Sampling strategies in bioarchaeology

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What is a sample?

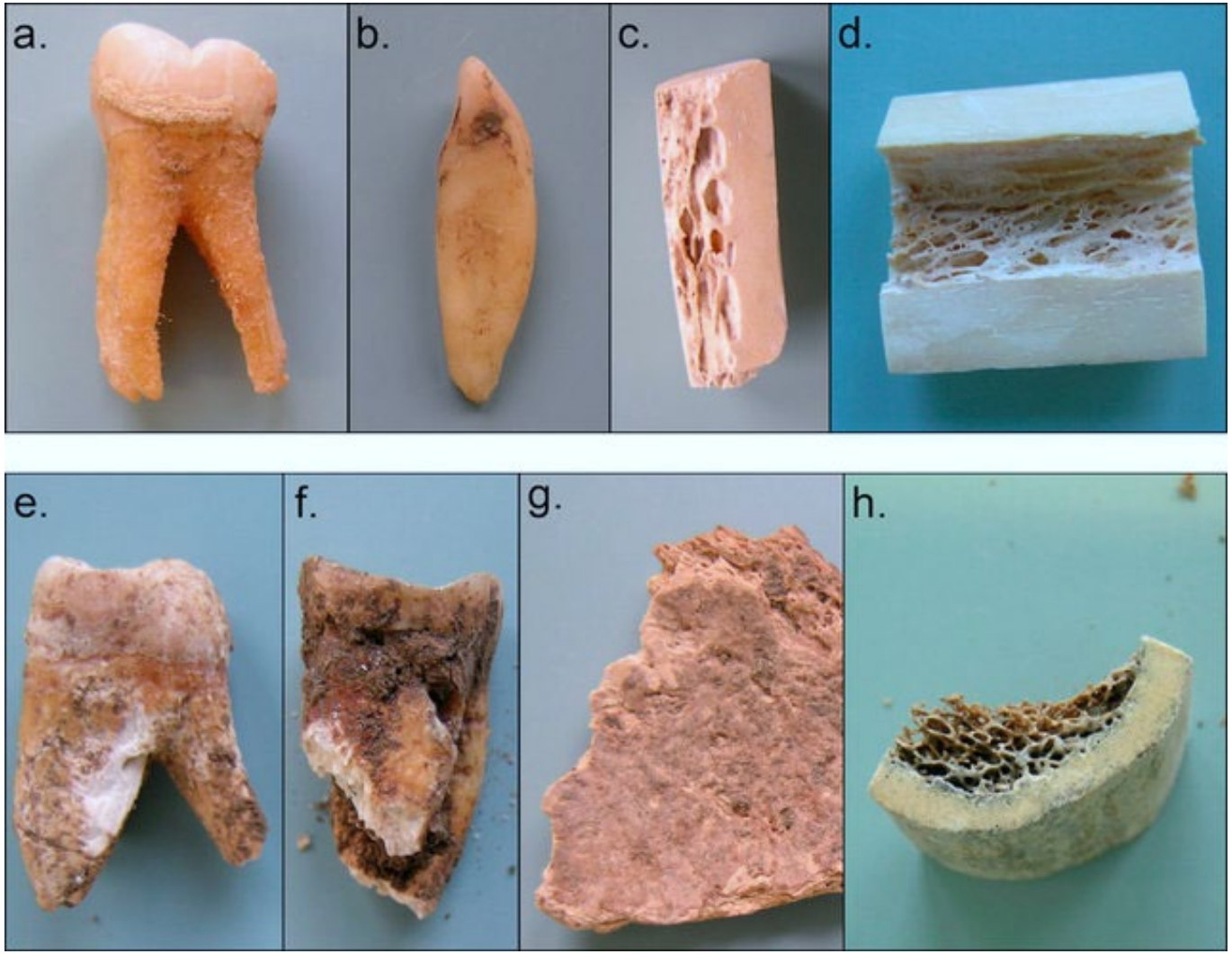
In bioarchaeology, a sample encompasses everything we work with, as we seldom or never have access to an exhaustive site or collection.

A sample typically refers to a group of human individuals, a specific specimen, a skeletal remain, or a portion thereof, that is selected for further analysis.

Today, the lecture's emphasis is on destructive analysis techniques.

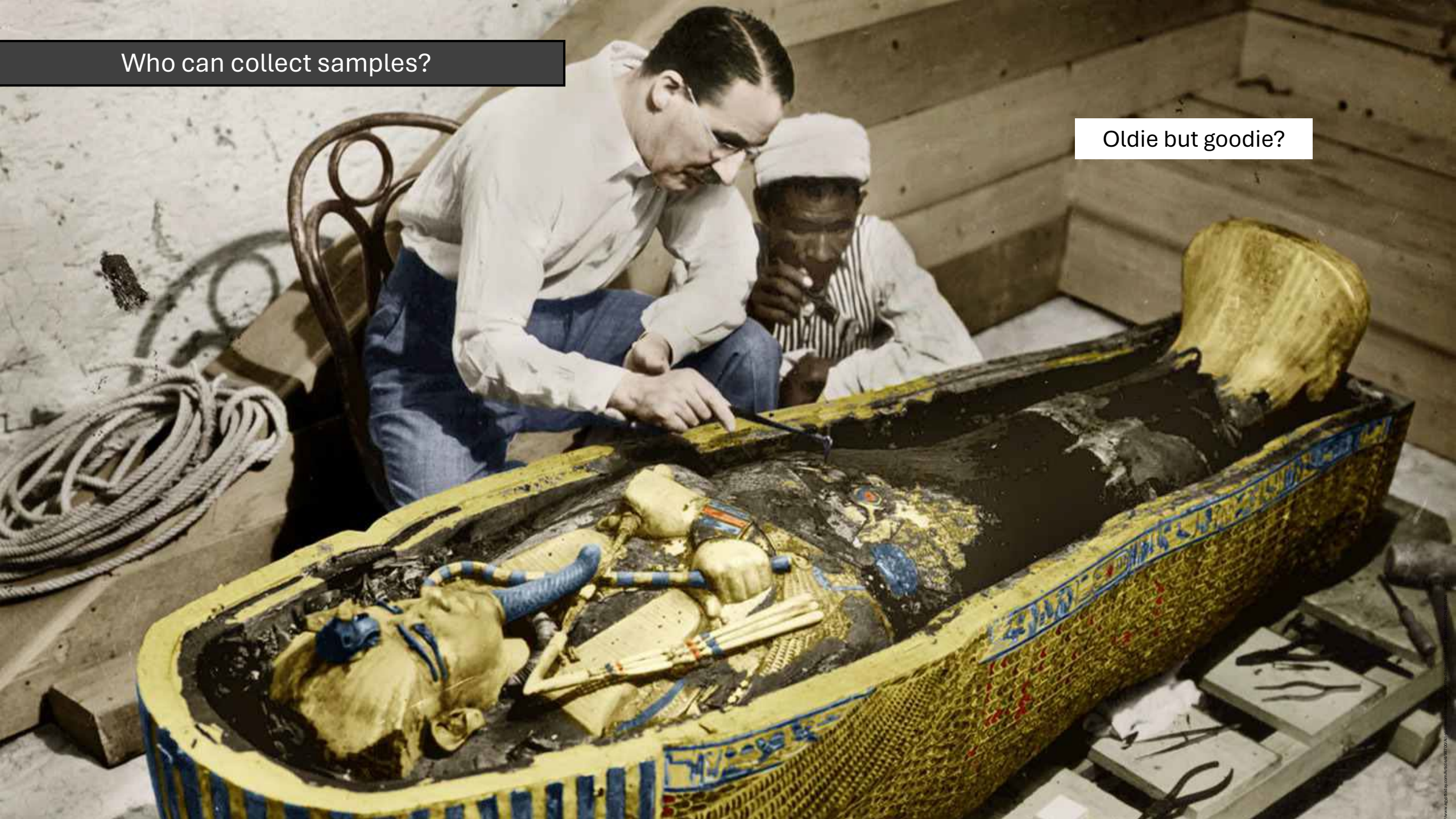
What is a sample?

In most cases, a non-processed sample resembles this:



Llamas et al. (2016)
https://doi.org/10.1080/20548923.2016.1258824

Who can collect samples?



Oldie but goodie?

Who can collect samples?

The classy way...


Who can collect samples?
The nerdy way...

Who can collect samples?

If trained…

When to sample?

Sampling can take place during excavation by focusing on a subset of the total skeletons being excavated.

Alternatively, sampling can occur during the post-excavation assessment by selecting a subsample of all the archaeologically excavated skeletons.

The approach chosen generally depends on the intended analysis and the preservation of the targeted samples.

If the analysis allow it, choosing skeletons for further study during the post-excavation assessment is often simpler and preferable because the evidential value of the site will be known.

Osteologists and other relevant specialists involved in the fieldwork project should collaborate in devising any sampling strategy.

When to sample?

Introducing a sampling strategy at the excavation stage can save costs, time and efforts at all subsequent stages, but great care is needed in designing and implementing it.

The sampling strategy should be tailored to the specific context, objectives and purposes of the excavation project.

Sampling for the sake of sampling should be approached with careful consideration.

Sampling in the field might be crucial for minimizing contamination and retrieving certain information.

https://moundsland.com/app/uploads/2018/04/8-Correct-numbering-and-labelling-of-bag-contents-is-essential.jpg

What to sample?

Might be sample
Not only human remains…

What to sample?

Significant considerations arise before conducting destructive sampling of human remains.

Can the research question(s) be addressed using non-destructive techniques? Destructive sampling should only be contemplated if this is not so.

Any program of destructive analysis on human remains should take place within a planned research program and should have a realistic prospect of producing useful knowledge.

If the feasibility of a technique is questionable but it is nevertheless deemed worthy of further investigation, consideration should be given to conducting a pilot study on a small number of samples before permission for a full program entailing destruction of larger amounts is given.

Only the quantity of material considered necessary to address the research questions should be taken as a sample. Any material removed but not destroyed during analysis should be returned to the collection.

https://s39671.pcdn.co/wp-content/uploads/2023/04/lXksMayOW0MR-Safe-and-Sound-Social-Media-Ethics-in-a-Changing-World-1.png

What to sample?

Significant considerations arise before conducting destructive sampling of human remains.

The location in the skeleton from which the sample(s) is taken should be carefully considered. For example, avoid sampling from areas of known osteological landmarks as this will reduce the information obtainable from the collection by future workers. Unless the study is specifically of diseased bone, sampling from pathological bone should be avoided.

All sampling should be fully documented so future researchers will know what has been taken.

The skeletal element sampled should be fully recorded and measured prior to sampling. Under some circumstances (e.g. if the skeleton is intended for museum display) consideration should be given to producing a cast or scan and 3D print of parts which will be damaged or destroyed.

How to sample?

Skeleton selection strategies typically include four main approaches.

Random selection: It avoids the introduction of additional bias into the data but prevents targeting the more osteologically or archaeologically interesting skeletons.

Selection of burials by osteological information content: Well-preserved, complete skeletons generally have a greater osteological information content (sex, age, etc.).

Selection of burials according to osteological features of interest: Skeletons might be selected because they show certain pathologies or they are a specific demographic subgroup (e.g. children).

Selection of burials according to archaeological aspects: Skeletons may be chosen based on dating or grave goods for investigating mortuary practices.

Methods

Ancient human DNA

Ancient pathogen DNA

Parasite analysis

Radiocarbon dating

Stable isotope analysis

Ancient human DNA

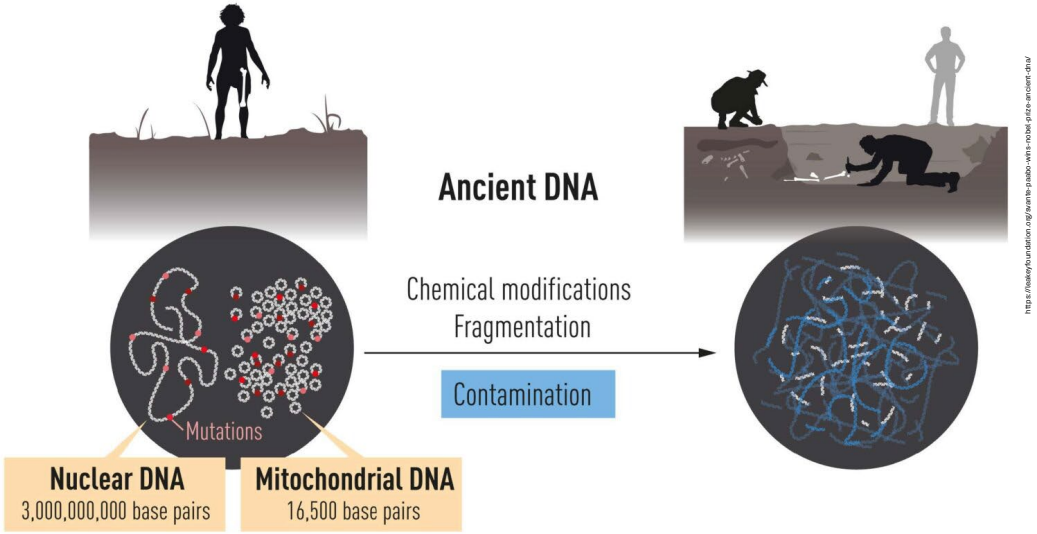
DNA, the carrier of an organism's genetic blueprint, resides within the organic matrix of bones and teeth.

DNA is not present in dental enamel!

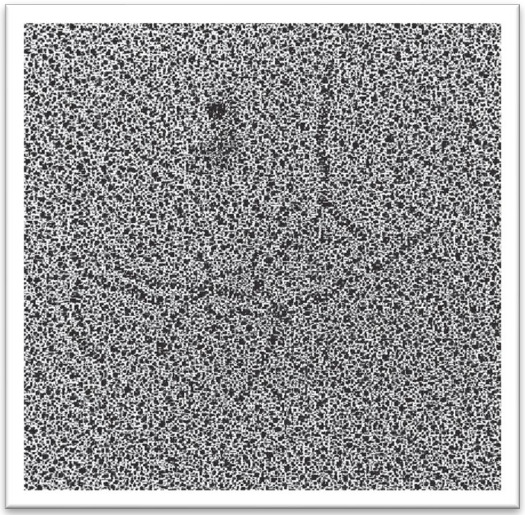
Studies of ancient DNA (aDNA) in archaeological human remains primarily target human DNA and pathogen DNA from contemporaneous infections.

DNA molecules degrade rapidly in soil, undergoing fragmentation and damage over time, leading to instances where analyses of human remains yield limited or unusable data.

Advances in aDNA techniques have lessened these limitations. Next-Generation Sequencing (NGS) has improved the likelihood of obtaining valuable information despite material degradation.



DNA is localized in two different compartments in the cell. After death, DNA is degraded over time and ultimately only small amounts remain.



Cross-linked DNA extracted from 4,000-year-old liver of an ancient Egyptian priest called Nekht-Ankh

Ancient human DNA

At a broad level, study of ancient human DNA can tell us about relationships between different human populations, and about migrations and population history.

At a smaller scale it can be used to reconstruct patterns of kinship within burial grounds and communities.

aDNA analysis probabilistically determines the sex of skeletons when osteological methods are inconclusive, such as with highly fragmented remains or those of children.

aDNA analysis can provide probabilistic insights into phenotypic traits like hair, skin, or eye color, as well as factors such as lactase persistence (affecting milk digestion) and genetic predispositions to diseases.

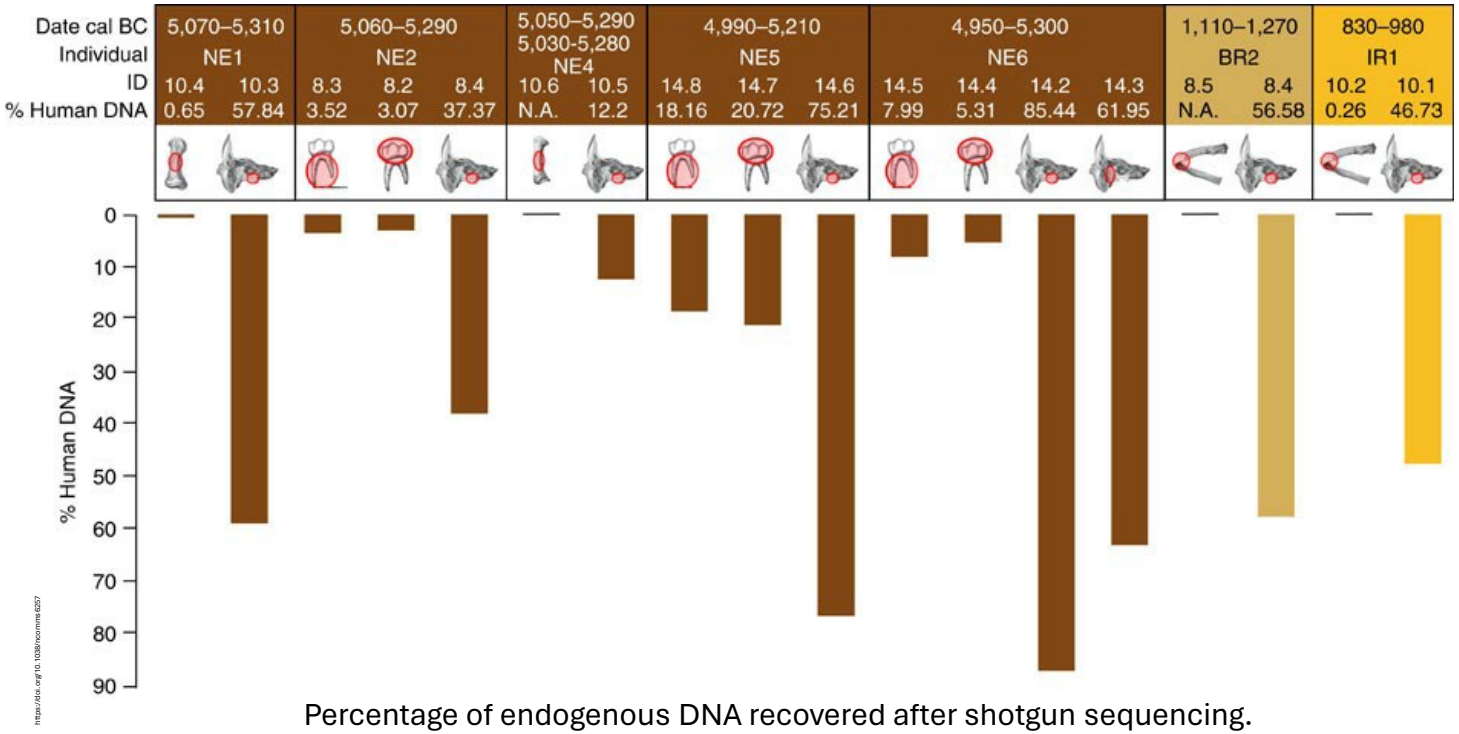
https://www.nih.gov/news-events/nih-research-matters/ancient-viral-dna-may-help-humans-fight-infections

Ancient human DNA

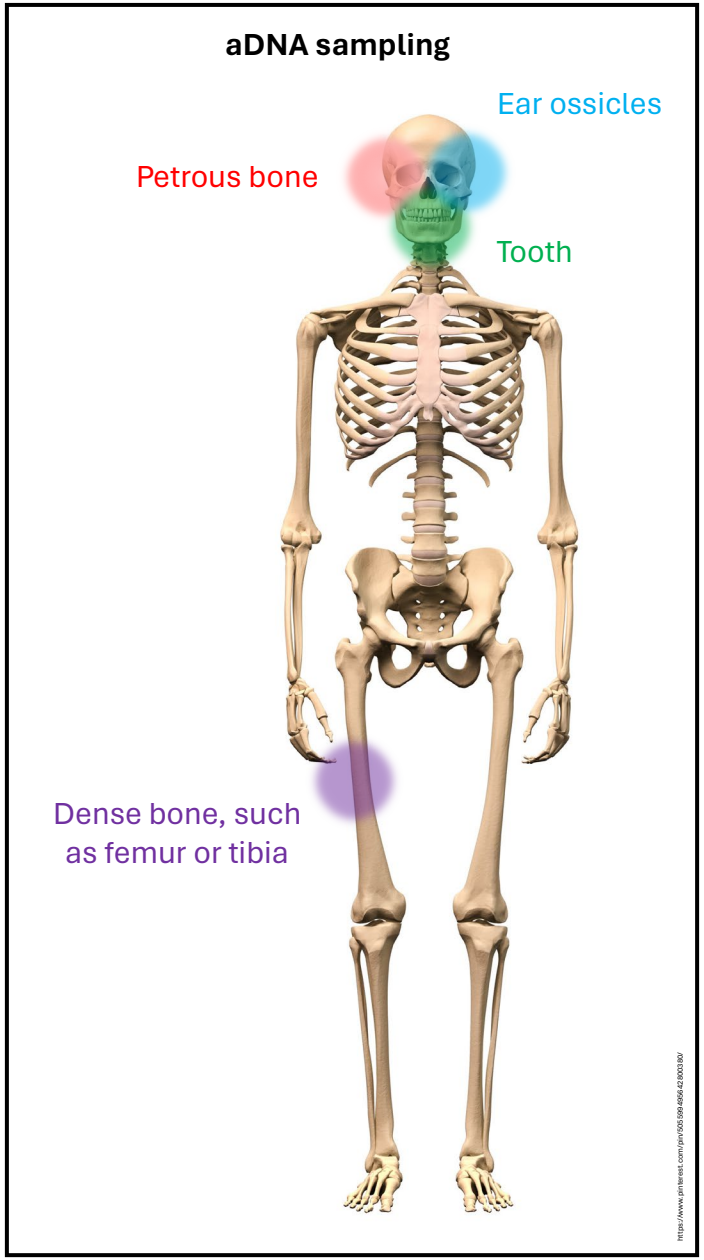
For aDNA, it is possible to sample:

- a dense bone, such as femur or tibia,
- The dentine par of a well-preserved tooth (complete, closed root, uncracked, without cavity),
- the petrous part of a temporal bone (= best sampling site),
- the ear ossicles.

Cremated bone is not generally suitable for DNA studies.



Percentage of endogenous DNA recovered after shotgun sequencing.



Ancient human DNA

aDNA sampling on the field look like this…

https://scroll.in/article/979693/how-archaeology-has-stripped-india-s-imagination-of-itself

Precautions to control or minimise both environmental and human contaminating DNA should be taken to preserve sample integrity.

Ancient human DNA

Llamas et al. (2017)

In situ sampling is preferable if conditions allow.
In this situation, the following procedures should be implemented:

If the skeletal material is **articulated** and the orientation of a body can be identified, the skeletal remains to be sampled for aDNA analysis should be freed from the soil and collected first, before excavating the rest of the skeleton.

