

Part 1:

Traveling the past in **ancient DNA** time capsules



Lorlando@snm.ku.dk

Disclaimer

What this talk will not be about



Disclaimer

What this talk will not be about



Ancient DNA Research

Source Material





Higuchi et al. Nature 1984
229bp mtDNA

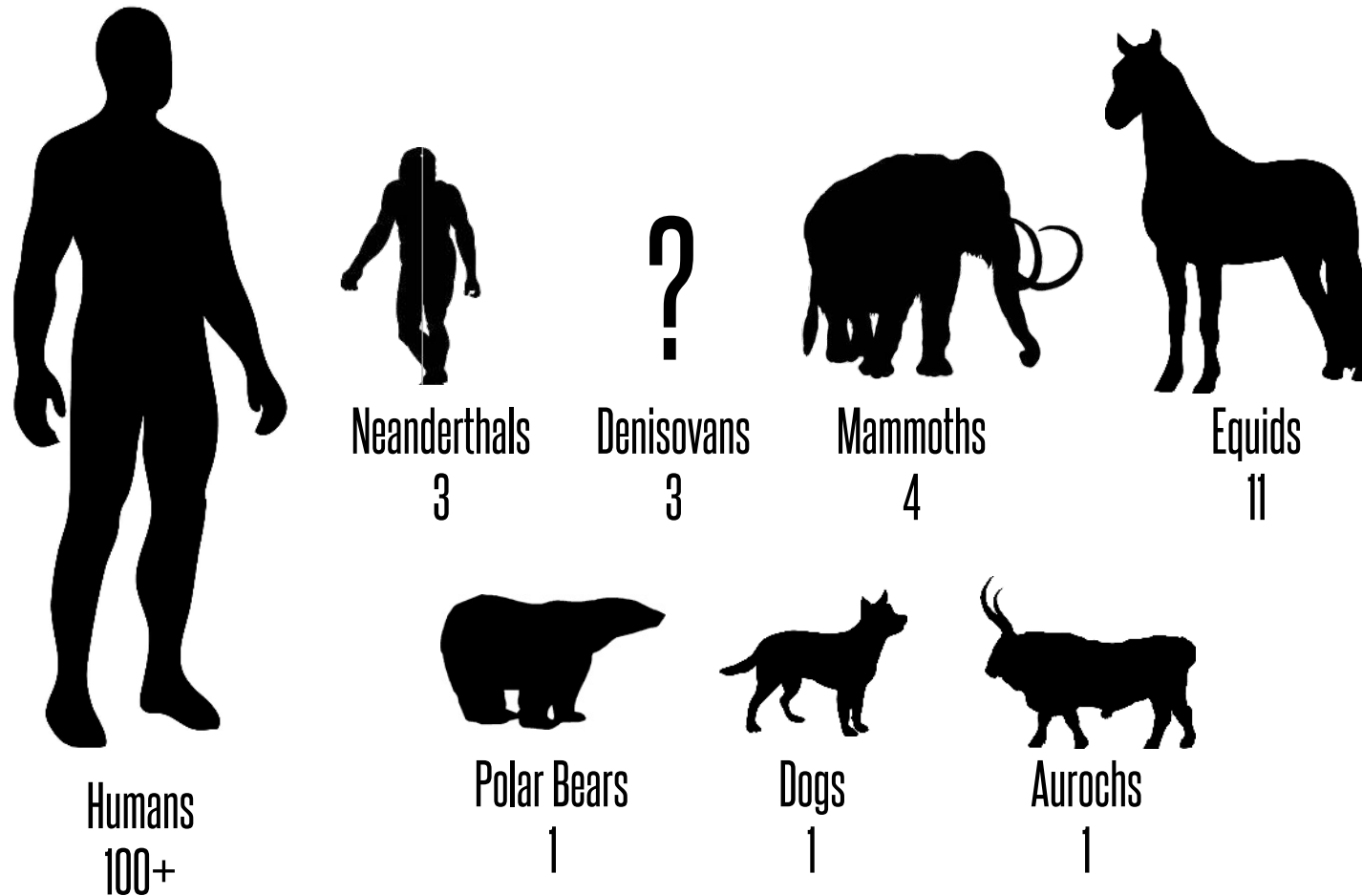
Jonsson et al. PNAS 2014
19,683,412,251bp ~ 7.9X
19,054,421,191bp ~ 8.1X

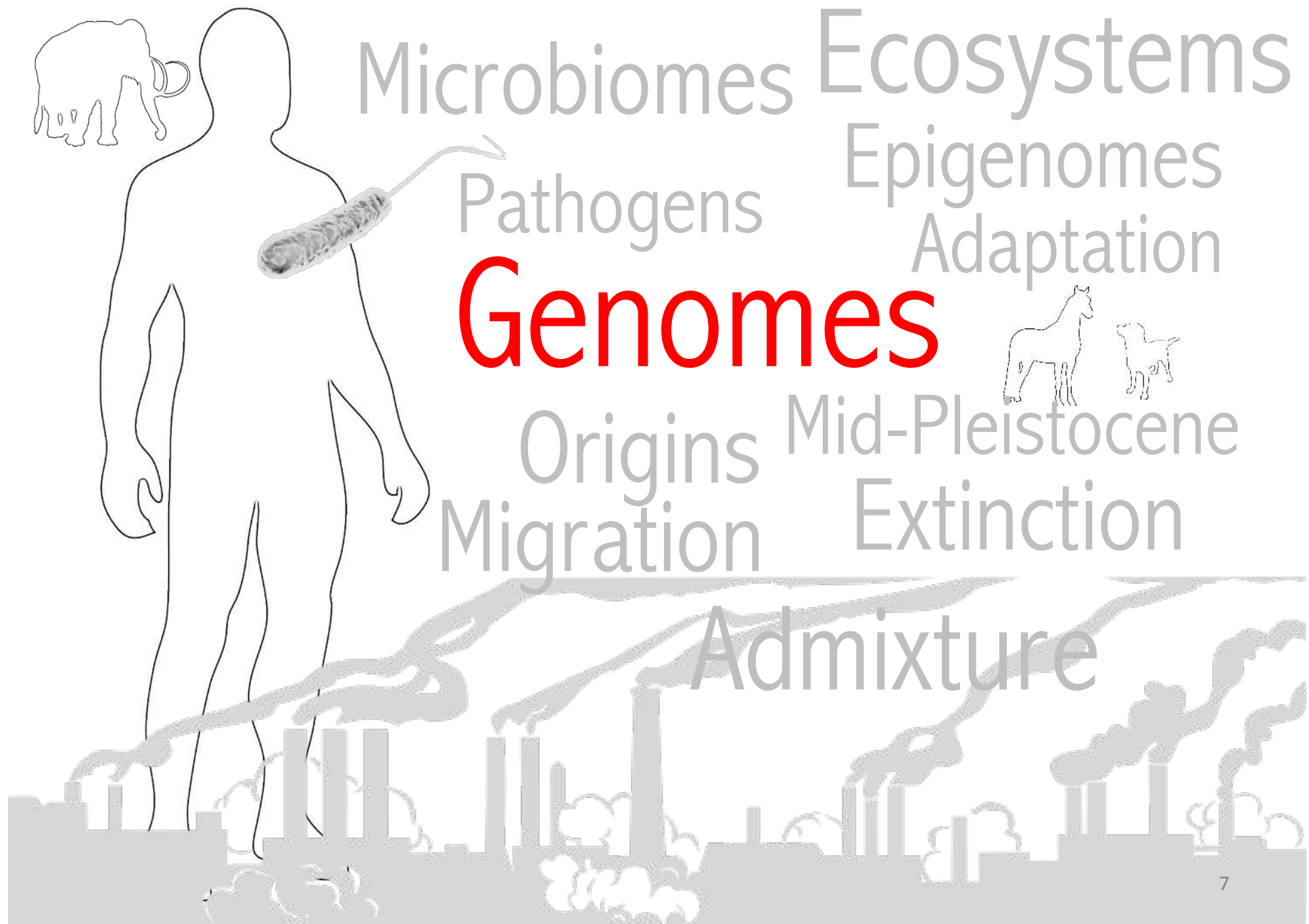
30 Years of Ancient DNA Research

The Extinct Quagga Zebra

Ancient Genomics

>100 Ancient Genomes Have Now Been Characterized



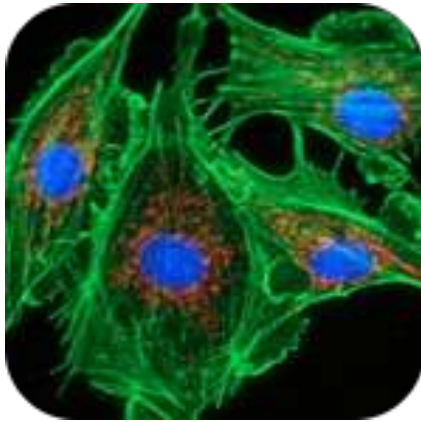


Ancient Genomics

Really?

800 mg - 379 bp

16,500
3,000,000,000



From Krings et al. Cell 1999

Ancient Genomics

Really?



Ancient Genomics

Really?

6.6 tons

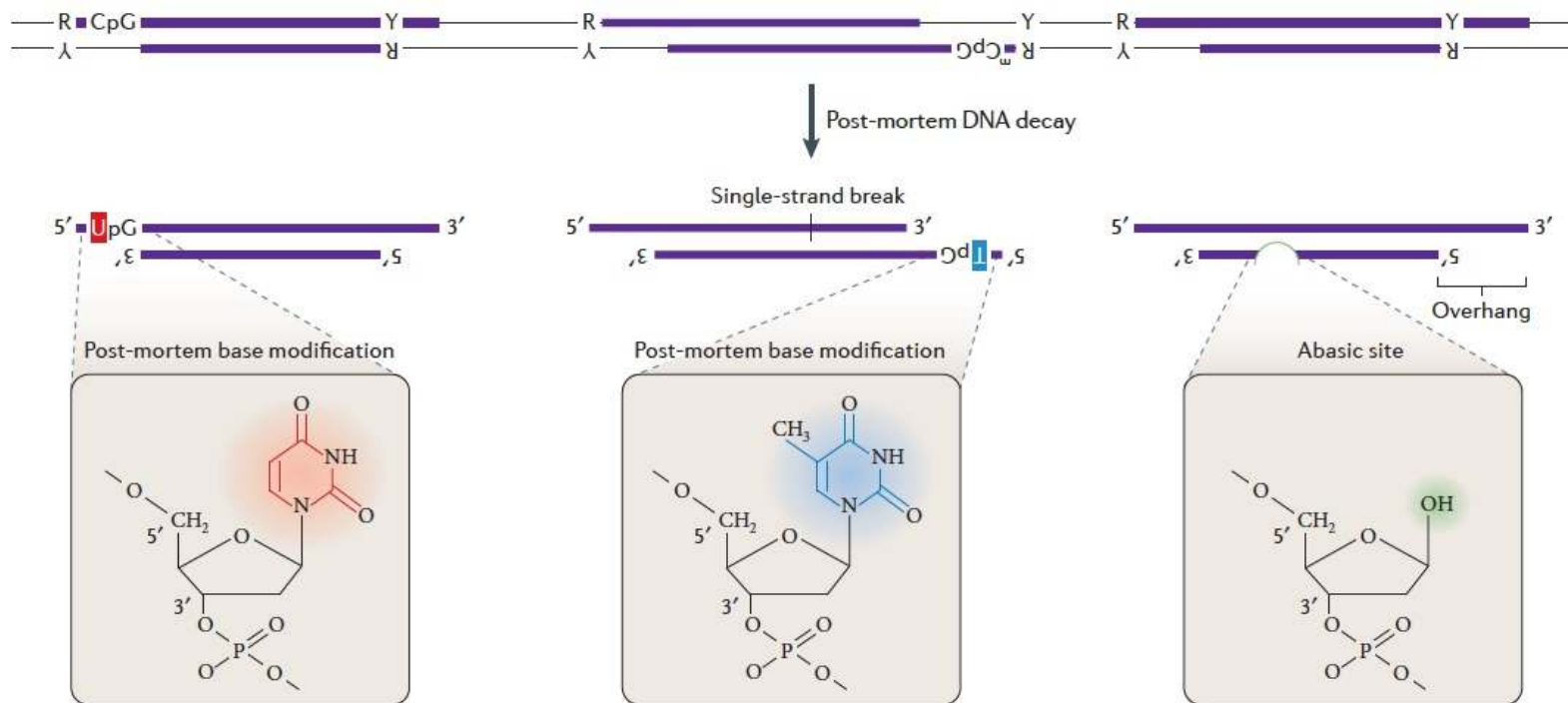
NATURE | Vol 456 | 20 November 2008

Ancient Genomics

Post-Mortem DNA decay

Ancient Genomics

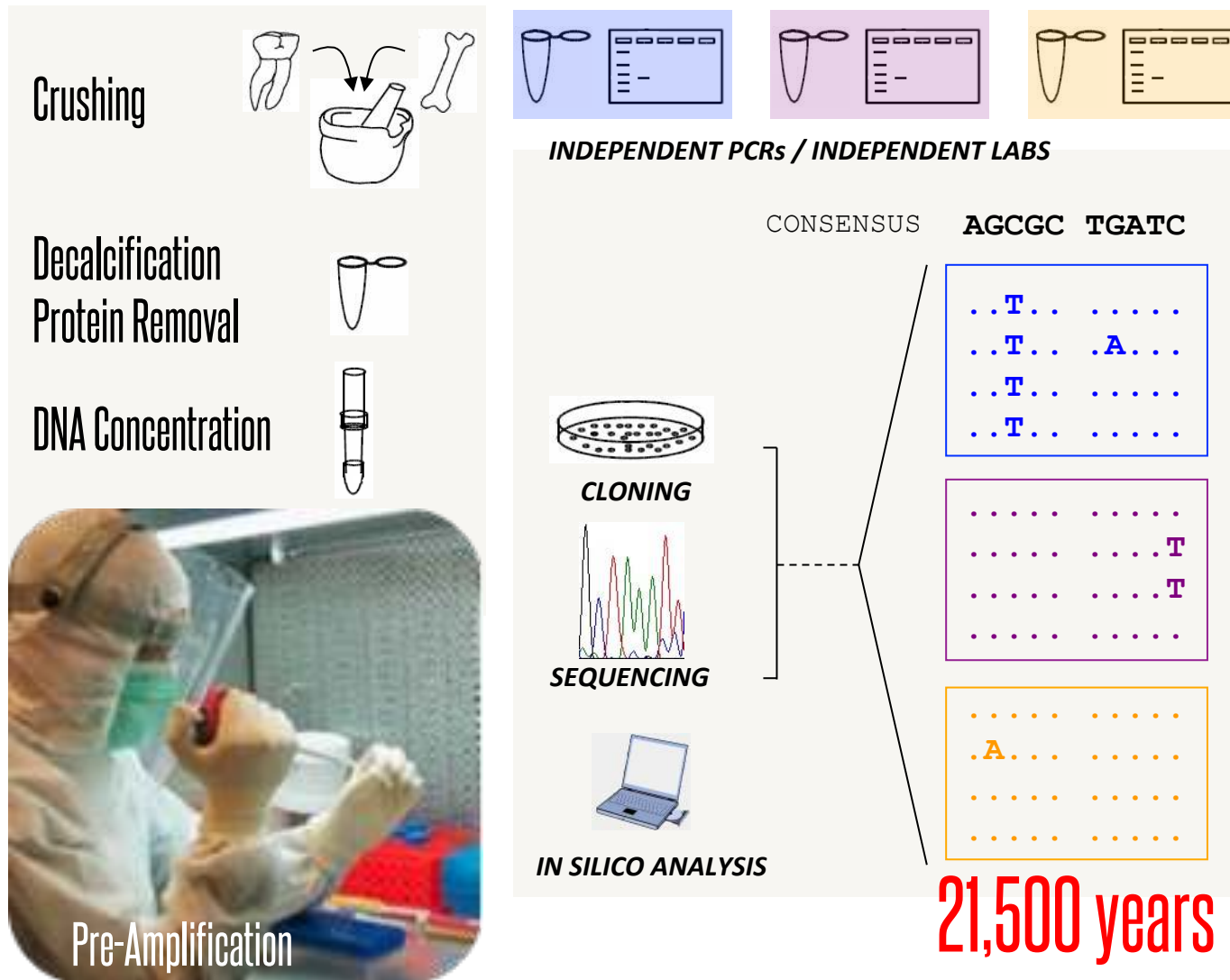
Post-Mortem DNA decay



Orlando et al. Nature Rev Genet 2015

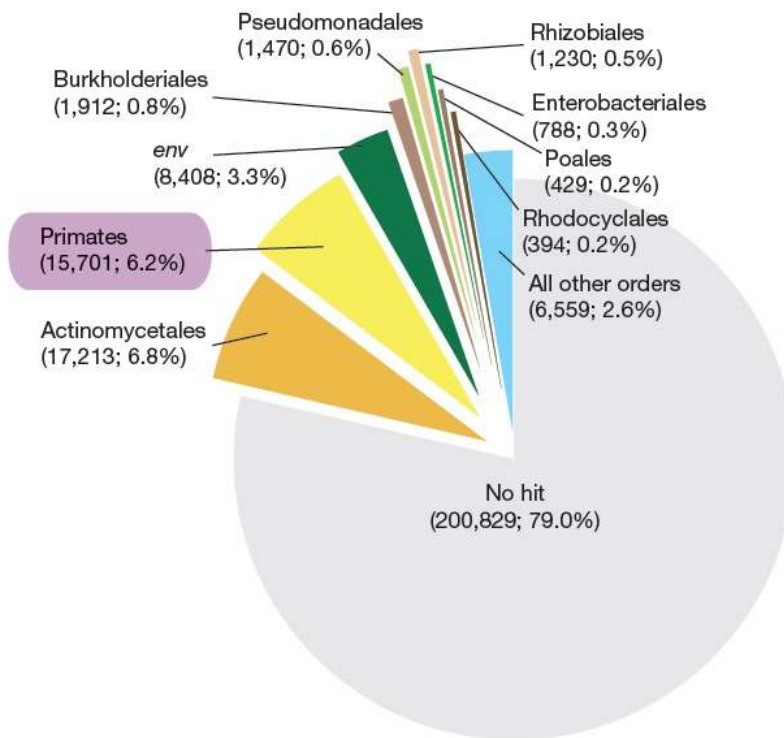
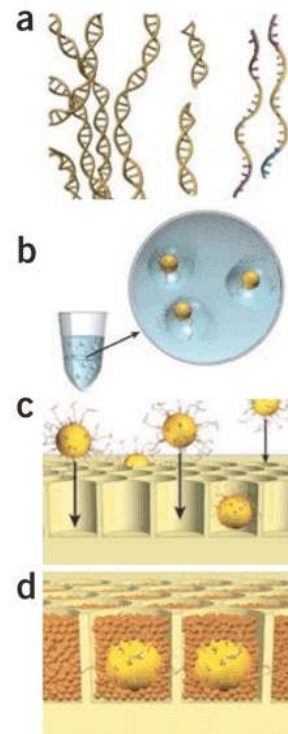
Ancient Genomics

