Ancient DNA

Next Generation Sequencing Analysis
DTU, 10/1/2025

Martin Sikora, PhD
Globe Institute
University of Copenhagen
martin.sikora@sund.ku.dk

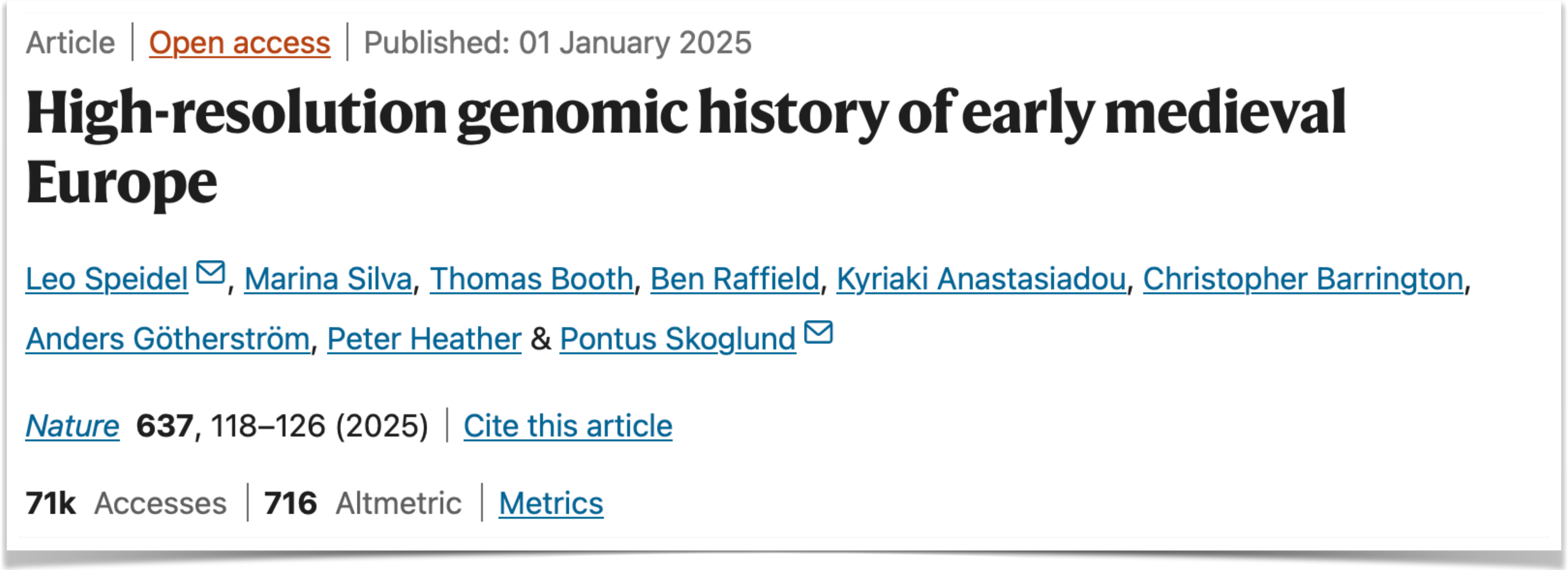
The week in ancient DNA

News > Science > Archaeology

DNA study reveals secrets of migrations across Europe 2000 years ago

Most of the migrations involved people speaking three main branches of Germanic languages

Vishwam Sankaran • Thursday 02 January 2025 11:11 GMT • Comments



Article | Open access | Published: 01 January 2025

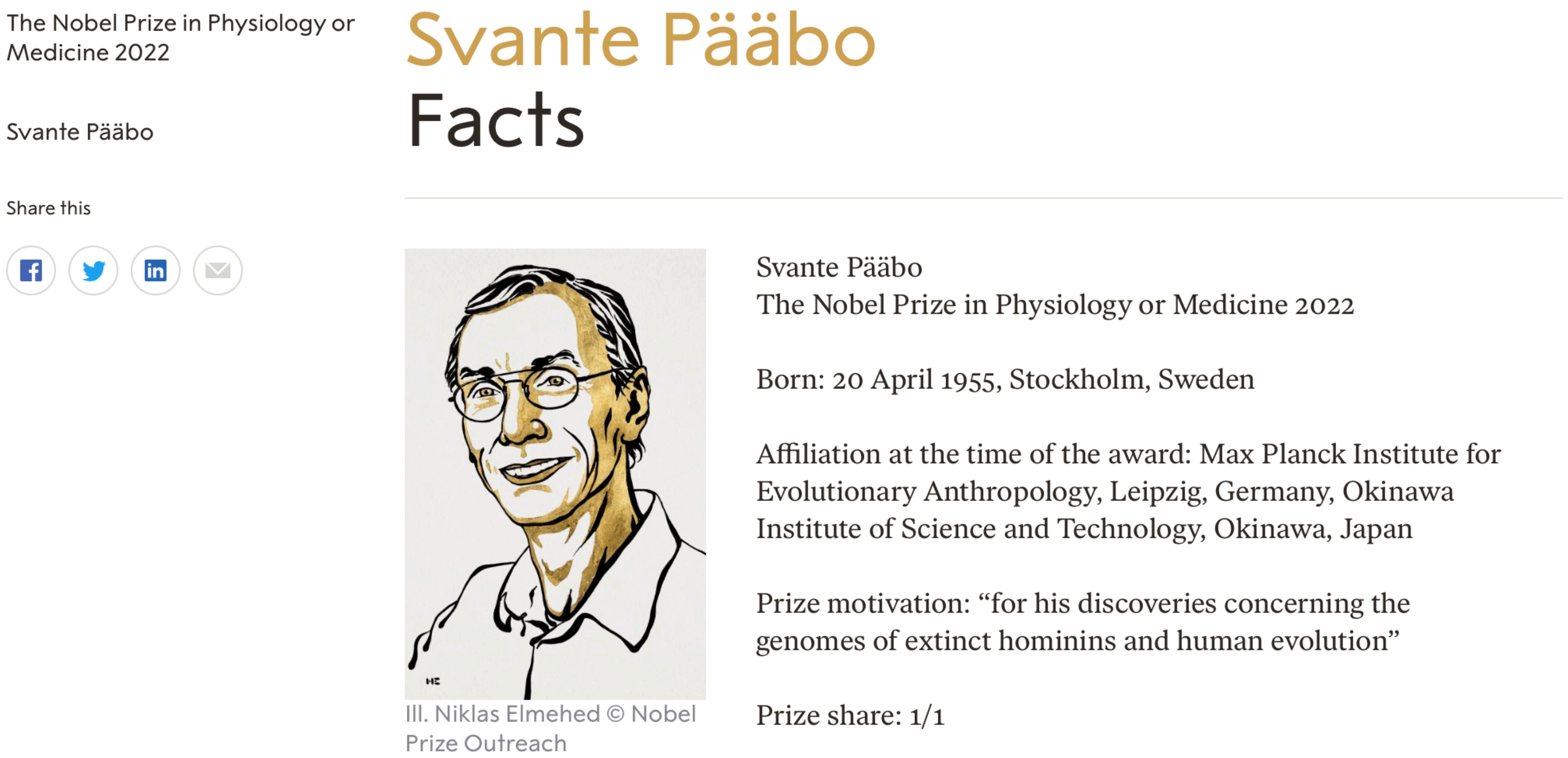
High-resolution genomic history of early medieval Europe

Leo Speidel, Marina Silva, Thomas Booth, Ben Raffield, Kyriaki Anastasiadou, Christopher Barrington, Anders Götherström, Peter Heather & Pontus Skoglund

Nature **637**, 118–126 (2025) | Cite this article

71k Accesses | **716** Altmetric | Metrics

Nobel prize in Medicine 2022



The Nobel Prize in Physiology or Medicine 2022

Svante Pääbo

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Svante Pääbo

Facts

Ill. Niklas Elmehed © Nobel Prize Outreach

Svante Pääbo
The Nobel Prize in Physiology or Medicine 2022

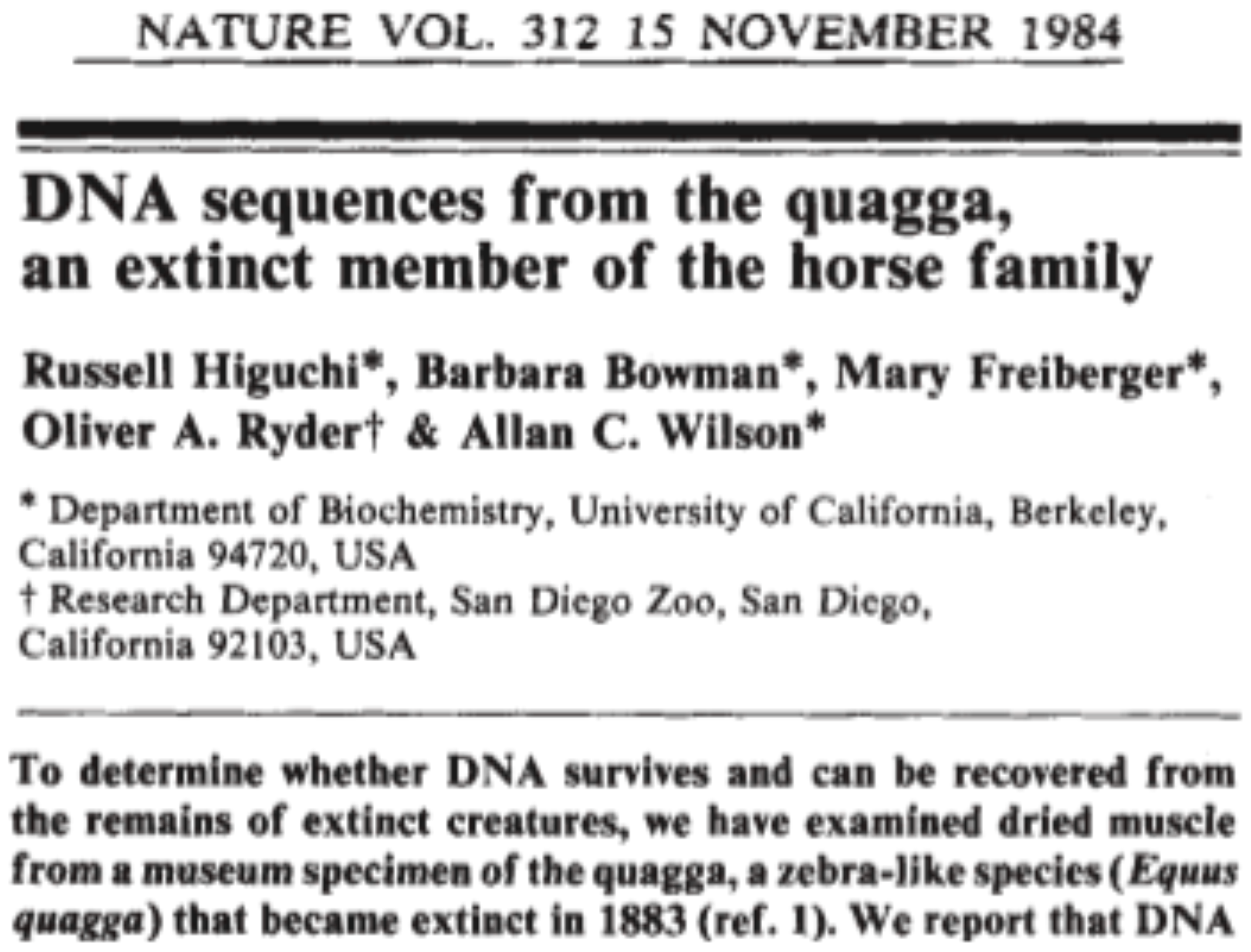
Born: 20 April 1955, Stockholm, Sweden

Affiliation at the time of the award: Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany, Okinawa Institute of Science and Technology, Okinawa, Japan

Prize motivation: “for his discoveries concerning the genomes of extinct hominins and human evolution”

Prize share: 1/1

1984 - the birth of ancient DNA



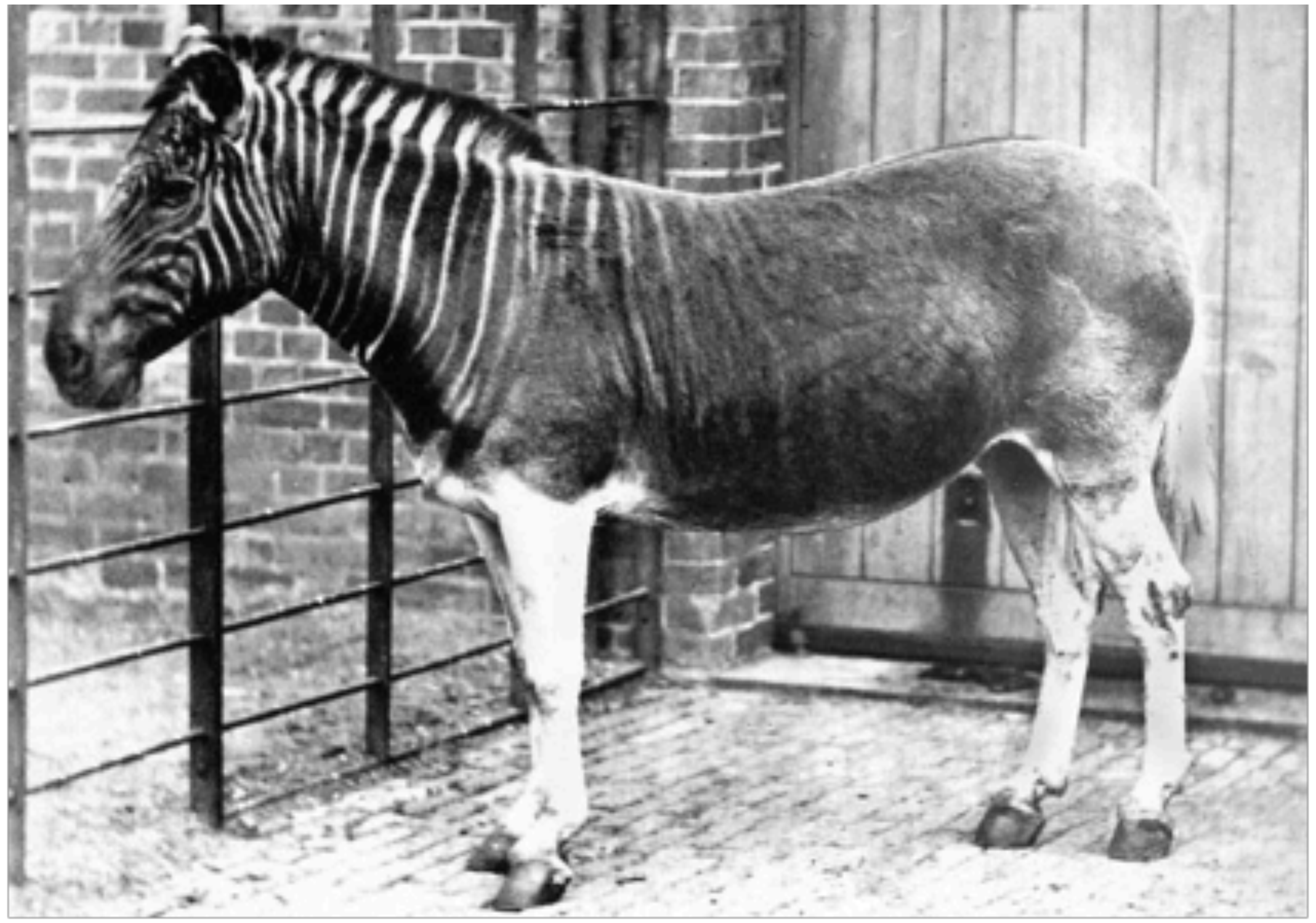
NATURE VOL. 312 15 NOVEMBER 1984

DNA sequences from the quagga, an extinct member of the horse family

Russell Higuchi*, Barbara Bowman*, Mary Freiberger*, Oliver A. Ryder† & Allan C. Wilson*

* Department of Biochemistry, University of California, Berkeley,
California 94720, USA
† Research Department, San Diego Zoo, San Diego,
California 92103, USA

To determine whether DNA survives and can be recovered from the remains of extinct creatures, we have examined dried muscle from a museum specimen of the quagga, a zebra-like species (*Equus quagga*) that became extinct in 1883 (ref. 1). We report that DNA



Unidentified reading frame 1

```
Quagga  C CCA ATC CTG CTC GCC GTA GCA TTC CTC ACA CTA GTT GAA CGA AAA GTC TTA GGC TAC ATA CAA CTT CGT AAA GGA CCC AAC ATC GTA GGC CCC TAT GGC CTA CTA CAA CCC ATT AC
 Zebra  . ... ... ... ..T ... ..G ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .G*
```

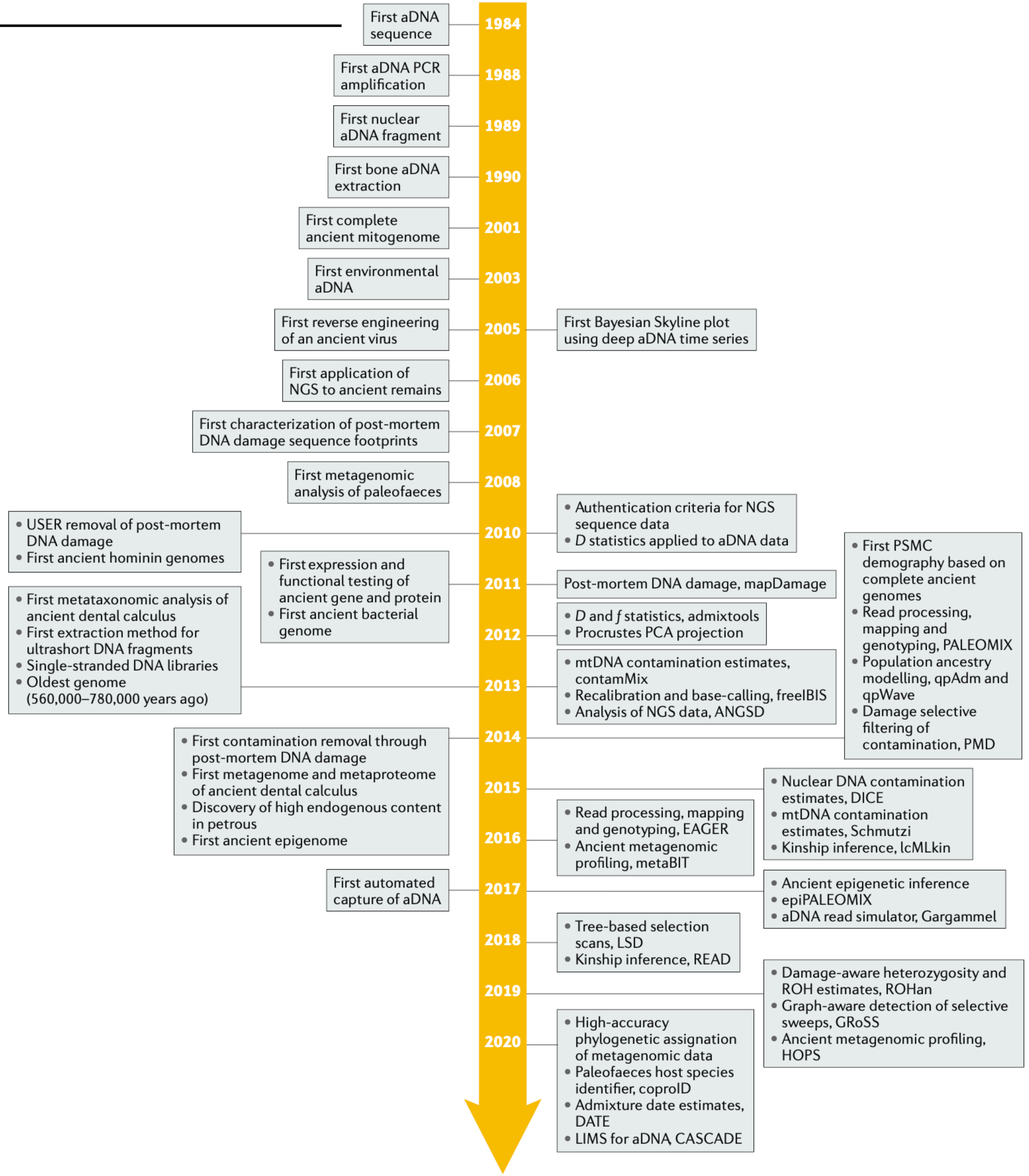
Cytochrome oxidase I

```
Quagga  A GGA GGA TTC GTT CAC TGA TTC CCT CTA TTC TCA GGA TAC ACA CTC AAC CAA ACC TGA GCA AAA ATT CAC TTT ACA ATT ATA TTC GTA GGG GTC AAC ATA ATT TTC TTC CCA
 Zebra  G ... ... ..T ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ..A ... ..T ... .C*. ... ... ...
```

Fig. 1 Sequences of the coding strands determined for two pieces of quagga mtDNA. The sequences are arranged in triplets corresponding to the amino acids that they encode. At 12 positions, the quagga sequences differ from those of mtDNA from a mountain zebra; only for these positions is the nature of the base specified for the zebra. The two asterisks identify triplets at which the zebra and quagga differ by an amino acid replacement.

Milestones in ancient DNA

1984 - First aDNA sequence



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Orlando et al (2021) Nat Rev Methods Primers

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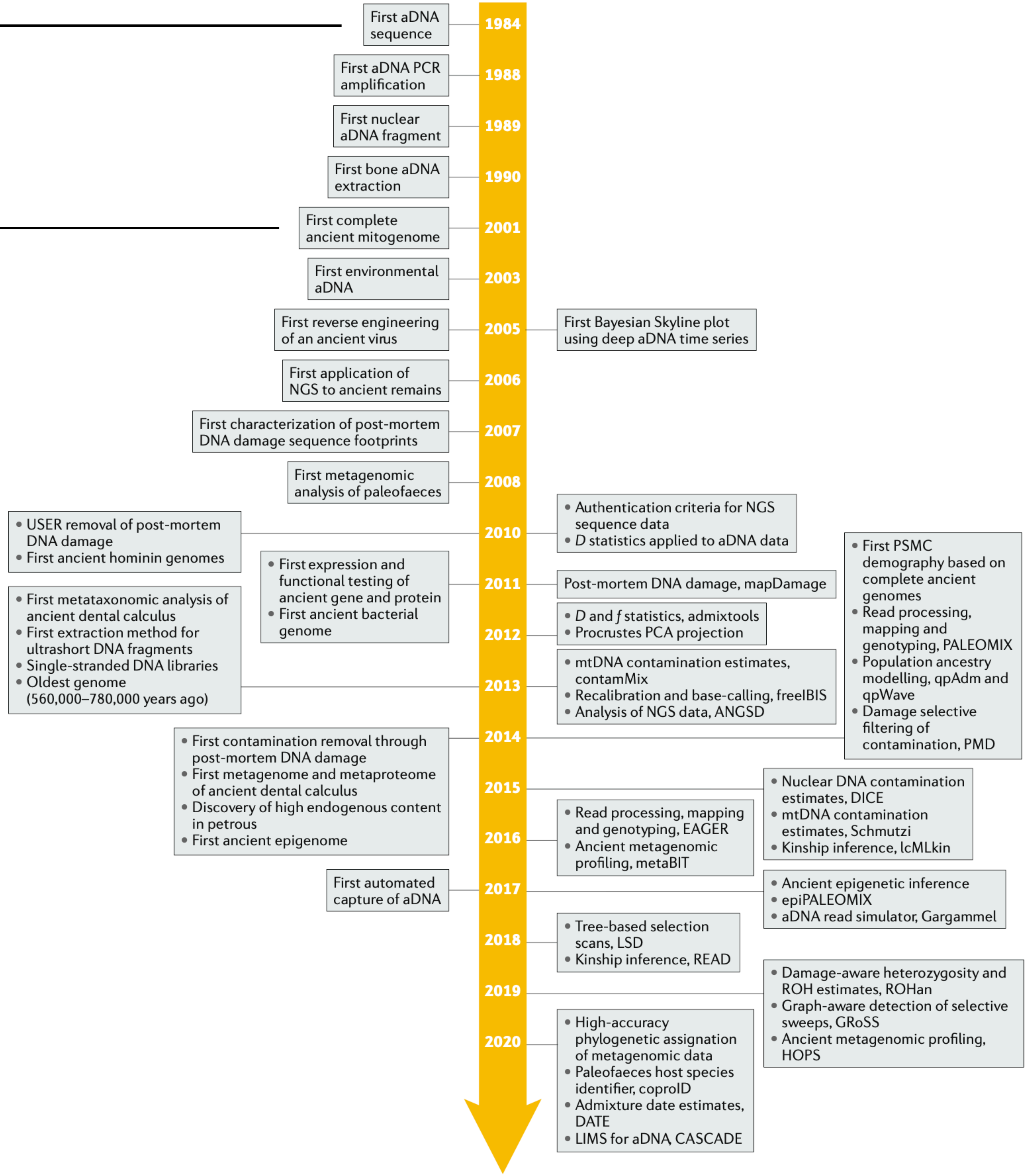

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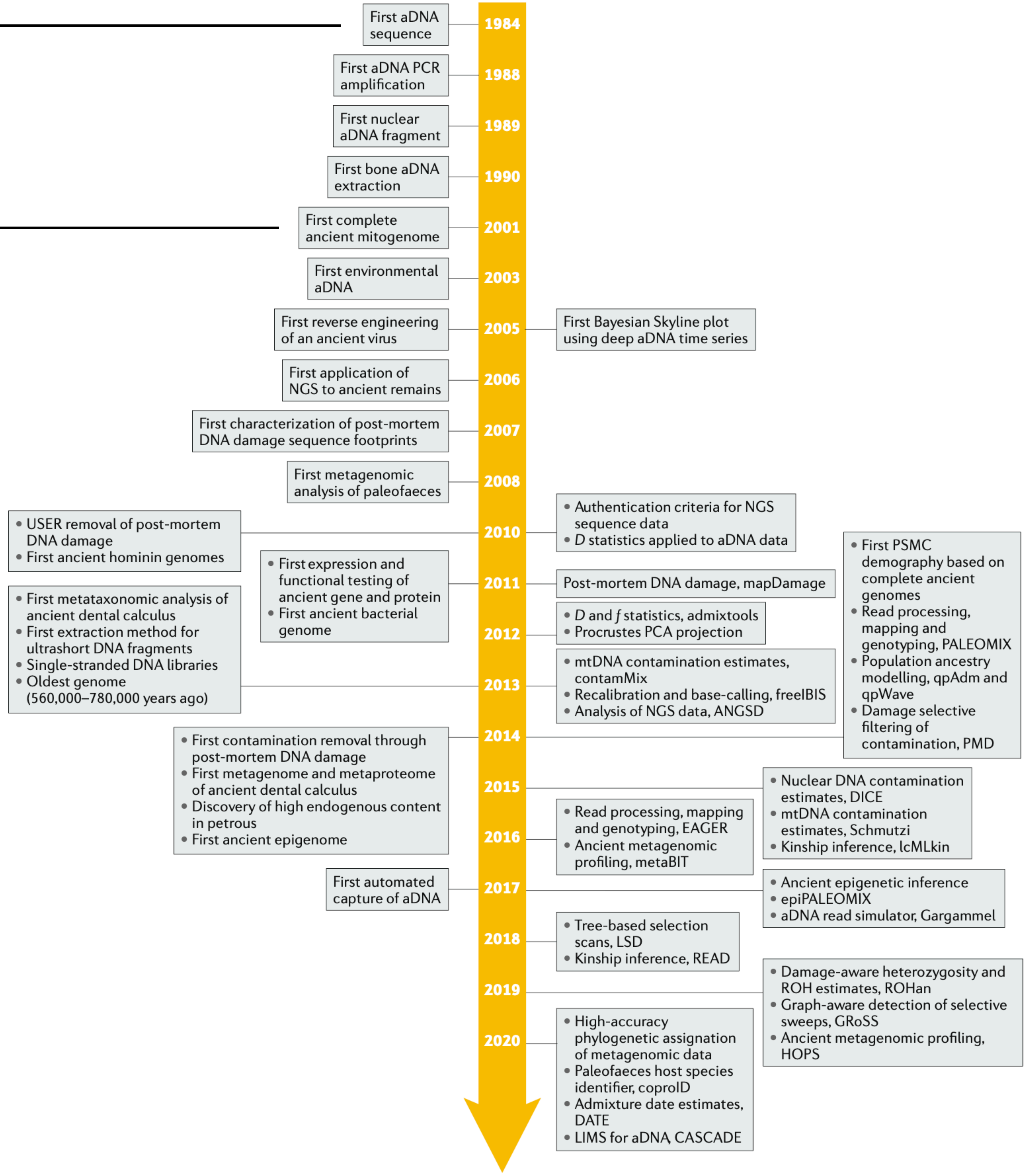

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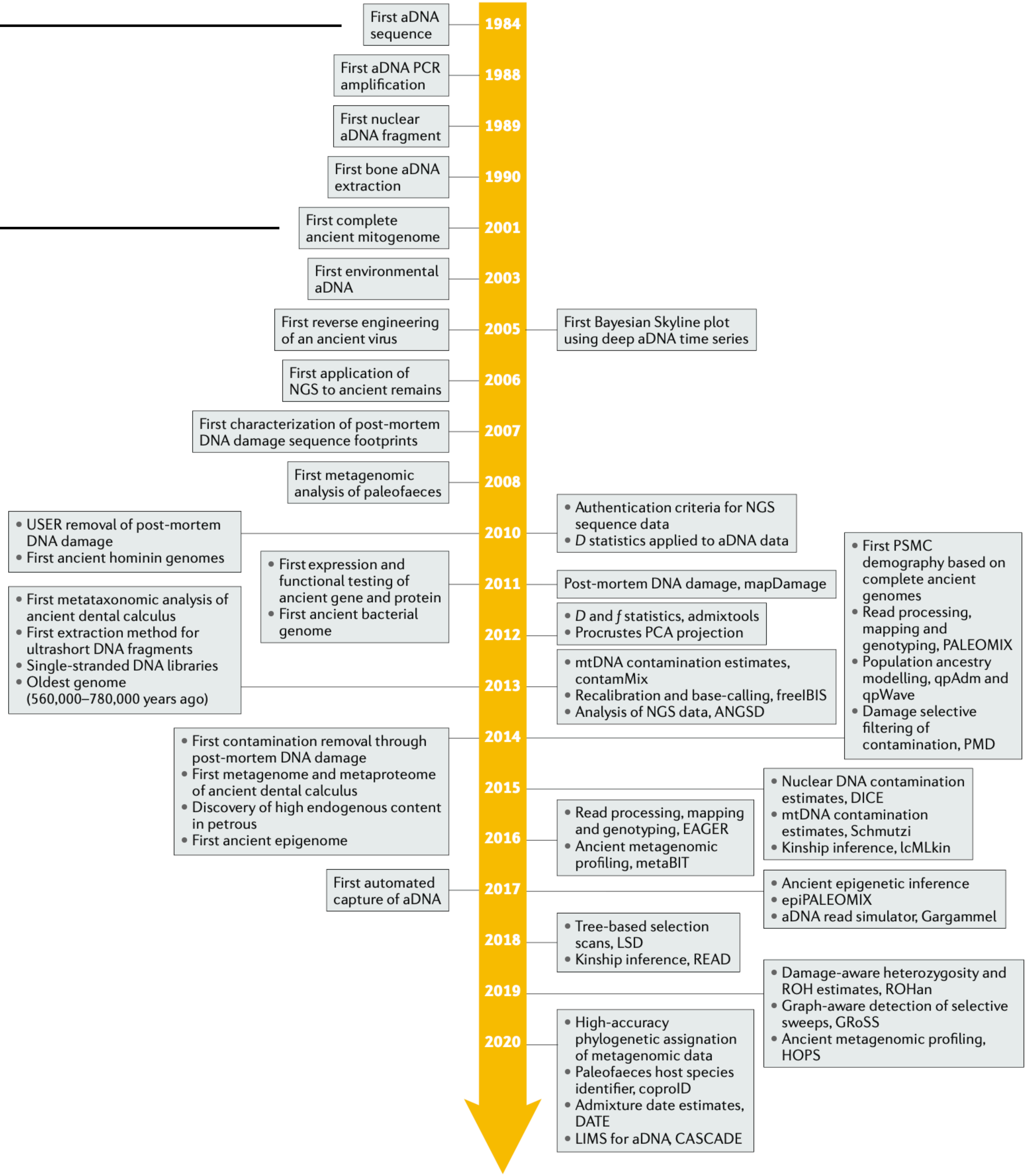
The “big data” era of ancient genomics



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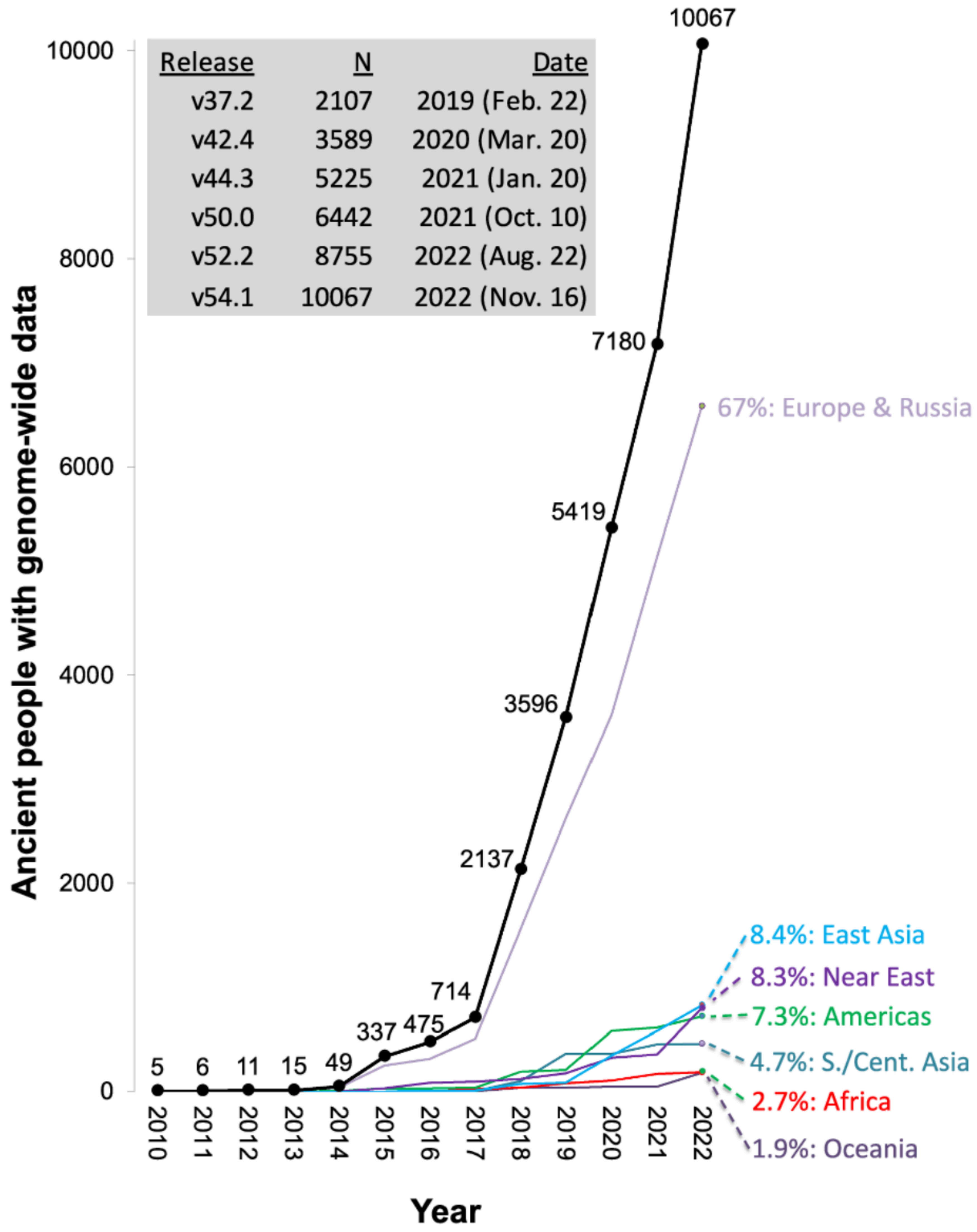
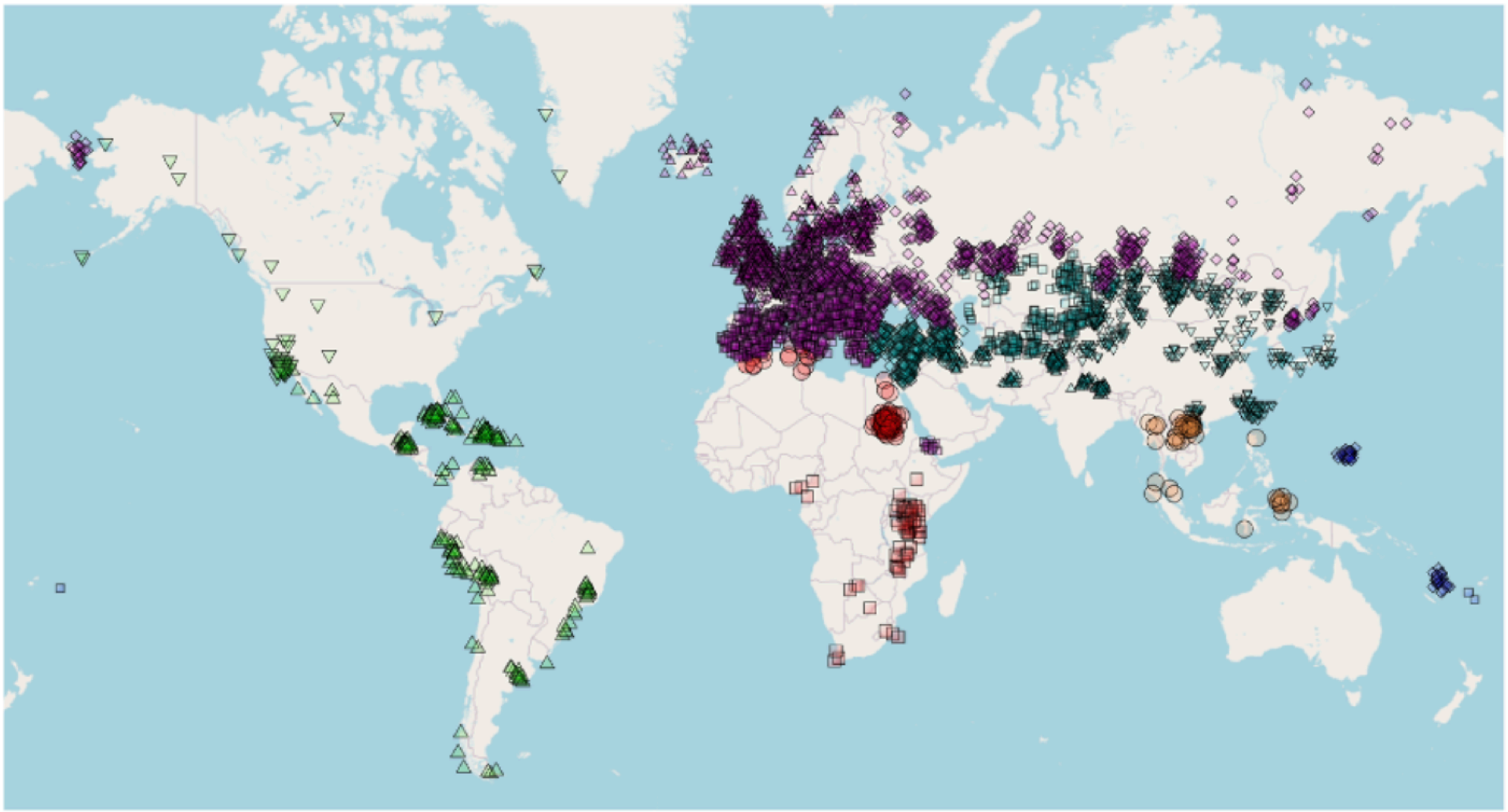
Orlando et al (2021) Nat Rev Methods Primers