If several specimens are **mingled** (e.g. mass grave or collective burial), unearthed skulls should be protected while excavating until all of the individuals have been identified. Samples that are unequivocally assigned to each individual can then be collected (most likely teeth or cranial elements).

Ancient human DNA

Llamas et al. (2017)

Precautions that can potentially make a big difference

Disposable gloves: Wear disposable medical gloves during excavation and when handling specimens to protect samples from human DNA contaminants. Change gloves between specimens, after touching hair or face, or after touching communal items (e.g. trowels, pens).

Do not wash specimens with water: Water contains contaminating bacterial DNA, and can deeply penetrate into the sample and cause unwanted hydrolytic damage to the endogenous DNA. Light brushing of specimens with a dry brush is preferred to washing with water.

Storage: Samples should either be completely dry to avoid further contamination with microbial DNA (microbial growth) and damage (hydrolysis), or stored in a cold, dry place as soon as possible (e.g. cooler, fridge/freezer).

Ancient human DNA

Llamas et al. (2017)

Optional precautions for maximal effect

Protective gear: In addition to gloves, other (optional) disposable protective gear may include surgical mask, hair cover/net, and sleeves to cover the arms (Figure 1). If budget and conditions allow, a clean disposable surgical gown or body suit and goggles are desirable.

Protect the site: Protect the site from dust, rain and direct sunlight to limit hydrolysis, irradiation, and further contamination with environmental DNA once the specimen is exposed.

Dedicated trained staff: If possible, assign one or two members of the excavation team to be formally trained and solely responsible for collecting the 'contaminating-modern-DNA-free' samples for aDNA analysis.

Clean tools: Clean tools (e.g. trowels, dental picks, and brushes) with ≥3% bleach between samples, and dry them prior use on the next sample.

Ancient human DNA

But the realm of genetics often is a storage room…

https://asaigroup.it/it/product_solutions/archivio-file-box-storage-shelving/

This falls far short of the ideal scenario..

Specimens stored in museums or in other facilities are extremely valuable for aDNA research, despite the potential lack of contextual information, storage at room temperature, and extensive manipulation with bare hands.

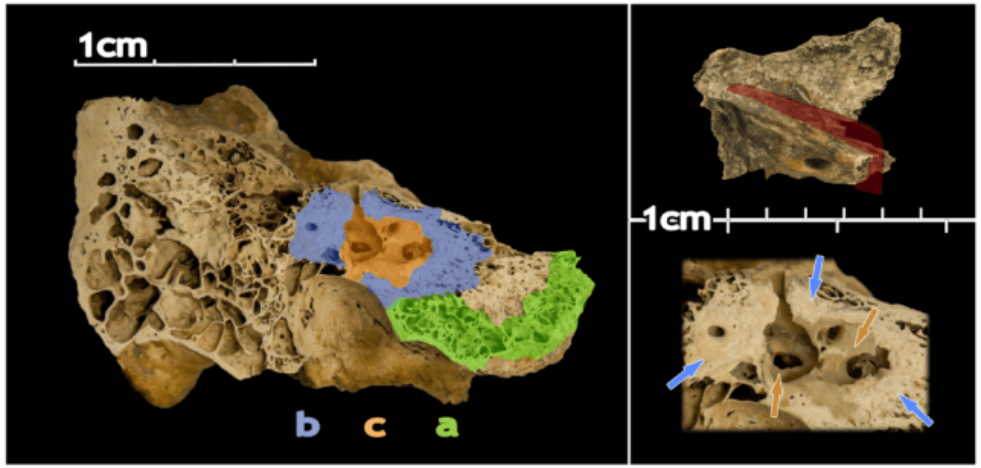
When sampling museum specimens, we strongly advise the routine implementation and use of protective gear and appropriate sampling protocols as outlined above.

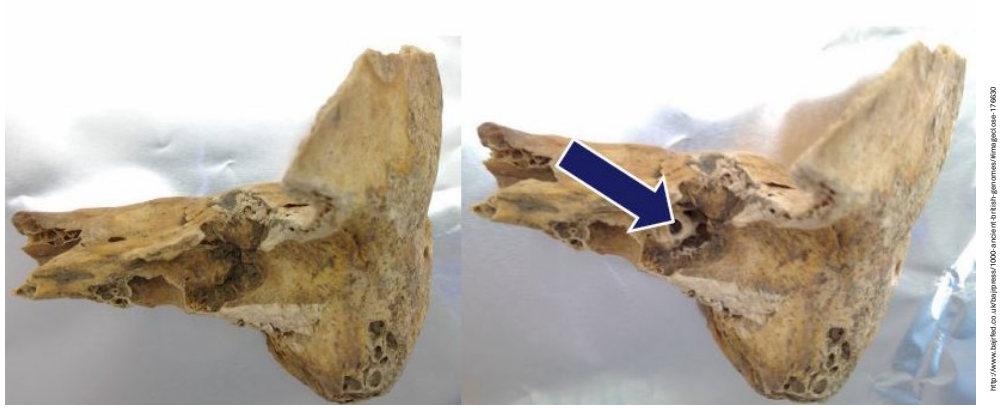
Remains with preservative agents, hardeners, varnish glue, adhesive tape, or any other chemicals should be avoided, as they can inhibit experimental enzymatic reactions or introduce contaminating DNA.

Ancient human DNA

Lab sampling

The denser the bone (A < B < C), the higher the proportion of endogenous DNA





Impact of sampling on the petrous portion of the temporal bone before (left) and after (right)

A diamond bur attached to a high-speed dental handpiece creates a hole on the crown surface of the tooth to access the pulp chamber/dentine.

Bone drilled in a clean facility

Ancient human DNA

Human aDNA detection

The workflow outlines techniques for detecting human DNA in ancient remains.

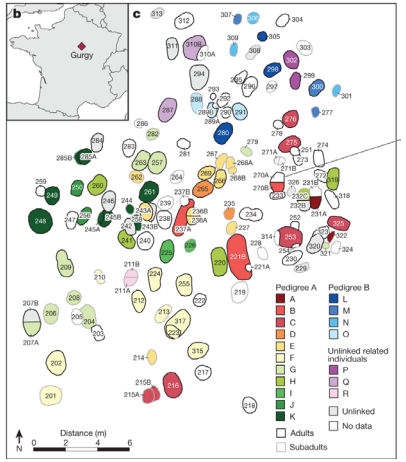
The different wet-laboratory procedures must be carried out in specific aDNA facilities, minimizing environmental contamination, and include all pre-amplification, amplification and post-amplification steps.

You can do computational work outside those facilities.

Spyrou et al. (2019)
https://doi.org/10.1038/s41576-019-0119-1

Let’s close this blackbox!

Ancient human DNA



The study involved analyzing various data sources from 94 individuals buried at Gurgy. They used genome-wide ancient DNA data, mitochondrial DNA (maternal lineages), Y-chromosome data (paternal lineages), and genetic sex.

nature

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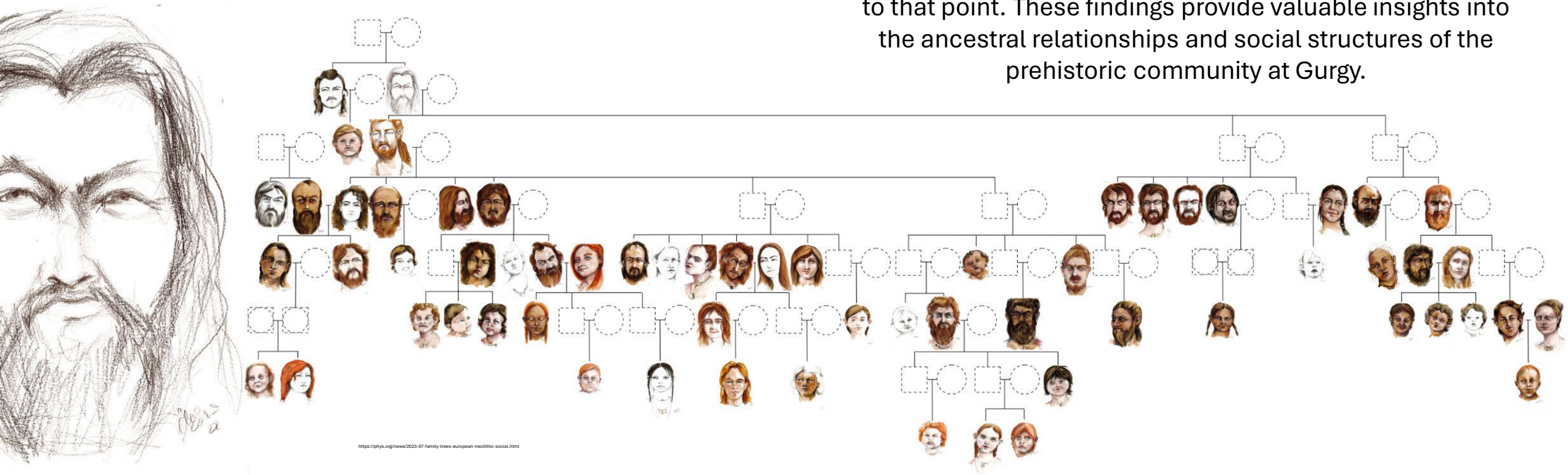
https://doi.org/10.1038/s41586-023-06350-8

Article | Open access | Published: 26 July 2023

Extensive pedigrees reveal the social organization of a Neolithic community

Maïté Rivollat, Adam Benjamin Rohrlach, Harald Ringbauer, Ainash Childebayeva, Fanny Mendisco,

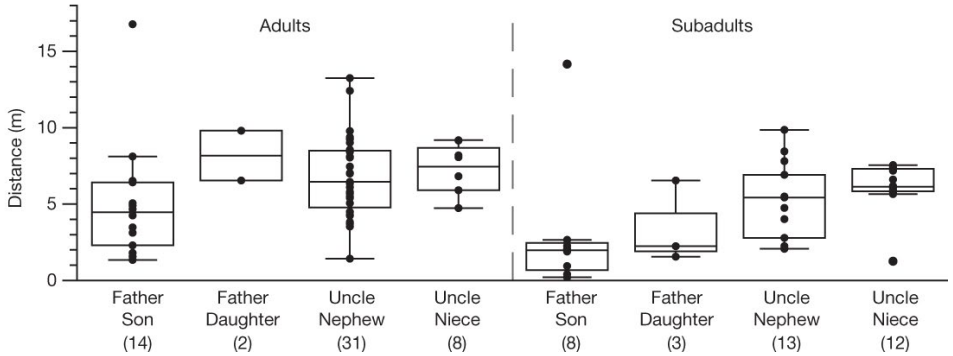
The data revealed that approximately two-thirds of the buried individuals belonged to a single family, making it the most extensive pedigree reconstructed from ancient DNA up to that point. These findings provide valuable insights into the ancestral relationships and social structures of the prehistoric community at Gurgy.



https://phys.org/news/2023-07-family-trees-european-neolithic-social.html

Ancient human DNA

Photograph of female individual GLN270A (no genetic results) with the reburied remains of the main male ancestor GLN270B of pedigree A.



The spatial distances of father–offspring and uncle–nephew/niece pairs.

nature

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The exploration of pedigrees at Gurgy revealed a strong patrilineal pattern, with each generation predominantly connected through the biological father.

Mitochondrial lineages and strontium isotopes suggest women were mostly non local, implying patrilocality, where sons stayed in Gurgy and married outside.

Interestingly, the adult daughters in the lineage were mostly absent, suggesting female exogamy and a potential reciprocal exchange system.

A standout among the discoveries was a grave featuring the nearly intact skeleton of a woman, alongside scattered bones purposefully moved from another burial. DNA analysis revealed these bones belonged to the founding male ancestor.

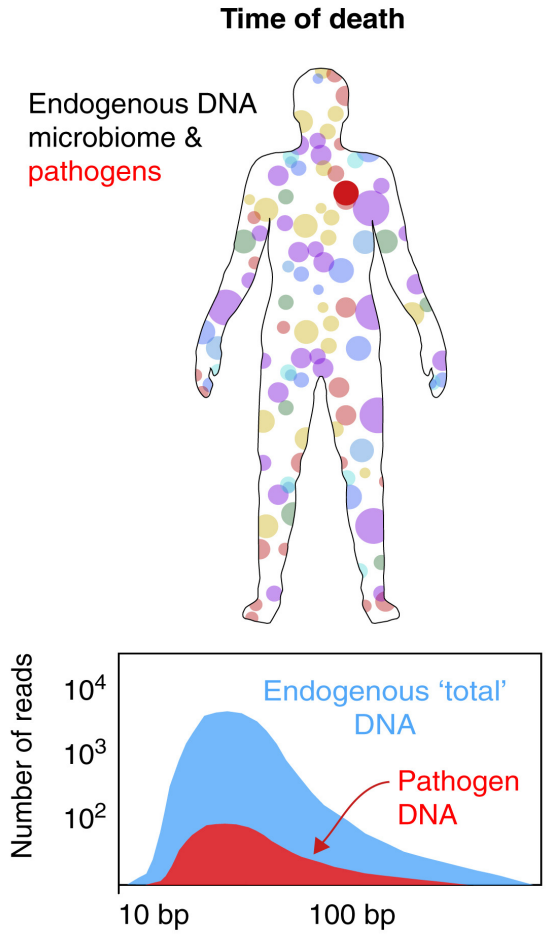
Ancient pathogen DNA

For a long time, the study of infectious diseases relied on traditional paleopathological assessment of ancient human skeletons unearthed during archaeological excavations.

However, thanks to recent and ongoing advancements in paleogenomics, a novel field of research has emerged: **molecular paleoepidemiology**.

This field of research focus on:

- Confirming the presence of infectious pathogens,
- Identifying extinct pathogen lineages,
- Refining the timeline of pathogen emergence in human populations,
- Reconstructing the evolutionary history of pathogens.



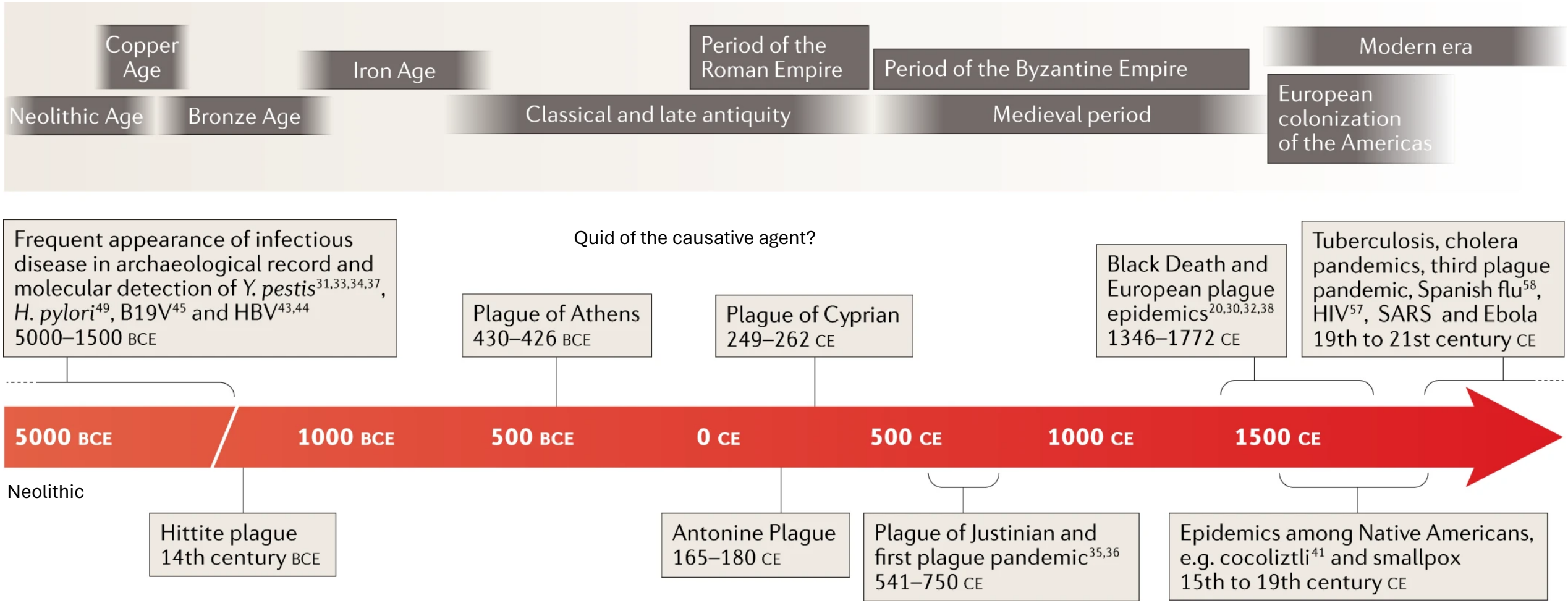
Pathogen DNA at the time of death

Duchêne *et al.* (2020)
https://doi.org/10.1016/j.cub.2020.08.08

Ancient pathogen DNA

Spyrou et al. (2019)
https://doi.org/10.1038/s41576-019-0119-1

Timeline of key events in Eurasian history which have overlapped with major historical epidemics.

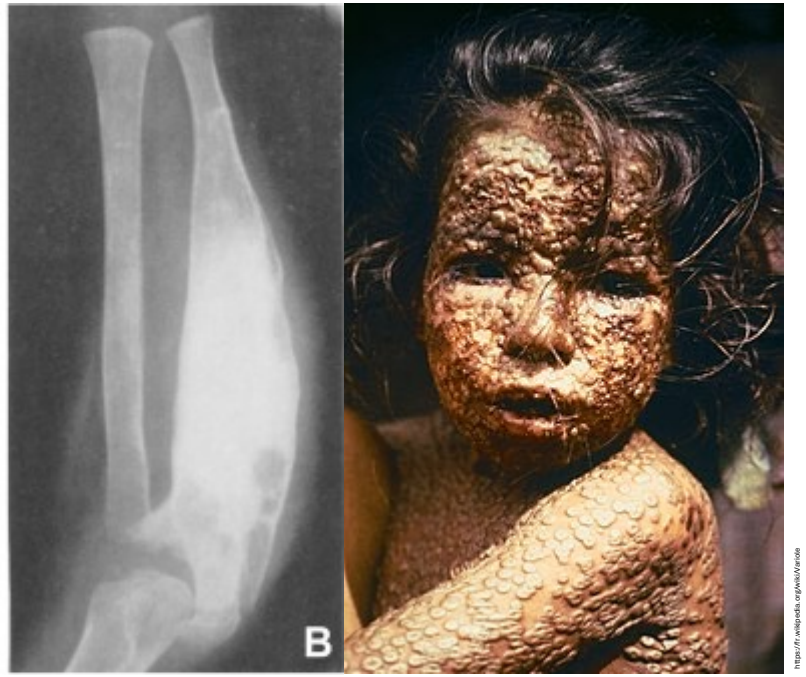


The respective citations are indicated, in which whole-genome or low-coverage genome-wide data from pathogens implicated in those events have been reconstructed by ancient DNA analysis. B19V, human parvovirus B19; BCE, before current era; CE, current era; HBV, hepatitis B virus; H. pylori, Helicobacter pylori; SARS, severe acute respiratory syndrome; Y. pestis, Yersinia pestis.

Ancient pathogen DNA

Crawford et al. (2024)
https://doi.org/10.1016/j.ijpp.2023.12.002

Radiographs of smallpox patients admitted to University College Hospital, Ibadan, Nigeria, 1957.
B) Severe involvement of the forearm and elbow joint in a two-year-old child, two months after infection onset.



Smallpox

In **2011**, a significant milestone was achieved with the publication of the **first** successful publication of the entire genome of an ancient pathogen retrieved from skeletal human remains.

This groundbreaking achievement focused on *Yersinia pestis*, the well-known bacterial causative agent of **plague**.

By now, the largest amount of genomic data is obtained for:
- *Yersinia pestis* (**plague**),
- *Variola virus* (**smallpox**),
- *Vibrio cholerae* (**cholera**),
- HBV (**hepatitis B** virus),
- *Mycobacterium tuberculosis* (**tuberculosis**),
- *Mycobacterium leprae* (**leprosy**),
- *Treponema pallidum* (**syphilis**).

This list is not exhaustive!

Leprosy

Roberts (2018)
Loss of the anterior nasal spine, widening of the nasal aperture, and remodeling of the nasal aperture edges of a person who had experienced leprosy during life.

Ancient pathogen DNA

Providing rationale for a sampling initiative

https://www.archaeology.org/news/10848-220922-slovakia-mass-grave

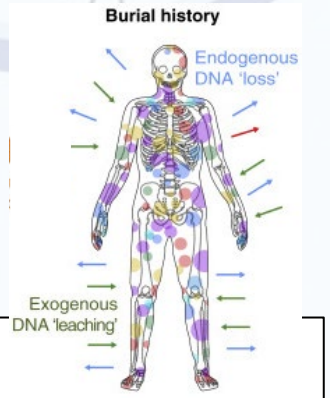
Contextual evidence

Considering the subsistence strategy, population density, geography, ecology, climate, and social organization, type of site, etc., *is it probable that the pathogen of interest will be present in this location*?

https://bonesdontlie.wordpress.com/2012/11/13/on-the-trail-of-tuberculosis/

Paleopathological evidence

In light of paleopathological investigations, *is it probable that there are cases of the disease under consideration at the archaeological site*, particularly regarding diseases that leave skeletal manifestations?



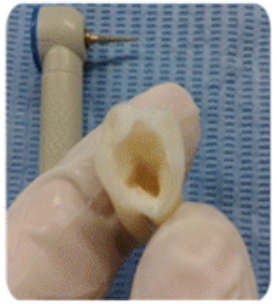
https://doi.org/10.1016/j.cub.2020.08.081

Pilot study

Is it possible to confirm the preservation of the targeted aDNA and determine if the level of preservation is sufficient for successful genome recovery?