Low-Throughput Approaches



Ancient Genomics

High-Throughput Approaches

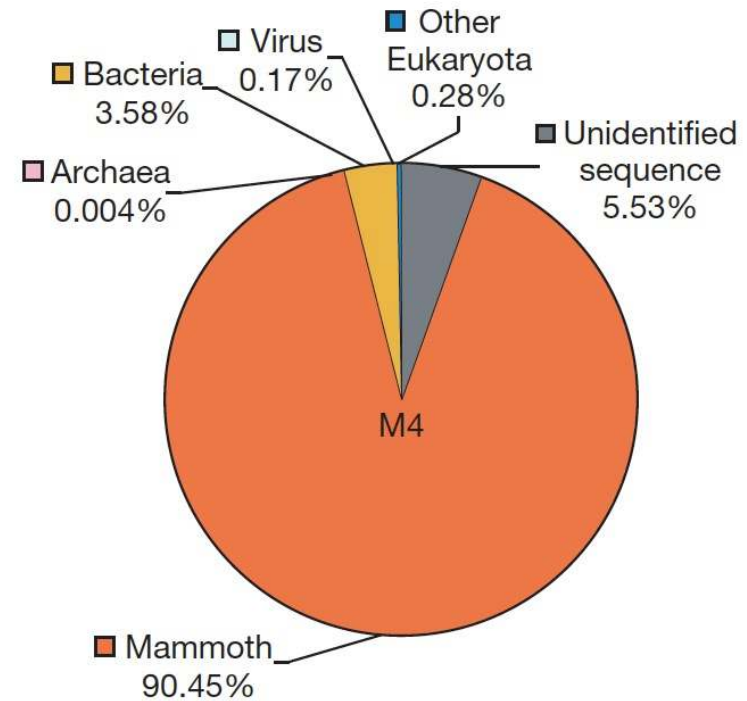


From Green et al. Nature 2006

138.6 yrs

Ancient Genomics

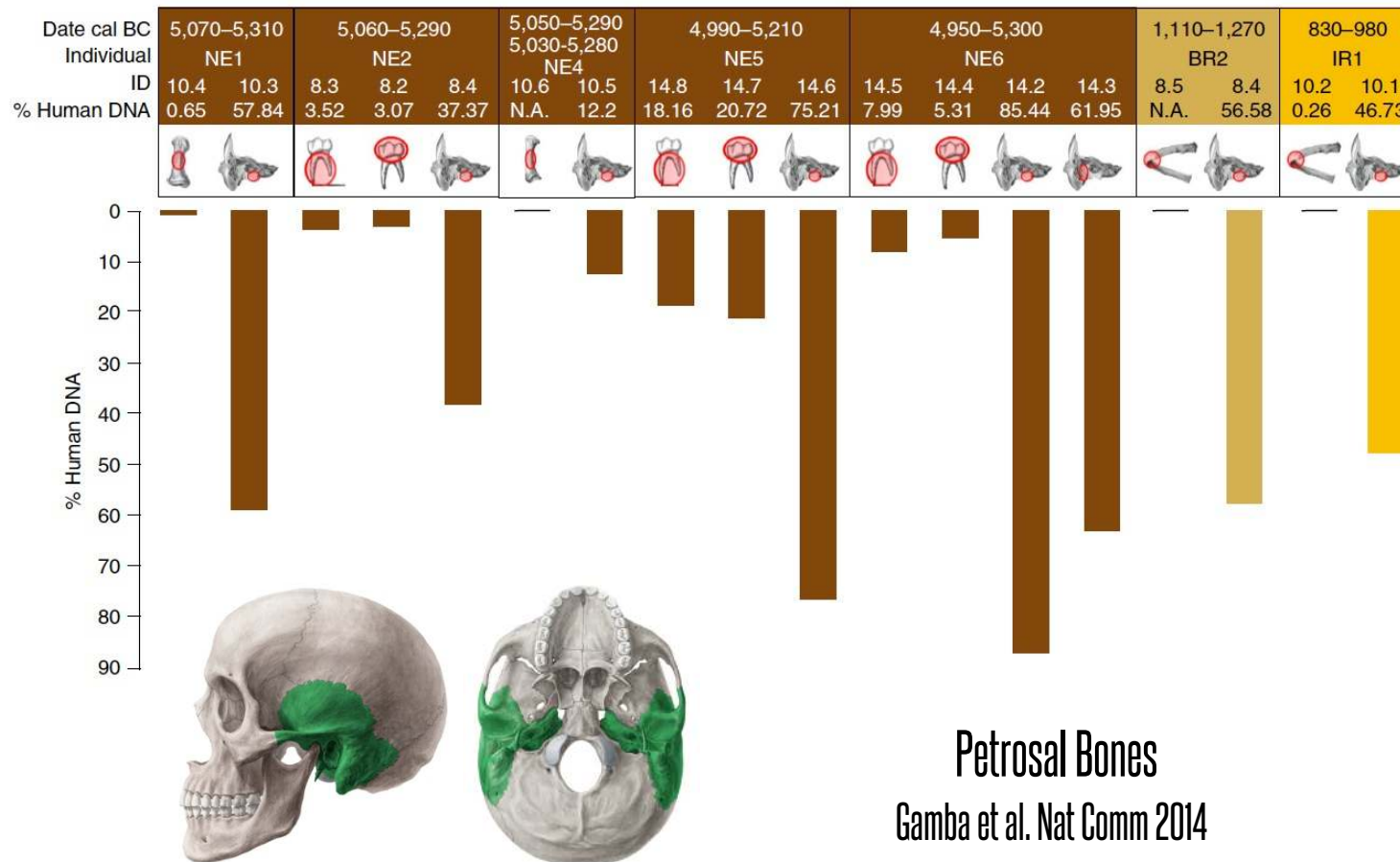
Solution #1: New DNA Reservoirs



From Miller et al. Nature 2008

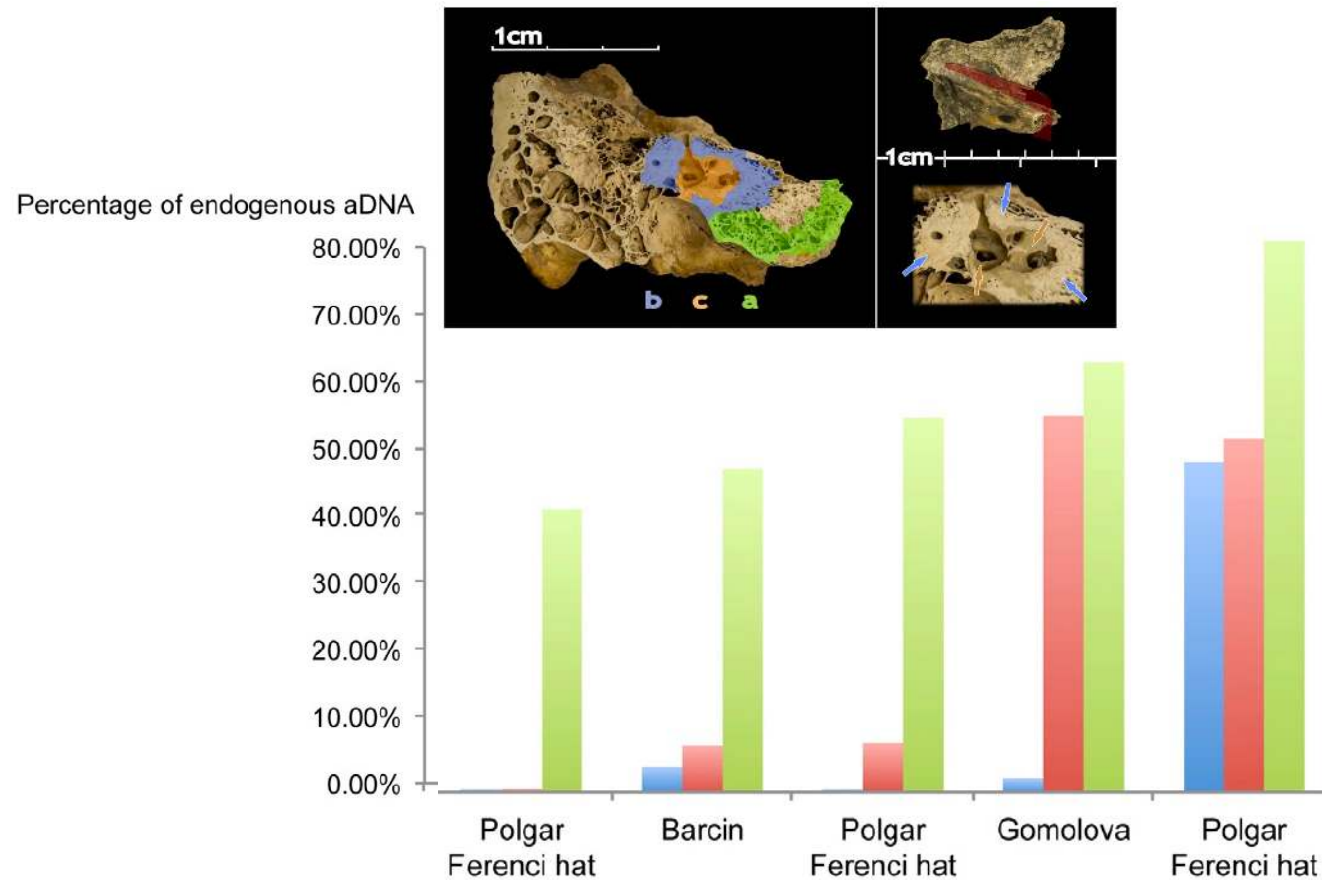
Ancient Genomics

Solution #1: New DNA Reservoirs



Ancient Genomics

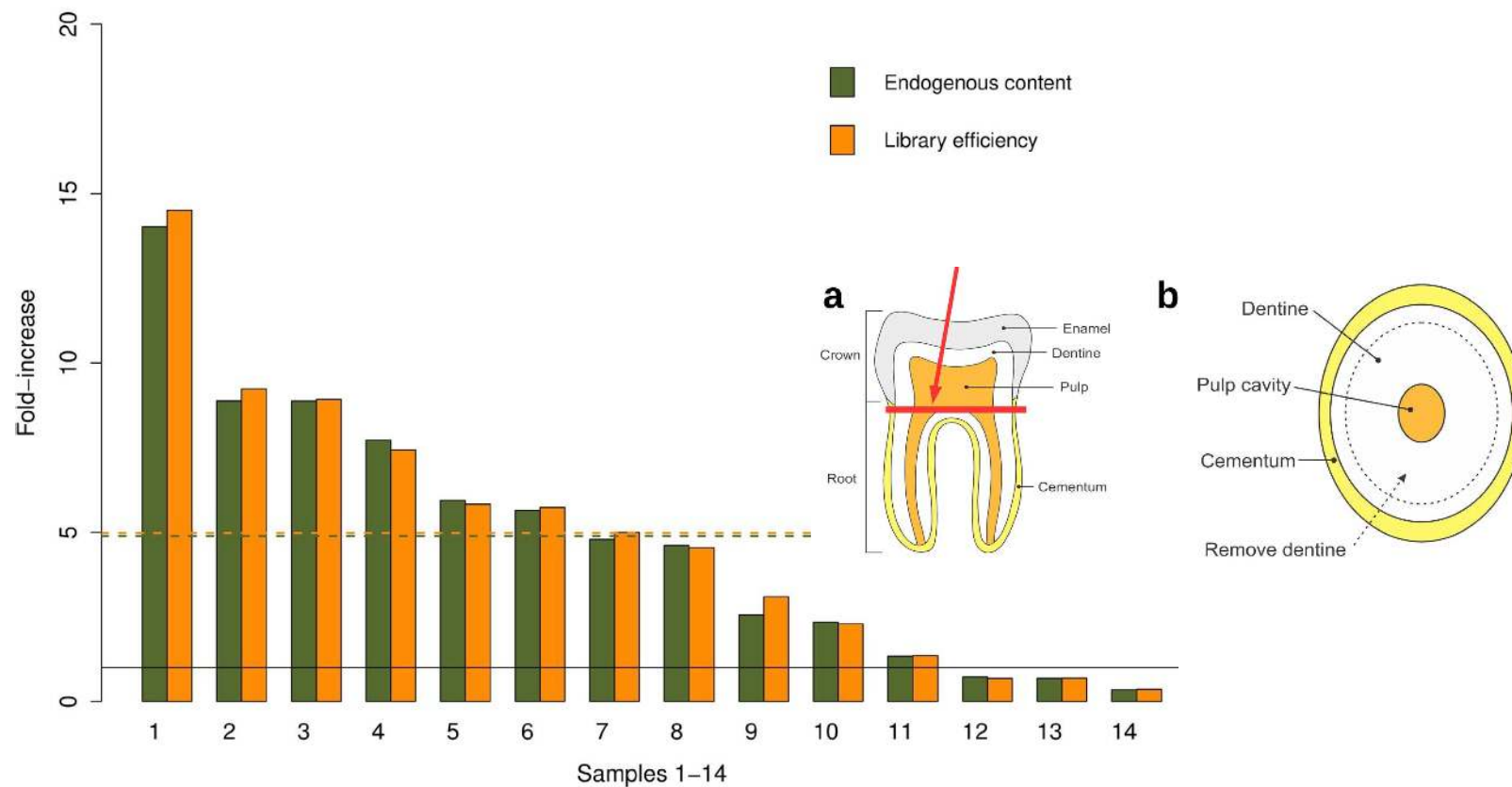
Solution #1: New DNA Reservoirs



Petrosal Bones
Pinhasi et al. PLoS One 2015

Ancient Genomics

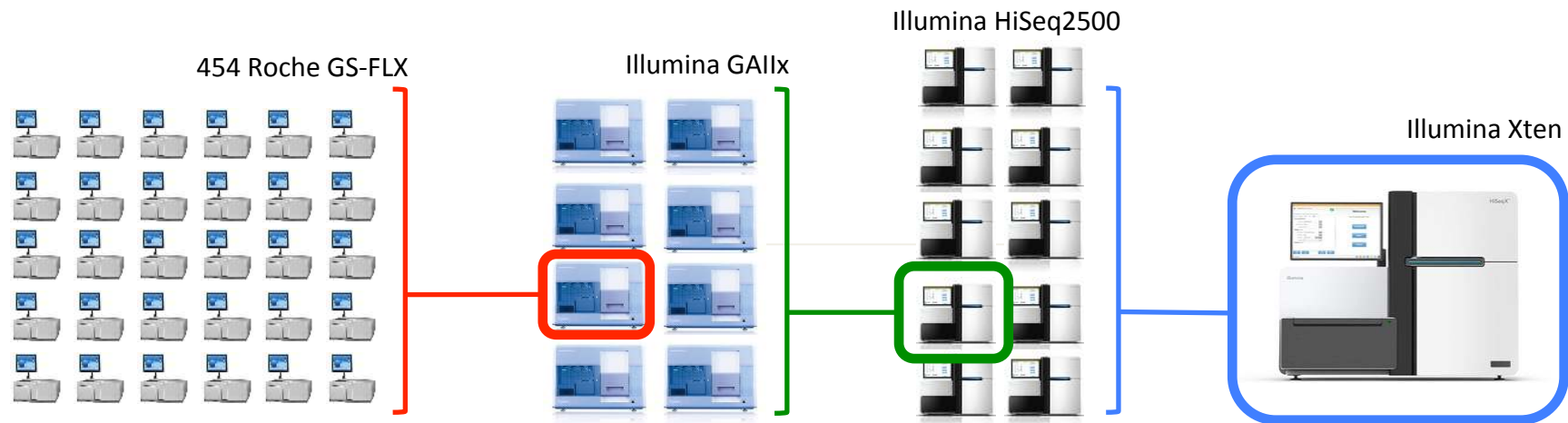
Solution #1: New DNA Reservoirs



Tooth Cementum
Pinhasi et al. PLoS One 2015

Ancient Genomics

Solution #2: Enhanced Sequencing Power



0.5M

250M

4G

30G

0.5d

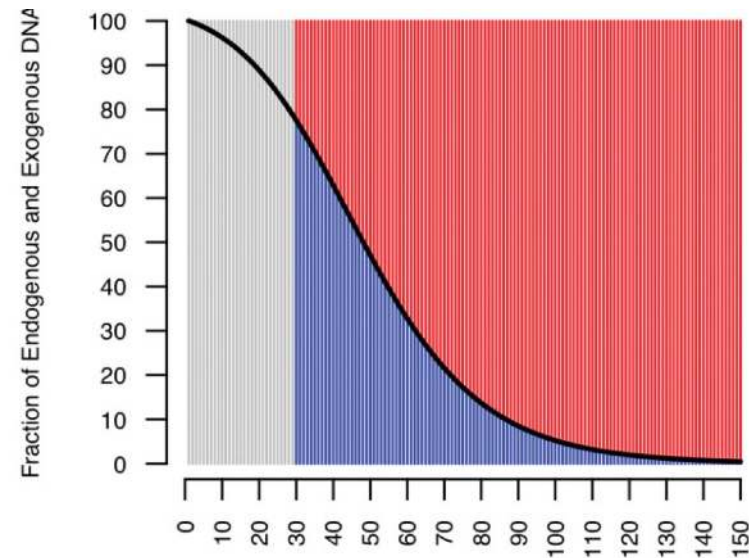
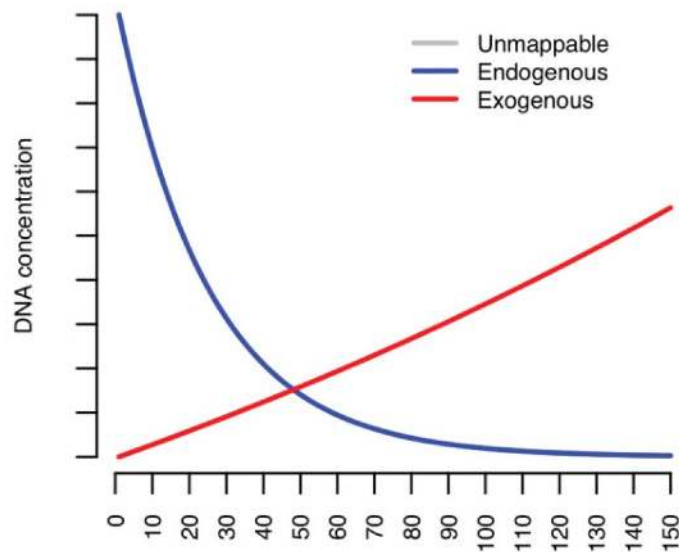
5d

5d

3d

Ancient Genomics

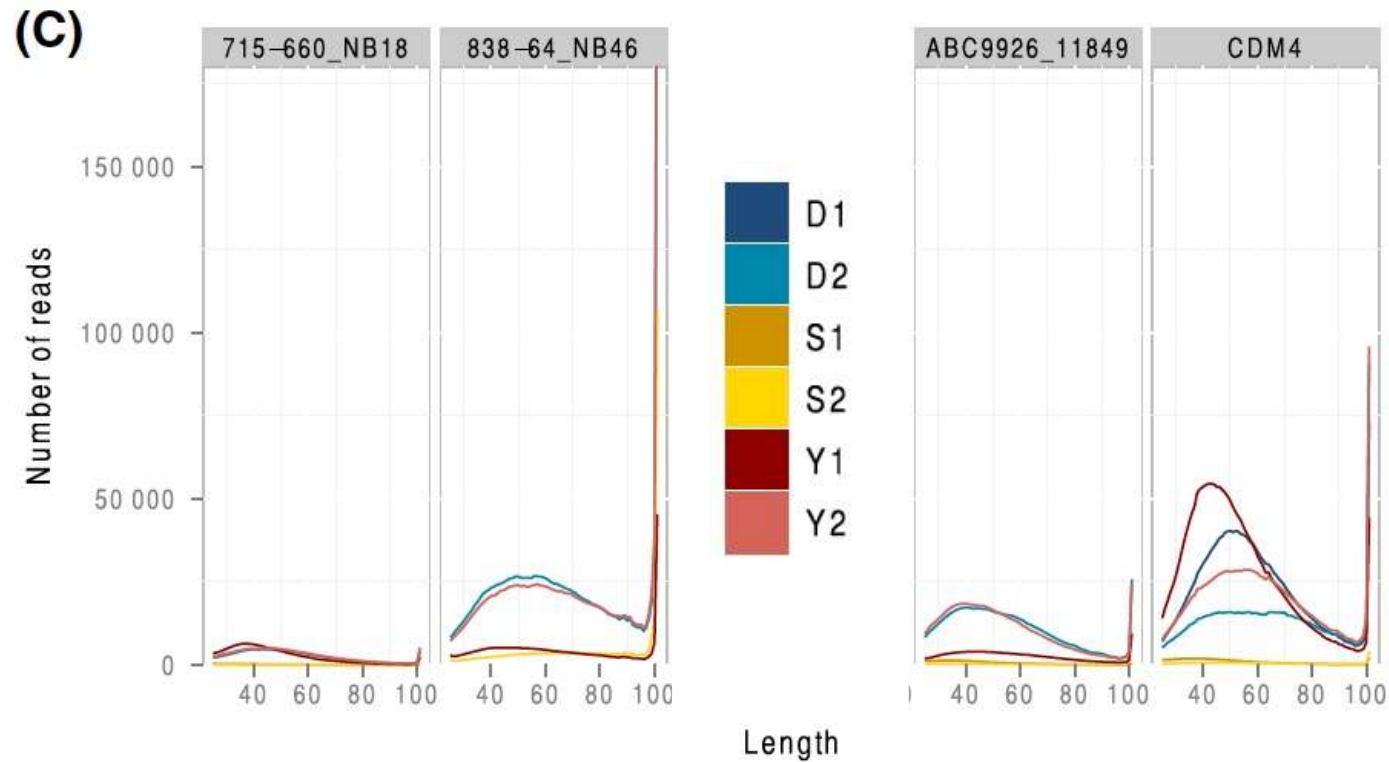
Solution #3: Develop Ultra-Sensitive Molecular Tools



Extraction Methods Tailored to the Ultra-Short
Orlando L. Bioessays 2014

Ancient Genomics

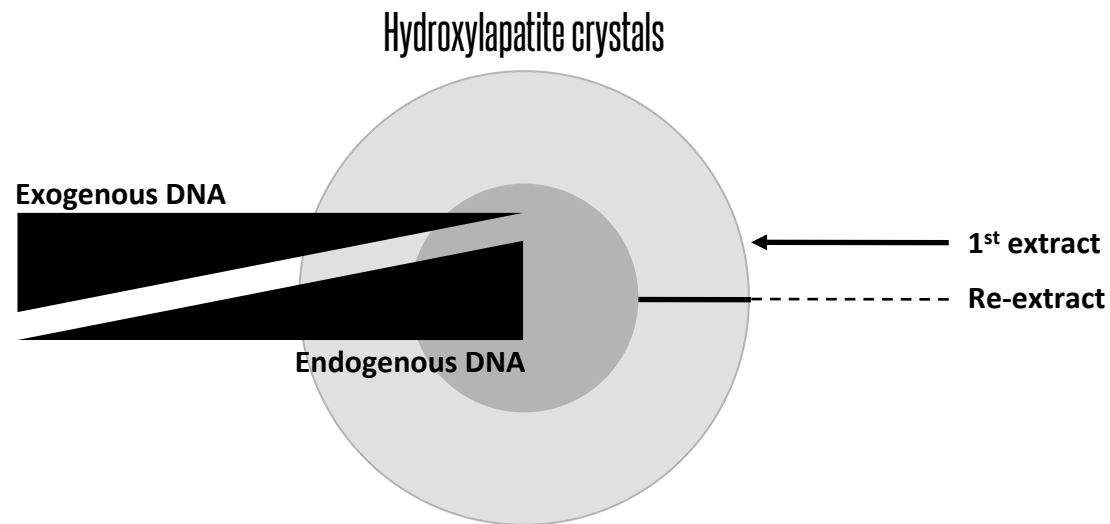
Solution #3: Develop Ultra-Sensitive Molecular Tools



Extraction Methods Tailored to the Ultra-Short
Gamba et al. Mol Ecol Res 2015

Ancient Genomics

Solution #3: Develop Ultra-Sensitive Molecular Tools

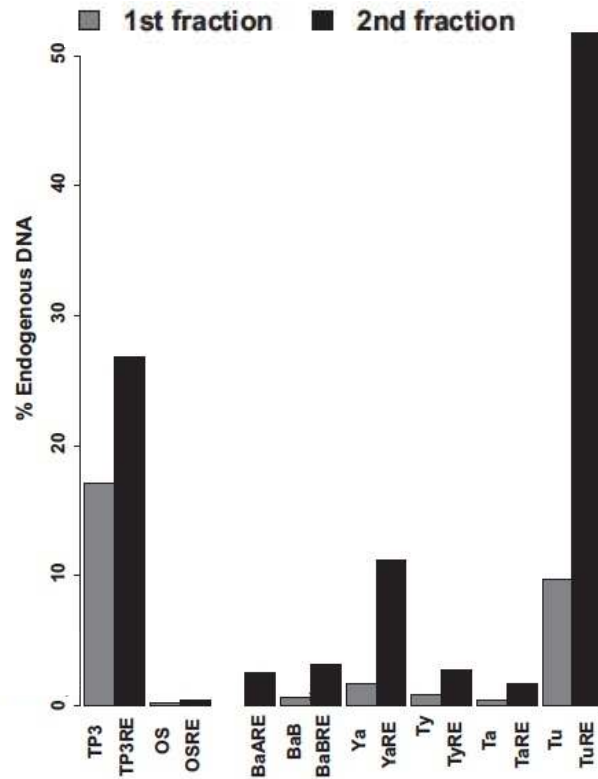


Extraction Procedures Removing Part of the Contaminating Fraction

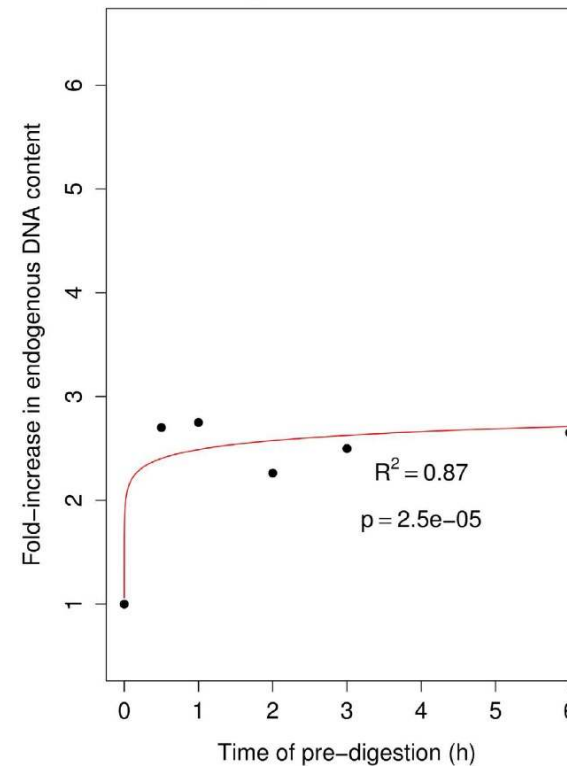
Der Sarkissian et al. Mol Ecol 2014

Ancient Genomics

Solution #3: Develop Ultra-Sensitive Molecular Tools

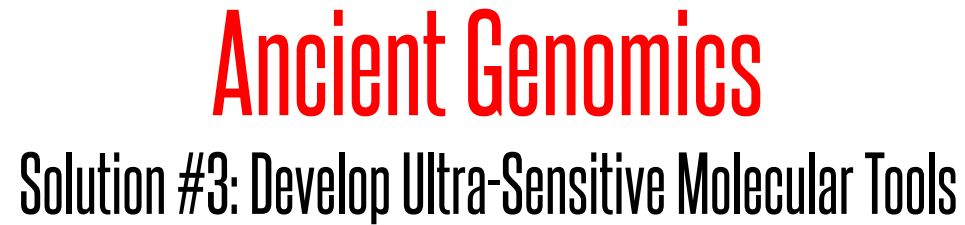


Der Sarkissian et al. Mol Ecol 2014



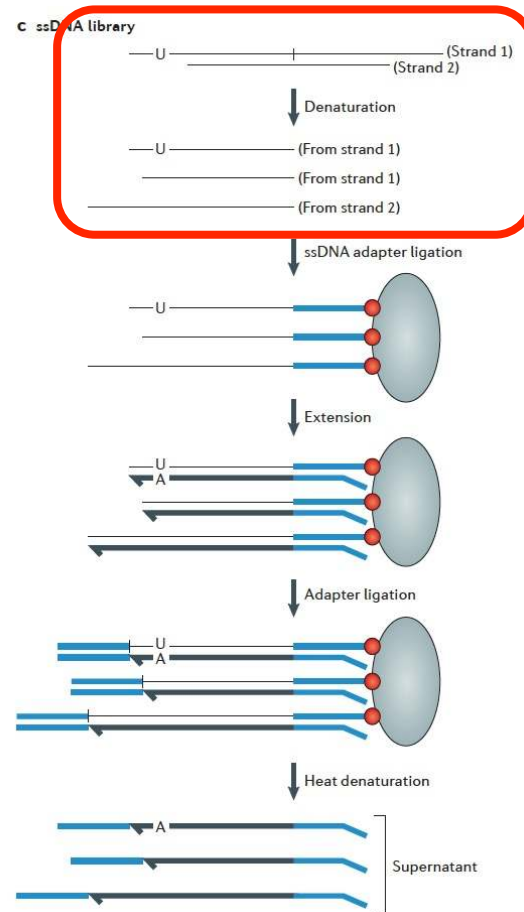
Damgaard et al. Sci Rep 2015

Extraction Procedures Removing Part of the Contaminating Fraction



Ancient Genomics

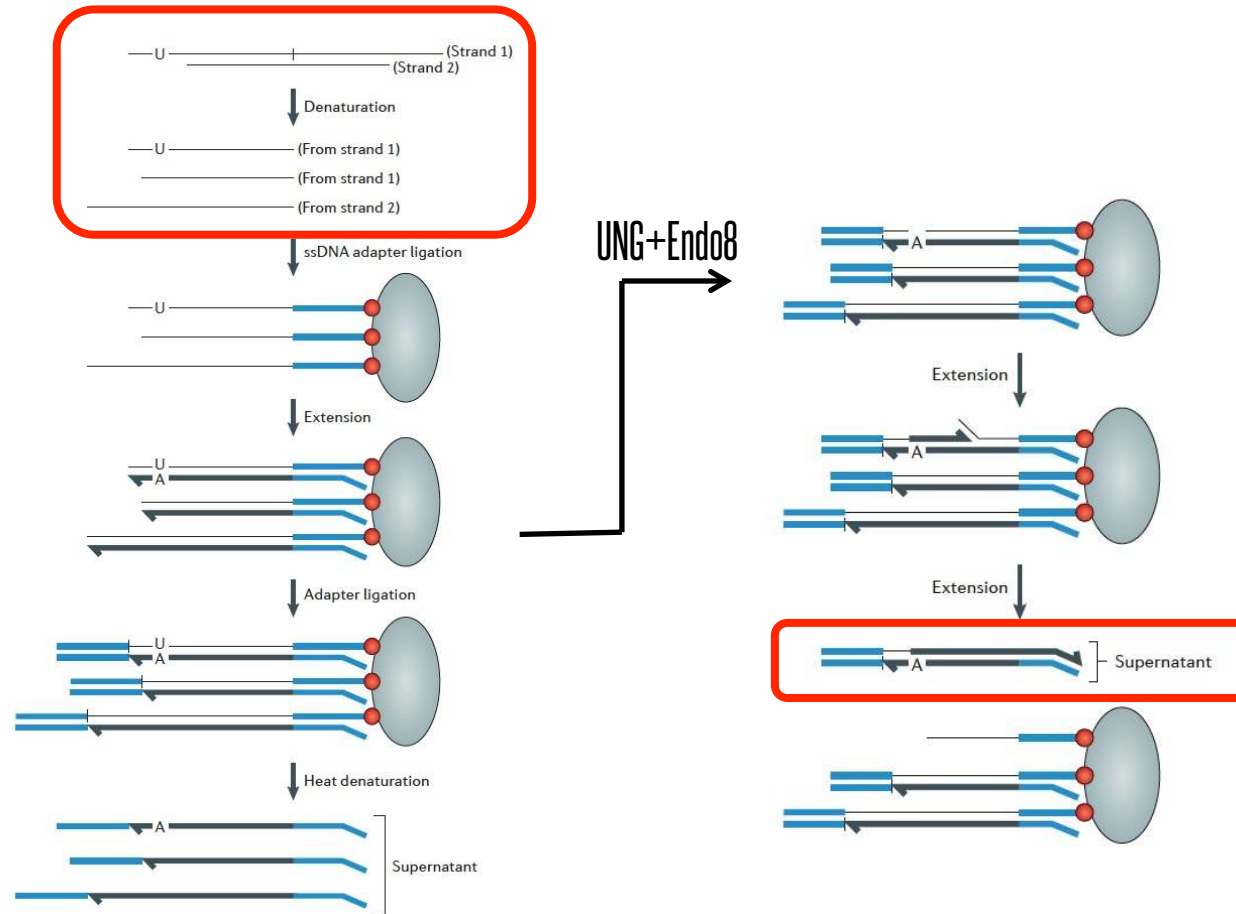
Solution #3: Develop Ultra-Sensitive Molecular Tools



Target ssDNA Templates
Gansauge & Meyer Nat Protoc 2013

Ancient Genomics

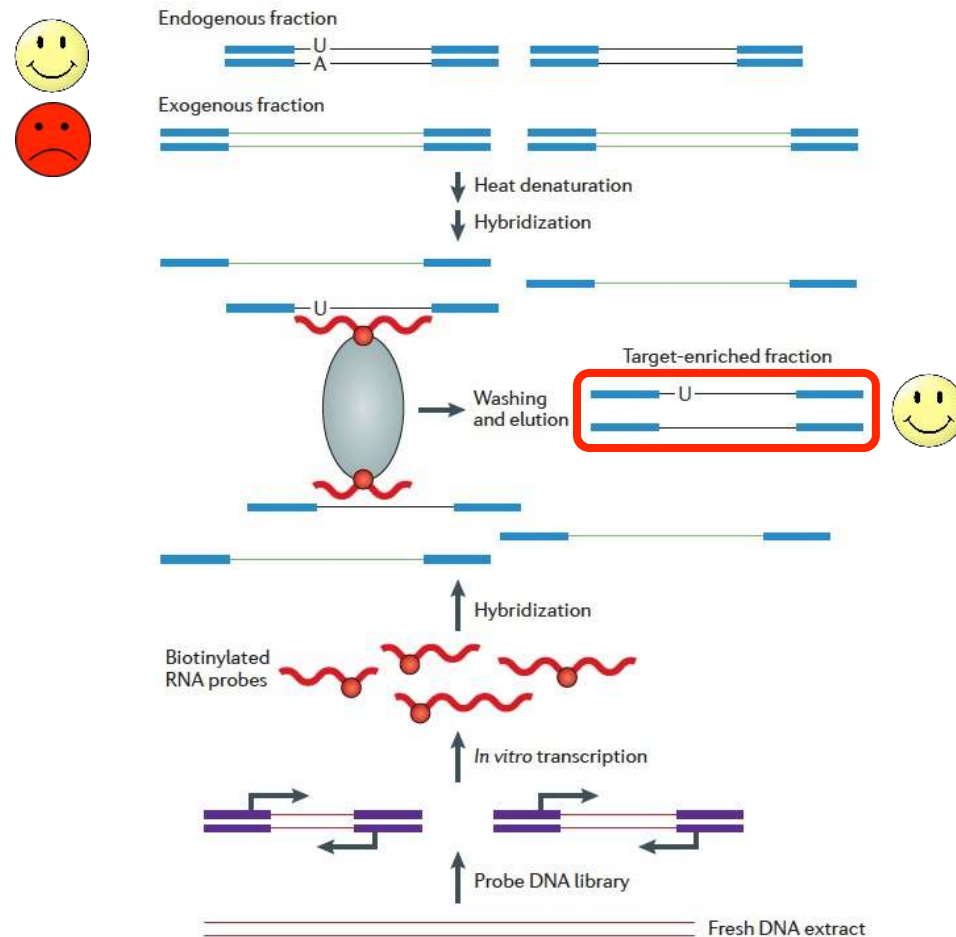
Solution #3: Develop Ultra-Sensitive Molecular Tools



Enrich DNA Libraries for Damaged DNA
Orlando et al. Nat Rev Genetics 2015

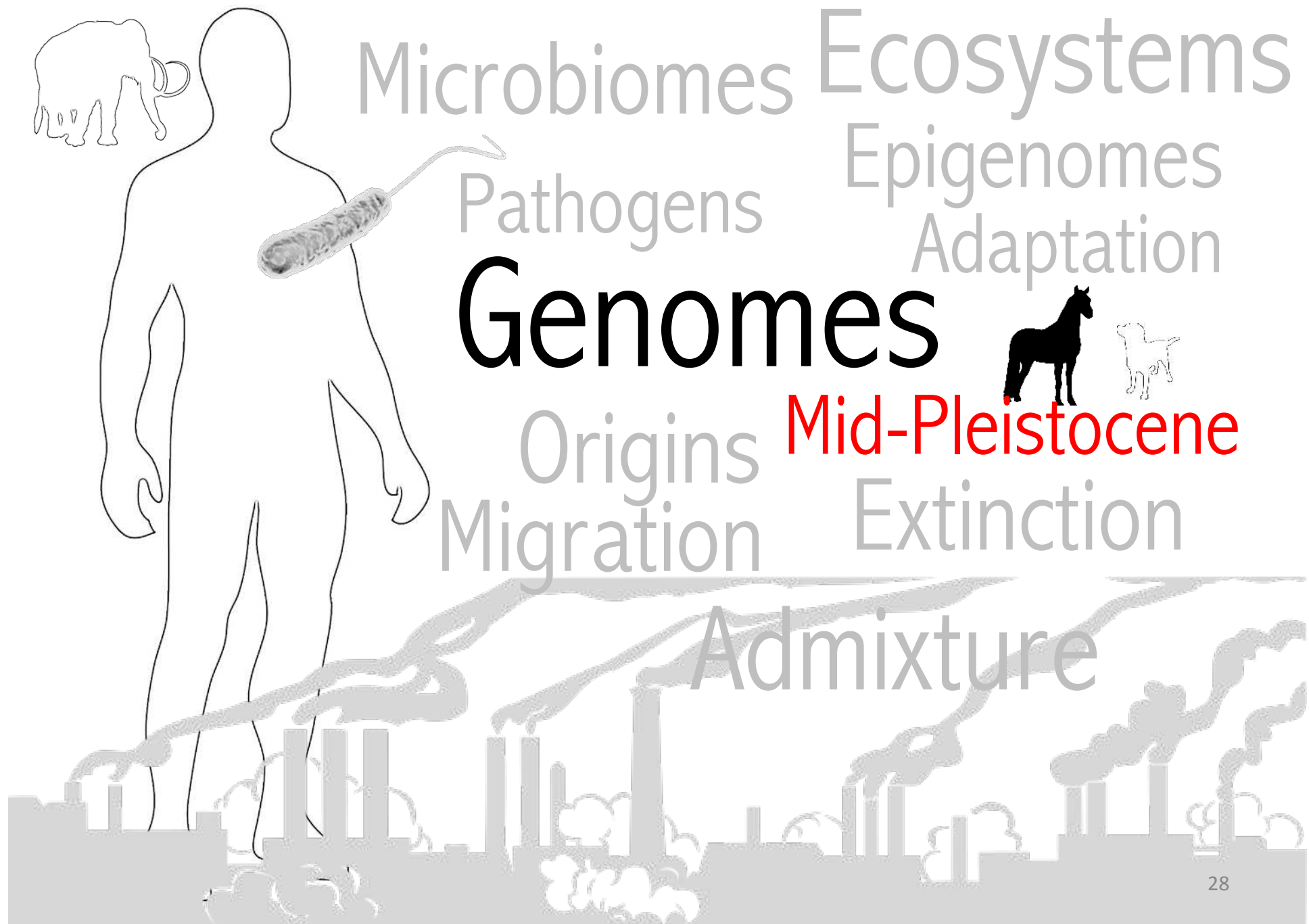
Ancient Genomics

High-Throughput Approaches: Leveraging on Close Relatives



Enrich DNA Libraries for Target DNA

Orlando et al. Nat Rev Genetics 2015

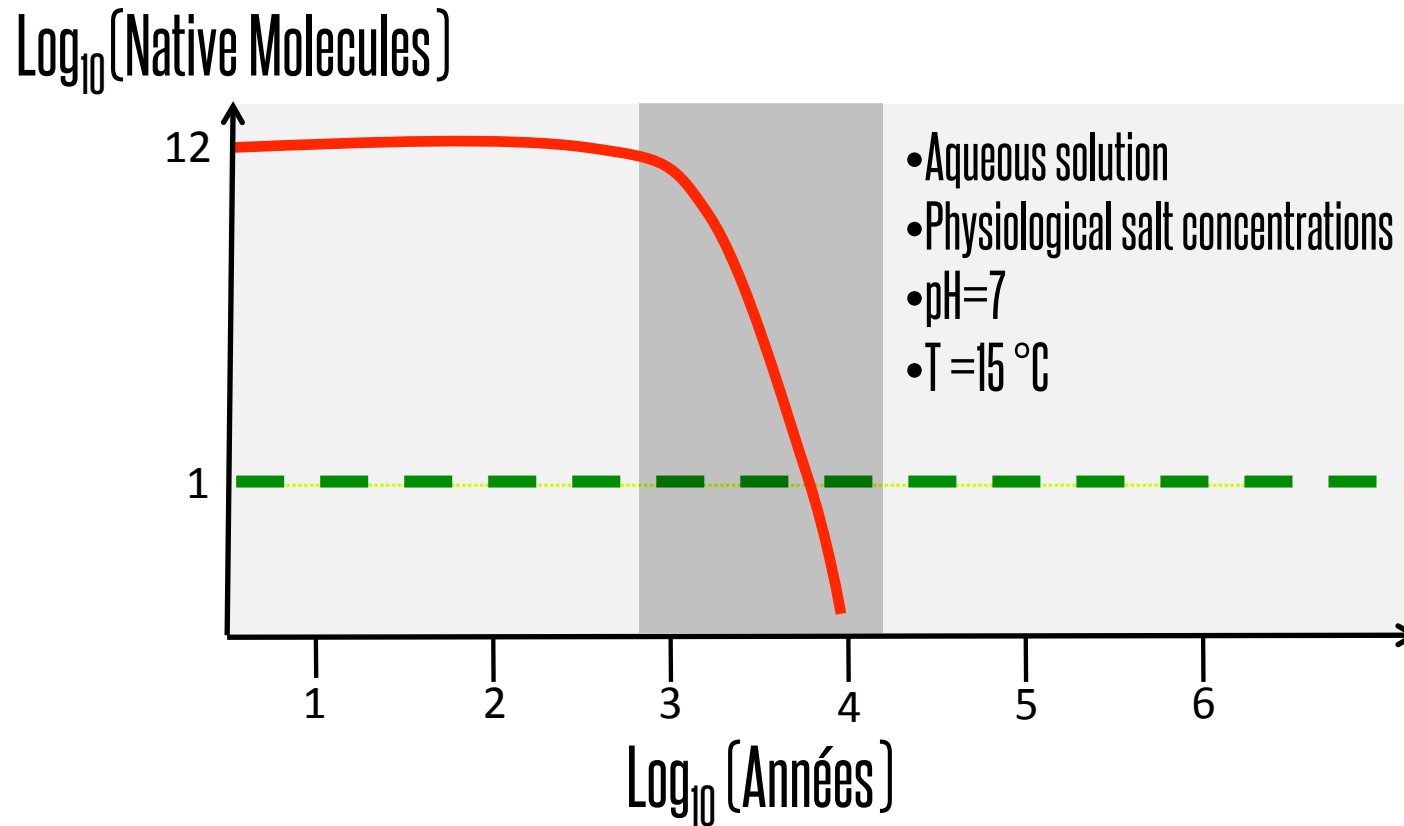


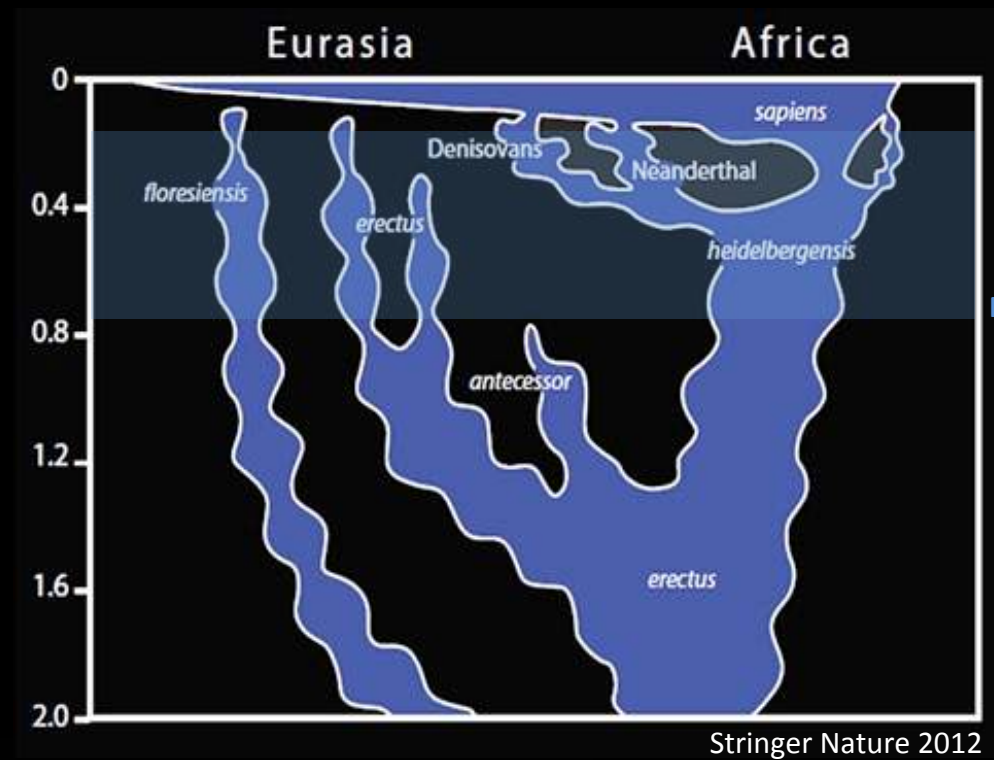
Mr Big-Shot-In-The-Field (2005)

*“So, barring some unimaginable technical advance, diachronical studies of DNA sequences are confined to the past one million years, and **more probably to the past 100,000 years.**”*

Ancient Genomics

DNA decay





MIDDLE PLEISTOCENE



Ancient Genomics

From the Anthropocene to the Middle Pleistocene

Osseous Horse and Hominin Material



560-780 kyBP
Permafrost
1.1X Nuclear Genome



400 kyBP
Temperate Cave
Complete mtDNA

OK but come on, really?