The ancient human DNA revolution



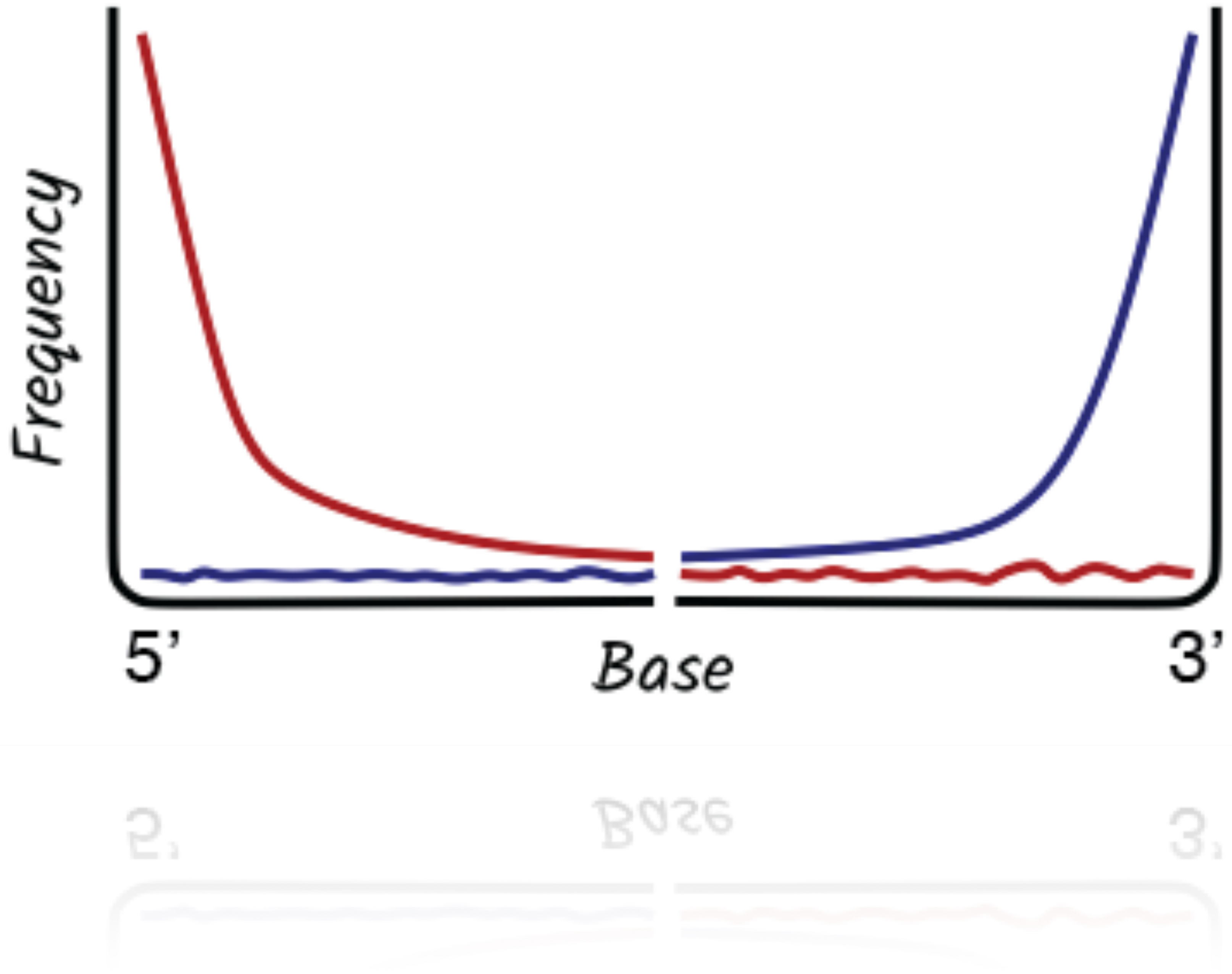
Release	N	Date
v37.2	2107	2019 (Feb. 22)
v42.4	3589	2020 (Mar. 20)
v44.3	5225	2021 (Jan. 20)
v50.0	6442	2021 (Oct. 10)
v52.2	8755	2022 (Aug. 22)
v54.1	10067	2022 (Nov. 16)



Over 10,000 ancient humans with genome-wide data since 2010

Mallick et al (2024) Sci Data

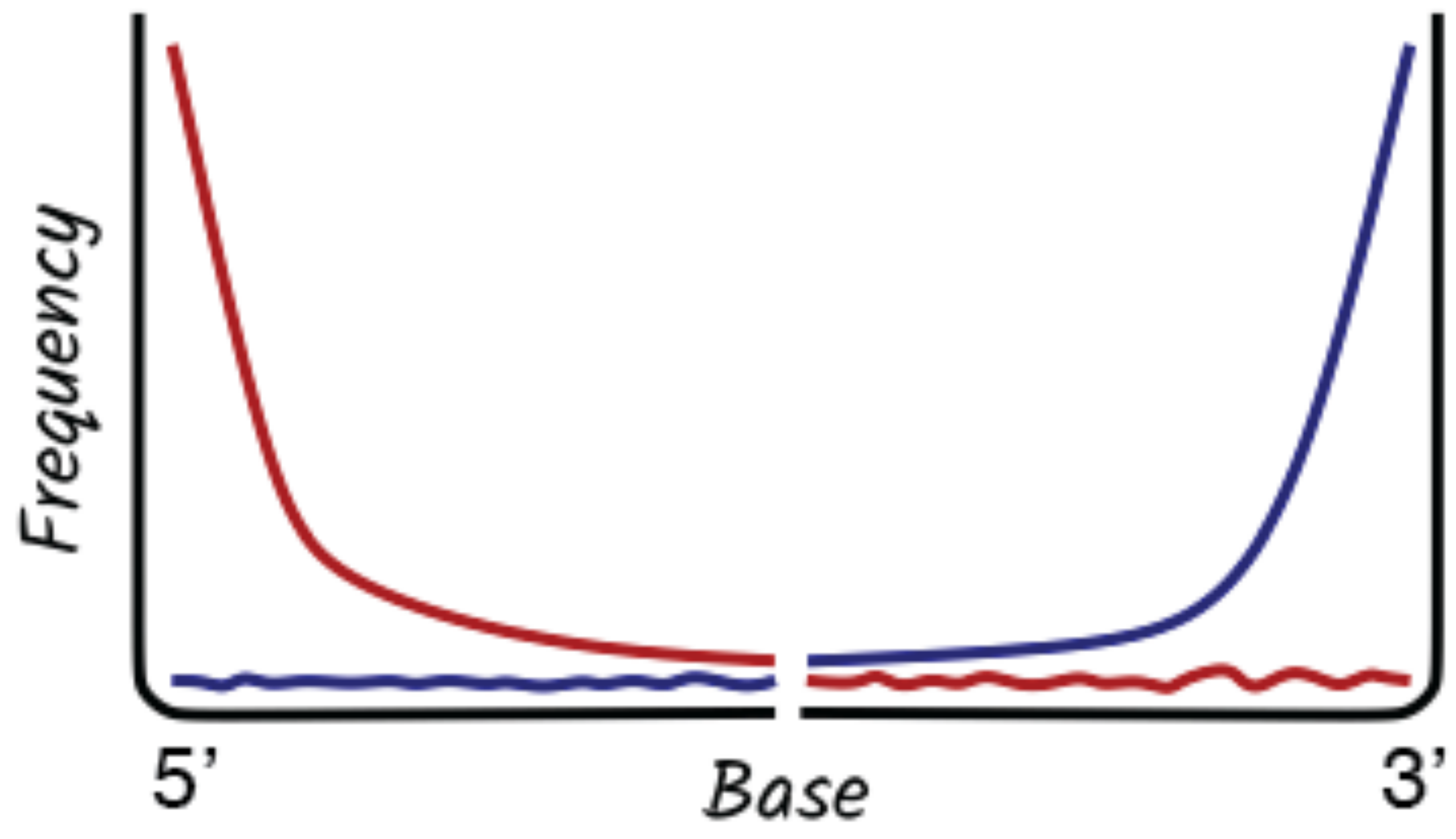
Topics for today's lecture

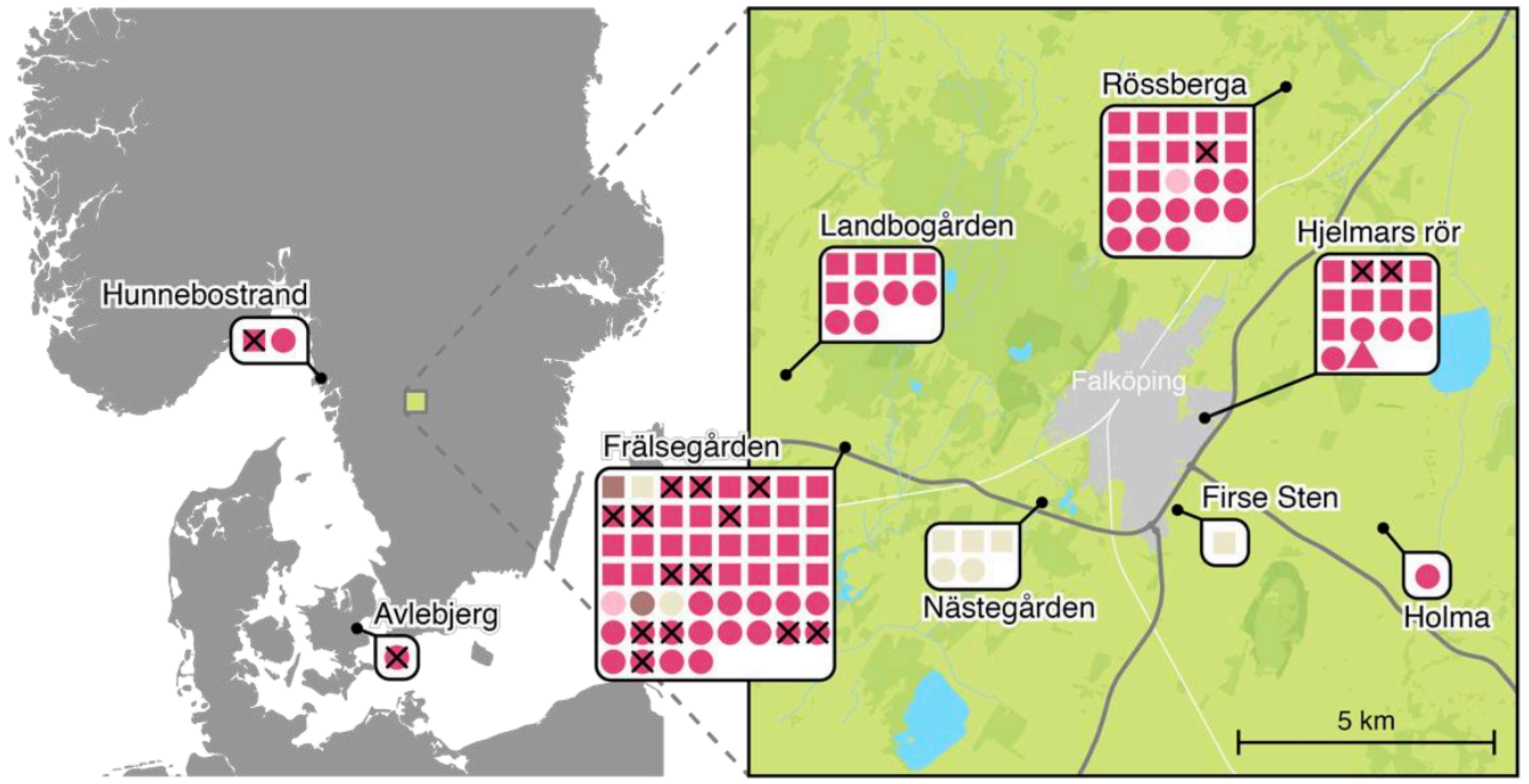


Ancient DNA primer

Characteristics and challenges

Topics for today's lecture





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Ancient DNA showcase

Kinship and plague in Stone Age Scandinavia

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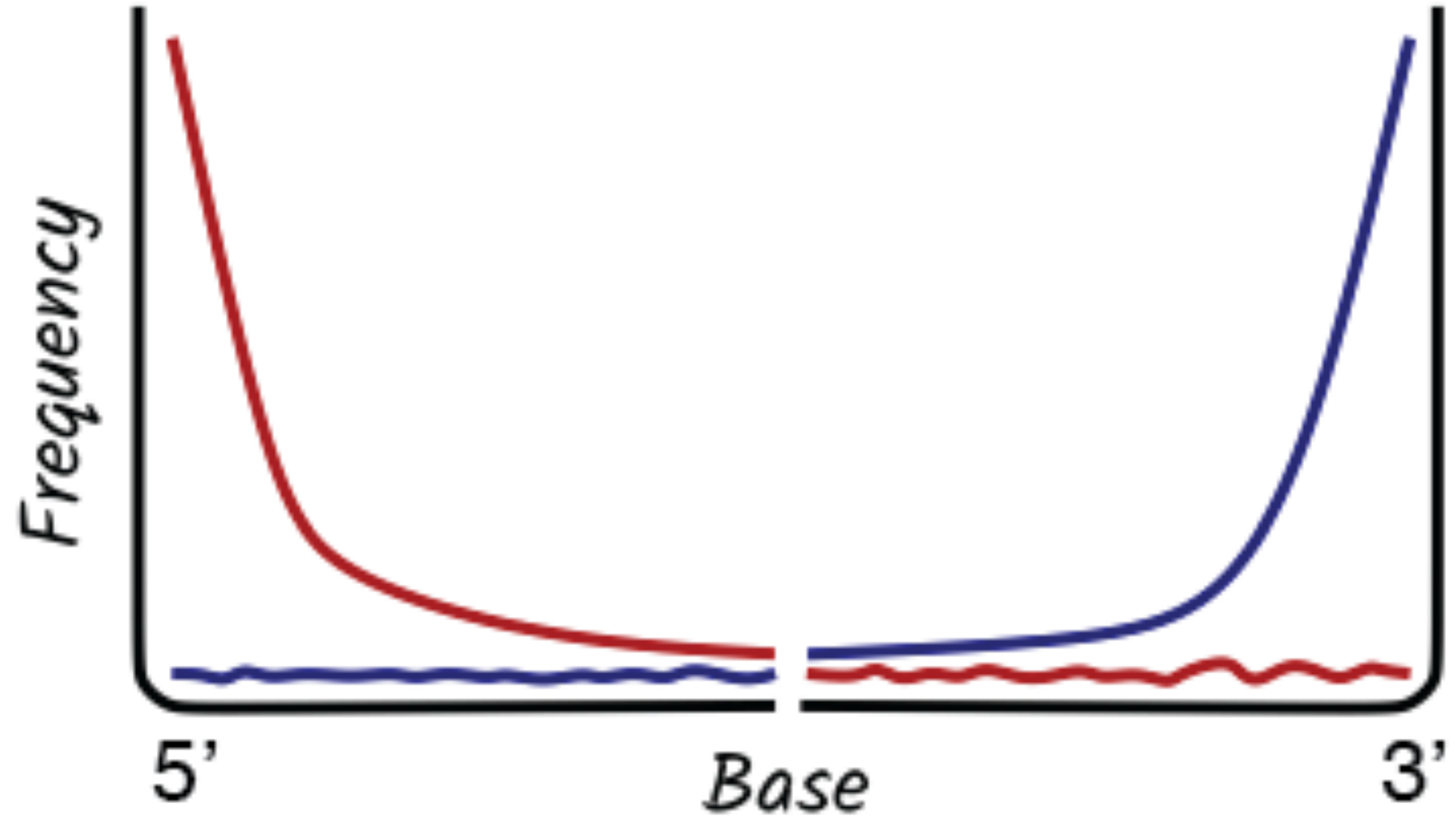
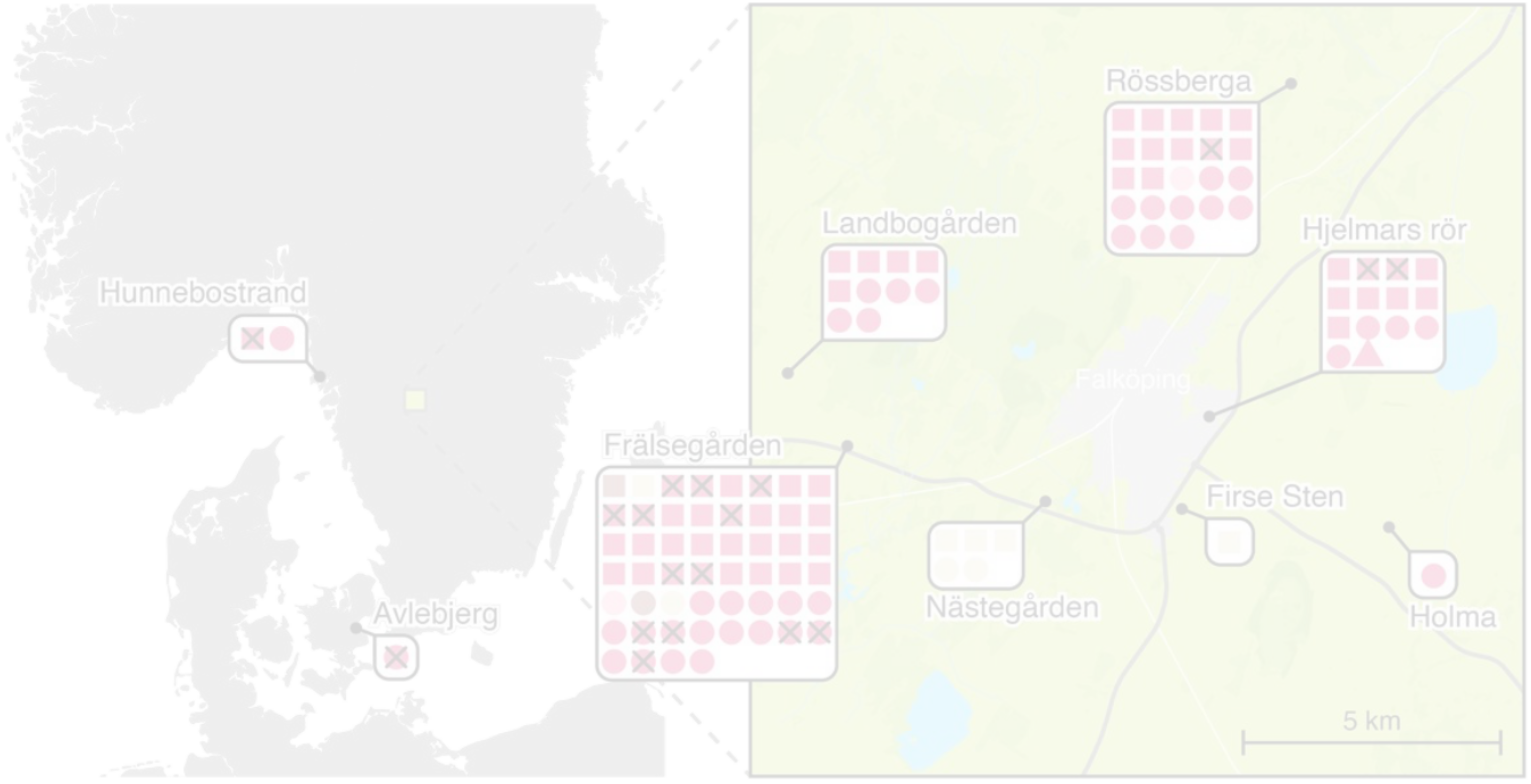




Ancient DNA primer

Characteristics and challenges

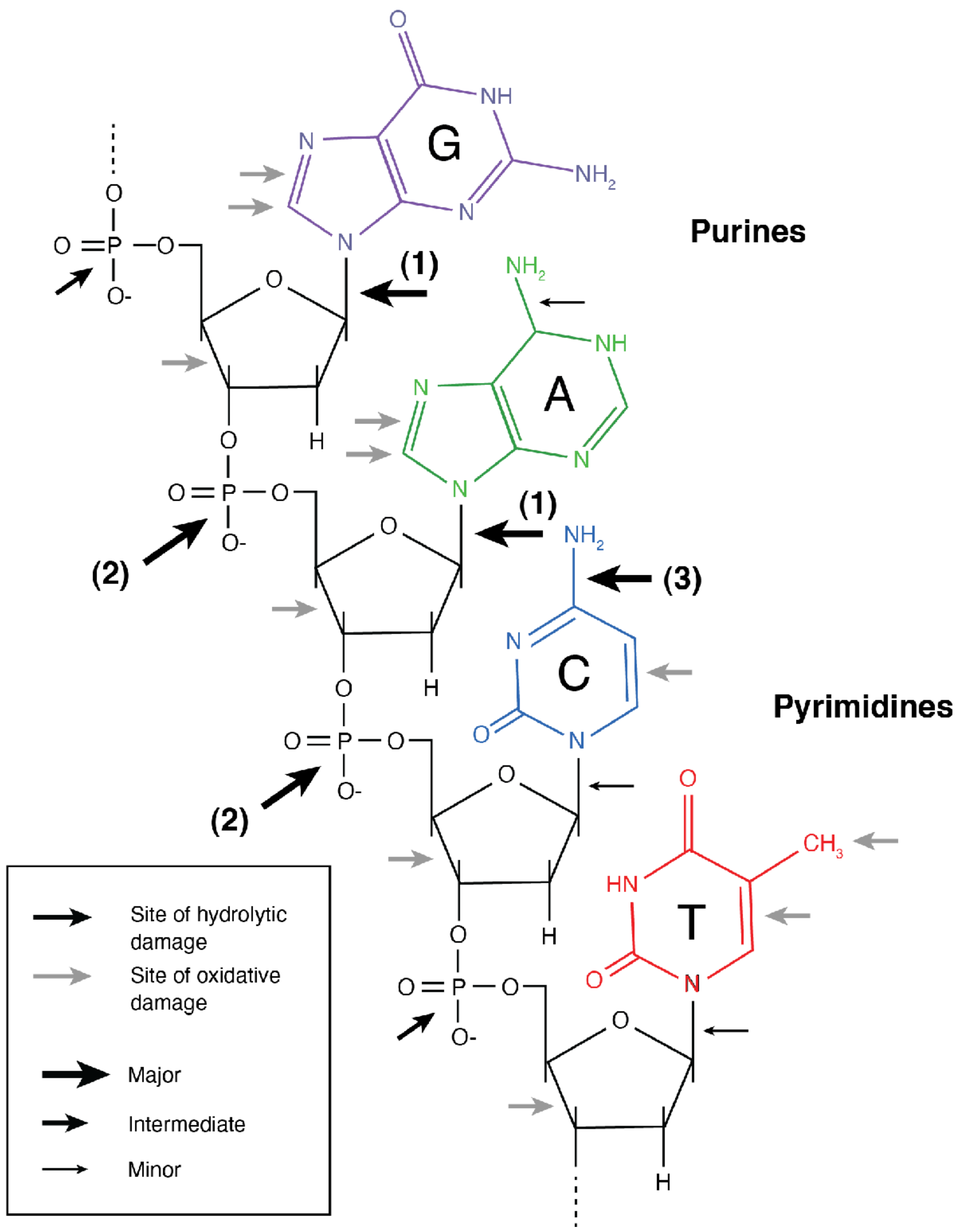
Ancient DNA showcase

Kinship and plague in Stone Age Scandinavia

Characteristics of ancient DNA

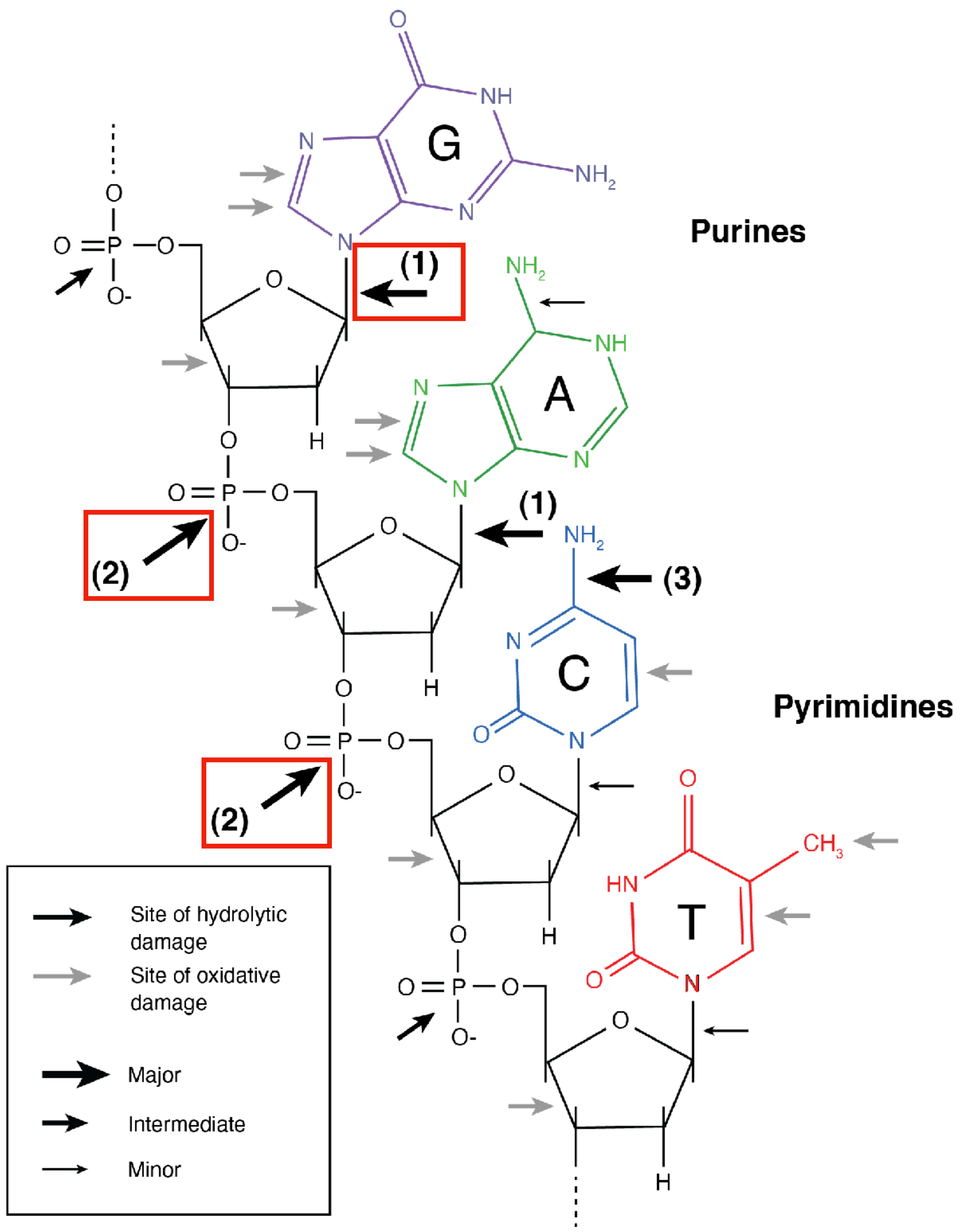
5,000 year-old human remains from the Provadia salt mine, Bulgaria

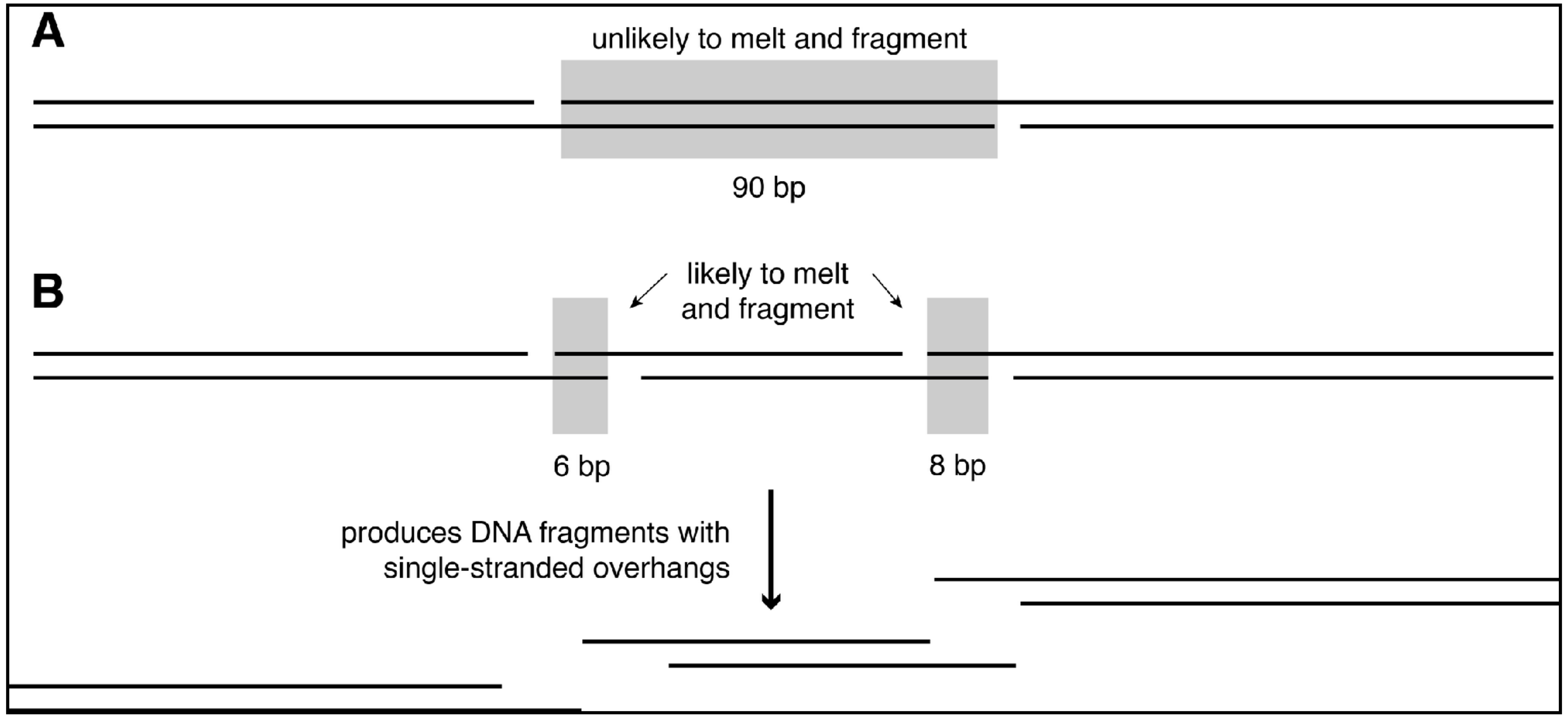
How does DNA degrade?