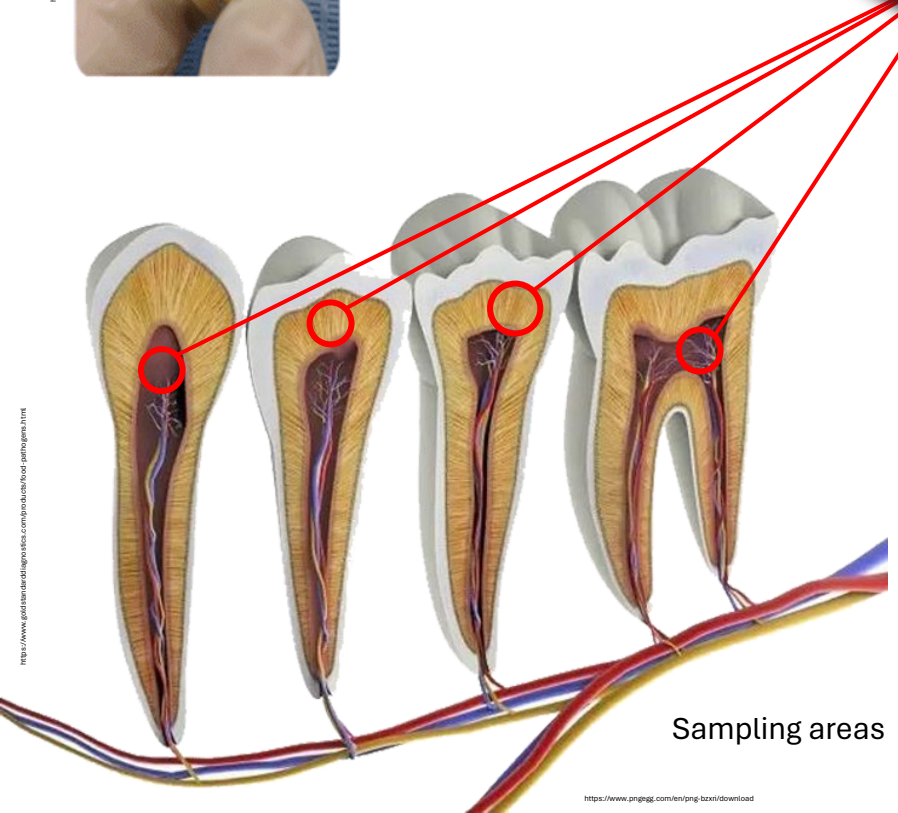
Ancient pathogen DNA

Tooth after access pulp chamber



https://doi.org/10.1186/s41933-017-0030-x

Side note: Only the organic fraction is of interest. Enamel is not sampled for aDNA recovery!



https://www.gpddstandardsdiagnostics.com/products/food-pathogens.html

Sampling areas

https://www.pngegg.com/en/png-bzxri/download

Sampling strategy

It may be obvious, but we can only recover what was there.

There are 3 main substrates.

1st substrate

Pulp chamber (or dentin)

- Pathogens that cause septicemia (e.g. *Yersinia pestis* for the septicemic plague);
- Pathogens that infect oral cavity and rhinomaxillary area (e.g. *Mycobacterium leprae* for the leprosy).

Residual blood cells are effectively shielded within the tooth.

Ancient pathogen DNA

Sampling strategy

It may be obvious, but we can only recover what was there.

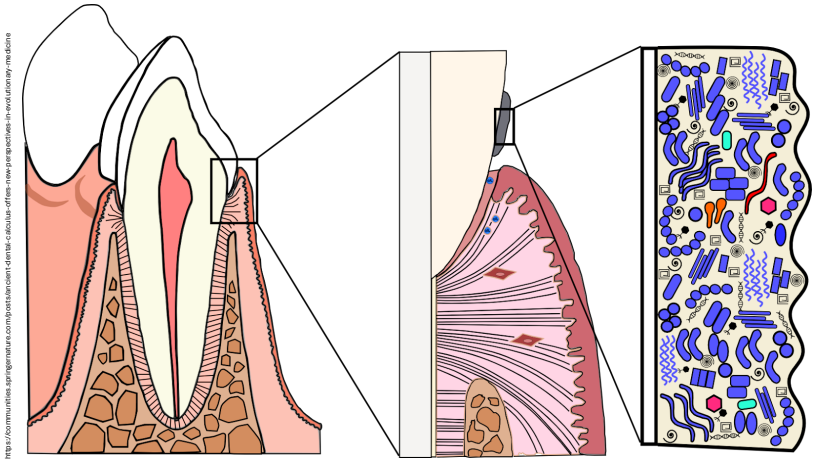
2nd substrate

Dental plaque or calculus

- Pathogens that infect oral cavity and rhinomaxillary area (e.g. *those responsible for eliciting the inflammatory response characteristic of periodontal disease).*

https://communities.springernature.com/posts/ancient-dental-calculus-offers-new-perspectives-in-evolutionary-medicine

Ancient dental calculus on teeth used in aDNA study prior to sampling.

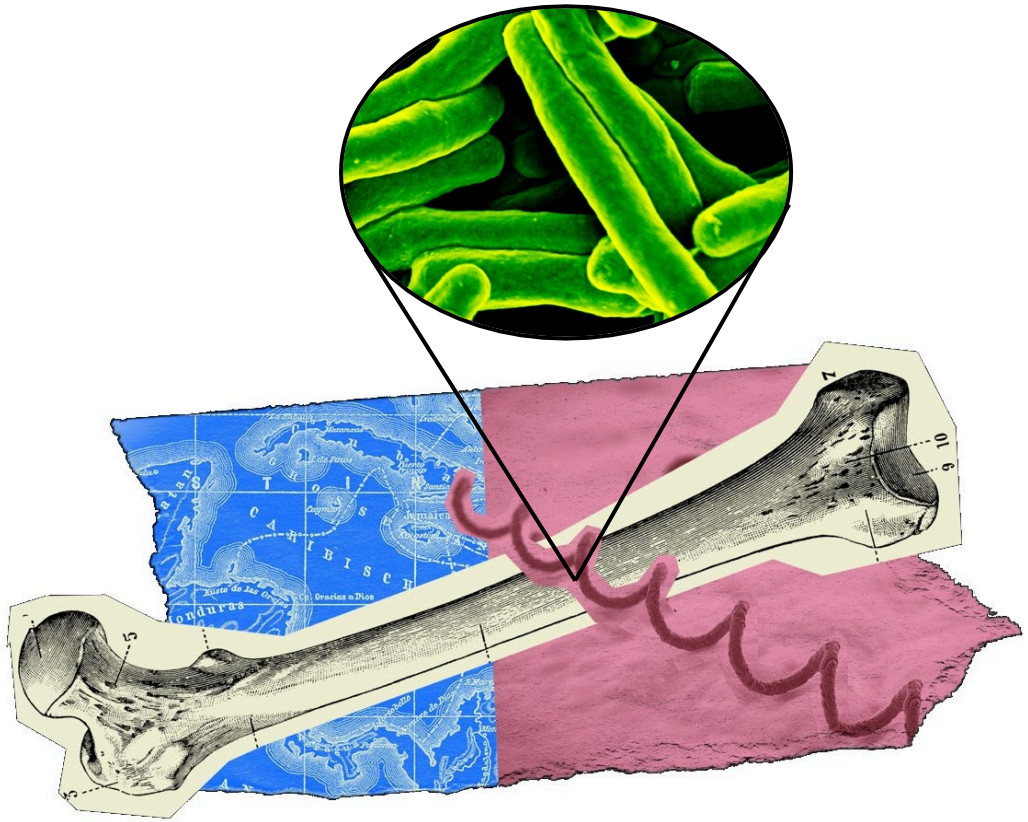


https://communities.springernature.com/posts/ancient-dental-calculus-offers-new-perspectives-in-evolutionary-medicine

Dental plaque biofilm contains multitudes of microbes including bacteria, archaea, and viruses, as well as DNA, proteins, and metabolites from the microbes and the host.

Ancient pathogen DNA

Side note: If there is no indication suggesting the presence of pathogens, refrain from sampling.



https://www.sciencealert.com/a-bone-eating-pathogen-from-thousands-of-years-ago-is-still-circulating-inside-us
https://www.wired.com/story/a-medieval-french-skeleton-is-rewriting-the-history-of-syphilis/

Sampling strategy

It may be obvious, but we can only recover what was there.

https://www.mdpi.com/1424-2818/9/4/43

Bone specimens from seven different individuals with apparent signs of osteomyelitis

3rd substrate

Bone

- Pathogens from infections (e.g. Staphylococcus aureus for osteomyelitis), and more rarely from septicemia.

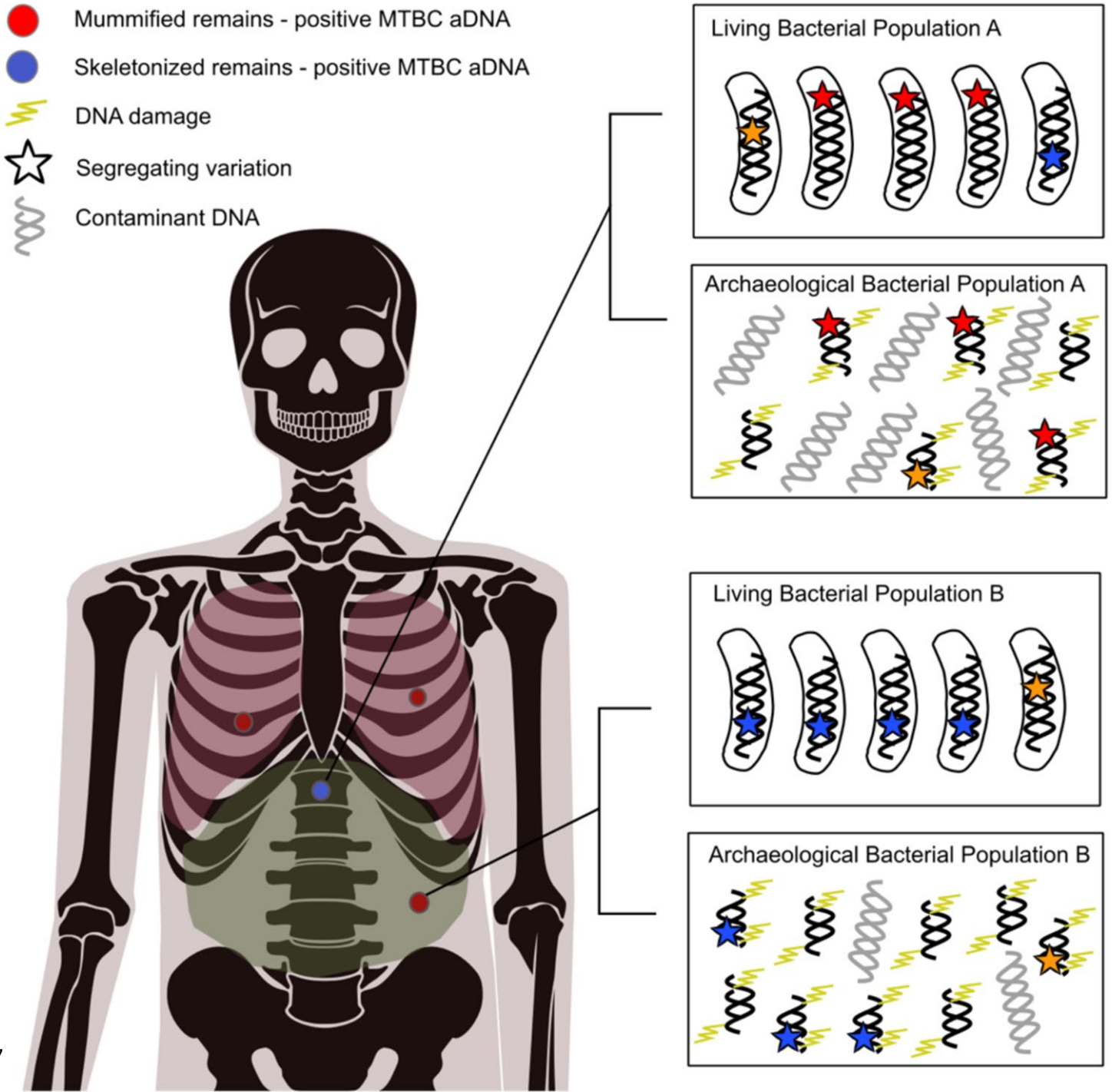
Ancient pathogen DNA

Let's take *Mycobacterium tuberculosis* as an example, that is a causative agent of tuberculosis (TB).

TB is infection diseases cause by a bacteria and spreads through the air when infected people cough, sneeze or spit.

The symptoms people get depend on where in the body TB becomes active. While TB usually affects the lungs, it also affects the kidneys, brain, spine and skin.

Therefore, TB is not distributed in an infected body homogeneously.



Morales-Arce et al. 2021
https://doi.org/10.1038/s41437-020-00377-7

Ancient pathogen DNA

Where to sample?

When dealing with localized infections, it is important to consider the skeletal manifestations, particularly for people living in the era before the advent of antibiotics.

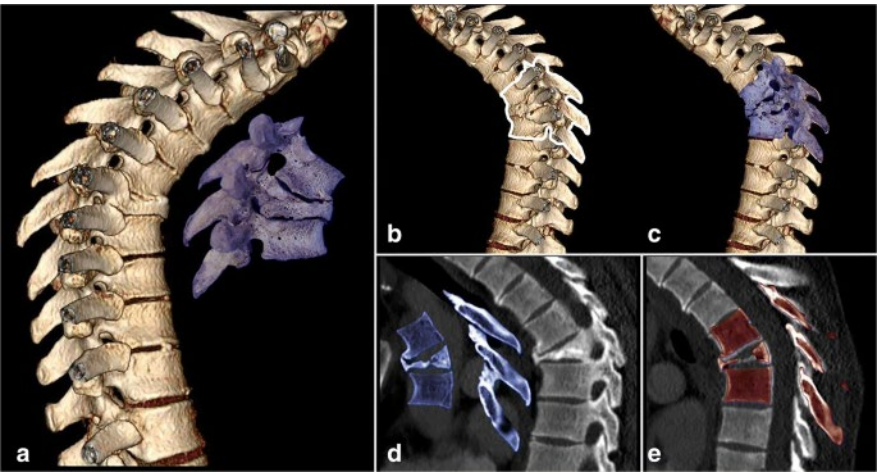
These manifestations serve as flags or signposts, indicating that the pathogen was there during the individual's lifetime or at their death. Therefore, they represent the most suitable locations for sampling.

Ancient pathogen DNA

Where to sample?

In the case of TB, the bacteria can spread throughout the body via the bloodstream, favoring blood-rich areas such as spongy bones for proliferation.

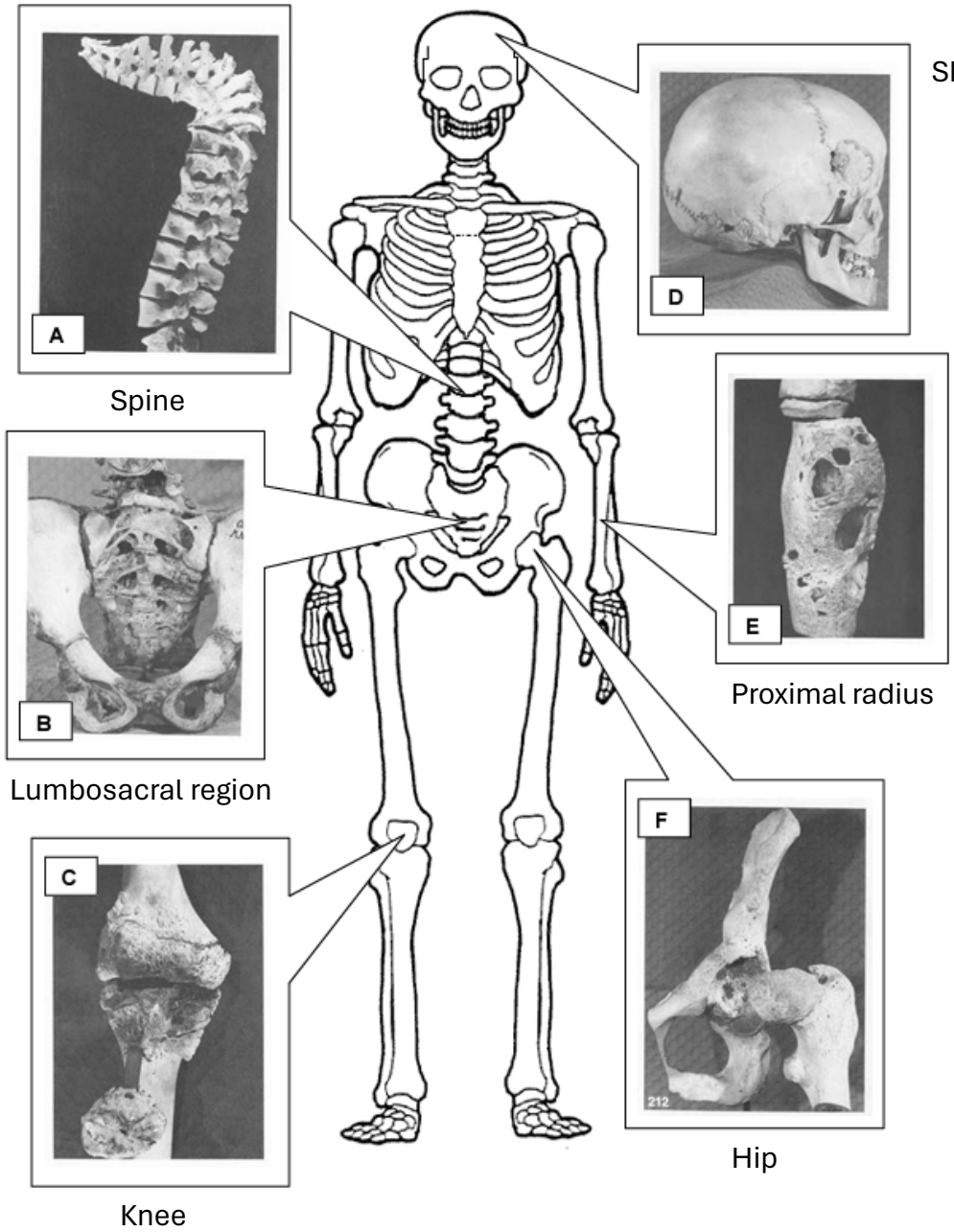
The manifestations of TB on bones are diverse. They can be preserved and identified in archaeological individuals. Hence, these are the specific sites to be targeted.



Collapsed vertebrae

Martrille *et al.* (2020)
https://doi.org/10.1007/s00414-020-02348-3

Classical tuberculosis lesions



Giacon (2008) based on Ortner and Putschar (1985)

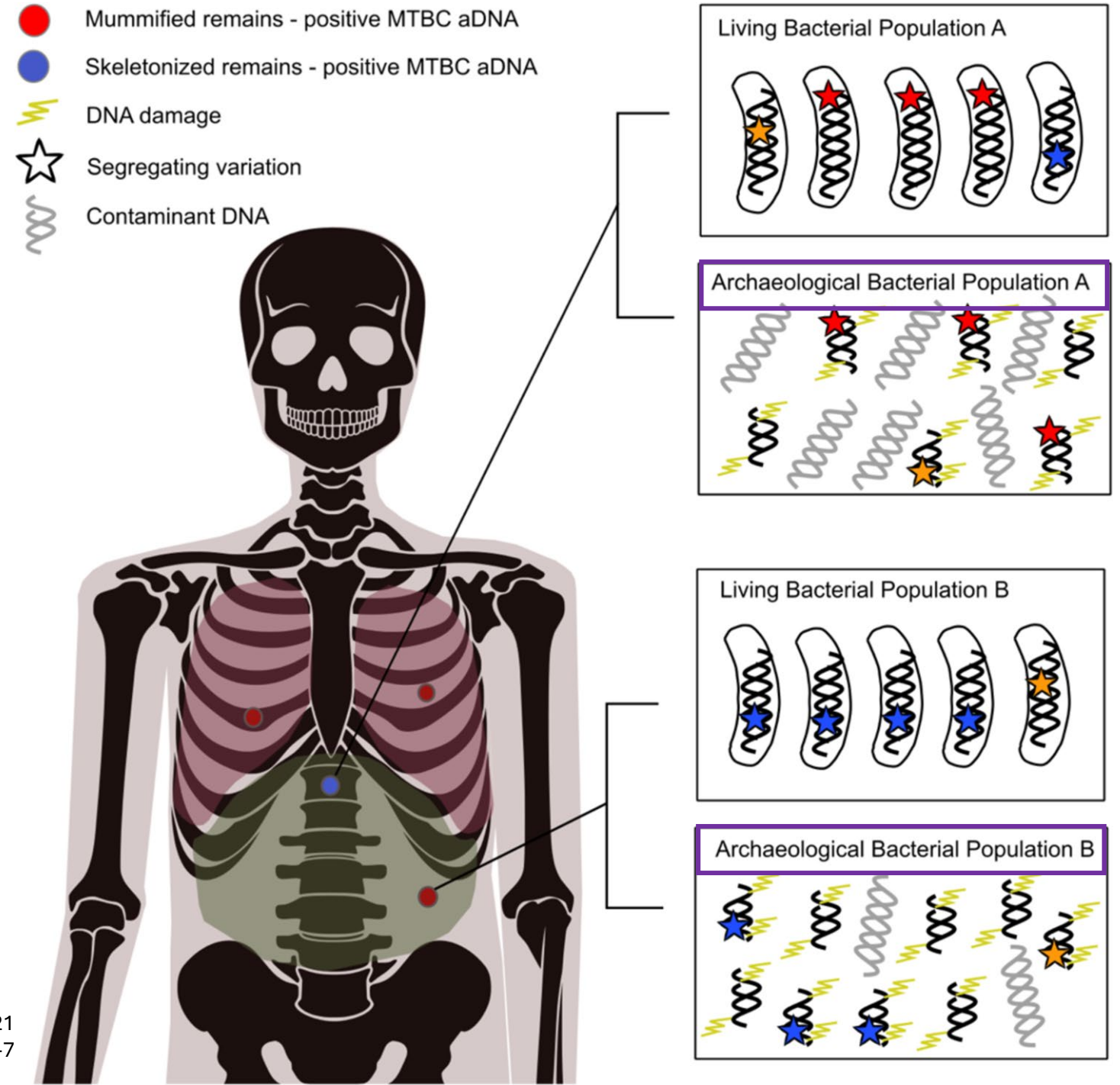
Ancient pathogen DNA

The red and blue dots indicate sites from which ancient TB DNA has been successfully recovered.

- Vertebrae
- A rib bone
- A calcified lung nodule
- Soft tissue from the lungs and abdominal cavity

In the majority of cases, only one sampling site is represented per individual.

The diversity discovered in one sampling site may not be representative of the total infection population (i.e. subpopulations) within a host.