The First Genome From the Early Middle Pleistocene

Post-Mortem DNA damage

AAAGTCA CAGTCGCGAATGCGCATAGCGAT
LLLCAG LGLCAGCGCLLVCGCGLVLCGGLV

The First Genome From the Early Middle Pleistocene

Post-Mortem DNA damage

The diagram illustrates post-mortem DNA damage in a double-stranded sequence. It consists of two horizontal lines representing the DNA strands, with vertical tick marks indicating base positions. The top strand contains the sequence: AAAGTC CAGTCGCGAATGCGCATAGCGAT. The bottom strand contains the sequence: LLLGAG LGTGAAGG CLLVCGCGLVLCGGLV. The first 10 base pairs (AAAGTC / LLLGAG) are shown in light gray, while the remaining 20 base pairs (CAGTCGCGAATGCGCATAGCGAT / CLLVCGCGLVLCGGLV) are in black. The bottom strand sequence is the reverse complement of the top strand, with the first 10 bases (LLLGAG) being the reverse of the first 10 bases (AAAGTC) and the remaining 20 bases (LGTGAAGG CLLVCGCGLVLCGGLV) being the reverse of the remaining 20 bases (CAGTCGCGAATGCGCATAGCGAT).

AAAGTC CAGTCGCGAATGCGCATAGCGAT
LLLGA LGLGAGG CLLVCGCGLVLCGGLV

The First Genome From the Early Middle Pleistocene

Post-Mortem DNA damage



The diagram illustrates a DNA double helix. The top strand is a continuous sequence of 16 nucleotides: CAGTCGCGAATGCGCATAGCGAT. The bottom strand is a complementary sequence of 16 nucleotides: ATCGGATCGGATCGGATCGGATC. A vertical gap is present in the bottom strand between the 8th and 9th positions, where the sequence changes from ATCGGAT to CGGATCGGATC. This gap represents a site of post-mortem DNA damage, such as a strand break or a deletion.

CAGTCGCGAATGCGCATAGCGAT
ATCGGATCGGATCGGATCGGATC

The First Genome From the Early Middle Pleistocene

Post-Mortem DNA damage

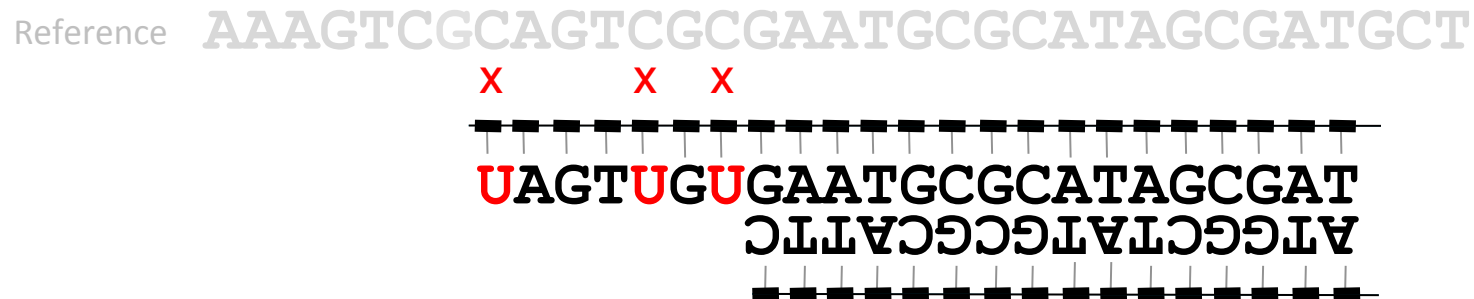
Reference AAAGTCGCAGTCGCGAATGCGCATAGCGATGCT

X
- - - - -
UAGTCGCGAATGCGCATAGCGAT
 ATCGGCTAGGCT
- - - - -



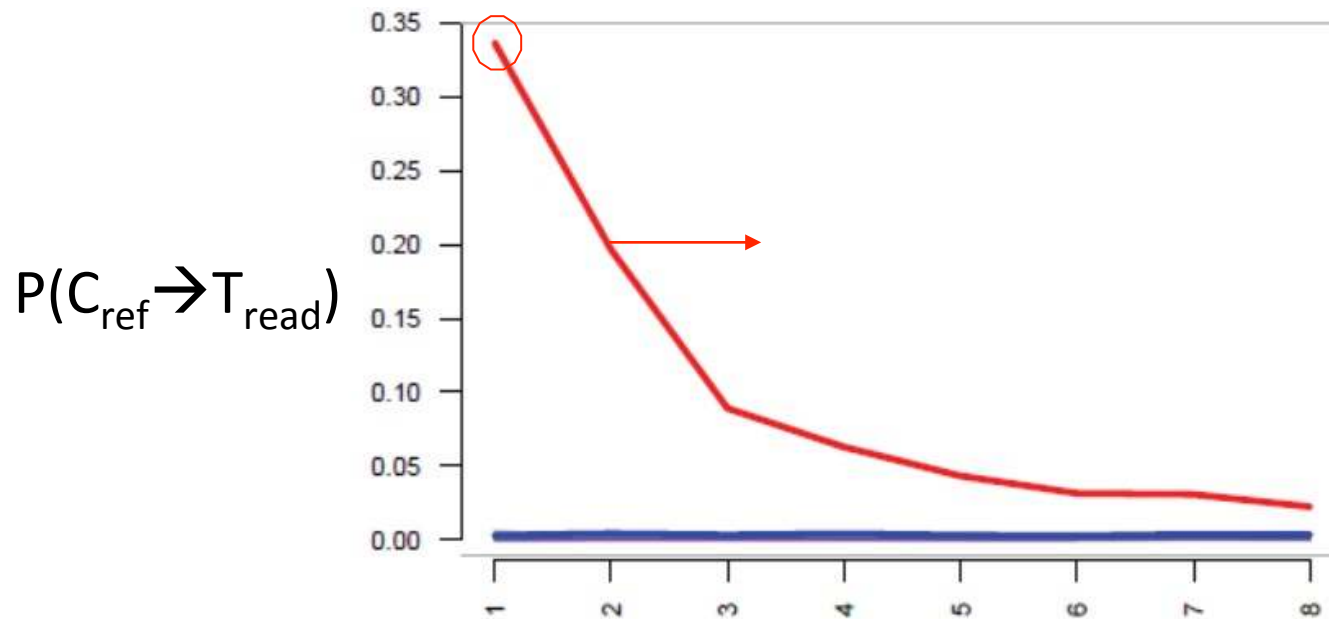
The First Genome From the Early Middle Pleistocene

Post-Mortem DNA damage



The First Genome From the Early Middle Pleistocene

Post-Mortem DNA damage

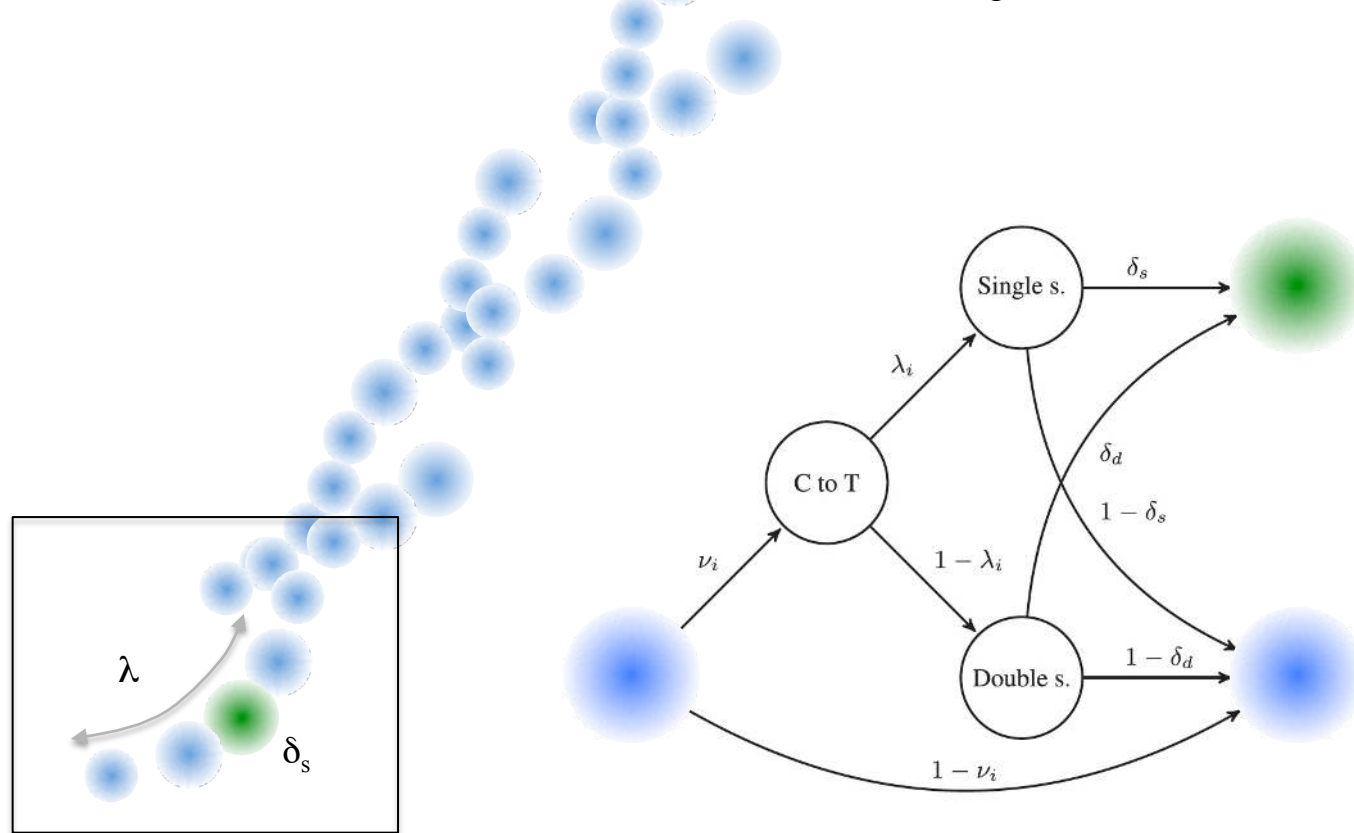


NNNNNNNNNNNNNNNNNNNN**AAGTCGCGAATGCGCATAGCGAT**NNNNNNNNNNNNNNNNNNNN

Diagram illustrating a DNA double helix structure. The top strand (coding strand) is shown with the sequence: AAAGTC AAGTUGCGAATGCGUATAGCGAT. The bottom strand (template strand) is shown with the sequence: LLLGAC LLLGACGGC LLLVCGCGLVLCGGIV. The mutation is highlighted in red in the top strand, showing a C to U change. A red arrow points to the left below the template strand, indicating the direction of transcription.

The First Genome From the Early Middle Pleistocene

Post-Mortem DNA damage

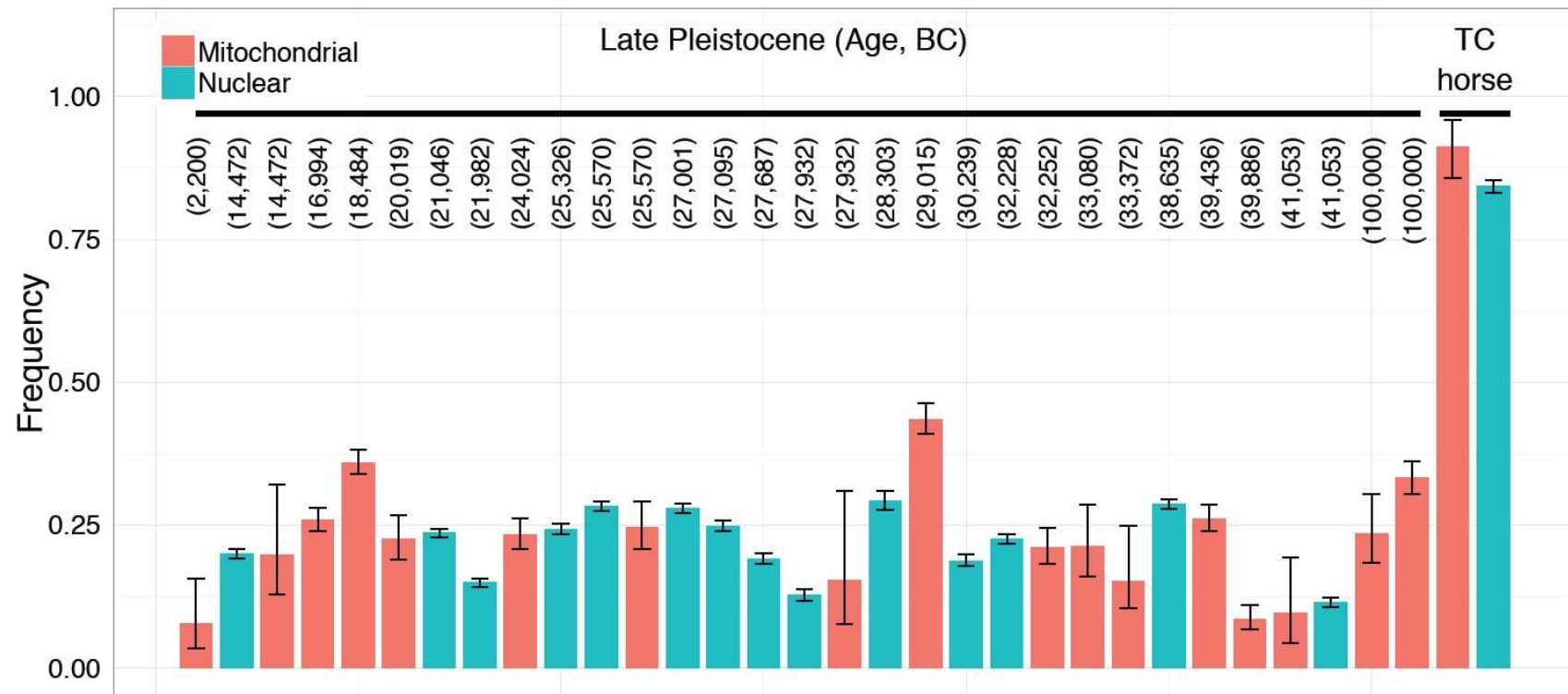


$$S_{A,i} \sim \text{Mul}(D_A, (1, 0, 0, 0) \cdot \Theta(\mu, \rho) \cdot P_{\text{dam}}(\delta_d, \delta_s, \lambda, \nu, i))$$



The First Genome From the Early Middle Pleistocene

Cytosine Deamination Levels

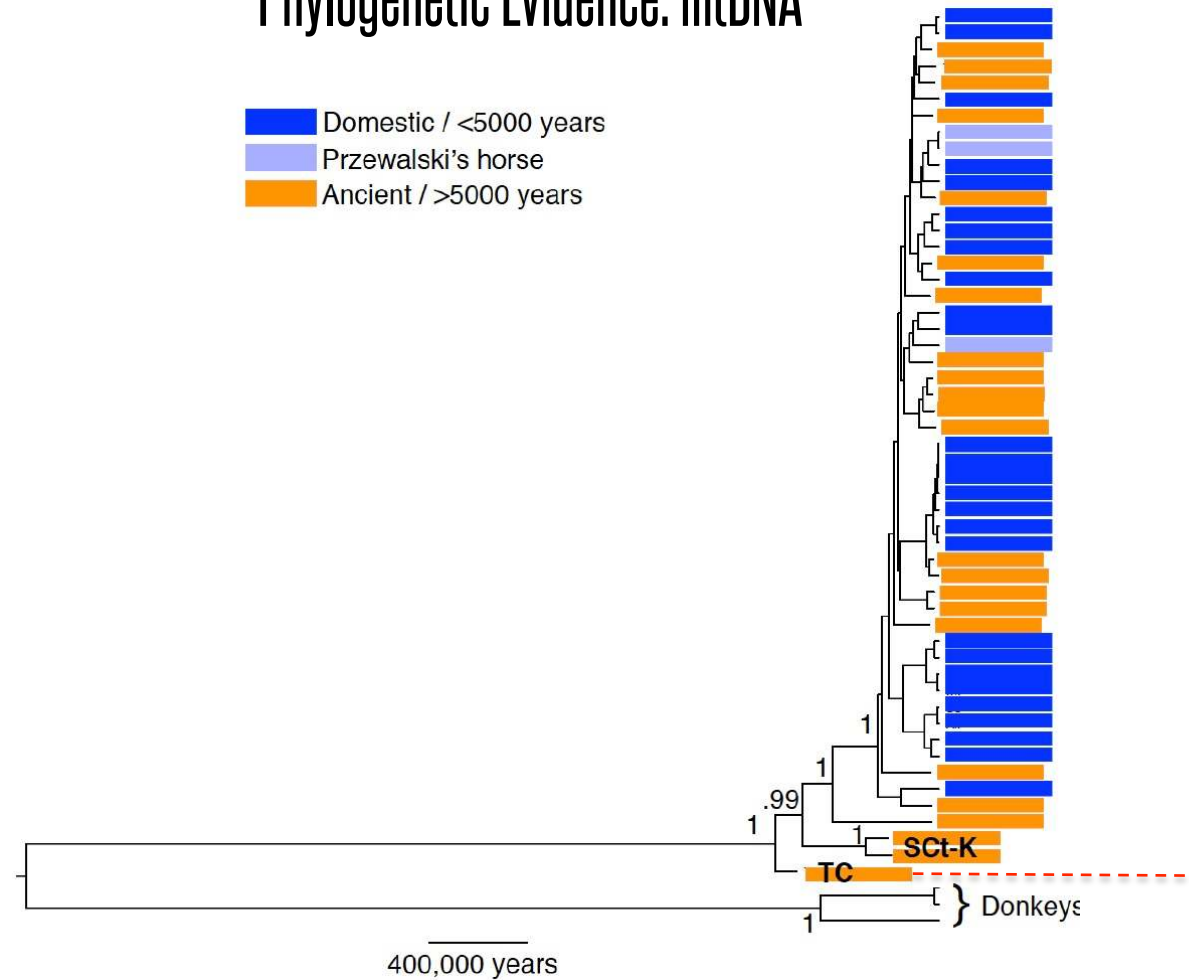


OK but come on, really?



The First Genome From the Early Middle Pleistocene

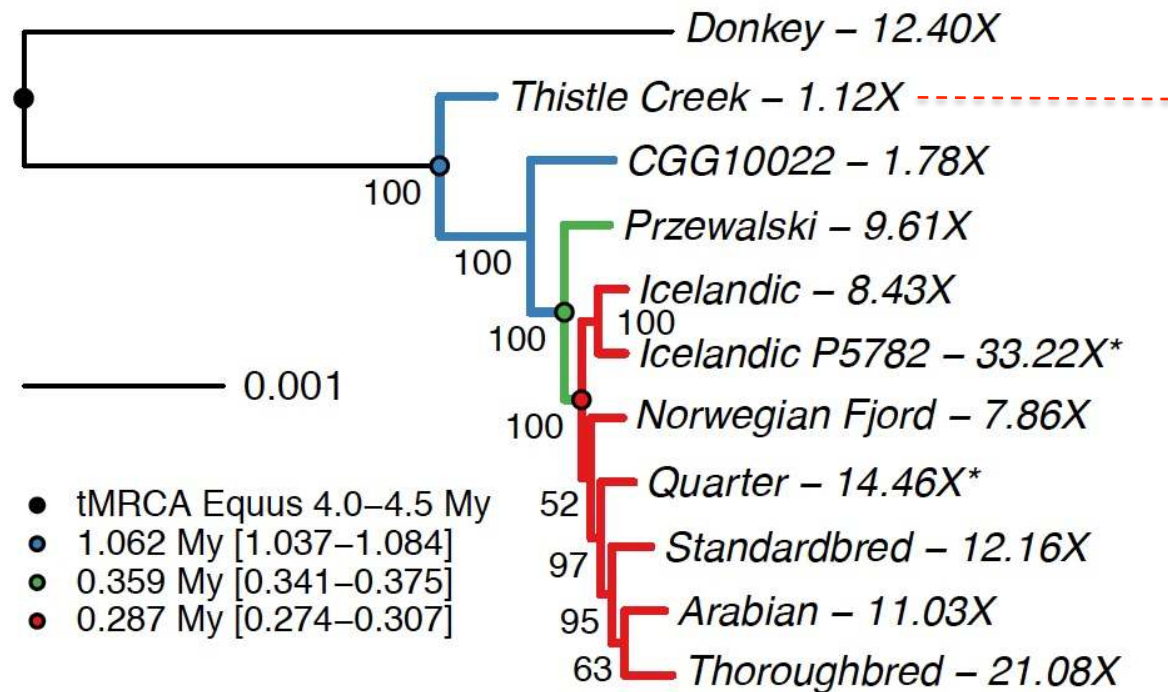
Phylogenetic Evidence: mtDNA



29 novel ancient complete
mitochondrial genomes

The First Genome From the Early Middle Pleistocene

Phylogenetic Evidence: nucDNA



DONKEY



43 KY BP



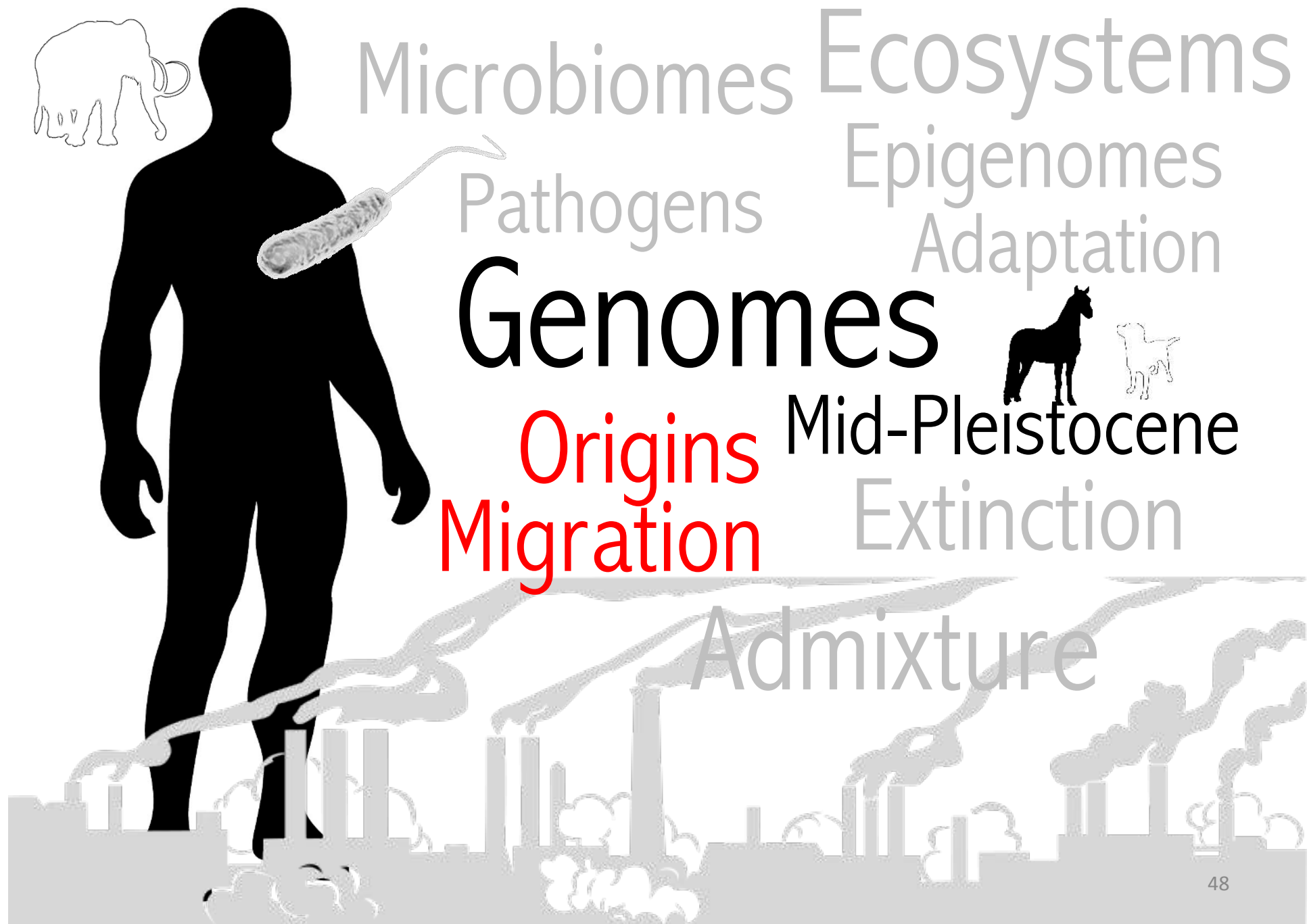
PRZEWALSKI



DOMESTIC BREEDS

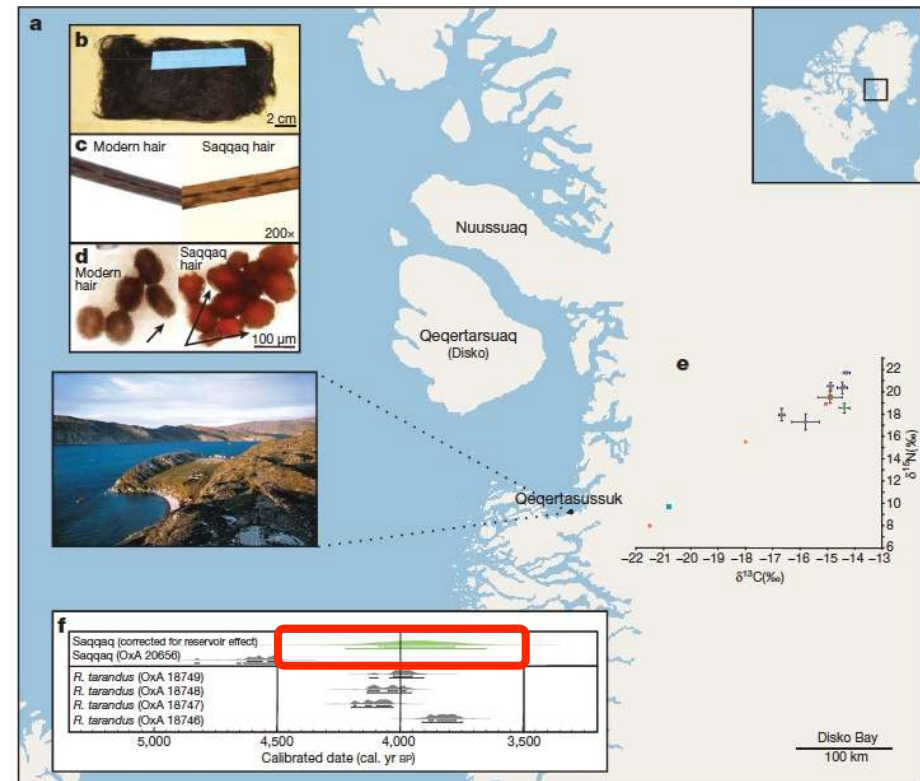


Really.



