onward, we observed relocation trends, driven by the movements of both the newcomers and the earlier resident populations. This led to a notable increase in genetic connections across microregions.

Our comprehensive methodology sheds light on complex demographic processes that shaped the population of the Carpathian Basin in this period, providing valuable insights into the genetic and cultural integration of the medieval period.

Genetic Origins of the Kiritimati Population from Central-Eastern Micronesia

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Keywords: Population genetics, Oceania, Kiritimati

The migration of Austronesian-speaking populations through Oceania has intrigued researchers for decades. The Kiribati islands, situated along the boundaries of Micronesia and Polynesia, provide a crucial link in this migration. We analyzed the genome-wide data of the Kiritimati population of Kiribati to uncover their genetic origins and connections with other Oceanian groups. Our study reveals that the Kiritimati population primarily exhibits Remote Oceanian-related ancestry associated with ancient Lapita and present-day Polynesian populations. In addition, our identity-by-descent analysis identifies populations from Philippines as their closest relatives in Island Southeast Asia. The genetic links between Kiritimati, ancient Lapita, and modern Polynesians underscore the shared ancestry and continuous gene flow across these regions. This genetic continuity and ongoing links are supported by linguistic and cultural evidence, illustrating a complex history of migration and admixture in Oceania.

Tracing Pigeon Dispersal through Time: Ancient mtDNA from Roman to Post-Medieval Oxfordshire

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Keywords: Hybridization, Migration, Genetics

The application of mtDNA to understand genetic change over time through hybridization, or the merging of multiple species, is fundamental for broadening our understanding of population change within a species over time. *Columba livia* or the rock dove is the progenitor of the more widely known feral pigeon *Columba livia domestica*, or the pigeon you see populating the street. The pigeon was likely the first bird domesticated in Western Eurasia. Dovecotes, archaeologically seen structures, could house over a thousand pigeons, and provided squabs, eggs, and crucial fertilizer—up to 15 tons annually. In addition to its role in agriculture, the pigeon contributed to evolutionary studies, with Darwin using a dovecote to study their remarkable phenotypic variation, which led to the development of fancy breeds.

Despite the feral pigeons' ubiquity and presence on every continent except for Antarctica, and its use as a model organism for domestication, no comprehensive studies have been done on the ancient genetics of pigeons. This study analyses mtDNA of pigeon bones from the Roman, Early, High, Late and Post-Medieval periods to better understand when rock doves and feral street pigeon hybridization occurred. Studying these waves of occupation through clades of pigeon reintroduction in Britain broadens our understanding of human migration, displacement, and replacement as the success of pigeons co-evolved with humans. This study looks at the migrations and translocations of pigeons within these dovecotes by establishing where the progenitor for each new clade of pigeons, associated with each new wave of human migration, originated.

Fifteen Millennia of Mitogenome Evolution in Sicily

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Keywords: Ancient and modern DNA, Mitochondrial variation, Sicily

Sicily, situated at the heart of the Mediterranean Sea, has been a crossroads of people of different origins since the Paleolithic. To gain further insight into the genetic history of the island from a matrilinear viewpoint, we investigated fifteen millennia of mitogenome evolution. A unique Sicilian mitochondrial dataset, represented by 116 ancient mitogenomes (including 2 newly sequenced) collected in 16 archaeological sites dating from 14,700 to 545 years before present (BP), was compared with a novel collection of 236 modern mitogenomes covering all districts of the island. By integrating demographic modeling with phylogeographic analyses for the first time, we were able to identify a statistically supported genetic discontinuity between the Paleolithic/Late Mesolithic and Early Neolithic periods, as well as two mtDNA lineages (U5b and U8b/K) that specifically mark this transition, which is characterized by a certain degree of population replacement. Specifically, Paleolithic hunter-gatherer groups carrying U5b1d1, U5b2b, and U5b3 arrived and expanded on the island until the Late Mesolithic. These groups survived in various refugia in western Eurasia and were partially replaced by farming communities migrating from the Near East via the Mediterranean and inland routes. These farming communities introduced the Neolithic lineages U8b1b1, K1a3, and K2b into the island. The high mtDNA variation and lack of genetic differentiation among modern mitogenomes suggest the presence of continuous gene flow from diverse regions of Western Eurasia (since the Paleolithic) and Africa (since the Bronze Age).

Inhabitants of the Iron Age Scythian Settlement: Where Did They Come from?

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Keywords: Ancient DNA, Scythians, Mobility

The Bilsk fortified settlement is a Scythian site in Ukraine with a rich archaeological context. It dates to VIII-III cc BCE and represents one of the largest Iron Age complexes in Eastern Europe. Archaeological evidence indicates several waves of migrants arriving over the four centuries of the settlement's history. Burial customs and archaeological artefacts allow distinguishing elite and non-elite as well as a temporal stratification of elite groups into early and late. Our goals were to reveal the genetic composition of the settlement's population and its demic sources, trace its change over time, study interaction between immigrants and local population, find out about life-trajectories of individuals.

We compiled a collection of osteological samples with well-characterized context information and generated genomic sequences of ancient individuals (n=26). A screening shotgun sequencing (10/20M reads per sample) was performed, samples with low coverage and endogenous DNA >10% were re-sequenced, genotypes with coverage of >0.3 were imputed. The mobility and diet were studied based on strontium isotope ratio $^{87}\text{Sr}/^{86}\text{Sr}$, stable carbon and nitrogen isotopes from tooth enamel. We compared ancestry profiles of early and late elite individuals, and non-elite ones to reveal their genetic affiliation, interactions and admixture. Preliminary results show extremely high genetic heterogeneity of the settlement population, discontinuity of early and late elites, distant migration for at least some early settlers.

Christian Pioneers: $^{87}\text{Sr}/^{86}\text{Sr}$ isotope analysis at Kata Gård, an early Christian site in Varnhem, Sweden

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Keywords: Strontium isotopes, Mobility, Christianity

The Christianization of Sweden (8th–11th centuries) was a period of political, social, and religious change, and it implies a period of movement and mobility. Excavations at the Varnhem estate church grounds (Kata Gård), with a church originally constructed around AD 1000 in Västergötland, Sweden, have yielded information about this period. Key information findings include a large socially stratified cemetery, burial practices that indicate remnants of pre-Christian burial customs, and grave goods suggesting contact with other areas. This study uses strontium isotope analysis to investigate the lives of these early Christians buried at Kata Gård and to identify the non-locals. Our results indicate that a large part of the adult population was non-local and had spent their childhoods in other geological areas, whereas the children had mostly local strontium isotope signatures.

The Royal Ship *Kronan* Crew: Revealing the Demographic Structure through Archaeogenomics

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Keywords: *Kronan* ship crew, Ancient genomics, Demographic patterns

The Swedish Royal Ship *Kronan*, one of the largest warships of its time, tragically exploded and sank near Öland Island in the Baltic Sea in 1676, claiming the lives of over 800 crew members. Designed for both warfare and the display of power, the ship carried a diverse crew of sailors and soldiers, including mercenaries bound for the continent. As such, the *Kronan* crew provides a unique snapshot of 17th-century Sweden. Archaeological excavations have recovered extensive human remains from the wreck, including those of several children.

Building upon these archaeological findings, we conducted archaeogenomic analysis of the recovered human remains to investigate the genetic structure and demographic composition of the *Kronan* crew. We generated genomes from 64 individuals (54 adults and 10 children) and applied state-of-the-art methods, including Identity-by-Descent (IBD) analysis. Our research focused on identifying the crew's demographic origins, exploring kinship patterns among them, and examining their genetic affinities with present-day Swedish populations.

The results provide new insights into the genetic diversity and social dynamics of the *Kronan* crew, enriching our understanding of the Swedish navy in the 17th century. This study also complements existing archaeological findings and generates valuable genomic data for future interdisciplinary research within the archaeogenomic and archaeological communities.

Investigating Shovel-Shaped Incisors in Northern Italy's Bronze Age Populations: Preliminary Results of the “SHOVELING” Project

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Keywords: Bronze Age, Non-metric traits, EDAR

"SHOVELING" is an interdisciplinary project supported by the PRIN (Italian Research Projects of Significant National Interest – NextGenerationEU) program that investigates the evolutionary origins and prevalence of shovel-shaped incisors in Bronze Age populations from Northern Italy. This study integrates anthropological, genomic, and isotopic analyses to test hypotheses regarding the presence of a non-metric dental trait. Shovel-shaped incisors, common among extant Asians and Native Americans, are rare in other human groups. Recent findings suggest a mutation in the EDAR gene, which may have arisen in China or Beringia around 20,000 years ago, contributing to the trait's prevalence.

Our research focuses on individuals with and without shovel-teeth from the Middle to Recent Bronze Age (ca. 1300-1200 BCE) in Northern Italy, a key region for ancient population movements. We analysed samples from several sites, including Castello del Tartaro and Bovolone (Verona), and Ballabio (Lecco).

Employing ancient DNA techniques tailored to highly degraded samples, we analysed the genetic makeup of these individuals, alongside stable isotopes to assess their mobility and dietary patterns. Our primary goal was to test whether the potential link between EDAR-gene variants and the presence of shovel-shaped incisors was due to isolation, migrations or local selective pressures.

We will present the preliminary results from this project, which aims to elucidate population dynamics and adaptive strategies of Bronze Age communities in Northern Italy, contributing to our understanding of human evolutionary history and the interplay between genetics, environment, and cultural behaviours.

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Integrating ancient biomolecules and archaeological theory

Understanding the Complex Social Relations in Pre-Roman Umbrian Communities with an Integrated Approach of Bioarchaeology

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Keywords: Pre-Roman Umbrians, Stable isotopes, Palaeogenomic.

In pre-Roman times, Central Italy witnessed the rise of various populations characterized by distinctive languages and traditions. Despite numerous works on the Etruscans and the Latins, little is known about the Umbrians. This research aims to fill this gap in bioarchaeological research by examining the necropolis of Piazza d'Armi in Spoleto (Umbria), dated to the 8th-6th century BCE. This necropolis is a unique opportunity to understand the complex social structures of an Umbrian pre-Roman population, due to the rich grave goods found in the burials, particularly those associated with women, children, and possibly foreigners.

Both morphological and morphometric methods, applied prior to molecular analysis, confirmed that the sample consisted of 17 non-adults and 21 adults. Sr, N and C stable isotopes data of 36 individuals and faunal remains have been used to evaluate mobility and dietary patterns, considering potential sex and age differences.

Forty samples (petrous bones and/or teeth) were collected for palaeogenomic analyses. Double-stranded genomic libraries were sequenced in depth to obtain low-coverage genome-wide data and to assess the degree of DNA conservation. Specific bioinformatics pipelines for ancient DNA have been used to identify ancestry variants, informative kinship and pathological markers.

Combined bioarchaeological approaches will strengthen hypothesis related to the origins of the people from Spoleto, their family relationships, social links and human mobility (particularly of women and their children), based on excavation findings.

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Targeted Sampling of Microcontexts for SedaDNA at the Late Pleistocene Site, Satsurblia Cave, Georgia

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Keywords: Microcontextual, sedaDNA, Archaeology

The study of ancient sedimentary DNA (sedaDNA) is a young, fast-paced and constantly evolving field with the potential to reveal a new level of archaeological information. However, in archaeology spatial information (context) is crucial for connecting variable datasets, hence to associate sedaDNA with archaeological and paleoanthropological remains we need to control the (micro)context of sedaDNA¹. A methodology employed by archaeologists to provide context is micromorphology, where an undisturbed block of sediment is removed from the site, impregnated with resin and cut for thin section (glass slides with ~35 µm of material) analysis under a petrographic microscope. Here, we present results of a targeted microsampling methodology² that was developed and employed to understand DNA integrity and preservation at the Upper Palaeolithic site, Satsurblia Cave, Georgia. The site was intensively occupied by humans before, during and after the Last Glacial Maximum, which created a complex microstratified sequence. A sediment sample from Satsurblia has previously yielded both nuclear and mitochondrial DNA of a wolf, bison and human from 25,000 years ago³. To provide microcontextual information to the sedaDNA samples we drilled them from the impregnated blocks, with corresponding thin sections and µXRF data. We obtained samples from microcontexts where DNA was expected to preserve and others where it was not, along with areas affected by different post-depositional processes that we could identify, such as freeze-thaw. This approach will provide a firmer grasp on the source, movement and preservation dynamics of sedaDNA in archaeological cave sites.

References

1. Aldeias, V. & Stahlschmidt, M. C. Sediment DNA can revolutionize archaeology - if it is used the right way. PNAS 121, (2024).
2. Massilani, D. et al. Microstratigraphic preservation of ancient faunal and hominin DNA in Pleistocene cave sediments. PNAS 119, (2021).
3. Gelabert, P. et al. Genome-scale sequencing and analysis of human, wolf, and bison DNA from 25,000-year-old sediment. Curr. Biol. 31, 3564-3574.e9 (2021).

Beyond the Massacre: Genomic Insights into Ancestry, Identity, and Change in Early Medieval Bohemia (9th-11th century)

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Keywords: Ancient DNA, Social stratification, Early Medieval Bohemia

The Early Middle Ages in Bohemia were a period of profound transformation, driven by the rise of the Přemyslid dynasty and Christianity. The archaeological sites of Budeč (N=153) and Brandýsek (N=38) provide a unique opportunity to explore community relationships, power dynamics, and mobility through a combination of archaeological, isotopic, and genetic data. At Budeč, three key sites - the Rotunda of St. Peter and Paul with its elite cemetery, the mass grave at Na Týnici (outside the fortress), and the surrounding burial ground - reveal complex social structures. Uniform Manifold Approximation and Projection (UMAP) was employed to explore genetic diversity and population structures, while kinship relations suggest genealogical relationships and possible social stratification. Isotopic analysis of nitrogen, carbon, and strontium, has provided insights into mobility and diet, and ancestral profiling aims to trace the origins of individuals with unusual burial patterns, such as prone women or individuals with poorer grave goods, possibly representing subordinate groups. The elite cemetery highlights Budeč's political and religious significance, corroborated by historical records, while the mass grave raises questions about whether the individuals were outsiders, possibly attackers, or locals involved in dynastic struggles. Brandýsek, located 6 km from Budeč, further complicates the question of whether these populations were distinct or engaged in migration, cultural exchange, or kinship. This study integrates archaeological, isotopic, and genomic methods to shed light on Budeč's role as a power center and offers new insights into Early Medieval Bohemia.

Childhood Adversities Across Time: Integrating Skeletal and Genomic Analyses from Ancient Rome to the Middle Ages

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Keywords: Infant, Bioarcheology, Ancient DNA

The study of childhood in past populations has recently expanded, particularly regarding high infant mortality and the challenges caused by harsh living conditions such as high population density, poor hygiene, and repeated exposure to stressors. This project examines early-life adversities through morphological and MSCT (Multi Slice CT 128s) of the skeletal remains and ancient DNA (aDNA) analysis focusing on two archaeological sites: a Roman site (Casal Bertone, Rome, 1st–3rd centuries CE) and a medieval site (Leopoli-Cencelle, Rome, 9th–16th centuries).

At Casal Bertone 202 individuals were recovered, 53% of whom are non-adults. The most represented age group is 1–6 years. At Leopoli-Cencelle, 38% of 877 individuals were non-adults. A selection of 85 well-preserved children was morphologically analyzed, and the most represented age group was 7–12 years. While only dental stress markers were identified at the Cencelle site, hyperostosis, periostitis, and biomechanical stress markers are very common in the roman community.

Additionally, MSCT scan was performed, which allowed virtual reconstruction providing information on biomechanical stress and physical activity levels. DNA was extracted from 79 samples from both sites, and preliminary screening sequencing results show good endogenous content.

This will contribute to more detailed investigations of complex genotype-phenotype correlations providing deeper insights into the biological impacts of early-life adversity. By focusing on whole-genome of individuals with stress traits that are high SNP-based heritability, using UK Biobank, we will utilize imputed genomic sequences to refine the risk loci correlations and provide deeper insights into the biological consequences of childhood stress.