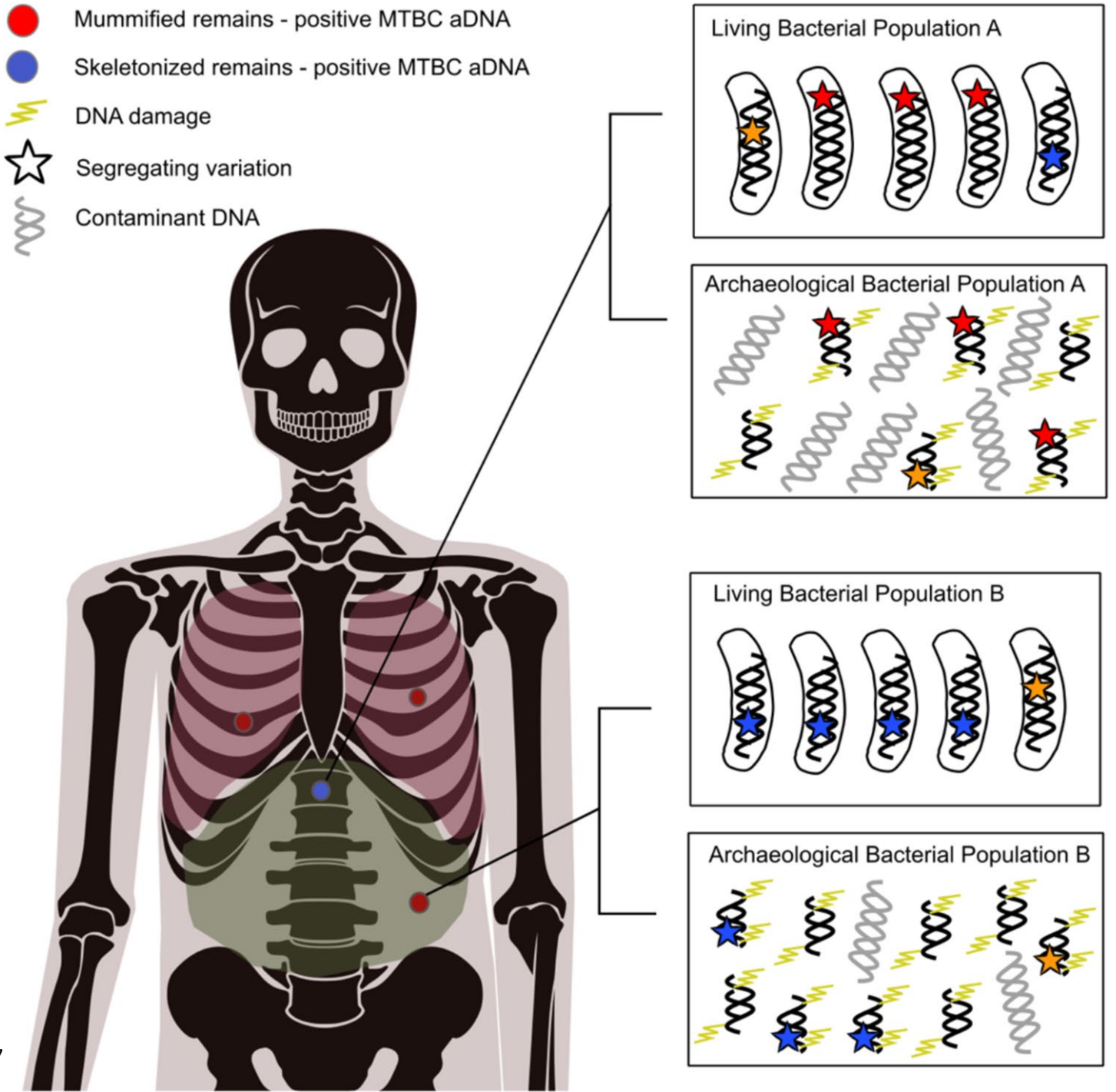
Morales-Arce et al. 2021
https://doi.org/10.1038/s41437-020-00377-7

Ancient pathogen DNA

The stochasticity of DNA preservation in terms of ubiquitous contamination, fragmentation, and cytosine-to-uracil deamination poses barriers to accurate reconstruction of bacterial population diversity during life.

<u>Stochastic effect</u> is a fluctuation of results between replicate analyses.

<u>Preservation</u> complicates this issue as time passes, leading to DNA degradation and diminishing the likelihood of recovering pathogens.



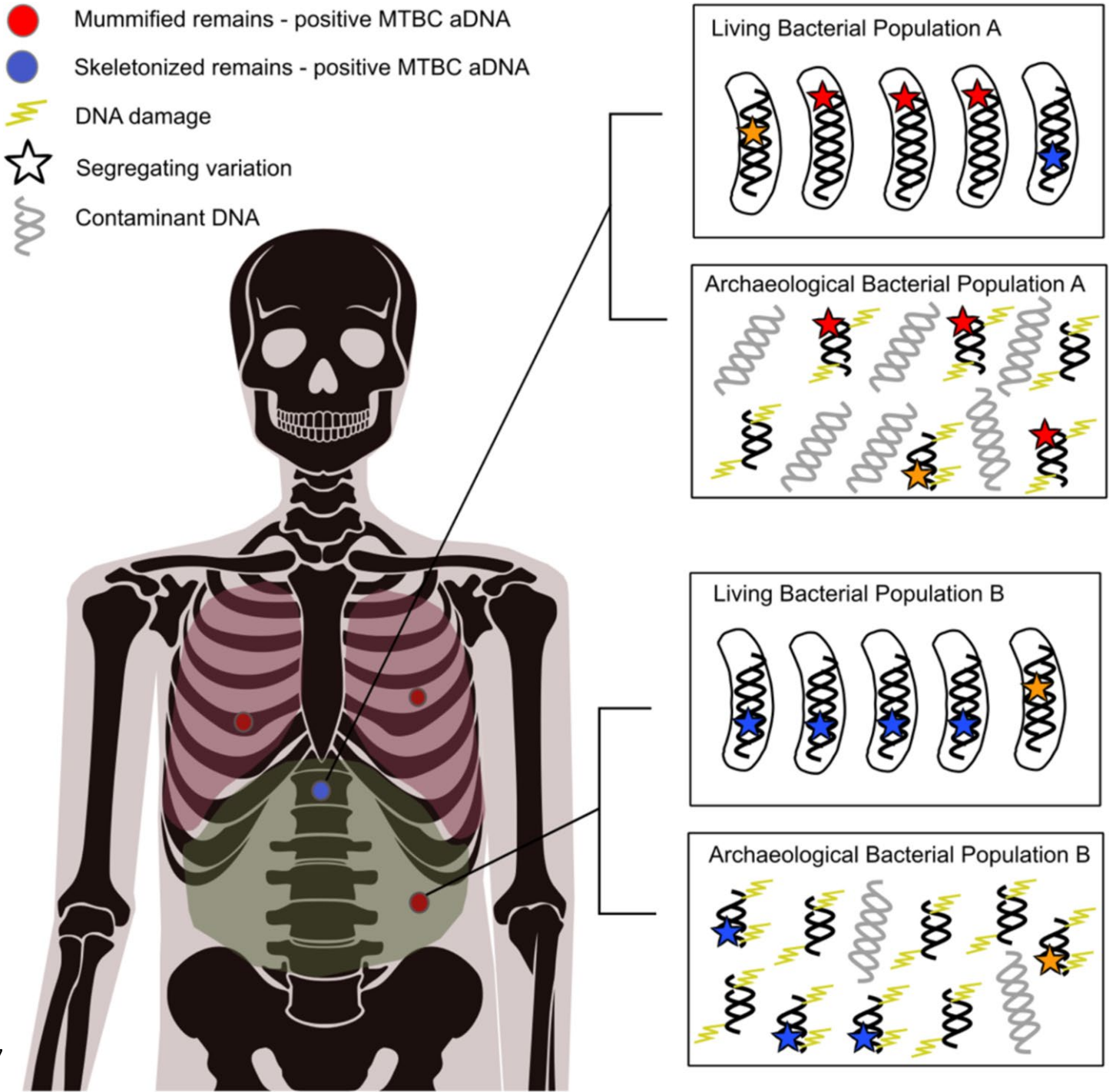
Morales-Arce et al. 2021
https://doi.org/10.1038/s41437-020-00377-7

Ancient pathogen DNA

If an individual has tuberculosis in their lungs, it does not necessarily imply that bacteria were present throughout every part of the lungs.

Hence, there exists intra-element variation.

This same rationale applies to the skeleton. The presence of a bone lesion caused by tuberculosis does not mean that bacteria can be retrieved from every bone of this skeleton.



Morales-Arce et al. 2021
https://doi.org/10.1038/s41437-020-00377-7

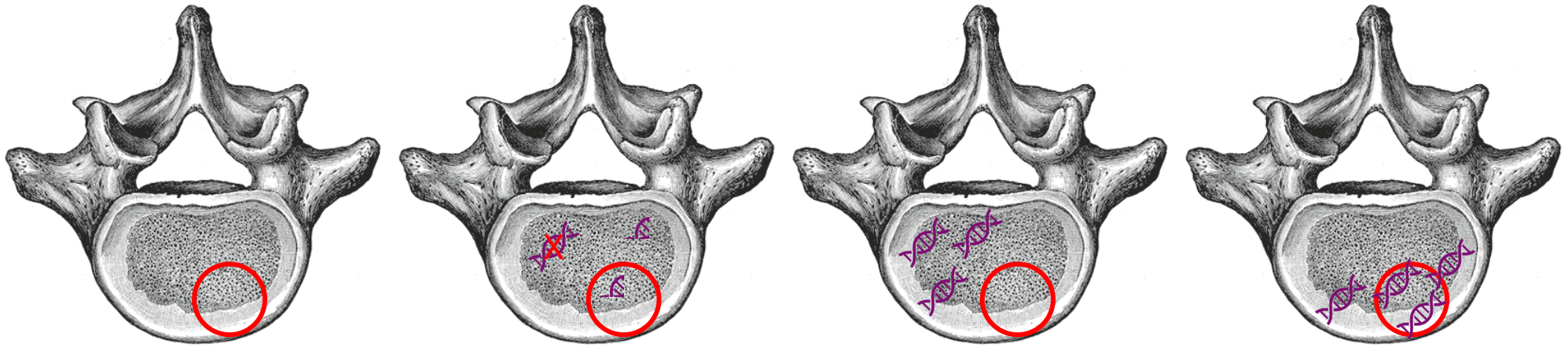
Ancient pathogen DNA

Mycobacterium tuberculosis aDNA recovery

4 scenarios

Negative results

Positive result



Not present

Not preserved

Preserved but wrong site

Preserved & recovered

Cannot be differentiated

https://www.sciencedirect.com/science/article/pii/S1879981719301536#fig0020

The dream scenario

Ancient pathogen DNA

Pathogen aDNA detection

The diagram outlines techniques for detecting pathogen DNA in ancient remains, categorizing them into laboratory and computational methods.

Spyrou et al. (2019)
https://doi.org/10.1038/s41576-019-0119-1

Blackbox?

A blackbox is a system which can be viewed in terms of its inputs and outputs, without any knowledge of its internal workings.

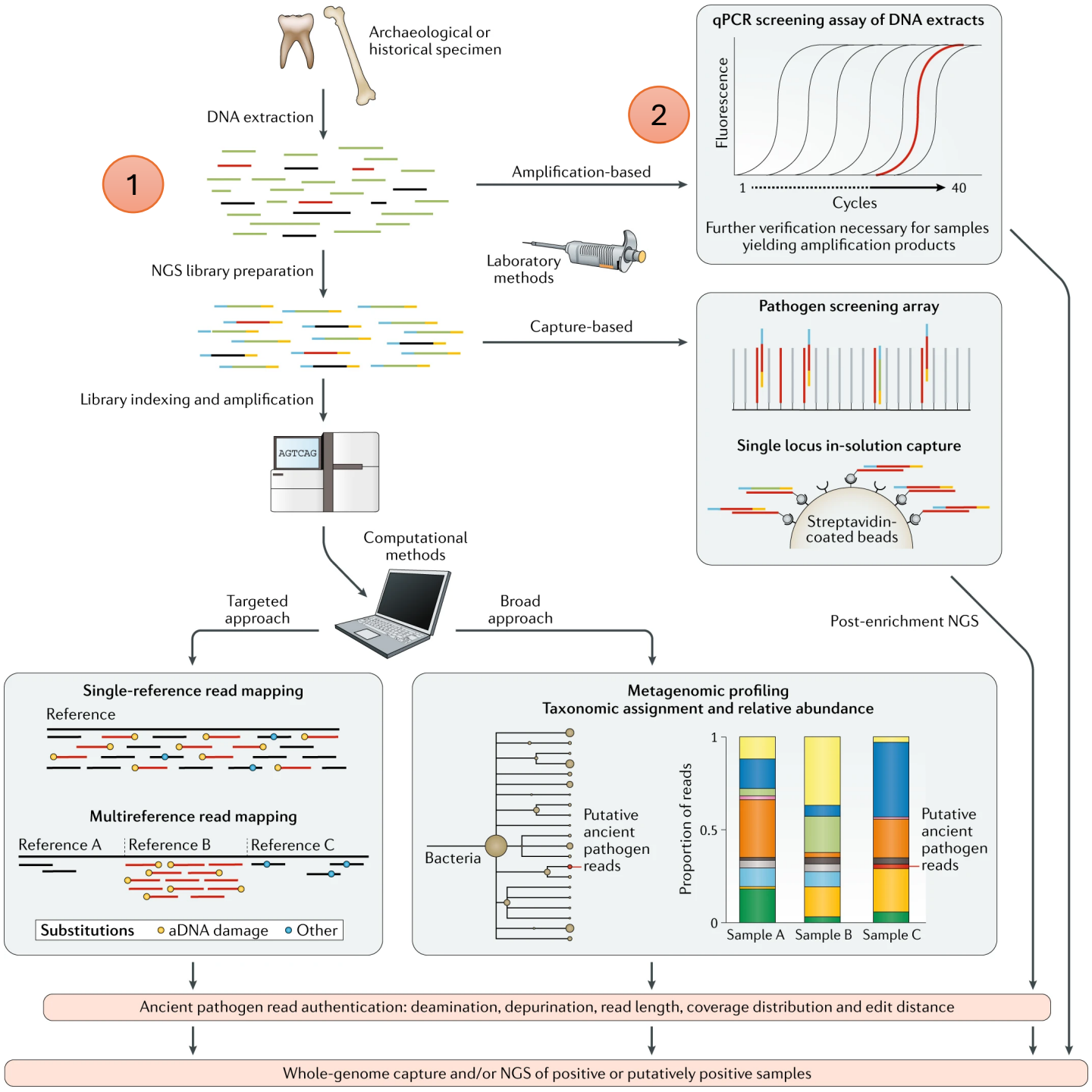
Ancient pathogen DNA

Pathogen aDNA detection

1. The process commences with the extraction of DNA from ancient specimens.

2. Extracts can undergo direct screening via PCR targeting species-specific genes.

PCR techniques alone, however, can suffer from frequent false-positive results.



Spyrou et al. (2019)
https://doi.org/10.1038/s41576-019-0119-1

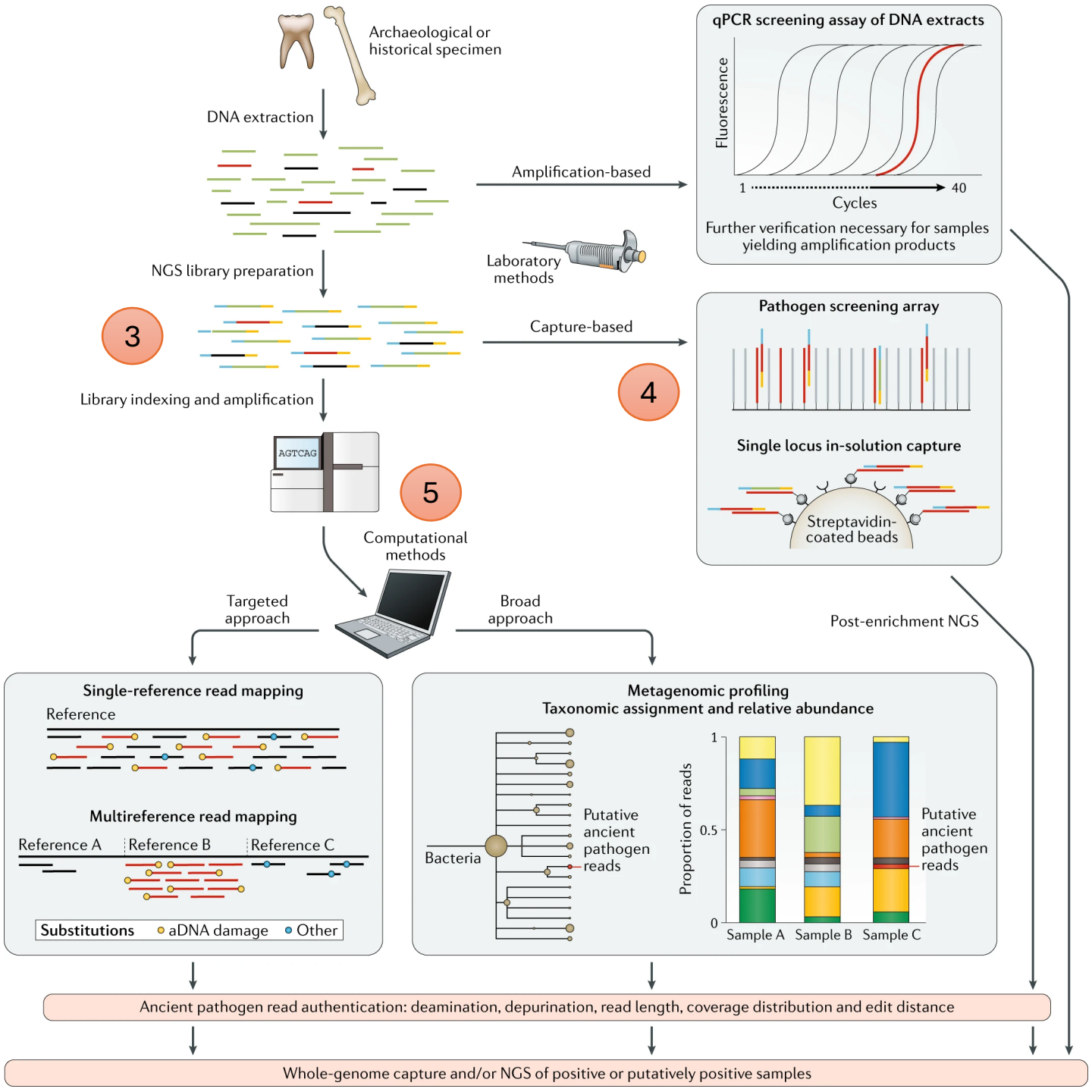
Ancient pathogen DNA

Pathogen aDNA detection

3 Then, NGS libraries can be constructed as part of the process.

4 These libraries enable pathogen screening through fluorescence-based detection on microarrays and DNA enrichment approaches.

5 These libraries can also be utilized for pathogen screening through computational tools.



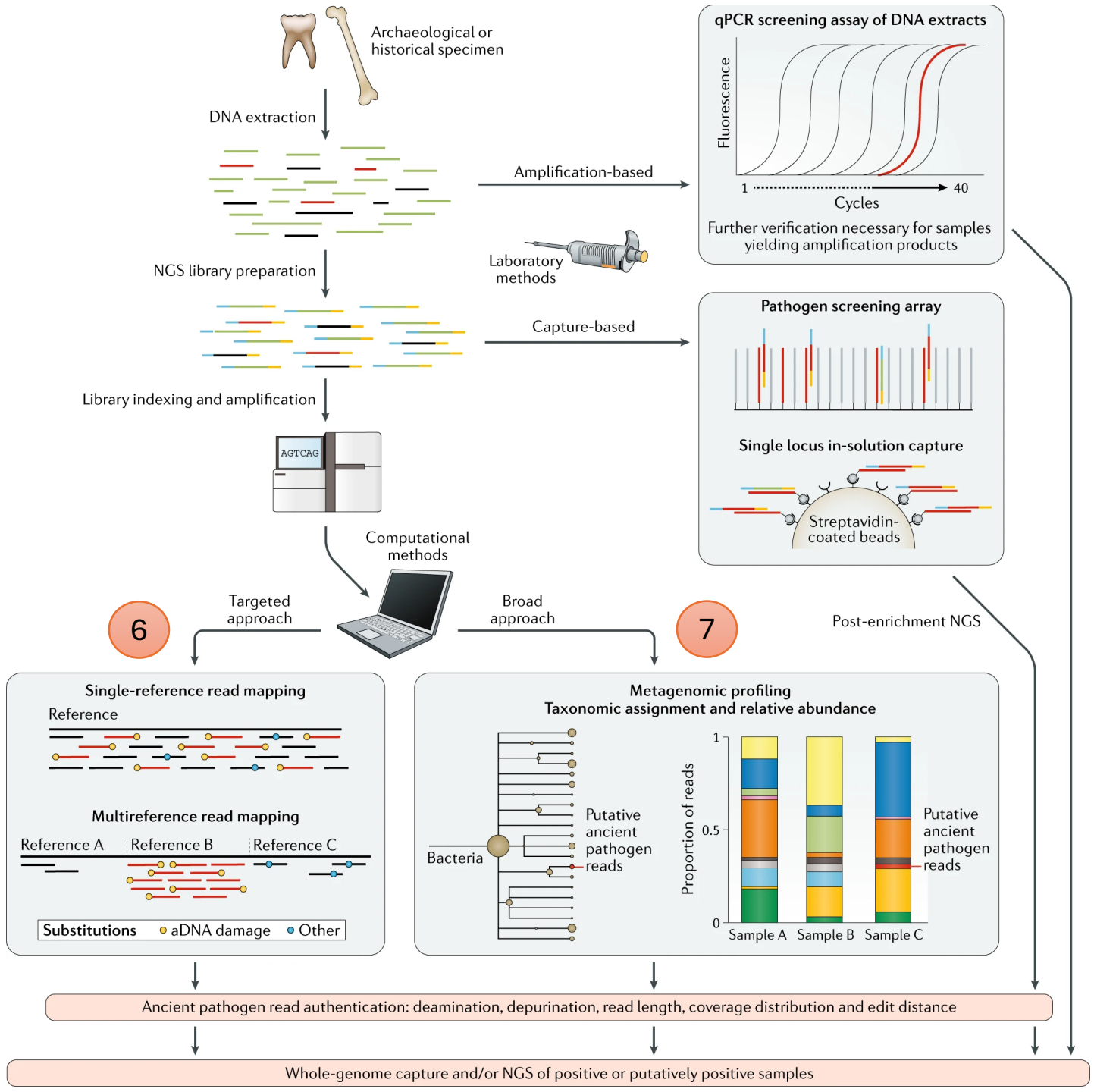
Spyrou et al. (2019)
https://doi.org/10.1038/s41576-019-0119-1