Ancient Genomes Help Refining Our Origins

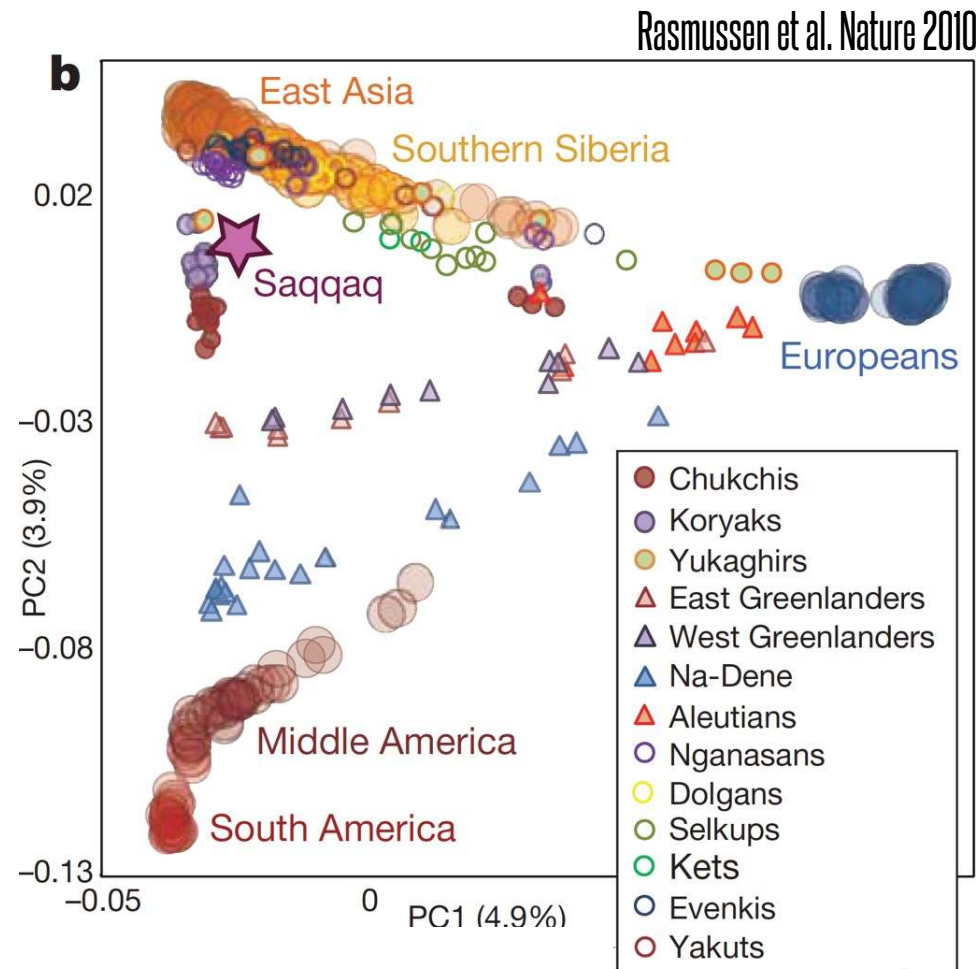
The First Ancient Human Genome



Rasmussen et al. Nature 2010

Ancient Genomes Help Refining Our Origins

The Peopling of the New World Arctic



Ancient Genomes Help Refining Archaeological Models

Complementing Cultural Information

3000BC → 800BC → 1350AD → Now

Migration 1



Culture 1 - Saqqaq

Culture 2 - Dorset

Culture 3 - Inuit



Migration 1



Culture 1 - Saqqaq

Culture 2 - Dorset

Replacement

Migration 2



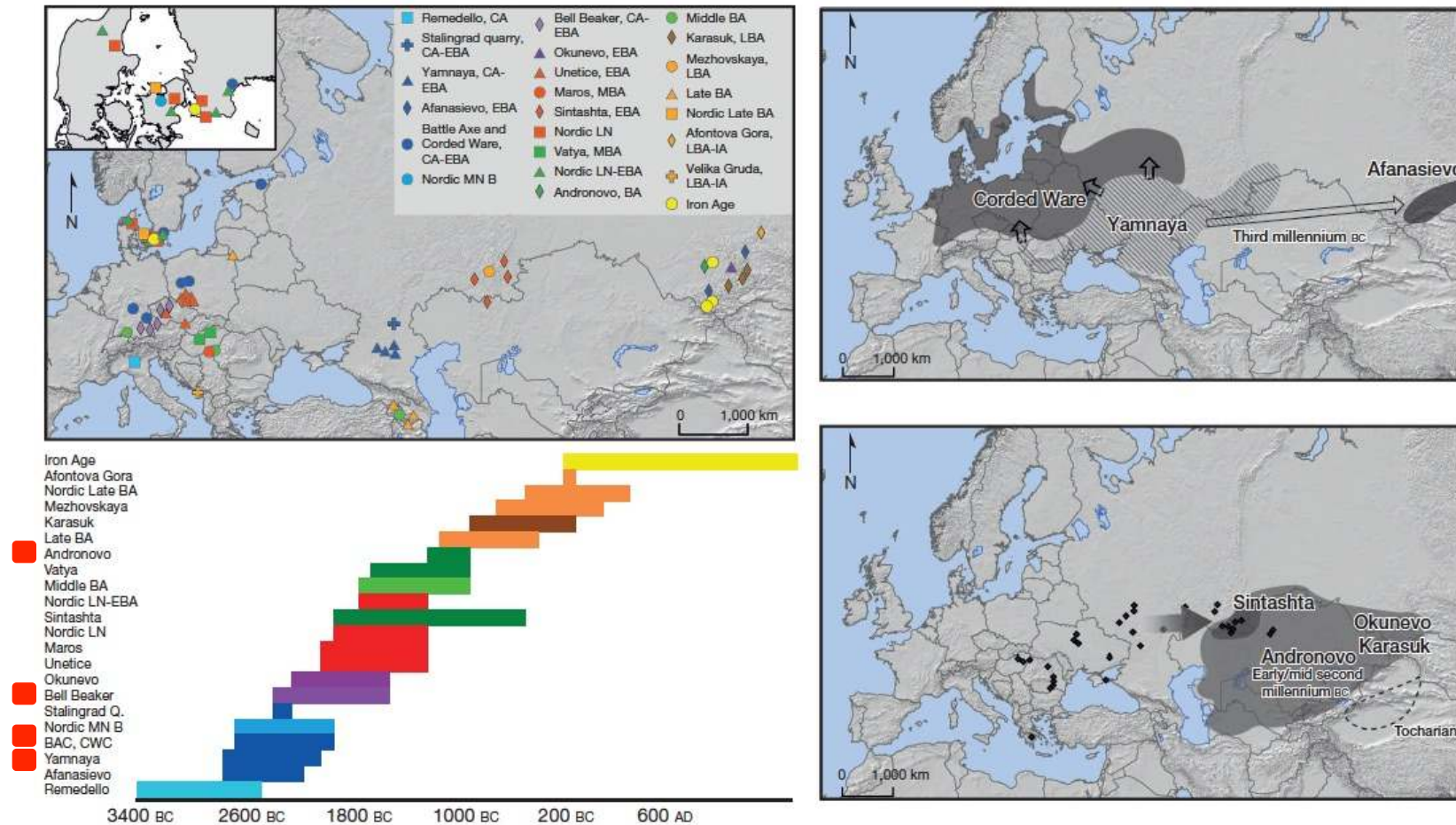
Culture 3 - Inuit



Raghavan et al. Science 2014

Bronze Age Genomics

>100 Ancient Human Genomes

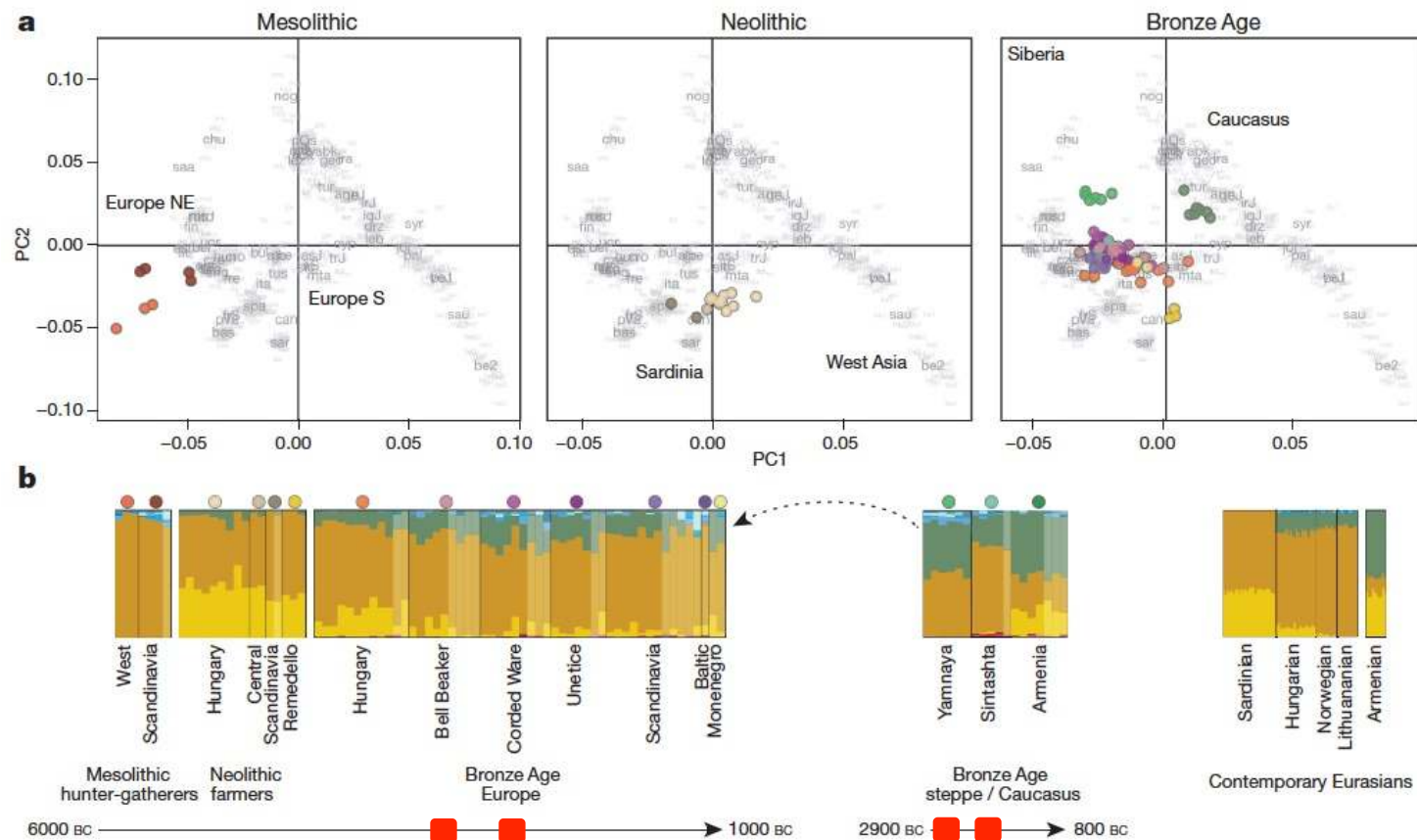


Allentoft et al. Nature 2015

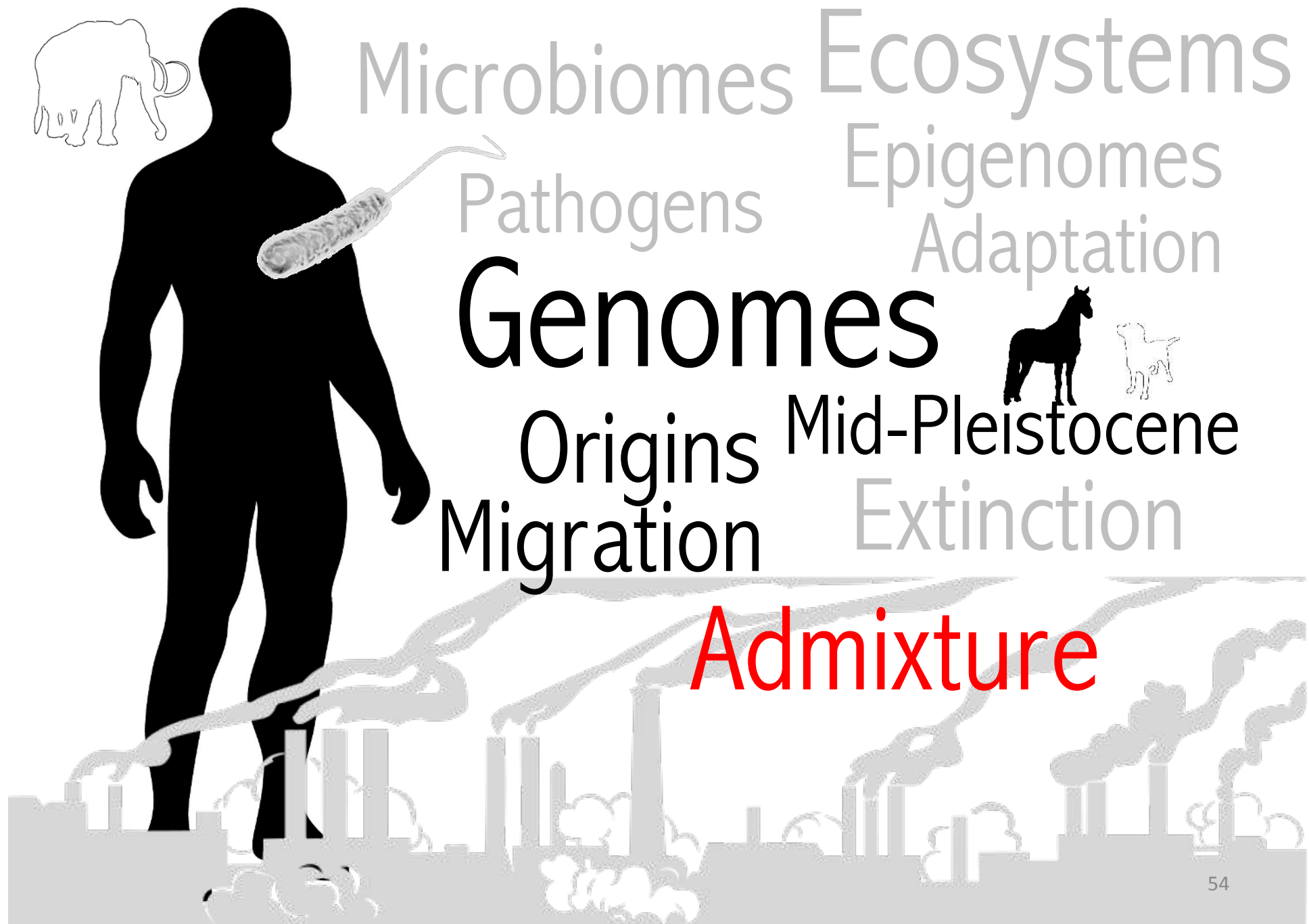
Bronze Age Genomics

>100 Ancient Human Genomes

Europe

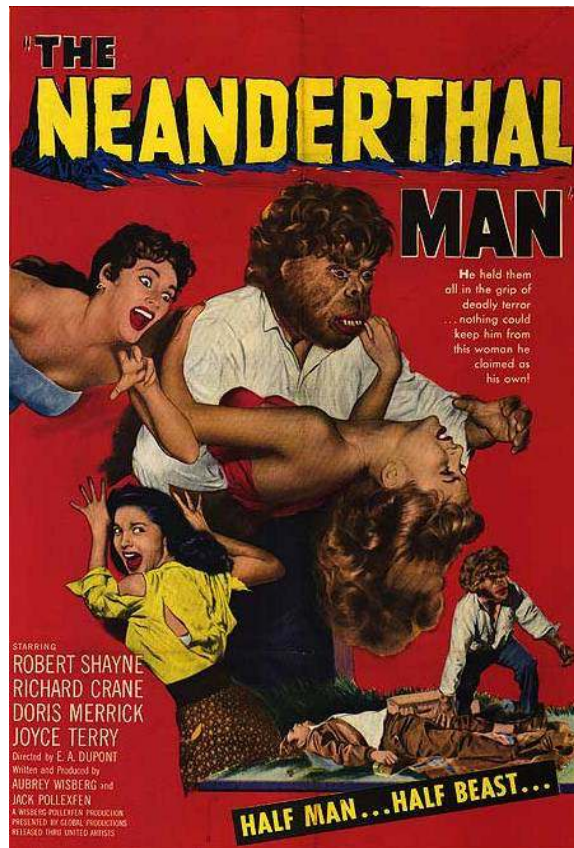


Allentoft et al. Nature 2015



Archaic hominins

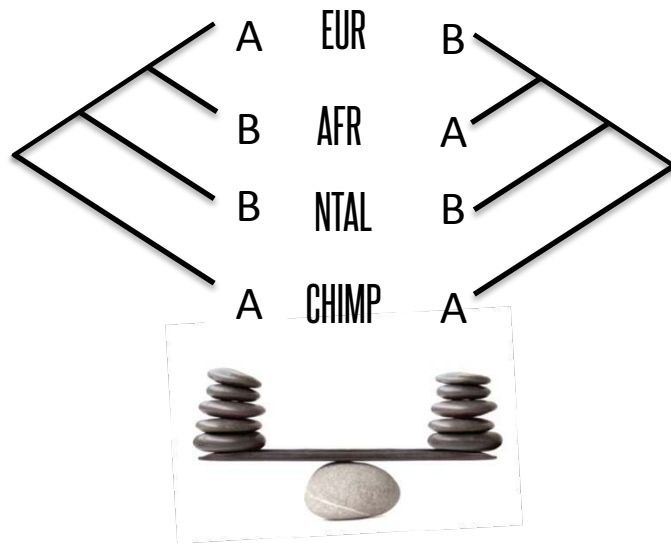
Neandertal Genomics



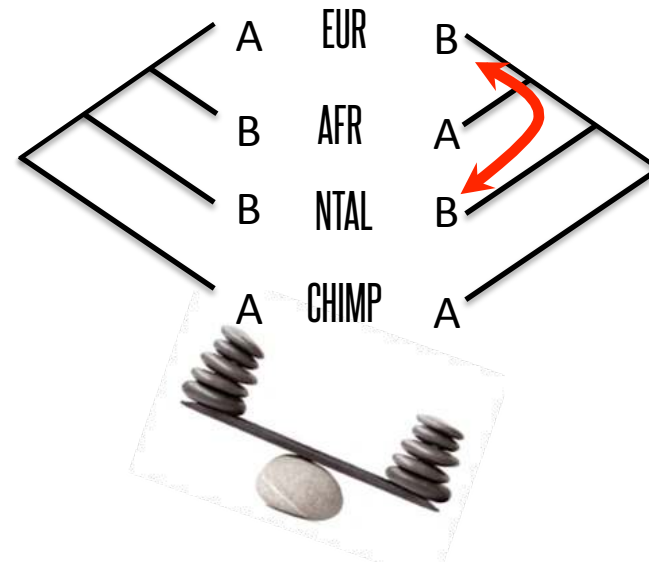
Archaic hominins

Neandertal Genomics

D-Statistics



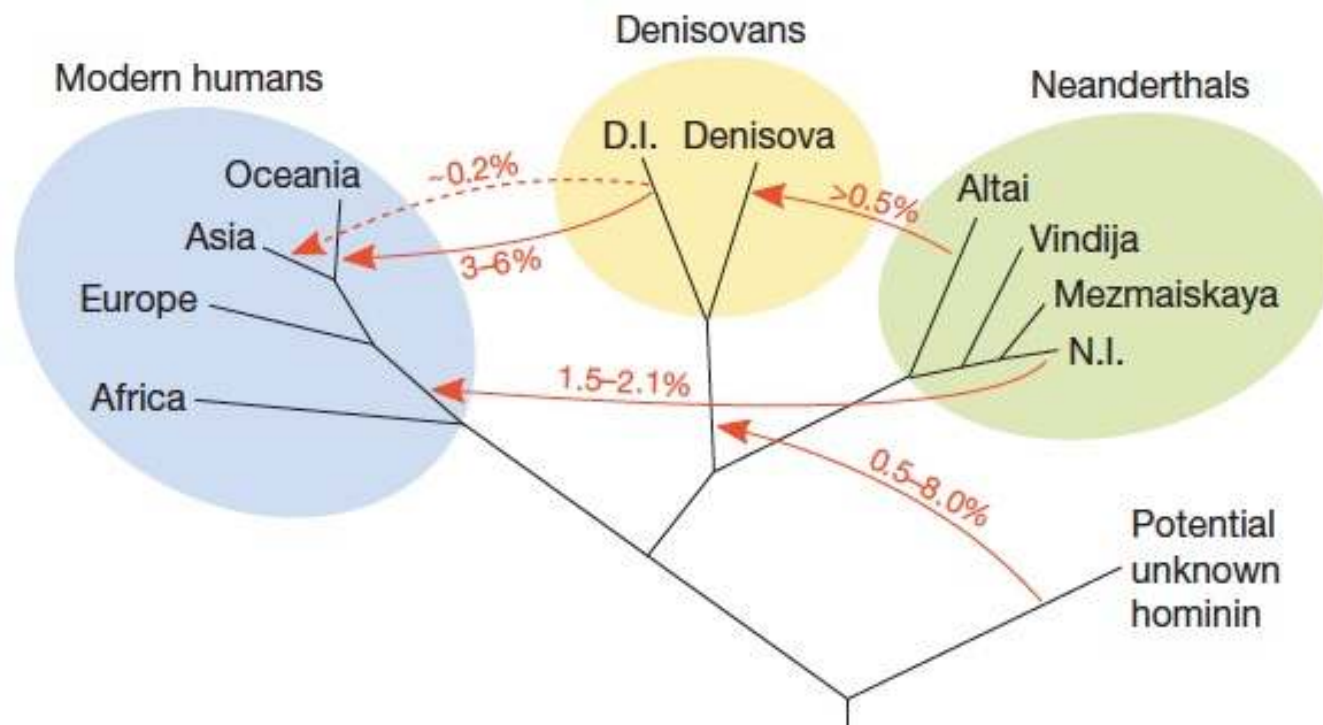
NO Admixture



Admixture

Archaic hominins

Neandertals, Denisovans, Ghosts
Admixture

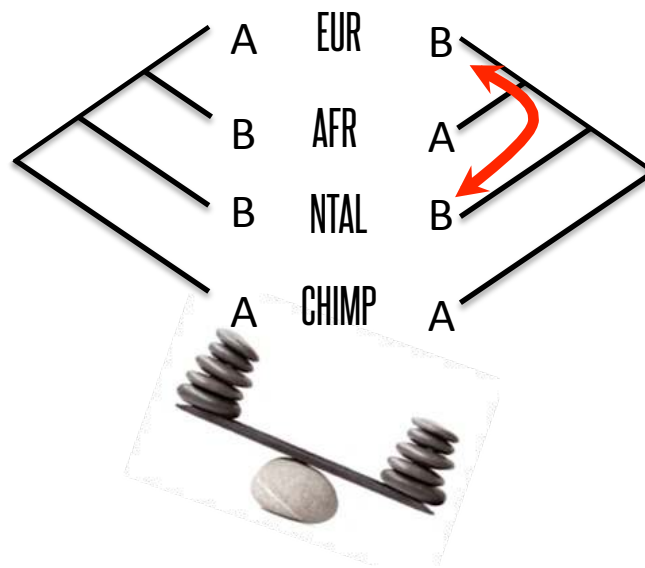
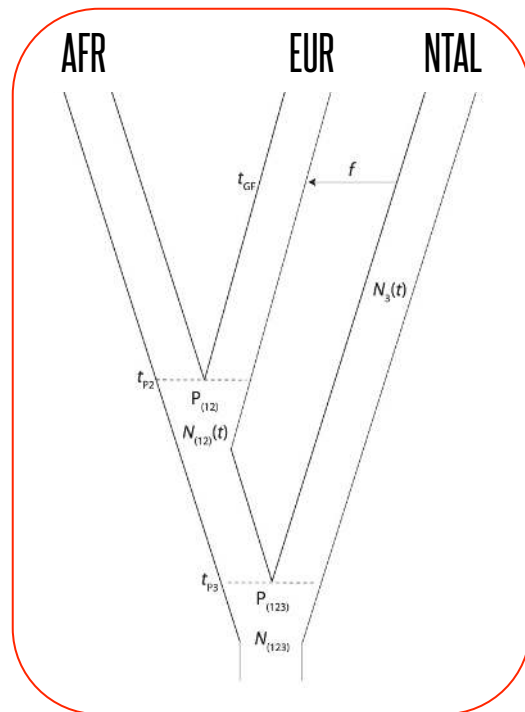


From Prufer et al. Nature 2014

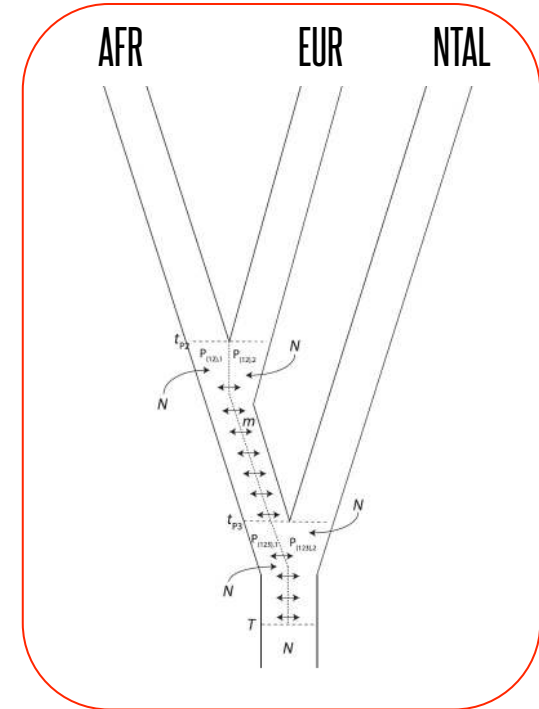
Archaic hominins

Neandertals, Denisovans, Ghosts

Admixture



Admixture



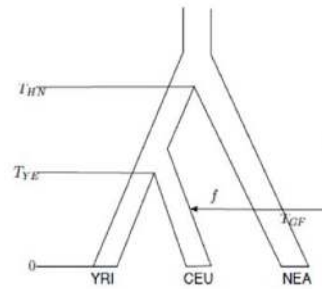
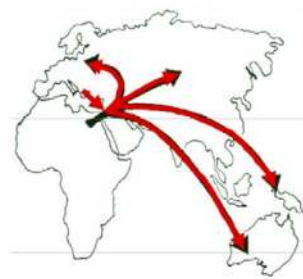
From Durand et al. Mol Biol Evol 2011

Archaic hominins

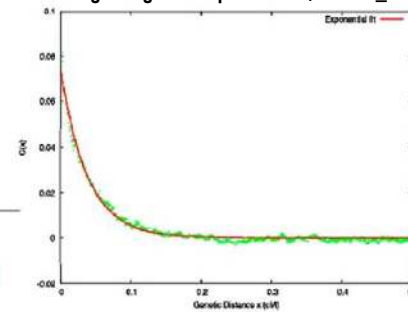
Neandertals, Denisovans, Ghosts

Admixture

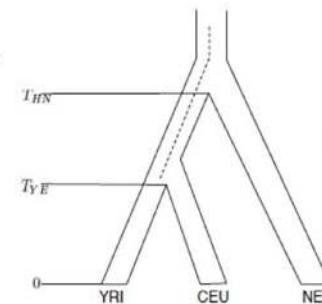
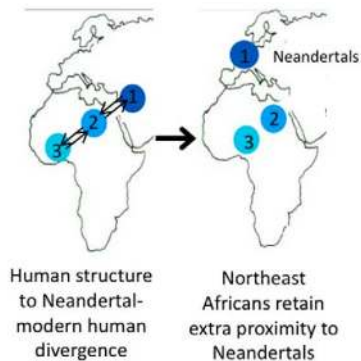
(a) Gene flow <100,000 years ago



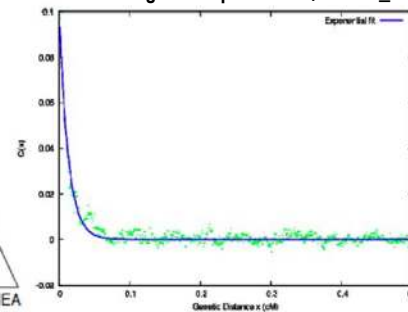
Long-Range LD expectation | Neand_Der



(b) Ancient structure >230,000 years ago



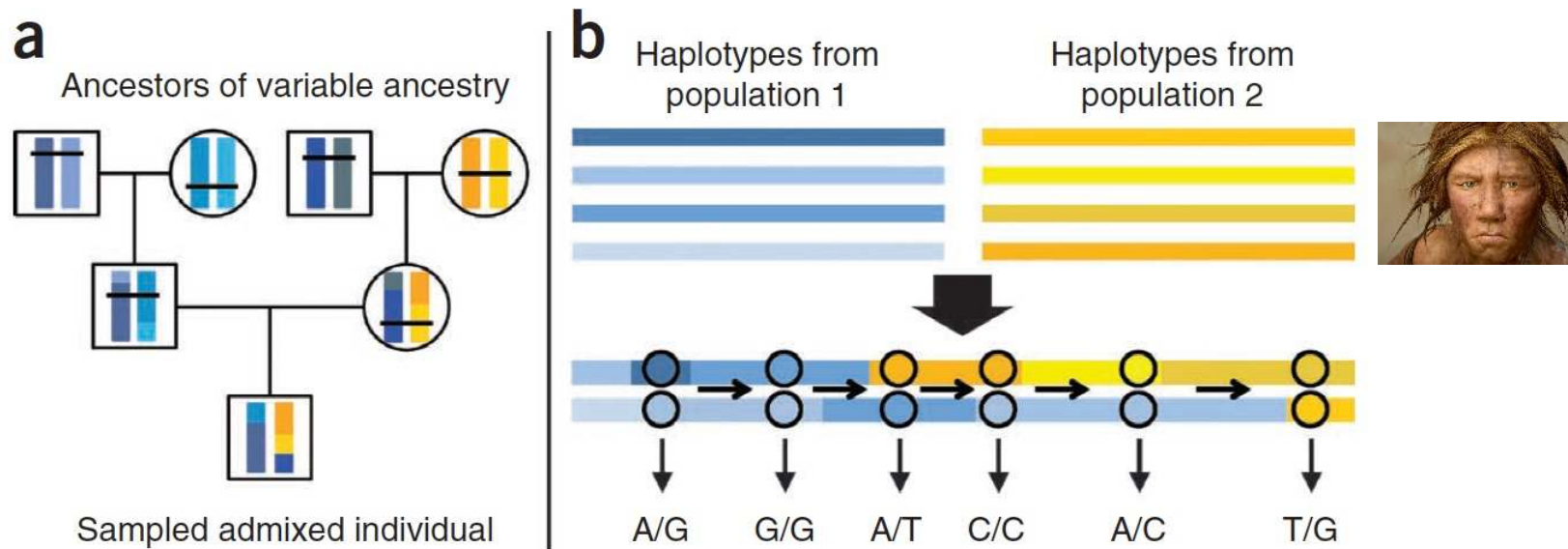
Short-Range LD expectation | Neand_Der



Sankararaman et al. PLoS Genet 2012

Archaic hominins

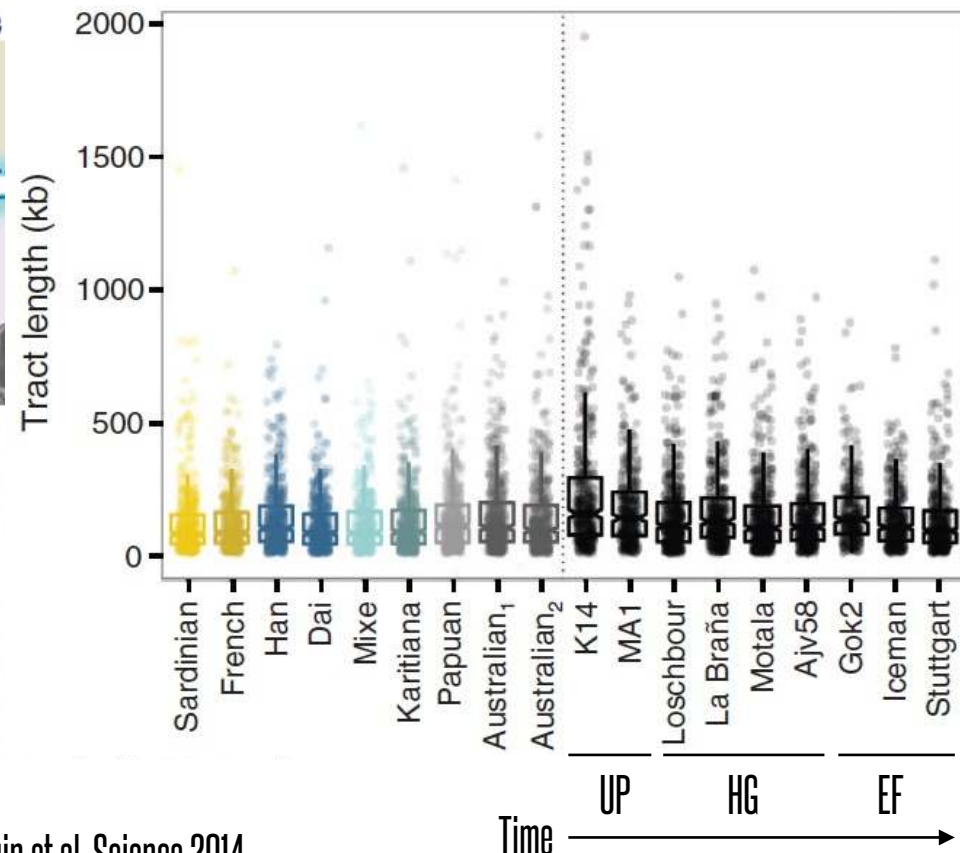
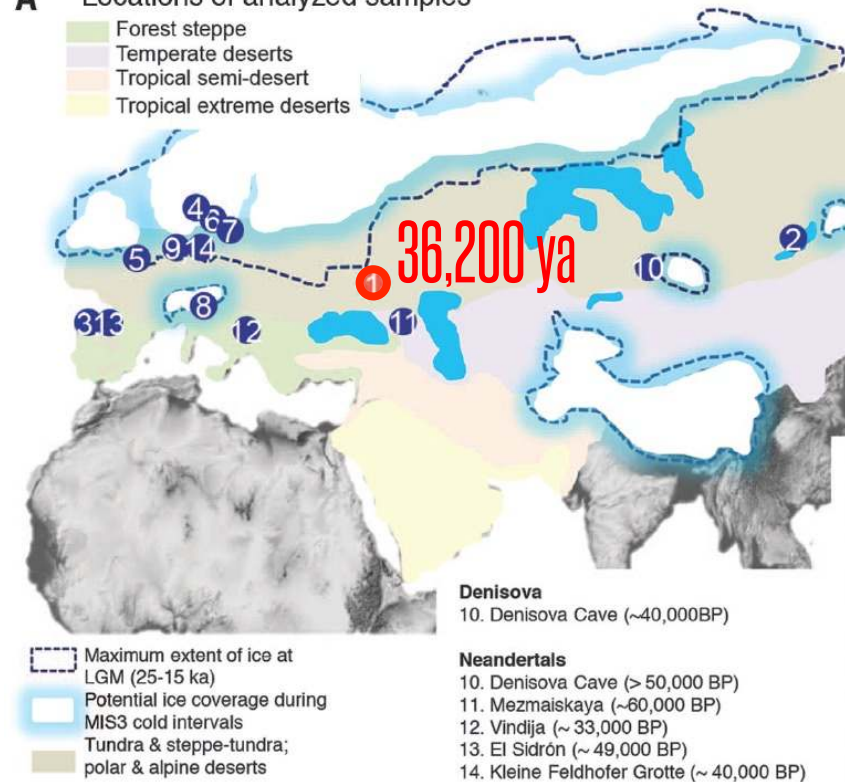
Dating the Admixture with Neanderthals (~55 kyr ago)