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Omics Analyses Suggest Diversity within the Lowest Social Strata-Associated Individuals of Late Antiquity in Italy

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Keywords: Ancient DNA, Paleopathology, Pathogens

Late antiquity and the early medieval ages are associated with significant political and social changes, characterized by the fall of the Western Roman Empire and the arrival of foreign non-Romanized groups in Southern Europe. These processes also resulted in dramatic changes in societies in Northern Italy, where the Longobards established a kingdom. Little is currently known about the living conditions of the lowest strata of society at that time and how much several biological determinants, including sex or ancestry, may have been associated with inequality in these societies. The wealth of information from archeological research on this period allows us to study a case scenario of social stratification and how this affects the lives of past societies.

Here, we present an interdisciplinary work that combines archeology, faunal and isotopic analysis, genomics, metagenomics, and paleoanthropology to study hundreds of individuals from 12 archaeologically well-characterized sites in Northern Italy. The analyses have revealed diversity in the ancestry of these populations, and we pioneered the integration of metagenomic analyses with archaeological markers. Screening the samples for pathogens yielded five complete *Hepatitis B virus* genomes and one *Human betaherpesvirus 6B* genome, the latter of which is one of only six genomes of this species ever found in ancient individuals. We also used dental calculus to describe the ancient oral microbiome of 69 different individuals and identify newly described MAGs.

Similar methodologies can be implemented in the future to understand how cultural changes affected health status and social organisation in past societies.

Dietary Heterogeneity and Private Ownership in the Lower Yangtze River Valley During the Middle Neolithic

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Keywords: Dietary heterogeneity, Private ownership, Songze culture

In the Lower Yangtze River Valley of China, the Songze Culture (c. 6000–5300 BP) is when societal complexity emerged and rice cultivation became more intensive. There is now little direct evidence of human diets and dietary changes among the human population due to the poor preservation of bones. The isotope analysis (C, N) of 151 human and 100 animal bones was conducted in this study at the Xuecheng site in Jiangsu, China. This was the biggest sample size in the Lower Yangtze River Valley to date. The results demonstrate significant isotopic heterogeneity across individuals, suggesting that a variety of subsistence practices, such as hunting and fishing, gathering and/or rice farming, and substantial millet consumption, coexisted. There was significant isotopic variance among pig populations. Most significantly, the trophic level is linked to the isotopic spacing between the majority of humans and pigs in the same grave, which had different isotopic values. This strongly suggests that the pigs used for sacrifice were grown in households. Therefore, our study offers the first concrete proof of a customized animal-raising industry, indicating the existence of private ownership. The beginnings and growth of private ownership in China, particularly in the Lower Yangtze River Valley, were examined under the theoretical framework of privacy.

aDNA and Bio-Molecular Research on Early Islamic Stained Textiles from the Negev Desert Along the Ancient Trade Routes of the Silk Roads

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Keywords: aDNA, Organic residues, Textiles

The 2022 excavation at the small village and waystation Nahal Omer (6th -8th centuries CE), an open-air site in a hyper-arid environment located in the Negev Desert of Southern Israel, unearthed a striking amount of organic material evident of local and long-distance trade networks: basketry, ropes, date palm, papyri, leather artifacts, and thousands of textiles (made of silk, cotton, linen, and wool). Among the expansive textile assemblage excavated from refuse middens rich in organic materials, an unprecedented subsection of exceptionally well-preserved textiles contains stained fabrics with ink and dye, pronounced discolorations and, most notably, visual markers resembling blood and traces of human body remains. Each of the stained fabrics from various garments, including a sock and sleeve, small pouch bags, and cut strips of various standard sizes provide new sets of information of the specific cultural activities or behaviors related to textile production, use and reuse, disposal, and preservation.

aDNA and ZooMS analyses provide tangible evidence to identify and characterize the origin of the stains and, ultimately, the use and purpose of the fabric itself by correlating specific residues with specific activities or behaviors (i.e. bandages, menstrual products, bags). These data sets provide new information about the biogeographic origins of the textiles and provide new insights on the uses of the disposed stained fabrics. The study also contributes to current wet-lab standard protocols by exploring sampling and extraction protocols specifically for organic residues on archaeological textiles to investigate the viability of such multidisciplinary approaches in future textile and bio-molecular research.

Reference

Bar-Oz, G. *et al.* Treasures in the trash mounds of Nahal 'Omer: An Early Islamic village in Wadi Arabah, Israel. *Carmel* 3, 1-24 (2024).

On-Site Plant-Wax Biomarker Analysis for Assessing Soil-Organic-Matter Preservation in Archaeological Contexts: A Case Study from Southern-India

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Keywords: Human-environment relationship, Proxy-integrity, Plant-wax biomarkers

Stable carbon isotope ($\delta^{13}\text{C}$) analysis of soil organic matter (SOM) from archaeological context is commonly used to reconstruct the past vegetation and environmental conditions of that particular site. However, the accuracy of $\delta^{13}\text{C}_{\text{SOM}}$ as a proxy for past vegetation is affected by physical and chemical alterations and isotopic fractionation, leading to an increased focus on biomarker approaches that target specific organic sources. Our research at the Palaeolithic site of Jwalapuram in India demonstrates this issue, where plant-wax biomarker data indicate the breakdown of plant-derived lipids, casting doubt on the reliability of $\delta^{13}\text{C}_{\text{SOM}}$ for reconstructing past vegetation in this context. To further explore SOM degradation, we examined topsoil samples from central Andhra Pradesh, revealing varying degrees of SOM preservation across the area, which were closely linked to sedimentary and vegetation contexts. This study underscores the necessity of combining source-specific indicators, such as plant-wax biomarkers, with bulk measurements like $\delta^{13}\text{C}_{\text{SOM}}$ to address preservation biases and ensure accurate reconstructions of past vegetation, particularly in challenging or extreme conditions.

Sedimentary Ancient DNA at the Archaeological Site of Kvalvika in Lofoten, Norway

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Keywords: Sedimentary ancient DNA, Metabarcoding, Open-air archaeological sites

Archaeological science has recently seen a boom in sedimentary ancient DNA (sedaDNA) studies from caves and lake deposits, but so far few open-air archaeological sites have been studied. This study analyses sediments from the medieval residential site of Kvalvika in Lofoten, Norway. The coastal site was rapidly being lost due to erosion at the time of excavation. The rescue excavation over two seasons revealed a structure with a central oven, well preserved cultural layers and high amounts of faunal remains. Archaeological sediments from multiple features and profiles at the site as well as the natural sediments at the site were analysed for sedaDNA. The preservation of the cultural layers and faunal material made Kvalvika ideal for understanding how sedaDNA compares to the zooarchaeological and palaeobotanical record at the site and how it can be applied at other open-air sites with poor preservation of organic materials to better understand species presence and site use. The DNA results demonstrate a clear appearance of marine species in the medieval period throughout the site which agrees with the faunal record and further includes species not found in the faunal record. Domesticated plant and animal species are also present. Through successful metabarcoding of mammal, fish, bird, and plant DNA, the study further uncovers information hidden in these sediments that can contribute to archaeological interpretations about past lifeways and subsistence practices. The current challenges and future directions for the use of this method at archaeological sites will be discussed.

Applying the Bioarchaeological Analyses to Reconstruct Origins and Mobility of Human Populations in the Late Bronze and Early Iron Age Central Europe: Project Introduction

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Keywords: Ancient DNA, Gene flow, Bronze and Iron Age in Central Europe

During the Late Bronze Age and Early Iron Age, the widespread practice of cremation in Central Europe—characterized by the Urnfield phenomenon—has led to significant gaps in our understanding of the genetic history of the Lusatian, Knovíz-Štítary, and Hallstatt cultures. To address this gap, we have assembled a unique collection of human skeletal samples, primarily from biritual archaeological sites where inhumations predominate, located in Upper Silesia (Poland), and Moravia and Bohemia regions (Czech Republic).

Our aim is to assess the genetic backgrounds of populations associated with these cultures, gene flow between populations favoring inhumation practices. Kinship analysis among densely sampled sites will offer further insights into marriage patterns, social structures, and inheritance practices. To achieve these objectives, we employ a multidisciplinary approach, integrating ancient DNA (aDNA) analysis (160 individuals), radiocarbon dating, and isotopic measurements ($\delta^{15}\text{N}$, $^{87}\text{Sr}/^{86}\text{Sr}$, $\delta^{18}\text{O}$, and $\delta^{13}\text{C}$) (70 individuals).

In our report we will introduce the first results from sequencing screening of our sample set. The results provide new insights into migration patterns, gene flow, and population structure during the pivotal period in Central European prehistory. Our research aims to fill critical gaps in our understanding of the genetic and cultural dynamics of populations associated with the Lusatian, Knovíz-Štítary, Hallstatt cultures, and beyond, contributing to broader discussions about human mobility and cultural exchange in prehistory.

Biomolecular Humanities: Moving on and Beyond

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Keywords: Inter- and transdisciplinarity, Biomolecular humanities

‘It is not philosophy but the life sciences which are leading an epistemic change in our relationship to the human’ wrote Niklas Rose in 2013¹. Indeed, biomolecular methods, archives, technologies and datasets present a challenge to, and opportunity for, humanities scholarship and practice, and this is something that archaeology has vividly experienced over the last decades. This paper explains the concept of “biomolecular humanities” as an integration of approaches from biomolecular science and the human (and social) sciences, but instead of simply seeking an interzone between these disciplines, we see it as a praxis, research process, purpose, and technical methodology. The talk will focus on highlighting major challenges and perspectives of integrating and merging natural sciences and humanities touching upon epistemological, educational and institutional constraints and opportunities. We argue that the latter can aid us in moving beyond simplified science+humanities, to a paradigm change and practice that sees biomolecularity as theoretically and practically foundational, assimilating and evolving.

Reference

1. Rose, N. The Human Sciences in a Biological Age. *Theory, Culture & Society* 30, 3–34 (2013).

Plague and Other Infectious Diseases in Viking to Early Modern Sigtuna, Sweden

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Infectious diseases are not easy to detect in paleopathology. Long-lasting diseases such as tuberculosis, leprosy, syphilis, and brucellosis can leave visible traces on bones, but most infectious diseases do not. With the development of next-generation sequencing techniques, it is now possible to recover not only ancient human genomes but also those of the pathogens they harboured upon death, such as *Yersinia pestis*, the agent of plague.

Here, we focus on the impact of urbanization on infectious disease circulation in Sigtuna, Sweden, spanning 500 years from the Late Viking Age early urban center to the Early Modern town. Using ancient metagenomic screening, we reconstruct the pathogen landscape of Sigtuna to explore how urban growth influenced disease dynamics. Over 60 individuals were subjected to shotgun sequencing and screened for potential pathogens using aMeta, an ancient metagenomics workflow (Pochon *et al.* 2023).

As a general microbial screening pipeline, aMeta enables unbiased detection of both well-known and understudied pathogens. We detected Hepatitis B virus, leprosy, plague and *Yersinia enterocolitica*. Today, *Y. enterocolitica* primarily causes foodborne zoonosis, but transmission via close contact with cattle cannot be excluded in an early urban context.

Both *Y. pestis* and *M. leprae* underwent capture enrichment. Notably, we identified a plague outbreak affecting an adult and an infant buried together in one grave dating from c. 1465 to 1640 (calibrated C14). Phylogenetic analysis of *Y. pestis* indicates their closest strain was found in 1463–1475 Vilnius, Lithuania (Giffin *et al.*, 2020). Given both were Baltic harbor cities, plague transmission by water routes seems plausible.

Coastal or Continental Culture? The French Atlantic Coast during the End of the Neolithic Period

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Keywords: Ceramic use, Atlantic coast, Late Neolithic

Reconstructing ancient sea levels is tricky on the French Atlantic Coast because of the flat landforms: in some places it is difficult to determine whether we are standing on an ancient coastline or a few kilometres inland. In this region, acidity, limited sedimentation, and erosion hinder the preservation of archaeological and zooarchaeological data. Bio-organic materials are exceptional outside marshes, which limits any evidence of fishing or basketry (i.e.). However, several sites provide clear evidence of fishing or shellfish gathering, demonstrating their proximity to the seashore. Ceramics at these sites are difficult to study due to complex cultural mosaic, simple forms, rare decoration and high fragmentation.

A PhD study on the lipid content of a large series of ceramics (n=500) from 23 archaeological sites explore the lifestyles of the populations of the end of the Neolithic and the beginning of the Bronze Age (4300-1500 BCE), their relationship with the coastal territory and the exploitation of maritime resources. Main findings revealed strong ties to livestock (dairy and ruminant meat) and land use with beeswax, pine resin, and vegetable wax – which seems to reflect specific local practices. The rarity of aquatic biomarkers found in ceramics suggest that fish were treated differently, even though they were prepared on the site.

This study offer insight into the Late Neolithic maritime cultural facies of the Atlantic coast, in terms of environmental exploitation and site proximity to the barrier beach. The behaviours highlighted contrast to those known via ORA studies i.e. in Northern Europe or Japan.

Genetic Diversity and Population Structure of Xiongnu Period Individuals from the Bayanbulag Site, Mongolia

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Keywords: Ancient DNA, Xiongnu, Han

The Bayanbulag site, located in Nomgon sum, Umnugovi province, Mongolia, is an ancient city believed to date back to the Xiongnu period (end of the 3rd century BC to 2nd century AD). It features a square-shaped earthen wall and has been the subject of archaeological research since the 1950s. Excavations have uncovered artifacts, such as pottery, coins, and tools, linked to both Han and Xiongnu cultures. Initially thought to be a Xiongnu fortress, later studies suggest it may be Shouxiangcheng, a Han Dynasty border fortification. Notably, human remains discovered in 2011 indicate a battle scenario and mass burial, raising questions about the identities of those buried.

To address this, we conducted whole genome analysis on 15 individuals from the site. DNA was extracted from petrous bones, yielding high-quality data (92%–98% endogenous content, 2.8–6.8x coverage), enabling robust population genomic analysis. By comparing their genomes with published ancient and modern populations from Central and East Asia, we assessed their population structure and genetic affinities.

Our results provide key insights into the ancestry and genetic diversity of these individuals, determining whether they align more closely with the Xiongnu, Han, or a mixed population. By integrating biomolecular data with archaeological evidence, this research offers new genetic perspectives on the site's historical affiliation, demonstrating the role of ancient DNA in refining archaeological interpretations of during the Xiongnu period.

Before the Romans. Addressing the Interplay Between Genetic and Cultural Variation in Pre-Roman Italy

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Keywords: Ancient DNA, Italic people, Iron Age

Over the last decade, studies of the European Bronze Age and Iron Age have experienced a paradigm shift, driven by interdisciplinary approaches that integrate archaeological, linguistic, and genetic analyses to uncover groundbreaking insights. Ancient Italy offers a rich repository of archaeological and textual evidence, highlighting significant cultural and linguistic diversity prior to the Roman Era. In this study, we combine archaeology and genetics in a synergic interdisciplinary effort to characterize the genetic makeup of ancient Italic peoples during the Iron Age. To address the research focus, we employed a shotgun sequencing genetic approach to analyze 495 individuals dating to the first millennium BCE from approximately 50 archaeological sites across the Italian peninsula. We successfully obtained 306 low-coverage genomes with coverage >0.1 , which were imputed and merged with a reference panel of thousands of previously published Eurasian shotgun genomes. This dataset was rigorously filtered and analyzed using methods such as PCA and identity by descent (IBD-sharing) to provide an overview of genetic variability across Iron Age Italy. We argue that the obtained temporal and regional differentiation can be better understood when seen through the lenses of the ‘imagined community’ conceptual framework. Considering communities are fundamentally characterized by shared values, which ideological narratives make specific and unique in their own combination, we propose to analyse the temporal and spatial relation between the archaeological, linguistic, isotopic, and biomolecular data as the evidence of choices defining the way different pre-Roman populations perceived themselves.

One-Pot Reaction: Decoding the Chemical Transformations of Lipids During Cooking in Pottery

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Keywords: Organic residue analysis, Experimental archaeology, Thermal degradation products

The analysis of organic residues in archaeological ceramics provides a unique insight into past culinary practices. These lipid residues may undergo thermal and oxidative degradation reactions, producing more stable compounds which serve as important indicators of vessel use and past environments. However, their mechanisms of formation and the conditions favoring it are often poorly understood. Some chromatographic peaks even remain unaddressed or simply unidentified. In addition, these reaction products may resemble naturally occurring lipids (e.g., hexadecanedioic acid), thus introducing a potential for misinterpretation. In this study, we present the results of replicated thermal degradation experiments using individual lipid compounds heated with various metal oxides. Sealed ampoules under controlled atmospheric compositions were used to investigate the formation of thermal decomposition products (TDP). The experimental setup is designed to explore the pathways of fatty acid degradation while isolating variables such as carbon chain saturation, metal ions, availability of oxygen, and temperature. Preliminary results reveal a variety of TDP, some of which have not been previously reported in organic residue analysis studies. Their occurrence is dependent on the tested conditions, indicating that metal catalysis in particular plays a crucial role. Insights into identified mechanisms could allow us to interpret data obtained from archaeological pottery more reliably, expanding the scope of diagnostically valuable biomarkers, and distinguishing TDP and diagenetic markers. This research aims to explore some of the basics of ceramic composition in order to better understand the role of the ceramic matrix and the associated formation and distribution of TDP.