Fellows Yates et al (2024) Zenodo. https://doi.org/10.5281/zenodo.13784555

Fragmentation of DNA

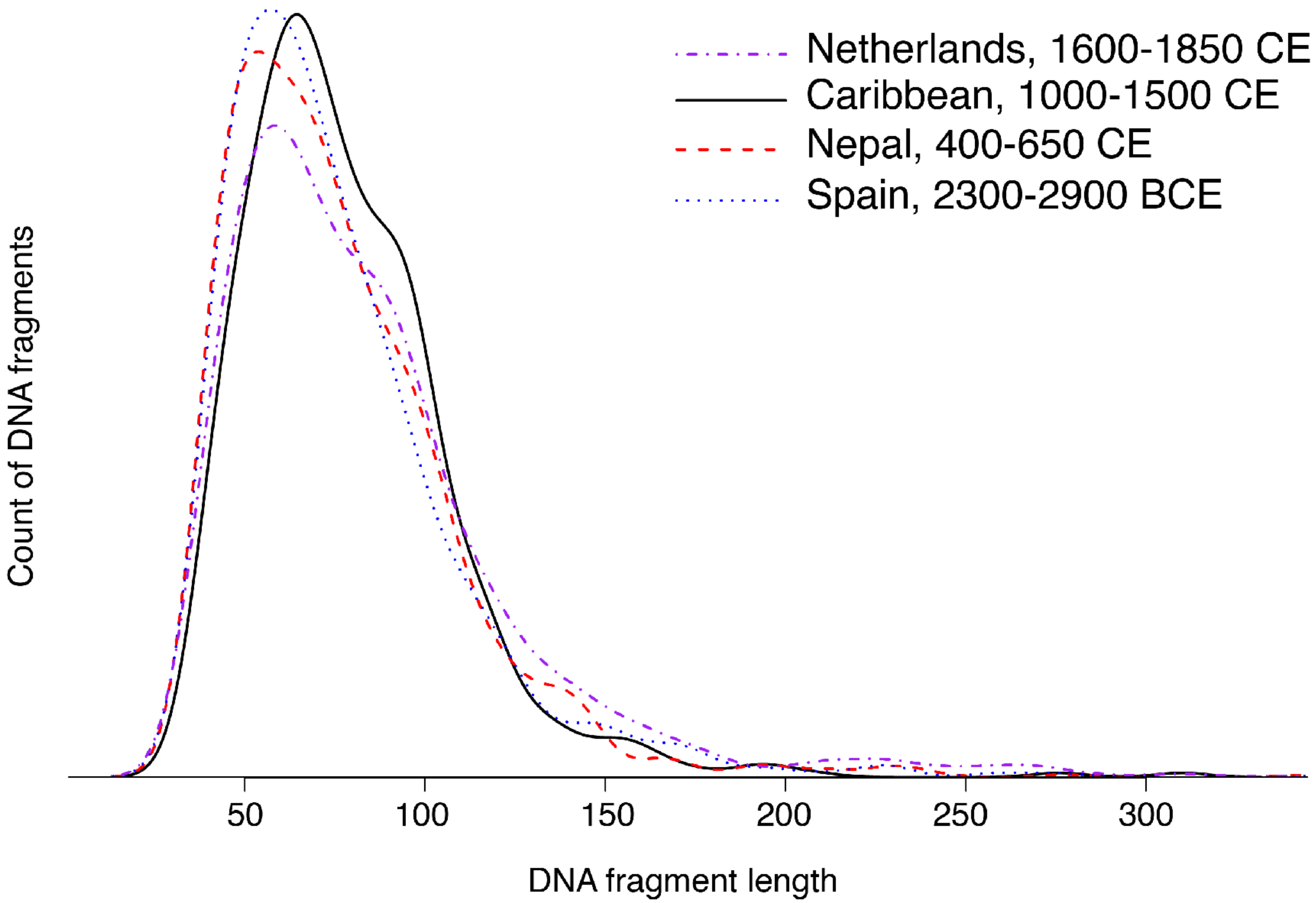




Depurination and nicking results in DNA fragmentation

Fellows Yates et al (2024) Zenodo. https://doi.org/10.5281/zenodo.13784555

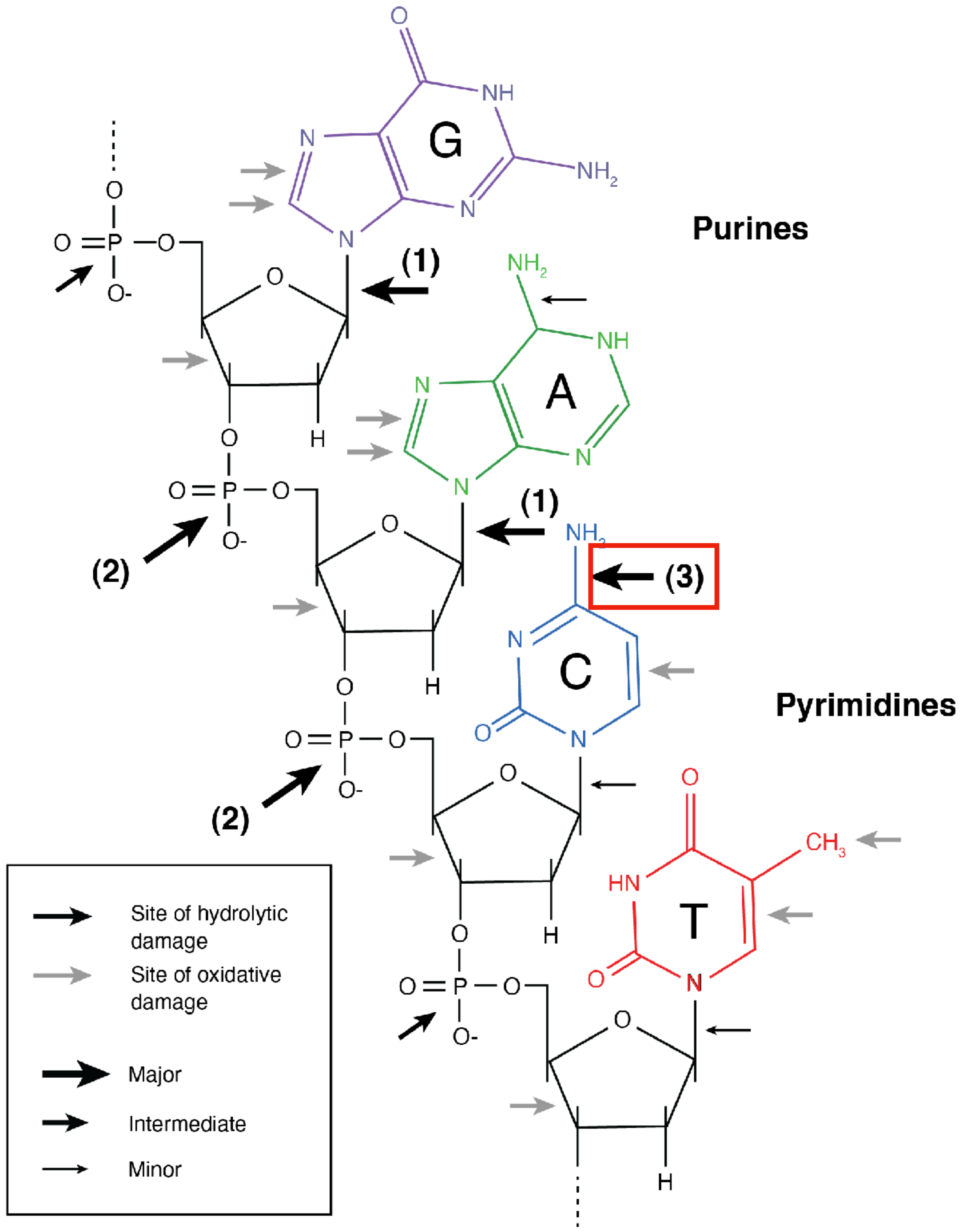
Characteristics of ancient DNA

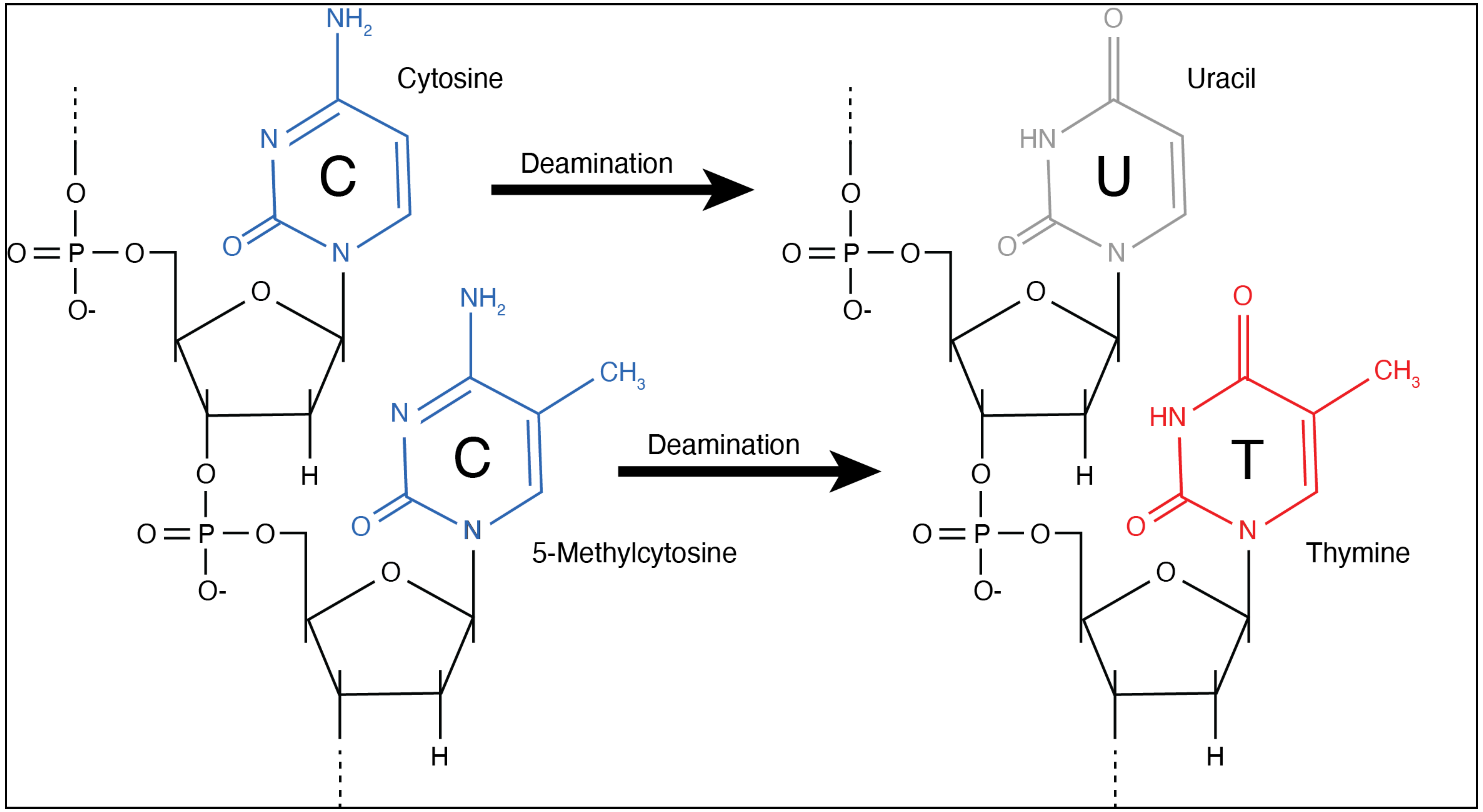


Ancient DNA is short and fragmented

Fellows Yates et al (2024) Zenodo. https://doi.org/10.5281/zenodo.13784555

Accumulation of DNA damage

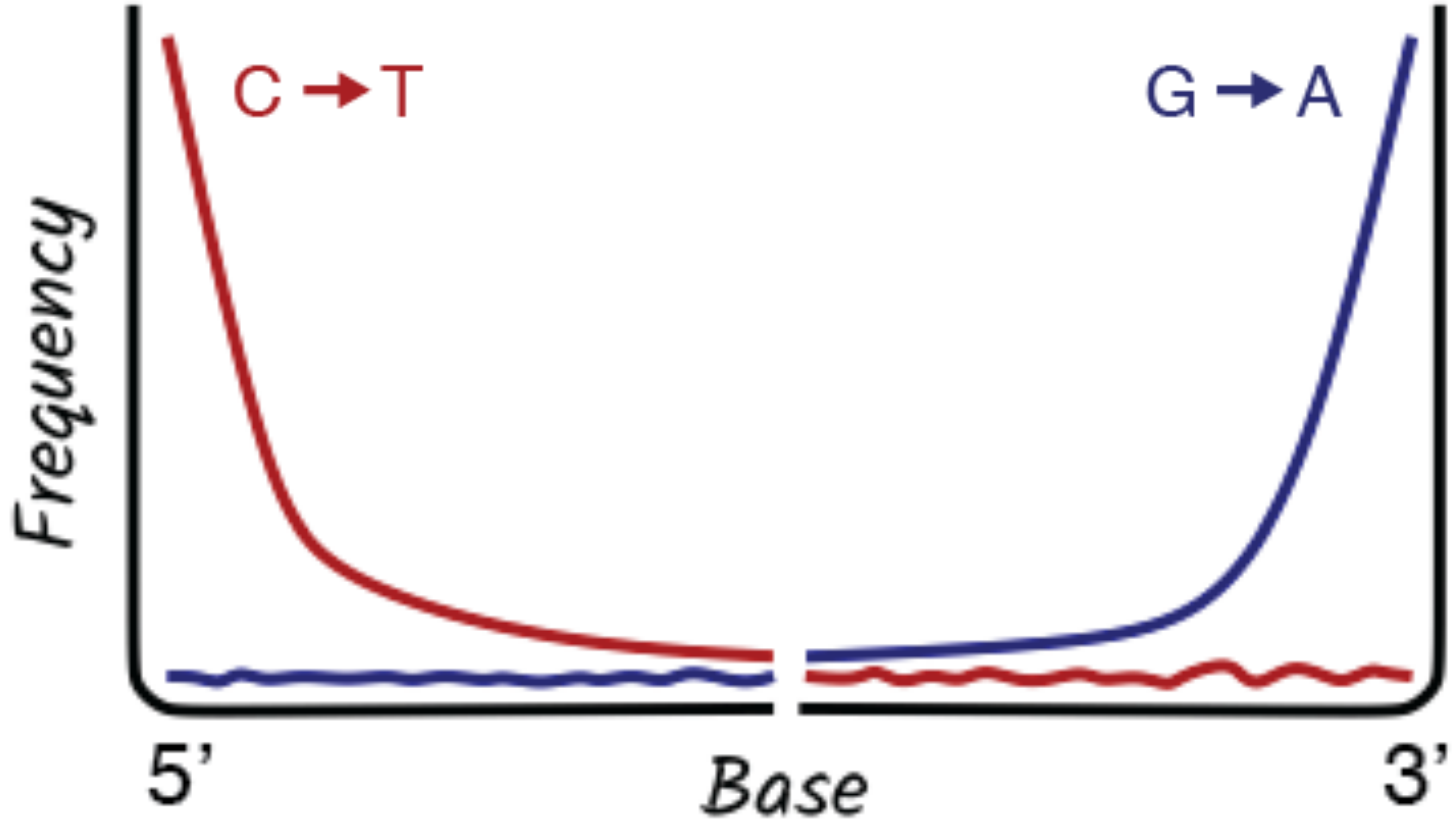




Deamination results in base substitutions

Fellows Yates et al (2024) Zenodo. https://doi.org/10.5281/zenodo.13784555

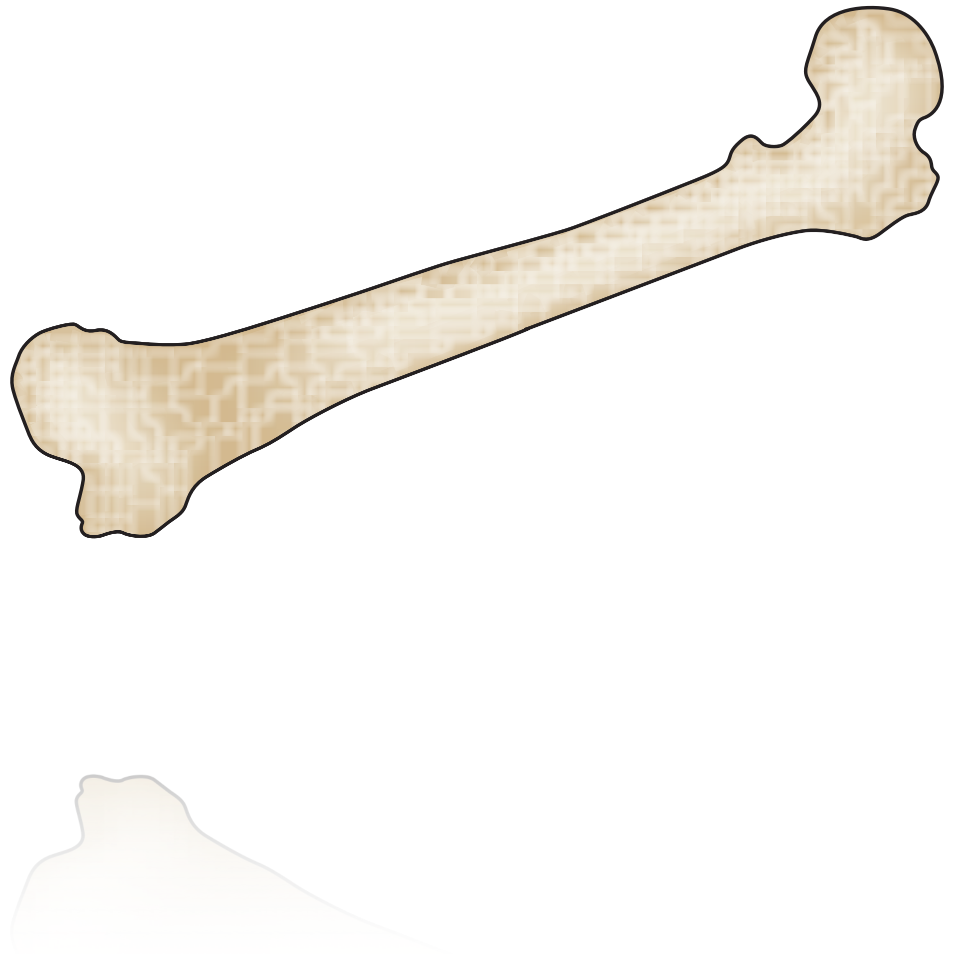
Characteristics of ancient DNA



Ancient DNA is damaged with characteristic substitution patterns

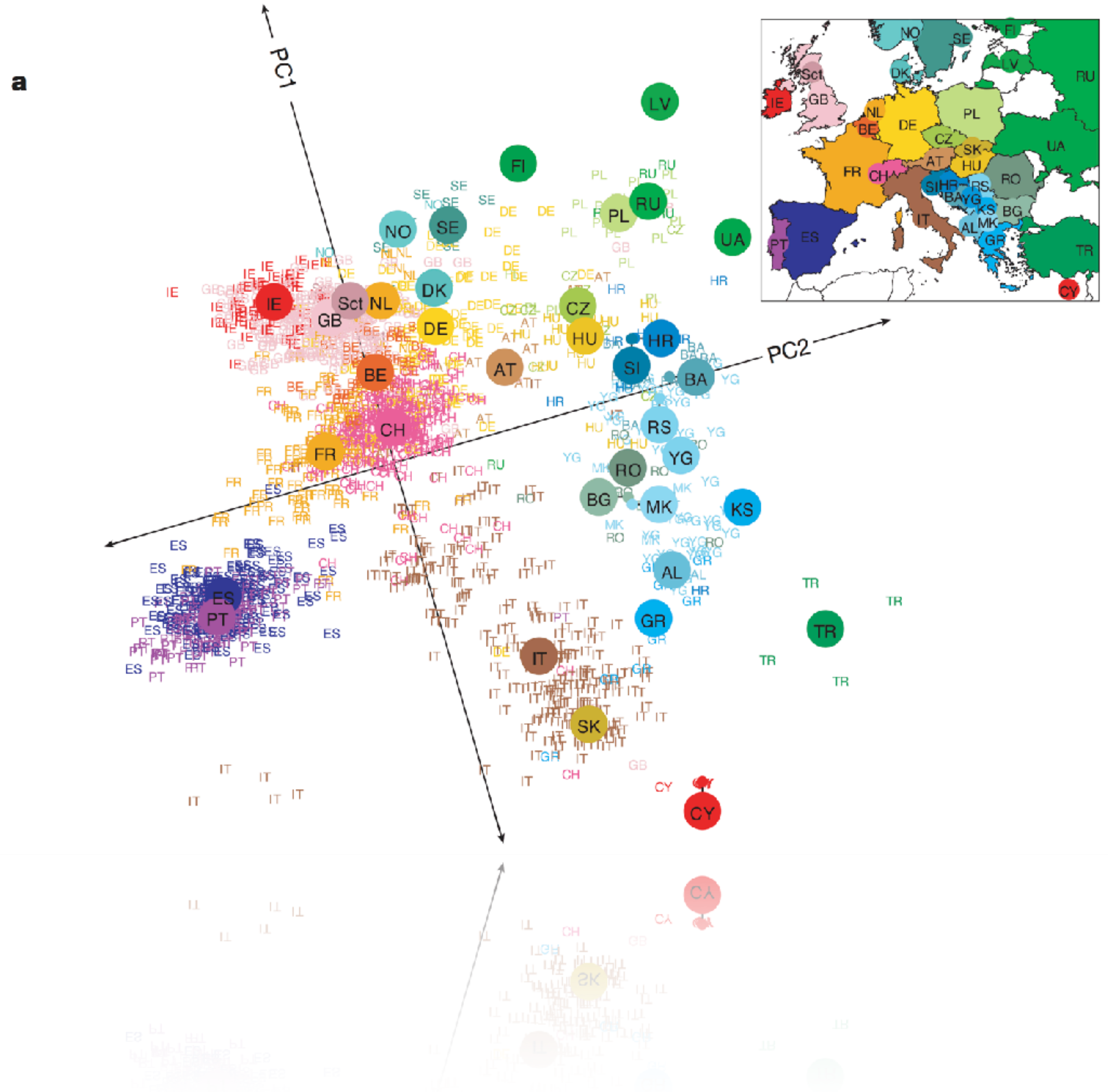
Ancient DNA challenges

Sample



DNA sequences

Analysis results



Stoneking and Krause (2011) Nat Rev Genet; Novembre et al (2008) Nature

Ancient DNA challenges

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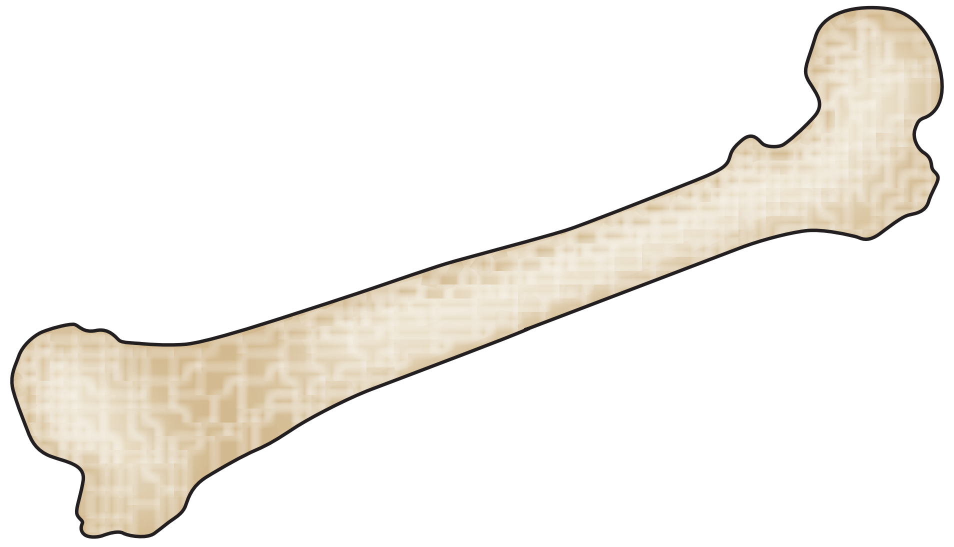

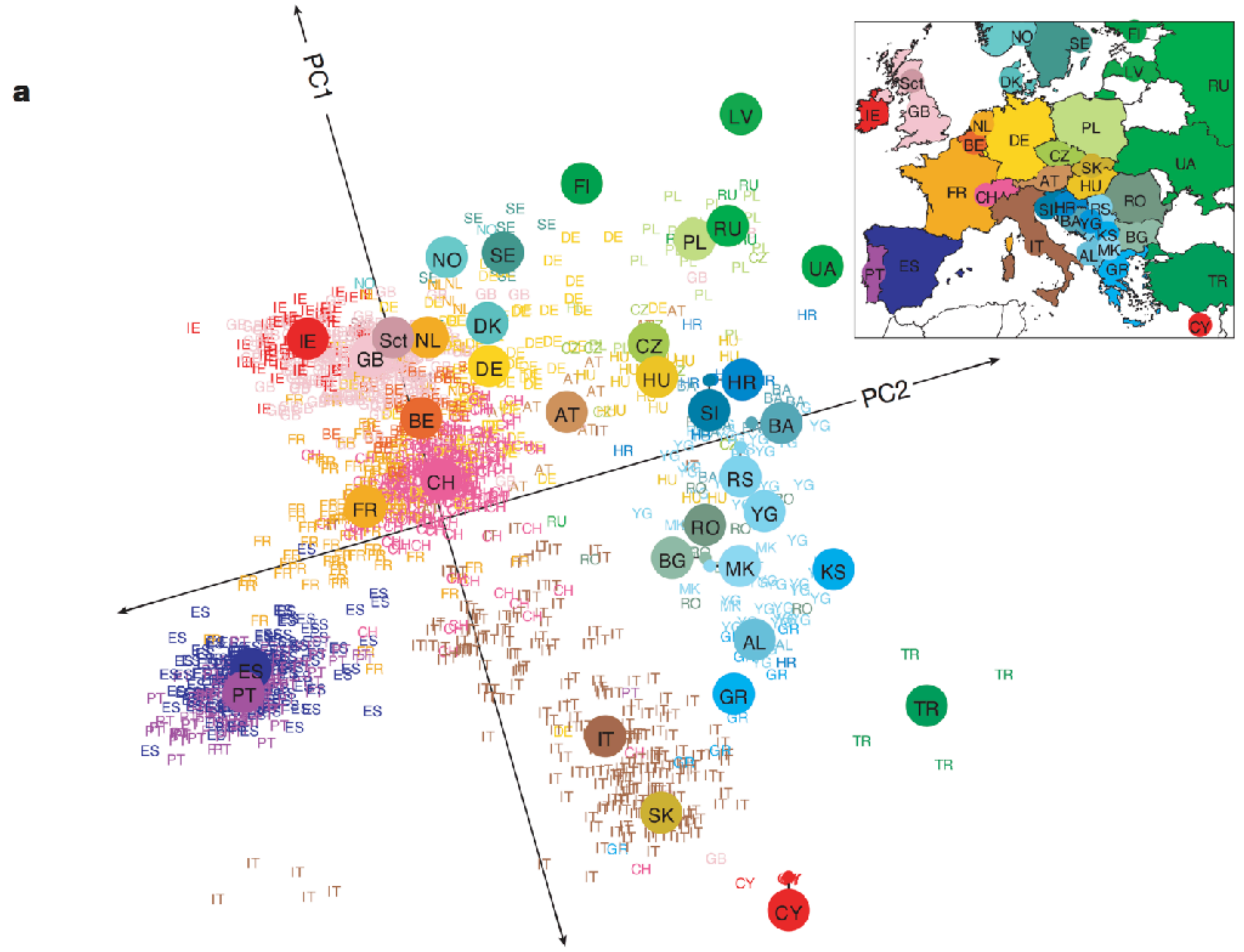

DNA recovery

Preservation
Sample material

Data quality

Contamination
Genome coverage
Error rates

Stoneking and Krause (2011) Nat Rev Genet; Novembre et al (2008) Nature

Ancient DNA challenges

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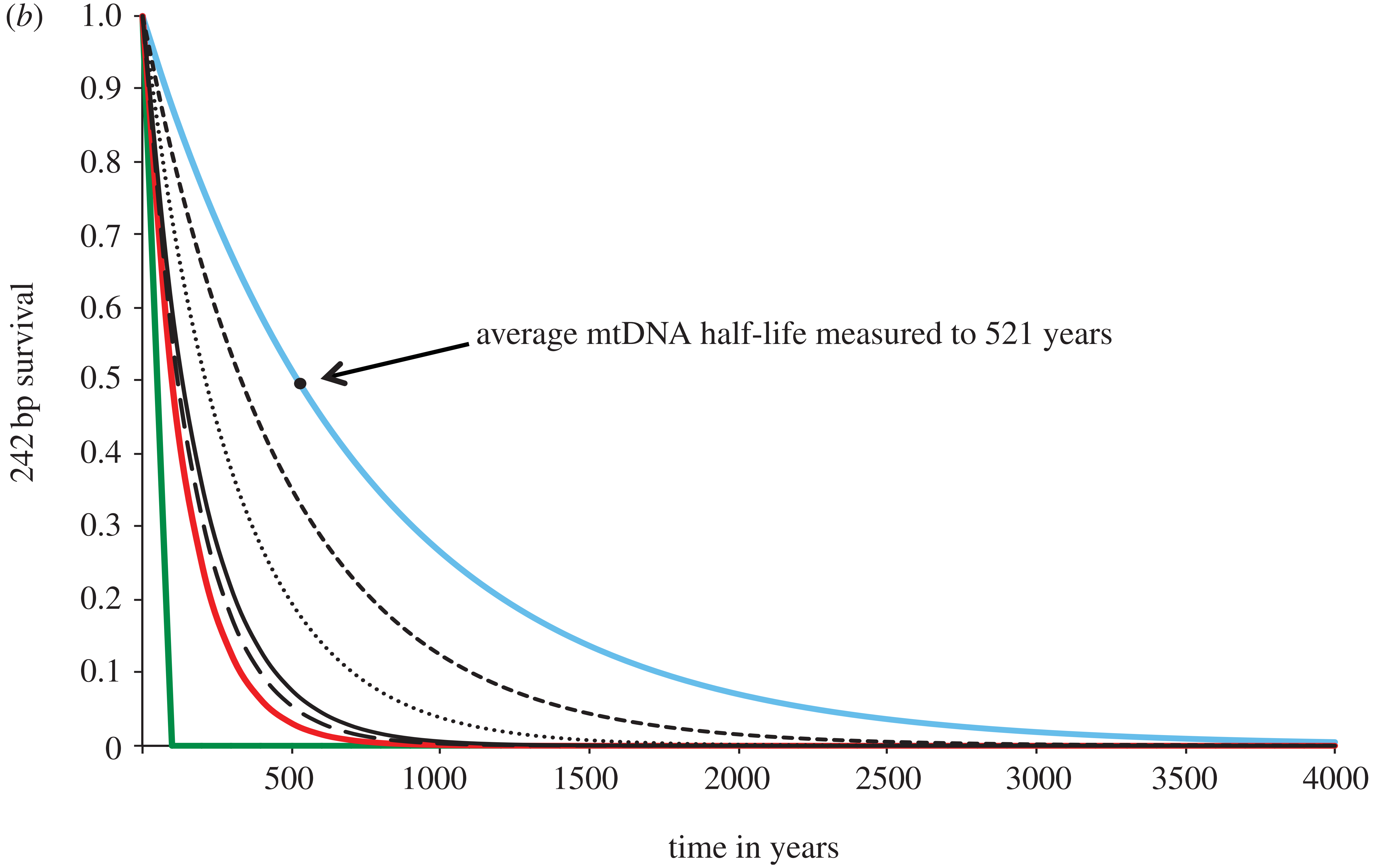
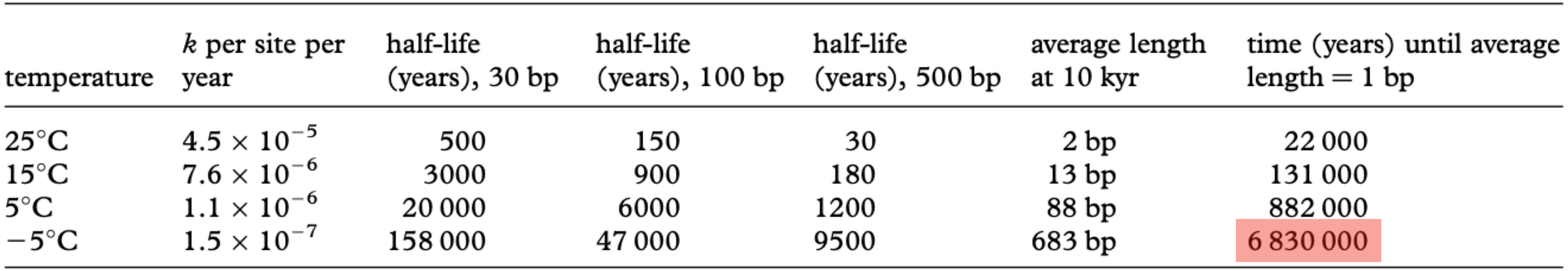
Challenges - Ancient DNA preservation



Ancient DNA fragmentation follows exponential decay dynamics

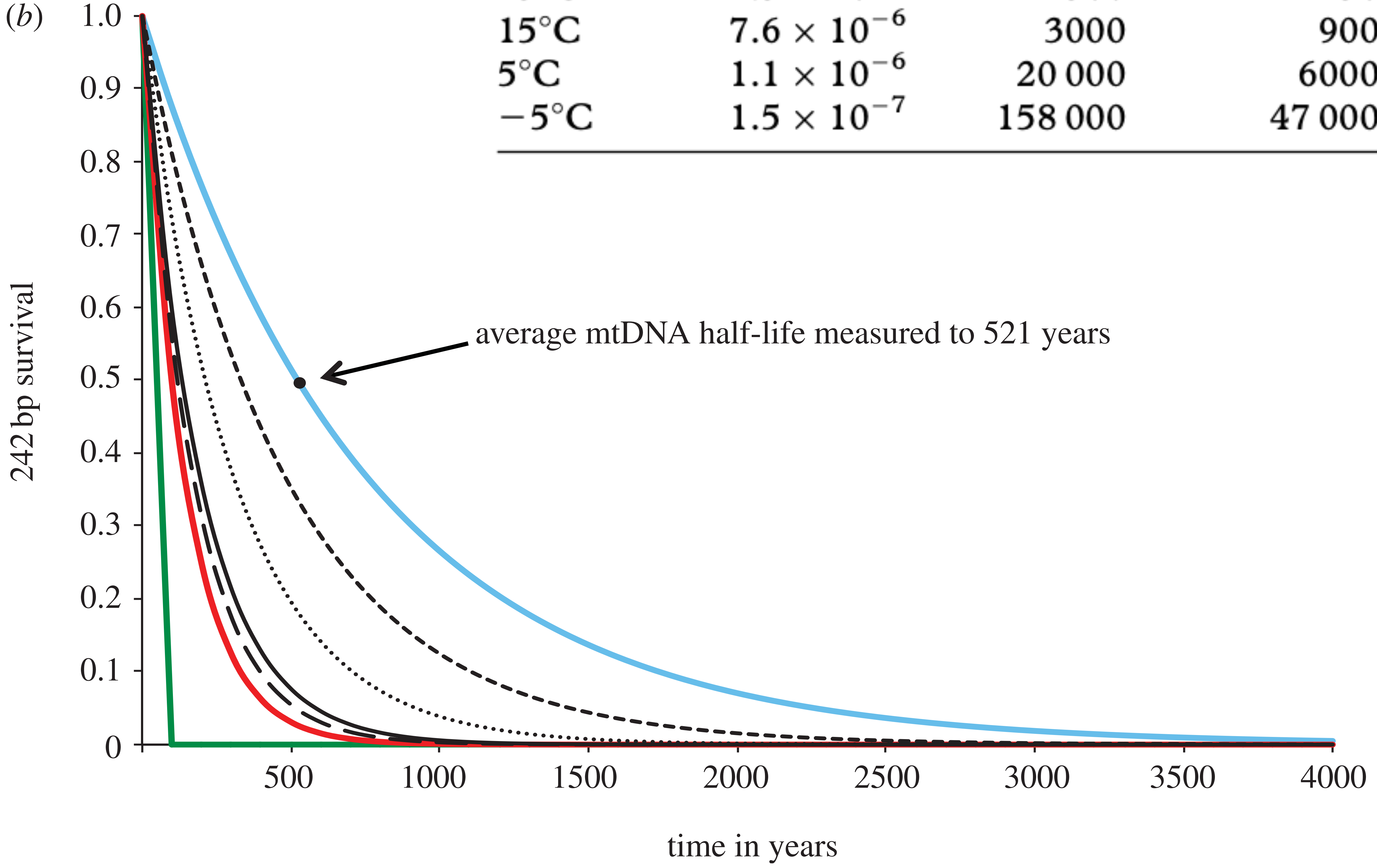
Allentoft et al (2012) Proc R Soc B

Challenges - Ancient DNA preservation



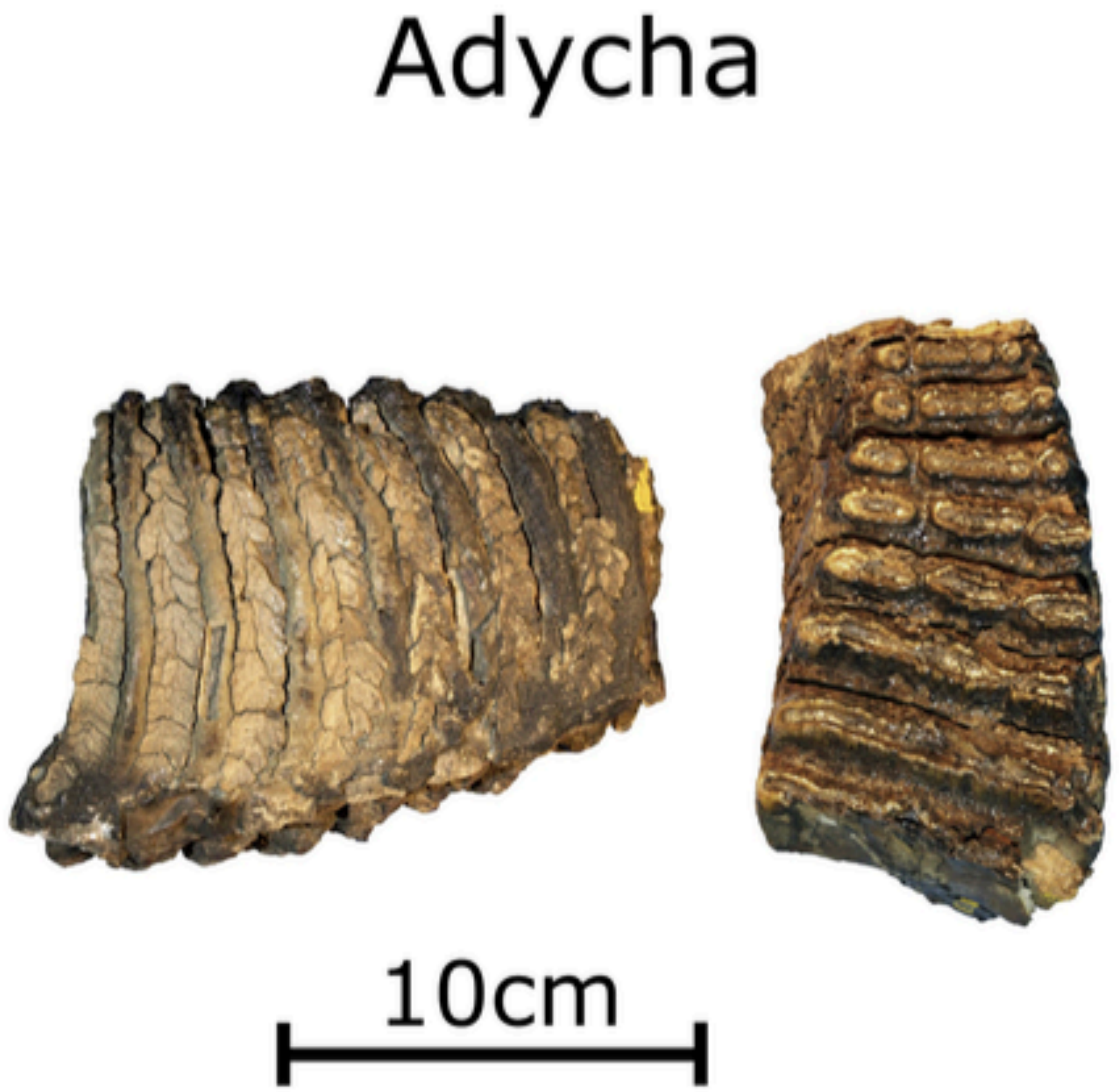
temperature	*k* per site per year	half-life (years), 30 bp	half-life (years), 100 bp	half-life (years), 500 bp	average length at 10 kyr	time (years) until average length = 1 bp
25°C	4.5×10^{-5}	500	150	30	2 bp	22 000
15°C	7.6×10^{-6}	3000	900	180	13 bp	131 000
5°C	1.1×10^{-6}	20 000	6000	1200	88 bp	882 000
−5°C	1.5×10^{-7}	158 000	47 000	9500	683 bp	6 830 000