Archaic hominins

Dating the Admixture with Neanderthals

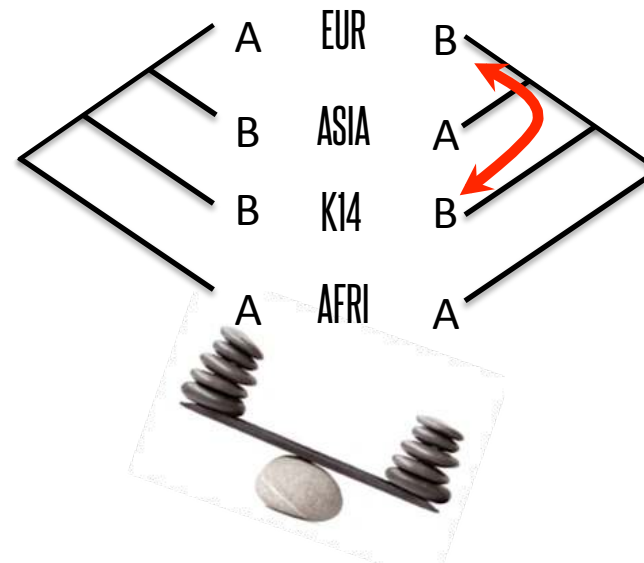
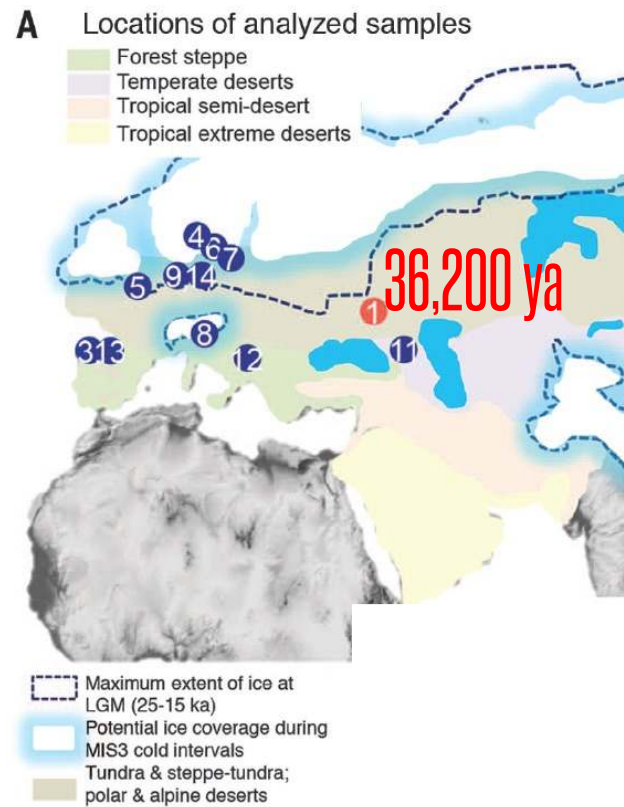
A Locations of analyzed samples



Orlando-Sequin et al. Science 2014

Upper Paleolithic Humans

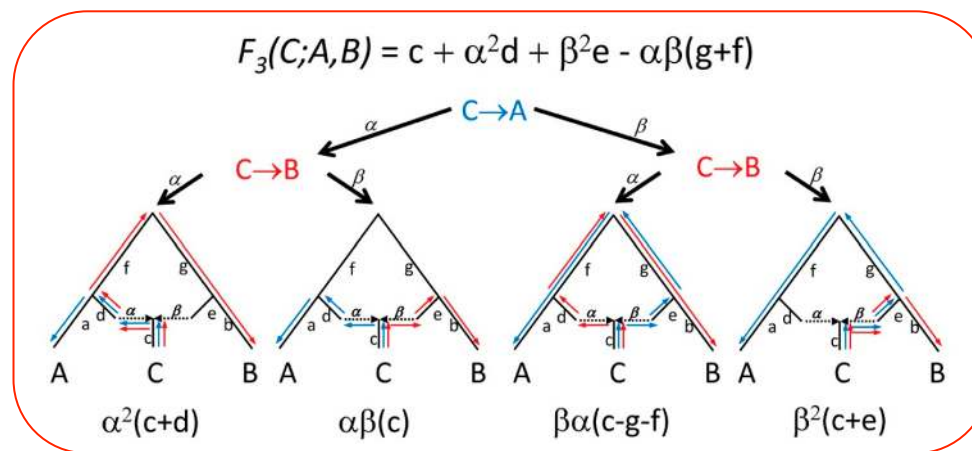
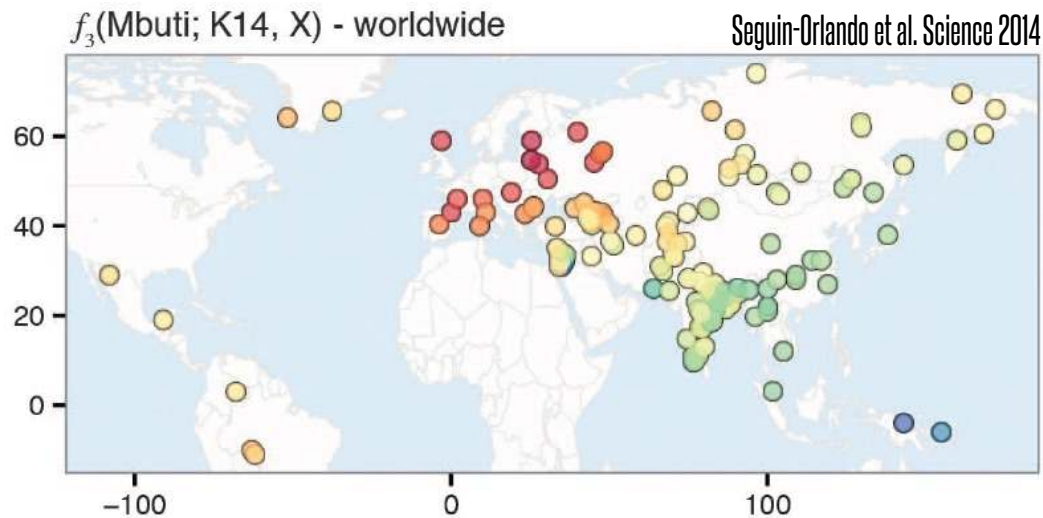
Kostenki K14 (f3- and D-statistics)



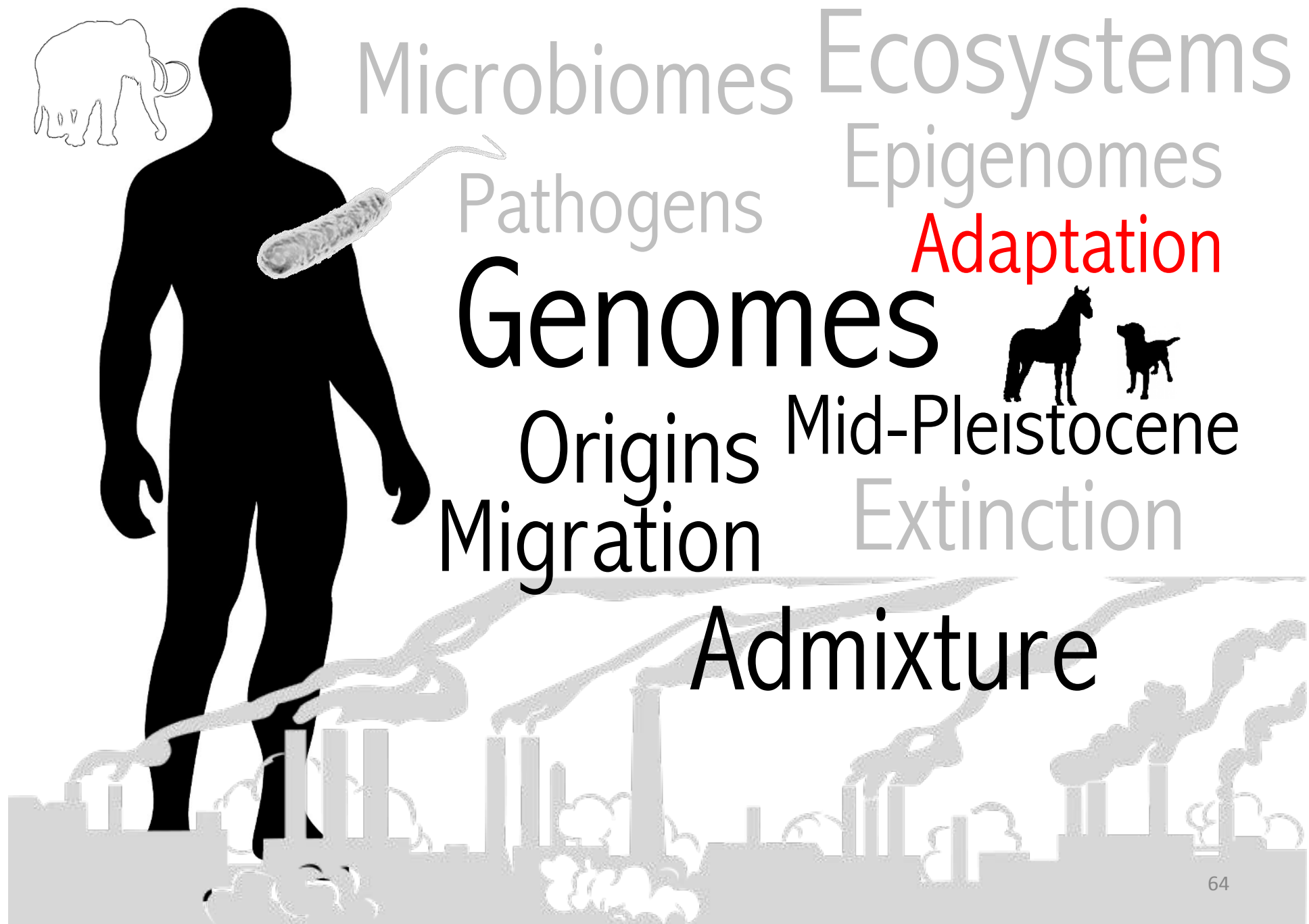
Seguin-Orlando et al. Science 2014

Upper Paleolithic Humans

Kostenki K14 (f₃- and D-statistics)



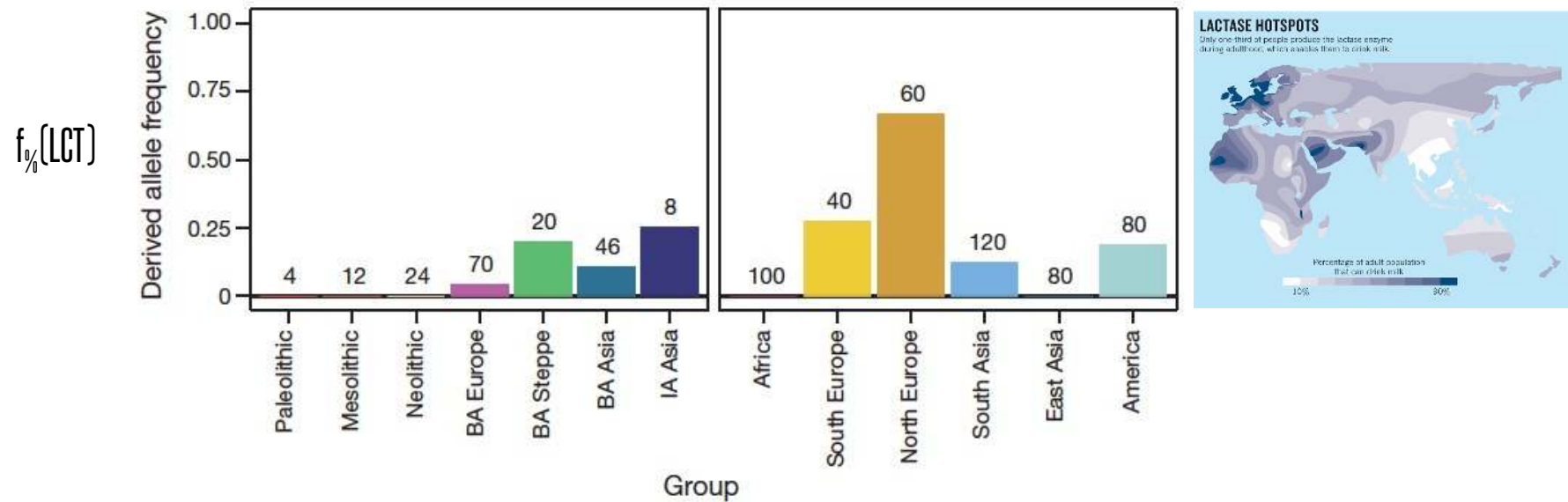
Patterson et al. Genetics 2012



Bronze Age Genomics

>100 Ancient Human Genomes

Lactose Tolerance

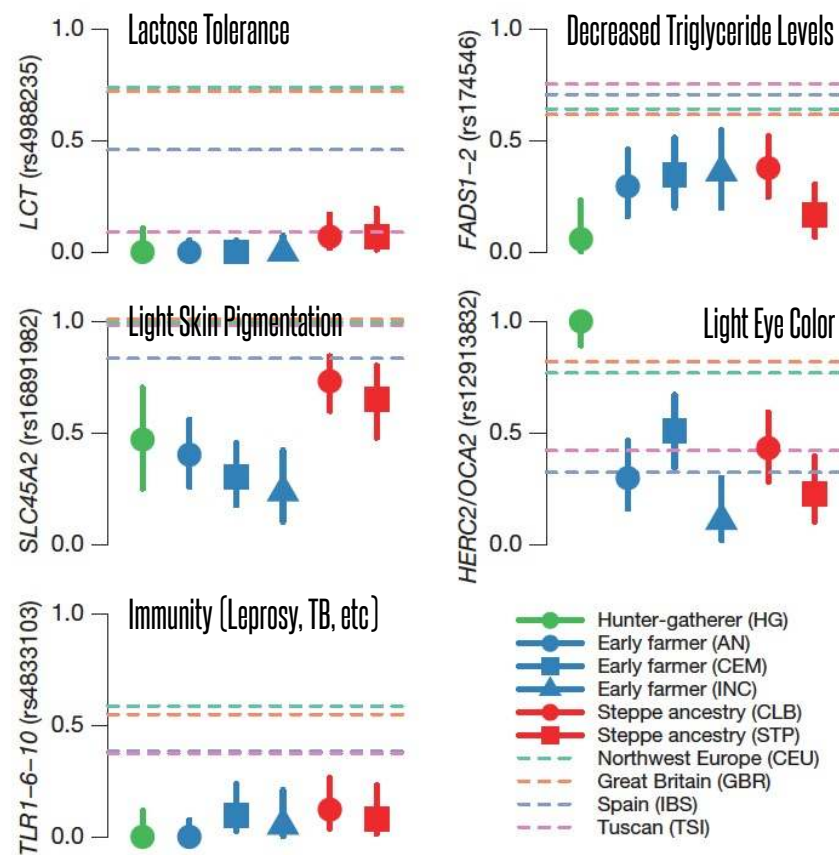


Allentoft et al. Nature 2015

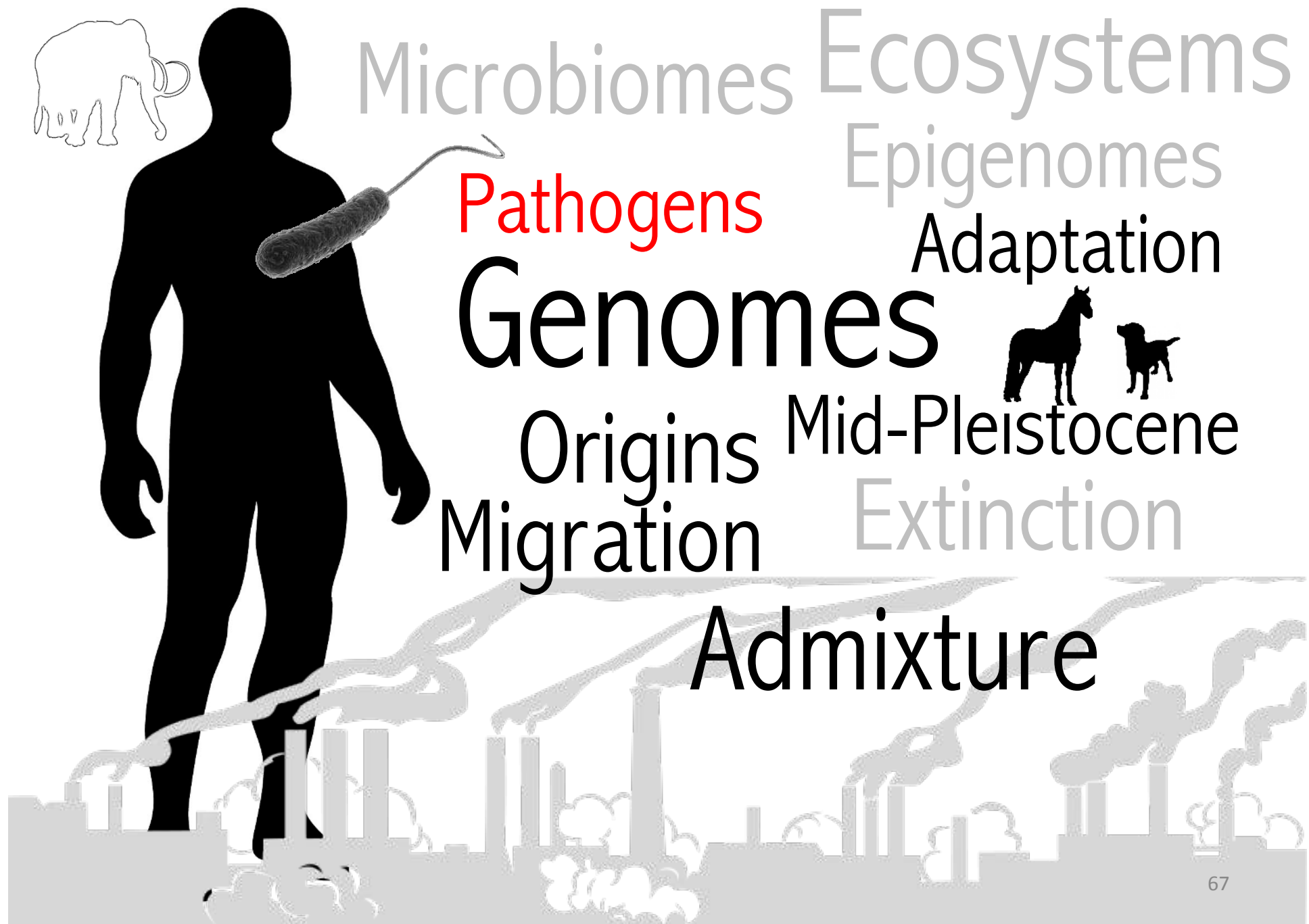
Bronze Age Genomics

>100 Ancient Human Genomes

Selection Patterns

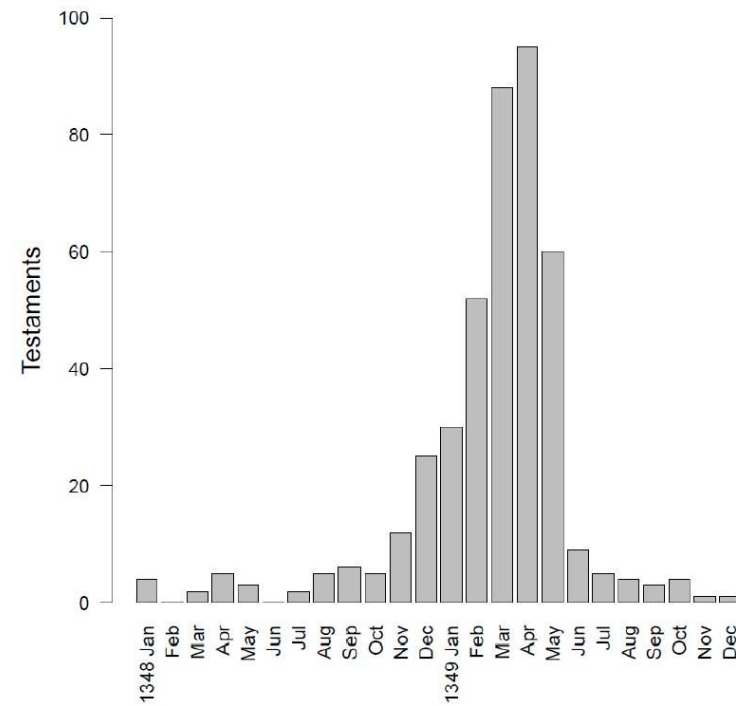


Mathieson et al. Nature 2015



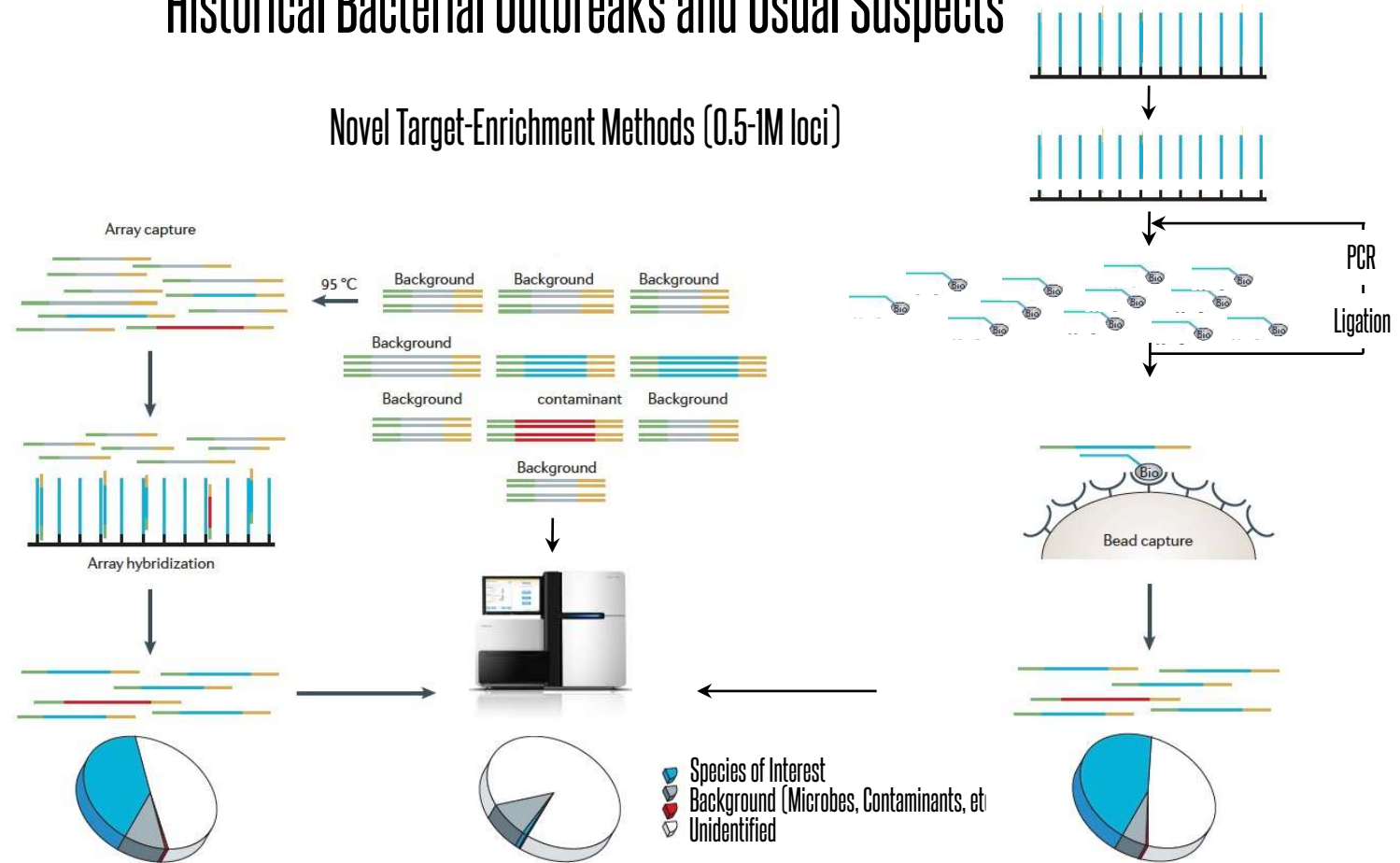
Beyond Genomes, Microbiomes

Historical Bacterial Outbreaks and Usual Suspects



Beyond Genomes, Microbiomes

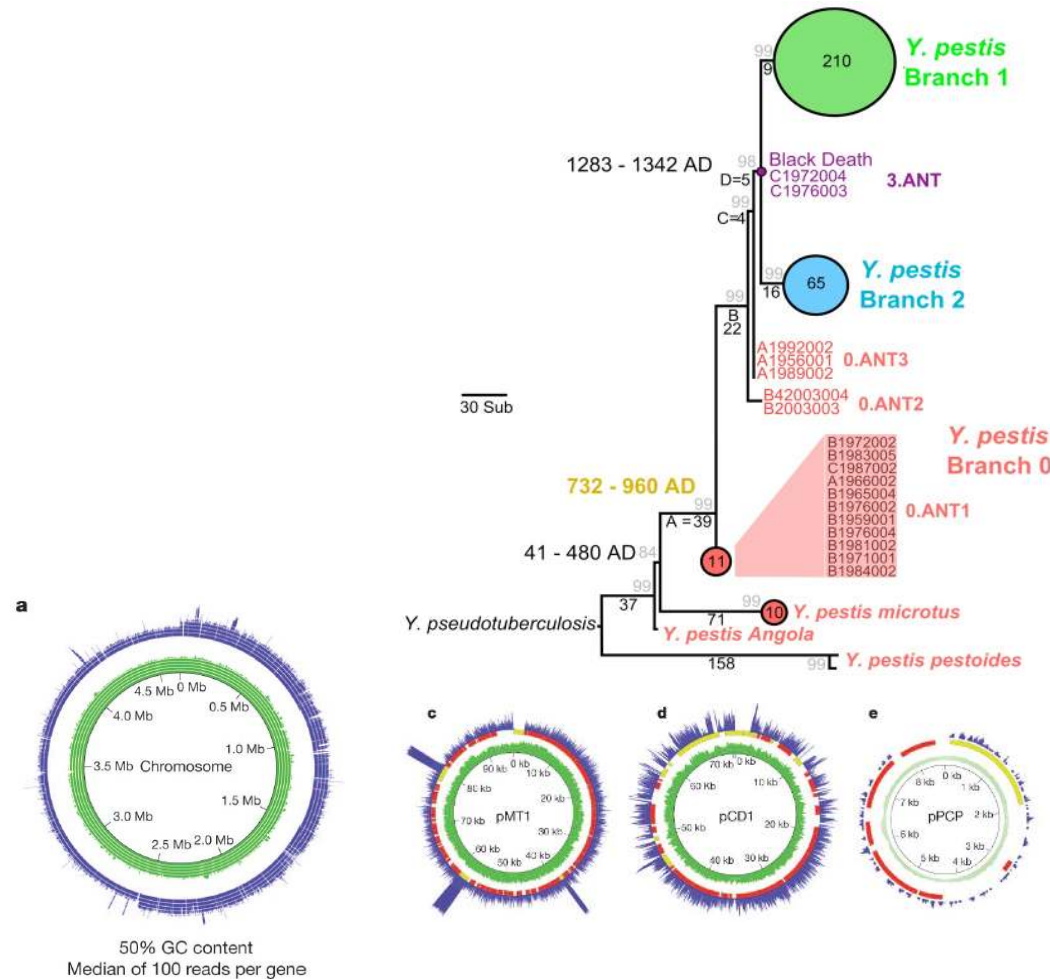
Historical Bacterial Outbreaks and Usual Suspects