Ancient pathogen DNA

Pathogen aDNA detection

6 Next, reads can be directly mapped against a target single- or multi-reference genome.

7 Metagenomic profiling methods can also be employed to detect ancient pathogen DNA.

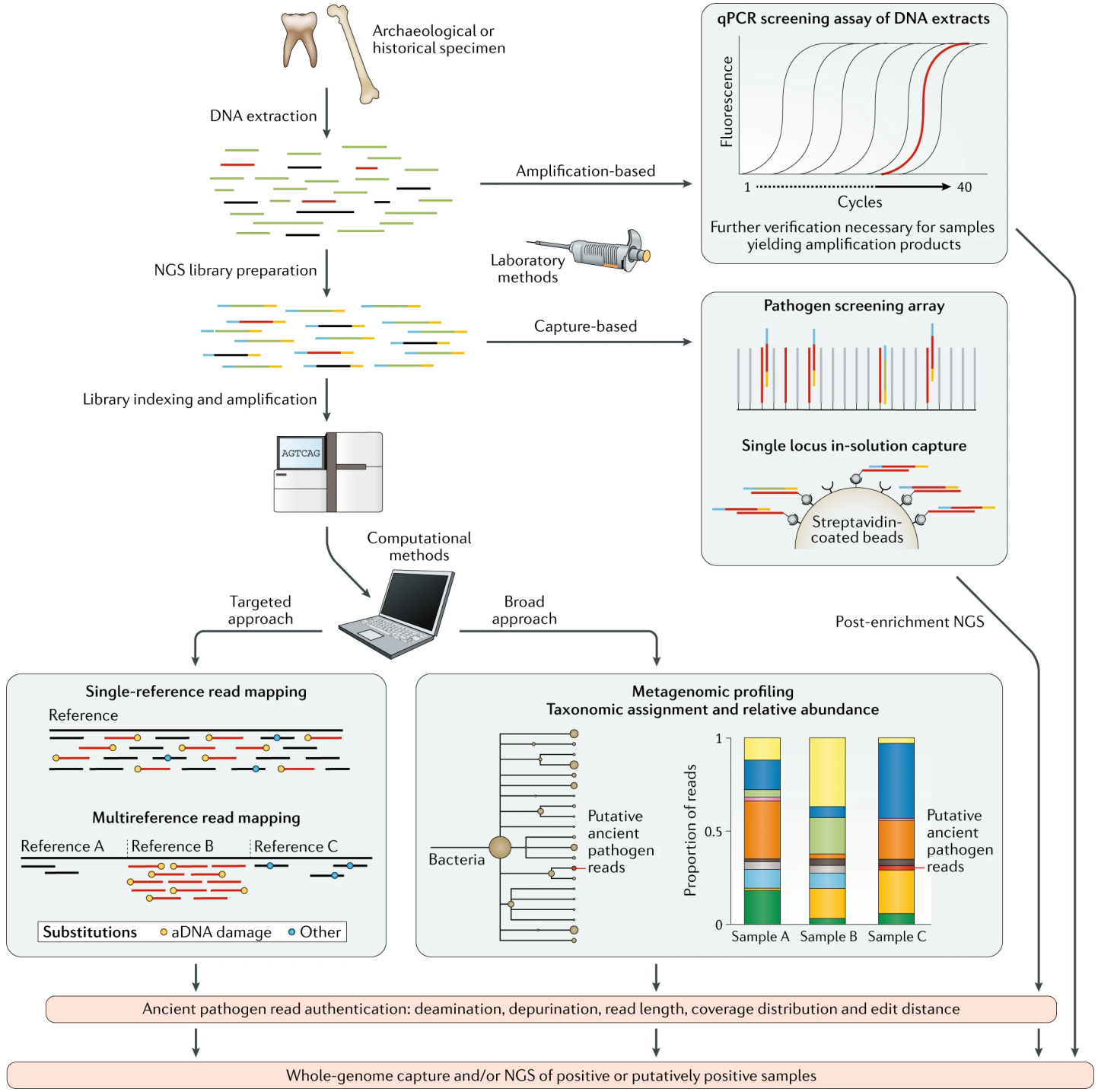


Spyrou et al. (2019)
https://doi.org/10.1038/s41576-019-0119-1

Ancient pathogen DNA

Pathogen aDNA detection

These approaches enable the assessment of aDNA authenticity and can be followed by whole pathogen genome retrieval through targeted enrichment or direct sequencing of positive sample libraries.



Spyrou et al. (2019)
https://doi.org/10.1038/s41576-019-0119-1

Ancient pathogen DNA

PNAS

Genomic blueprint of a relapsing fever pathogen in 15th century Scandinavia

Meriam Guellil[a,1], Oliver Kersten[a], Amine Namouchi[a], Egil L. Bauer[b], Michael Derrick[b], Anne Ø. Jensen[b], Nils C. Stenseth[a,1], and Barbara Bramanti[a,c,1]

[a]Centre for Ecological and Evolutionary Synthesis, Department of Biosciences, University of Oslo, N-0316 Oslo, Norway; [b]Norwegian Institute for Cultural Heritage Research, N-0155 Oslo, Norway; and [c]Department of Biomedical and Specialty Surgical Sciences, Faculty of Medicine, Pharmacy and Prevention, University of Ferrara, 35-441221 Ferrara, Italy

Contributed by N. C. Stenseth, August 13, 2018 (sent for review May 4, 2018; reviewed by Sally J. Cutler and Albert R. Zink)

Guellil *et al.* (2018)

https://doi.org/10.1073/pnas.1807266115

Osteoarchaeologist:

One individual had been marked as a potential **case of leprosy**.

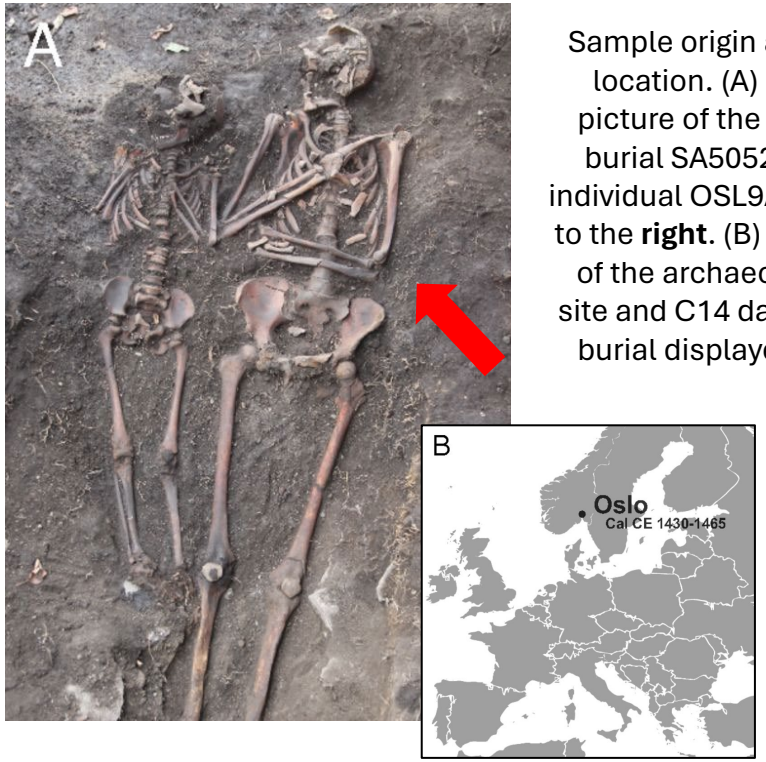
The skeleton displayed distinctive pathogenic lesions on its lower limbs. The skull was recovered heavily fractured, which made the confirmation of facial leprotic lesions impossible.

Sampling:

Two well preserved molars from each of nine individuals were sampled on the field. One exception was an incisor for one individual.

Geneticist:

No reads corresponding to *Mycobacterium leprae* were identified, but reads matching *Borrelia recurrentis* were detected.



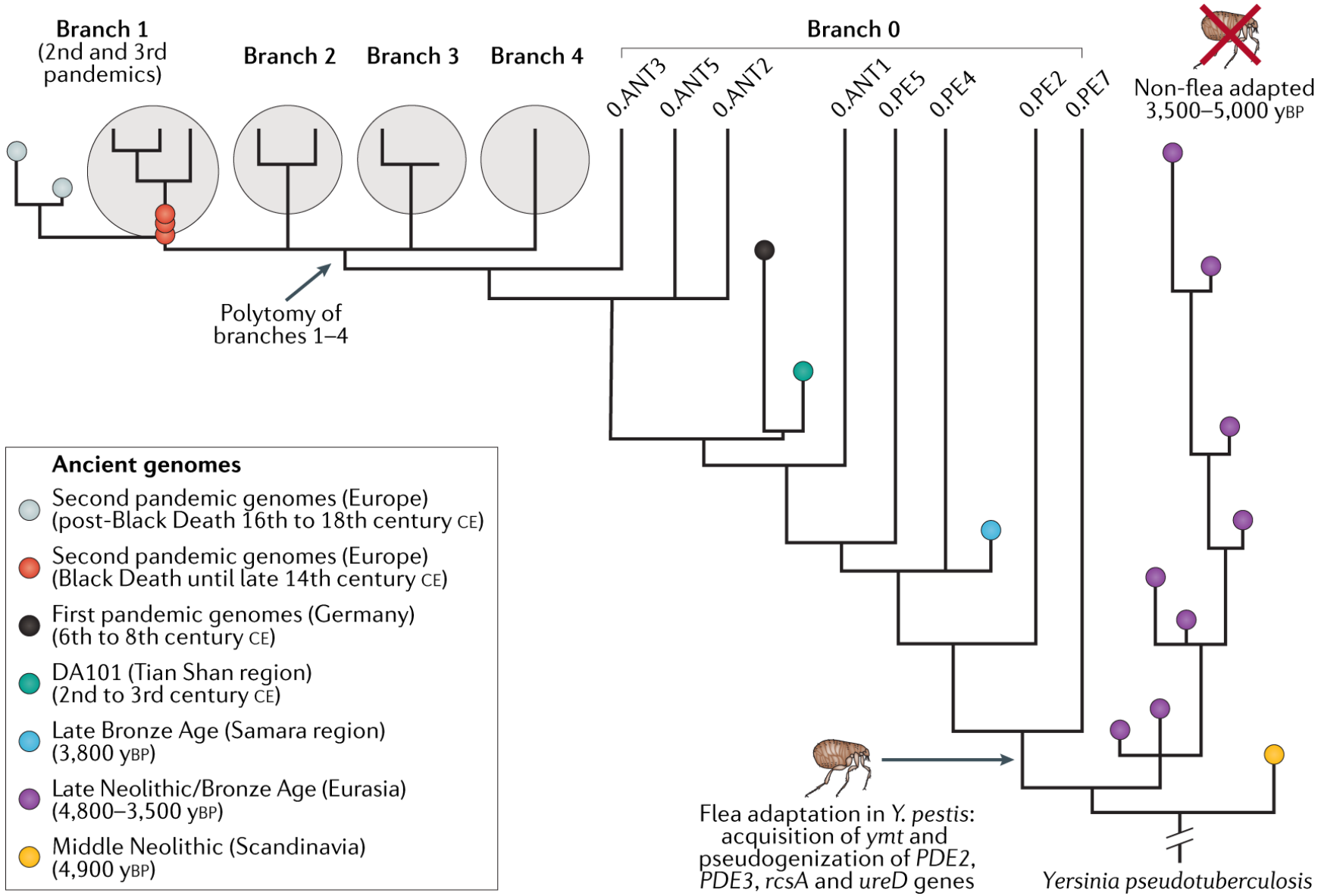
Sample origin and site location. (A) In situ picture of the double burial SA50521 with individual OSL9/SZ50522 to the **right**. (B) Location of the archaeological site and C14 date of the burial displayed in A.

The discovery of *Borrelia recurrentis* in a 15th-century individual from Norway suggests that, in addition to Yersinia pestis, other vector-borne pathogens were also circulating in medieval Europe.

Ancient pathogen DNA

A phylogenetic tree graphic depicting the evolutionary history of *Yersinia pestis* based on both ancient and modern genomes.

Ancient strains that have been previously characterized by phylogenetic analysis are represented with colored circles among the tree branches



Spyrou et al. (2019)
https://doi.org/10.1038/s41576-019-0119-1

Parasite analysis

Paleoparasitology is a discipline that studies human parasites.

Paleopathologies, hygiene, food habits, or waste management are among topics that can be addressed by paleoparasitology.

It also allows to better understand the history of parasites and the evolution of the host-pathogen relationships.

Modern humans for instance are known to be potentially infected by **179 eukaryotic parasites**, including 35 specific ones

The emphasis is generally on **gastrointestinal parasites**, which can be recovered from soil samples taken from ancient graves, where remnants of most intestinal parasites can persist for thousands of years.

Parasite analysis

Sampling

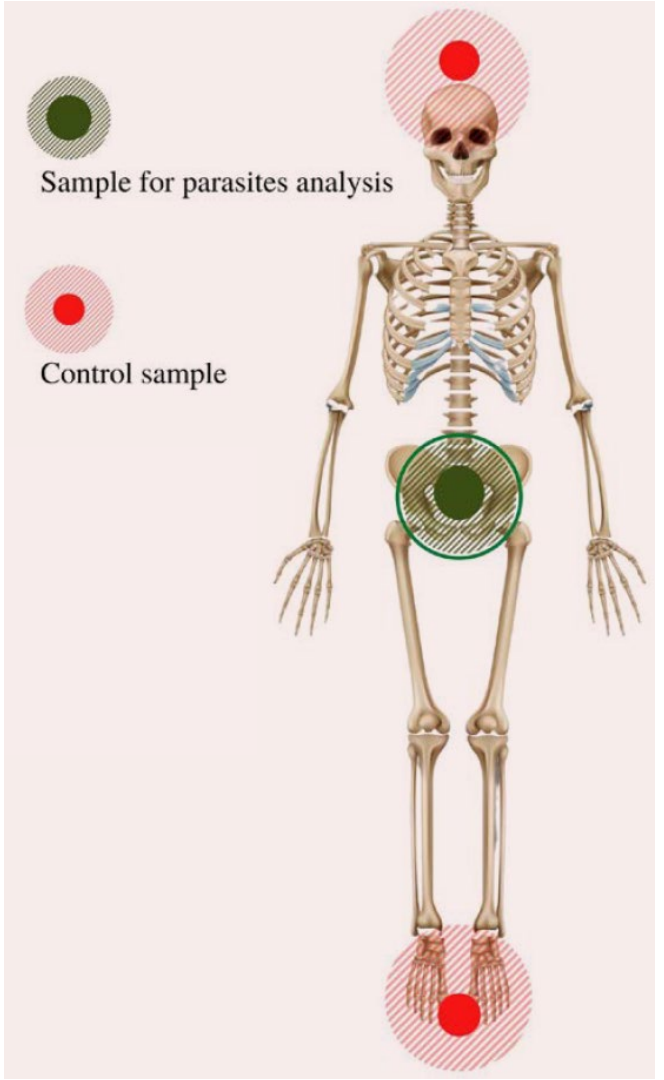
The sampling strategy in the field is simple and samples are taken by the archaeologist or the anthropologist during the excavation.

When collecting samples, concentrate on the pelvic girdle area, excluding any extraneous topsoil or soil not directly linked to the skeleton.

In paleoparasitology from graves, maintaining the archaeological integrity of samples is crucial, necessitating the inclusion of control samples. At least two are required: one from under the skull and one from under the feet.

Use a clean spoon to fill the container with 20-100 grams of sediment for multiple analyses.

Radini (2023)



Location of samples for paleoparasitology

Parasite analysis

The identification of parasites in ancient contexts is possible through the observation of preserved markers that can be classified into 3 categories: **macroremains**, **dissemination and reproduction forms**, and **biomolecules**.

1st category: it corresponds to the body parts of adult worms or larvae. Observations of digestive parasite macroremains are rare.

2nd category: it includes eggs produced by parasitic helminths during reproduction and cysts of parasitic unicellular organisms. Examples of preserved cysts are very rare.

3rd category: it refers to parasite-specific biomolecules, namely antigens (immunology) and nucleic acids (ancient DNA).

Parasite analysis - Macroremains

Forensic Sci Med Pathol (2016) 12:113–114
DOI 10.1007/s12024-015-9722-4

LESSONS FROM THE MUSEUM

https://doi.org/10.1007/s12024-015-9722-4

Schistosomiasis in the mummified viscera of Saint-Louis (1270 AD)

Philippe Charlier[1] · Françoise Bouchet[2] · Raphaël Weil[3] · Bruno Bonnet[4]

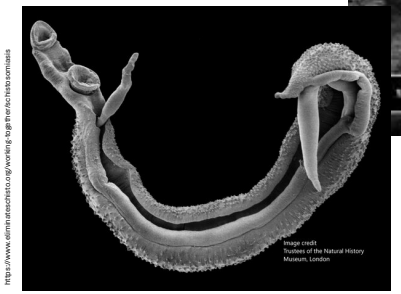
Microscopic analysis of embalmed viscera from Louis IX, King of France from 1226 to 1270 AD, revealed various parasitic formations, including adult male *Schistosoma* worms, indicating a diagnosis of schistosomiasis.

When adult worms are present, the eggs they produce typically migrate to the intestine, liver, lungs or bladder, leading to fever, chills, cough, muscle ache, inflammation, cancer, and/or scarring.

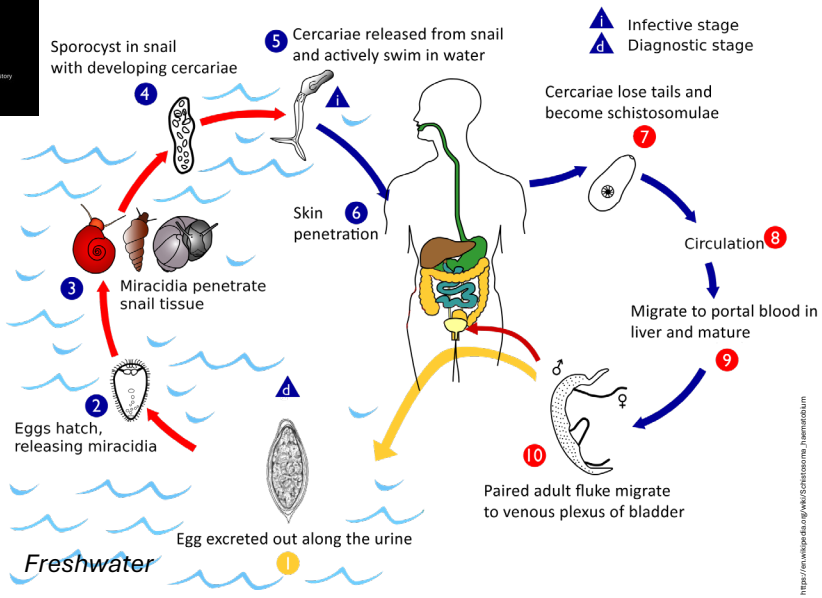
Symptoms of schistosomiasis are caused by the body's reaction to the eggs produced by worms, not by the worms themselves.

Gross aspect of the St Louis embalmed viscera material under binocular lenses (magnification ×40)

One adult Schistosoma worm from the St Louis mummified viscera under SEM examination (magnification ×1700)



Modern adult Schistosoma worm



Life cycle of *S. haematobium*

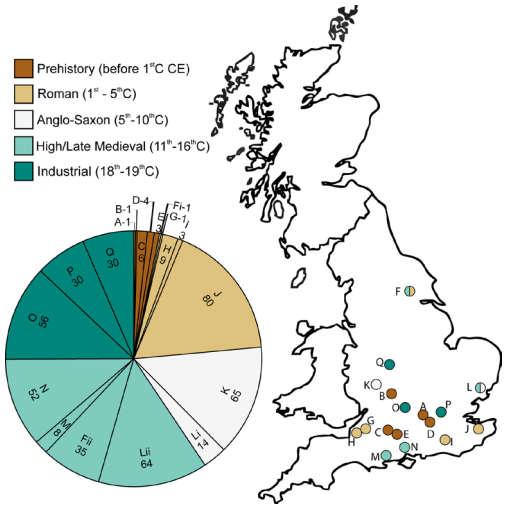
Parasite analysis - Reproduction forms

Helminth species (worms), capable of producing thousands of microscopic eggs per day, and each host being possibly infected by hundreds of worms.

Helminth eggs are identified based on morphological criteria (shape, the presence or not of an operculum, or the ornamentation sometimes present on the shell), and their dimensions (essentially length and width).

A general overview in the UK suggests that the impact of helminth infections on past populations fluctuated over time, with certain regions experiencing a significant decrease in parasite prevalence during the industrial era (18th-19th century).

The changing prevalences of nematode infections can be attributed to changes in effective sanitation and the presence of cestode infections reflect dietary practices.



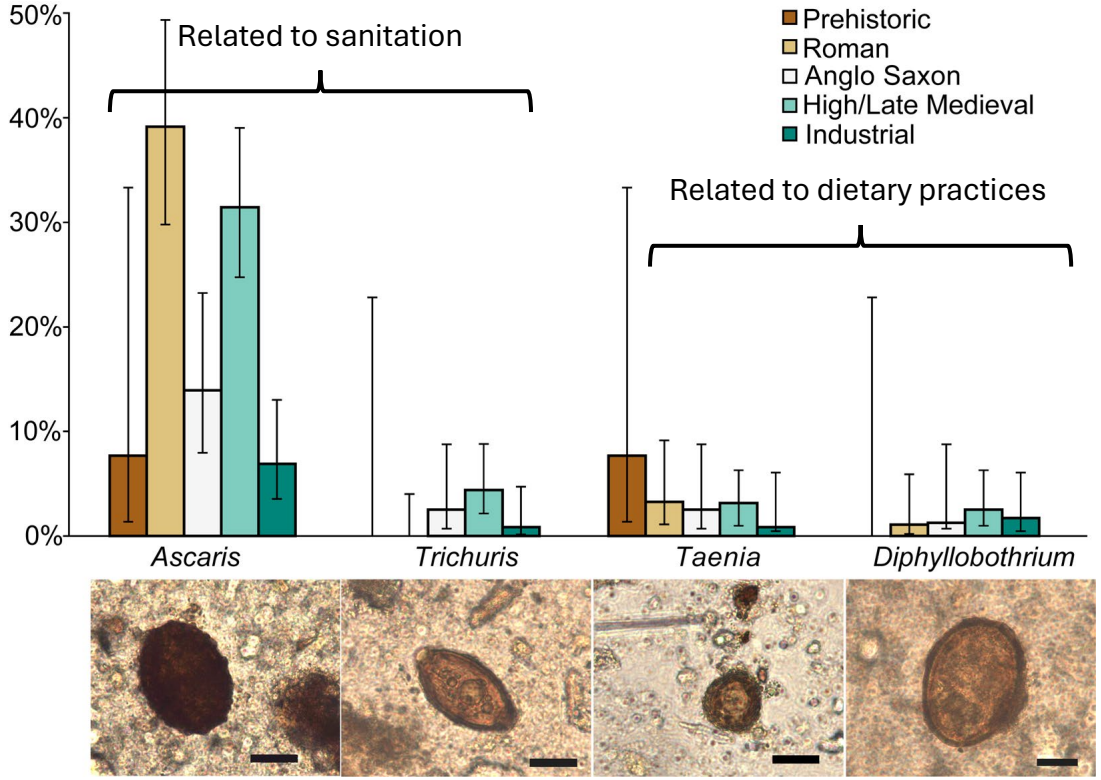
PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Reconstructing the history of helminth prevalence in the UK

Hannah Ryan[1], Patrik G Flammer[2], Rebecca Nicholson[3], Louise Loe[3], Ben Reeves[4], Enid Allison[5], Christopher Guy[6], Inés Lopez Doriga[7], Tony Waldron[8†], Don Walker[9], Claas Kirchhelle[10], Greger Larson[1], Adrian L Smith[2*]

https://doi.org/ 10.1371/journal.pntd.0010312



Parasite analysis - Biomolecules

Sediment samples were collected from the abdominal cavities of 18 out of the 75 individuals discovered at the Uffizi Gallery (Florence, Italy) to search for intestinal parasites.