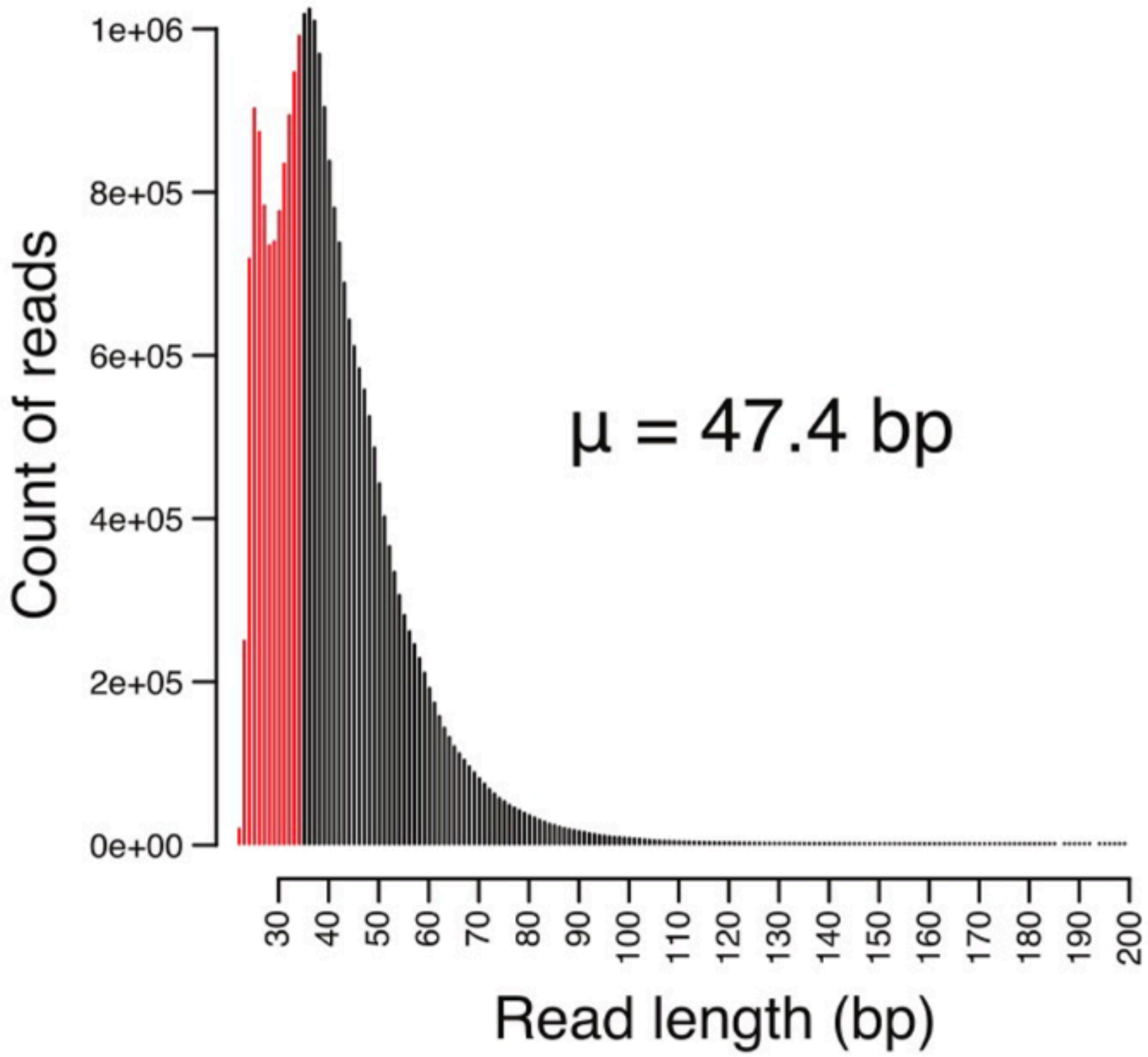
Average fragment length of 6.8 million-year old DNA
1 base pair



Allentoft et al (2012) Proc R Soc B

Challenges - Ancient DNA preservation





DNA from a 1.2 million year old mammoth tooth is highly fragmented

van der Valk et al (2020) Nature

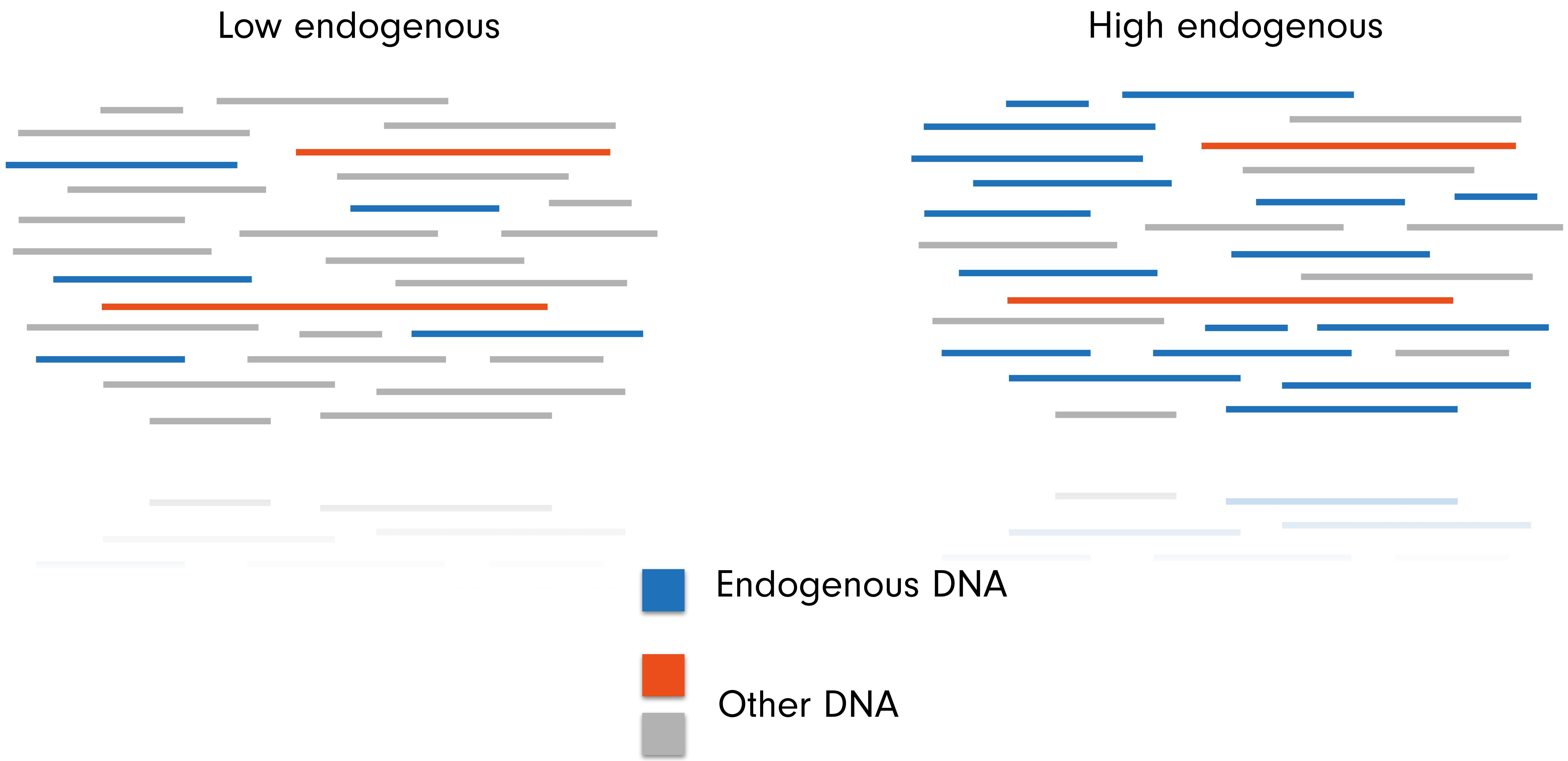
Challenges - Endogenous DNA content

Low endogenous

🟦 Endogenous DNA

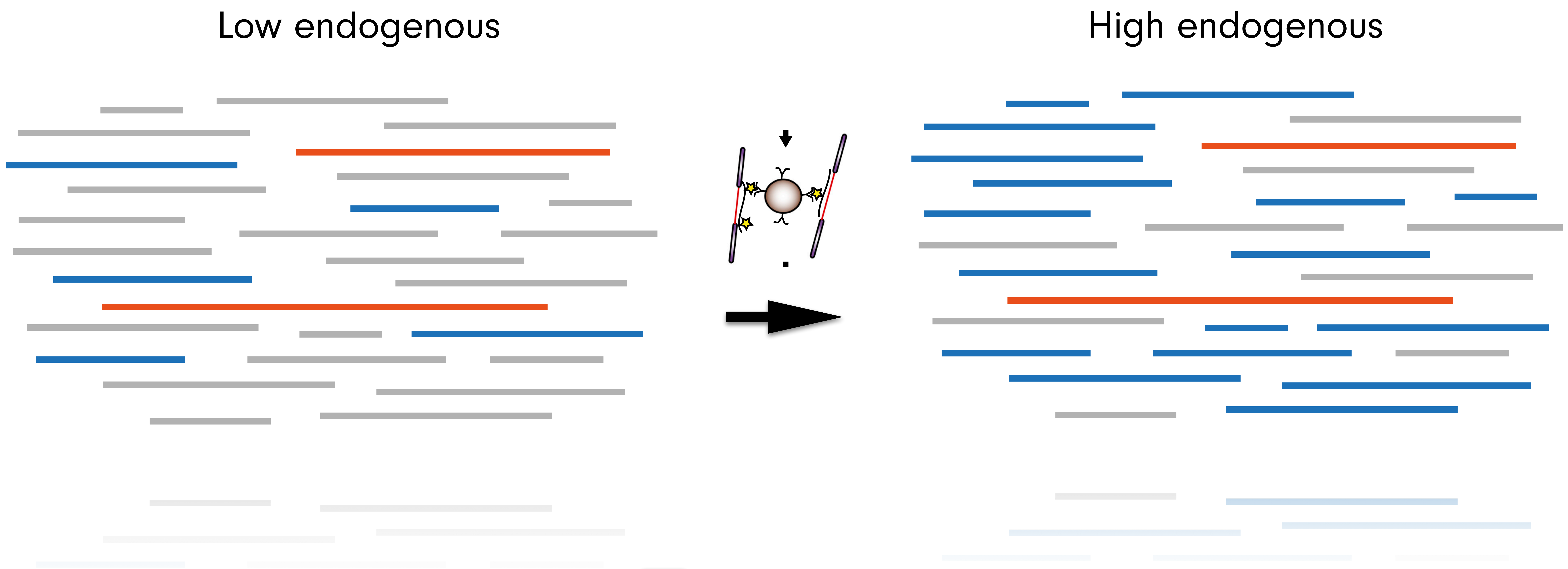
🟧
⬜ Other DNA

Challenges - Endogenous DNA content



Endogenous DNA content varies substantially between samples

Challenges - Endogenous DNA content



Low endogenous

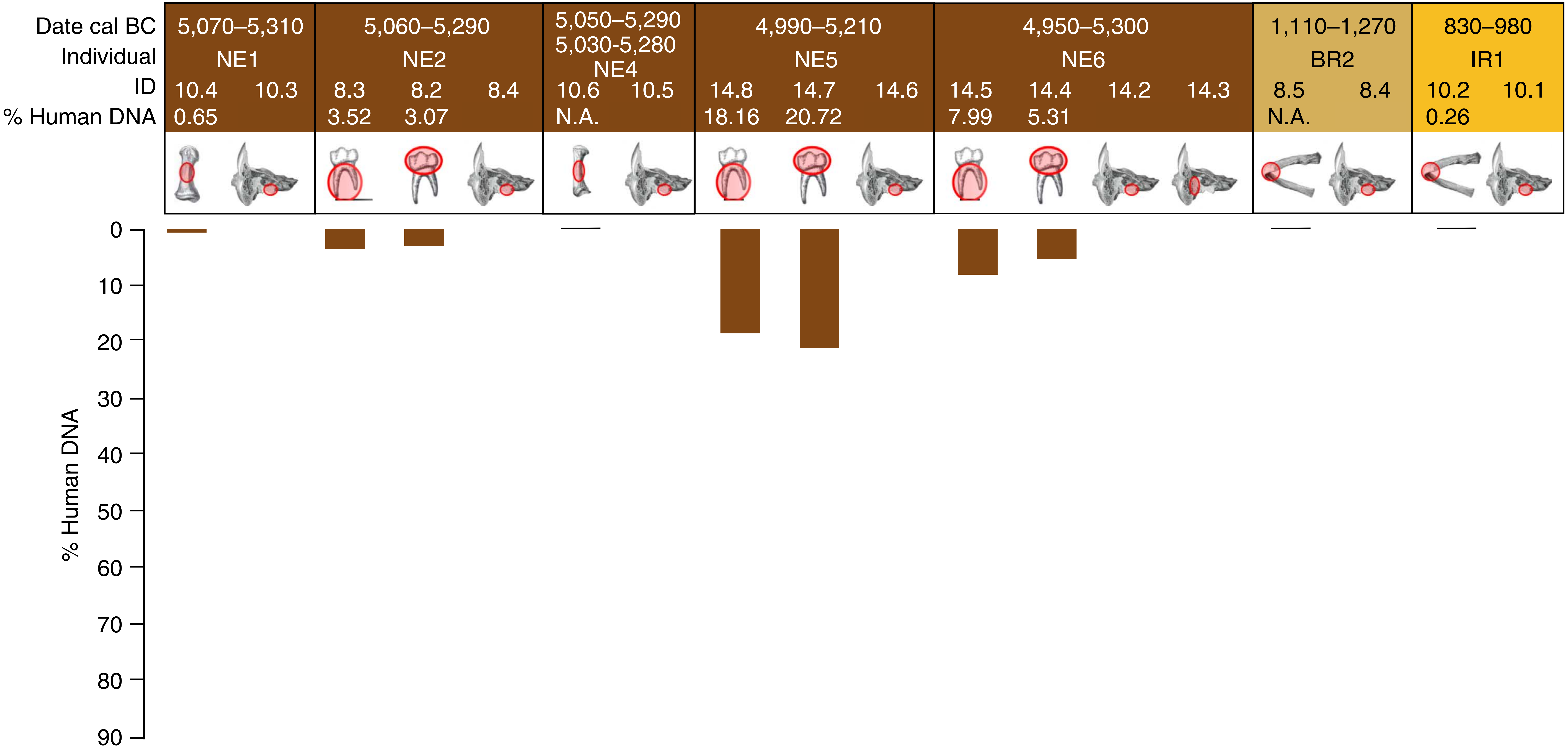
High endogenous

🟦 Endogenous DNA

🟧 ⬜ Other DNA

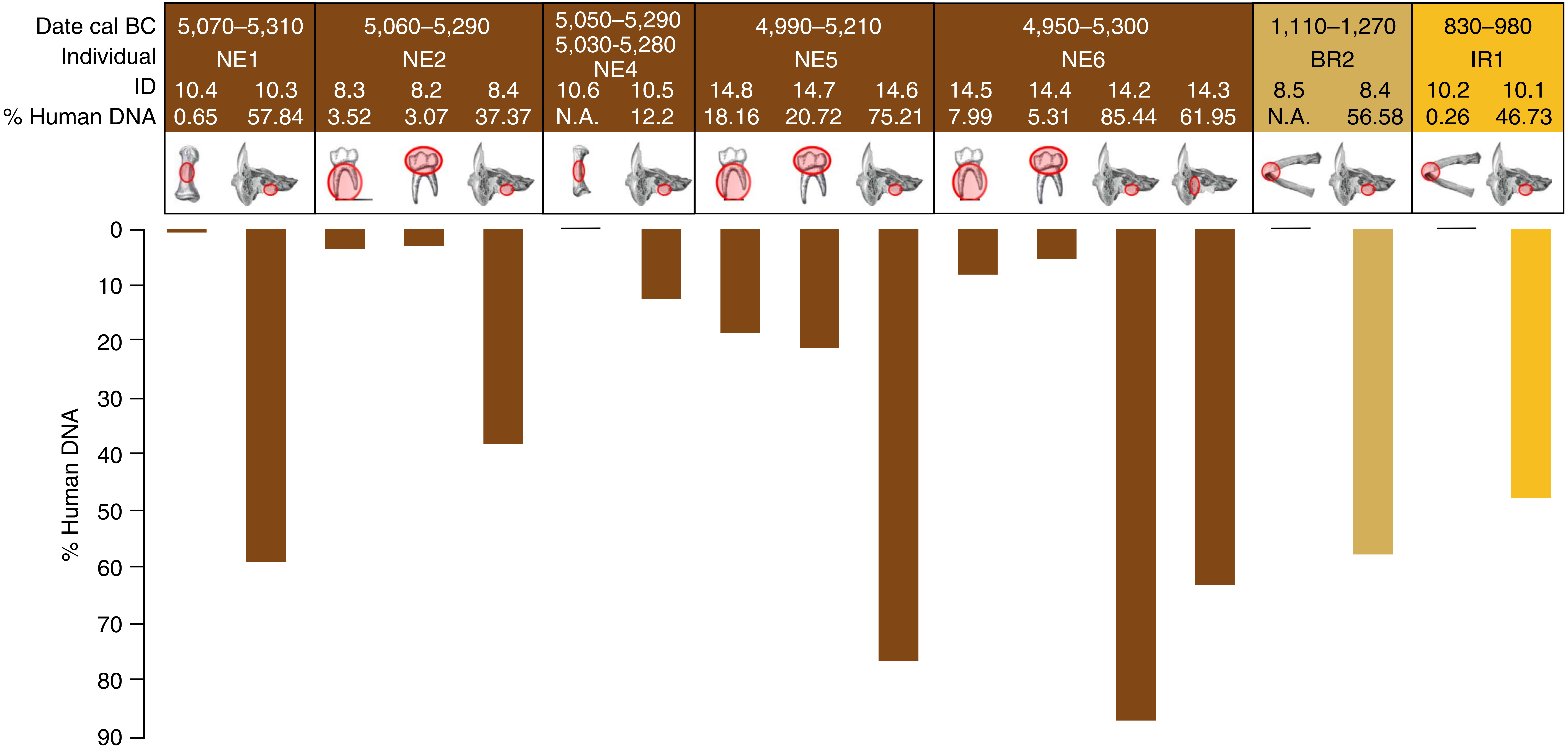
DNA capture enrichment (targeted or whole genome) to increase endogenous DNA

Impact of sample material



Gamba et al (2014) Nat Comm

Impact of sample material



Date cal BC	5,070–5,310		5,060–5,290			5,050–5,290 5,030-5,280		4,990–5,210			4,950–5,300				1,110–1,270		830–980	
Individual	NE1		NE2			NE4		NE5			NE6				BR2		IR1	
ID	10.4	10.3	8.3	8.2	8.4	10.6	10.5	14.8	14.7	14.6	14.5	14.4	14.2	14.3	8.5	8.4	10.2	10.1
% Human DNA	0.65	57.84	3.52	3.07	37.37	N.A.	12.2	18.16	20.72	75.21	7.99	5.31	85.44	61.95	N.A.	56.58	0.26	46.73

Gamba et al (2014) Nat Comm

Ancient DNA challenges

Sample

DNA sequences

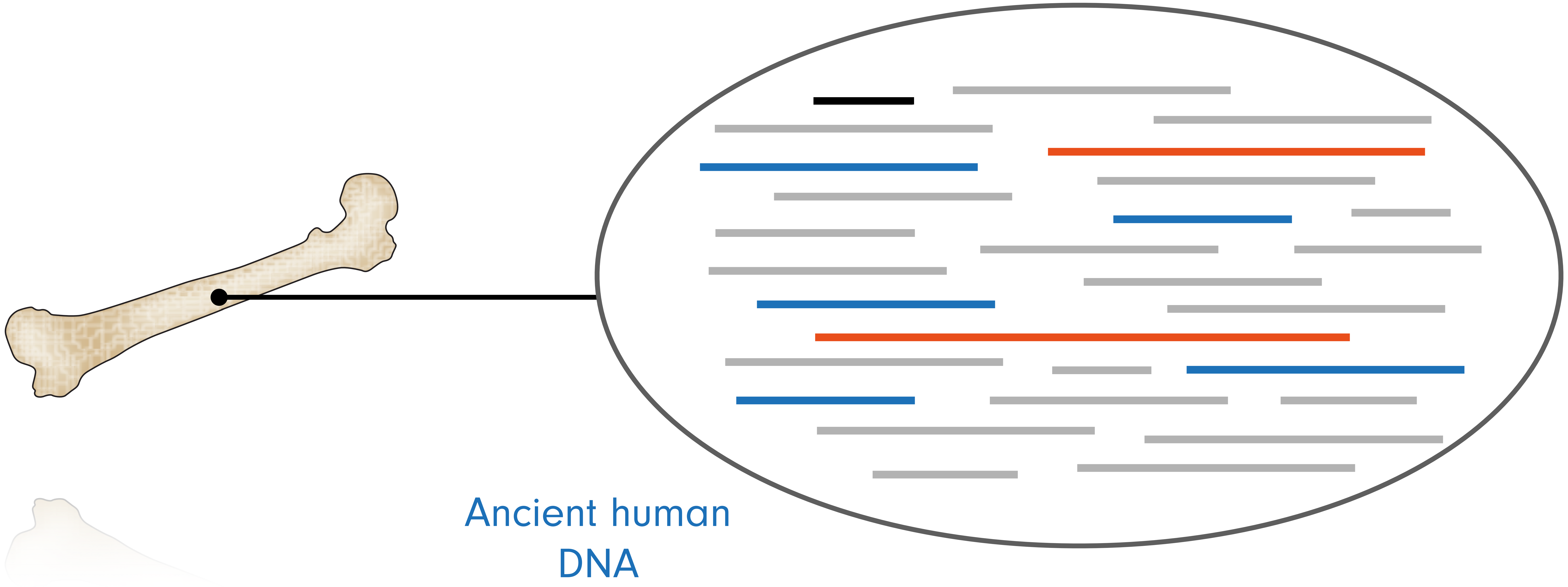
Analysis results

Data quality

Contamination
Genome coverage
Error rates

Stoneking and Krause (2011) Nat Rev Genet; Novembre et al (2008) Nature

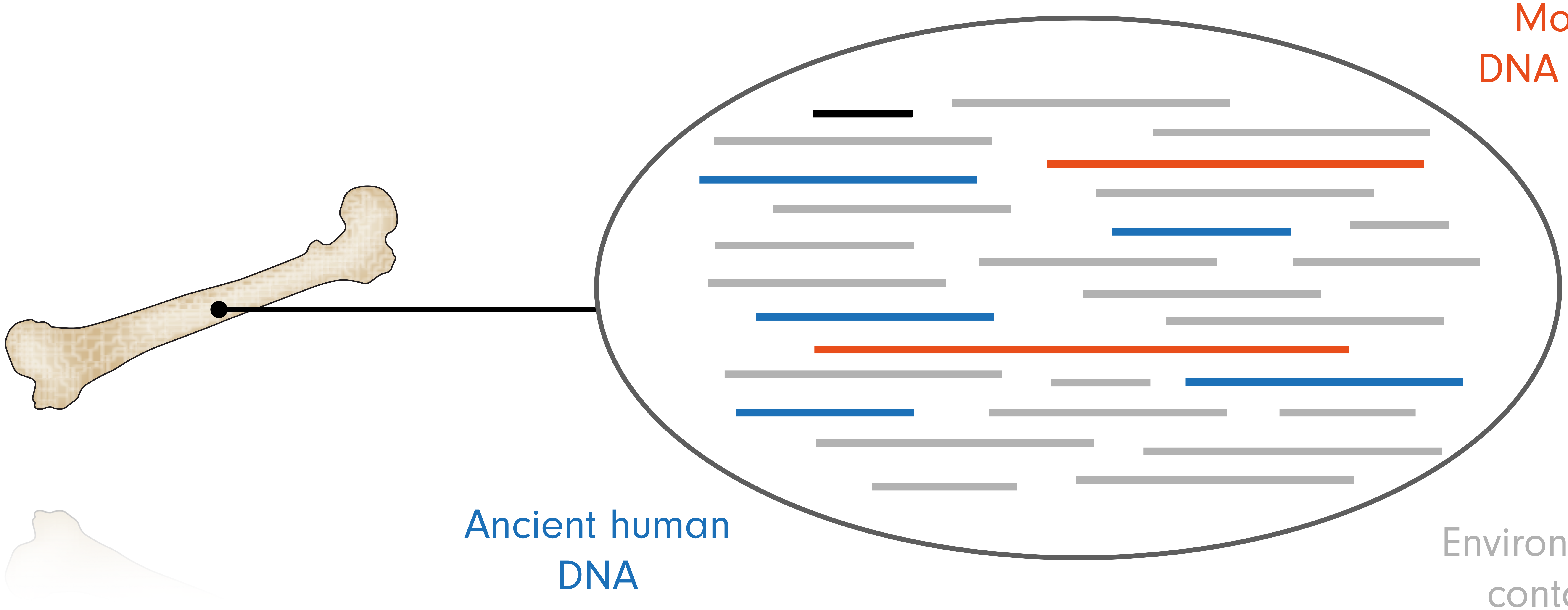
Challenges - Contamination



Ancient human DNA

Stoneking and Krause (2011) Nat Rev Genet

Challenges - Contamination



Modern human
DNA contamination

Ancient human
DNA

Environmental DNA
contamination

Ancient DNA data is a complex mixture of DNA from different sources

Stoneking and Krause (2011) Nat Rev Genet

Challenges - Contamination

Barcelona lab

Florence lab

```
Monti Lessini (L906-H924)
                                                            TCATCTACGCCTTCCACAGCCAGGAGCTCCGCAGGACGCTCAAGGAGGTGCTGACAT
B.2.1                                                                           ............G....
B.2.2                                                                           ............G....
B.2.3                                                                           ............G....
B.2.4                                                                           .................
B.2.5                                                                           .................
B.2.6                                                                           .................
B.2.7                                                                           .................
B.2.8                                                                           .................
B.2.9                                                                           .................
B.2.10                                                                          .................
B.2.11                                                                          .................
B.2.12                                                                          .................

Monti Lessini (L884-H936)
                                      TGCAATGCCATCATCGACCCCCTCATCTACGCCTTCCACAGCCAGGAGCTCCGCAGGACGCTCAAGGAGGTGCTGACATG
CTCCTGGTGA
F.1.1                                                     ..................................G................
F.1.2                                                     ..................................G................
F.1.3                                                     ..................................G................
F.1.4                                                     ..................................G................
F.1.5                                                     ..................................G................
F.1.6                                                     .......C...........................................
F.1.7                                                     ..C................................................
F.1.8                                                     ...................................................
F.1.9                                                     ...................................................
F.1.10                                                    ...................................................
F.1.11                                                    ...................................................
F.1.12                                                    ...................................................
F.1.13                                                    ...................................................
F.1.14                                                    ...................................................
F.1.15                                                    ...................................................
F.1.16                                                    ...................................................
F.1.17                                                    ...................................................
F.1.18                                                    ...................................................
F.1.19                                                    ...................................................
F.1.20                                                    ...................................................
F.1.21                                                    ...................................................
F.1.22                                                    ...................................................
F.1.23                                                    ...................................................
```

MC1R gene fragments amplified from Monti Lessini Neandertal DNA extract

Lalueza-Fox et al (2007) Science

Challenges - Contamination

Barcelona lab

```
Monti Lessini (L906-H924)

          TCATCTACGCCTTCCACAGCCAGGAGCTCCGCAGGACGCTCAAGGAGGTGCTGACAT
B.2.1                         ............G....
B.2.2                         ............G....
B.2.3                         ............G....
B.2.4                         .................
B.2.5                         .................
B.2.6                         .................
B.2.7                         .................
B.2.8                         .................
B.2.9                         .................
B.2.10                        .................
B.2.11                        .................
B.2.12                        .................
```

Neandertal sequences

Florence lab

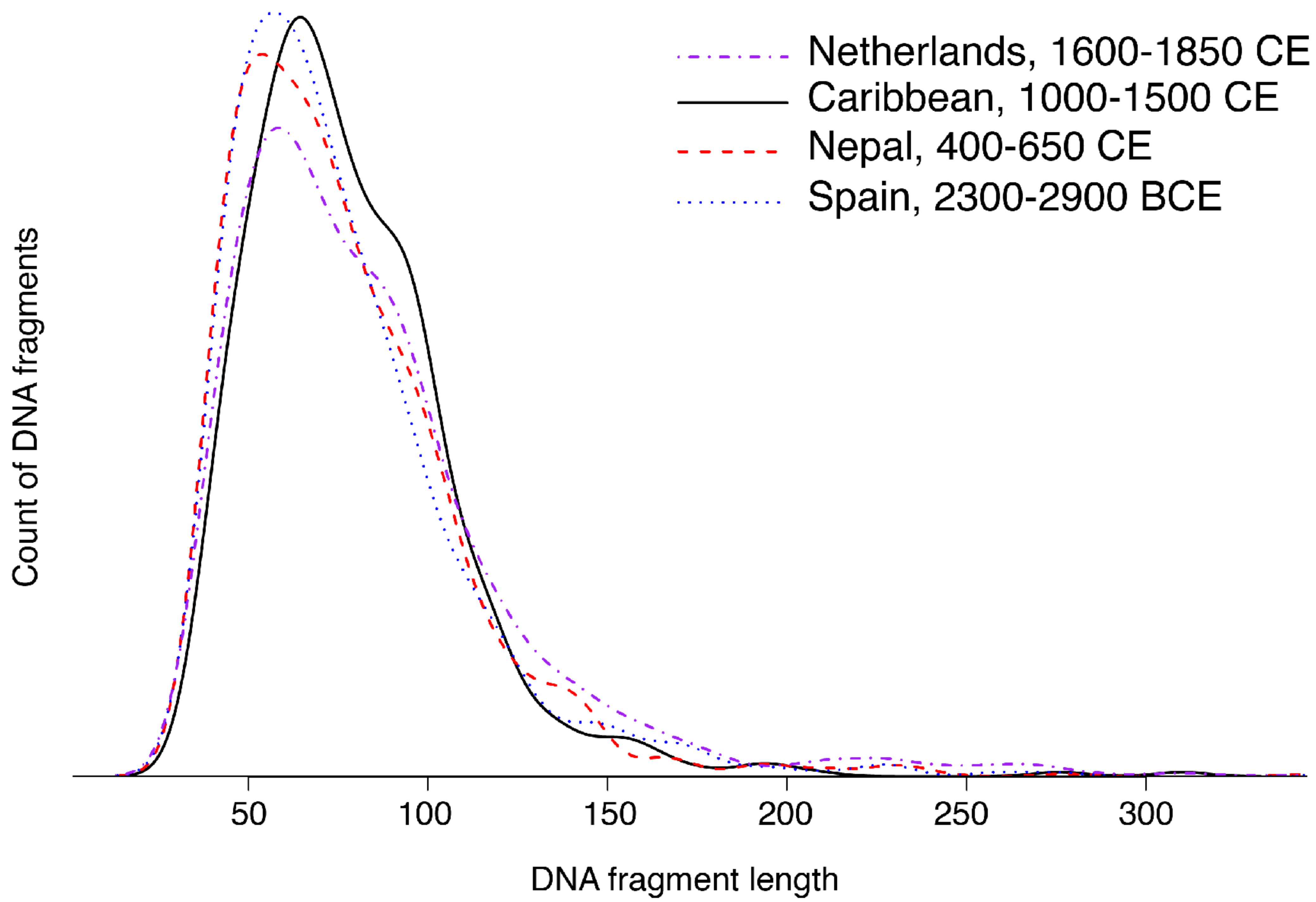
```
Monti Lessini (L884-H936)
          TGCAATGCCATCATCGACCCCCTCATCTACGCCTTCCACAGCCAGGAGCTCCGCAGGACGCTCAAGGAGGTGCTGACATG
CTCCTGGTGA
F.1.1                         ..................................G................
F.1.2                         ..................................G................
F.1.3                         ..................................G................
F.1.4                         ..................................G................
F.1.5                         ..................................G................
F.1.6                         .......C...........................................
F.1.7                         ..C................................................
F.1.8                         ...................................................
F.1.9                         ...................................................
F.1.10                        ...................................................
F.1.11                        ...................................................
F.1.12                        ...................................................
F.1.13                        ...................................................
F.1.14                        ...................................................
F.1.15                        ...................................................
F.1.16                        ...................................................
F.1.17                        ...................................................
F.1.18                        ...................................................
F.1.19                        ...................................................
F.1.20                        ...................................................
F.1.21                        ...................................................
F.1.22                        ...................................................
F.1.23                        ...................................................
```

Neandertal sequences

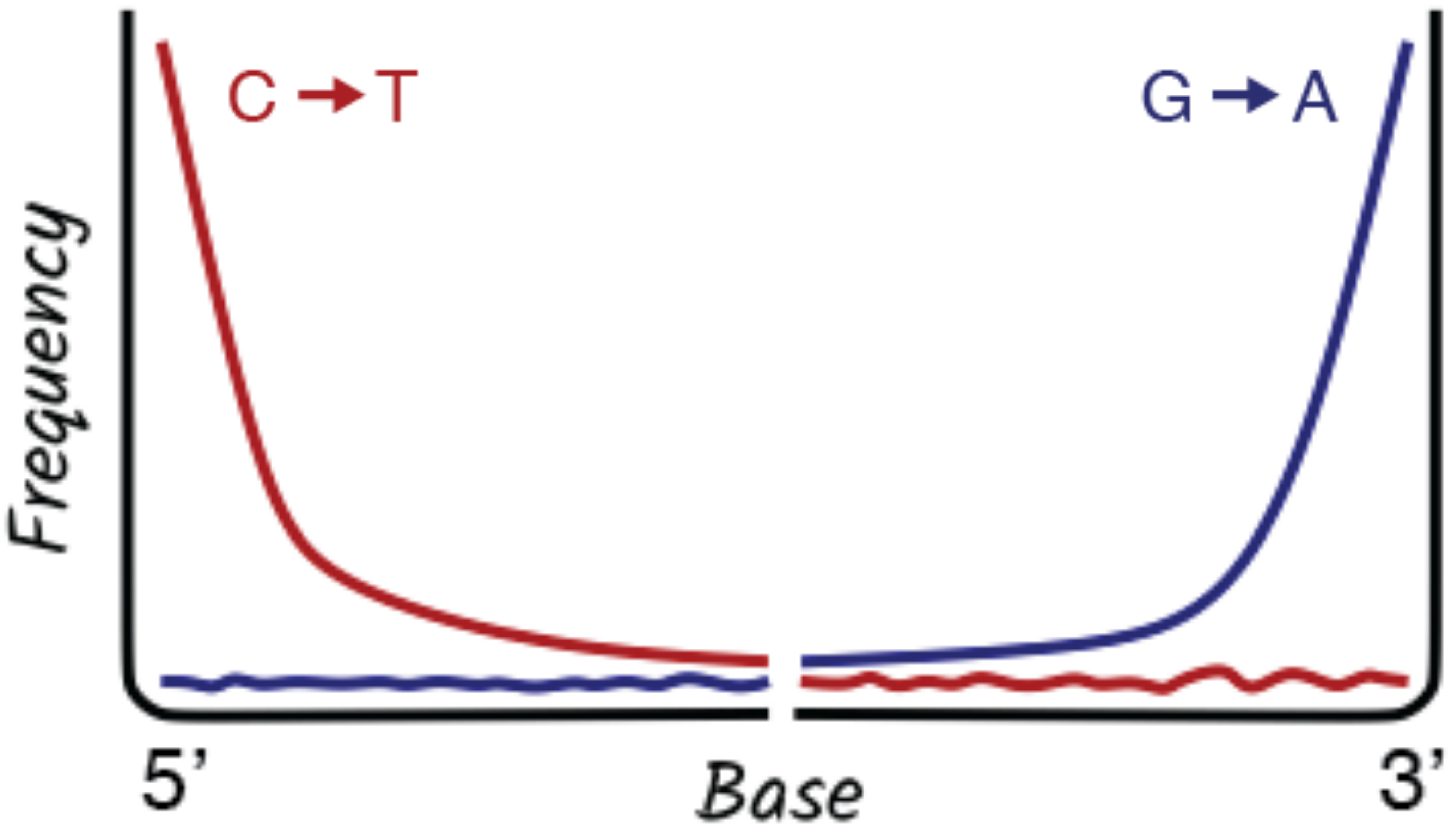
MC1R gene fragments amplified from Monti Lessini Neandertal DNA extract