Modified from Krause & Stoneking Nat Rev Genet 2011, Fu et al. PNAS 2013

Beyond Genomes, Microbiomes

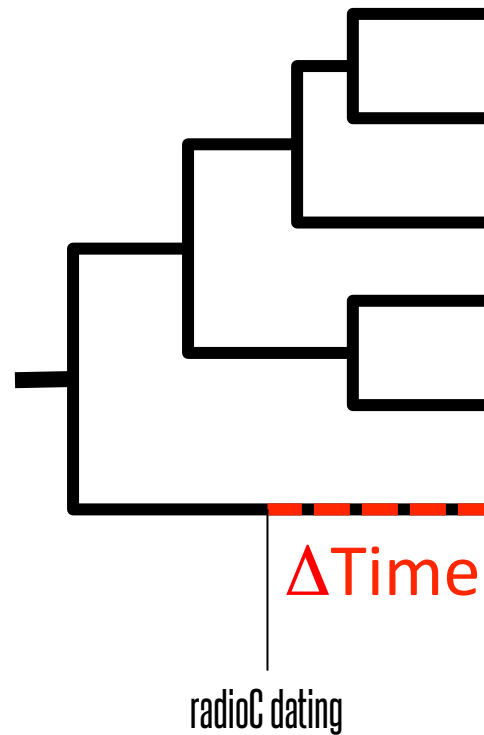
Historical Bacterial Outbreaks and Usual Suspects



Bos et al. PNAS 2011, Nature 2011, PLoS One 2012

Beyond Genomes, Microbiomes

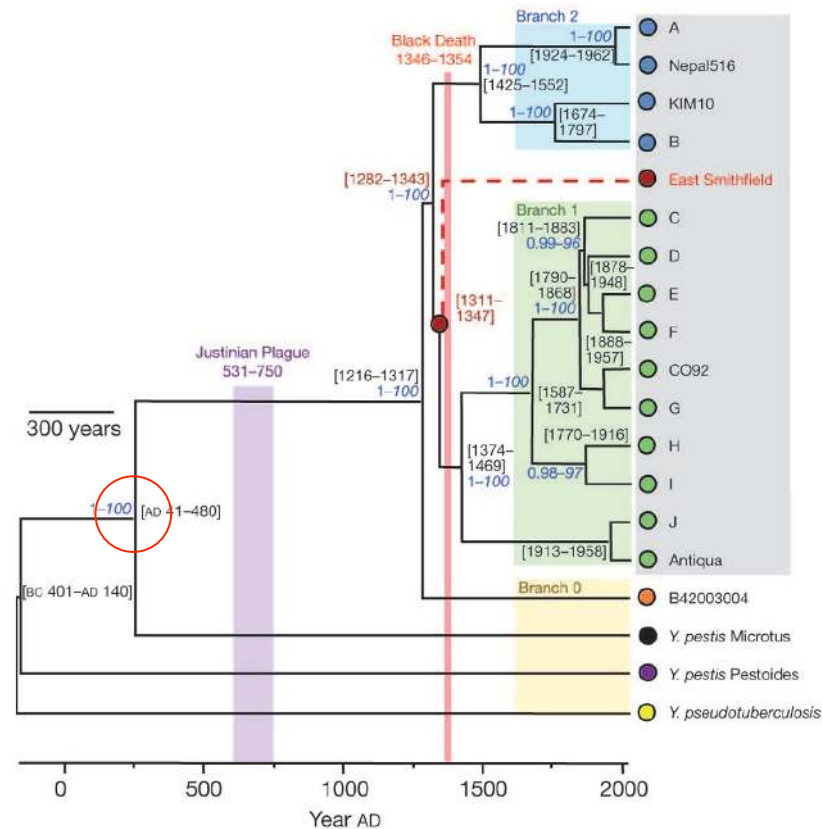
Historical Bacterial Outbreaks and Usual Suspects



Branch-Shortening based Calibration of Molecular Clocks

Beyond Genomes, Microbiomes

Historical Bacterial Outbreaks and Usual Suspects

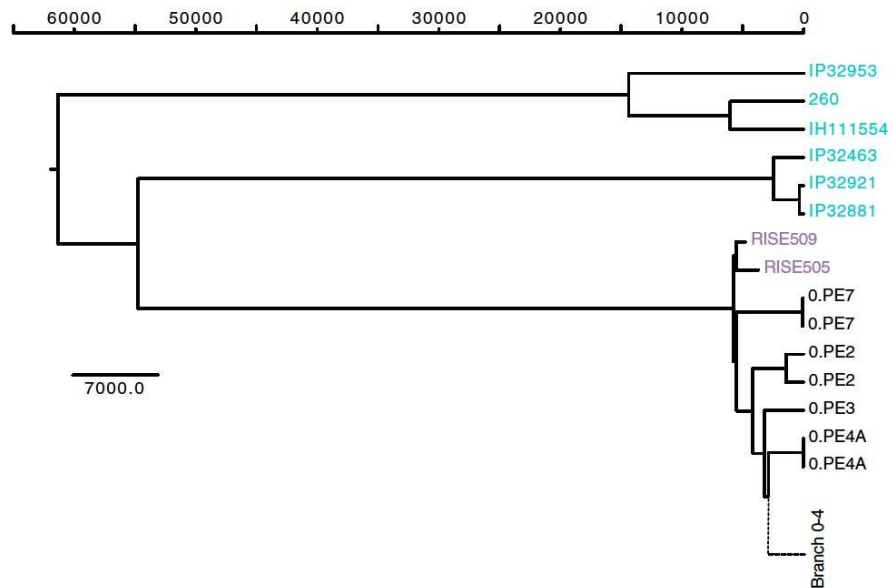


Branch-Shortening based Calibration of Molecular Clocks

Beyond Genomes, Microbiomes

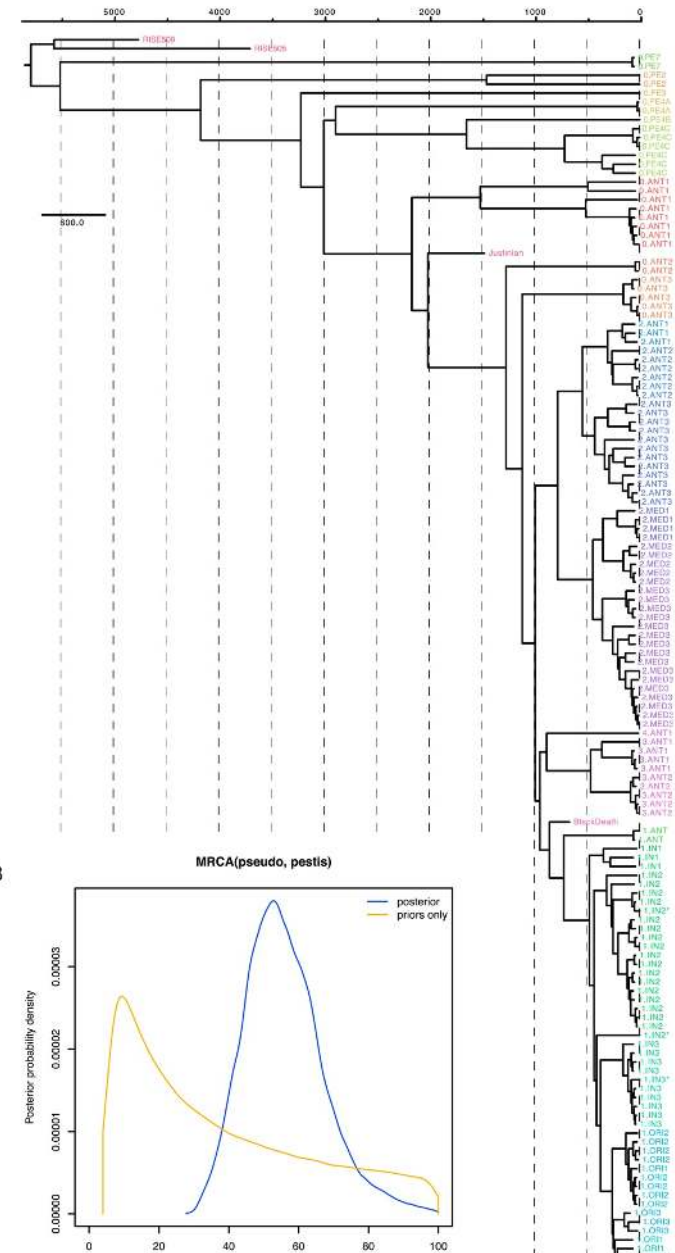
Historical Bacterial Outbreaks and Usual Suspects

Phylogenetic Reconstruction and Tip-Dating



Sample	Country	Site	Culture	Date (cal BC)	CO92	pMT1	pPCP1	pCD1
RISE00	Estonia	Sope	Corded Ware	2575–2349	0.39	0.36	1.40	0.66
RISE139	Poland	Chociwel	Unetice	2135–1923	0.14	0.24	0.76	0.28
RISE386	Russia	Bulanovo	Sintashta	2280–2047	0.82	0.96	1.12	1.60
RISE397	Armenia	Kapan	EIA	1048–885	0.25	0.40	6.88	0.50
RISE505	Russia	Kytmanovo	Andronovo	1746–1626	8.73	9.15	34.09	17.46
RISE509	Russia	Afanasievo Gora	Afanasievo	2887–2677	29.45	16.96	31.22	50.32
RISE511	Russia	Afanasievo Gora	Afanasievo	2909–2679	0.20	0.24	1.19	0.60

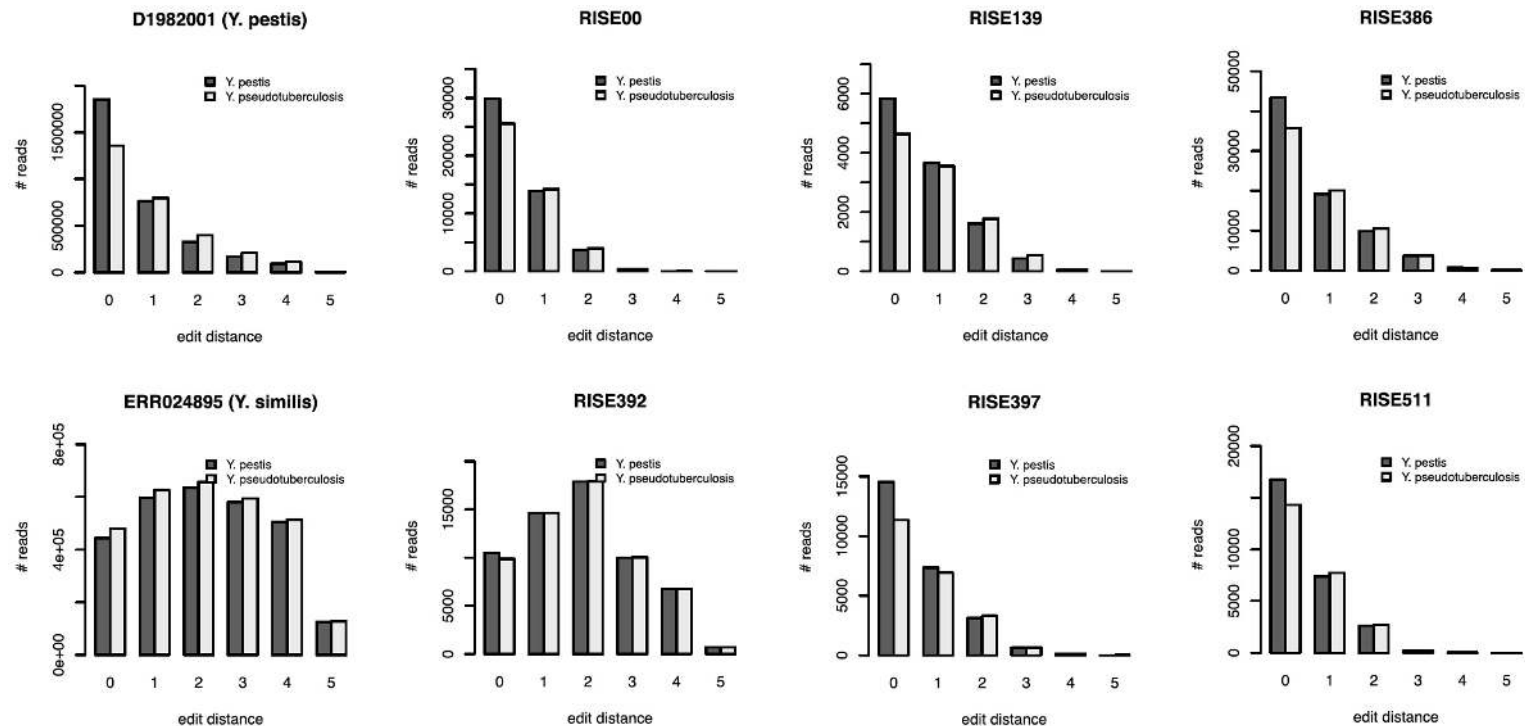
Rasmussen et al. Cell 2015



Beyond Genomes, Microbiomes

Historical Bacterial Outbreaks and Usual Suspects

Edit-Distance Distribution

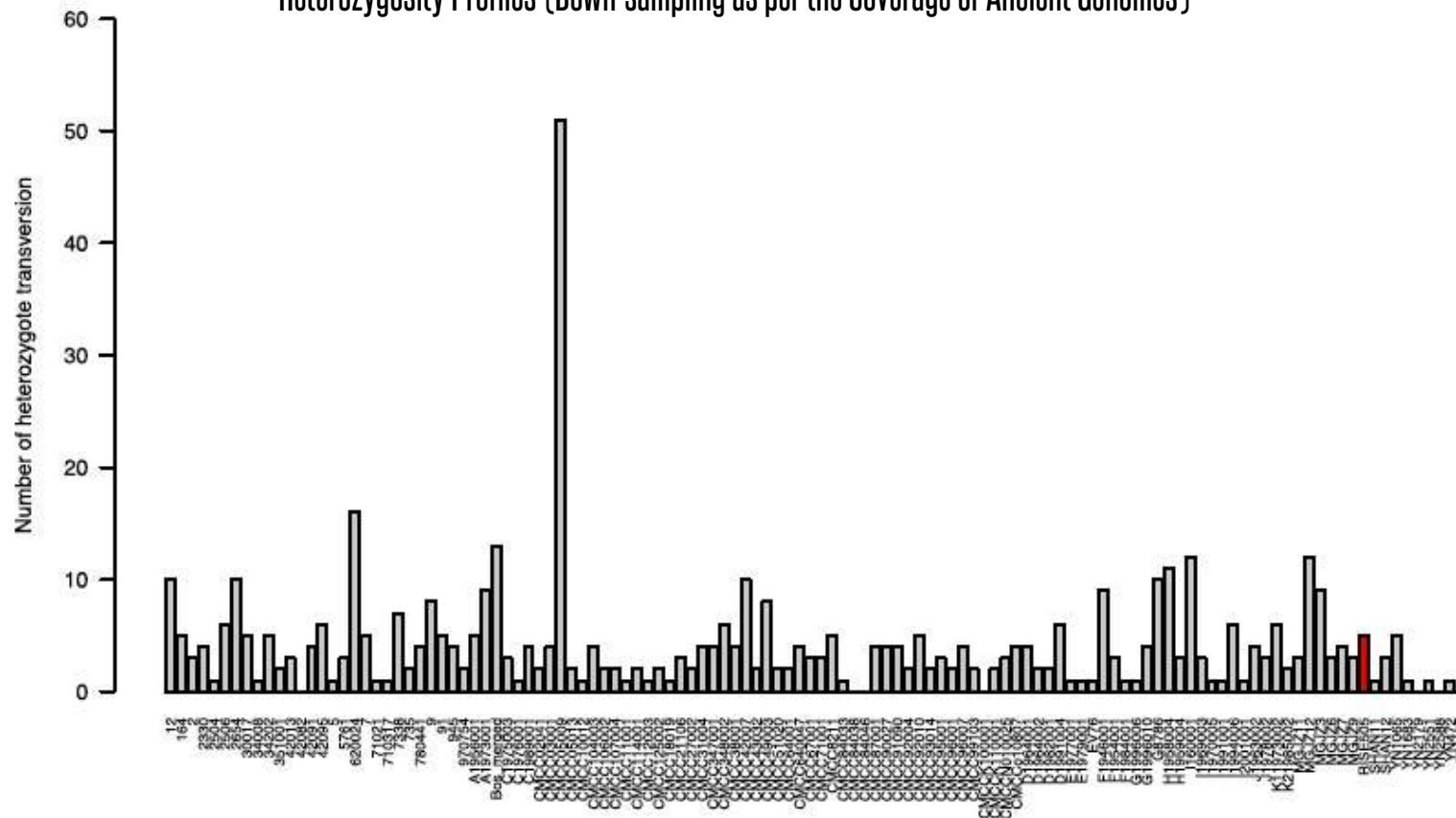


Rasmussen et al. Cell 2015

Beyond Genomes, Microbiomes

Historical Bacterial Outbreaks and Usual Suspects

Heterozygosity Profiles (Down-sampling as per the Coverage of Ancient Genomes)

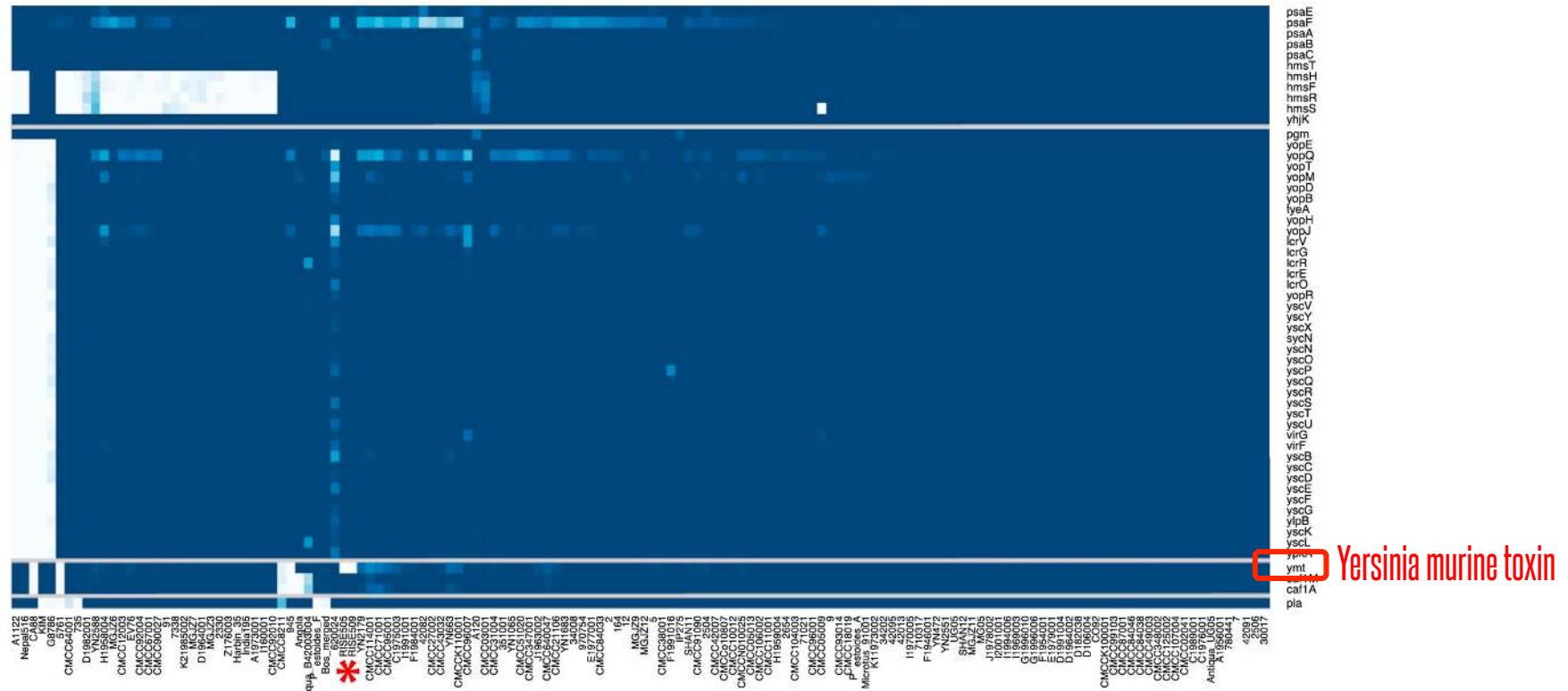


Rasmussen et al. Cell 2015

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Historical Bacterial Outbreaks and Usual Suspects

Average coverage at 55 Virulence Genes

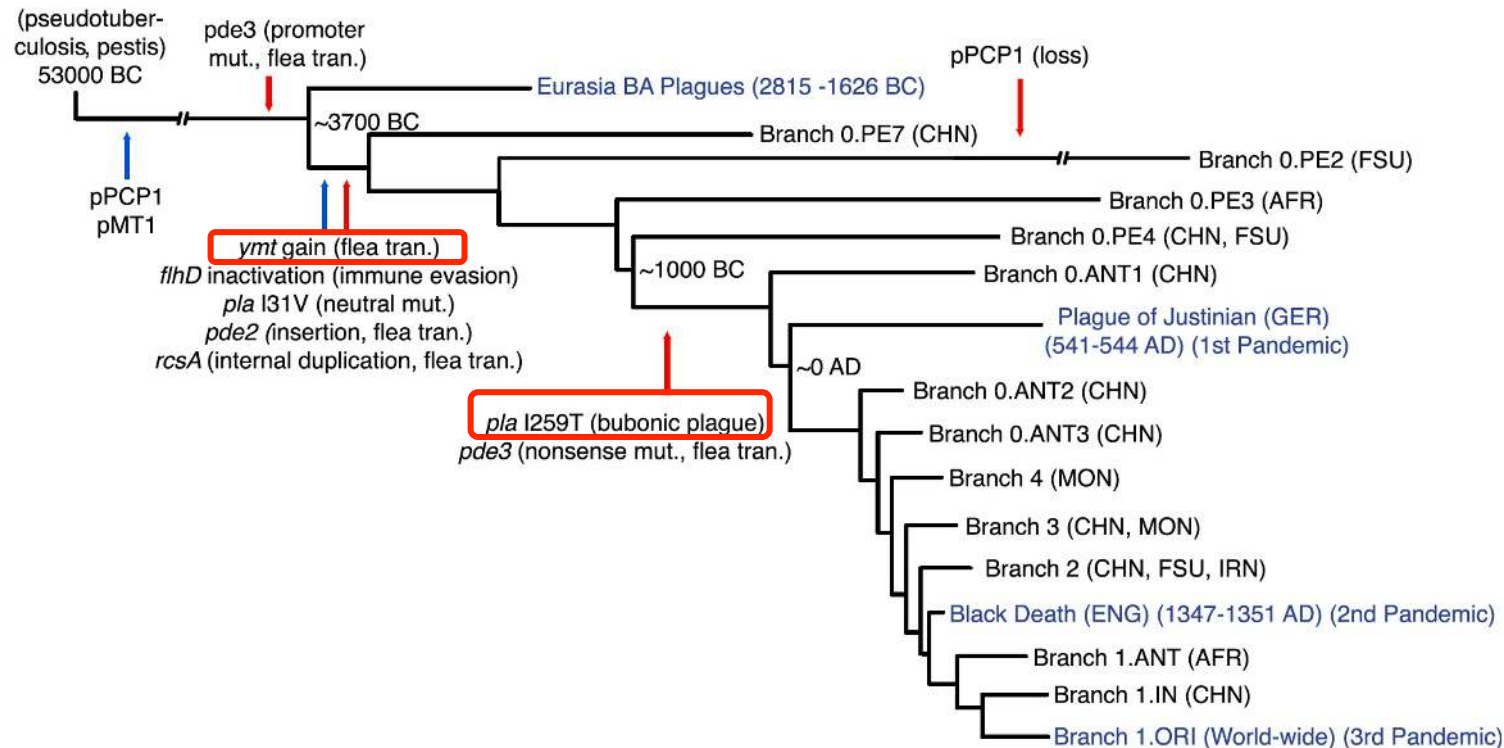


Rasmussen et al. Cell 2015

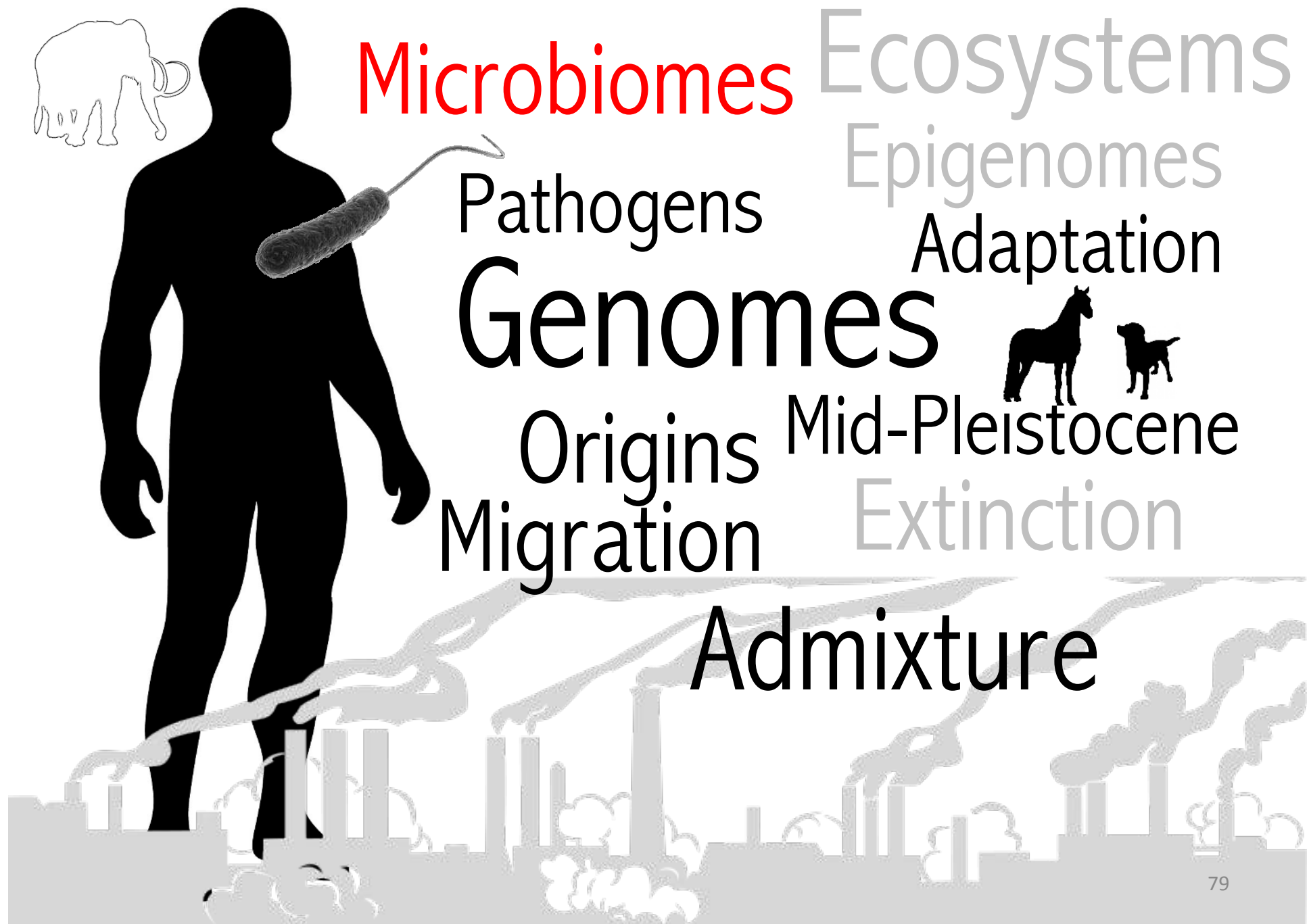
Beyond Genomes, Microbiomes

Historical Bacterial Outbreaks and Usual Suspects

The Genetic Makeup of a Deadly Human Pathogen



Rasmussen et al. Cell 2015



Beyond Genomes, Epigenomes

Ancient Microbiomes



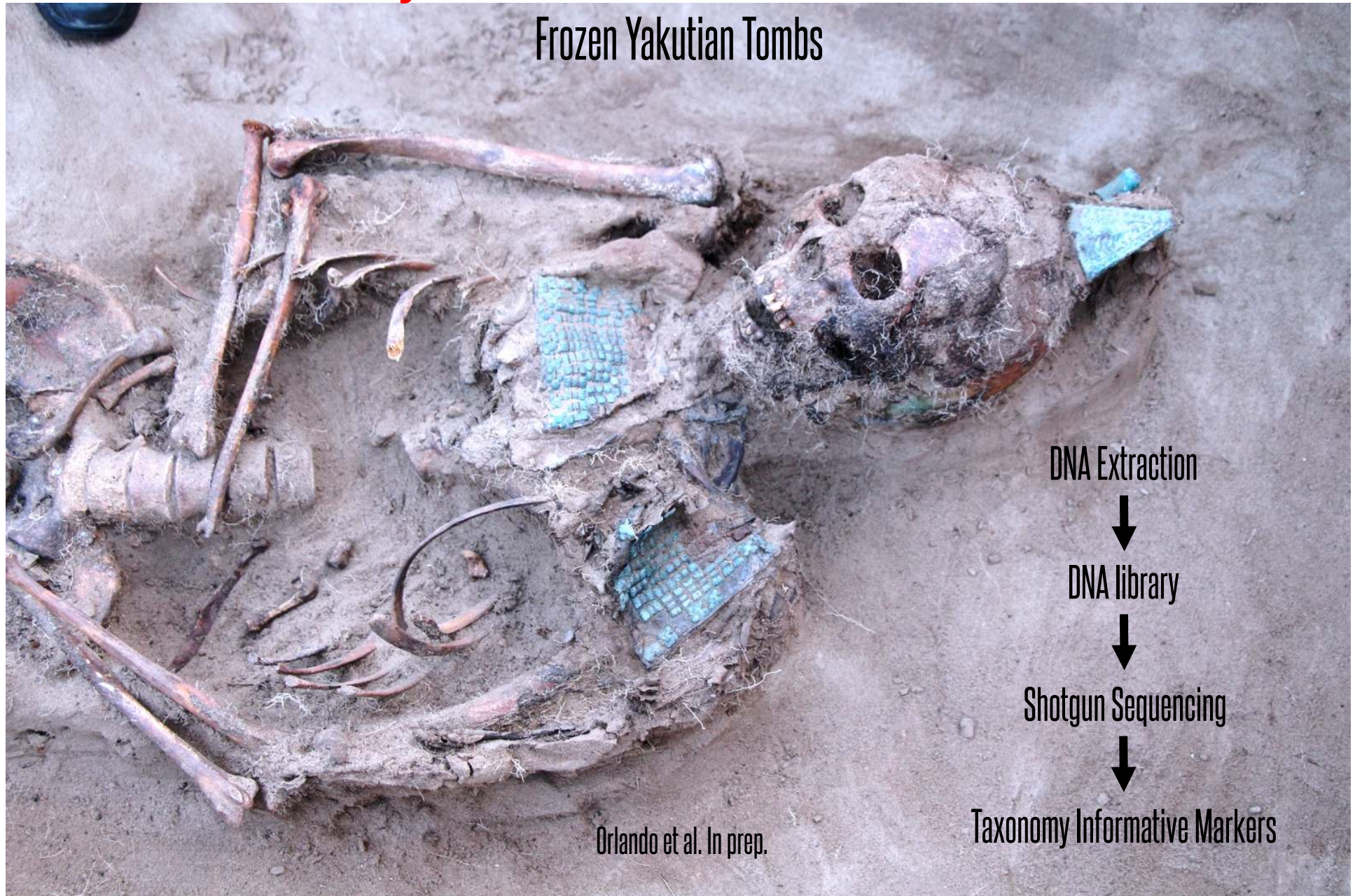
Warriner et al. Nature Genetics 2013

The Dental Plaque Metagenome Is Extremely Rich in Ancient DNA

Past Diet, Diseases, And Behaviour

Beyond Genomes, Microbiomes

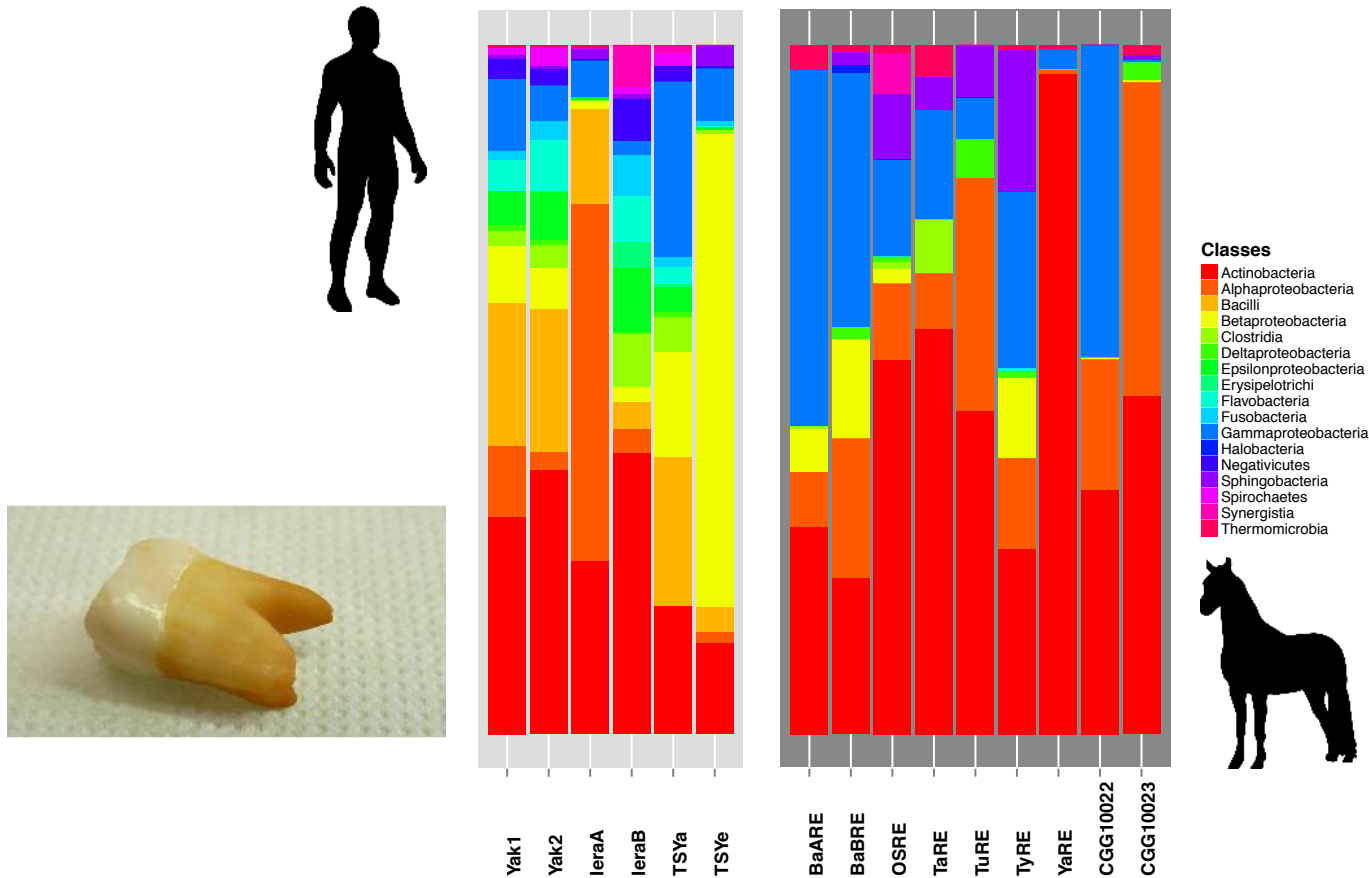
Frozen Yakutian Tombs



Orlando et al. In prep.