5 individuals showed *Ascarid*-type specimens, most probably related to *Ascaris lumbricoides*.

A targeted aDNA approach allowed the authors to detect two nematodes and one trematode aDNA fragments, namely *Ascaris sp.* (confirmation), *Trichuris trichiura*, and *Dicrocoelium dendriticum* (discovery).

This suggests a very widespread parasitic burden related to the faecal pollution of soils, drinking water and food in ancient Florence, including co-infection by possibly debilitating nematodes (= making someone very weak and infirm).

This burden may have played a role in aggravating a mortality crisis that plagued this community.

Infection, Genetics and Evolution 90 (2021) 104713

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

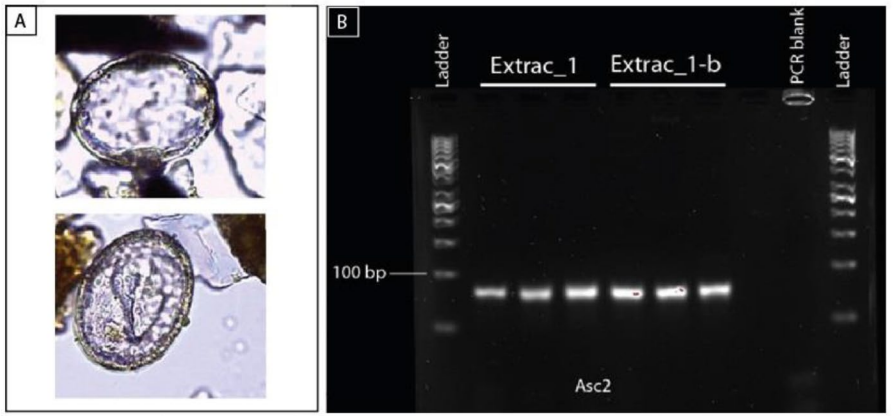
journal homepage: www.elsevier.com/locate/meegid

Research paper https://doi.org/10.1016/j.meegid.2021.104713

Gastrointestinal parasite burden in 4th-5th c. CE Florence highlighted by microscopy and paleogenetics

Kévin Roche [a,b,*], Nicolas Capelli [b], Elsa Pacciani [c], Paolo Lelli [d], Pasquino Pallecchi [c], Raffaella Bianucci [e,f,g], Matthieu Le Bailly [b,*]

Multiple grave of epidemic origin from Uffizi Gallery, Florence, Italy (4th-5th c. AD)



Targeted species	Targeted gene	Size of amplicons (bp)	Obtained sequence	Homology after BlastN
A. lumbricoides *A. suum*	Cytochrome b (cytb)	74	GGTATGGTTTTGGGTTT TCAGATTTTGACTGGTA CTTTTTTGGCTTTTTATT ATTCTAATGATGGTGCT TTGGC	100%

Obtained amplicons from 6 PCRs reactions in 2 sediment DNA extractions showing a clear positive reaction for *Ascaris sp.*

Radiocarbon dating

Isotopes are atoms of a chemical element with different masses.

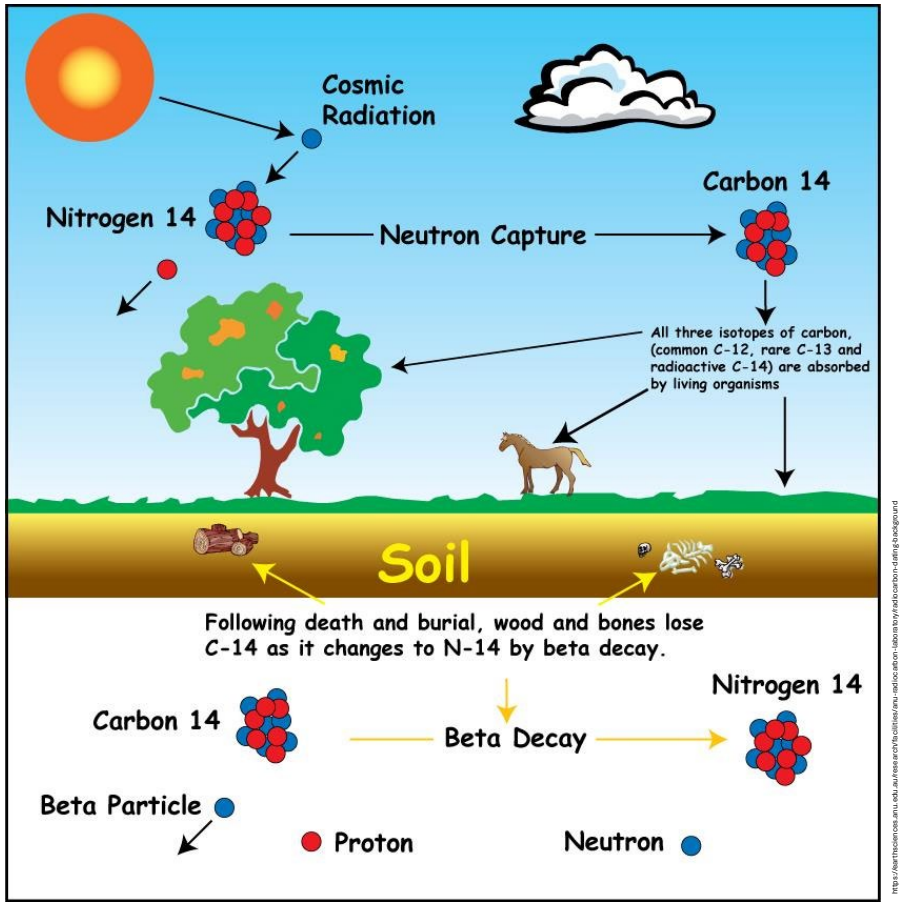
Some are radioactive and steadily decay, transmuting into other elements. This is the case of the radiocarbon (^{14}C).

^{14}C has a half-life of 5,730±40 years and can date object up to 55,000 year before present.

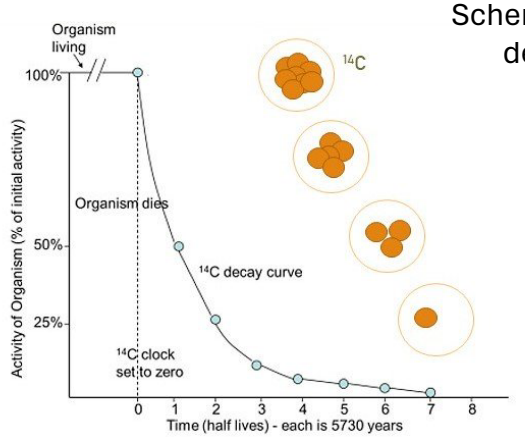
When a plant, human, or animal dies, it no longer takes in ^{14}C and thus, over time, the proportion of ^{14}C falls at a rate that is determined by the law of radioactive decay.

By measuring the proportion of ^{14}C that remains, it is possible to estimate the time since the organism died.

Due to the varying production of radiocarbon in the atmosphere, a year in radiocarbon age does not correspond directly to an interval in the calendar timescale. Thus, calibration is necessary.



Schematic of ^{14}C production and decay in the atmosphere.



Decay of ^{14}C

Radiocarbon dating

While unburnt human bone is frequently employed for radiocarbon dating, the dating of tooth (and preserved soft tissues) is also viable.

Collagen serves as the primary matrix for ^{14}C dating due to established quality criteria ensuring date reliability. However, in cases where collagen preservation is lacking, dating the apatite is also feasible.

Calcined bones, devoid of organic carbon due to high temperatures, can now be dated by analyzing the inorganic bone matrix (re-crystallized bio-apatite). This advancement allows dating of this prevalent burial practice during its historical dominance.

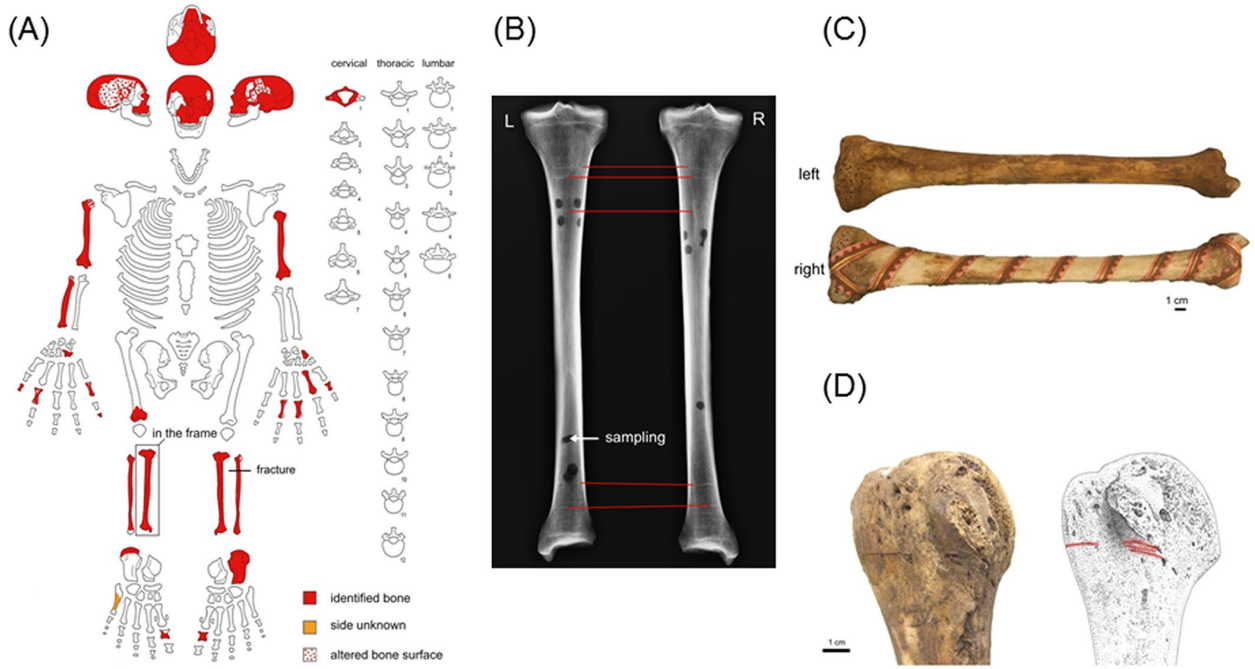
Typically, bone or tooth sampling is conducted within the laboratory. Samples are cut using a rotary tool, labeled, documented via the registration form of the 14C laboratory (typically online), and then shipped.

Unburnt vs burnt bones

Radiocarbon dating

Results of anthropological, isotopic and genetic analyses provided evidence that the likelihood that the remains found in the reliquary are those of Jacques de Vitry is very high.

Especially, the 4 ^{14}C dates are very similar and gave an average date of 919±13 BP, which led to a skeleton date between 1040 and 1170AD (95.4% probability) after calibration.



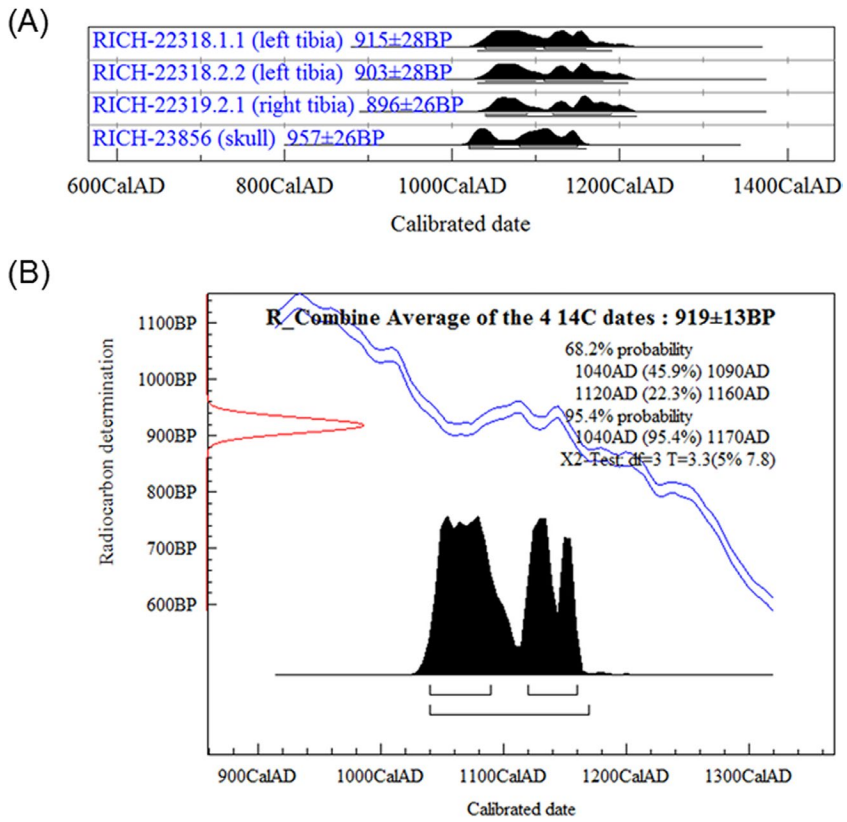
PLOS ONE

OPEN ACCESS PEER-REVIEWED
RESEARCH ARTICLE

https://doi.org/10.1371/journal.pone.0201424

An interdisciplinary study around the reliquary of the late cardinal Jacques de Vitry

Ronny Decorte, Caroline Polet, Mathieu Boudin, Françoise Tilquin, Jean-Yves Matroule, Marc Dieu, Catherine Charles, Aurore Carlier, Fiona Lebecque, Olivier Deparis



(A) Calibrated ages (2σ) of the left tibia, right tibia and skull. (B) Average age of the four 14C dates determined for these remains.

Stable isotope analysis

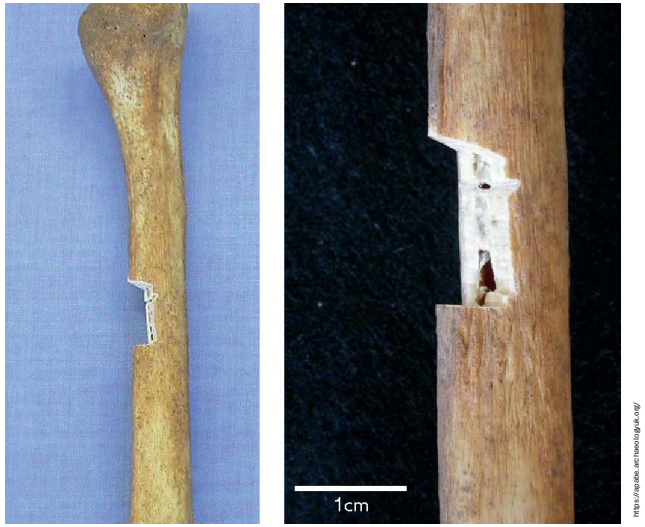
Most chemical elements exist as mixtures of two or more stable isotopes. For some elements, the stable isotope ratios differ in different classes of foods, and these differences are passed on to the tissues of the consumer.

Hence measurement of stable isotope ratios in skeletal remains can be used to study ancient diets. The most widely used elements in this respect are carbon and nitrogen.

Dietary stable isotope studies normally focus on collagen from bone or tooth dentine.

Researchers often find it useful to have local isotopic values from archaeological faunal remains (and plant remains if possible), to provide a baseline to help interpret the human data.

Enamel is drilled for stable carbon analysis



A sample for carbon and nitrogen stable isotope analysis has been removed from the shaft of the bone.

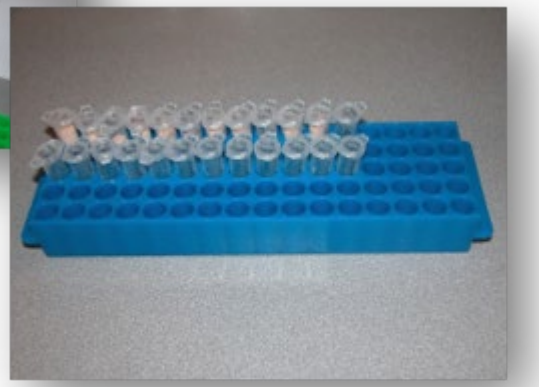
Stable isotope analysis

Enamel preparation for isotope analysis.



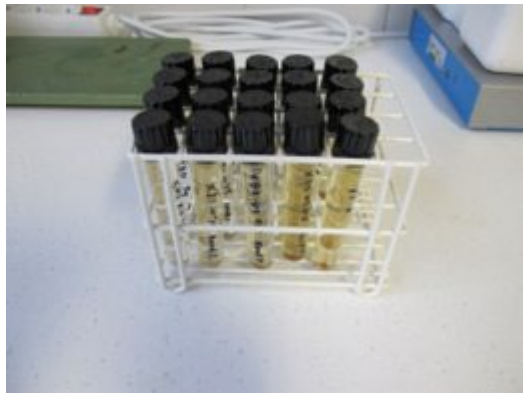






Stable isotope analysis

Collagen preparation for isotope analysis.



Stable isotope analysis

Isotope Ratio Mass Spectrometers (IRMS).

Stable isotope analysis

Dentine

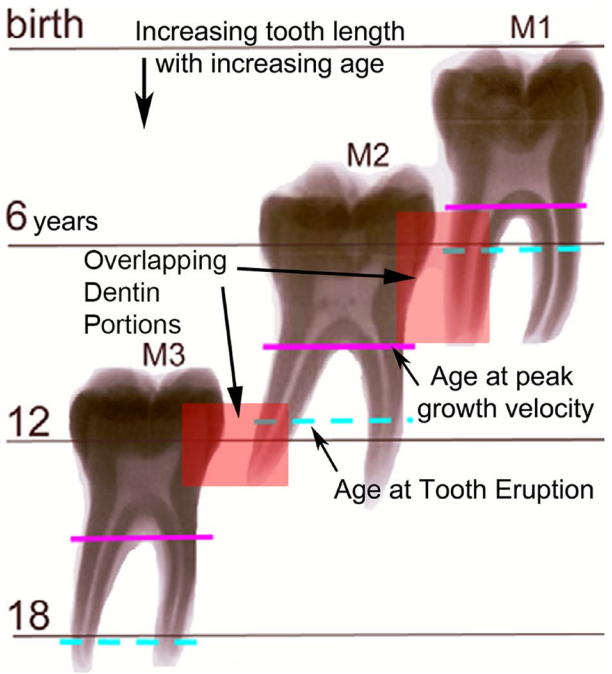
Dentine is not as completely mineralized as enamel (95 % HA). Its chemical composition is more similar to bone.

We can extract collagen.

We need c. 0.3 mg for C-N isotope analysis, and c. 1.2 mg for synchronous C-N- S isotope analysis.

It is possible to obtain a high number of time-series measurements (up to 15-20) from a single adult human tooth.

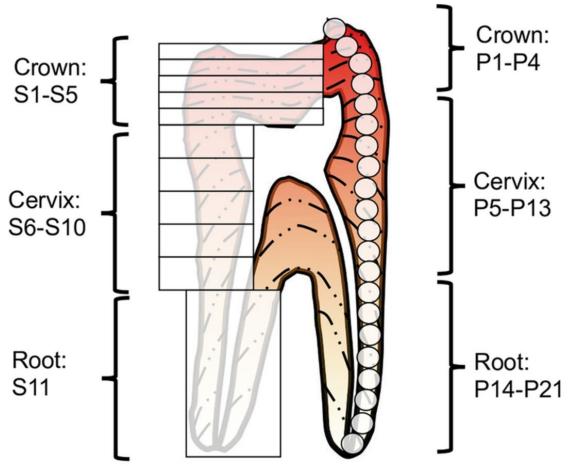
There are two common approaches for sequential sampling of human dentine: micro-punches vs. micro-slices



Scharlotta et al. (2018)
https://doi.org/10.1002/ajhb.23163

Sequence of initiation of crown and root mineralization through to root completion compared for 3 molars, with approximate ages (years). Overlapping portions of dentin highlighted. Age at peak growth velocity and tooth eruption times following Dean and Cole (2013).

(A)



Schematic of the sampling site. P refers to micro-punches; S refers to micro-slices. A total of 21 micro-punches and 11 micro-slices are obtained from this tooth.