Lalueza-Fox et al (2007) Science

Authentication of ancient DNA

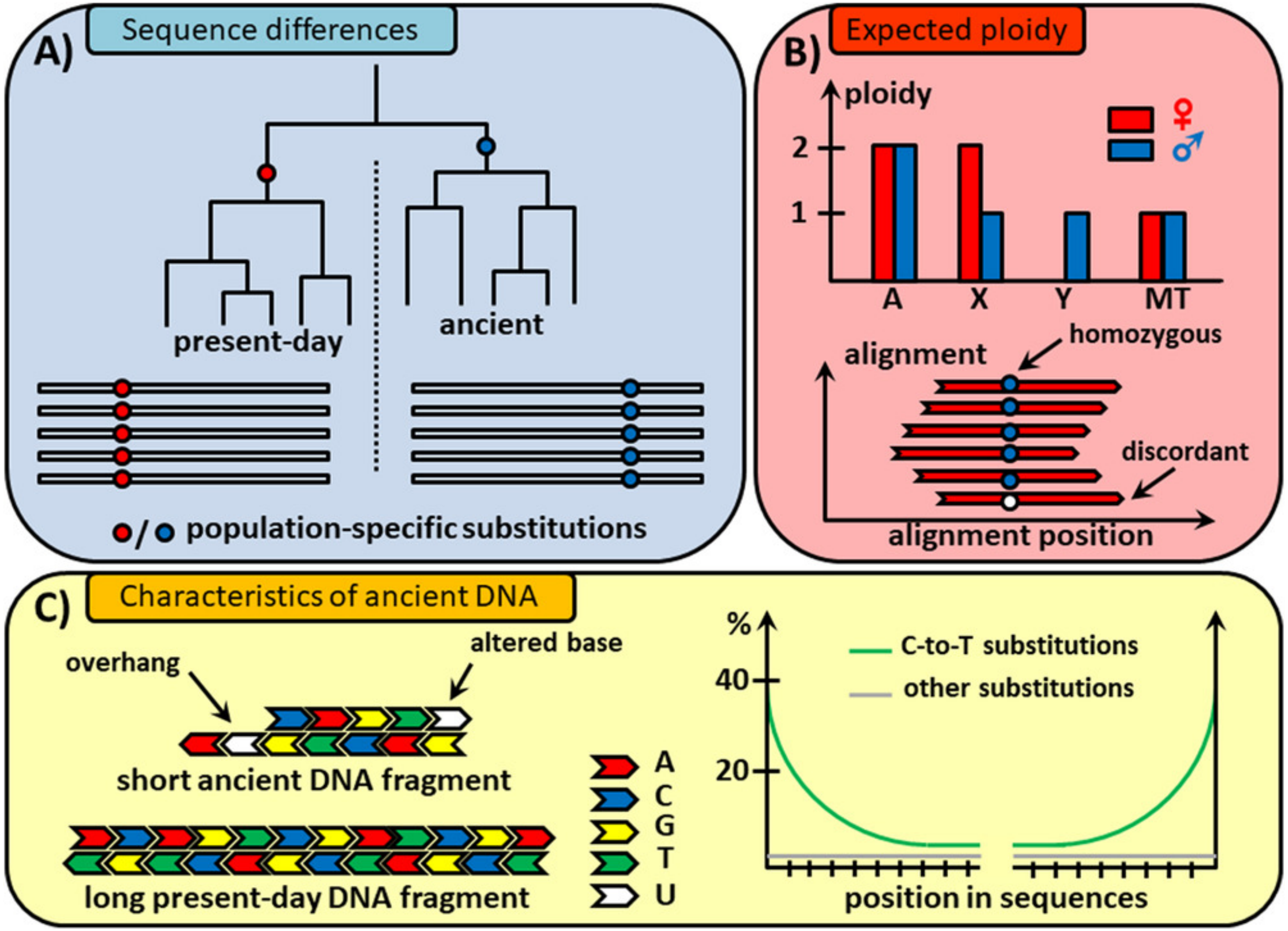


Ancient DNA is short and fragmented



Ancient DNA is damaged
with characteristic substitution patterns

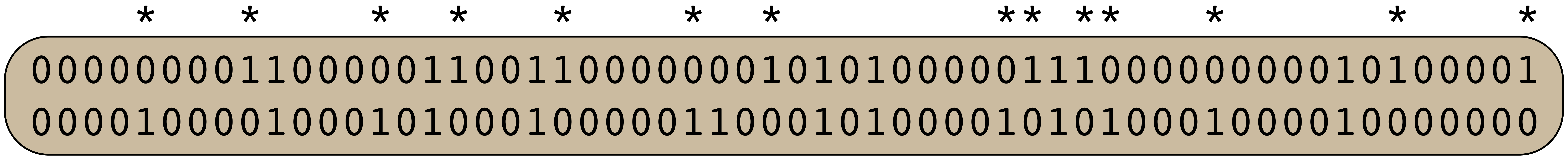
Authentication of ancient DNA



Approaches to estimate contamination levels

Peyrégne and Prüfer (2020) BioEssays

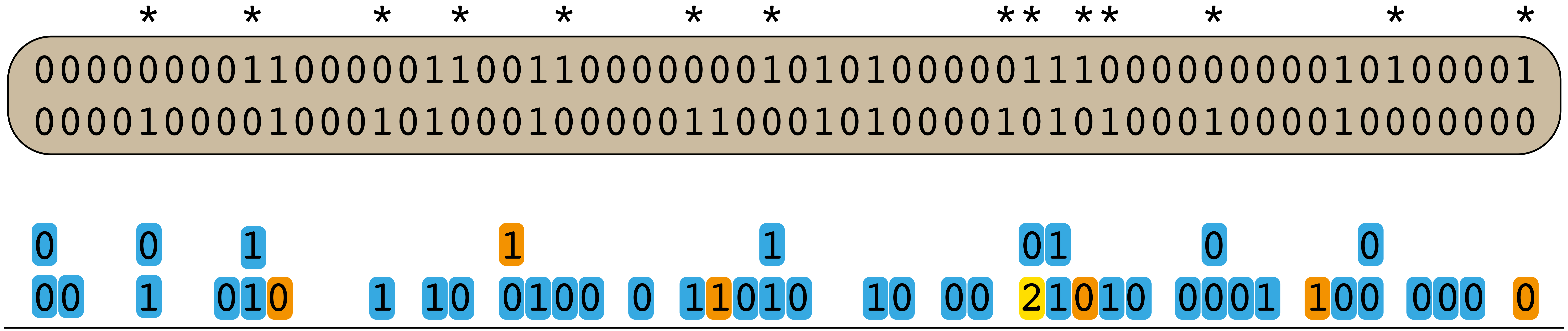
Challenges - Genome coverage and errors



```
    *   *    *  *   *    *  *         ** **   *      *    *
00000000110000011001100000000101010000011100000000010100001
00001000010001010001000000110001010000101010001000010000000
```

Diploid individual

Challenges - Genome coverage and errors



* * * * * * * ** ** * * *

0000000011000001100110000000101010000011100000000010100001

0000100001000101000100000110001010000101010001000001000000

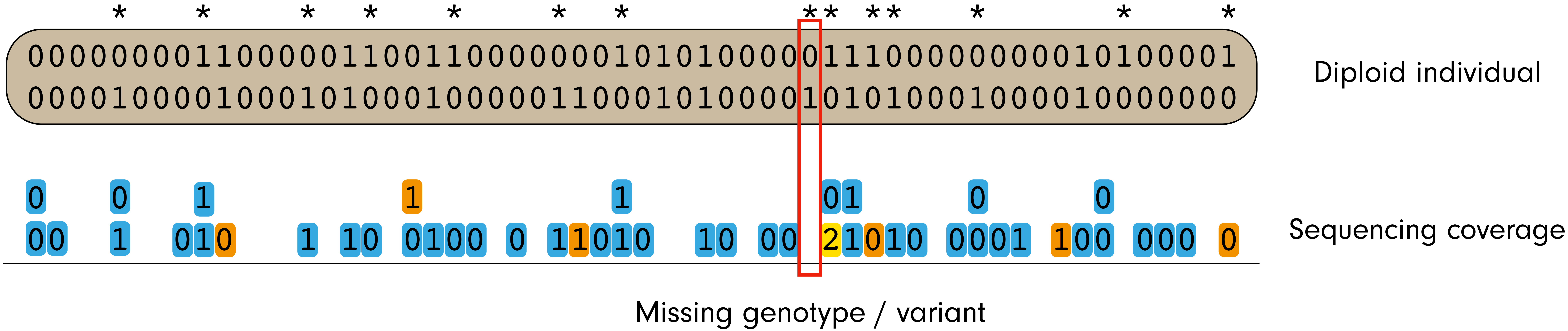
Diploid individual

0 0 1 1 1 01 0 0

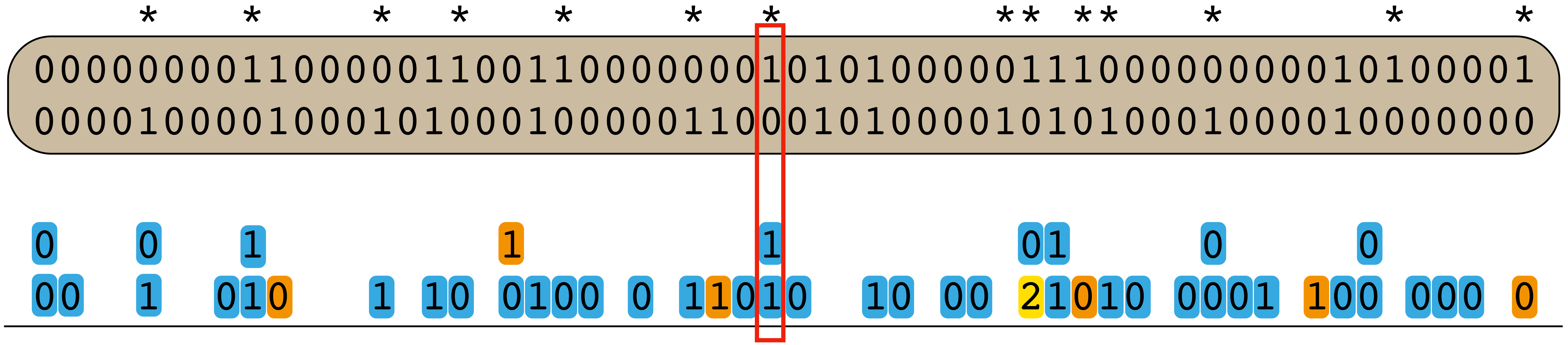
00 1 010 1 10 0100 0 11010 10 00 21010 0001 100 000 0

Sequencing coverage

Challenges - Genome coverage and errors



Challenges - Genome coverage and errors

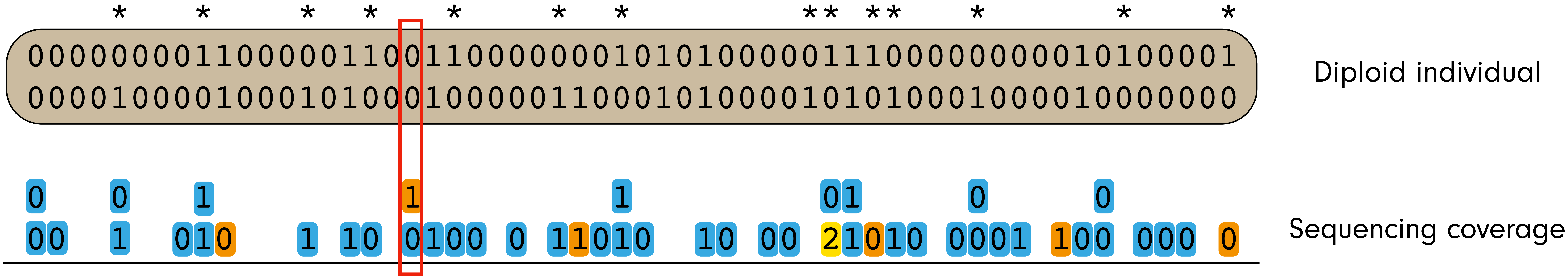


Diploid individual

Sequencing coverage

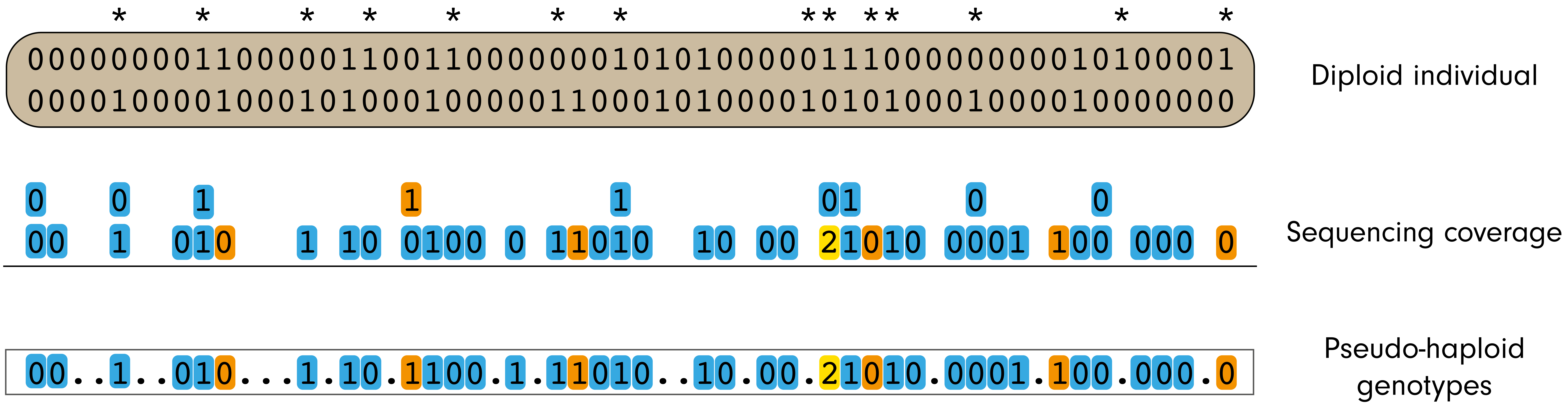
Missing allele at heterozygous site

Challenges - Genome coverage and errors

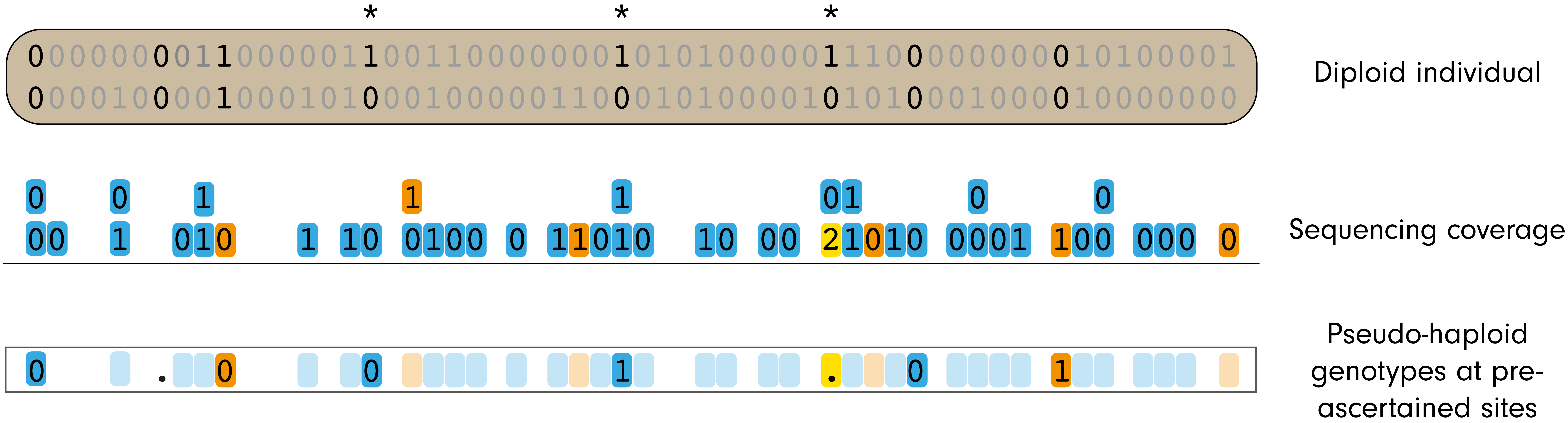


Erroneous genotype

Challenges - Genome coverage and errors

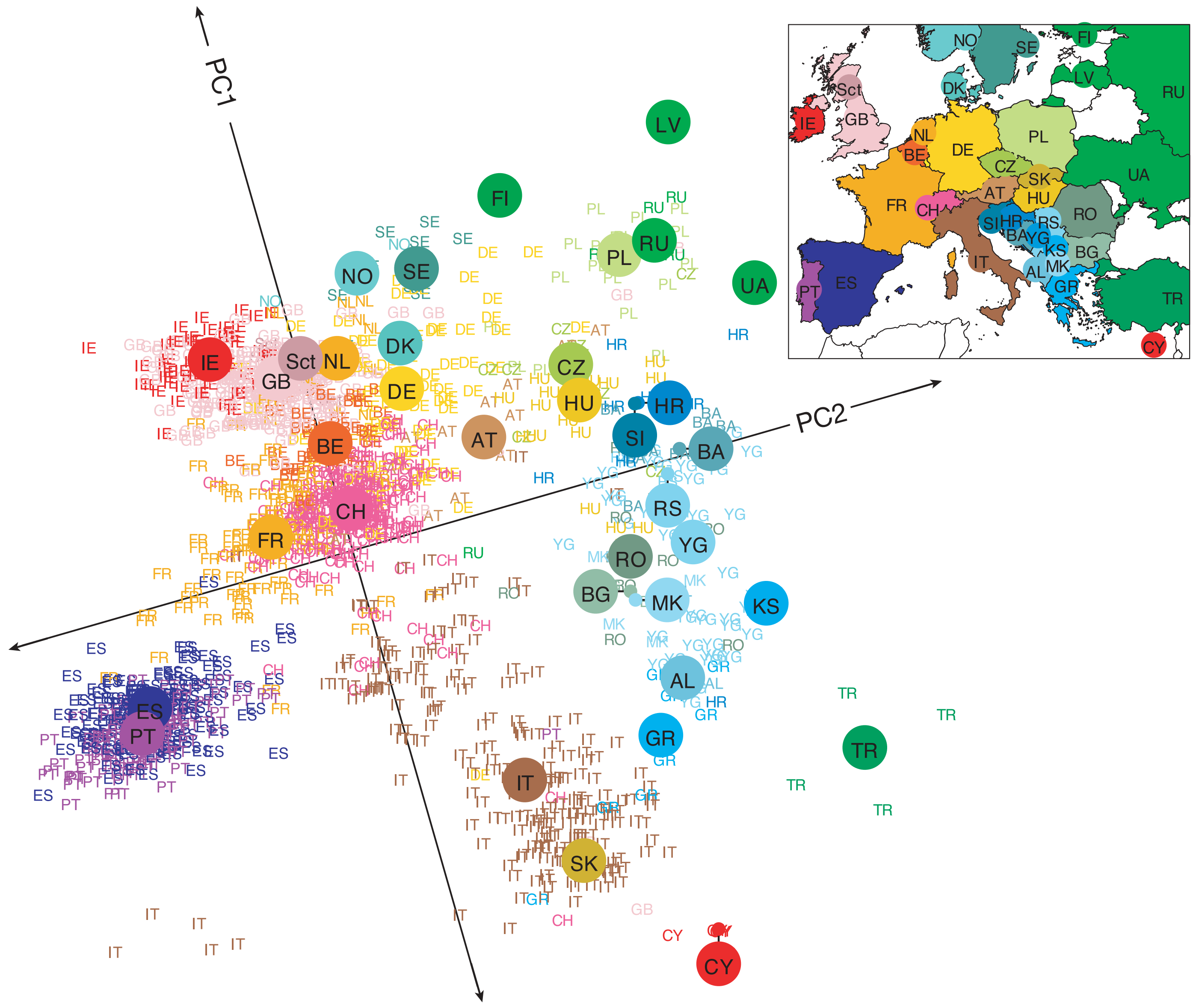


Challenges - Genome coverage and errors

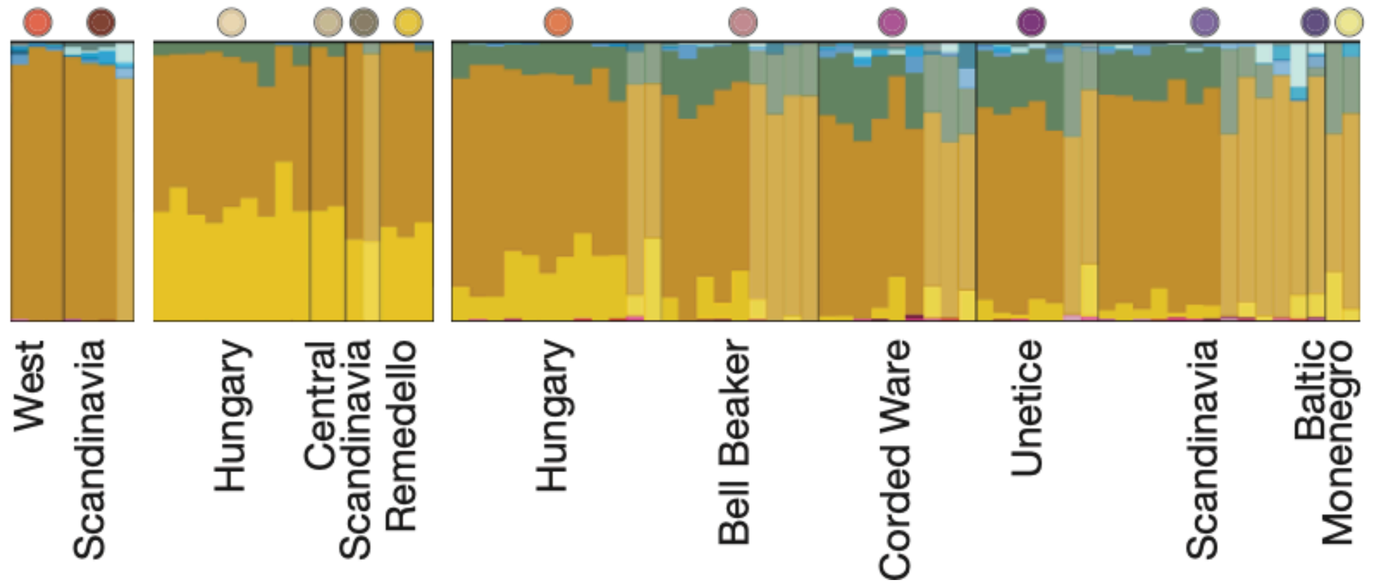


Population genetic analysis of ancient DNA data

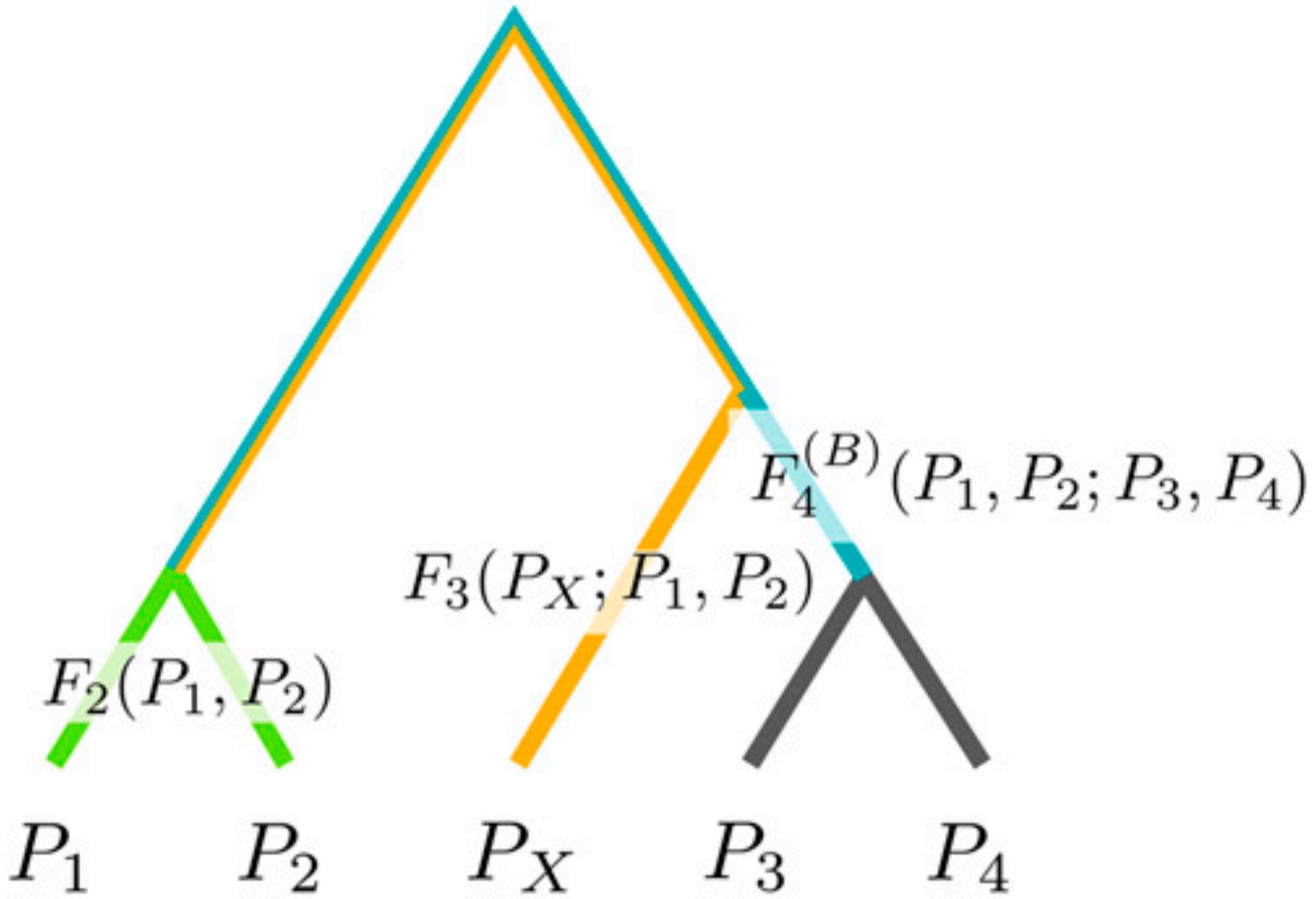
Principal component analysis



Model-based clustering

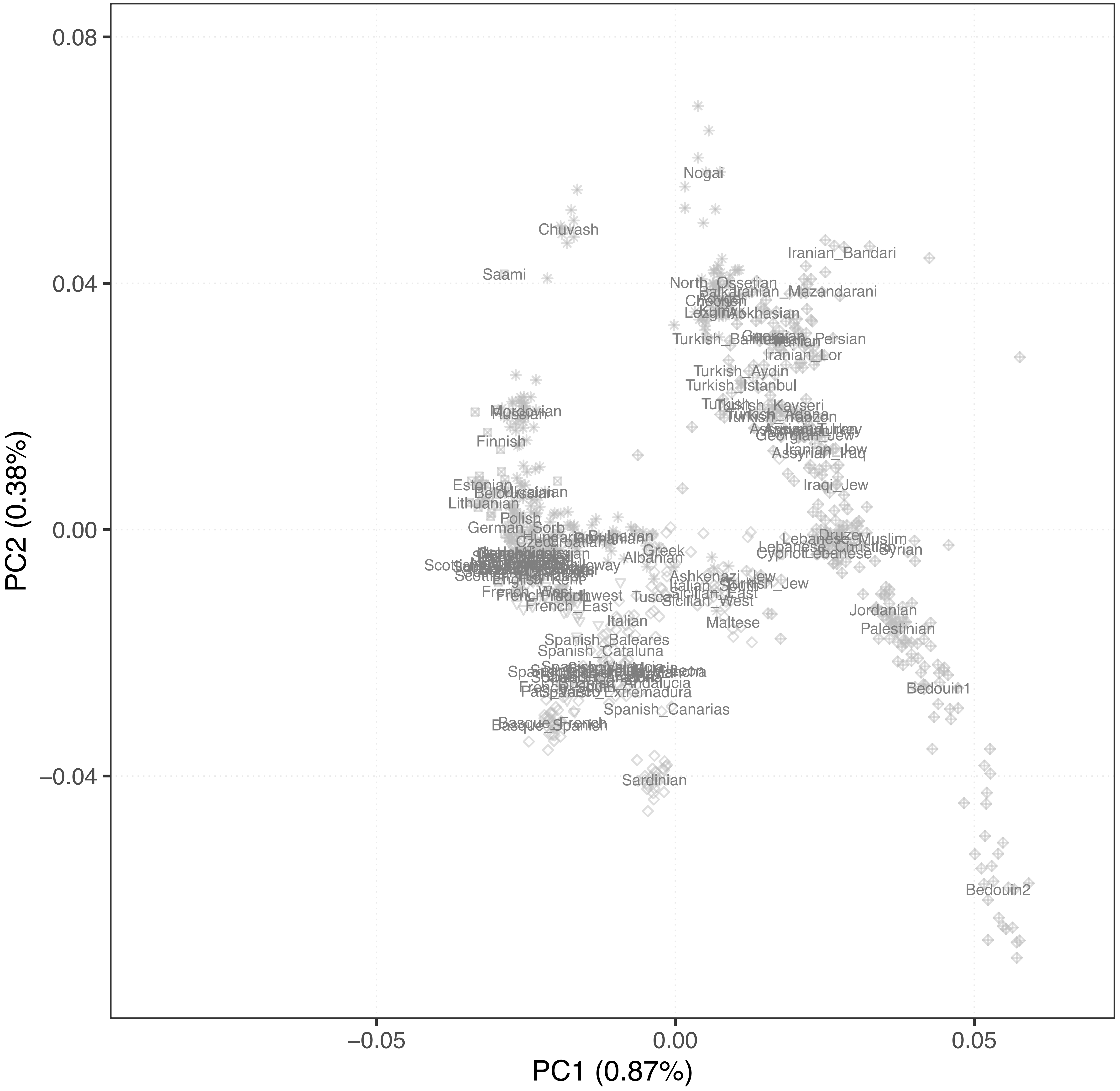


f-statistics



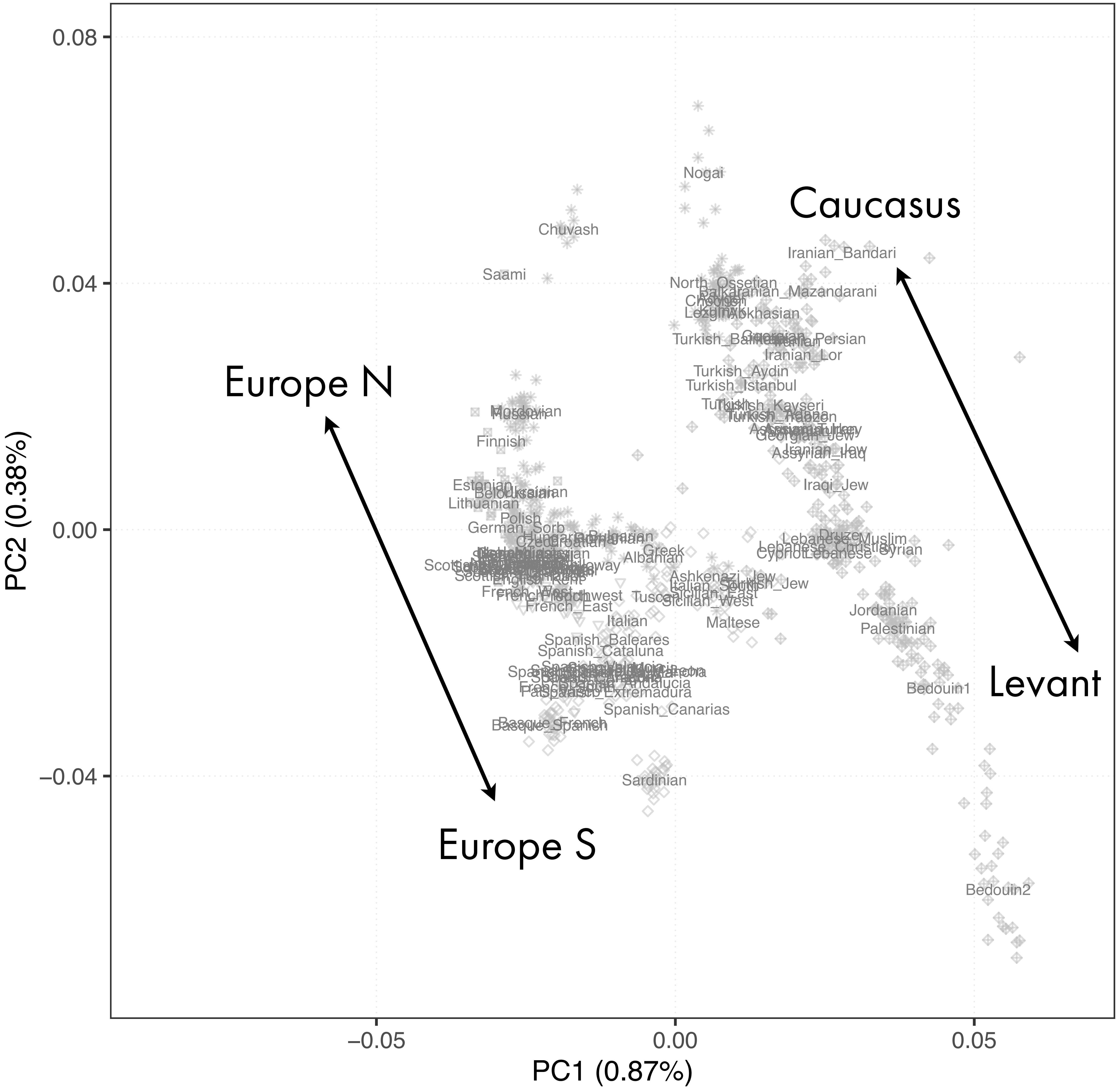
Allele-frequency based methods suited for low coverage / pseudo-haploid data