A Multidisciplinary Approach to Fragmented Bone from the Late Neanderthal Site of Cioarei Boroșteni (Romania)

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Keywords: ZooMS, Radiocarbon dating, Late Middle Palaeolithic

Due to advances in ancient DNA studies and recent archaeological discoveries, the coexistence between late Neanderthals and early *Homo sapiens* is now established¹. However, there is currently a limited understanding of when and where these groups met. To address this, we present new data from Romania, as part of the wider COEXIST project focussed on central and southeastern Europe.

Although Late Pleistocene human remains have been discovered in Romania, the chronological framework of this Palaeolithic occupation is still poorly understood, with many sites lacking reliable radiocarbon chronologies². Using a multidisciplinary approach, combining both Zooarchaeology by Mass Spectrometry (ZooMS) and radiocarbon dating, our aim is to harness data from unidentifiable bone fragments from the region.

Our main focus is Cioarei Boroșteni, a cave less than 100 km from the notable sites of Peștera cu Oase and Peștera Muierii³. We undertook a pilot study of 64 ZooMS samples and five ¹⁴C samples from the late Middle Palaeolithic layers J and K excavated in 1994-5. Initial results indicate excellent collagen preservation across both techniques, with 62/64 specimens identified through ZooMS. While there is a dominance of Ursidae, a range of prey species were also identified, such as Cervid/Saiga, *Bos/Bison*, and Caprinae.

This study explores the potential of these methods to refine our understanding of human presence and subsistence by re-analysing museum collections. These preliminary data represent the first step in gaining a more detailed understanding of late Neanderthal and early *Homo sapiens* coexistence in the Southern Carpathians of Romania.

References

1. Mylopotamitaki, D et al. *Homo sapiens* reached the higher latitudes of Europe by 45,000 years ago. *Nature* 626, 341-346 (2024)
2. Chu, W., Doboș, A., Soressi, M. Interactions in bones but not stone: Anomalous cultural transmission gaps in Romania's Middle to Upper Paleolithic Transition. *Quaternary Science Reviews* 329, 108546 (2024)
3. Carciumaru, M., Colan, M. The Middle and Upper Palaeolithic from the Cioarei Cave – Boroșteni (Romania). Târgoviște : Cetatea de Scaun Editura, ISBN 978-606-537-692-2, https://www.researchgate.net/publication/381551548_M_Carciumaru_E_E_Colan_TH

E_MIDDLE_AND_UPPER_PALAEOLITHIC_FROM_THE_CIOAREI_CAVE_-
_BOROSTENI_ROMANIA-1pdf (2024)

Genetic History of a Frontier Roman City During the Migration Period

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Keywords: aDNA, Viminacium, Late Antiquity

The Roman city of Viminacium was located in present-day Serbia, strategically positioned on the Danube and at the crossroads of the Balkan Peninsula, the Black Sea and the western part of the Roman Empire. It acted both as a commercial hub and a military stronghold, eventually developing into one of the largest cities in the northern provinces and becoming the capital of the province of Upper Moesia. During Late Antiquity, this region of the Roman Empire underwent profound political and social transformations. In Southeastern Europe, these changes were accompanied by increasing migration into the empire. For Viminacium, these developments had devastating consequences, ultimately culminating in the city's destruction by the Huns in 441. Viminacium was then rebuilt by Emperor Justinian, only to be sacked again by the Avars in 582. To study the history of the city beyond the available historical records, and to investigate whether the aforementioned changes had an effect on its demography, we generated more than a hundred new genomes from the Viminacium necropoleis, focusing on the 4th, 5th and 6th centuries. We document a significant shift in the ancestry of Viminacium in the 5th century, marked by a decline in genetic diversity with respect to the previous period, potentially reflecting the mutated policies of the Late Roman Empire or the effect of the Huns' destruction of the city.

Environmental DNA as a Tracer for Past Behavior

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Keywords: eDNA, Behavior, Forensics

Ancient Environmental DNA (eDNA) has been used to study past ecosystems, evolutionary change, ancient resource utilization and even human population dynamics. Ancient DNA from skeletal elements has dramatically reshaped our understanding of the past, but this DNA is limited to the location and time of death of an individual. In contrast, eDNA is deposited throughout the life of an organism, and under appropriate conditions can be preserved for hundreds of thousands of years. However, in many instances the source of this eDNA is unknown, clouding interpretation. To determine the conditions under which DNA is released into the environment, and as part of a practical course at the University of Leipzig, we investigated the deposition of eDNA in a real life context: in the offices and hallways of the Max Planck Institute for Evolutionary Anthropology. We recovered primarily DNA from humans and dogs, and found that this DNA is tied almost exclusively to extended physical presence. By comparing eDNA samples with DNA from individual dogs sharing an office, we found that eDNA can be used to reconstruct behavioral characteristics. This investigation suggests that eDNA can be used to reconstruct past behavior, and demonstrates that "experimental archaeology" projects will be useful for the interpretation of eDNA archives from archaeological and possibly other contexts.

Burials in the Caves of the Franconian Jura: Unravelling Genetic Diversity, Social Roles and Ritual Practices

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Keywords: Ancient DNA, Kinship, Iron Age

The Franconian Jura in Southern Germany is rich in caves containing archaeological finds and prehistoric human remains. The use of these caves as burial sites has long been debated: Do they represent regular burials or rather burials for selected individuals? Did they serve as burial sites for families, marginalized groups such as migrants or people with a different diet or pathological anomalies, or even victims of ritual or warlike violence?

To answer these questions, we integrated analyses of archaeological context, skeletal material, stable isotopes, radiocarbon dating and 100 palaeogenomes to compare individuals buried in caves with individuals from regular burials from grave fields. The individuals studied are chronologically heterogeneous, with the oldest being from the Mesolithic period, but predominantly date to the Late Bronze Age and Iron Age (1300-150 BC).

The genetic diversity is very high, both within sites and when grouped chronologically across sites. In the entire data set, only three cases of familial relationship (1st-3rd degree) were found, so there is little indication that the caves were used as family burial sites. When comparing individuals buried in caves and contemporaneous grave fields in the region, there are no clear differences in terms of sex ratio, genetic diversity and ancestry signals, and therefore no genetic evidence to suggest they did not belong to the population of the region or that they represent selected individuals.

Establishment of a New Power Center After Fall of The Avar Empire - Tracing Social Changes With Archaeogenetic Research

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At the turn of the 8th and 9th centuries, the Avar Khaganate, which encompassed the entire Carpathian Basin, collapsed. On its ruins, the Moravian Principality was established north of the Danube, the Croatian Principality south of the Sava, the remnants of the Avar Khaganate were pushed back east of the Danube, while the Danube and the Drava-Sava basin became part of the Carolingian Empire, its easternmost province, known as Pannonia. One of the latter's regional political and ecclesiastical centers was Mosaburg (Zalavár), founded around 840 by Priwina, who was expelled by the Moravian prince Moimir and made a Frankish vassal (made count of the Carolingian Empire in 847). He gathered his followers from 'everywhere' spanning the Carolingian Empire to the Byzantine Empire and their environs.

To investigate this transition, we studied biomolecular data from 350 individuals buried in five cemeteries between the 7th and 9th centuries CE, complemented by reanalysis of 678 isotopic samples. Our findings suggest that Mosaburg's development was not a simple act of foundation, but rather a complex formation of a completely different social and power structure.

Comparative analyses with neighboring settlements demonstrate clear differences in social structure and genetic ancestry, but also evidence of strong connections between chronologically divided communities, shedding light on the reconfiguration of social hierarchies and integration of local populations following the fall of the Avar Khaganate. Evidence also points to close biological connections stretching up to 300 km, revealing how people and ideas moved across the regions.

Methodological advances in biomolecular archaeology

Region-Specific Reference Panel for Genotype Imputation in Ancient Human Genomes from Eastern Europe

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Keywords: Genotype imputation, aDNA, Haplotype analysis

As sequencing technologies advance, more sequences are retrieved from ancient samples. However, due to the high degradation of these samples, obtained sequences result in highly fragmented and contaminated DNA. This issue, in turn, causes low genomic coverage and, therefore, makes it almost impossible to do diploid genotype calling on these ancient samples. Genotype imputation effectively addresses this challenge by inferring missing sites through the utilization of extensive reference panels.

Although this sounds straightforward to use, there are several aspects on which imputation accuracy depends. These include coverage limitation, usage of a representative (population- or region-specific), and a big enough reference panel that also sufficiently represents the rare variants. In this study, we first created a region-specific enriched reference panel by merging and phasing the available high-coverage genome sequences from the Estonian Biobank, Haplotype Reference Consortium, and The Thousand Polish Genome Project to be used for genotype imputation in ancient DNA samples from the Baltic region. We then cross-validated the newly created reference panel to ensure it can be used for imputation. This will be followed by evaluating the performance of enriched and “single-source” panels in genotype imputation accuracy for low-coverage ancient genomes from various archaeological contexts and locations around Europe, focusing on the Baltic region. Further, the enriched reference panel will be used to impute genotypes and perform haplotype- and allele frequency-based analyses to study the fine-scale genetic structure and its dynamics in Eastern Europe during the last two millennia.

Comparison of Different Methods of Decontamination and DNA Extraction from Fossils (Dental Roots)

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Keywords: Ancient DNA, Decontamination, Wetlab

Contamination of ancient DNA by modern or environmental DNA is a major problem in paleogenetics, increasing both project costs and sequence reliability. Methods have been developed to reduce the extent of this problem. The aim of this study is to compare several methods from the recent literature for decontaminating bone fragments and powder in order to increase the percentage of endogenous DNA and the complexity of libraries. Here, we focus on treatment with bleach (proven method in our lab), phosphate buffer applied with a 3-step temperature gradient (Essel et al.) and pre-digestion with EDTA/proteinase K buffer applied hot (Shapiro et al.) on ancient boar (*Sus scrofa*) and goat (*Capra hircus*) teeth. After extraction using the modified Korlevic' et al/ Dabney et al/ Roche method, DNA fragments are integrated into ILLUMINA-type libraries and sequenced on NovaSeq 6000 S4. After bioinformatics processing, it appears that the percentage of endogenous DNA is higher in bleach-treated extracts than in those treated with phosphate buffer. Similarly, the percentage of endogenous DNA is higher in extracts treated with phosphate buffer than those treated with pre-digestion. This was true for all extracts and species studied.

Influence of the Ceramic Matrix on Lipid Retention and Extraction from Archaeological Potteries

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Keywords: Calcium carbonate, Ceramic matrix, Lipids

Triacylglycerols, plant sterols, and terpenoids are valuable biomarkers in Organic Residue Analysis. They are generally well preserved within porous unglazed ceramics due to their hydrophobic and chemically stable nature, which help prevent leaching and microbial breakdown. The entrapment and retention of these residues are influenced by the ceramic matrix's porosity and composition and recent studies have highlighted the potential role of calcium carbonate, commonly used as a temper in archaeological ceramics, in lipid retention and extraction.^{1,2} The amount of calcium carbonate present could lead to differential recovery of lipids and thus bias the extracted lipidic profile.

Our project combines traditional mineralogical characterization methods (XRD, XRF, SEM-EDX, Hg μ porosimetry) with AFM-IR³, which allows for precise mapping of residues within ceramic matrices, to investigate the influence of the ceramic matrix on lipid extraction. Additionally, we are constructing a reliable model of the cooking processes in different ceramic matrices, in view of obtaining detailed temperature profiles. This will enable to work with a broader dataset and a better understanding of the thermal history of archaeological ceramics, further refining our ability to interpret artefact use and cultural practices.

References

¹Hammann, S., Scurr, D.J., Alexander, M.R. & Cramp, L.J.E. Mechanisms of lipid preservation in archaeological clay ceramics revealed by mass spectrometry imaging. *PNAS* 117, 14688–14693, (2020).

²Cayme, J-M., Vahur, S., Teearu, A., Oras, E., Leito, I. The impact of mineral composition on the yield and preservation of selected fatty acids in replicate archeological ceramics. *Journal of Chemical Metrology* 18, 95-113 (2024).

³Mathurin, J. et al. AFM-IR nanospectroscopy of nanoglobule-like particles in Ryugu samples returned by the Hayabusa2 mission. *Astronomy & Astrophysics* 684, 1-14 (2024).

Molecular Age Estimation of Rodent Remains as a Means to Date Late Pleistocene Sites.

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Keywords: Molecular dating, Late Pleistocene, Mitochondrial DNA

Absolute dating of artefacts and sites is one of the cornerstones of palaeontological and archaeological research. This enables the reconstruction of the chronology of evolutionary events, archeological cultures or faunal successions. Radiocarbon dating (^{14}C) and thermally or optically stimulated luminescence (T/OSL) are the most popular methods for absolute dating of materials of the Late Pleistocene age. They provide a relatively accurate ages for the organic samples and sediments. However, both methods have several limitations and cannot always be used.

An alternative or supplement to the above methods may be the molecular estimation of the sample ages based on their phylogenetic position. This method was previously used to estimate the ages of numerous Late Pleistocene human and animal remains. In the case of animal remains, analyses are based on mitochondrial DNA, and because of the relatively low mutation rate in large mammals, the results are of limited accuracy and resolution. Rodents which exhibit higher mutation rates owing to short generation times may provide a higher resolution and accuracy of age estimates. Their remains are also much more abundant on palaeontological sites than remains of large mammals. I will showcase the age estimates based on mitogenomic datasets of four rodent species widespread in Eurasia during the Late Pleistocene, from a range of sites such as Hohle Fels and Ranis (Germany), Łabajowa cave and Pietraszyn 11 (Poland) or Denisova cave (Russia) and discuss the applicability of this approach to date multilayer palaeontological sites.

Maximising Ancient DNA Recovery from Dental Remains: A Density-Based Pre-Treatment Method

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Keywords: Pre-treatment, Density separation, Method development

Density separation has recently emerged as a promising pre-treatment method to enhance the recovery of endogenous ancient DNA (aDNA) from petrous bone. This study evaluates its application to teeth, which differ chemically and are valuable reservoirs of aDNA. We developed a modified pre-treatment protocol tailored to the unique composition of teeth to assess its effectiveness compared to standard protocols without pre-treatment. Thirteen teeth samples from diverse spatial and temporal contexts were pulverized and density-separated into five intervals using a non-toxic heavy liquid. DNA extraction and library preparation were conducted on both pre-treated and untreated samples. Seven specimens yielded high read counts across all intervals and were further analysed. Our results demonstrate that lower-density intervals contained significantly higher proportions of endogenous DNA, with up to a 12-fold increase in recovery from pre-treated samples. Notably, the second density interval (2.10–2.20 g/cm³) consistently outperformed standard controls, yielding higher unique endogenous reads while preserving authenticity markers. This study highlights the potential of density separation as a resource-efficient pre-treatment protocol for teeth, significantly improving aDNA recovery while reducing time and equipment requirements. These findings broaden the applicability of density separation, offering a robust approach to enhance aDNA studies from dental specimens.

Investigating Inhibition in Ancient DNA Extracts from Bones and Teeth

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Keywords: Inhibition, aDNA, Library preparation

Inhibition, caused by the co-extraction of substances greatly reducing, misleading, or completely preventing enzyme activity, has been recognized as problematic in ancient sedimentary DNA extracts, allowing for the compilation of guidelines to reduce the effect of inhibition on downstream methods¹. However, for ancient DNA (aDNA) extracts generated from non-sedimentary contexts (e.g. bones and teeth), the presence and effects of co-extracted inhibitors during library preparation and the influence on next generation sequencing data have mainly been ignored.