Beyond Genomes, Microbiomes

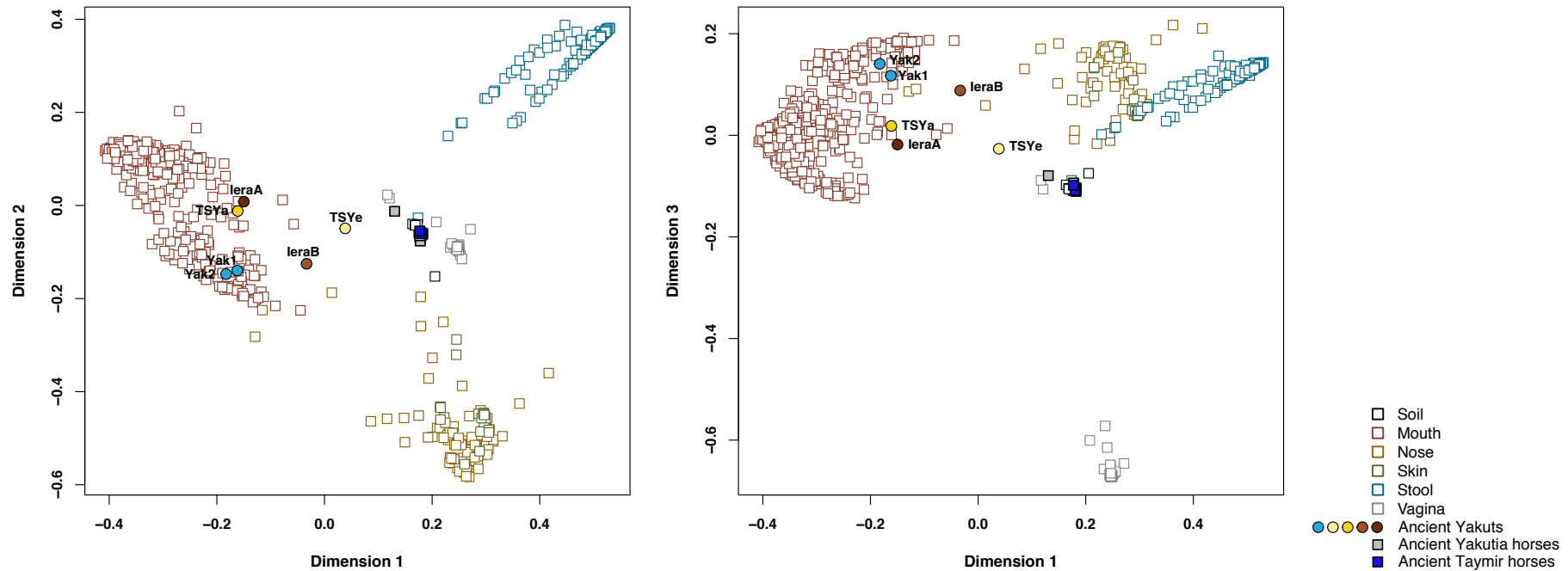
Frozen Yakutian Tombs



Orlando et al. In prep.

Beyond Genomes, Microbiomes

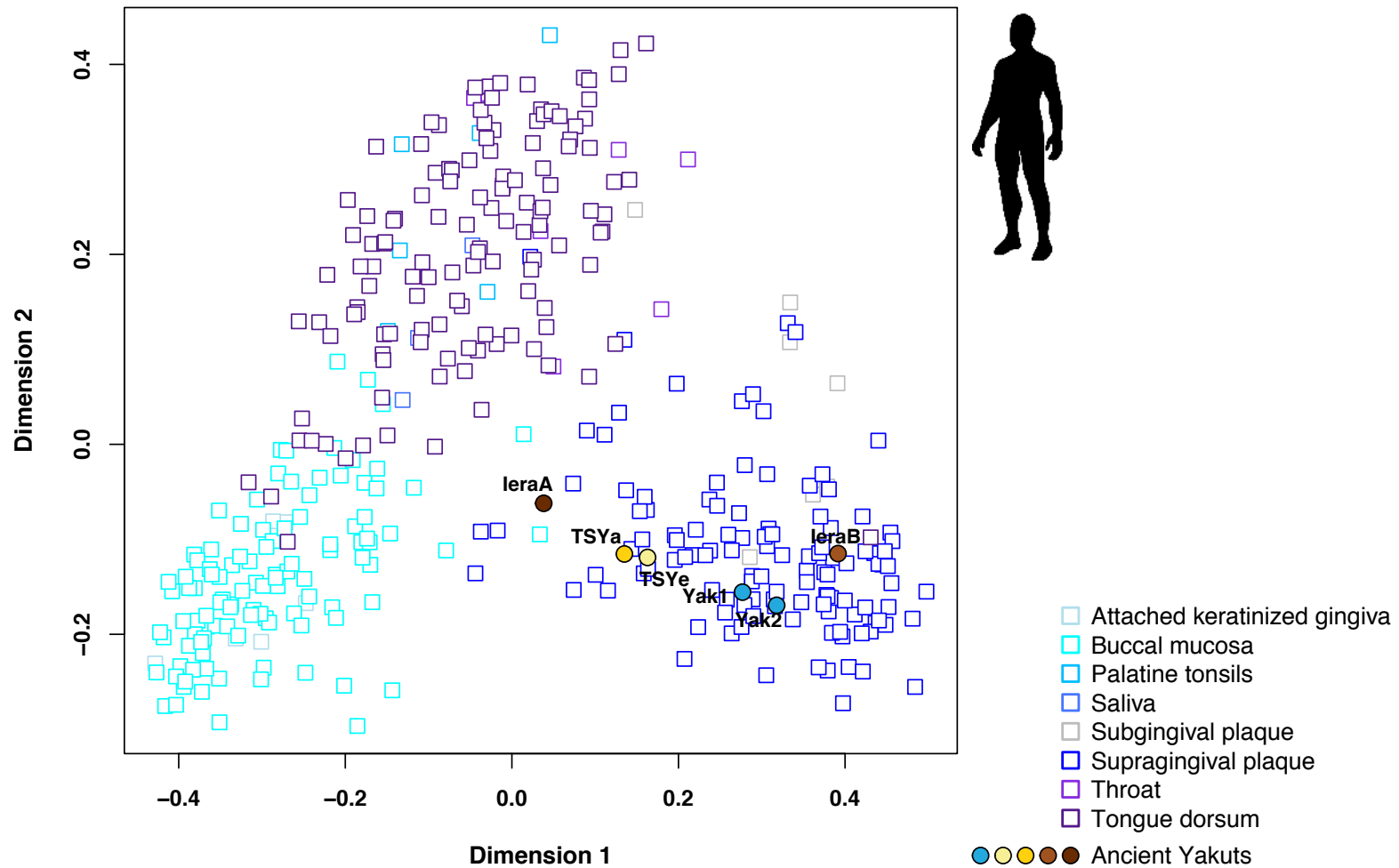
Frozen Yakutian Tombs



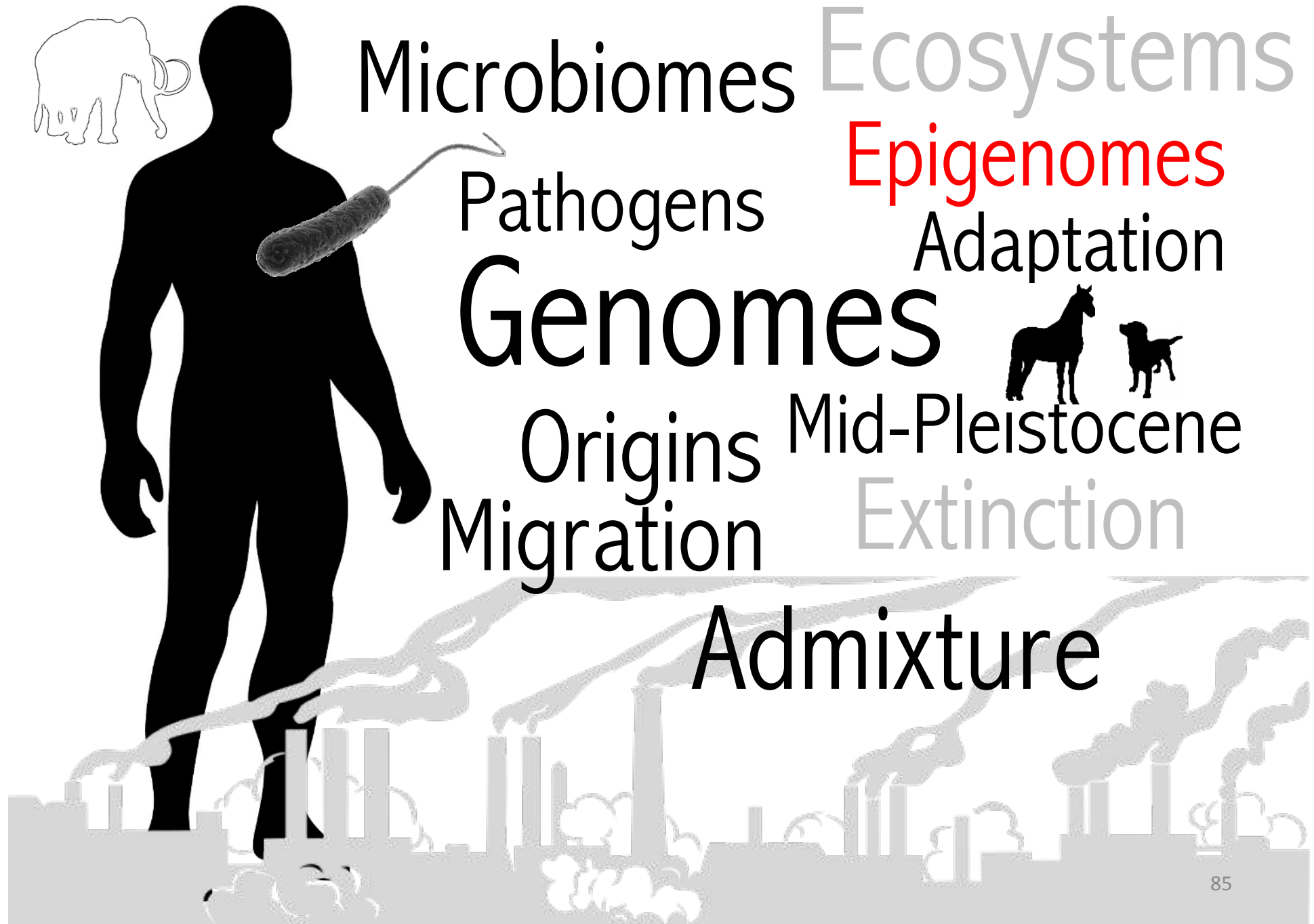
Orlando et al. In prep.

Beyond Genomes, Microbiomes

Frozen Yakutian Tombs



Orlando et al. In prep.



Microbiomes

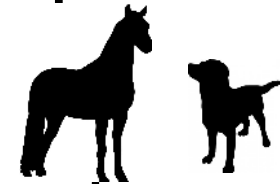
Ecosystems

Epigenomes

Pathogens

Adaptation

Genomes



Origins

Mid-Pleistocene

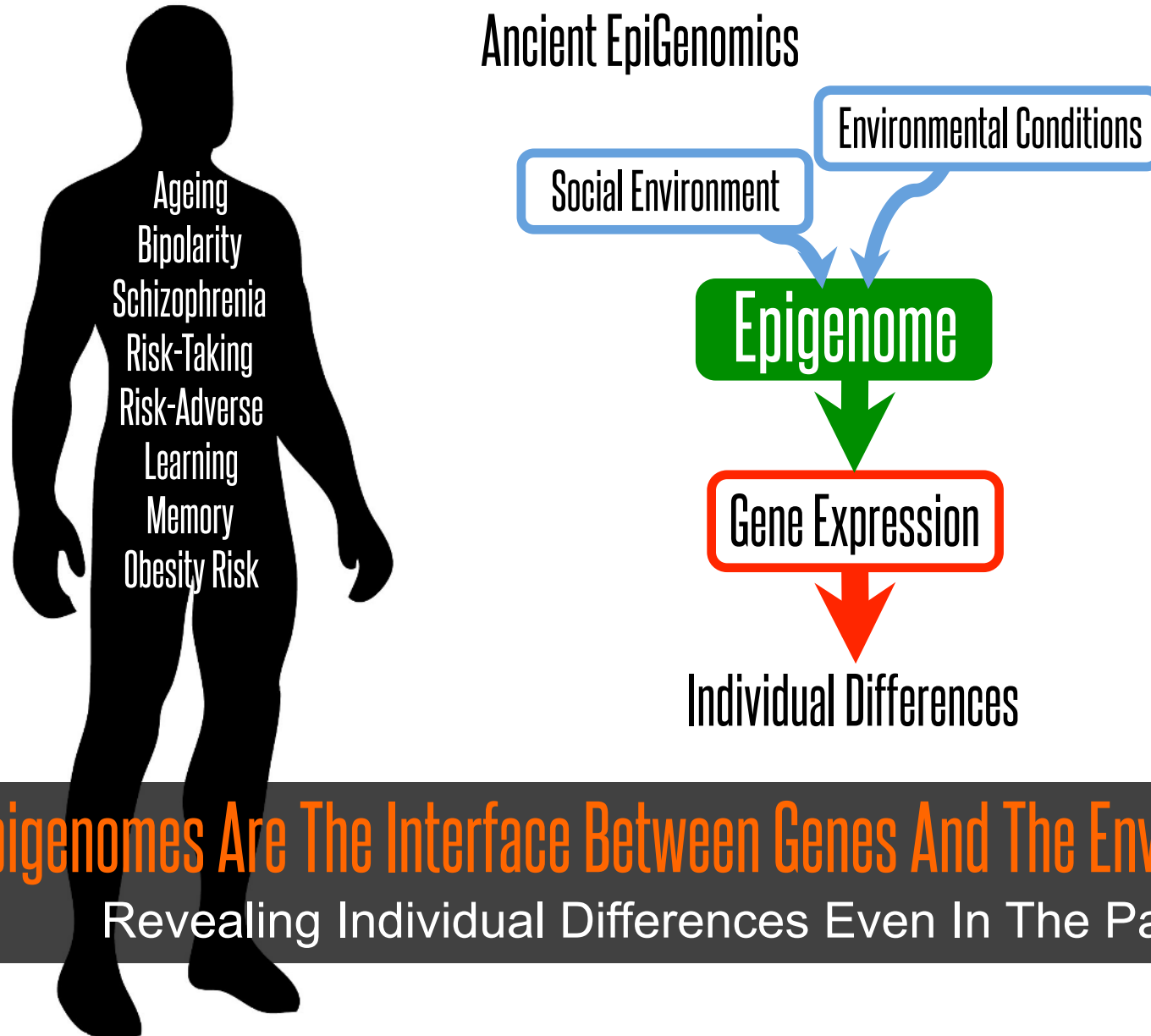
Migration

Extinction

Admixture

Beyond Genomes, Epigenomes

Ancient EpiGenomics

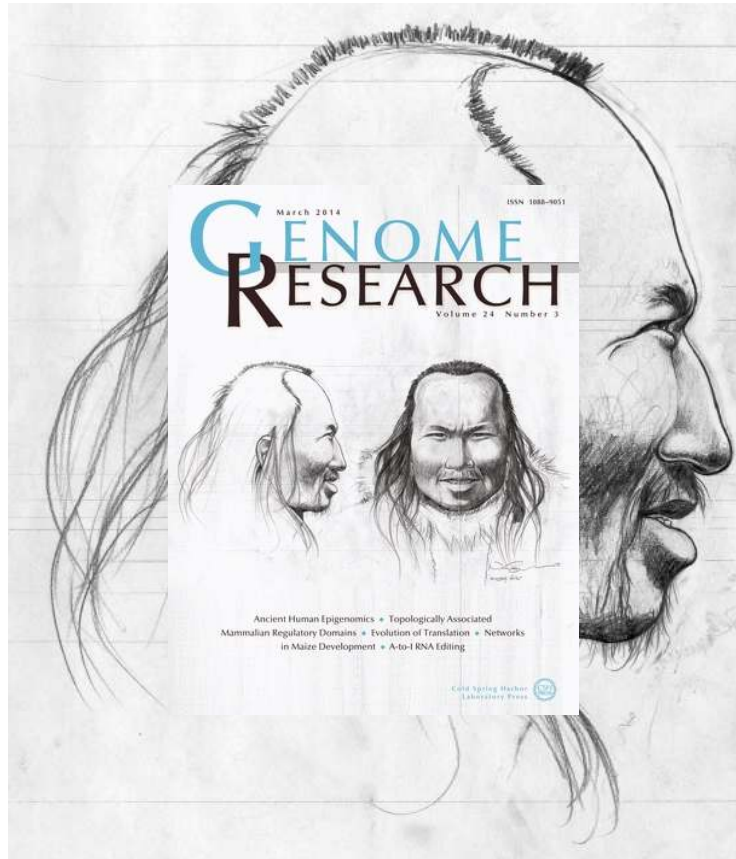


Epigenomes Are The Interface Between Genes And The Environment

Revealing Individual Differences Even In The Past

Beyond Genomes, Epigenomes

Ancient EpiGenomics



EpiGenome

Pedersen et al. Genome Res 2014

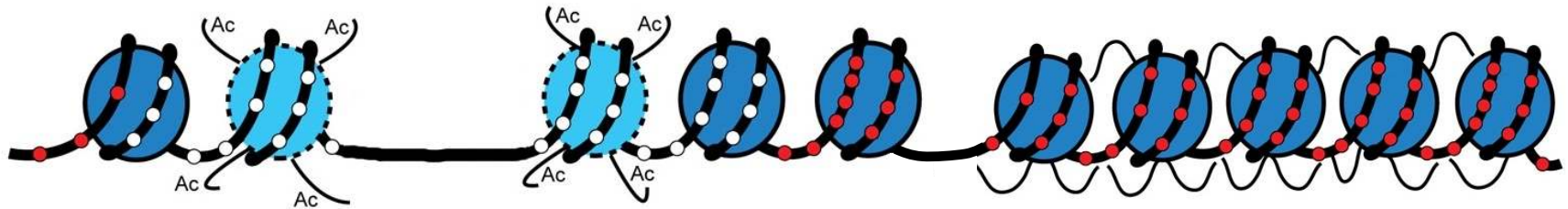


Genome

Rasmussen et al. Nature 2010

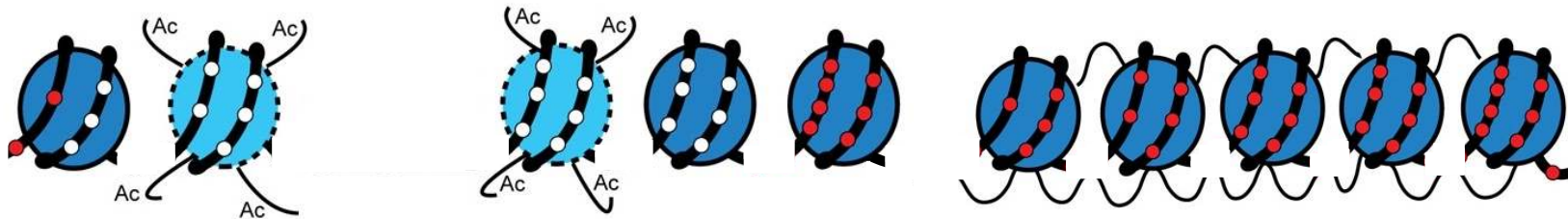
The First Ancient Epigenome

Rationale: Nucleosome Protection After Death



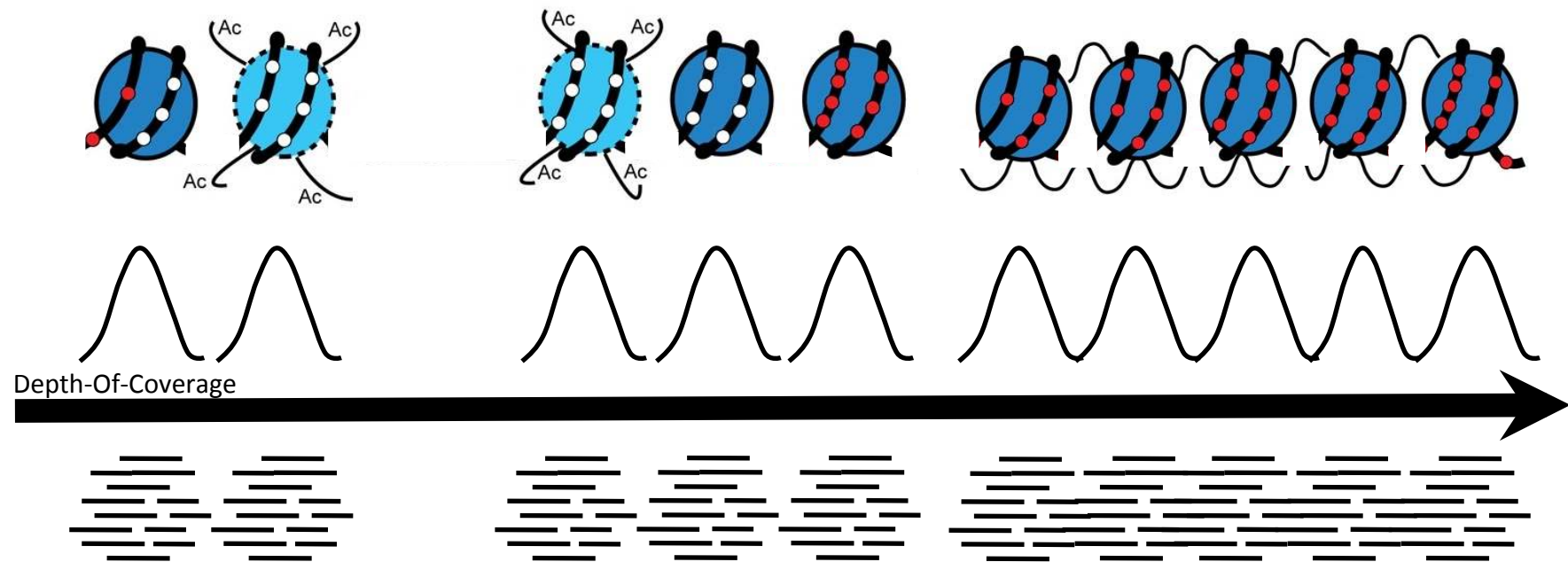
The First Ancient Epigenome

Rationale: Nucleosome Protection After Death



The First Ancient Epigenome

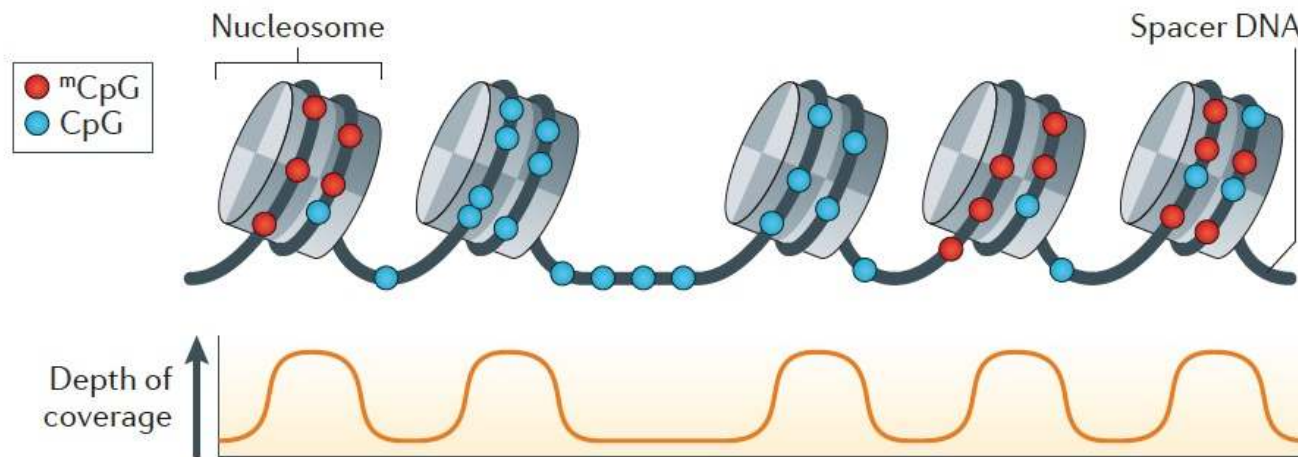
Rationale: Nucleosome Protection After Death