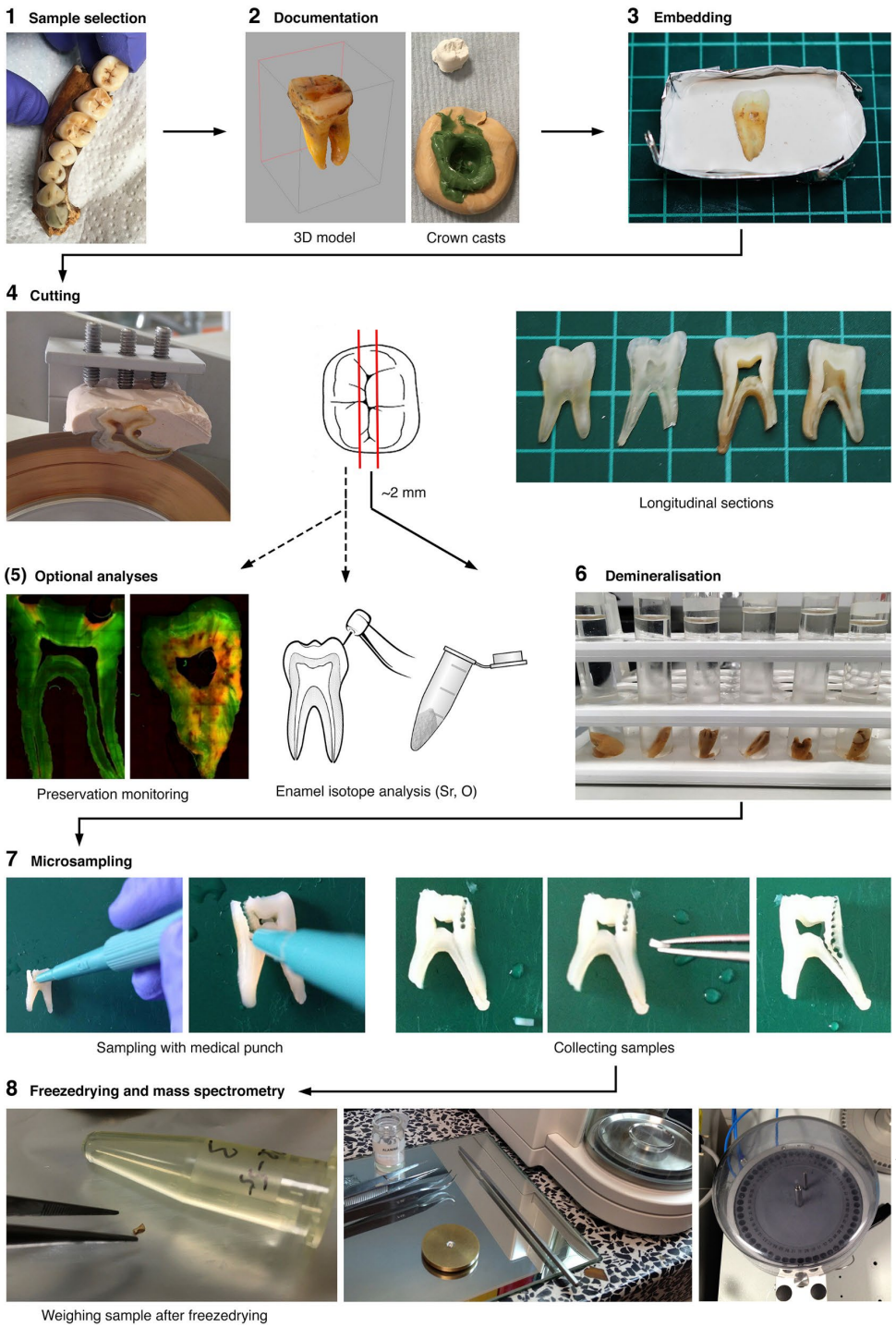
Cheung et al. (2022)
https://doi.org/10.1002/rcm.9380

Stable isotope analysis

Dentine

Solubilized collagen sample from dentine.

Cheung et al. (2022)
https://doi.org/10.1002/rcm.9380



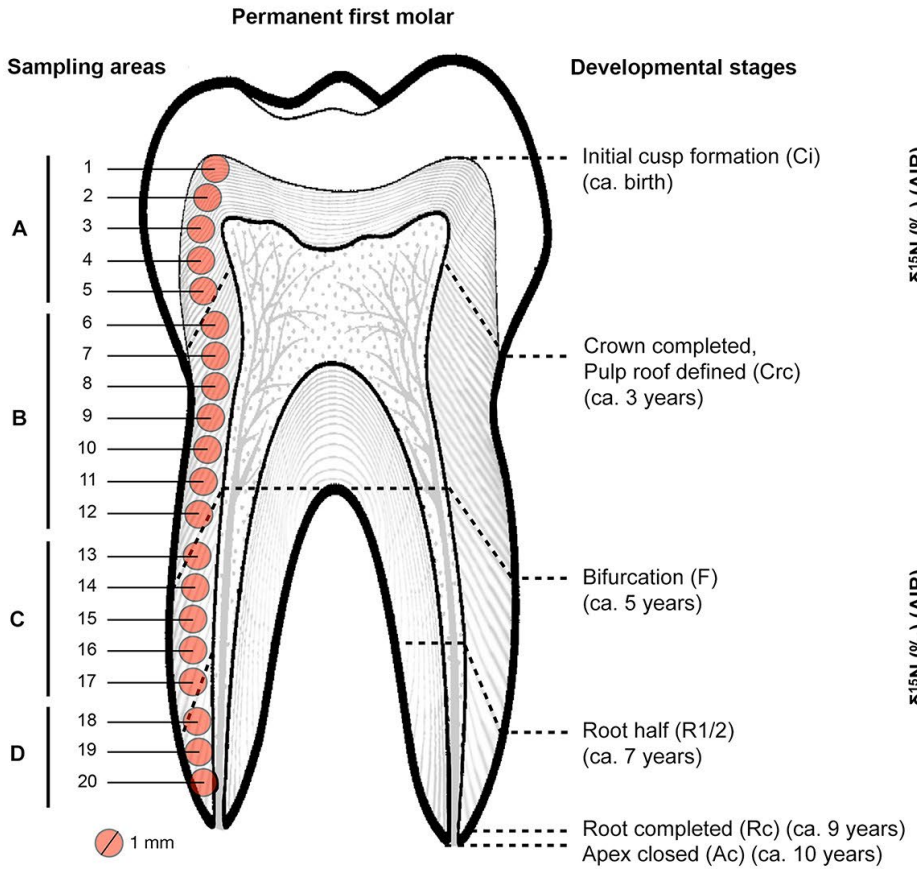
https://doi.org/10.1002/ajpa.24126

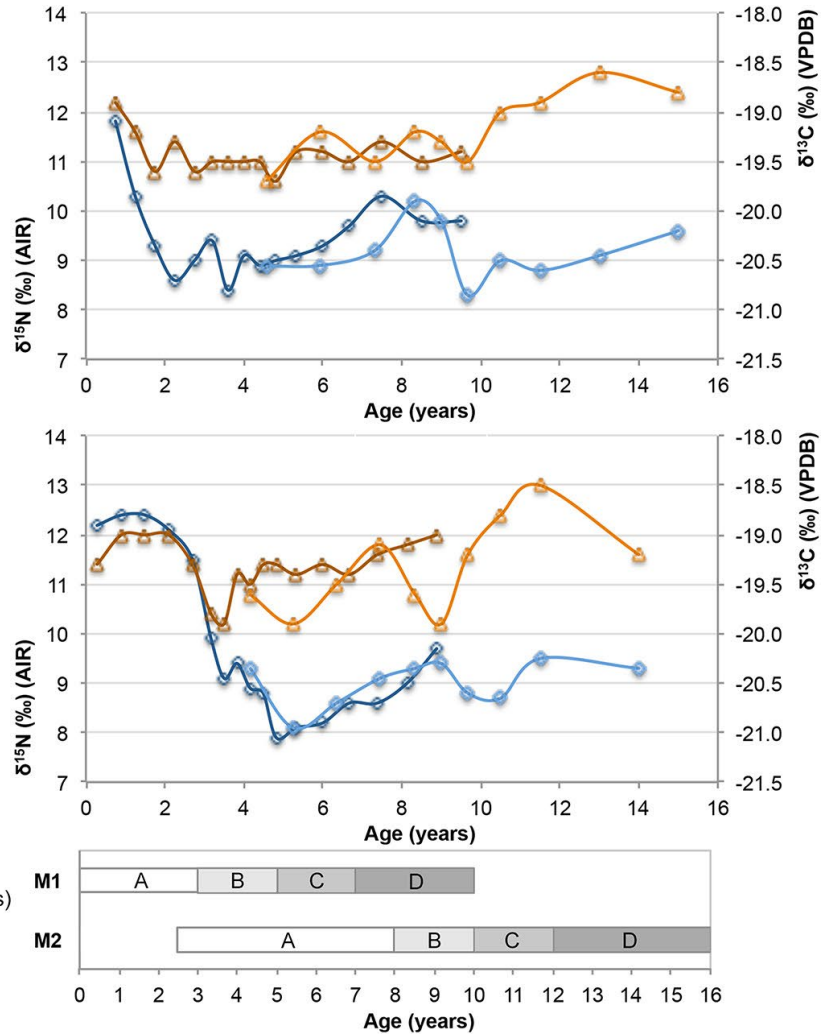
Microsampling workflow proposed by Czermak et al. (2020).

Stable isotope analysis

Dentine

Sampling map and exemplary display of isotope analyses. Left panel: Scheme for taking microsamples from anatomical areas (M1, AlQahtani et al., 2010). Right panel: Examples for age alignment of isotope values measured of samples taken from anatomical areas indicated in the left panel. Data plots of dentine collagen $\delta^{13}C$ (orange triangles) and $\delta^{15}N$ (blue squares) profiles of first (M1, darker color) and second (M2, lighter color) molars from two individuals. The bottom graph illustrates the correspondence between anatomical areas and tooth developmental ages used to assign age to each dentine sample



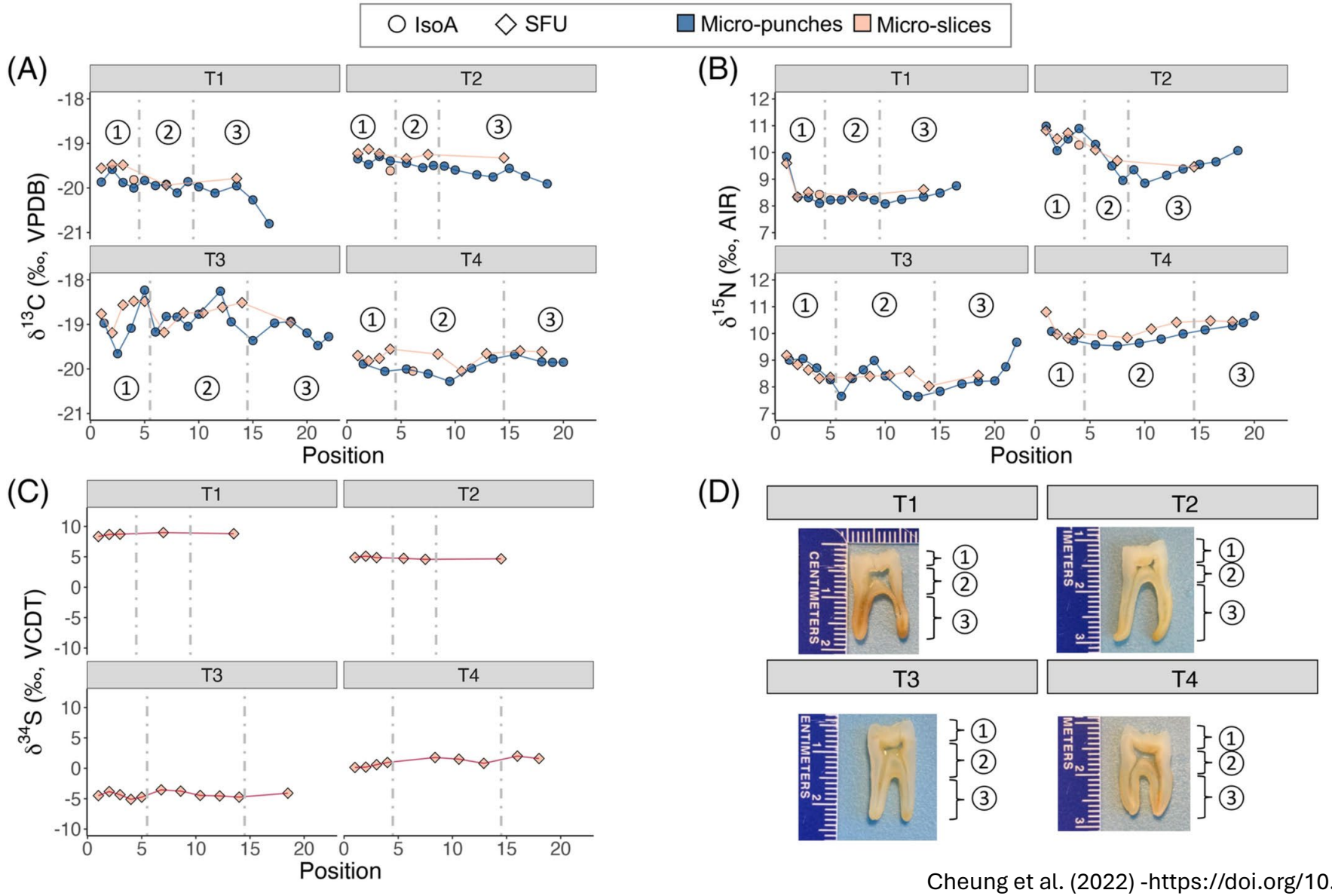


Czermak et al. (2020) - https://doi.org/10.1002/ajpa.24126

Stable isotope analysis

Stable isotope compositions of all four Early Modern teeth (T1–T4). The subsections, indicated by ①, ②, and ③, refer to the three anatomical sections: crown, cervix, and roots, respectively. developmental ages used to assign age to each dentine sample

Dentine

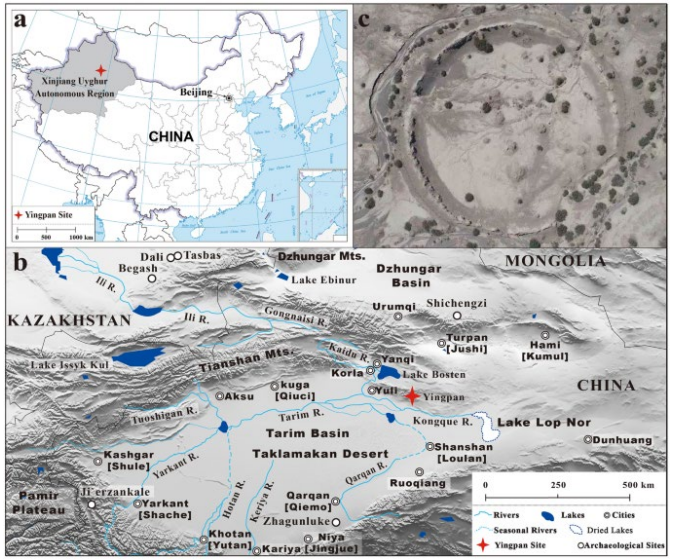


Cheung et al. (2022) -https://doi.org/10.1002/rcm.9380

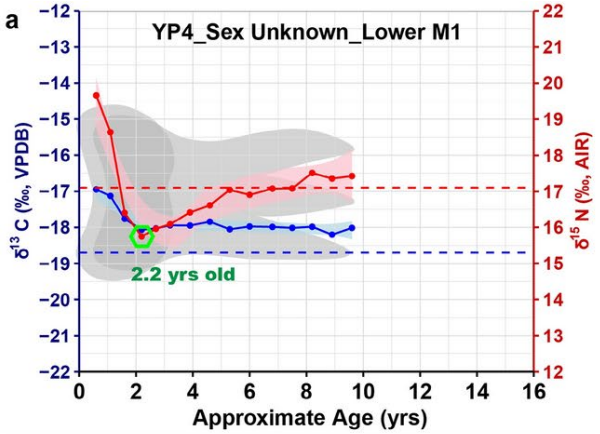
Stable isotope analysis

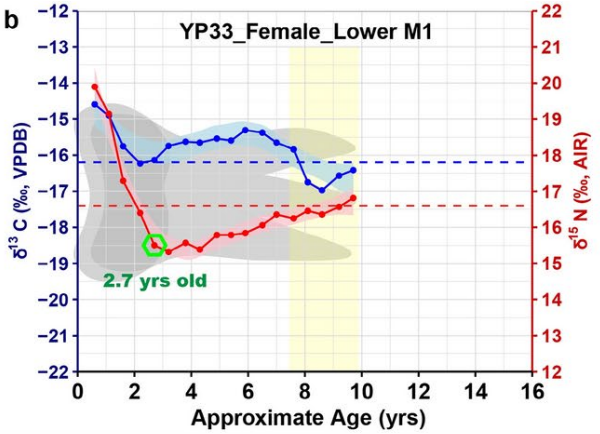
Dentine

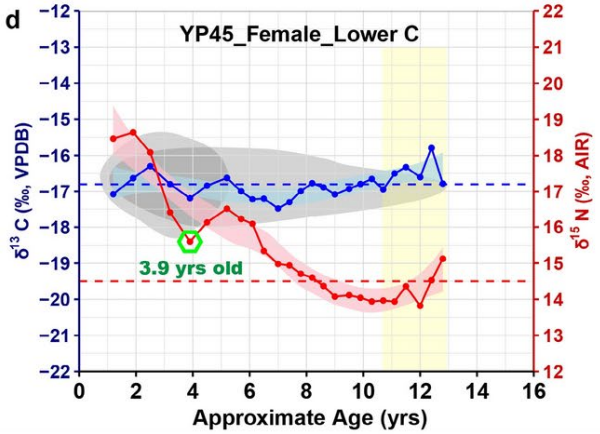
$\delta^{13}C$ (blue) and $\delta^{15}N$ (red) results of serial dentine (solid line) and bone collagen (dashed line) from individuals of the Yingpan cemetery (~ 25–420 AD). Note: approximate age of breastfeeding cessation is labelled with green hexagon.

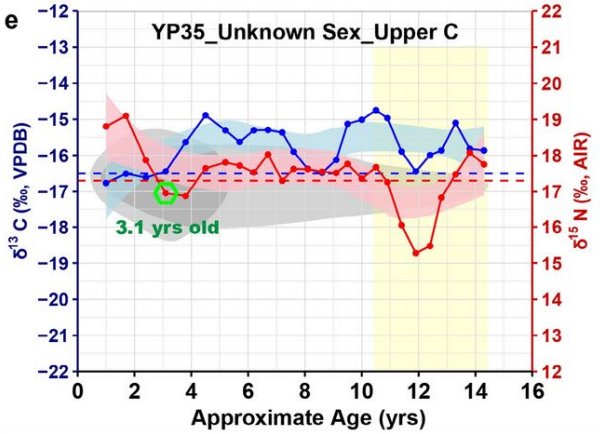


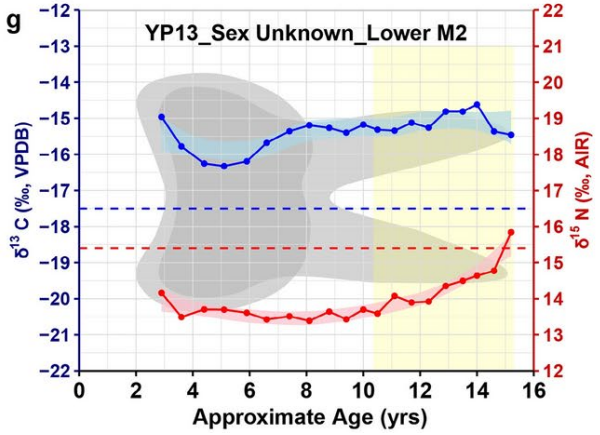
Wang et al.
https://doi.org/10.1038/s41598-022-24119-3

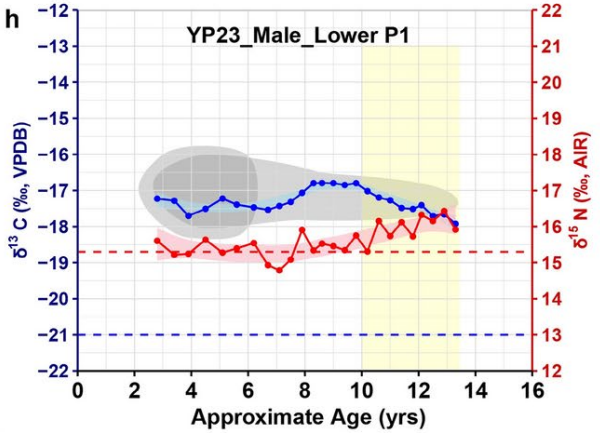












Stable isotope analysis

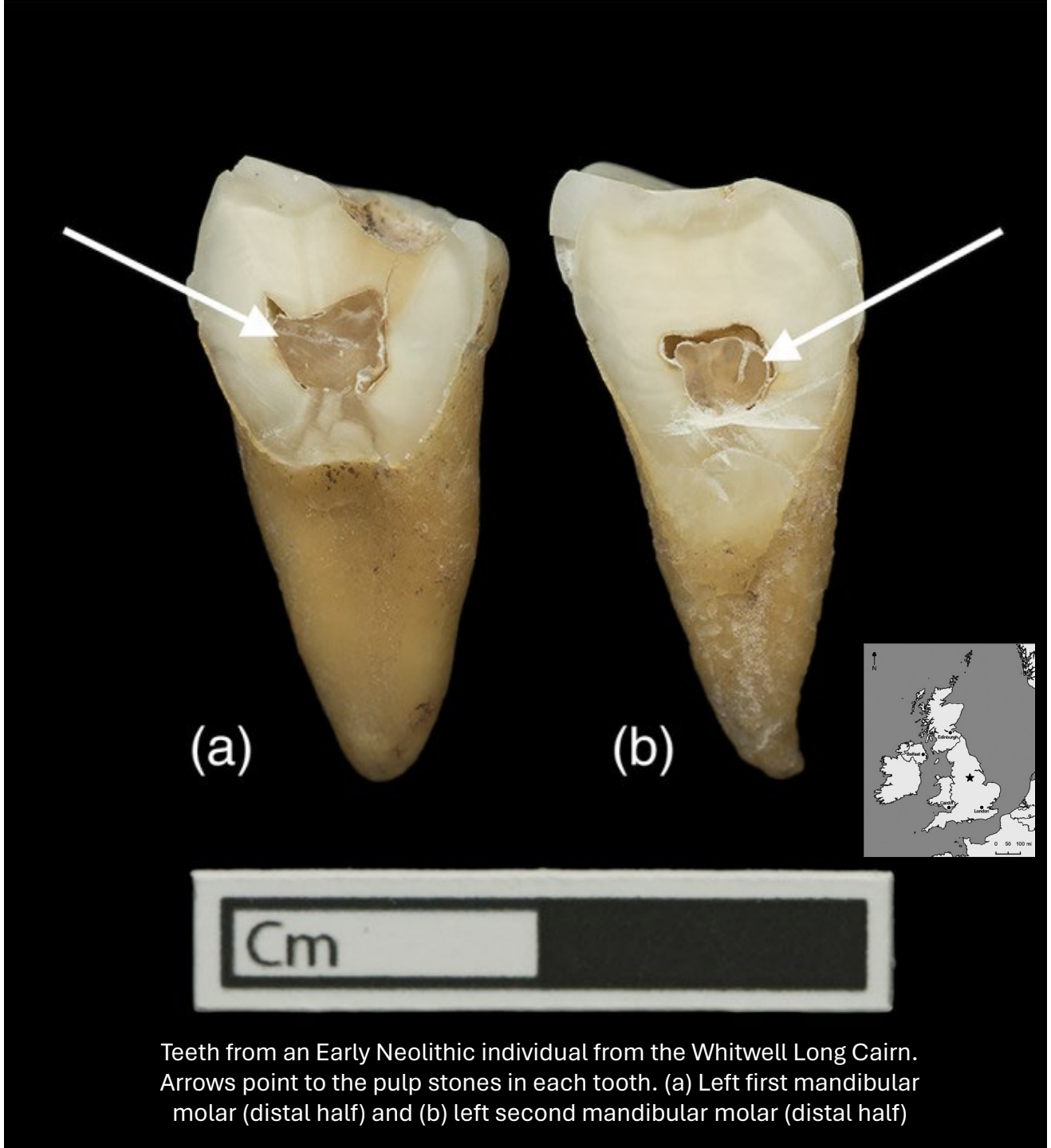
Pulp stone or ‘denticles’

Pulp stones are mineralized masses that can form within the dental pulp, and can occur in all types of teeth, but are most prevalent in molars.