We are currently investigating the presence of inhibitory substances in aDNA extracts from bones and teeth using a qPCR approach. Preliminary results show the presence of inhibitors in aDNA extracts from different ages, geographic locations, and skeletal elements, with some extraction methods being more prone to the co-extraction of inhibitors than others. These inhibitors can heavily affect library preparation to the point of complete failure. Since inhibition is concentration dependent, we varied the volume used as input for double-stranded library preparation. Although a reduction in volume affects the total amount of molecules available for library preparation as well as the conversion rate, in heavily inhibited samples it seems to be a viable approach that doesn't involve additional clean up steps.

Additionally, preliminary results show that inhibitory substances can still be present in the final library, influencing amplification during indexing.

Reference

¹ Heintzman, P. D., Nota, K., Rouillard, A., Lammers, Y., Murchie, T. J., Armbrrecht, L., ... & Vernot, B. (2023). The sedimentary ancient DNA workflow. In *Tracking Environmental Change Using Lake Sediments: Volume 6: Sedimentary DNA* (pp. 53-84). Cham: Springer International Publishing.

Exploring the Utility of Secondary Analyses in Ancient Pathogens Research: A Bioanthropological Perspective

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Keywords: Ancient pathogens, Secondary study, Ethical research

Ancient DNA (aDNA) analysis and other biomolecular methods in archaeological and bioanthropological research can offer novel insights into the dynamics of ancient diseases, such as pathogen evolution, human–pathogen–environment interactions, and contexts of disease transmission. However, a growing awareness among public stakeholders and researchers of the ethical imperative to preserve human remains wherever possible has encouraged the development of non- and minimally-destructive methods in biomolecular archaeology. Given these ethical considerations and the pragmatic limitations of time and resources when sequencing aDNA, secondary analyses present a potential alternate avenue for investigating questions about ancient diseases. To test the possibilities and limitations of such approaches, this exploratory study screened archaeological metagenomes published in the NCBI Sequence Read Archive (SRA) for evidence of ancient pathogenic content—namely, *Brucella*, the causative agent of brucellosis, a zoonosis whose presence in the archaeological record may offer insight into human behavioural ecology and past lifeways. Ancient samples containing the target pathogen from disparate archaeological contexts were identified and investigated with reference to the available archaeological and molecular data. Some key considerations in genetic data publishing and accessibility are reported, including opportunities for cross-disciplinary collaboration. This study engages an anthropological commitment to holistically integrating archaeological, bioarchaeological, palaeopathological, environmental, and genomic data. As bioanthropologists increasingly grapple with questions of consent, ethics, accessibility, and preservation in aDNA research, secondary approaches can maximise the utility of previously sequenced genetic data to creatively explore anthropological research questions about human health and disease in the past.

Optimization of Nanopore Sequencing Technologies for Ancient DNA

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Keywords: Nanopore, Methods, Methylation

Recent advancements in nanopore sequencing technologies have become increasingly accessible for ancient DNA (aDNA) research, offering the potential to greatly enhance our understanding of gene regulation and expression through methylation patterns in historic and ancient populations, both human and non-human. The comparison of modern and ancient methylation profiles would allow for exploration of and inferences about potential gene regulated adaptations in the past in response to rapid environmental fluctuations. Here, we utilize Oxford Nanopore's PromethION platform to evaluate the efficacy of nanopore sequencing for the recovery of methylation profiles from aDNA under three different conditions. Using archaeological bear samples (N=4) from the Aleutian Islands, Alaska (5500 cal BP – 2500 cal BP), three treatments were applied prior to sequencing – no treatment, partial uracil-DNA glycosylase (UDG), and full UDG – to assess their ability to mitigate A/T ligation issues and improve the quality of the data obtained. In addition, we sequenced two contemporary bears for comparative purposes. Our successful recovery of ancient methylation profiles highlights the potential of amplification-free sequencing to address the limitations of short-read methods, offering new avenues for exploring how ancient organisms responded to past climate fluctuations and other environmental pressures.

Rapid Palaeoproteomic Amelogenin Sex Estimation of Cattle Remains

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Keywords: Amelogenin, Sex estimation, Zooarchaeology

Amelogenin is an enamel-specific protein that is encoded on the sex chromosomes of many mammals; consequently, the X- and Y-isoforms of the protein differ in many mammalian species. These sequence differences can be exploited by mass spectrometry (MS) to provide palaeoproteomic sex estimation of human and many mammalian teeth. This approach presents some advantages over aDNA sex estimation (hindered by the relative instability of aDNA), and osteology (limited by skeletal variability between the sexes, and inapplicability to non-adults). To fully understand population-wide demographics, employment of rapid and cheap analytical techniques to enable sex estimation of large numbers of samples is essential.

Here, a 2.9-minute MS method is implemented, giving a total sample throughput time of less than 20 minutes per sample (including sampling, preparation and data analysis). This high-throughput method is employed on cattle teeth from all the skulls recovered from an Iron Age site in Yorkshire, England. Sex estimations were correlated with complementary analyses (such as age-at-death estimation and burial locations), revealing the role biological sex plays in the observed mortality profiles. Furthermore, this method is employed for the proteomic analysis of cattle enamel from Saudi Arabia. Extreme climatic conditions in Saudi Arabia preclude analysis of many biomolecules, but here we demonstrate the survival of amelogenin peptides from as early as the Neolithic. These two case studies demonstrate the power of a rapid sex-estimation method in combination with zooarchaeological analyses, revealing insights into human-animal interactions, as well as peptide survival in hot climates.

Ancient DNA and Protein Preservation and Transfer in Concretions Covering Human Remains

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Keywords: aDNA retrieval, Metaproteomics, New substrates

The preservation of ancient DNA (aDNA) and proteins is strongly influenced by environmental factors such as temperature, pH, moisture and soil chemistry. In specific environmental conditions, sediment can become adhered to the surface of human remains, potentially affecting the preservation of biomolecules in the bones they encase. In certain regions, concreted bones are regularly encountered by archaeologists, yet both the concretions and the bones they contain remain underexplored for DNA and protein content.

Therefore, we investigated whether sediment concretions could preserve ancient biomolecules. We used a combined aDNA and proteins extraction protocol to test ancient human teeth and the surrounding concretions, as well as non-concreted teeth and dental calculus from the same archaeological context. Concretions were viable for microbial aDNA and protein analysis, but proved poor sources of endogenous human aDNA, limiting their utility for population genetics. Importantly, oral bacteria like *Anaerolinaceae bacterium oral taxon 439* found in the tested concretions, revealed high phylogenetic affinity to genomes retrieved from the same site. Palaeoproteomics analysis revealed consistent human peptides across all analysed substrates. Additionally, specific peptides from the human oral microbiome have been recovered from teeth, calculus and concretions.

Thus, we show that concretions tend to form in conditions less favourable for human aDNA preservation but can preserve microbial aDNA. They are also suitable for ancient protein conservation. These findings suggest concretions could serve as supplementary sources of ancient biomolecules, particularly when substrates like dental calculus are unavailable.

Towards Personalised Bioarchaeology: Molecularly Imprinted Polynorepinephrine for the Detection of Amelogenin in Human Teeth

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Keywords: Amelogenin, Biosensing, Molecularly imprinted polymers

Amelogenins (AMG) are crucial for sexing archaeological human and animal remains, yet standard Mass Spectrometry analysis is often complex and costly¹. To address these issues, a simple and effective digestion-free protein extraction protocol from mg-sized enamel powder samples was established. LC-MS/MS enabled the characterisation and semi-quantification of modern and archaeological human enamel extracts, supporting the development of an easy-to-use, cost-effective biosensing tool for detecting AMG. We present an innovative approach to imprint mimetic receptors based on Molecularly Imprinted Polynorepinephrine able to detect and bind full-length AMG from standard solutions and AMG residues from enamel extracts. Expanding upon single Epitope Imprinting^{2,3}, an original "Finger-Imprint" (FI) approach was attempted by directly imprinting the whole protein extracts from human dental enamel. The biomimetic receptor was coupled with Surface Plasmon Resonance and Bio-Layer Interferometry platforms to assess imprinting efficiency, kinetic rates and affinity parameters of the system towards the target protein. The successful results prove the effectiveness of the FI-based biomimetic receptor, which was specifically tailored to the extract and requires a quick and simple preparation involving bio-compatible reagents.

References

1. Buonasera, T. *et al.* A comparison of proteomic, genomic, and osteological methods of archaeological sex estimation. *Sci Rep* 10, (2020).
2. Sestaioni, D. *et al.* Sustainable and effective reconditioning of SPR gold chips functionalized with molecularly imprinted polynorepinephrine. *Anal Chim Acta* 1321, (2024).
3. Ventisette, S. *et al.* An antibody-free bio-layer interferometry biosensor for immunoglobulin G1 detection in human serum by using molecularly imprinted polynorepinephrine. *Biosens Bioelectron* 271, (2025).

Rethinking Ancient Proteomic Analysis: Developing Valley Indexing (Vidx) for Complex Mixtures

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Keywords: Palaeoproteomics, Bioinformatics, Mass spectrometry

In palaeoproteomics, the analysis of complex mixtures, such as textiles, binders, and foodstuffs, is commonplace. When proteins from multiple sources or species must be disentangled, challenges arise, as proteins of the same family share significant sequence identity.

Under the principle of Occam's razor, current analytical pipelines typically assume that all peptides derive from a single protein source, assigning them to the most abundant protein. While this assumption may suffice for modern samples of known origin, it is problematic for ancient protein mixtures. Attempting to disentangle these signatures requires a fundamentally different approach.

I will introduce a new strategy Valley Indexing (Vidx), designed to address the complexity of ancient protein analysis. This approach challenges conventional assumptions, offering an alternative pipeline that accommodates the diverse and degraded nature of ancient proteomic data. Furthermore, it integrates the use of multiple software packages to enhance analytical flexibility and accuracy.

The presentation will outline Vidx's conceptual development over the past year and demonstrate its potential applications through case studies. While the software remains under development, this paper aims to provide a framework for rethinking ancient protein analysis and inspire further methodological advances in the field.

Evaluating Imputation Accuracy for Ancient Low-Coverage Domestic Cat Genomes

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Keywords: Genome imputation, Cat domestication, Ancient DNA

Recent studies on animal domesticates (e.g., dogs, cattle, donkeys) have shown that imputing ancient low-coverage genomes can achieve high accuracy, enabling finer-scale population genomic analyses based on haplotypes. However, these studies underscore the lack of a standard imputation strategy, as species-specific factors—such as genetic architecture, introgression from wild relatives, and reference panel composition—critically influence accuracy.

Despite being one of humanity's closest companions, the domestic cat (*Felis catus*) remains underrepresented in genomic research, leaving many aspects of its evolutionary history unresolved. Ancient and modern genomic datasets for both wild and domestic cats are sparse, with population allele frequencies often inferred from single or few individuals, resulting in potential biases. Addressing these biases requires haplotype-based approaches and thus a tailored imputation pipeline.

Imputing cat genomes poses several challenges, including the absence of a high-density genetic map essential for phasing the reference panel. Additionally, only three high-coverage genomes of *Felis lybica lybica*, the domestic cat's wild ancestor, are currently available. Including more genomes of wild relatives as well as ancient high-coverage genomes in reference panels has proven to enhance imputation accuracy. To enrich the reference panel, we thus generated novel modern and ancient high-quality genomes (>10X) of both wild and domestic cats. By constructing a fine-scale genetic map and testing various imputation filtering pipelines, we aim to establish a gold standard for cat imputation, enabling robust haplotype-based analyses. This will provide unprecedented insights into the domestication, adaptation, and evolutionary history of domestic cats.

Ancient DNA Preservation in Archaeological Sediment from Tropical Africa

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Keywords: Ancient DNA, Archaeological sediment, Africa

Research on ancient DNA (aDNA) in Africa has been limited by environmental conditions that hinder the preservation of archaeological remains, resulting in geographic biases. The use of archaeological sediment as a source of aDNA presents an unprecedented opportunity to overcome these challenges. Sediment is the most abundant material excavated from archaeological sites; this approach has the potential to unlock a profusion of samples from any location where humans once lived. Additionally, because all living organisms present at a site could have left traces of their DNA in the environment, aDNA from sediment could provide a comprehensive understanding of past ecological contexts. However, the preservation of aDNA in sediments from challenging environments remains poorly understood. Here, we explore the integrity and abundance of DNA preserved in sediment from tropical Africa. We present the results of our extraction and targeted sequencing of ancient mammalian mitochondrial DNA from over two hundred ancient environmental samples, dated from the late Pleistocene to the Holocene, collected at ten sites in tropical Africa, including some with prior evidence of aDNA preservation in skeletal remains. These sites vary in temperature, elevation, and environmental characteristics and include caves, rock shelters, open-air sites, and burials in Gabon (Central Africa), Kenya (East Africa), and Malawi (East Africa). Our data provides insights into the potential of aDNA research in challenging environments, expanding access to regions previously considered inaccessible. This study paves the way for further research representing human evolutionary history and past ecological interactions through ancient environmental DNA in Africa.

Comparative Approaches for Ancient Virus Detection

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Keywords: Ancient DNA, Bacteriophages, Ancient Microbiomes

Ancient DNA (aDNA) provides an invaluable window into past ecosystems, offering insights into microbial communities and their viral components, including bacteriophages. Detecting phages in aDNA samples is critical for reconstructing ancient microbiomes and understanding host-phage dynamics over evolutionary timescales. However, phage identification remains challenging due to degradation, contamination, and methodological biases inherent to ancient samples. In this study, we compare multiple complementary techniques for phage detection, including taxonomic profiling, reads mapping, and *de novo* assembly. Our findings reveal that taxonomic profiling enables the identification of broad viral categories, while reads mapping provides higher-resolution insights into which phages are present in the samples. *De novo* assembly proves essential for uncovering novel and fragmented phage genomes. These approaches, when used in combination, offer a robust and comprehensive framework for characterizing phage diversity and dynamics in ancient microbiomes, paving the way for new discoveries in aDNA.

Archaeobotanical Insights from The Roman Villa Rustica of Costigliole Saluzzo (Southern Piedmont): Crossing Macroremains with Metabarcoding Investigations

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Keywords: Archaeobotanical macroremains, Metabarcoding, Modern contamination

Archaeological plant remains of the Roman period have been widely obtained and investigated from several sites of Northern Italy¹, but less knowledge is available for settlements of its western border. In this work, some preliminary insights are given on the archaeobotanical heritage of a *villa rustica* (I-III AD) discovered in southern Piedmont at the periphery of Costigliole Saluzzo (Cuneo), a past strategic transport hub at the outlet of Varaita valley, investigated since 2003 and still under excavation². Abundant and well preserved, carbonized macroremains (grains, seeds, nuts) obtained from different parts of the villa (*pars urbana*, *pars rustica*, *pars fructuaria*) and adjacent constructions provide evidence of agricultural products of the rural settlement before its destruction by a momentous fire, and are well related with the discovery of cereal milling and wine plants. These findings are here compared with results from metabarcoding analyses on sediments from a putative sewage system, focused on plants, but also extended to fungal and bacterial diversity. Notwithstanding that some contaminations from modern pollens are clearly recognizable, OTUs retrieved from the archaeological samples (and not in controls from the ground layer) in part correspond to dominant macroremains, but also to taxa poorly or not represented by the macroremains. This pattern remarks the complementarity of the two approaches and some potentiality of metabarcoding in the absence of macroremains.

References

¹Bosi, G., Castiglioni, E., Rinaldi, R., Mazzanti, M., Marchesini, M., & Rottoli, M. *Vegetation History and Archaeobotany*, 29, 681-697 (2020).

²Elia, D., & Meirano, V. *Revue Archéologique des Alpes du Sud*, 2, 150-165 (2023).