A PCA tour of western Eurasia



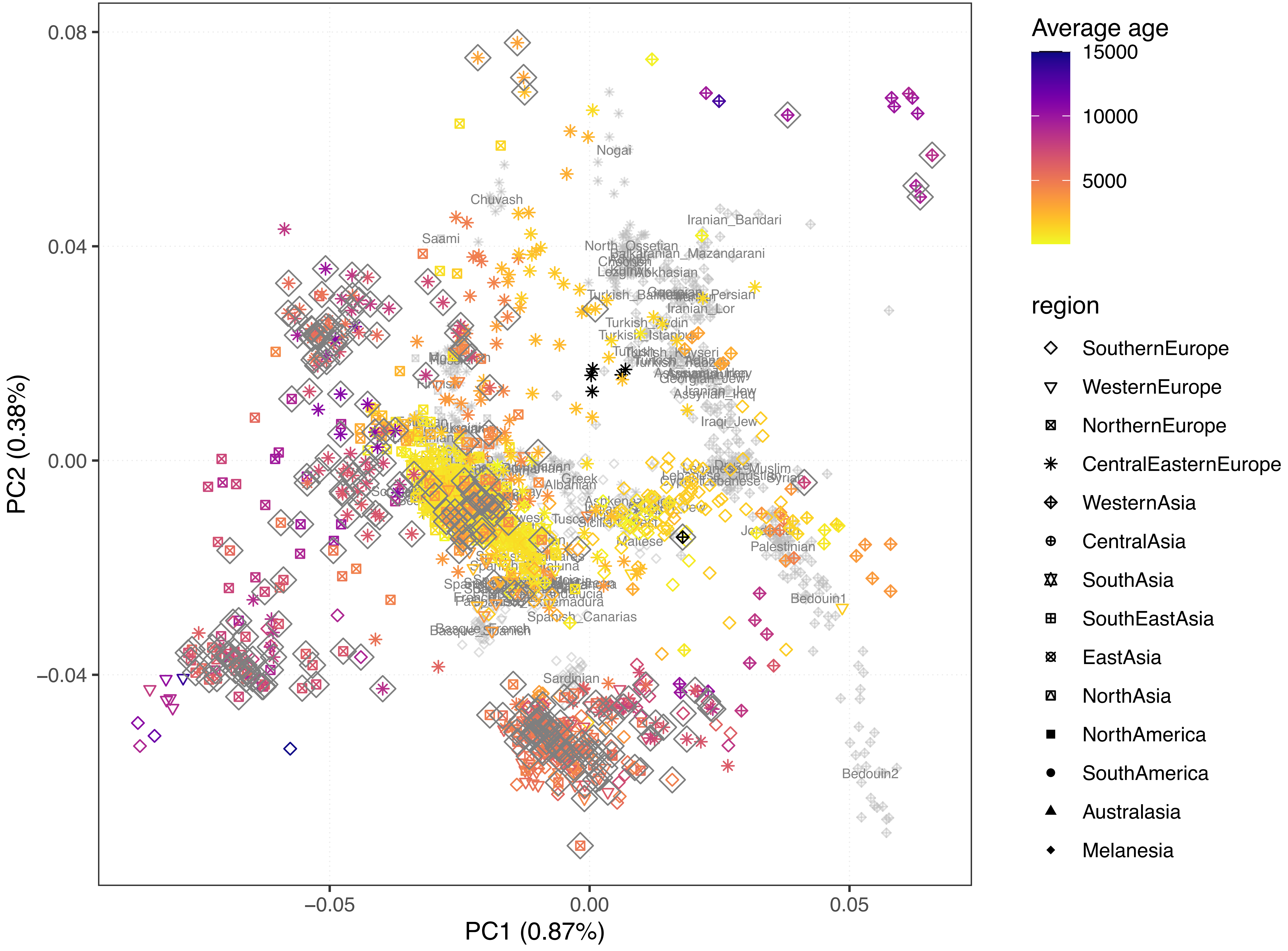
Allentoft*, Sikora* et al (2024) Nature

A PCA tour of western Eurasia



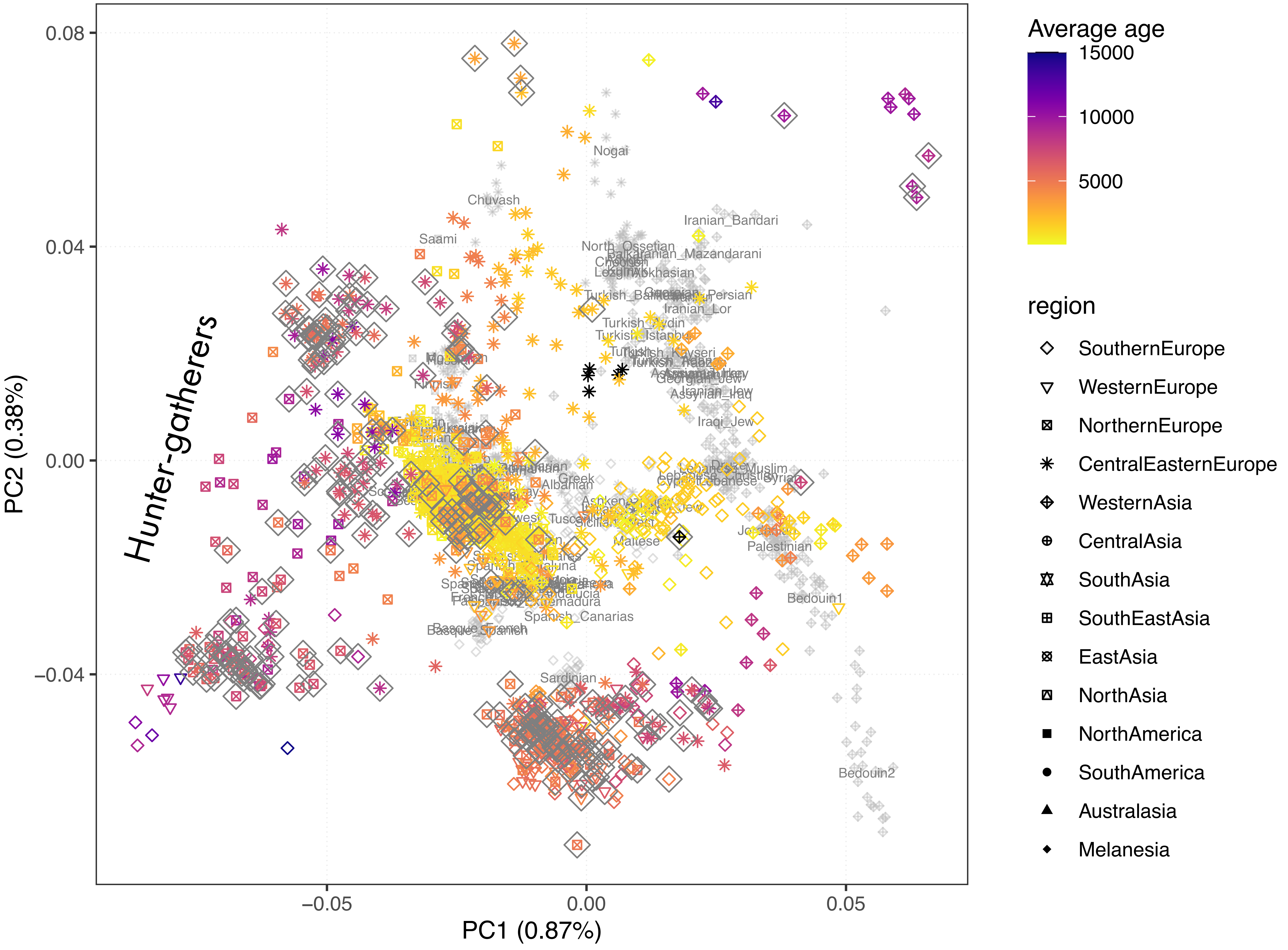
Allentoft*, Sikora* et al (2024) Nature

A PCA tour of western Eurasia



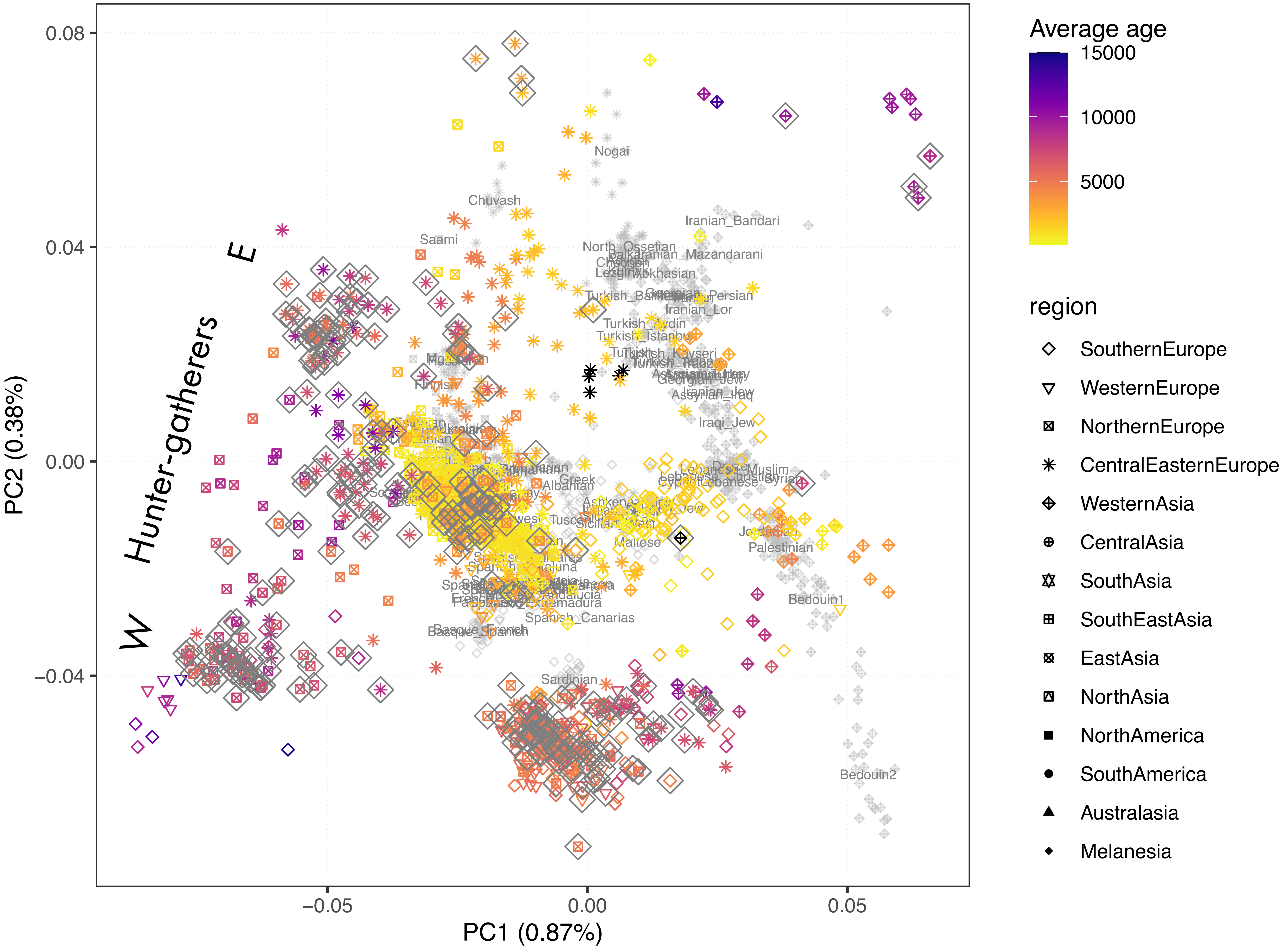
Allentoft*, Sikora* et al (2024) Nature

A PCA tour of western Eurasia



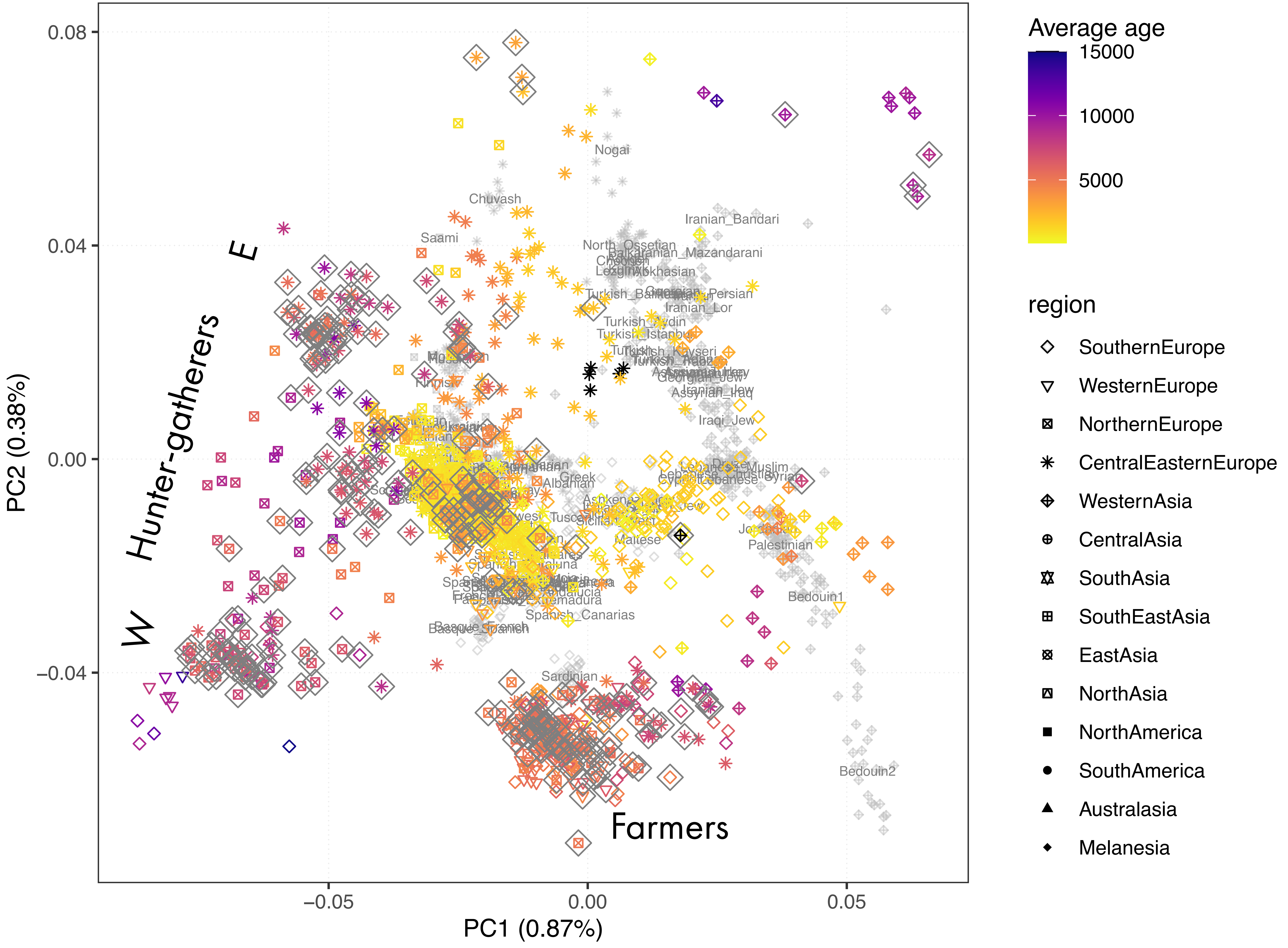
Allentoft*, Sikora* et al (2024) Nature

A PCA tour of western Eurasia



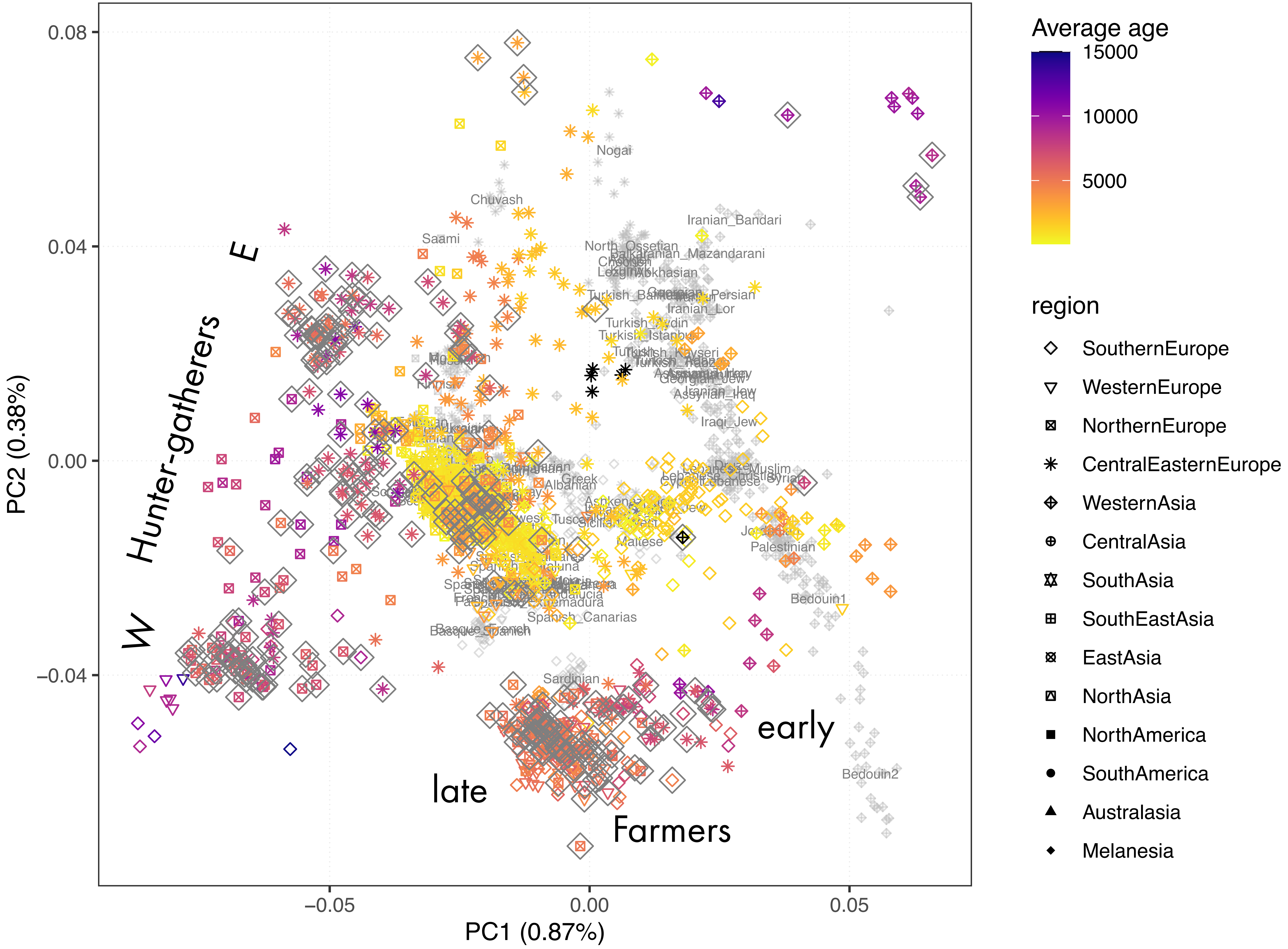
Allentoft*, Sikora* et al (2024) Nature

A PCA tour of western Eurasia



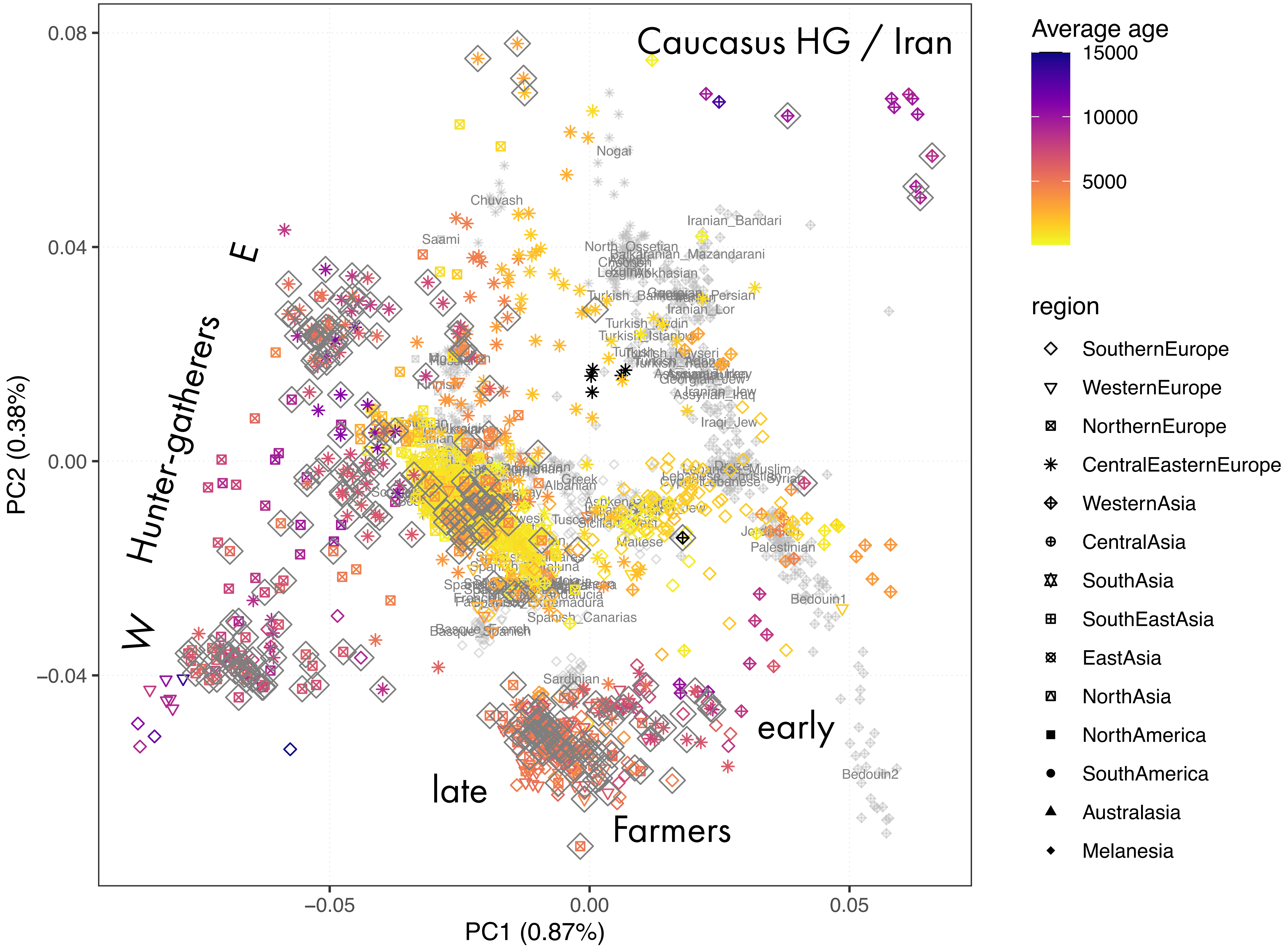
Allentoft*, Sikora* et al (2024) Nature

A PCA tour of western Eurasia



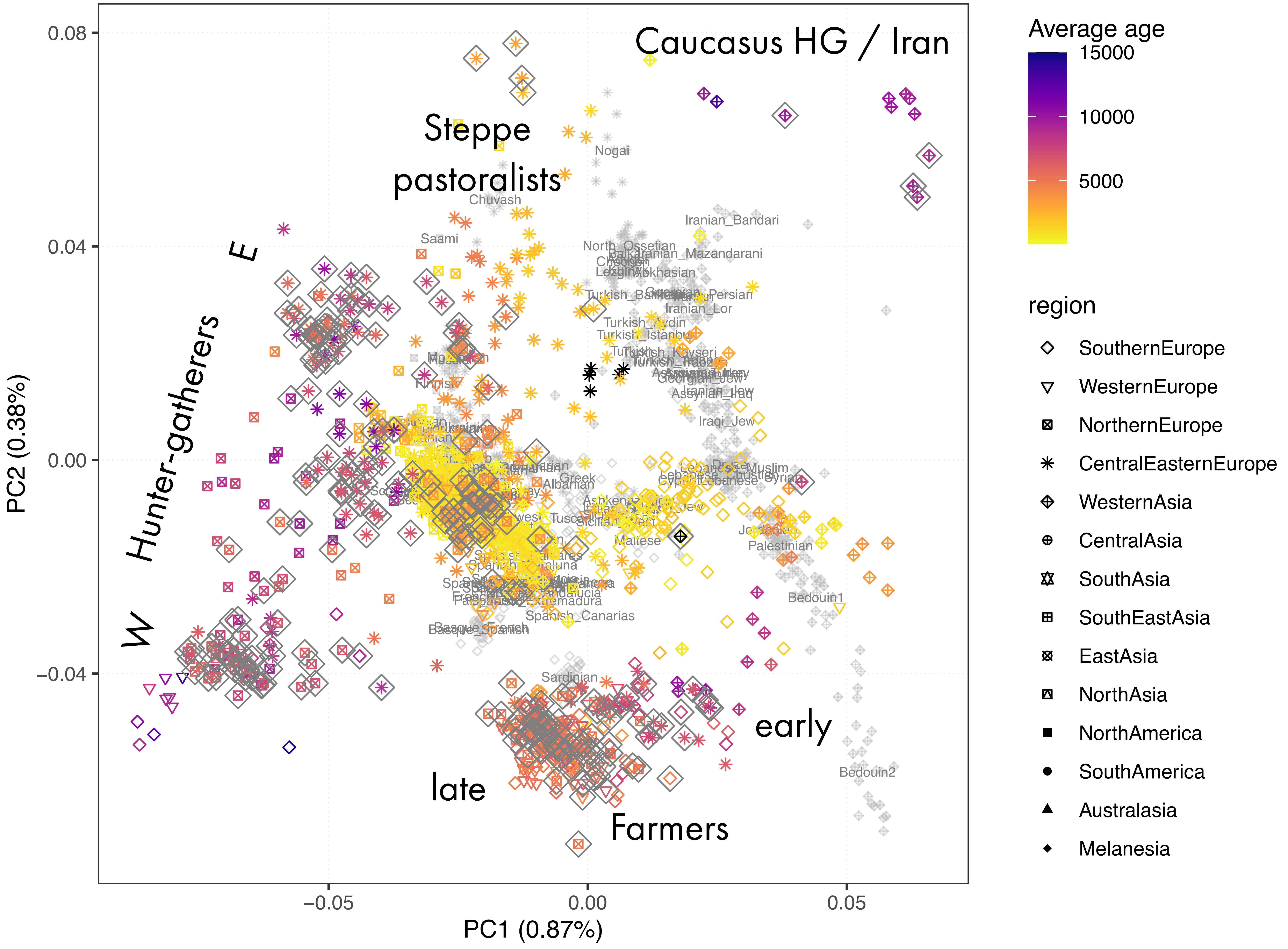
Allentoft*, Sikora* et al (2024) Nature

A PCA tour of western Eurasia



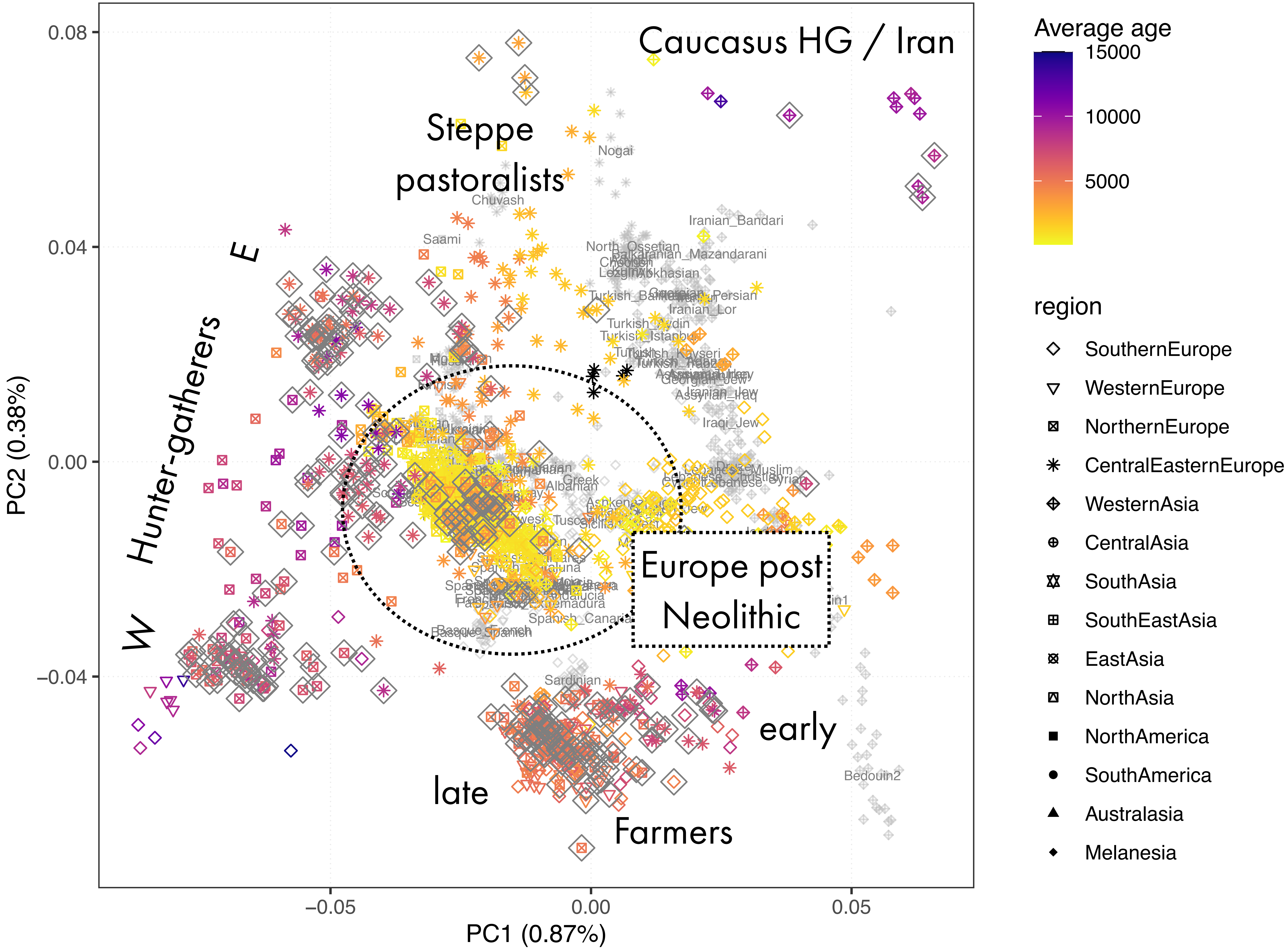
Allentoft*, Sikora* et al (2024) Nature

A PCA tour of western Eurasia



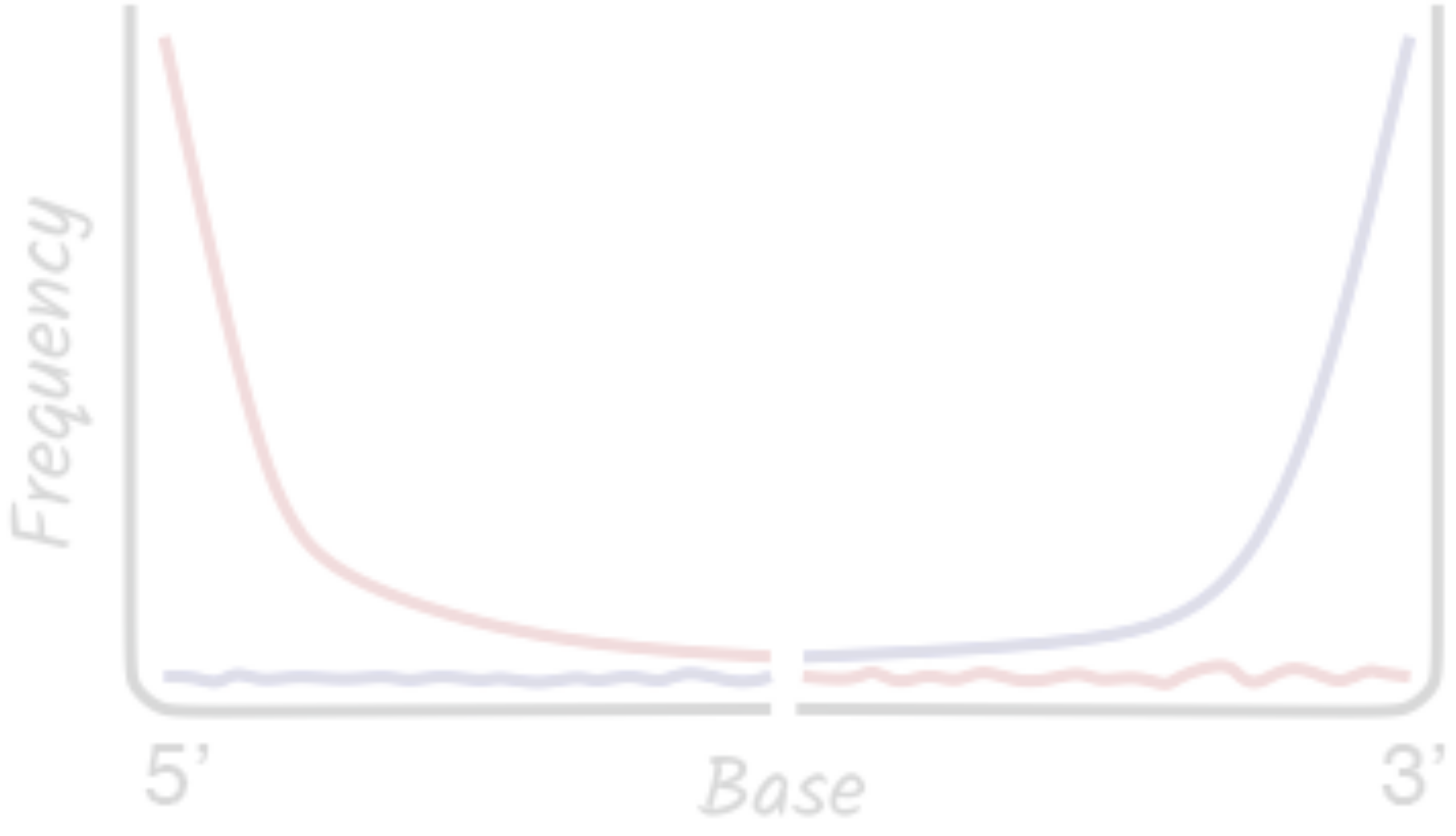
Allentoft*, Sikora* et al (2024) Nature

A PCA tour of western Eurasia



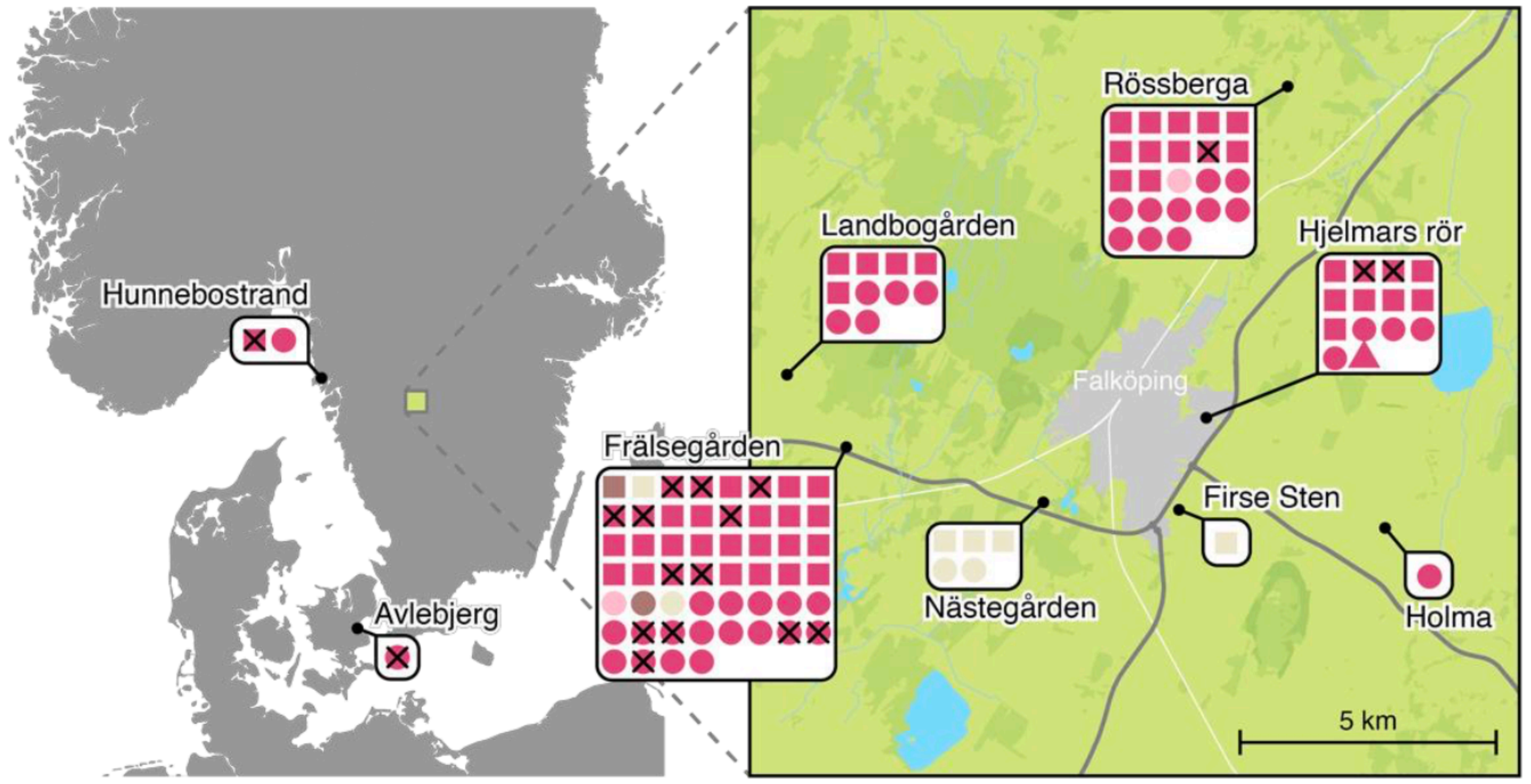
Allentoft*, Sikora* et al (2024) Nature

Topics for today's lecture



Ancient DNA primer

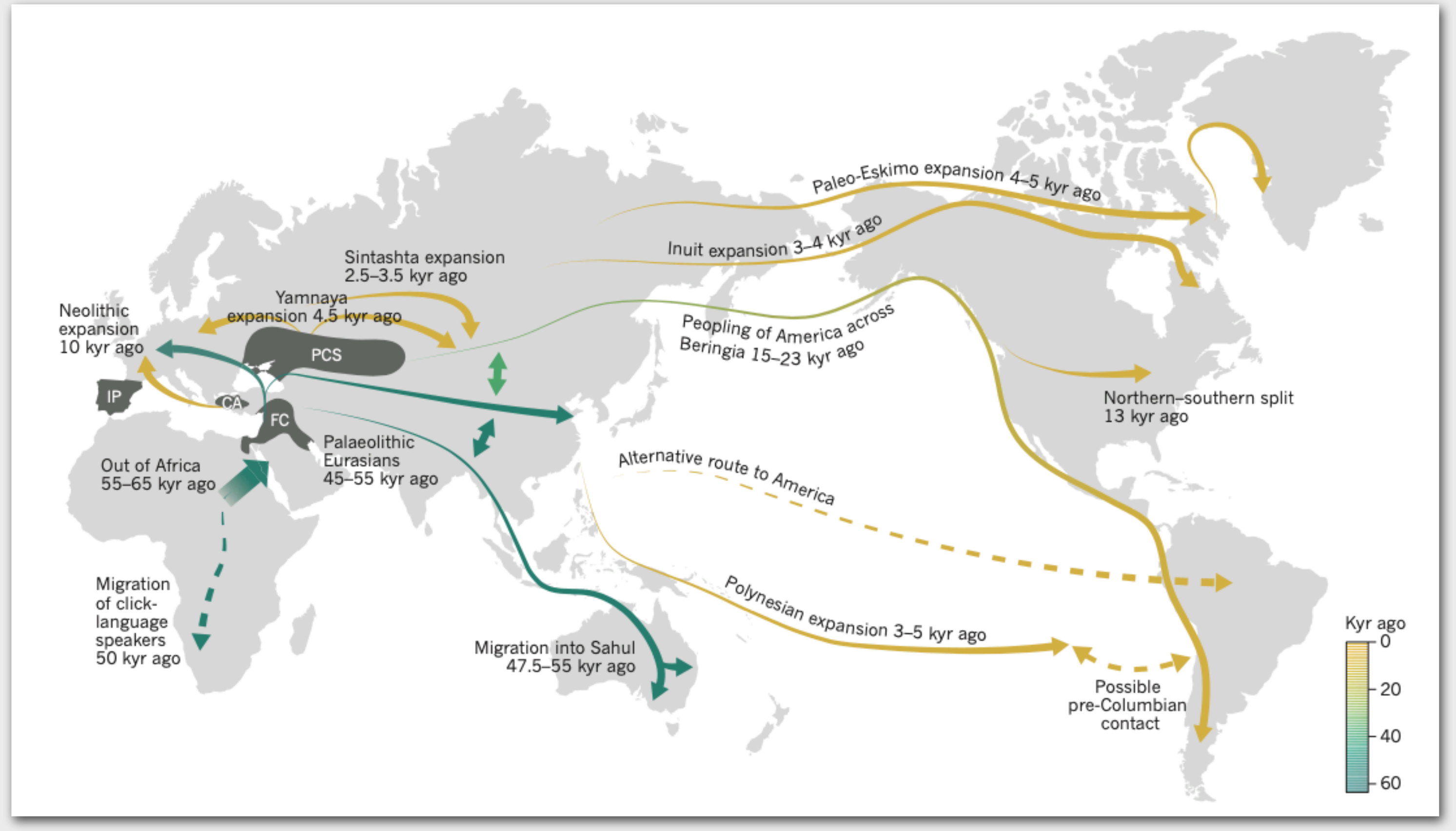