Structurally, pulp stones consist of collagen and bioapatite.

Paleodietary information can be obtained. It has been tested on collagen.

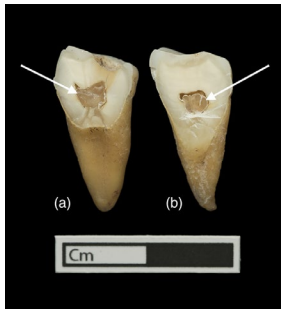
There are, however, significant challenges in interpreting this data, particularly as to inferring the timing and duration of their formation.



Teeth from an Early Neolithic individual from the Whitwell Long Cairn. Arrows point to the pulp stones in each tooth. (a) Left first mandibular molar (distal half) and (b) left second mandibular molar (distal half)

Ostrum et al. (2022)
https://doi.org/10.1002%2Fajpa.24479

Stable isotope analysis



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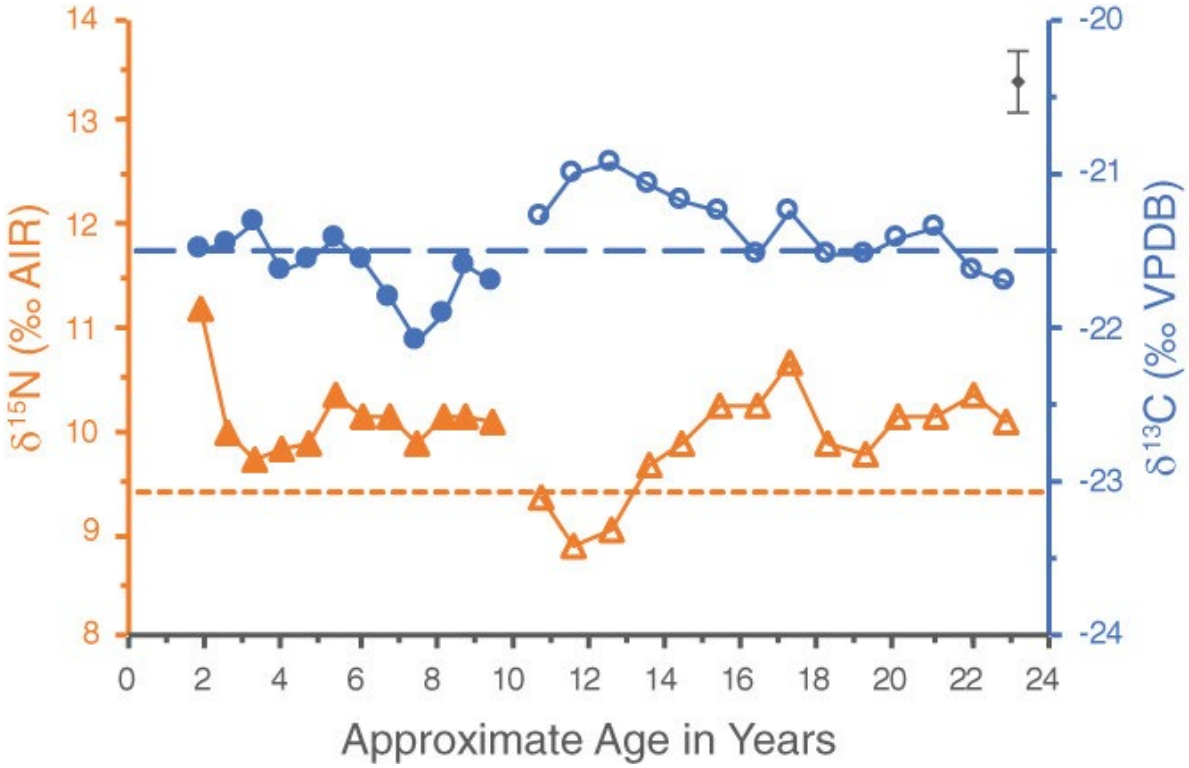


Incremental dentine series of WHIT 0219 comprised of the left first (closed symbols) and third (open symbols) mandibular molars. Circles represent $\delta^{13}C$ values, whereas triangles represent $\delta^{15}N$ values. The pulp stone, represented by the dashed lines, is from the first molar. The wider dashed line represents the pulp stone $\delta^{13}C$ value, and the narrower dashed line represents its $\delta^{15}N$ value.

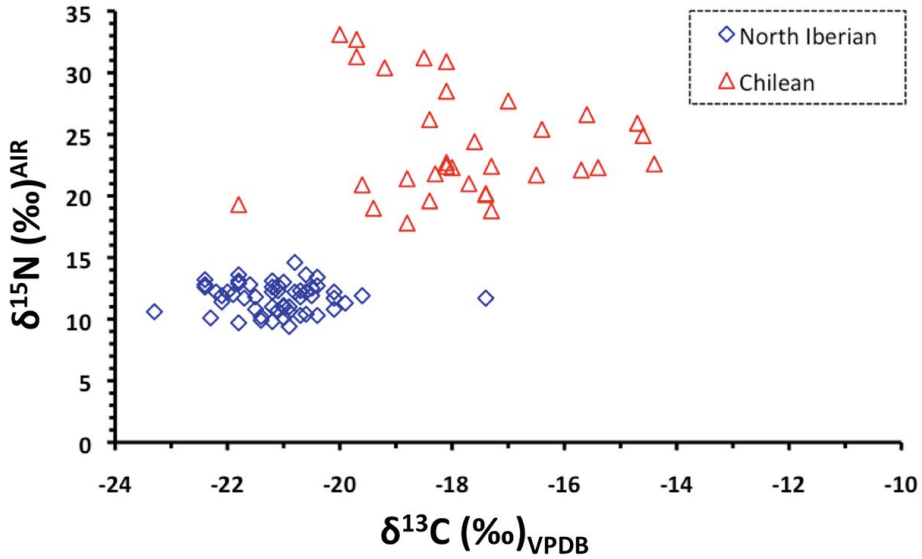
Ostrum et al. (2022)
https://doi.org/10.1002%2Fajpa.24479

Stable isotope analysis

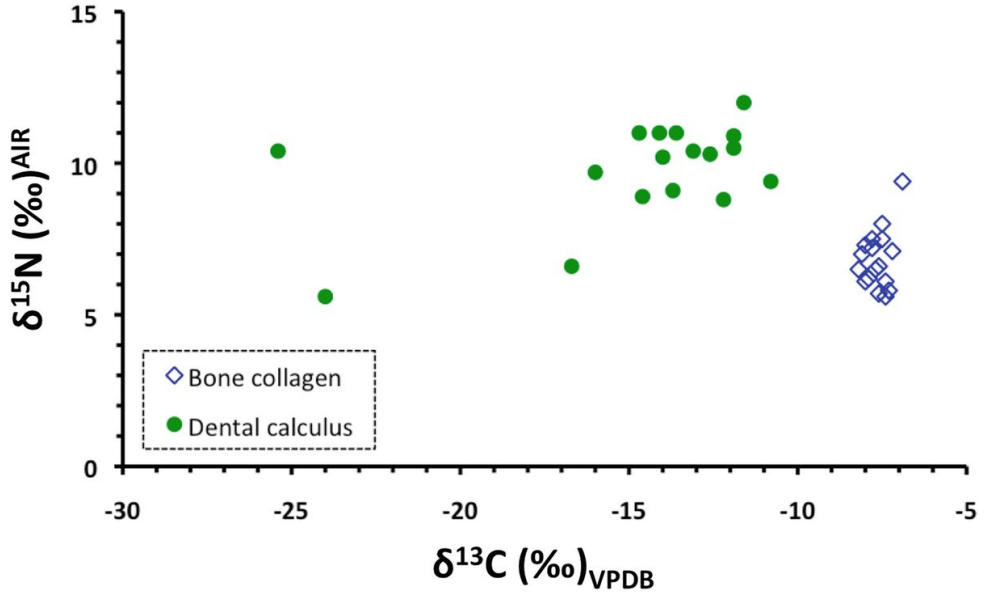
Calculus

There are many pitfalls in isotopic analysis of dental calculus. Numerous researchers have fruitlessly delved into this method.

Forget about it!



First studies: Plot of published bulk dental calculus $\delta^{13}C$ and $\delta^{15}N$ values from Northern Iberian and Chilean populations (Scott et al., 2013; Scott & Poulson, 2012).



Comparison of $\delta^{13}C$ and $\delta^{15}N$ values obtained from paired bulk dental calculus and bone collagen samples from a Basketmaker II population at the Grand Gulch site.

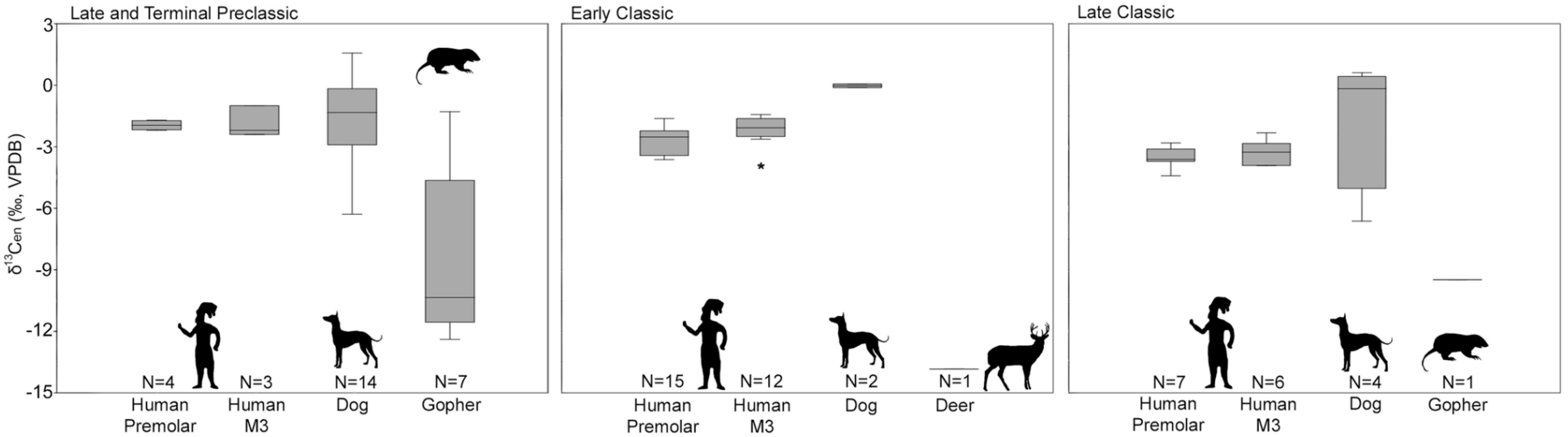
Note the lack of concordance between bulk dental calculus and bone collagen with respect to $\delta^{13}C$ and $\delta^{15}N$ values and sample isotopic variance

Salazar-García et al. (2023)
https://doi.org/10.1007/978-3-031-32268-6_6

Stable isotope analysis

Enamel

Human and dog dietary $\delta^{13}C_{enamel}$ are most similar during the Late/Terminal Preclassic period, but dog diets show elevated $\delta^{13}C_{enamel}$ in later periods. This may indicate that maize was more prevalent in dog diets, on average, compared to humans during the Early and Late Classic periods.



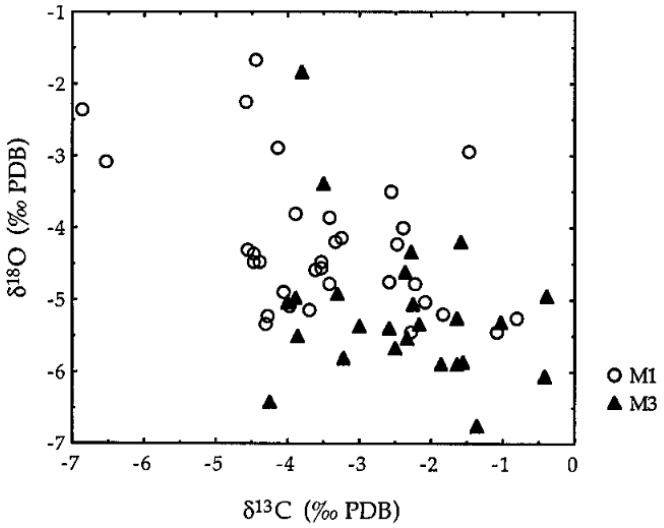
Map of the Maya region and the Maya Site of Kaminaljuyu, Guatemala (800 BCE-1525 CE).

Sharpe et al. (2022)
https://doi.org/10.1007/s12520-021-01498-8

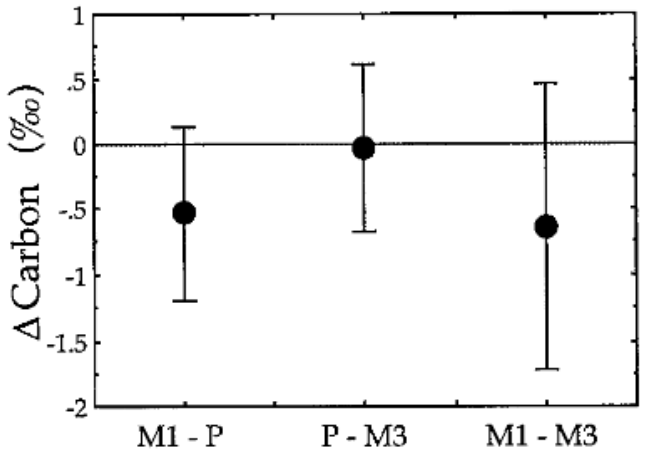
Stable isotope analysis

Enamel

Wright and Schwarcz (1998) comparing the $\delta^{13}C_{enamel}$ of multiple teeth within the same individual found that the $\delta^{13}C_{enamel}$ of the first molars of humans was generally lower than permanent second premolars and third molars, which have later enamel formation. The latter contain the $\delta^{13}C_{enamel}$ values after the introduction of solid foods into the diet, which largely consisted of maize.



Stable isotopic ratios of first molar and third molar enamel carbonate from Kaminaljuyu śkeletons for which more than two teeth were sampled.



Mean difference in stable isotopic composition between pairs of teeth. Stable carbon isotopic composition.

Map of the Maya region and the Maya Site of Kaminaljuyu, Guatemala (800 BCE-1525 CE).

Wright and Schwarcz (1998)
https://doi.org/10.1002/(SICI)1096-8644(199805)106:1<1::AID-AJPA1>3.0.CO;2-W

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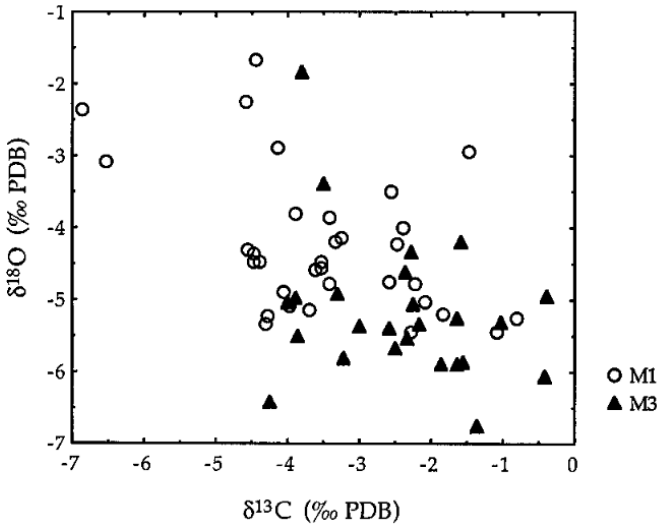

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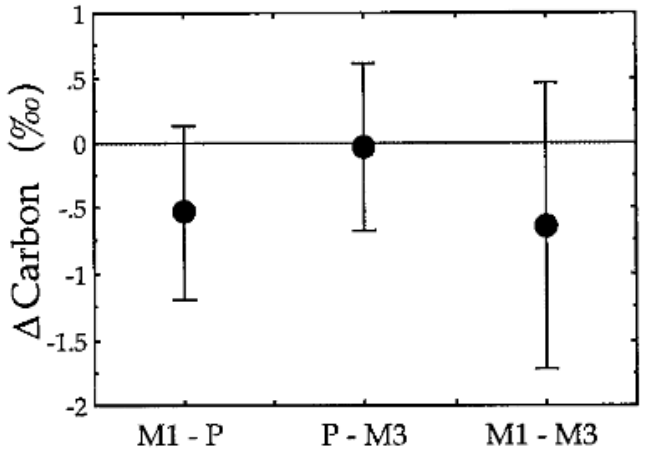

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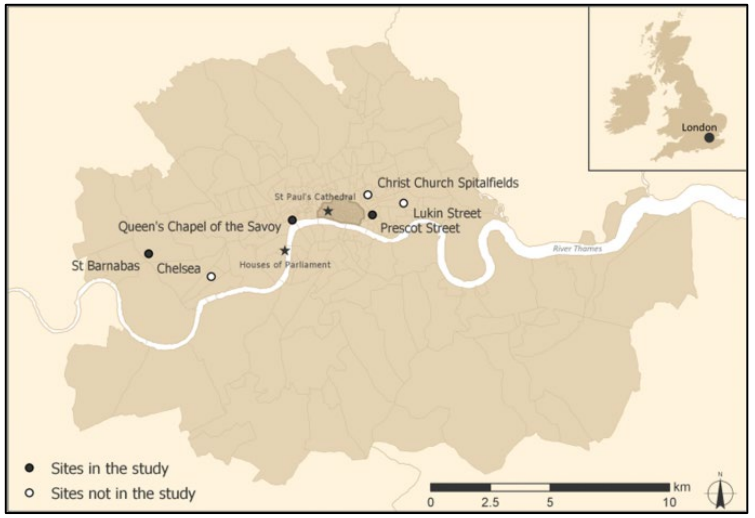
Stable isotope analysis

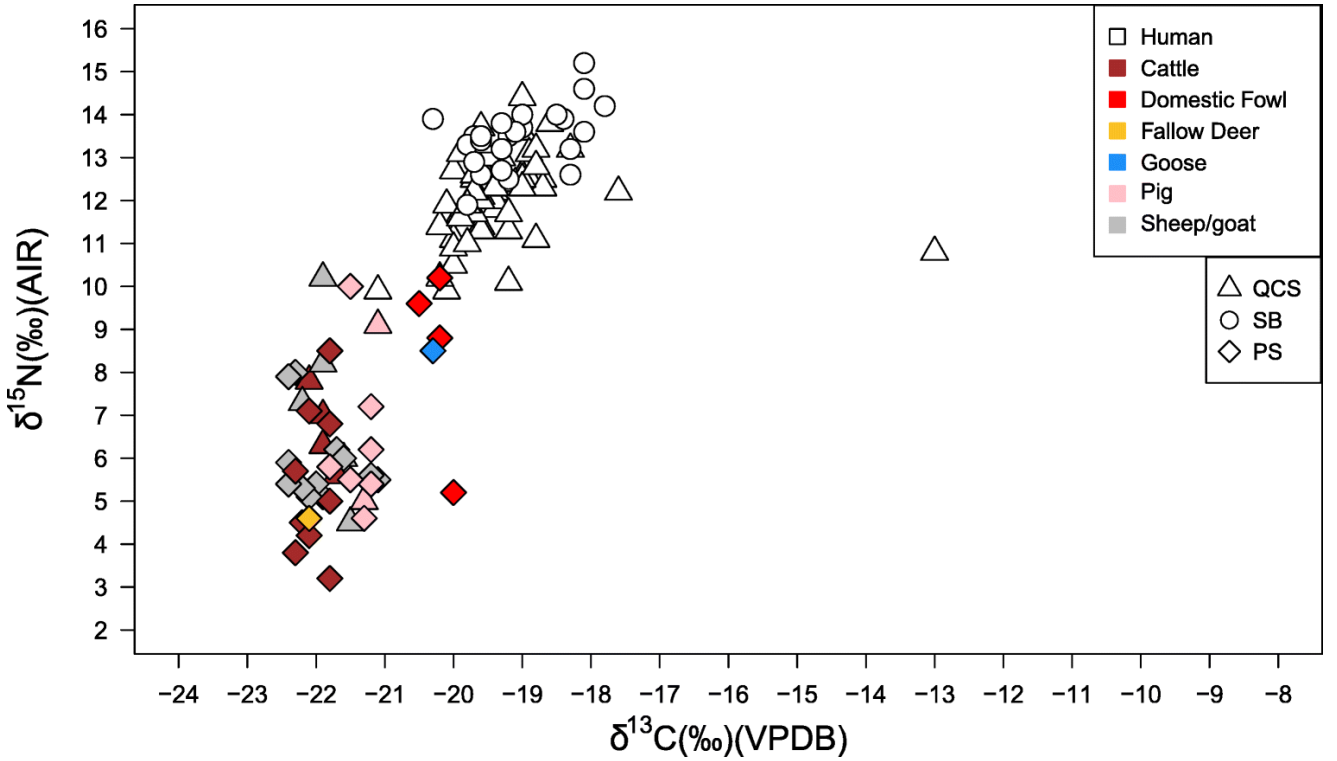
Bone

Overall, isotopic results suggest a largely C3-based terrestrial diet for both populations, with the exception of QCS1123 who exhibited values consistent with the consumption of C_4 food sources throughout childhood and adulthood.

The differences exhibited in $\delta 15N_{coll}$ across both populations likely reflect variations in diet due to social class and occupation, with individuals from SB likely representing wealthier individuals consuming larger quantities of animal and marine fish protein.

Map of Victorian London showing the locations of the sites of study, post-medieval sites mentioned in the text and landmarks. The city of London is shaded





Dietary investigation of post-medieval populations from London using stable isotopes

Bleasdale et al. (2019)
https://link.springer.com/article/10.1007/s12520-019-00910-8

Thank you