AMPHY: A Phylogenetic Workflow for Ancient and Modern Microbial Genomes

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Keywords: Phylogenomics, Nextflow, Workflow

Whole genome phylogenetics has become a common tool to analyze microbial genomes – including commensal and viral and bacterial pathogens– from ancient and modern strains. Although different labs established somehow converging methodologies to perform this type of analysis, there is still a lack of open-source automated pipelines to this end. To fill this gap, we developed AMPHY (Ancient and Modern PHYlogenomics), a Nextflow workflow that enables the reconstruction of phylogenetic trees of microbial species of interest, starting with raw sequencing data–metagenomic or genomic– from ancient DNA or modern sources, and even assembled microbial isolates. The AMPHY workflow performs all necessary steps from raw data to variant calling, including adapter removal and quality trimming, mapping into a reference genome, merging of bam files from the same sample, duplicates removal and correction of ancient DNA damage. AMPHY also includes several strategies to filter mapped data (BAM files) and genotypes (VCF files) that are routinely used in ancient DNA research, such as, filters based on breadth of coverage and sequencing depth, clipping of terminal bases, exclusion of heterozygous sites or custom-defined regions, etc. AMPHY places SNPs into a reference genome backbone, retrieves coding sequences, and concatenates core genes estimated during the workflow. It then reconstructs phylogenies using maximum-likelihood. It also includes a phylogenetic placement option for low-coverage strains, optimizations to be scalable to hundreds of genomes, and results can be automatically exported to iTol for visualization. We will illustrate its capabilities with real examples from ongoing projects of our lab.

SALEM: An Integrated Software Application for Stable Isotope Data Analysis

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Keywords: Paleodiet reconstruction, Computational archaeology, Bioarchaeology

SALEM is the first computational pipeline designed for analysing stable isotope ratios for paleodietary reconstructions. The application is developed in R and has a tab-based interface that facilitates a consistent and efficient analysis process.

SALEM is compatible with isotopic data derived from both dentine and bone collagen, offering broad applicability across various sample types in paleodietary research. To ensure the precision and reliability of isotopic measurements, SALEM integrates analytical methods that assess uncertainty and the accuracy of the data, aligning with universally recognized standards for quality control in isotopic analysis. For comprehensive data analysis, SALEM includes a variety of statistical models, such as the Kolmogorov-Smirnov test and the Shapiro-Wilk test, in addition to a range of graphical visualizations for trend and variance analysis. The application provides methods for estimating weaning duration, incorporating data from both bone (WARN¹) and dentine (WEAN²) measurements.

To assess dietary proportions, SALEM comprises existing models such as the MixSIAR R package³, enabling researchers to apply mixing models to isotopic data. The results are stored in CSV and Excel formats, ensuring full transparency, data reproducibility, and facilitating subsequent analyses.

The primary objective of SALEM is to integrate diverse isotopic methods into a unified, reproducible workflow for a complete and full-fledged data analysis. SALEM is an essential resource for bioarchaeologists and ecologists as well as new researchers to the field aiming to investigate dietary processes in past populations.

References

1. Tsutaya, T. & Yoneda, M. Quantitative Reconstruction of Weaning Ages in Archaeological Human Populations Using Bone Collagen Nitrogen Isotope Ratios and Approximate Bayesian Computation. *PLoS One* **8**, e72327 (2013).
2. Ganiatsou, E., Souleles, A. & Papageorgopoulou, C. WEaning Age FiNder (WEAN): a tool for estimating weaning age from stable isotope ratios of dentinal collagen. *Archaeol. Anthropol. Sci.* **15**, 50 (2023).
3. Stock, B. C. *et al.* Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *PeerJ* **6**, e5096 (2018).

Multi-isotopic ($\delta^{44/42}\text{Ca}$, $\delta^{88/86}\text{Sr}$, $\delta^{66/64}\text{Zn}$) Analysis from Single Bone and Enamel Aliquot of Upper Pleistocene Mammals from Camiac, France

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Non-traditional stable isotopes have been increasingly studied in the past decades in the archaeological and anthropological fields for their potential as ecological and dietary tracer. Measurements of modern bone and tooth enamel show that calcium ($\delta^{44/42}\text{Ca}$)(1), strontium ($\delta^{88/86}\text{Sr}$)(2) and zinc ($\delta^{66/64}\text{Zn}$)(3) isotopes are good indicators of diet and trophic position. These three elements can be recovered and analyzed from one single sample of less than 10mg. Despite this advantage, a multi-proxy study has not yet been attempted whereas it would allow less destructive analyses of precious Pleistocene samples. The present study consists in analyzing calcium, strontium and zinc isotopes in fossil bones and tooth enamel of carnivores and herbivores from the Camiac cave (Upper Pleistocene, Gironde, France) excavated by one of us (ML; 4). Usually, non-traditional isotope studies focus on fossil tooth enamel rather than bone, as dental enamel is more resistant to alteration and natural taphonomic processes. However, bones are usually more abundant in archaeological sites and give access to late life information, which make them an interesting material to study. Our study aims at 1) developing a protocol of Ca, Sr and Zn stable isotope analyses from a single sample 2) testing the preservation of biological isotopic signatures of diet in bone and tooth enamel, and 3) comparing the variations of the three isotopic systems in a fossil ecosystem. Results obtained for the $\delta^{88/86}\text{Sr}$ are very similar to $\delta^{44/42}\text{Ca}$ results and they display differences between two groups of herbivores (ruminant and non-ruminant herbivores). It is the first time that $\delta^{66/64}\text{Zn}$ results have been obtained in fossil bones and they are very encouraging as Zn isotope ratios in bone and teeth mirror one another and show a good separation between herbivores and carnivores. The concomitant analysis of these three isotopic systems on the two most abundant mineralized tissues of the fossil record allow to separate herbivores following their digestive system and allow a good separation between different trophic levels. Thus, it highlights the importance of analyzing several isotopic systems in a single sample and shows encouraging results for the preservation of biological isotopic signatures in fossil bone. This new methodology will surely be of use to study paleodiet in ancient human populations with limited sampling possibilities.

References

1. J. E. Martin, T. Tacail, V. Balter, Non-traditional isotope perspectives in vertebrate palaeobiology. *Palaeontology* **60**, 485–502 (2017).

2. K. J. Knudson, *et al.*, Introducing $\delta^{88}/^{86}\text{Sr}$ analysis in archaeology: a demonstration of the utility of strontium isotope fractionation in paleodietary studies. *Journal of Archaeological Science* **37**, 2352–2364 (2010).
3. K. Jaouen, M. Beasley, M. Schoeninger, J.-J. Hublin, M. P. Richards, Zinc isotope ratios of bones and teeth as new dietary indicators: results from a modern food web (Koobi Fora, Kenya). *Sci Rep* **6**, 26281 (2016).
4. M. Lenoir, La préhistoire ancienne en Gironde: apport des recherches récentes. *Gallia Préhistoire* **42**, 57-84 (2000).

Modeling Ancient DNA Preservation in Sediments through Empirical Bayesian Kriging

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Keywords: Sedimentary ancient DNA, Geospatial analysis, Biomolecule preservation

Sediments are excellent reservoirs of ancient DNA (aDNA), harboring unique amalgams of organic matter and minerals to adsorb and preserve biomolecules over millennia. aDNA is a highly informative component of the archaeological record, providing a glimpse into past environments and the individuals who inhabited them. Careful spatial documentation of features and artefacts is crucial when studying any archaeological site, and using aDNA to profile biogeography across strata should be no exception. Empirical Bayesian Kriging (EBK) is a geostatistical interpolation method which uses simulations to predict continuous values between point coordinates. All kriging interpolation methods simultaneously evaluate relationships between multiple points, but EBK best mitigates prediction error by producing semivariograms tailored to individual datasets. We are using ancient mammalian DNA signals from hundreds of sediment samples collected in a grid pattern along a ~2.5 m² stratigraphic profile of the Middle/Upper Paleolithic cave site Tsagaan Agui in Mongolia to build EBK models. These models will demonstrate how chronological sediment strata and biogeochemical properties (e.g., pH, organic carbon content, soil particle size) spatially overlap with sedaDNA, allowing us to determine the precise extent of their association. Spatial reconstructions of aDNA belonging to specific taxa could be used to contextualize human and non-human animal activity or burial patterns; and if applicable, artefacts or skeletal remains can be assessed for their role in aDNA preservation pattern. This method aims to define the optimal environmental conditions for biomolecule preservation, which can be further strengthened by increased documentation of sediment properties.

DORIAN: A Damage-aware Genome Reconstruction Tool to Improve the Analysis of Ancient Samples

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Keywords: aDNA, Deamination pattern, Bioinformatics

Ancient DNA (aDNA) samples provide valuable insights when working on evolutionary studies. However, these samples typically show low amounts of genetic material, short fragment sizes and patterns of chemical alterations in the DNA sequence (called damage) making it challenging to work with them. For many functional analyses used in evolutionary studies, such as molecular dating or phylogenetic inferences, a reliable genome reconstruction of the sample under investigation is crucial. Missing or inaccurate data as is characteristic for aDNA samples, can lead to incomplete or erroneous genome reconstructions resulting in imprecise or even false conclusions. DORIAN is a tool designed to perform a genome reconstruction for ancient mitochondrial samples. DORIAN accounts for the sample-specific damage patterns by utilising this information for a damage-aware reconstruction. DORIAN offers three reconstruction modes that differ in how damaged positions are identified and handled. This results in different degrees of leniency with which DORIAN corrects a putatively damaged position, enabling users to select the mode best suited to their specific needs. The method works regardless of the host species of the sample, therefore making it applicable to a wide range of samples. DORIAN was validated using simulated data. We show that the genome reconstructions performed with DORIAN are improved compared to uncorrected reconstructions, therefore leading to more accurate and reliable results in downstream analyses.

Phylogenetically Informed Radiocarbon Modelling of the Second Plague Pandemic

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Keywords: Ancient DNA, *Yersinia pestis*, disease

Ancient pathogen genomes reconstructed from human remains offer indispensable insights into the origin and spread of diseases in the past. With over a hundred published genomes dating between the 14th and 18th centuries, the second plague pandemic in Western Eurasia is arguably the pandemic with the densest palaeogenetic sampling so far. However, for many samples only relatively broad radiocarbon dating intervals of 100-150 years are available. This hampers an association of palaeogenomes with the rich historical datasets for detailed reconstructions of the temporal and geographic spread of sublineages due to the rapid succession of plague waves, afflicting regions and cities multiple times within a century or even a decade.

Here, we present a novel methodology to use relative-chronological information embedded in phylogenetic trees for radiocarbon modelling to refine the dating intervals of a large proportion of available second pandemic *Y. pestis* genomes. Along with this new dating approach, we present newly sequenced plague genomes from eight archaeological sites in England, Estonia, the Netherlands, Switzerland and western Russia, covering outbreaks from the Black Death (1349) up until the Plague of the Great Northern War (1710). Through in-depth historical contextualization of the plague genomes and their refined dating intervals, we are able to discuss their probable associations with documented plague waves. New data can be integrated into the model in the future to improve the results, and the same methodology can be applied on other clonally evolving pathogens.

Tracing the Earliest Chicken in the Korean Peninsula Using Zooarchaeology by Mass Spectrometry (ZooMS)

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Keywords: Chicken, ZooMS, Prehistoric Korea

The earliest presence of chicken (*Gallus gallus*) in the Korean peninsula remains uncertain. It is believed that chickens in East Asia initially originated from southern China and spread through Korea to Japan. During the initiative period of domestication in Korea, roosters seemed to symbolize royal authority and power, rather than being a food source. However, the exact period and process of this spread have remained unclear in East Asia. This is associated with the difficulty of separating chicken bones from indigenous pheasant (*Phasianus colchicus*) bones due to their morphological similarity. Recent advances in Zooarchaeology by Mass Spectrometry (ZooMS) analysis have improved our understanding of bird domestication in East Asia. In this study, we aim to identify the earliest chicken remains in Korea using Zooarchaeology by ZooMS analysis. We analyzed collagen peptides from Phasianidae remains (n = 14) from the Gungok-ri site (dated to the early 2nd century BCE) using MALDI-FT-ICR mass spectrometry. The analyzed collagen peptide spectra were compared to modern Phasianidae species. The MALDI-FT-ICR technique enabled precise differentiation between chickens and pheasants at the species level. Our study represents the first biomolecular evidence of the earliest chickens in the Korean peninsula. These findings not only highlight the spread of chicken on the Korean Peninsula but also provide valuable insights into the cultural significance of chicken within East Asia.

Towards the Application of Ambient Ionization to Ancient Protein Analysis

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Keywords: ZooMS, Methods development, Ionization

Zooarchaeology by Mass Spectrometry (ZooMS) has become a mainstay of biomolecular archaeology since its introduction over a decade ago. While improvements in methods and applications have been extensive, they have focused mainly on strategies for sample preparation. On the contrary, studies associated with instrumentation selection/optimization have been far less common. Different mass spectrometers have different characteristics, and adapting methods can allow for more optimal experimental tailoring for specific research goals. In this study we conducted ZooMS analysis on a LTQ XL mass spectrometer (ThermoFisher) using a combination of nano-ESI and paper spray ionization. We present three different methods that have been modified for this instrumentation. Modern bones (human, deer, and pig) were used to innovate and optimize each method and collect important secondary parameters, such as sample damage, cost, and accuracy. These parameters of the proposed methods will be analysed in a blind study on archaeological materials. Although the singly charged peptides produced by MALDI-TOF analysis allows for easier spectral interpretation, paper spray ionization furthers the application of ambient ionization to ancient protein analysis. Ambient ionization allows for reduced sample preparation saving time and cost of analysis; this opens the potential for in the field analysis of ancient proteins. Mass analysers that pair with these ionization methods also enable real time tandem mass spectrometry, providing peptide sequence confirmation alongside identification during analysis. The application of these instrumentations and ionization methods extends beyond ZooMS and can also be applied to lipids, metabolites, and other small molecules present in the archaeological record.

Insight into the domestication of cattle in the Neolithic Southern Levant

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Sex determination of modern and archaeological animal remains is crucial for understanding demographic patterns, mortality profiles, and domestication pathways. Osteological approaches, however, often face significant limitations due to preservation and morphological ambiguities. To address this, we developed an innovative protocol leveraging label-free quantification (LFQ)¹ of two unique Amelogenin-Y (AmelY) peptides, 'LRYPYP' ([M+2]²⁺ 404.7212 m/z) and 'LRYPYPSY' ([M+2]²⁺ 529.7689 m/z), exclusively found in male enamel. This method was validated on eight modern cattle (*Bos taurus*) of known sex, achieving 100% accuracy in sex assignment. Additionally, we applied this protocol to 27 Neolithic *Bos* samples from the Southern Levant, revealing sex ratios that suggest early cattle management strategies were primarily geared toward meat production rather than milk exploitation or hunting practices. Our findings highlight the potential of LFQ-based approaches in bioarchaeology, offering a robust solution for sex determination in ancient and modern bovine remains, particularly when dental tissues, which preserve better than bones, are available. This protocol not only enhances biomolecular archaeology methodologies but also contributes to understanding the complexities of Neolithic animal husbandry.

New Model for Estimating the Trophic Level in Mammalian Carnivores Based on Bone Collagen Individual Amino Acids Nitrogen Stable Isotopes

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Keywords: Paleoecology, Isotopic ecology, Trophic position

Estimation of the organism's trophic position within a food web is one of the most powerful implementations of stable isotopes in ecology. Currently, the most advanced tool is the difference of the nitrogen isotopic signal between 'trophic' and 'source' amino acids (AAs), in particular the glutamate and phenylalanine (Glu and Phe). Three parameters are needed: the Glu-Phe difference in a sample; the Glu-Phe shift between trophic levels (trophic discrimination factor, TDF); and the Glu-Phe difference at the baseline level of the ecosystem.

However, applying this method for fossil mammals was limited until now due to the lack of TDF for vertebrate collagen, which usually is the only available tissue in fossil assemblage. To address this concern, we designed a new methodological study. We analyzed the $\delta^{15}\text{N}$ values of individual Glu and Phe in bone collagen of two terrestrial carnivorans and their prey. We focused on recent wolf from the Białowieża Primeval Forest (eastern Poland), whose hunting preferences are well known due the intense ecological monitoring¹; and Late Pleistocene cave hyena from Perspektywiczna Cave (southern Poland), whose diet was studied through proteomic taxonomy of digested bone fragments². We found collagen-to-collagen Glu-Phe TDF in mammals lower than that determined previously in invertebrate proteins or in plant-to-vertebrate tissue systems³. This result allows for more accurate reconstruction of trophic position in ancient mammals, including hominins. We also propose a new way of the trophic level calculation, to minimize the assumptions and better anchor the model in the available isotopic data.