Characteristics and challenges



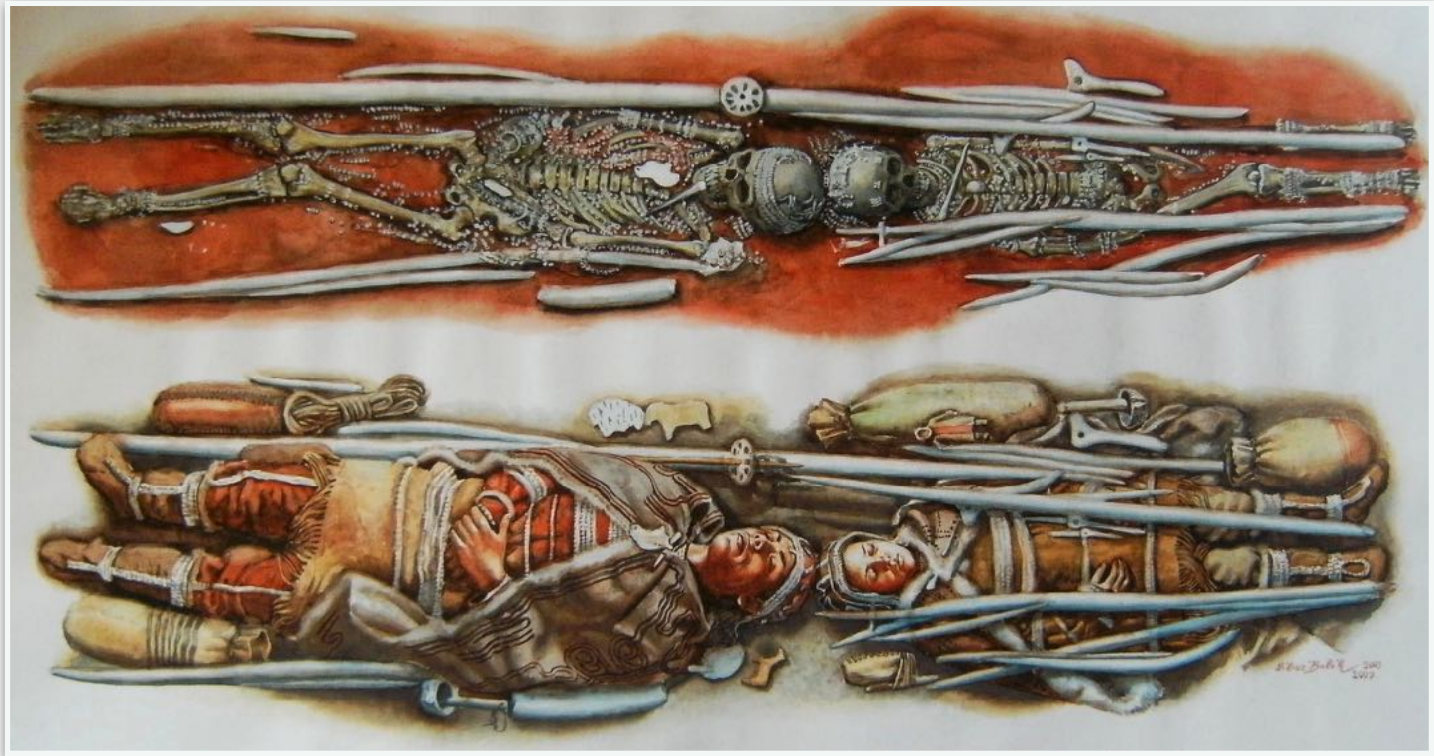
Ancient DNA showcase

Kinship and plague in Stone Age Scandinavia

Demography

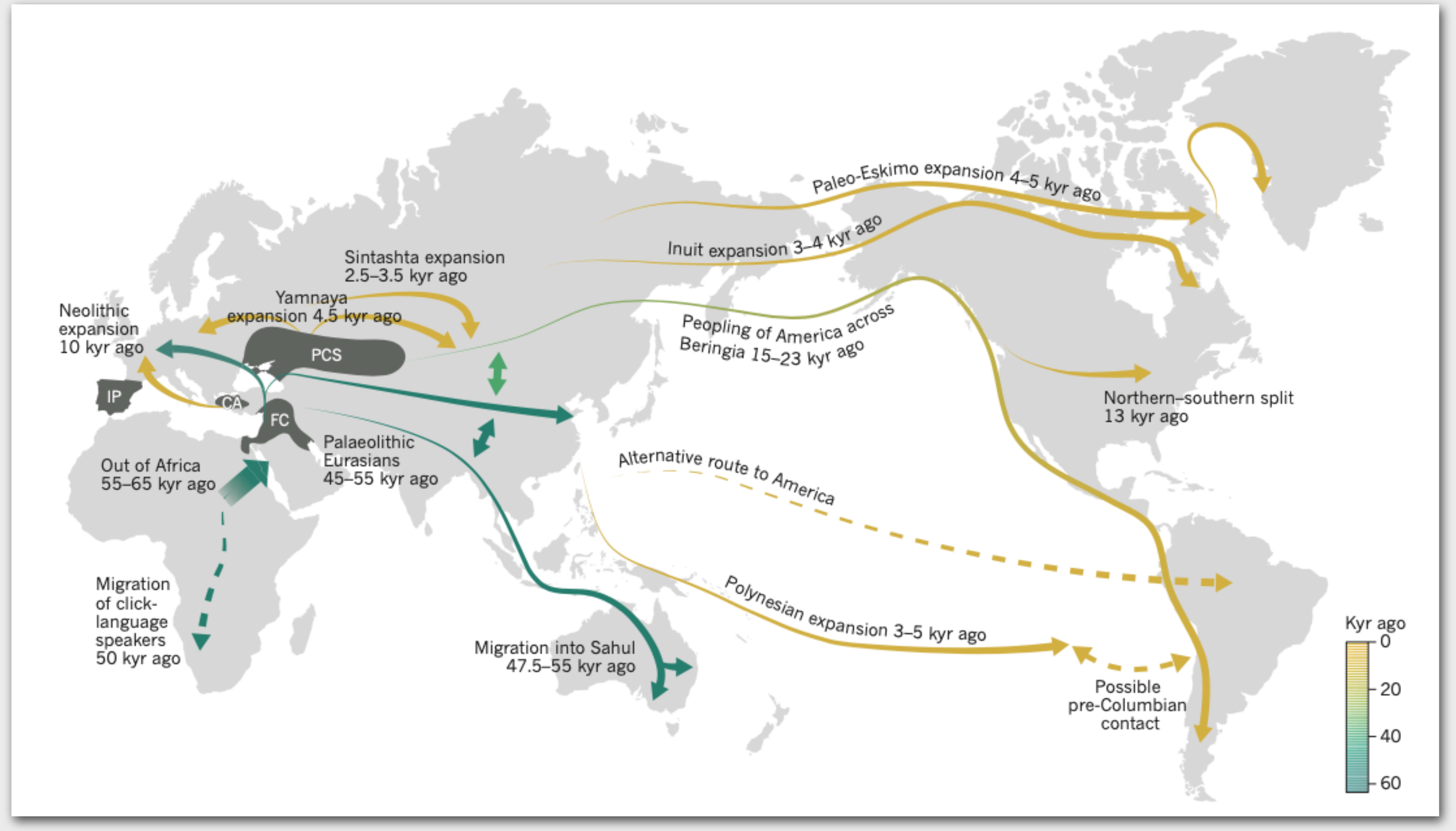


Social organisation



Endogenous host DNA

Demography

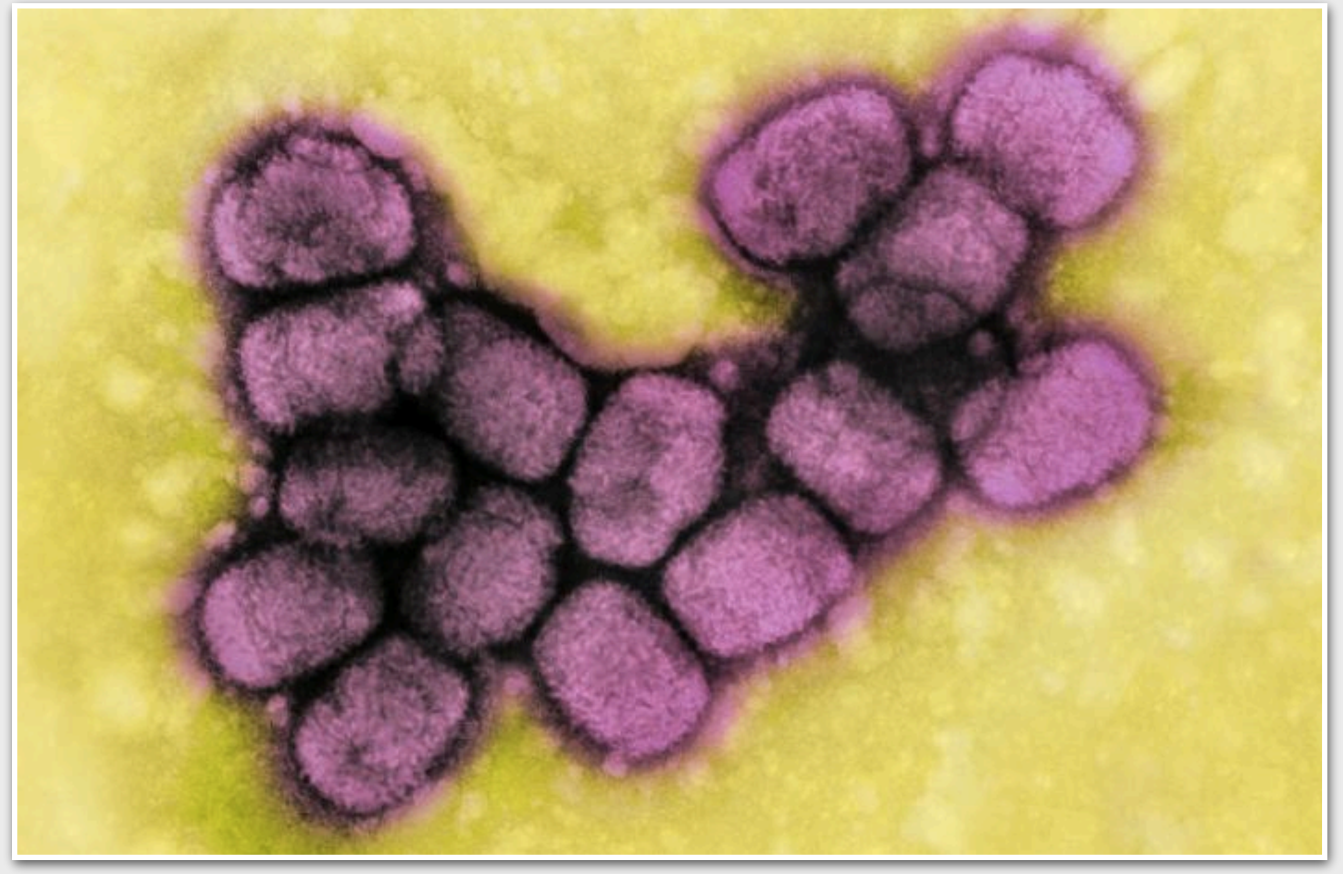


Social organisation

Endogenous host DNA

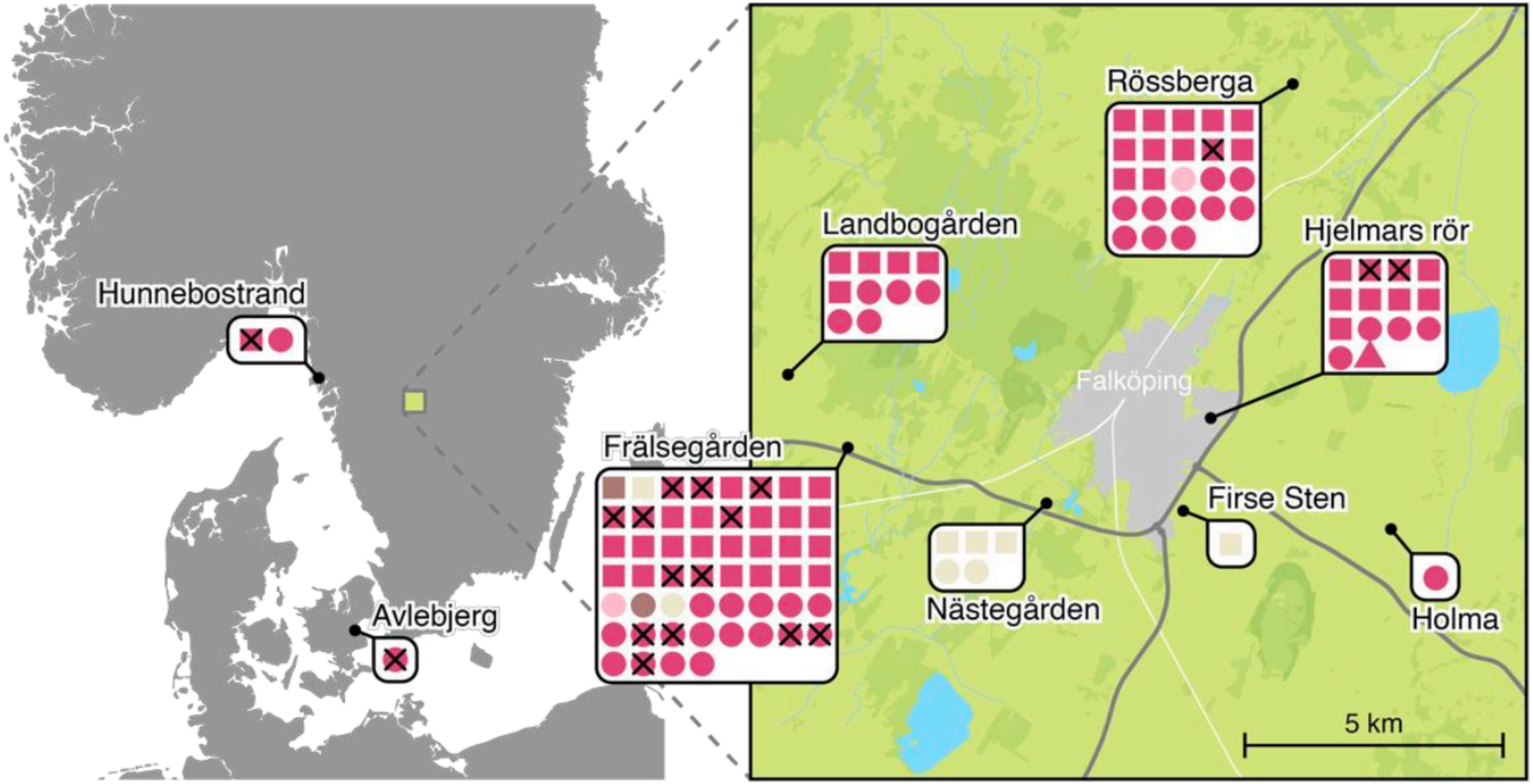
Diet and microbiome

Infectious diseases

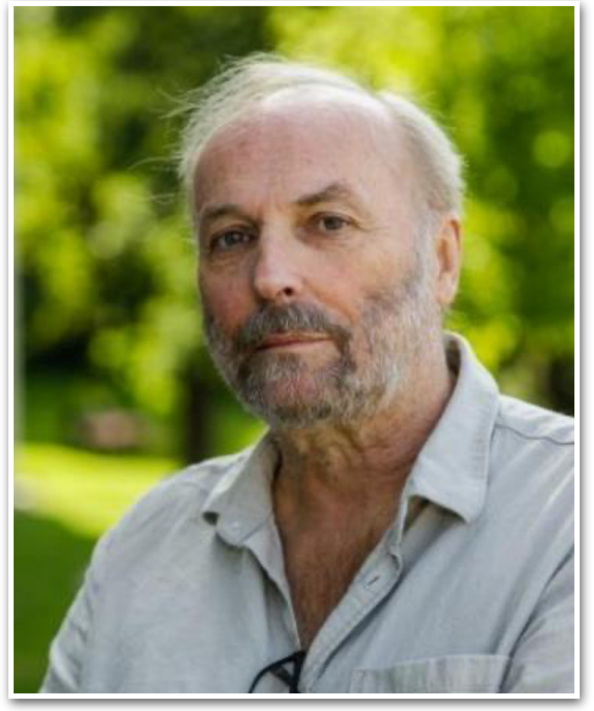


Non-host DNA

Population genomics of late Neolithic Scandinavia



Frederik Seersholm

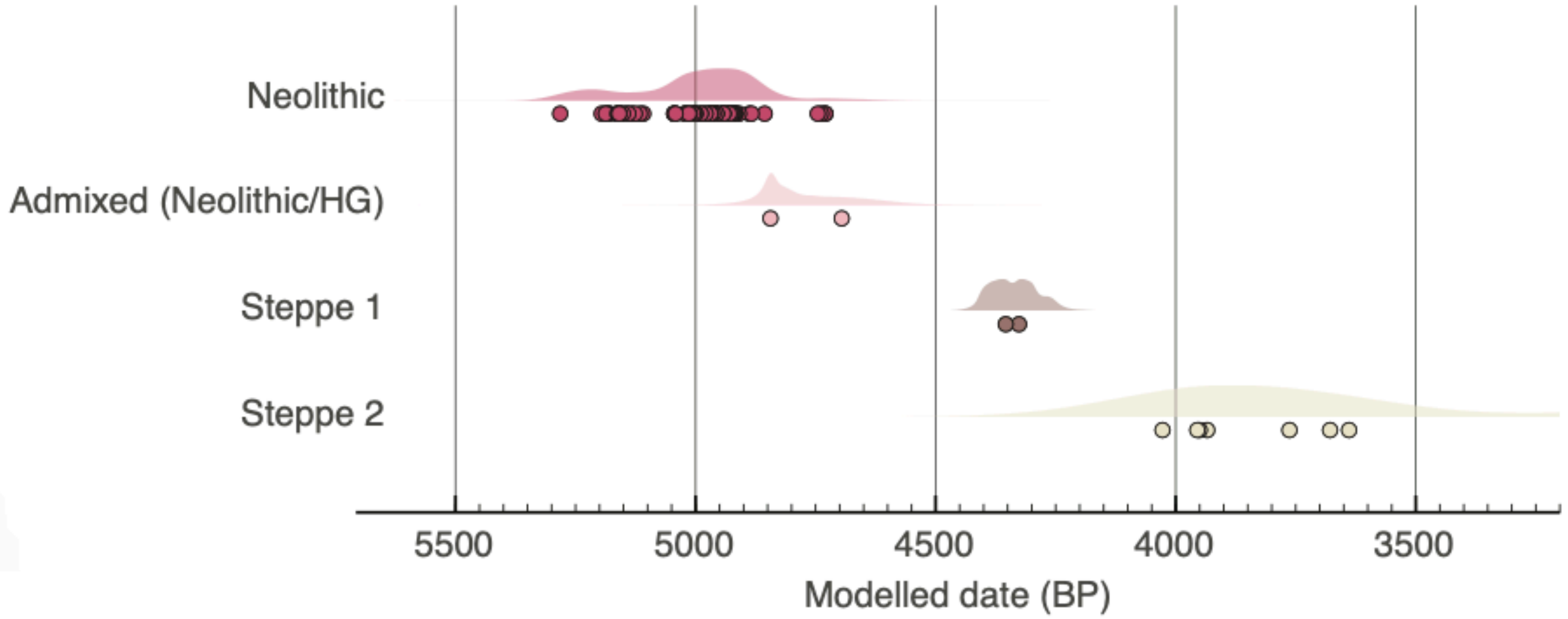


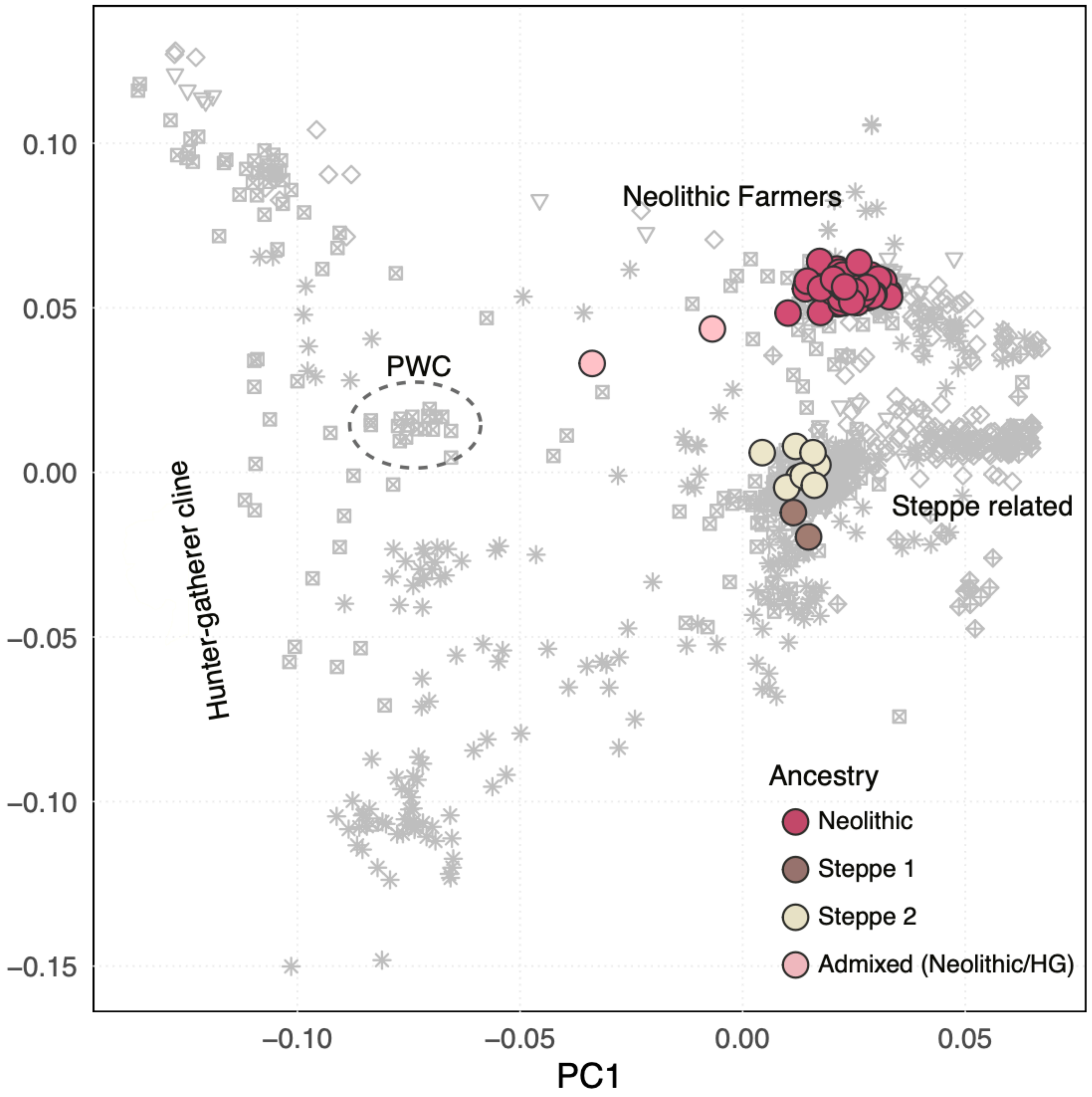
Karl-Göran Sjögren

109 ancient human genomes from megalithic graves in southern Scandinavia

Seersholm et al (2024) Nature

Genetic diversity of human populations

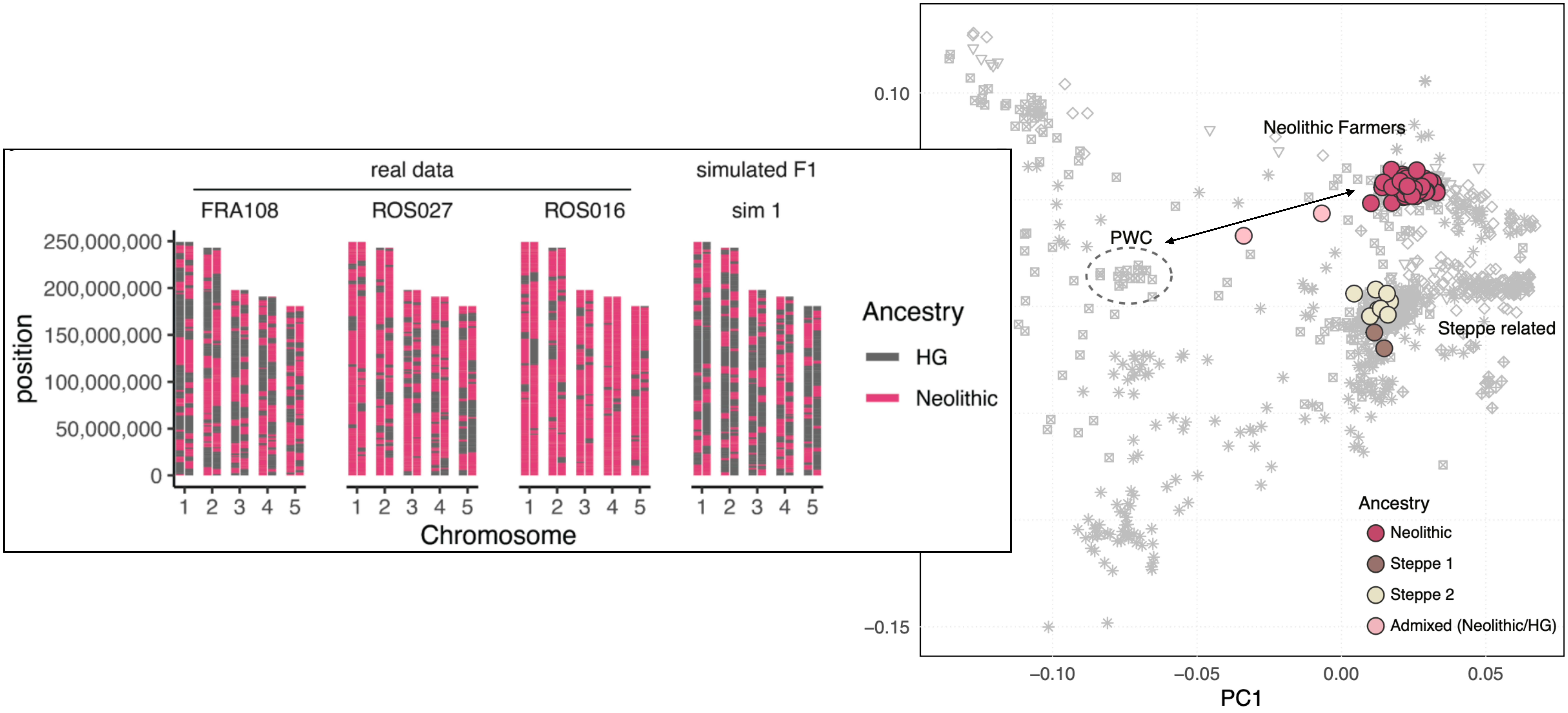




Repeated use of megalithic graves by peoples with different ancestries

Seersholm et al (2024) Nature

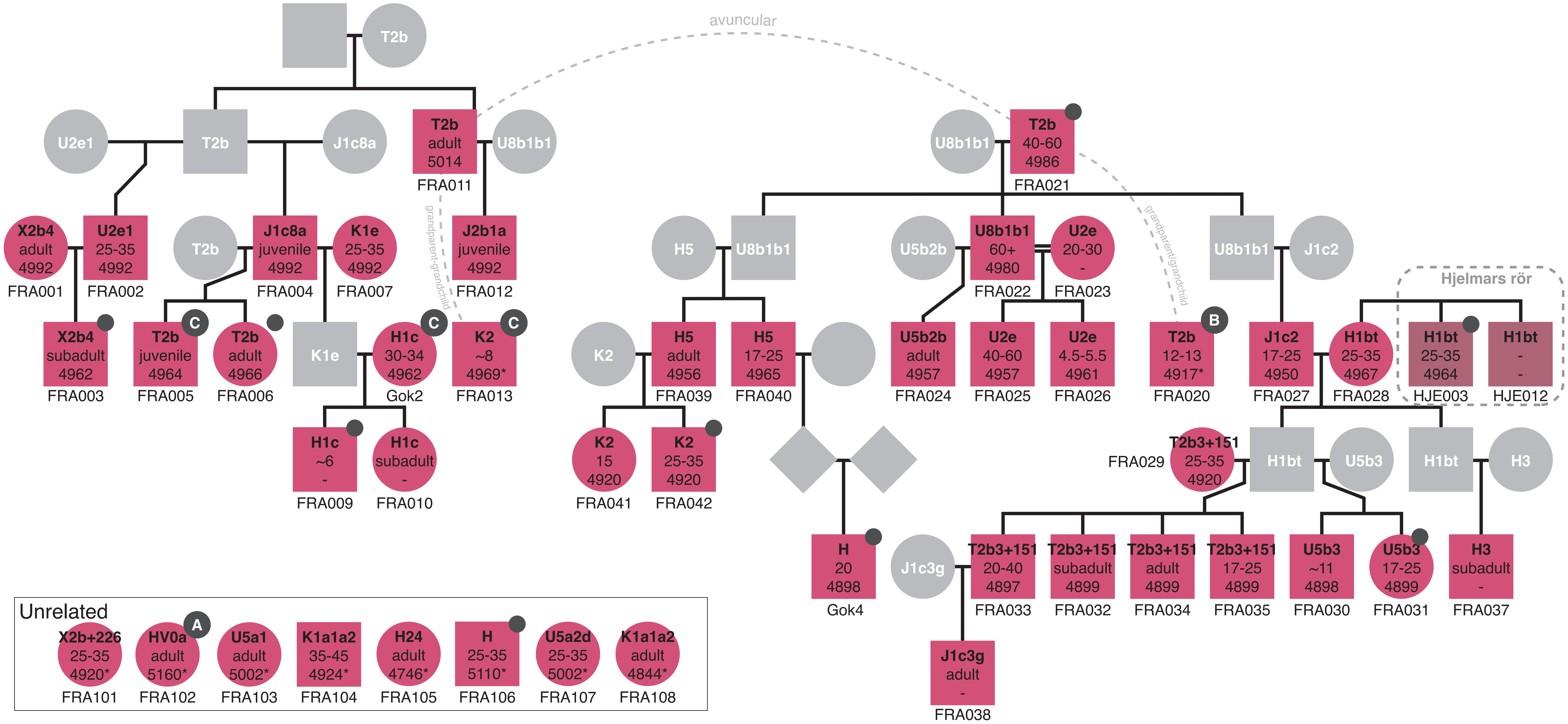
Recent admixture with Pitted-ware culture hunter-gatherers



Likely first- and second generation offspring at Fralsegarden and Rossberga

Seersholm et al (2024) Nature

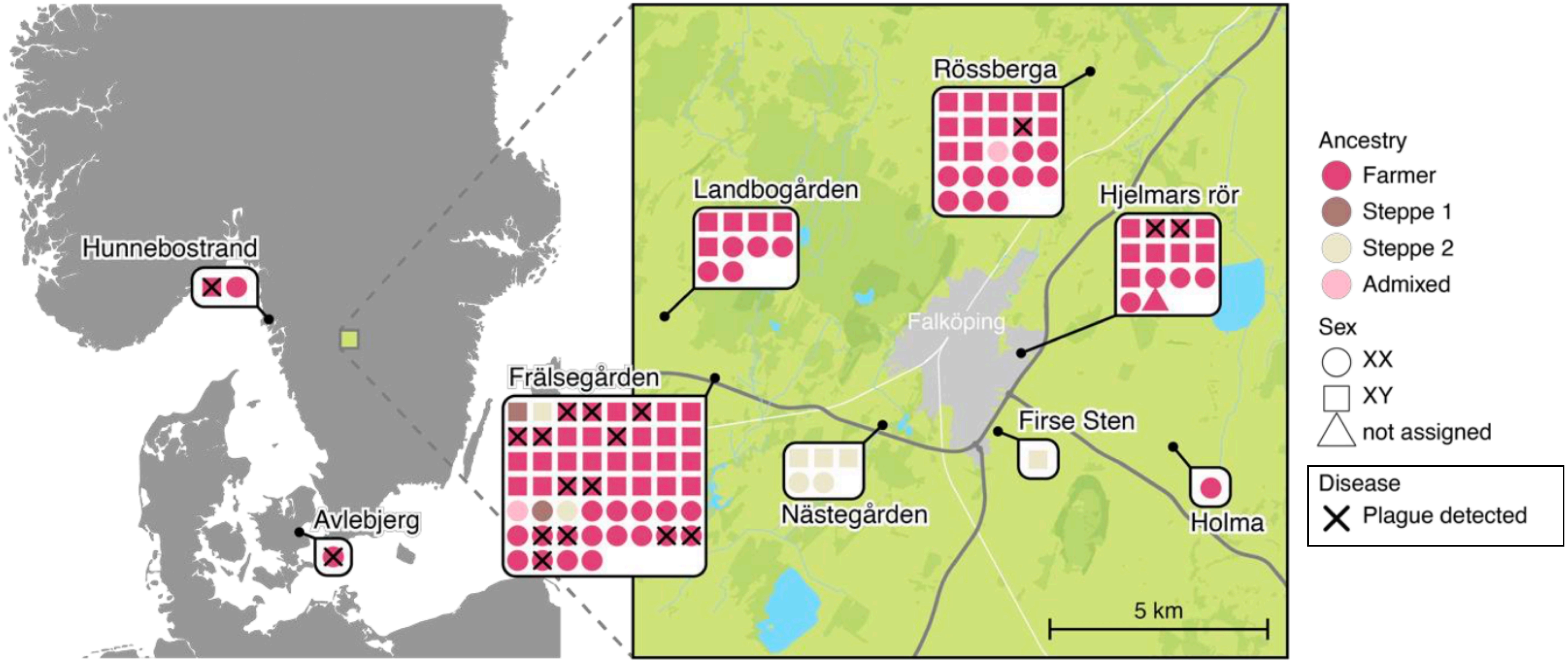
A five generation pedigree of Neolithic farmers