References

1. Jędrzejewski, W. *et al.* Prey selection and predation by wolves in Białowieża Primeval Forest, Poland. *J. Mammal.* 81, 197–212 (2000).
2. Krajcarz, M., Baumann, C., Bocherens, H., Presslee, S. & Krajcarz, M. T. Dietary preferences and collagen to collagen prey-predator trophic discrimination factors ($\Delta^{13}\text{C}$, $\Delta^{15}\text{N}$) in late Pleistocene cave hyena. *Quat. Res.* (2025) doi:10.1017/qua.2024.43
3. Chikaraishi, Y., Ogawa, N. O., Doi, H. & Ohkouchi, N. $^{15}\text{N}/^{14}\text{N}$ ratios of amino acids as a tool for studying terrestrial food webs: A case study of terrestrial insects (bees, wasps, and hornets). *Ecol. Res.* 26, 835–844 (2011).

Isotopic Study of Burned Animal Bone: Potential and Limitations

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Keywords: Non-human animals, Isotopes, Burned bone

The isotopic study of burned non-human animal remains has lagged behind that of burned humans which limits our understanding of animal-human interactions in communities of the past. This is exacerbated by the fact that animal remains are encountered frequently in burned form –in domestic, mortuary, or ritual contexts. Crucially, there are now several strands of enquiry available to push this research further. This poster presents new data from three different isotopic research avenues: a) strontium isotopes and concentrations ($^{87}\text{Sr}/^{86}\text{Sr}$ and $[\text{Sr}]$), b) stable strontium ($\delta^{88}\text{Sr}$), and c) Lead (Pb) isotopes. Experimental Pb isotope data indicate that besides the volatility of the element and diagenesis, problems persist regarding effective removal of contaminant Pb from burnt bone. Conversely, $^{87}\text{Sr}/^{86}\text{Sr}$ and $[\text{Sr}]$ are excellent proxies to investigate animal mobility and by extension animal-human relationships, trade, land management, etc., as exemplified by new data from the Late Bronze Age fortification and burnt animal bone offering site (*Brandopferplatz*) Stätteberg, Germany. Stable $\delta^{88}\text{Sr}$ has the potential to allow for the closer identification of comingled burned animal deposits by distinguishing between poly- and monogastric individuals, as demonstrated with data from several archaeological sites. These latter methods especially have the potential to add to our fundamental understanding of animal interactions in past communities.

Optimisation of Lipid Recovery from Pottery Vessels Using Microwave-Assisted Extraction

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Keywords: Organic residues analysis, Microwave, Extraction method

During the last decade organic residue analysis has become widely used and it is now deployed in studies looking at the content of hundreds, if not thousands, of pottery vessels. This change of scale has been possible with the use of a higher throughput protocol relying on a one-step acidified methanol extraction/derivatisation protocol that improves both extraction time and sample yield in comparison to conventional ultrasound-assisted solvent extraction.

Nevertheless, this protocol has limitations: Esterified compounds (i.e. triglycerides, waxes) are broken down during extraction, and highly polar compounds such as alkylresorcinols (a cereal biomarker) and sugars are not efficiently extracted.

Alternative extraction methods (soxhlet, supercritical fluid extraction, pressurised liquid extraction, focused ultrasonic-assisted extraction or microwave-assisted extractions) have been developed to improve analyte recovery or processing time. But their application for organic residue analysis of archaeological ceramic vessels is still uncommon.

Here we investigate the utility of microwave-assisted extraction (MAE) for releasing lipids from pottery. We report a series of experiments to determine the impact on yield, compound distribution, and processing time and we generate a series of recommended parameters for potential users.

Multidimensional Scaling (MDS) Analysis, Spectral Decomposition and Coalescent Theory

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Dimension reduction methods, such as Multidimensional Scaling (MDS) and Principal Components (PCA), are widely used in genomic analyses to understand and visualise genetic diversity between individuals and populations. In 2009, McVean [1] analytically derived the relationship between the eigenvectors of an MDS and the split time between two populations.

In this study, we extend this analytical framework by computing the split times of an arbitrary number of populations from simple demographic scenarios. We analyse and simulate the sensitivity of the split times to sequencing error, bottleneck, sample size, sample size heterogeneity and number of SNPs. We apply our method to real human data, namely the 1000 Genome Project [2].

Finally, we develop a user-friendly, open-source, software, to easily compute MDS or PCA. The input is flexible and includes vcf, eigenstrat or plink formats while the output is designed to allow for a graphically appealing exploration of the population structure. More specifically, the output is a self-contained dynamic html page that allows subsampling of individuals, time periods or geographical areas within the MDS/PCA plots.

References

[1] McVean, G., 2009. A Genealogical Interpretation of Principal Components Analysis. PLoS Genet 5, e1000686. doi: 10.1371/journal.pgen.1000686

[2] The 1000 Genomes Project Consortium (2015). "A global reference for human genetic variation". en. In: Nature 526.7571, pp. 68–74. issn: 0028-0836. doi: 10.1038/nature15393. url: <http://www.nature.com/nature/journal/v526/n7571/full/nature15393.html>.

Experimental Protein Extraction for the ZooMS analysis of bones with poor biomolecular preservation

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Keywords: Collagen, ZooMS, Palaeoproteomics

ZooMS (Zooarchaeology by Mass Spectrometry) has been applied broadly in archaeological and paleontological faunal assemblages, enabling species identification, especially in highly fragmented assemblages where morphological diagnostics are insufficient. However, the method faces significant challenges in tropical and warm environments, where biomolecular preservation—including collagen, the target biomolecule for ZooMS—is quite poor. Acid-based extraction protocols are known to yield better results with low-collagen samples, as shown by previous studies, but they require demineralisation of bone, a step that can negatively impact collagen preservation and recovery. This study introduces AmPhos, a novel protocol for ZooMS that does not rely on demineralisation and instead focuses on protein precipitation. Adapted from a protocol designed for LC-MS/MS applications (Cleland and Vashishth, 2015), AmPhos was tested alongside three established ZooMS protocols to evaluate its effectiveness in maximising collagen recovery. Samples were selected from four geographically and temporally diverse sites: the archaeological sites of Sakai Cave in Thailand (~50 - 100 years old), Wadi Madamagh (~25,000 - 23,000 BP) and Ghwair I (~8,880 - 8,390 BP) in Jordan, and the paleontological site of Tsiotra Vryssi (TSR) in Greece (~1.78 - 1.50 mya). This study introduces the AmPhos protocol as an alternative to acid-based protocols in ZooMS research, and it demonstrates the utility of exploring a demineralization-free approach for bones with low protein concentrations.

Reference

1. Cleland, T.P., Vashishth, D., 2015. Bone protein extraction without demineralization using principles from hydroxyapatite chromatography. *Anal. Biochem.* 472, 62–66.

Optimising Imputation and IBD Segment Retrieval in Ancient Genomes

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Keywords: Imputation, Ancient DNA, IBD

Ancient DNA has revolutionised the study of genetic variation and population movements over time. However, significant gaps in ancient genetic data, alongside geographic and temporal variations, hinder fine-scale characterisation of genomic structure and relatedness between individuals and/or archaeological sites. In this study, we implemented the imputation pipeline proposed by Hui et al. (2020), involving a two-step approach: (1) Conducting genotype imputation on individual ancient samples using the GLIMPSE software with the 1000 Genomes Project reference panel. (2) Constructing a multi-individual dataset of confident genotype calls from GLIMPSE for input into Beagle5, enabling a second round of imputation and phasing to improve genotype accuracy and minimise missing data. To benchmark our imputation pipeline, we downsampled high-coverage ancient genomes, including shotgun sequences and 1240k target enrichment data. We investigated each step of the pipeline testing different filter thresholds, including genotype posterior probability after the imputation steps. Besides the missingness, as heterozygote sites pose challenges for imputation, we prioritise heterozygote sensitivity as our primary metric for assessing imputation quality. Our findings highlight that a second round of imputation enhances the quality of imputed genotypes for both shotgun sequences and SNP capture samples. Moreover, we evaluate the performance of the second imputation with the identification of Identity-By-Descent (IBD) shared fragments between individuals using refinedIBD software, including long fragments, by examining known parent-offspring pairs. In conclusion, this study sheds light on improving ancient DNA analysis methodologies offering insights into population genetics and evolutionary dynamics.

Deep Solvents for Deep time: The Use of Deep Eutectic Solvents for Archaeological Bone Proteins

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Faunal assemblages, primarily consisting of animal bones, are widespread in archaeological contexts. The widespread adoption of biomolecular analysis over the past two decades have resulted in the retrieval of valuable information from previously unremarkable bone fragments. While collagen has been the primary focus of such biomolecular analysis owing to its abundance and excellent preservation potential, the study of non-collagenous proteins has the potential to add new information regarding phylogenetic relationships, physiology and diseases, and perhaps expand the success rate of radiometric dating.

The extraction of bone proteins has seen significant technological advances in the past few years, especially the introduction of magnetic bead-based extraction techniques (e.g. the SP3 method)¹. However, studies comparing the efficacy of various protein extraction agents and the various methods available for peptide purification remain rare. More recently, the use of Deep Eutectic Solvents (DES) was introduced for the analysis of ceramic-bound proteins, resulting in a threefold increase in the extraction of such proteins².

In the present study, we test the efficacy of DES in the extraction of proteins from Pleistocene-age bones excavated in 3 Eurasian caves and compare it to the amount and type of proteins extracted using traditional EDTA and GuHCl-based approaches. Additionally, we test the compatibility of DES to magnetic bead-based approaches and explore alternatives to the C₁₈-based solid phase extraction tips for peptide desalting and purification.

We show that the expansion and revision of protocols is required if we are to move palaeoproteomics back in time and in more challenging environments.

References

1. Cleland, T. P. 2018. Human Bone Paleoproteomics Utilizing the Single-Pot, Solid-Phase Enhanced Sample Preparation Method to Maximize Detected Proteins and Reduce Humics. *J. Proteom. Res.* 17, 11, 3976-3983.
2. Pal Chowdhury, M. Makarewicz, C., Piezonka, H., Buckley, M. 2022. Novel Deep Eutectic Solvent-Based Protein Extraction Method for Pottery Residues and Archaeological Implications. *J. Proteom. Res.* 21, 11, 2619-2634

Identification of Immune Response Signatures Associated with *Mycobacterium Tuberculosis* Infection in Past Individuals

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Keywords: Immune response, Palaeoproteomics, Biomarkers

The interdisciplinary approach in archaeological sciences of integrating diverse methods to identify past host-pathogen interactions has been shown to enhance the reliability of findings and can provide critical support when the results of a single method are equivocal. Additionally, a single method can serve as a reliable foundation for validating the use of other methods in tandem. While specific skeletal lesions may indicate an active immune response in past individuals infected with tuberculosis, ancient DNA analysis serves as a tool to identify the presence of the bacterium *Mycobacterium tuberculosis*, indicating an infection. With the addition of ancient protein analysis, our study aims to identify a specific immune signature associated with active tuberculosis infections in human bone samples from the 19th and 20th centuries. In order to identify biomarkers specific to this mycobacterial disease, we analyze immune response proteins of past individuals with and without documentary, osteological, and genetic evidence of tuberculosis infection at their time of death. In addition to providing insights into the health conditions of past populations, these distinct immune signatures have the potential to be utilized for proteomic screening of other possibly infected individuals in subsequent studies. A reliable screening method could serve as a powerful tool to prioritize for the labor- and cost-intensive analysis of ancient DNA to resolve other research questions such as the evolution of this pathogen.

Leveraging Ancient DNA and Finnish Biobank Data to Infer Fine-Scale Population Histories

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Keywords: Ancient DNA, Biobank, Finland, Common era, Identity-by-descent

Advances in the imputation of ancient genomes have enabled the analysis of genomic segments that are identical by descent (IBD) between pairs of ancient individuals. These segments originate from a common ancestor in the relatively recent past and provide insights into population history over the last tens to hundreds of generations. In ancient-DNA studies, IBD segments are most often used to detect biological relatedness between individuals, and IBD-based pedigrees and community network analyses have become increasingly popular tools for reconstructing past social structures. IBD sharing can also be harnessed to study population history from modern genetic data, but few studies have integrated IBD sharing across modern and ancient individuals.

Here, we explore the applications of IBD sharing between ancient individuals and present-day Finnish Biobank donors to infer population history in the Common Era. Finland is a promising region for such applications: the country has a history of isolation and small population sizes, which have resulted in an excess of shared haplotypes. The Finnish population is well-studied in the genetic literature, and the national Biobank hosts large collections of genetic data with fine-scale geographic origin information. Moreover, Finnish ancient-DNA studies are naturally limited to the Common Era due to the poor preservation of organic material in acidic soil. We present ancient genetic data from Early Medieval to pre-modern Finland and show how region-specific reference data can enhance the analysis of recent population history.

The Impact of Heating on Bone Protein Preservation and its Implications for Palaeoproteomics

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Keywords: Palaeoproteomics, Zooarchaeology by Mass Spectrometry, Fourier Transform Infrared Spectroscopy, Burned bone

In the field of palaeoproteomics, there is a general assumption that ancient proteins do not preserve in burned materials, due to the susceptibility of proteins to degradation at high temperatures. However, the successful recovery of ancient proteins from archaeological burned bone specimens has already been reported in a handful of studies. While many studies have focused on the structural, mechanical, and chemical changes in bone upon heating, investigations into the degradation and recovery of bone protein from heated bones are more limited. The only study to date that included a proteomics approach to address this question was conducted by Fellows-Yates¹, who used Zooarchaeology by Mass Spectrometry (ZooMS) to analyse experimentally burned bones. Since then, new extraction methods and analytical workflows have been developed, that have yet to be tested on burned bones. In this study, we tackle one main question: Is it possible to extract and identify proteins from burned bones, and, if so, until what temperatures? To address this question, we experimentally heated modern pig rib fragments in temperature increments up to 750°C. Fourier Transform Infrared Spectroscopy (FTIR) is used to assess structural and chemical changes to the bones. ZooMS and Species by Proteome INvestigation (SPIN) are then applied to qualitatively assess the degradation of proteins at different temperatures. Here, we present the preliminary results of our study that provide a deeper understanding of the impact of heating on (ancient) protein preservation in bone.

Reference

¹Fellows-Yates, J. `It will not be possible to use Zooarchaeology by Mass Spectrometry (ZooMS) to identify species in samples of cremated bone that have been burnt higher than 155°C. ` Bachelor thesis, University of York (2013).

Exploring Malformation in an Egyptian Foetus Mummy with Multi-Omics

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Keywords: Biomolecular Archaeology, Human remains, Multi-Omics

Our understanding of ancient Egyptian mummification is primarily based on the study of adult human mummies. However, recent research has uncovered human fetuses within raptor-shaped sarcophagi. A small lump of unknown material discovered during the conservation of a falcon-shaped mummy containing a human foetus at Maidstone Museum provided a unique opportunity to investigate biomarkers potentially linked to this unusual mummification practice.

A multi-omics approach—combining GC-MS, lipidomics, metabolomics, and proteomics—was applied to analyse the lump and samples from cartonnage. Lipid analysis identified saturated fatty acids, very long-chain fatty acids, and cholesterol degradation products, indicating the use of animal—and plant-based embalming materials. The presence of dehydroabietic acid and its oxidation products further supported artificial preservation.

Metabolites unique to the lump included glycerol and glycerol-3-phosphate (markers of well-preserved tissues), along with GABA and glutamic acid (critical to mammalian neural processes). Ceramides exhibited high intensity in the lump sample alongside sphingomyelins. Long-chain ceramides (C16:0, C18:0) are linked to apoptosis, stress signalling, and cardiovascular diseases. These ceramides, along with sphingomyelins, play broader roles in systemic health and disease processes known for resisting degradation. Proteomics revealed a unique protein profile dominated by collagen alongside immunoglobulin G and alpha-fetoprotein - biomarkers for preserved blood or plasma. Malaria-associated proteins were also detected.

A previous micro-CT scan of the foetus mummy revealed cranial and skeletal abnormalities. Our biomolecular results support these diagnostic observations, demonstrating the potential for multi-omics approaches to uncover insights into ancient embalming practices, congenital conditions, and diseases with modern relevance.

First Attempt to Apply Plant DNA Analysis to the Material Study of the Palm-Leaf Manuscripts

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Keywords: Palm-leaf manuscripts, Ancient plant DNA, DNA authenticity

Palm-leaf manuscript (PLM) production is a traditional practice in many South and Southeast Asian countries, yet it remains poorly documented. Various plants, often specific to regions, localities, or manufacturers are involved in the multi-step PLM creation process. Each step of the PLM production process involves close contact of the fresh palm leaves (merely of *Borassus flabellifer* L. and *Corypha umbraculifera* L.) with the other plants - from seasoning and boiling with various fruits and herbs to brushing (e.g. with grasses), oiling, inking and applying plant-based solutions for protection against insects. Sometimes, smoking with burned plant material and/or other worship practices are involved. Despite the known main steps, thoroughly documented practices of PLM creation are lacking as are lists of plants used regionally for PLM production and conservation. We applied a metabarcoding approach to reveal which plants were used in the production of five Indian manuscripts. About 100 plants were revealed. Although the studied manuscripts date from the 18th/19th centuries CE, we found that, apart from palm DNA, the presence of other extracted plant DNA was scarce. The DNA fragments were typically short and ultra-short molecules with extensive chemical damage that is typical of ancient DNA. Furthermore, the authenticity of the plant DNA remains largely uncertain, as it is unclear whether the plants whose DNA is present in small amounts were actually used in the PLM creation process or if this DNA represents contamination associated with the manuscript's creation process, even when it is not evidenced in the negative control.

High-resolution Oral Microbiome Analyses by Mapping Metagenomic Data on a New Curated Genomic Database of Oral Microbes

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Keywords: Ancient DNA, Oral microbiome

The oral microbiome situates at the interface between the external environment and the host, and influence both local and systemic health. Recent metagenomic studies have shed light on the taxonomic composition of the oral microbiome, but significant gaps remain in understanding its variation across multiple dimensions—including pangenomic, strain-level diversity, and encoded functions—as well as across different scales, from individual to population, and throughout temporal and geographical contexts. While *k-mer*-based approaches have been widely employed to taxonomically classify sequencing reads, their utility for more in-depth analyses at the above-mentioned levels is very limited. To move forward into this direction, we constructed a comprehensive and non-redundant database of oral microbial genomes that we used to map ancient and modern oral metagenomes. Our approach allows to capture genome-wide data from most species in the oral microbiome, which can be used to *i*) get better accuracy in detecting taxa, with strain-level resolution, *ii*) genotype hundreds of taxa simultaneously for evolutionary analyses (e.g., identify genes under selection), *iii*) perform phylogenomic and phylogeographic analyses, and *iv*) explore pangenome composition of each species and its genetic functional variation. Importantly, for the analysis of ancient oral metagenomes (dental calculus and birch pitch) our method showed a significant reduction of false positives and environmental noise, thus producing much cleaner oral microbiome profiles. Our approach represents a significant methodological advance to deepen our understanding of the oral microbiome's dynamics and composition, to investigate the evolutionary trajectories of its associated species, and its implications for human health and disease.

Evaluation of Resin Impregnation for Preserving Organic Residues in Archaeological Soils

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Keywords: Organic residues, Resin impregnation, Archaeological soils

This study explores the effectiveness of resin impregnation methods in preserving organic residues in archaeological soils, with a focus on enhancing the preservation of biomolecules such as proteins, amino acids, and fatty acids. Organic residues in soils provide valuable insights into ancient diets, plant and animal resource use, and ritual practices. However, these residues are prone to contamination and degradation, making their preservation a significant challenge. Existing methods like freezing and low-temperature preservation are often impractical in field archaeology. This research compares several preservation techniques, including room temperature storage, freezing, dehydration, and resin impregnation (both polyester and epoxy resins). The experiment uses soils containing degraded buried meat as a model system. After six months of aging, amino acid analysis, gas chromatography-mass spectrometry, and bicinchoninic acid assays are employed to evaluate the preservation quality of each method. Results show that epoxy resin impregnation significantly outperforms polyester resin impregnation and freezing in preserving organic residues, particularly in maintaining protein integrity and fatty acid content. These findings suggest that epoxy resin impregnation could become a key preservation method for archaeological sites lacking adequate refrigeration, contributing to more accurate analyses of ancient human activities. This study provides a practical solution to the preservation challenges in archaeological fieldwork and museum contexts.

Using MALDI-FTICR Mass Spectrometry to Enhance ZooMS Identifications of Pleistocene Bone Fragments Showing Variable Collagen Preservation

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Keywords: ZooMS, MALDI-MS, Pleistocene

Recent advances in high-throughput molecular analyses of collagen peptides, especially ZooMS (*Zooarchaeology by Mass Spectrometry*), have forced breakthroughs in the analysis of fragmented archaeological material. Despite these advances, the challenge of successfully analysing archaeological samples with poorer collagen preservation persists. This work examines the potential of two mass analysers, TOF (*Time of Flight*) and FTICR (*Fourier-transform ion cyclotron resonance*), and addresses how they can be used to optimise the ZooMS workflow.

Type 1 collagen (COL1) was extracted from 89 archaeological bones from the site of Le Piage (37–34 ka cal BP) in France. Three ZooMS extraction protocols were used, an acid-free buffer method (AmBic), and two methods of acid demineralisation (HCl and TFA). After analysing the specimens with MALDI-MS, we used bottom-up and PRM (*Parallel Reaction Monitoring*) LC-MS/MS, and MALDI-CASI-FTICR (*Continuous Accumulation of Selected ions*) to verify 26 less secure identifications.

Overall, 99 % of the samples could be identified to at least family level, with the rate of identification and precision varying by method. Despite challenges in detecting specific biomarkers with MALDI-FTICR—especially peptide A (COL1α2 978–990), which tends to be unstable—the high resolution of this method allowed the successful identification of more degraded specimens, including burnt bones.

Our work highlights the robustness of traditional MALDI-TOF for providing taxonomic identifications with low failure rates that is critical when processing large numbers of samples. MALDI-FTICR shows better potential for working with precious samples (e.g. artefacts) or degraded collagen. This study advances analytical peptide detection by optimising the ZooMS workflow in Pleistocene archaeological contexts, which often show variation in degree of preservation.

Distinguishing Hominin Species Using Proteomics: Potential and Limitations

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Keywords: Hominin, Proteomics, Evolution

Ancient genomics has significantly exceeded expectations and has led to an array of discoveries about human origins and migrations that would not have otherwise been known. Proteins bound to the hydroxyapatite matrix of bones and teeth can outlast DNA by hundreds of millennia, opening the door to similar molecular insight into the human story substantially deeper in time than DNA preserves. While we hope the performance of ancient proteomics will mirror that of aDNA to elucidate the origins of humankind, palaeoproteomics has thus far captured only a handful of evolutionarily informative polymorphisms, and its power to inform evolutionary discussions is yet undetermined. To ensure our use of the fossil record is responsible and productive, we set out to assess which questions can and cannot be addressed with this technique. By building a database that dynamically links genomic and proteomic data, we could simulate current state proteomics as well as different potential future advancements, and predict their power to distinguish between hominin species. We found that at the current state of the art, proteomics cannot reliably tell hominin species apart. Specifically, even the best ancient proteomes currently available cannot reliably discern neandertals from *Homo sapiens*. Here we will present the results of our simulations, define the edges of what is achievable with proteomics, and overview technological advancements that could increase the power of the method and allow it to reach its full potential.

Benchmarking Methods for Inferring Biological Relatedness in Ancient Datasets

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Keywords: biological relatedness, kinship inference, identity-by-descent

Understanding biological relationships in ancient populations is essential for reconstructing social dynamics, yet traditional anthropological and archaeological methods often face difficulties in identifying familial relationships. The rise of archaeogenetics has revolutionized our ability to reconstruct demographic changes, population structure and genealogical relationships, offering new insights into burial practices, familial structures, and societal organizations when integrated with osteological and archaeological evidence. Despite these advancements, challenges persist in ensuring the accuracy and reliability of archaeogenetic methods for inferring biological relatedness. Hence, this study systematically evaluates ten widely used tools for inferring biological relatedness from ancient DNA—Kennett 2017, TKGWV2, BREADR, KIN, READv2, correctKin, NgsRelateV2, lcMLkin, ancIBD, and GRUPS-rs—based on mismatch estimations, genotype likelihoods and/or identity-by-descent analysis. We apply these tools to a well-characterized Early Neolithic dataset from Britain ¹⁻³, analyzing 62 individuals, 10 of whom are part of an extended pedigree, providing a framework to test varying degrees of relatedness. Our results indicate that pseudohaploid-based methods have a higher accuracy than genotype likelihood-based approaches. However, the accuracy of most of them depends on coverage and the availability of a reference panel of unrelated individuals with a similar genetic background. While the inferred degrees of relatedness are generally consistent across most methods, many of them have constraints for accurately classifying distant relationships. Our work outlines the ideal use cases and limitations of each method, and our findings emphasize the need for using multiple complementary approaches to corroborate inferred levels of biological relatedness, ensuring robust kinship analysis in ancient datasets.

References

1. Fowler, C. *et al.* A high-resolution picture of kinship practices in an Early Neolithic tomb. *Nature* 601, 584–587 (2022).
2. Brace, S. *et al.* Ancient genomes indicate population replacement in Early Neolithic Britain. *Nat Ecol Evol* 3, 765–771 (2019).
3. Olalde, I., Haak, W., Barnes, I., Lalueza-Fox, C. & Reich, D. The Beaker Phenomenon and the Genomic Transformation of Northwest Europe. *Nature* 555, 190–196 (2018).

Unlocking the Potential of Formalin-Fixed Specimens for Ancient DNA Research

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Keywords: Methodological advances, Biomolecular archaeology, aDNA

Ancient DNA (aDNA) offers invaluable insights into the past of humans and their diseases, but its recovery remains challenging due to degradation, low endogenous content and often sporadic pathogen identification. Formalin-fixed specimens, historically used for preservation, represent a rich resource for aDNA investigations - containing often both: host and pathogen DNA. The vast number of formalin-fixed specimens available worldwide presents a unique opportunity to expand aDNA research. However, formalin fixation introduces cross-links and accelerates DNA fragmentation, making genomic analysis difficult. To overcome these barriers, it is necessary to refine DNA extraction, library preparation and enrichment protocols to collect the best possible data.

Here, we present an optimised, methodological framework for extracting aDNA from historical formalin-fixed human organs. Our approach optimises DNA recovery, single-stranded library preparation via the Santa Cruz Reaction, and in-solution capture for targeted pathogen DNA enrichment. By systematically addressing molecular damage caused by formalin fixation, we enhance the efficiency of library preparation and downstream bioinformatic analysis, enabling genomic reconstructions.

Our results demonstrate that formalin-fixed tissues can yield valuable genetic information when processed with tailored methods. This work highlights the importance of refining laboratory techniques for working with chemically treated historical specimens, unlocking new possibilities for studying past infections and human evolutionary history. By showcasing a viable strategy for leveraging formalin-fixed specimens' collections, we aim to engage in discussion and encourage further exploration of such as a rich but underutilized resource in biomolecular archaeology.

PCR-free Oxford Nanopore Technology Sequencing of aDNA and sedaDNA

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Keywords: Oxford Nanopore Technology, Ancient DNA, Methods advancements

Oxford Nanopore Technology (ONT) has recently enhanced its chemistry. Currently, it supports shorter DNA strands and has reduced error rates to a level comparable to Illumina, making ONT a viable option for ancient DNA (aDNA). Its portability and PCR-free protocols offer significant advantages for the aDNA field. We have advanced both laboratory and data analysis techniques to enable PCR-free sequencing of aDNA and sedimentary aDNA (sedaDNA) on ONT. First, we adapted a cell-free, PCR-free ONT protocol for aDNA. Second, we developed a capture method for PCR-free libraries, applied to aDNA from Roman petrous bones and sedaDNA from Satsurblia Cave, Georgia, and El Miron Cave, Spain, where prior studies indicated good DNA preservation. Our results confirm that capture of PCR-free libraries is feasible.

Finally, we adapted base ONT base calling methods to aDNA which contains cytosine (C) deamination to uracil (U), inferred via Illumina sequencing. ONT sequencing can directly identify U and DNA bases (C, T, A, G). However, current ONT base callers cannot differentiate all five bases simultaneously. To address this, we explored training networks using Incremental Learning (IL) and classical Anomaly Detection (AL) to distinguish U, C, T, A, and G. To generate the necessary dataset, we sequenced PCR-free aDNA libraries from Roman petrous bones and micro-synthesized 465,030 distinct oligonucleotides containing every possible base combination. These efforts lay the groundwork for robust, specialized ONT base callers for aDNA applications.

Comparing Bioaccessibility via UBM and Conventional Microwave Digestion for Sr Isotope and Elemental Analyses in Plants

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Keywords: Strontium, UBM, MC-ICP-MS

Strontium (Sr) isotopes and elemental concentrations are widely used as tracers of mobility and diet in archaeological and forensic studies, offering insights into past human behavior. The Sr isotopic composition of human teeth and bones reflects dietary intake and can be compared with environmental baselines, or isoscapes, which are often based on modern plant samples, making plants a critical link in tracing Sr sources.

This study compares two methods for extracting Sr from plant samples: the Unified Barge Method (UBM), a bioaccessibility protocol mimicking human gastrointestinal processes, and conventional microwave-assisted digestion using concentrated HNO₃ and HF. While the microwave method is designed for total Sr recovery, the UBM targets the bioaccessible fraction, representing the Sr likely to be absorbed and incorporated into human tissues.

This methodological comparison aims to evaluate whether rigorous total digestion might overestimate the bioavailable Sr fraction relevant for reconstructing dietary and environmental baselines. By simulating physiological processes, UBM could refine our understanding of how much Sr in plants becomes bioaccessible to humans, potentially aligning isotopic baselines more closely with observed Sr incorporation in human tissues. Such advancements are particularly relevant in archaeology and forensics, where accurate correlations between environmental baselines and human tissues are crucial for reconstructing mobility and dietary patterns.

Future work will assess the implications of these methods for Sr isotope studies and explore their potential to enhance our understanding of bioaccessibility, improving the precision of isotopic tracing for archaeological and forensic applications.

Improving Dietary DNA Identification from Ancient Dental Calculus Samples

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Keywords: Ancient diets, Paleometagenomics, Benchmarking

Insufficient clarity on ancient agricultural diet composition poses a challenge for understanding the magnitude of human dietary shifts since the Neolithic transition. Dietary analysis of ancient DNA (aDNA) fragments from dental calculus samples could elucidate detailed dietary composition information from past peoples. However, the recovery of ancient dietary DNA from metagenomic datasets is hindered by its low prevalence in contrast to microbial DNA (both from endogenous oral microbes and soil and laboratory contaminants), contaminated eukaryotic reference genomes, and cross-mapping between eukaryotic genome assemblies and prokaryotic sequences. Methodological advances to increase recovery of dietary aDNA and reduce the proportion of false-positive assignments are needed. Here, we generate a eukaryotic organelle genome database (DB) from NCBI's 2024 RefSeq and explore 18S metabarcoding as two alternative methods to increase the recovery of dietary aDNA from dental calculus. We assess performance of three taxonomic classifiers (KrakenUniq, Kraken2, and MALT/MEGAN) in accurately identifying dietary information from synthetic mock metagenomes that contain dietary sources and contaminants in different proportions. Using a combination of bioinformatics approaches (such as increasing DB k-mer size, using alignment-based tools, and DB masking), we then classify and reduce false-positive assignments, increasing classification precision. This research represents a marked improvement for dietary DNA analysis in ancient metagenomics datasets, with implications for detecting rare and low abundance species more broadly.

LOCUS: A User-Friendly Toolkit for Spatial Genetic Analysis of Ancient DNA

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Keywords: Genetic – Geographic correlation, Population structure, Paleogenetics

LOCUS is an easy-to-use software designed to incorporate spatial analysis into studies using ancient DNA (aDNA) data. While aDNA research has extensively explored temporal aspects like population divergence and gene flow, spatial dimensions have received less attention. LOCUS addresses this gap by allowing researchers to examine genetic-geographic correlations in ancient populations, helping to uncover how geography might have influenced genetic variation. The toolkit leverages f-statistics, widely used in aDNA research, to assess population relationships and genetic diversity. For spatial analysis, LOCUS incorporates statistical tests, including the Mantel test for genetic-geographic correlations, the Mantel correlogram for detecting nonlinear spatial patterns, and Moran's I for measuring spatial autocorrelation in univariate genetic measures like within-population diversity. To evaluate its effectiveness, we applied LOCUS to both simulated demographic scenarios and real-world datasets. These analyses demonstrated its ability to address diverse spatial questions, from reconstructing large-scale migration events like the Neolithic expansion to examining localized anthropological contexts, such as genetic patterns within prehistoric cemeteries. Overall, LOCUS enhances aDNA research by providing an efficient, automated approach to exploring the spatial aspect of human genetic variation, potentially revealing geographic features that shaped human dispersal and population history.