Pedigree of 38 sequenced individuals suggests patrilineal and patrilocal social organisation

Seersholm et al (2024) Nature

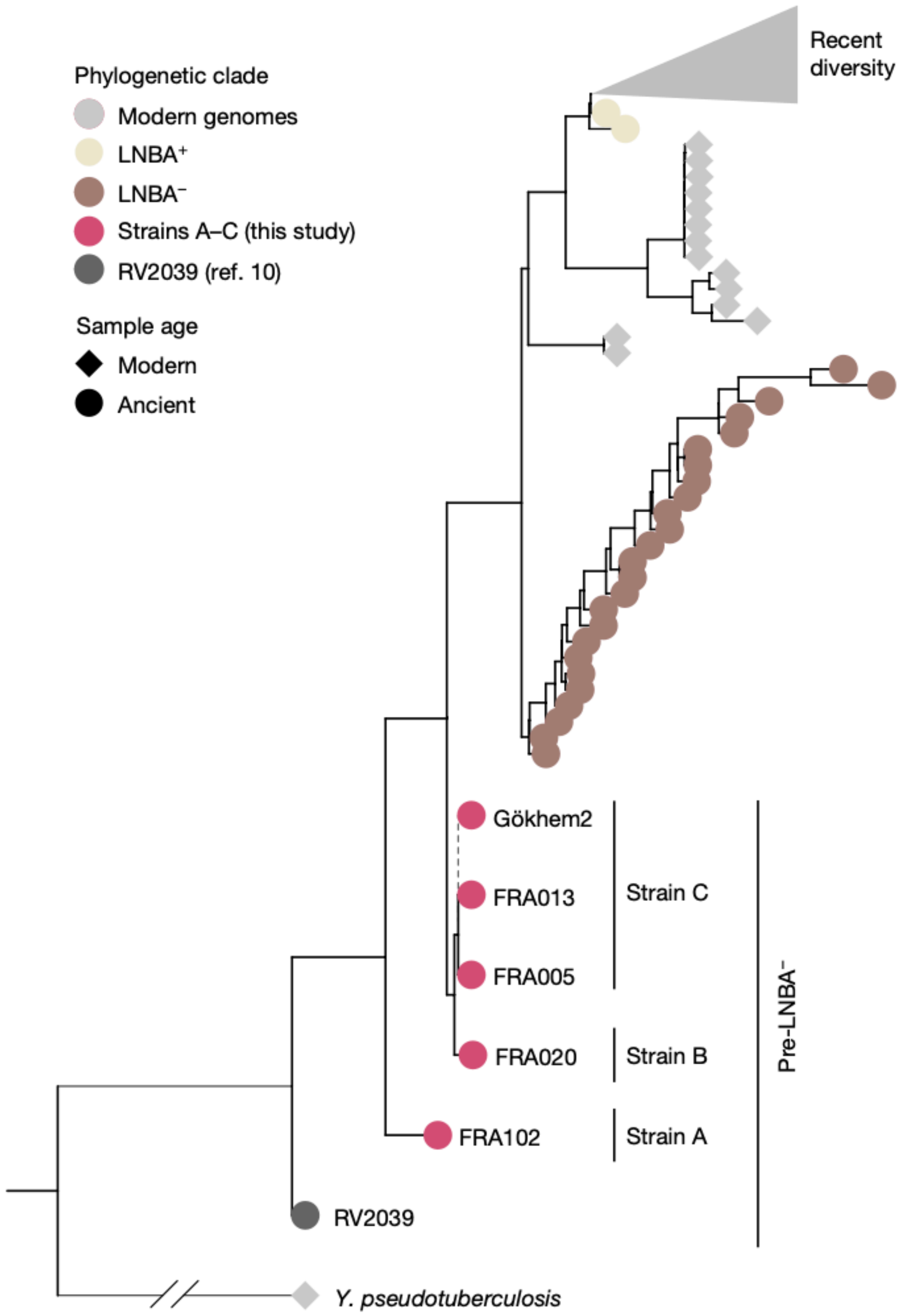
High prevalence of plague in late Neolithic Scandinavia



Plague detected in ~17% of individuals

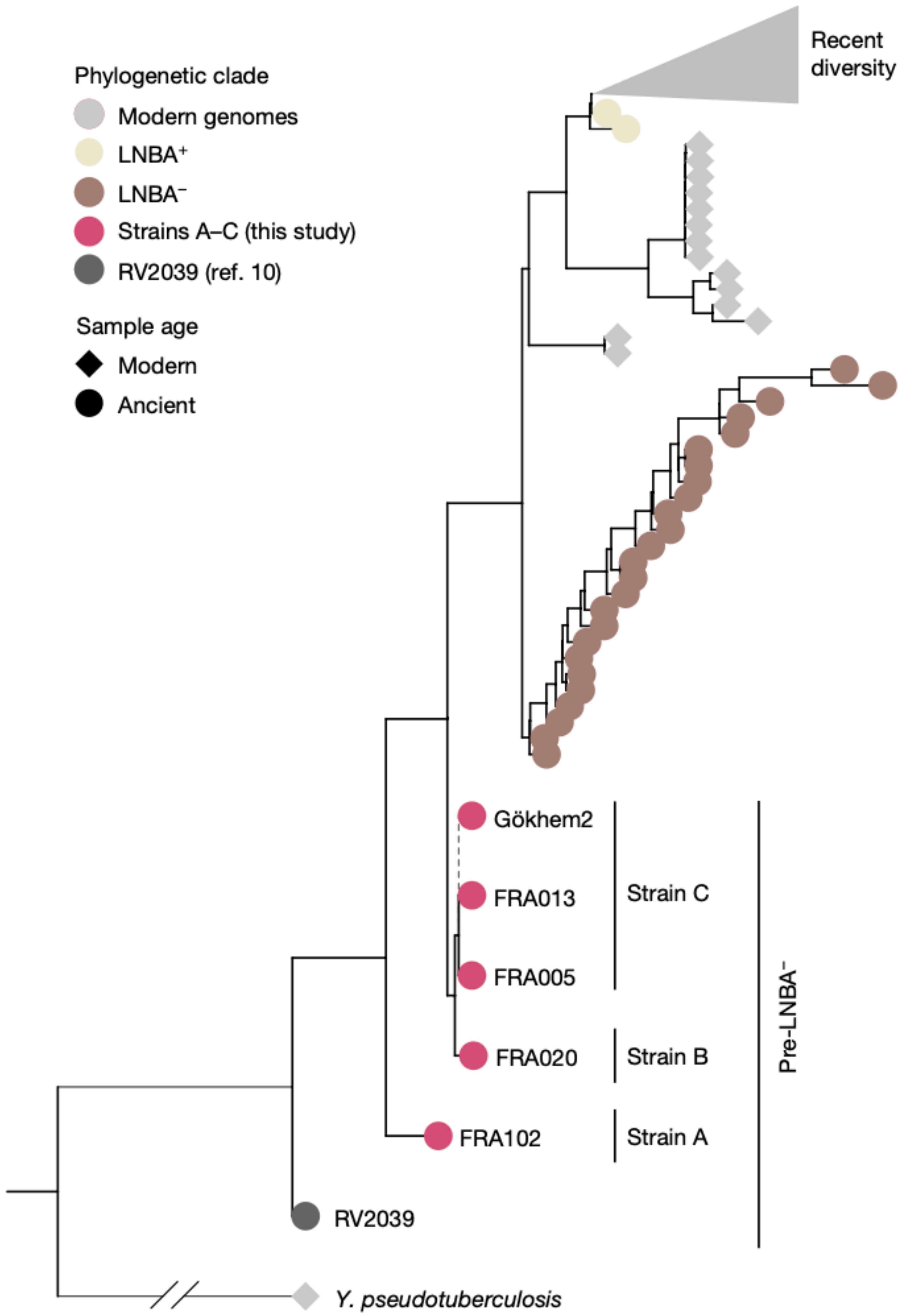
Seersholm et al (2024) Nature

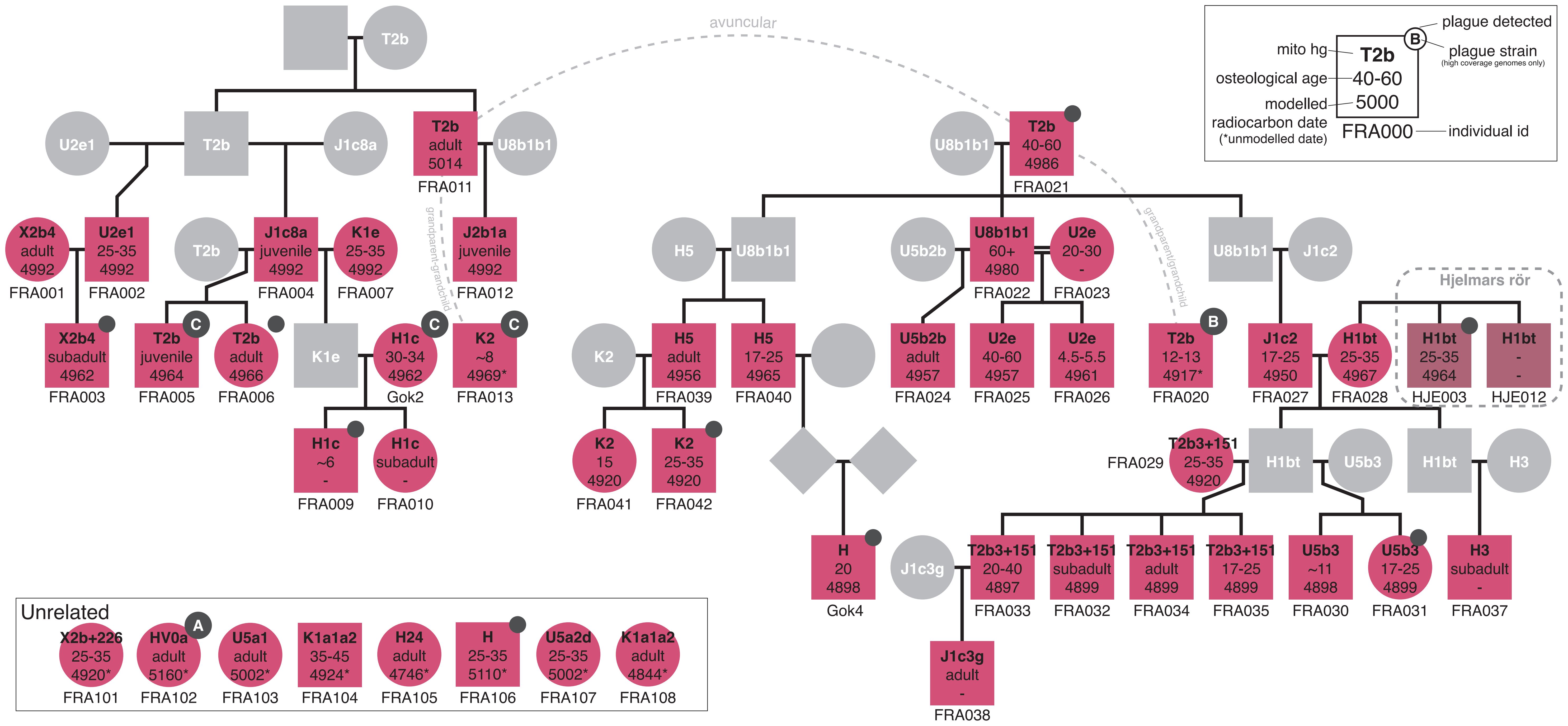
Multiple plague strains in Neolithic farmer families



Seersholm et al (2024) Nature

Multiple plague strains in Neolithic farmer families

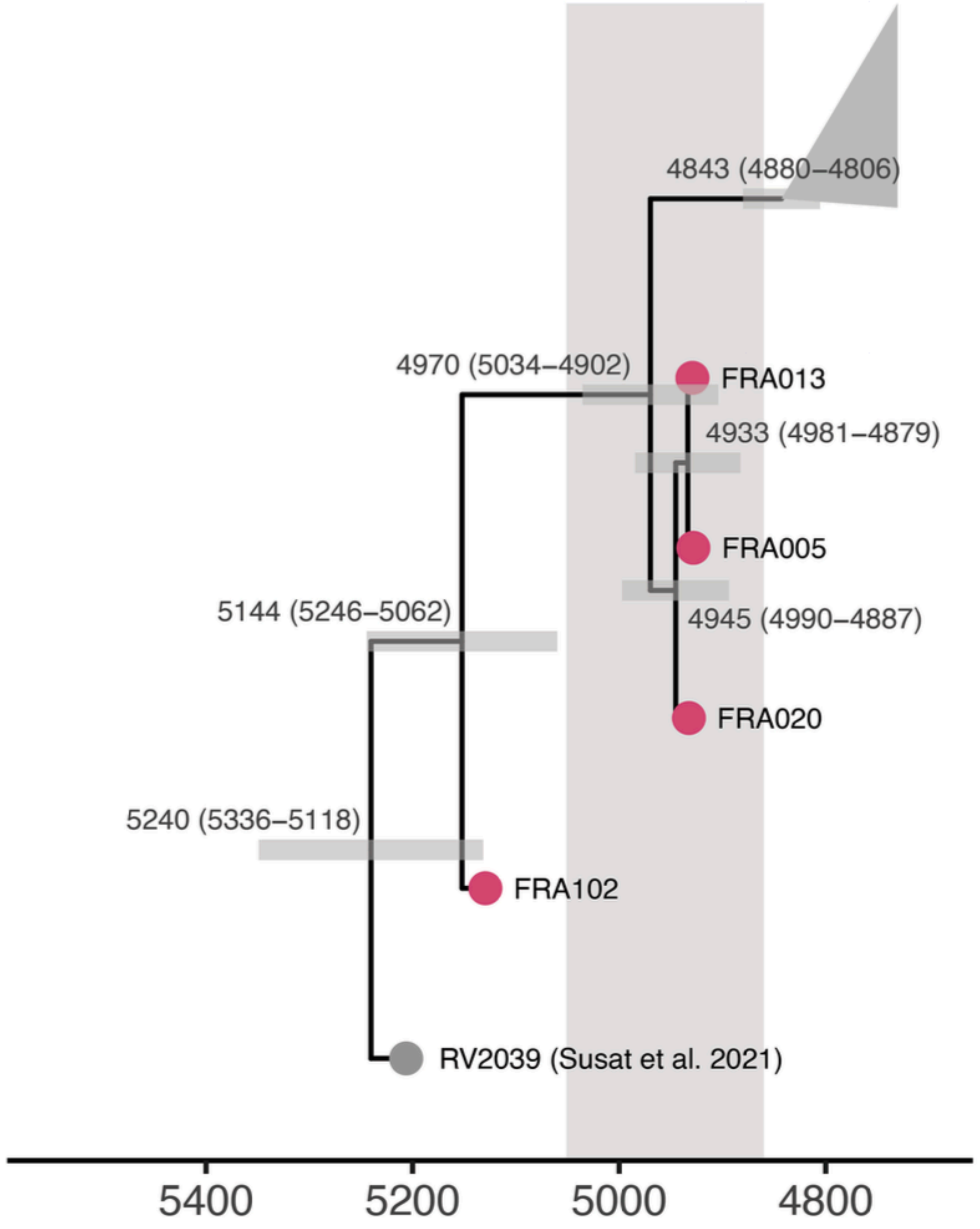


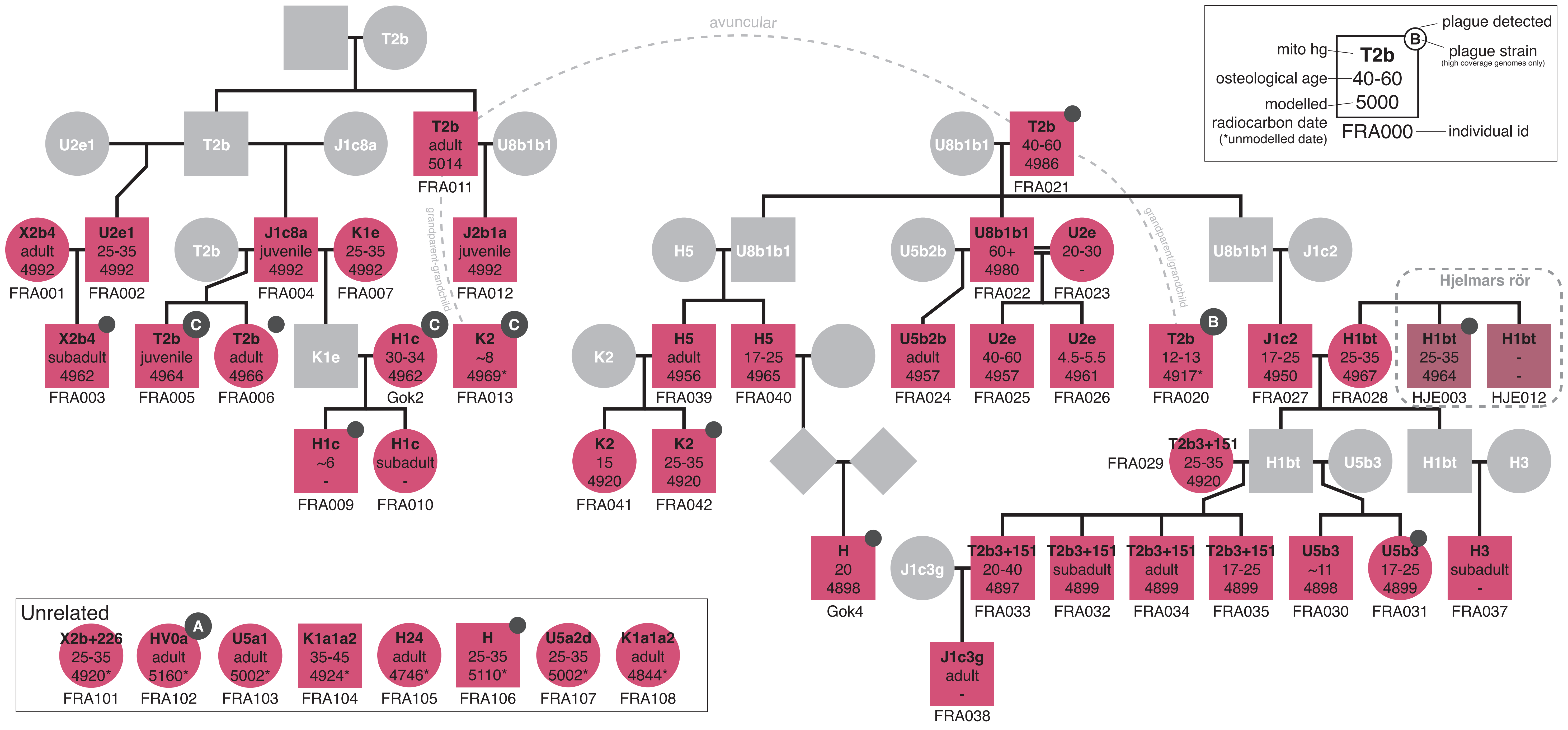


High prevalence of plague in Fralsegarden (28%) and multiple distinct strains

Seersholm et al (2024) Nature

Multiple plague strains in Neolithic farmer families

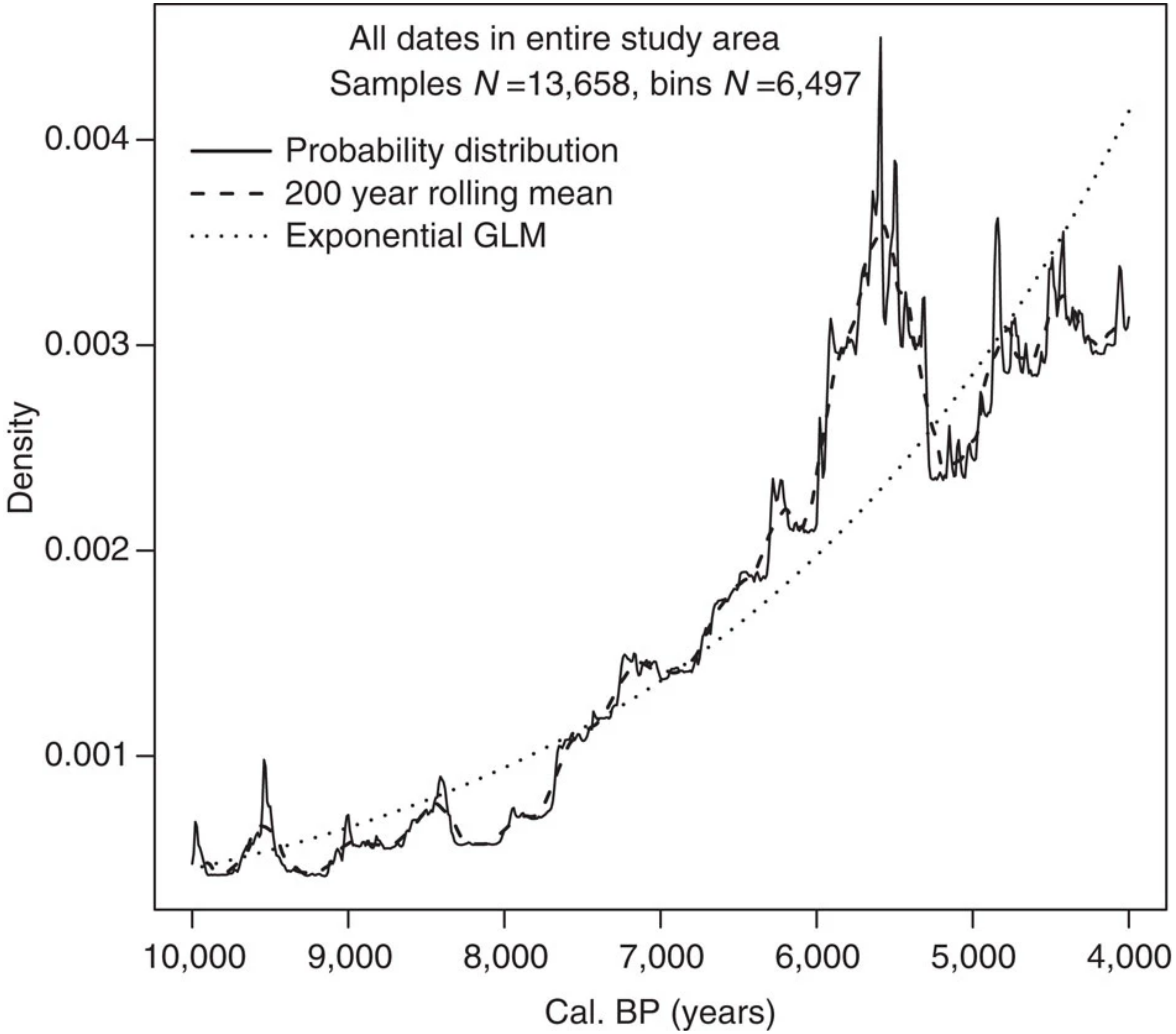




Divergence of strains A and B during Fralsegarden pedigree chronology

Seersholm et al (2024) Nature

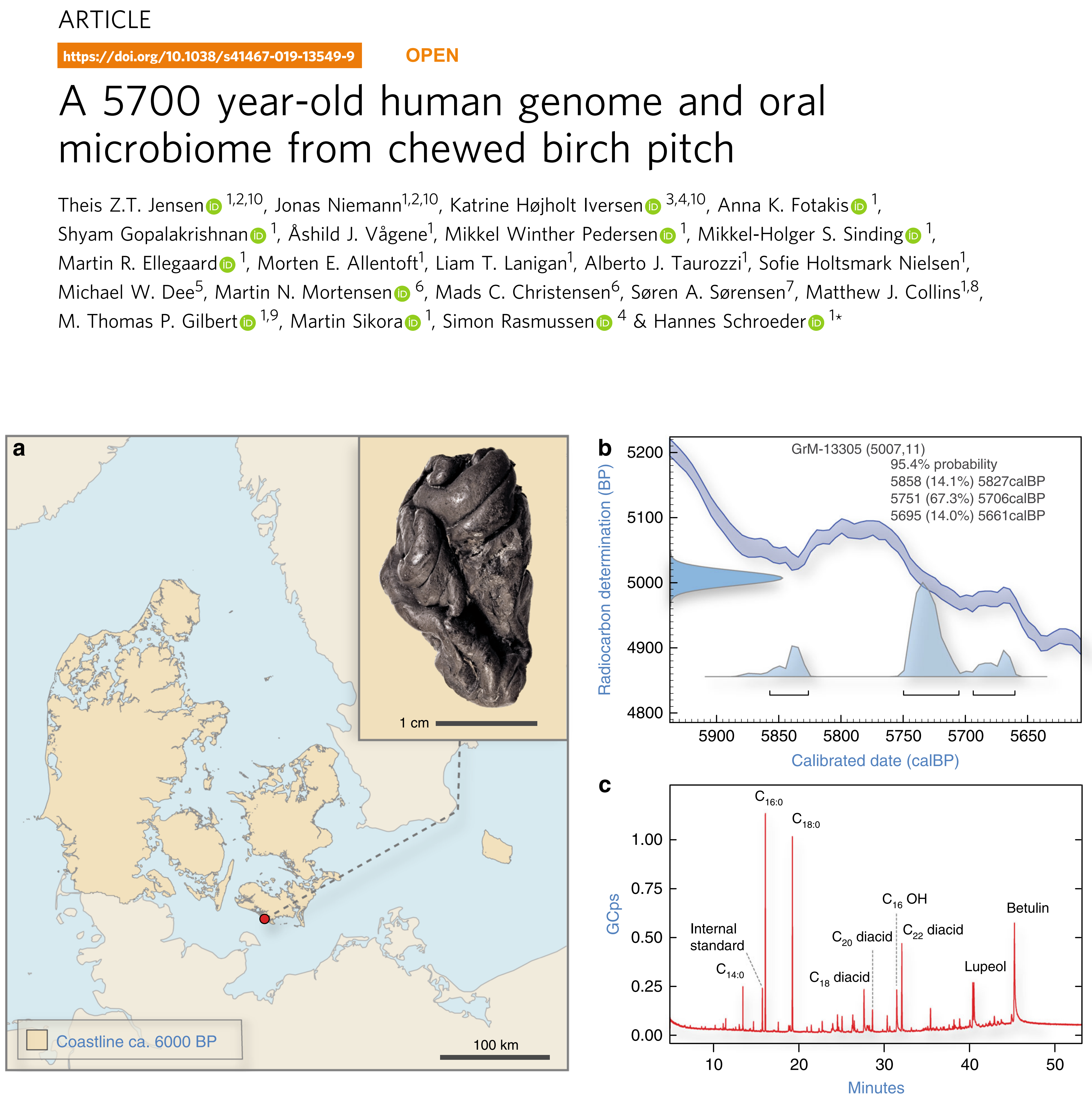
Did plague play a role in the Neolithic decline?



Population change in Western European history

Shannon et al (2013) Nat Comm

Novel sources for ancient DNA

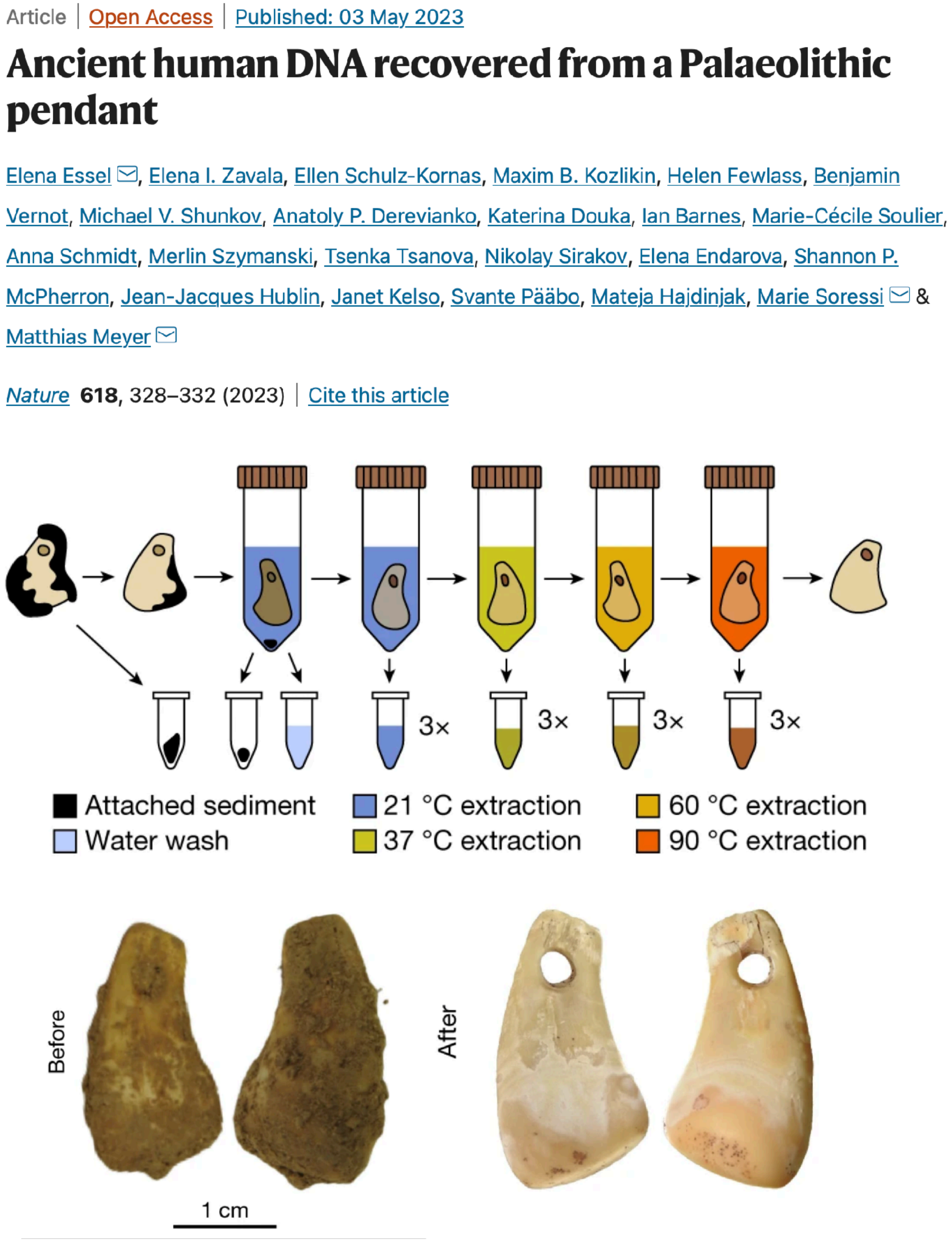


ARTICLE

https://doi.org/10.1038/s41467-019-13549-9 OPEN

A 5700 year-old human genome and oral microbiome from chewed birch pitch

Theis Z.T. Jensen[1,2,10], Jonas Niemann[1,2,10], Katrine Højholt Iversen[3,4,10], Anna K. Fotakis[1], Shyam Gopalakrishnan[1], Åshild J. Vågene[1], Mikkel Winther Pedersen[1], Mikkel-Holger S. Sinding[1], Martin R. Ellegaard[1], Morten E. Allentoft[1], Liam T. Lanigan[1], Alberto J. Taurozzi[1], Sofie Holtsmark Nielsen[1], Michael W. Dee[5], Martin N. Mortensen[6], Mads C. Christensen[6], Søren A. Sørensen[7], Matthew J. Collins[1,8], M. Thomas P. Gilbert[1,9], Martin Sikora[1], Simon Rasmussen[4] & Hannes Schroeder[1*]



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Ancient human DNA recovered from a Palaeolithic pendant

Elena Essel, Elena I. Zavala, Ellen Schulz-Kornas, Maxim B. Kozlikin, Helen Fewlass, Benjamin Vernot, Michael V. Shunkov, Anatoly P. Derevianko, Katerina Douka, Ian Barnes, Marie-Cécile Soulier, Anna Schmidt, Merlin Szymanski, Tsenka Tsanova, Nikolay Sirakov, Elena Endarova, Shannon P. McPherron, Jean-Jacques Hublin, Janet Kelso, Svante Pääbo, Mateja Hajdinjak, Marie Soressi & Matthias Meyer

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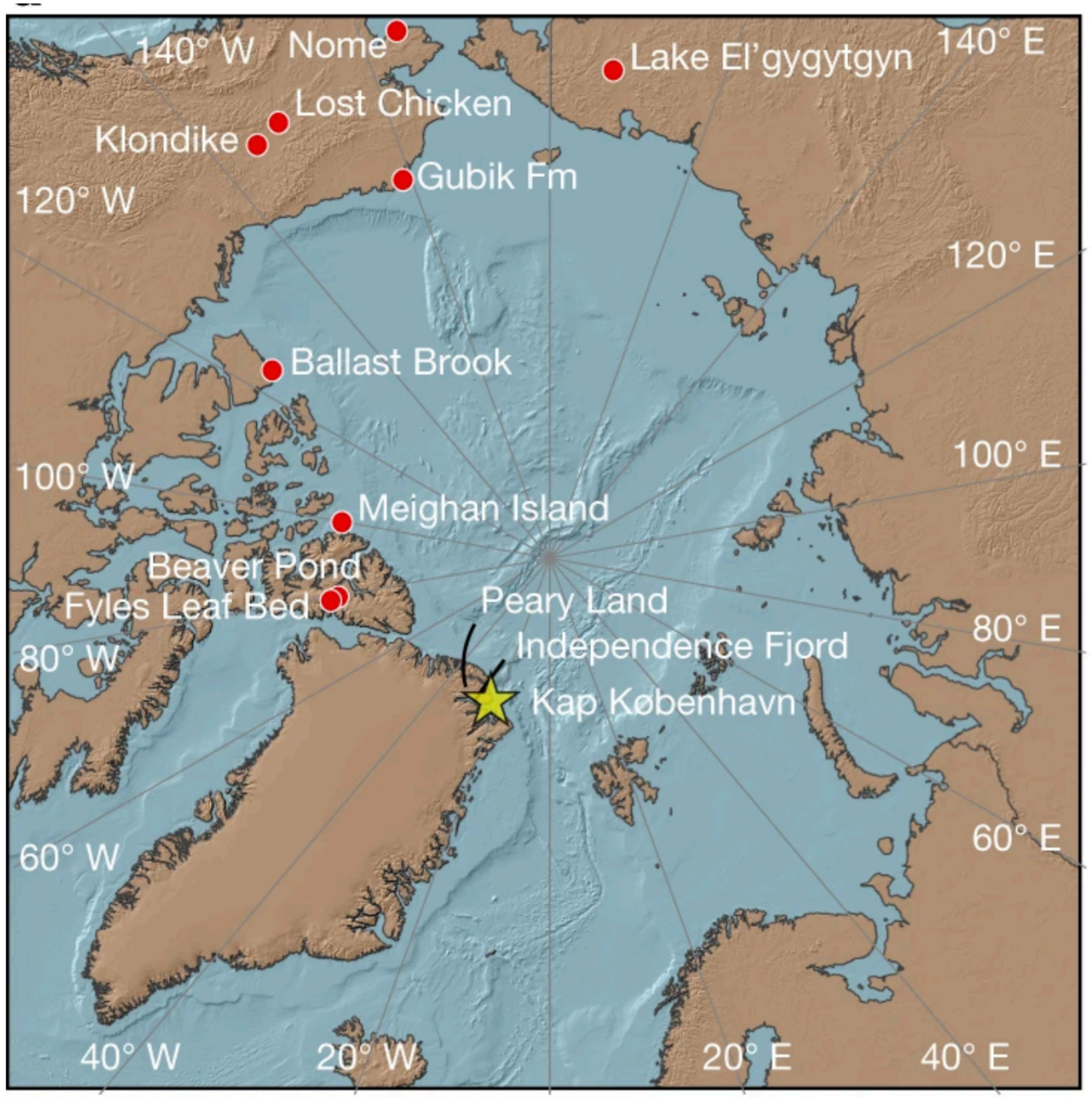

Reconstruction of a 2 million year-old ecosystem from ancient environmental DNA

Kjaer et al (2022) Nature

Ancient DNA

Next Generation Sequencing Analysis
DTU, 10/1/2025

Interested in a project?

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