Analyses of Amino Acids in Dental Enamel from Modern African Mammals

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Keywords: Dental enamel, Amino acid analyses, Dietary reconstruction

Dental enamel is the hardest material in the mammalian body, consisting of approximately 96% inorganic matter. Within its bioapatite crystallites, intact amino acids are preserved, making enamel an excellent bioarchive. The mineral-bound closed system protects organic matter from chemical or thermal diagenesis, allowing long-term preservation of endogenous amino acids. Compound-specific isotope analyses of these preserved amino acids could potentially be utilised for paleodiet reconstructions, including the assessment of trophic level, and tracing energy and nutrient flows, such as differentiating between meat and plant intake.

We developed a method to purify amino acids from dental enamel during which the organic matter is separated from the inorganic components. Seventeen amino acids were quantified via high-performance liquid chromatography (HPLC). This method has been established using in-house enamel and synthetic hydroxyapatite standards. Here, we present amino acid data obtained from the enamel of modern African fauna, including primates, bovids, suids and felids. Preliminary results indicate no substantial differences in amino acid composition across taxa or dietary groups, suggesting neither taxonomy nor diet influence amino acid profiles in diagenetically robust enamel. Our method of purifying and quantifying amino acids can be used as a screening methodology for the integrity of organic material in fossil samples.

Future work will expand these results by incorporating more species of different dietary groups as well as compound-specific amino acid carbon and nitrogen isotopic analyses, improving understanding of paleodiets and ecological relationships in the fossil records.

Accurate and Robust Inference of the Pedigree Relationship Between Two Individuals Using aDNA

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Keywords: Kinship, Machine-learning, IBD

Understanding the genetic kinship between individuals buried in geographically localised archaeological sites is important for understanding the behaviour and cultural practices of past human populations. Several methods exist that help to quantify the degree of biological relatedness (e.g., 1st degree, 2nd degree) using ancient genomes. However, currently published approaches have yet to distinguish between the exact type of relationship beyond the 1st degree, for example differentiating between commonly identified 2nd degree relationships such as grandparent-grandchild and half-siblings. Establishing this information is of vital importance for helping to easily untangle complex pedigrees.

Here we present a new method designed to accurately predict the type of 2nd and 3rd-degree relationships, including those more biologically complicated such as $\frac{3}{4}$ siblings. This method examines shared IBD segments, utilising segment length, number, and proportion to distinguish relationship types. We also use machine learning to identify IBD spatial patterning to support classification further. We demonstrate the power to infer pairwise relationships by combining real ancient genomes and simulated data. Through our application of this method to previously published ancient genomes, we also report updated solutions to pedigrees manually reconstructed from degrees of relationship. This method provides an efficient and powerful new tool to increase resolution when reconstructing biological pedigrees.

Promises and Limitations in Reconstructing Ancient Methylomes

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Keywords: Ancient DNA, DNA methylation, Epigenetic clocks

The fast response of the epigenome to environmental conditions helped building the assumptions that past changes in cultures, climate, and lifestyles could have left a detectable mark in our epigenome. Advancements in the fields of palaeogenomics and epigenetics led to the development of the field of palaeoepigenomics which has recently emerged as a complementary avenue of evolutionary research. The development of deamination-dependent computational tools that infer cytosine methylation is responsible for current, and probably upcoming advancements, in the field and subsequently led to the methylome reconstructions of ancient hominins (Sapiens, Neanderthals, and Denisovans). Progress has been made towards evolutionary adaptive transitions in humans, and there are some studies that identified potential epigenetic differences related to diet and immune function between different human groups. With such advancements, the logical next direction to this was their ability in using, or rather developing, epigenetic clocks. These statistical models would help in having better estimates of age-at-death, that would eventually lead to better understanding the dynamics of ancient and/or extinct populations and reviving the field of palaeodemography. Here, we try to make use of the abovementioned advancements and the availability of full and well-covered ancient genomes to explore the possibility of developing a computational method to estimate age-at-death from ancient human genomes. We show that the development of ancient epigenetic clocks is constrained by the reduced granularity of DNA methylation inference, due to the reliance on broader genomic windows rather than a site-specific one.

References

1. Gokhman, D. *et al.* (2014) 'Reconstructing the DNA methylation maps of the Neandertal and the Denisovan', *Science*, 344(6183), pp. 523–527.
2. Gokhman, D., Malul, A. and Carmel, L. (2017) 'Inferring Past Environments from Ancient Epigenomes', *Molecular biology and evolution*, 34(10), pp. 2429–2438.
3. Liu, X. *et al.* (2023) 'DNA methylation-based profiling of horse archaeological remains for age-at-death and castration', *iScience*. Elsevier, 26(3), p. 106144.

Sugar Mystery from the Ancient Eggshells?

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Keywords: Glycoprotein, Glycan, Eggshell

Glycosylation, the attachment of sugar moieties (glycans) to proteins, is the most abundant and diverse form of post-translational modification (PTM) in vertebrates. Bird eggshells are composed of inorganic components, primarily calcium carbonate, and organic components, which include proteins and glycosylated proteins such as proteoglycans. We have demonstrated that proteoglycans are preserved in ancient specimens, and we hypothesize that the glycans attached to these proteins remain intact in archaeological contexts. Proteoglycans are a unique class of evolutionarily conserved glycoconjugates, characterized by the presence of glycosaminoglycan (GAG) chains covalently attached at one or more sites to a core protein. These glycan chains can exist in both sulfated and non-sulfated forms of repeating disaccharides. GAGs are known to play a pivotal role in the biomineralization process of eggshells.

We extracted and purified GAGs from bird eggshells, digested them with enzymes, labeled the resulting GAG disaccharides with a 2-aminoacridone fluorescent tag, and analyzed them using ultra-performance liquid chromatography. Our results revealed that chondroitin sulfate is the most abundant type of GAG found in all the bird eggshells analyzed. Among these, chondroitin 4-sulfate was the most highly expressed GAG disaccharide in most bird eggshells, except ostriches and chickens. Heat modeling assays indicate that GAG disaccharides remain stable even after prolonged exposure to heat, suggesting their robustness and capacity for long-term preservation. We successfully recovered GAG disaccharides from ancient ratite bird eggshells, demonstrating that glycans could serve as a novel class of biomolecules for studying archaeological and paleontological specimens in the future.

Reconsidering How We Can Use Developmental Growth Patterns in Teeth to Improve Resolution of Palaeodietary and Palaeoclimate Studie

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Keywords: Stable isotope analysis, Tooth enamel, Animal mobility

Sequential analyses of tooth enamel stable isotope values have provided valuable insight into patterns of ancient animal mobility and behavior. However, the conventional sampling strategy used in these studies does not closely follow the geometry of tooth growth development. As a result, the biggest challenge to interpreting these hand-drilled enamel samples (commonly spaced ~1mm apart) is that each sub-sample represents a different and unknown unit of time. This talk will present the results of a mathematical simulation aimed at testing the resolution of distinct hand-drilling strategies. The simulations map isotopic dietary inputs across two teeth (one sheep and one pig) following their daily growth patterns visible on 50µm thick ground-sections. Different seasonal dietary patterns are used as the 'true' input and these are then compared to outputs generated by distinct hypothetical drilling strategies. Taking into consideration both the secretory and the maturation stages of enamel development, the outputs of the models allow us to test hypotheses regarding the feasibility of detecting geographically specific dietary and seasonal patterns. The talk will illustrate the applicability of the model to archaeological research using examples from distinct agropastoral settings and discuss the benefits of this approach for (a) refining inferences of already-published sequential isotope datasets, and (b) informing future research design and planning.

Non-Destructive Radiocarbon Dating of Archaeological and Paleontological Bone

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Keywords: Radiocarbon dating, Bone, Non-destructive

Radiocarbon dating is crucial for placing artifacts and human activities in a chronological framework, with bone being one of the main materials being analysed. By necessity, bone dating is a destructive process, involving drilling and sawing prior to chemical purification and ¹⁴C measurement. While other methods, e.g., ancient DNA and palaeoproteomics, have developed non-destructive strategies for analytical bone/teeth, similar techniques have not been devised for radiocarbon dating. We present the results of an experimental, non-destructive, hot water extraction protocol that eliminates destructive cutting or drilling of bone. Samples of whole bone were heated in ultrapure (MilliQ) water for several hours. We tested known-age bones, as well as archaeological bones of unknown age and succeeded in extracting a collagenous material comparable to collagen obtained through destructive, HCl-demineralization. Amino acid spectra, stable isotope values and atomic C/N ratios were indicative of collagen. The extracted collagen was subsequently purified using ultrafiltration or XAD-2 resin and AMS ¹⁴C dated. The radiocarbon determinations obtained through hot water extraction were statistically identical to radiocarbon ages obtained by the traditional destructive method. Other than variations in colour, no macroscopic changes to the bone were observed.

This experimental method presents new opportunities for research on rare and irreplaceable archaeological and paleontological bones with unique artistic value and osteological traits that would be destroyed by conventional dating methods.

Beyond Collagen: Evaluating the preservation and recovery of low molecular weight bone proteins

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Keywords: Osteocalcin, Non-collagenous protein, Diagenesis

Fossil bones recovered from warm and humid climates often lack collagen and DNA, hindering radiocarbon dating and biomolecular analyses. To address these limitations, this study investigates osteocalcin (OC), a gamma-carboxyglutamic acid (Gla)-containing non-collagenous protein, as an alternative biomolecule for dating and proteomic studies in heavily degraded bone. OC's strict localisation within the mineral-phase of bone offers potential protection against environmental degradation, making it a viable candidate for radiocarbon dating, despite the challenges encountered in earlier efforts during the 1980s and 1990s. This research represents the foundational phase in the effort to radiocarbon date OC in degraded bones, with a focus on quantifying its preservation and determining the optimal starting weight requirements for reliable radiocarbon analysis.

OC peptides were separated by an online reversed-phase HPLC connected to a Quadrupole Orbitrap mass spectrometer. OC preservation was quantified using a targeted approach through BCA antibodies and sandwich ELISA. Samples included well-preserved Palaeolithic bone (e.g., Denisova Cave, Russia) and poorly preserved bones with no to minimal collagen content (e.g., Tabarja, Lebanon). Preliminary results from Denisova demonstrated the successful detection and quantification of OC, validating the applied methodologies.

Ongoing analyses focus on testing the hypothesis that OC preservation occurs independently of collagen degradation. Additionally, OC quantification aims to establish a baseline for its future application in radiocarbon dating. This work advances our understanding of biomolecular preservation in fossil bone and by assessing OC stability under extreme environmental conditions lays the groundwork for novel approaches in dating and molecular analysis of degraded remains.

Stature Variation in Early Medieval Bavaria: Insights from Skeletal and Paleogenomic Analysis

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Keywords: Osteological height, Polygenic height scores, Childhood stress

Human stature serves as a crucial biological indicator of health, nutrition, and living conditions in past populations. This study analyzes skeletal remains from more than 100 adults excavated from early medieval burial sites in South Bavaria, combining multiple lines of evidence to understand achieved adult height and its determinants. We determine stature or long bone measurements and investigate genetic contributions to height through genome-wide ancient DNA analysis. To assess environmental influences on growth, we document linear enamel hypoplasia, a dental marker of childhood stress episodes, e.g. caused by disease or malnutrition. Our multidisciplinary approach integrates osteological and paleogenomic methodologies, contributing to a broader understanding of the interplay between genetics, environmental conditions and socio-economic factors in shaping human stature. Moreover, the results contribute to the controversial discussion of living conditions in the Early Middle Ages, the so called “Dark ages”, but also shed light on potential health disparities among different population groups during this period.

Extended Enamel Database for Hominin Palaeoproteomic Data Analysis

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Keywords: Palaeoproteomics, Database, Hominin

In palaeoproteomics, using an appropriate protein sequence database plays an important role. A protein database assists in accurately reconstructing an ancient protein sequence through de novo sequencing and determines the sequence information identified and retained during phylogenetic analysis. Palaeoproteomic studies of dental enamel have mainly focused on analysing a set of core proteins ($n \approx 13$). However, a large number of additional proteins have been reported in protein extracts from dental enamel, and these potentially contain single amino acid variants (SAAVs) that are informative for the taxonomic assignment of unidentified hominin teeth. To explore this potential, we characterise protein sequence variation among 86 proteins reported in the dental enamel literature for global, present-day human populations (1000 Genomes Project and HGDP) as well three Neanderthals and one Denisovan. We catalogued the number of proteins containing unique SAAVs for Denisovans, Neanderthals, and modern humans. The addition of present-day human variation to the database led to the recognition that several SAAVs are shared between modern humans and archaic hominins - these would otherwise not be identified by relying on the human protein reference alone. A deeper analysis and enrichment of the present database will assist in improving palaeoproteomic analysis of hominin skeletal material.

Arctic Marine Ecosystem Reconstruction Using sedaDNA: A Comparison of Shotgun Metagenomics and Metabarcoding

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Keywords: sedaDNA, Marine ecosystem change, Metagenomics

Our knowledge of past marine ecosystems is currently mostly based on rare finds of bones in caves, along beaches and at archaeological sites, and on microfossils of protists found in marine sediments. Sedimentary ancient DNA (sedaDNA) provides the opportunity to study past fish and marine mammal occurrences in a wider spatial context and on a more continuous time scale than possible from fossil remains. Furthermore, sedaDNA methods can detect soft-bodied organisms, like zooplankton and algae, and can therefore be used to study the effect of climate change and anthropogenic influences on lower trophic levels that are often invisible in the fossil record. Metabarcoding is currently the most common method for generating marine sedaDNA data, but it is difficult to apply to poorly preserved or deep-time samples. Shotgun metagenomics provides a more robust alternative although bioinformatics analysis is more complex and limited by the availability of suitable curated genome databases. We analysed DNA fragments from 35cm-long marine surface sediment cores and tested two different sedaDNA extraction protocols, a standard method based on silica columns, and a custom protocol, optimised for marine sedaDNA. We then aimed to reconstruct marine ecosystem responses to Holocene climate change in this area, using both shotgun metagenomics and metabarcoding approaches. In addition, we tested novel bioinformatics methods to screen the shotgun metagenomics data for a wide variety of marine organisms at a genome-wide level. We detected a broad range of taxa from across the ocean ecosystem, with a notable strong and consistent detection of whale DNA.

Metabolomics Reveals Diet and Health Profiles of Two Individuals from Kurgan Burials Near the Silk Road City of Bukhara in Southern Uzbekistan (3rd-6th Centuries CE)

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Keywords: Metabolomics, Diet, Silk Road

Metabolomics is the latest *omics* method being adapted for archaeology. It analyzes metabolites, small molecular products of cell metabolism, whose chemical composition can be determined by mass spectrometry. While most archaeometabolomics studies so far have focused on identifying ancient organic residues, a less explored variation of this method investigates metabolites within the human skeleton. Metabolites derived from endogenous biological functions and disease and from exogenous sources such as food, medicine, and infectious diseases become trapped in nanoscopic niches in bone as it develops. By extracting exogenous metabolites we can theoretically map them to the specific plant species that humans were consuming as well as to specific diseases.

This poster presents a pilot study on untargeted metabolomics of the osteological remains from two 3rd-6th centuries CE individuals, who were buried in *kurgans* (burial mounds) located on the edge of the Bukhara Oasis in southern Uzbekistan. This region in antiquity supported an important Silk Road city, Bukhara, as well as steppe pastoralists. While traditionally individuals buried in the ancient *kurgans* are assumed to be nomadic pastoralists, our metabolomics study reveals that they were potentially consuming large amounts of cultivated grapes. Also figuring prominently in their metabolomic profiles are markers for wild celery, licorice, and honey, foods that are typically invisible in the archaeological record. We have also found indicators of inflammation. These findings demonstrate the exciting potential of archaeometabolomics for characterizing ancient diets and diseases. This poster also presents our analytical pipeline as well as the current challenges of this work.