The First Ancient Epigenome

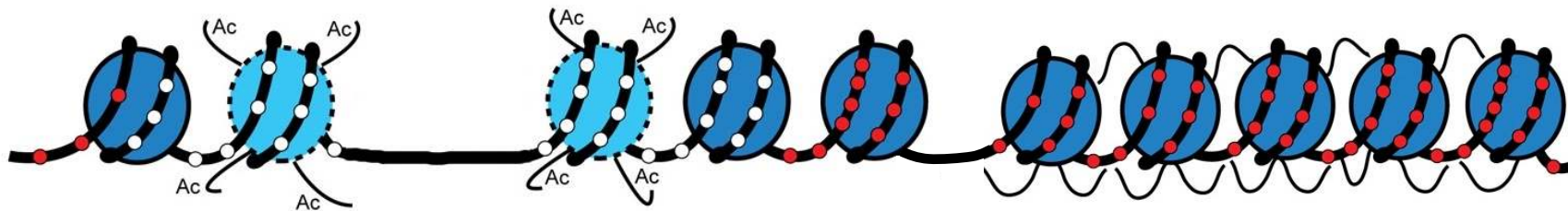
Prediction Summary

Genome-Wide Nucleosome and Methylation Maps



The First Ancient Epigenome

Rationale: Cytosine Deamination After Death

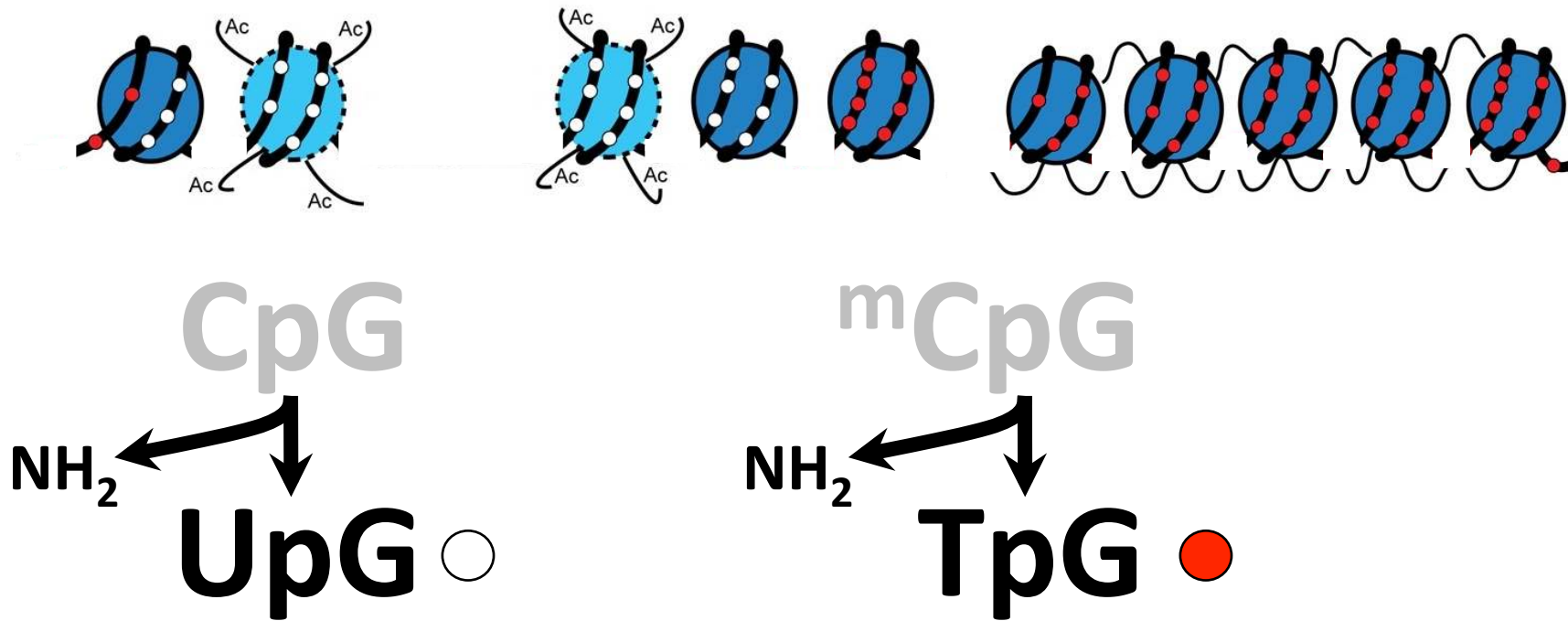


CpG ○

^mCpG ●

The First Ancient Epigenome

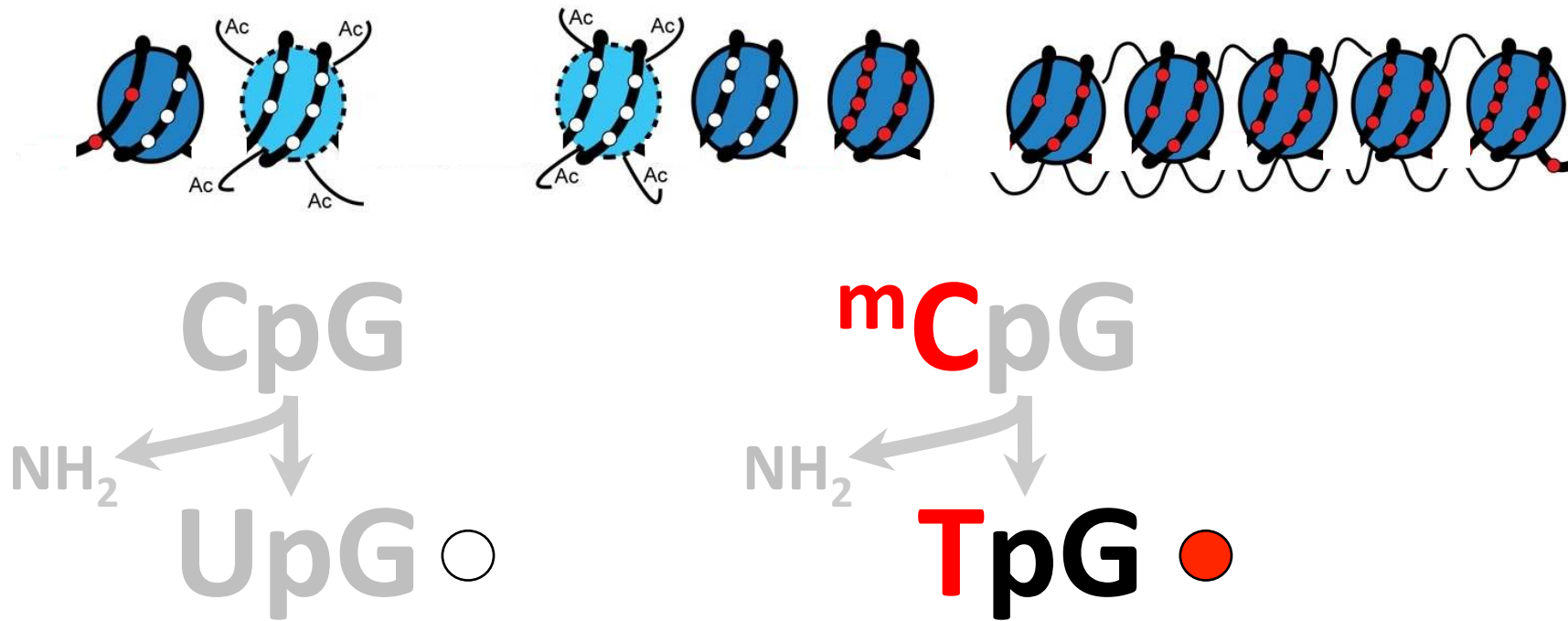
Rationale: Cytosine Deamination After Death



The First Ancient Epigenome

Cytosine Methylation and Gene Expression

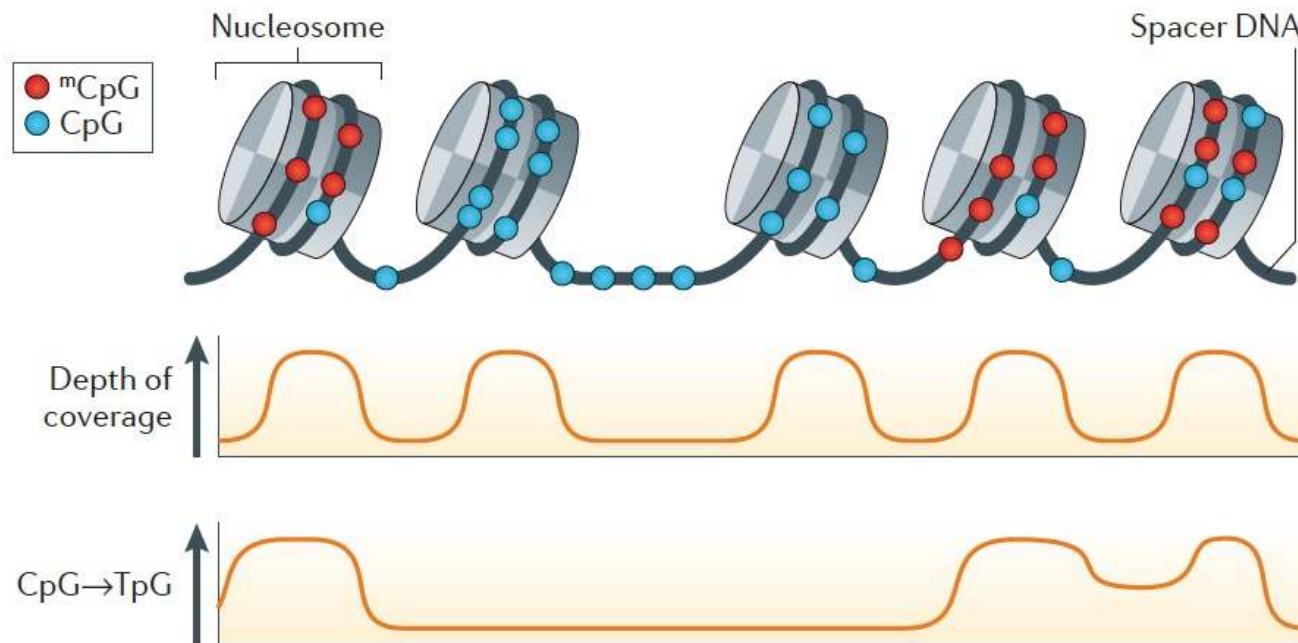
Post-Mortem Cytosine Deamination



The First Ancient Epigenome

Prediction Summary

Genome-Wide Nucleosome and Methylation Maps



The First Ancient Epigenome

Cytosine Methylation and Gene Expression

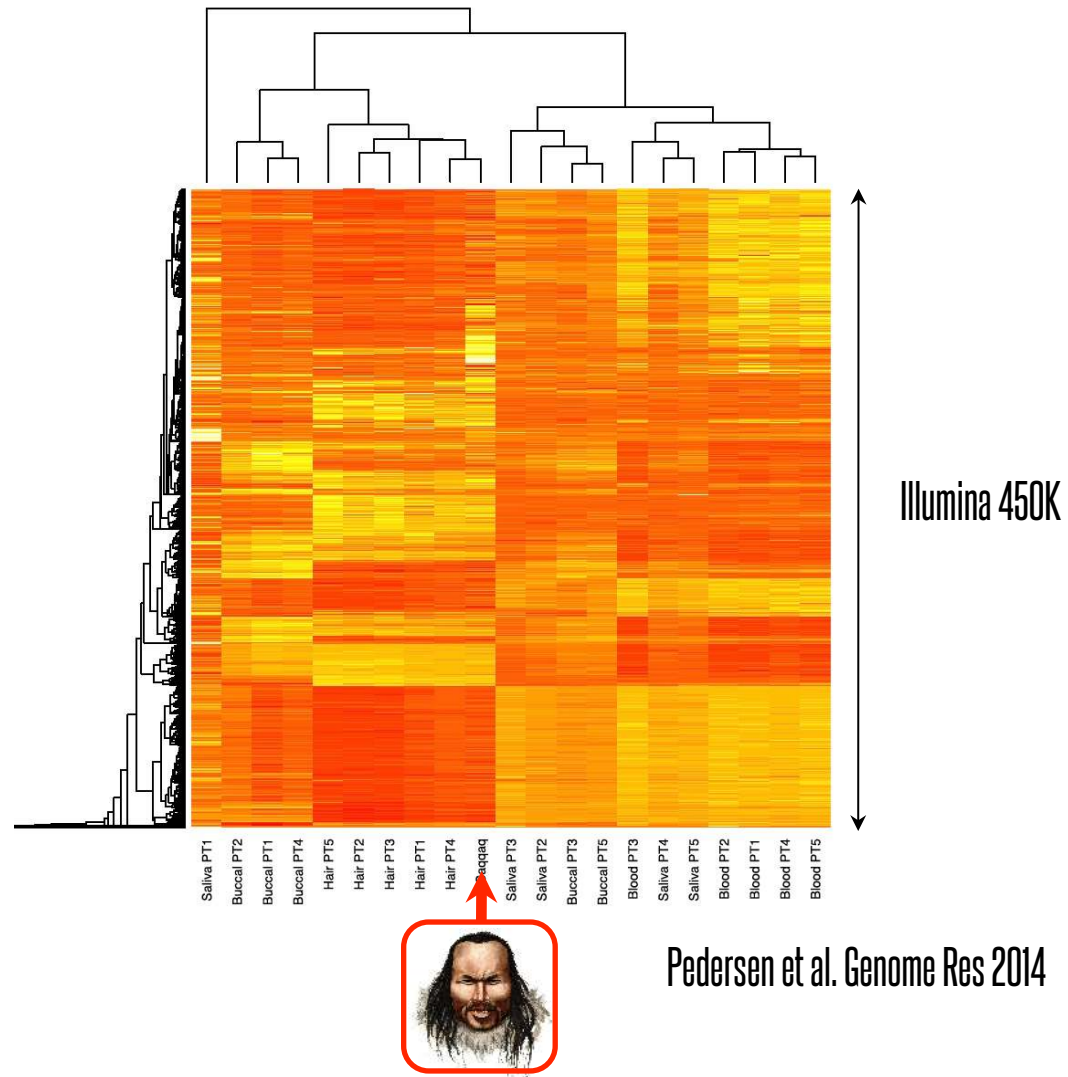
Post-Mortem Cytosine Deamination



From Briggs et al. Nuc Acids Res 2010

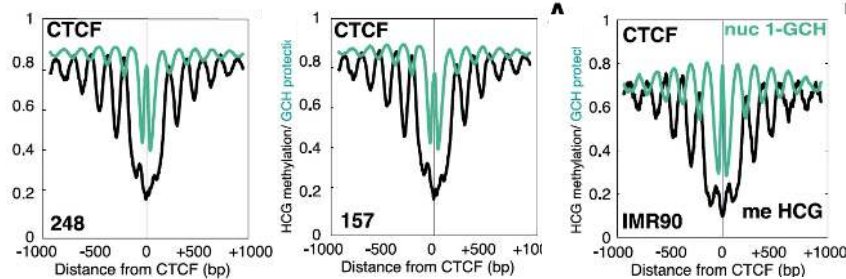
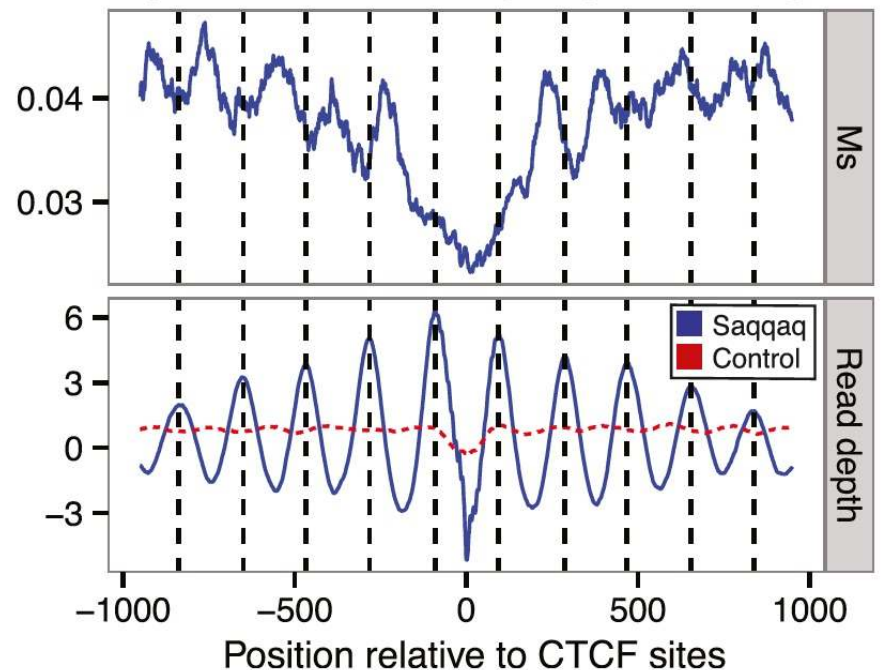
Ancient EpiGenomics

The First Genome-Wide Methylation Map



Ancient EpiGenomics

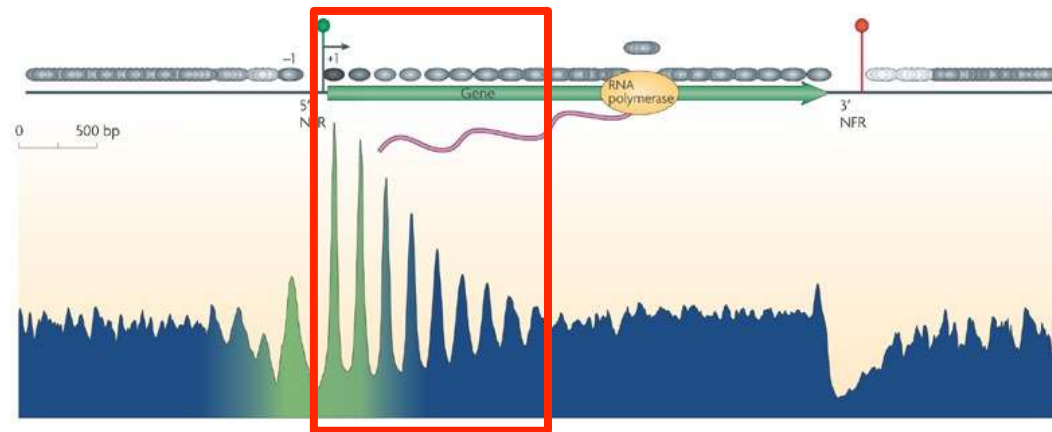
Confronting Nucleosome & Methylation Maps



Pedersen et al. Genome Res 2014

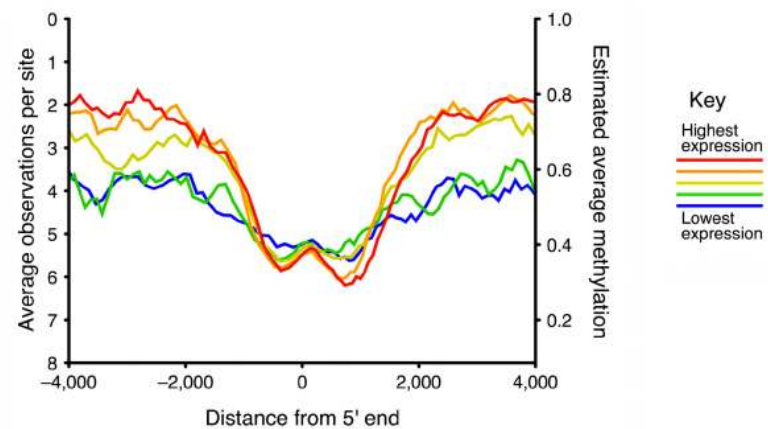
Ancient EpiGenomics

Predicting Ancient Gene Expression Levels



From Jiang & Pugh Nature Rev Genetics 2009

Nature Reviews | Genetics

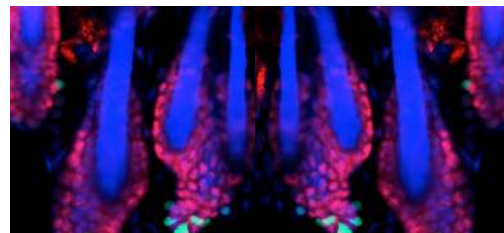
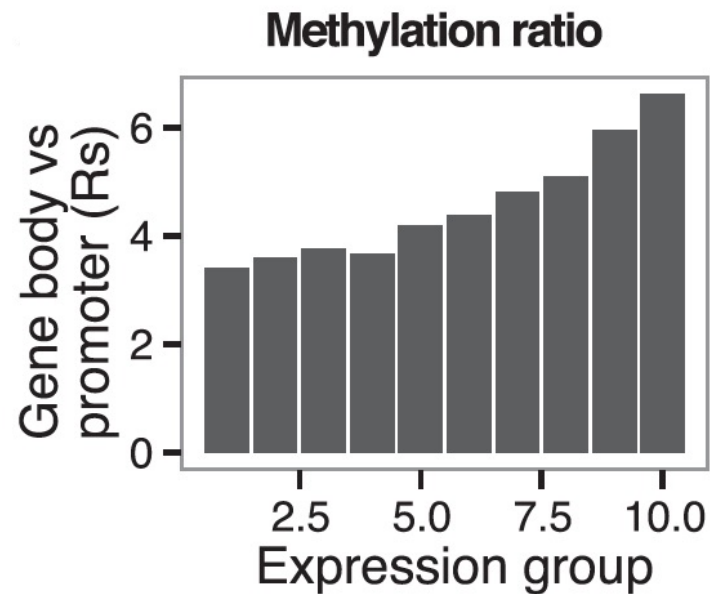


From Ball et al. Nature Biotech 2009

Ancient EpiGenomics

Predicting Ancient Gene Expression Levels (Methylation-based)

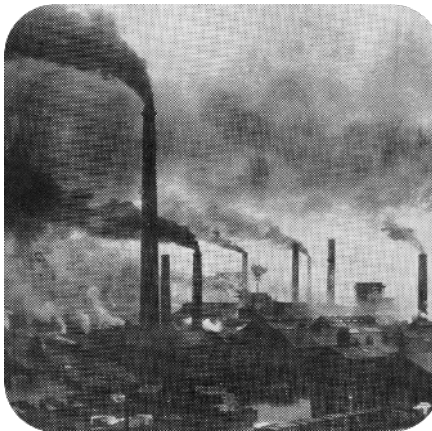
trichohyalin
keratin-71
keratin-85
plakophilin 1
plakophilin 3
desmoplakin
periplakin
plectin
keratin-79



Pedersen et al. Genome Res 2014

Beyond Genomes, Epigenomes

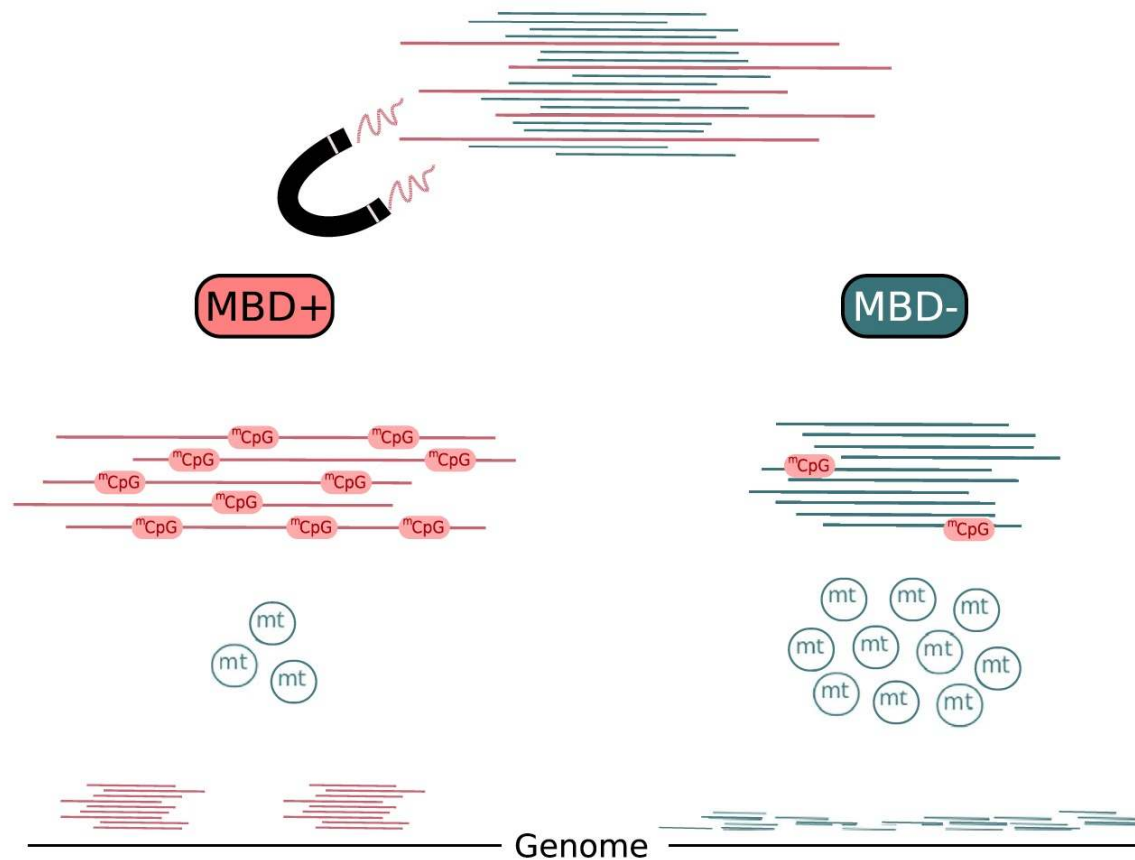
What Ancient EpiGenomics Could Reveal



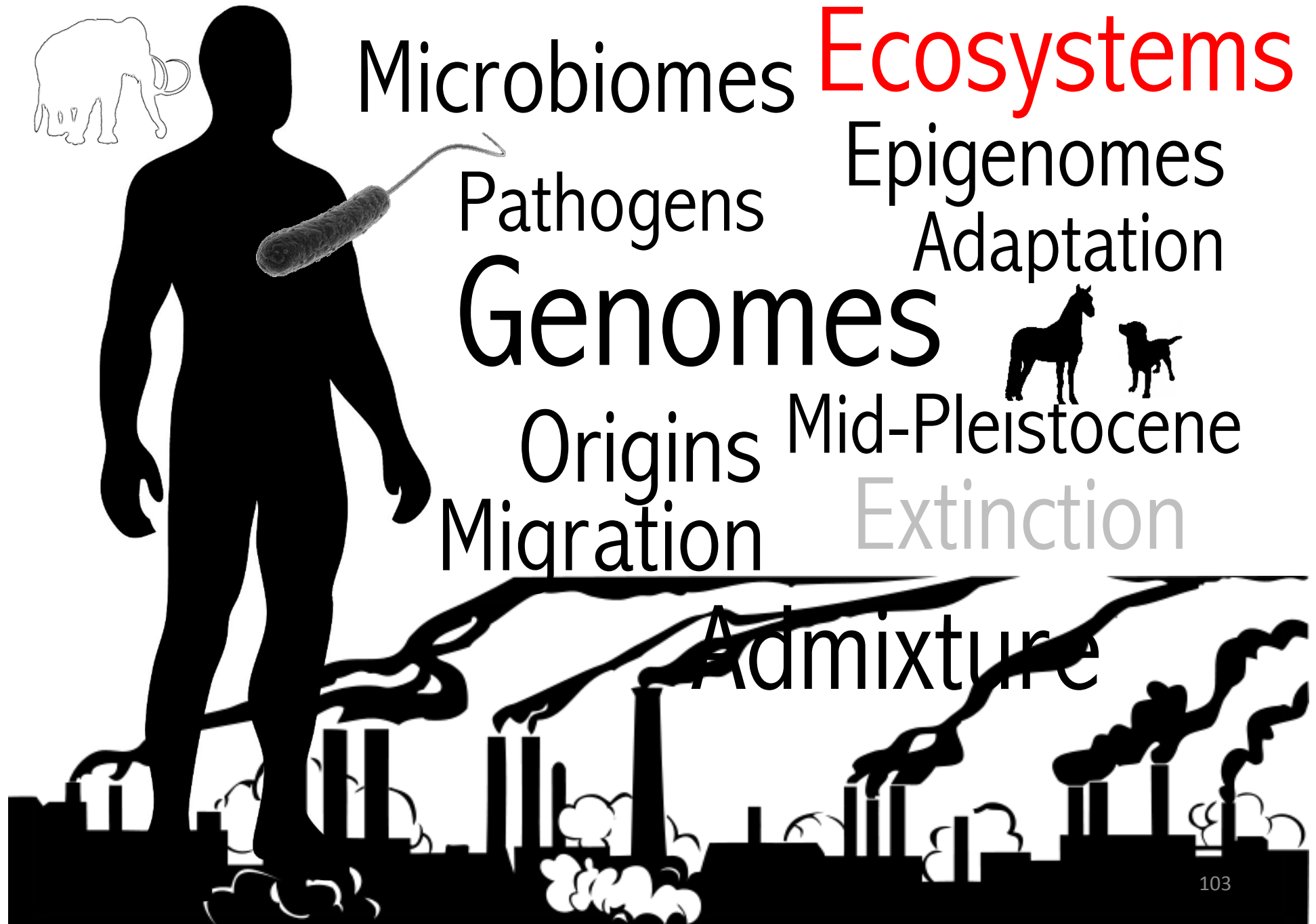
- Extinction
- Domestication
- Change in Life Style

Beyond Genomes, Epigenomes

Ancient Methylomes



Seguin-Orlando et al. Sci Rep 2015



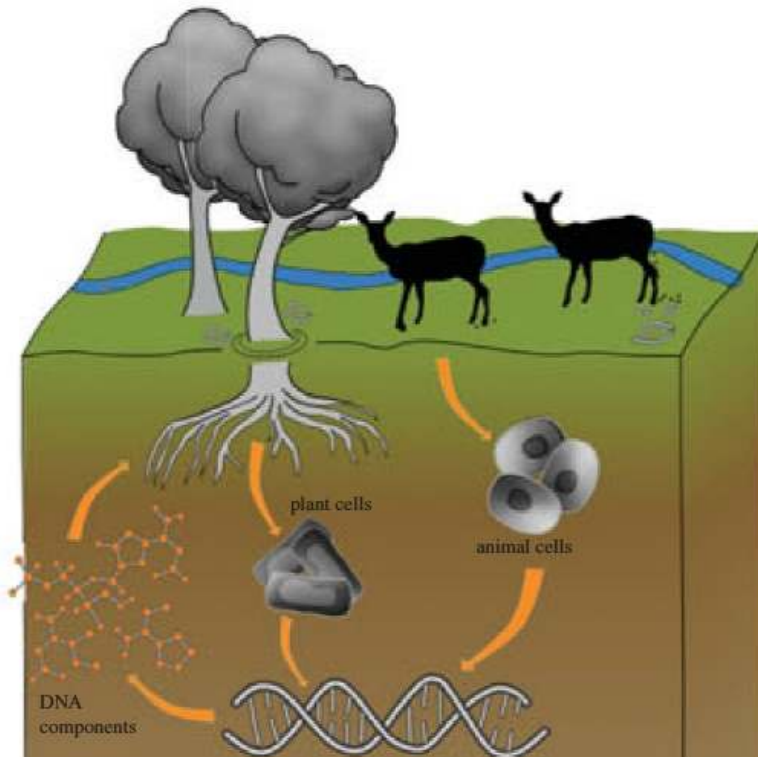
Beyond Genomes, Ecosystems

Mini-metabarcoding



Beyond Genomes, Ecosystems

Mini-metabarcoding



Winther-Pedersen et al. Proc B 2015



DNA Extraction

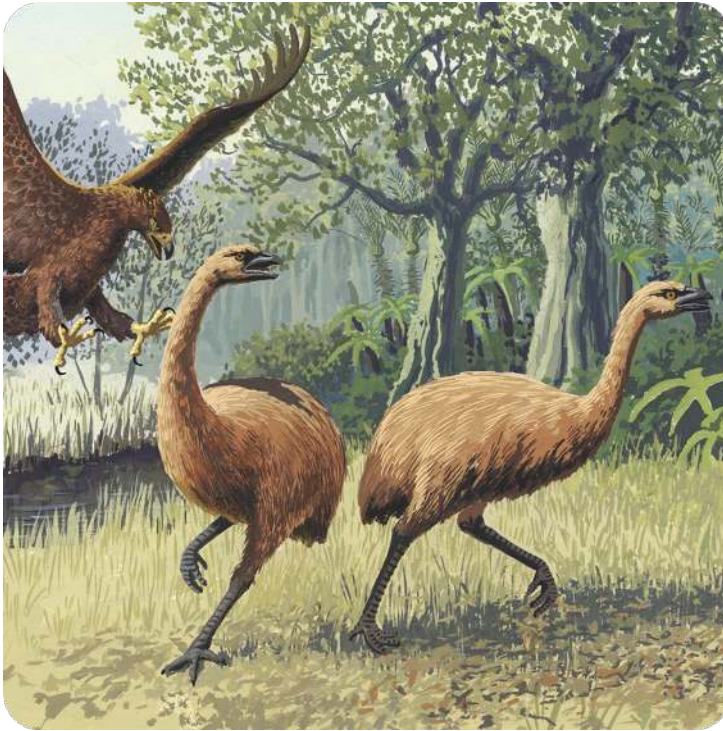
Minibarcoding PCR amplification

Deep Amplicon-Sequencing

Molecular Taxonomic Assignment

Beyond Genomes, Ecosystems

Mini-metabarcoding



New Zealand, 600 ya

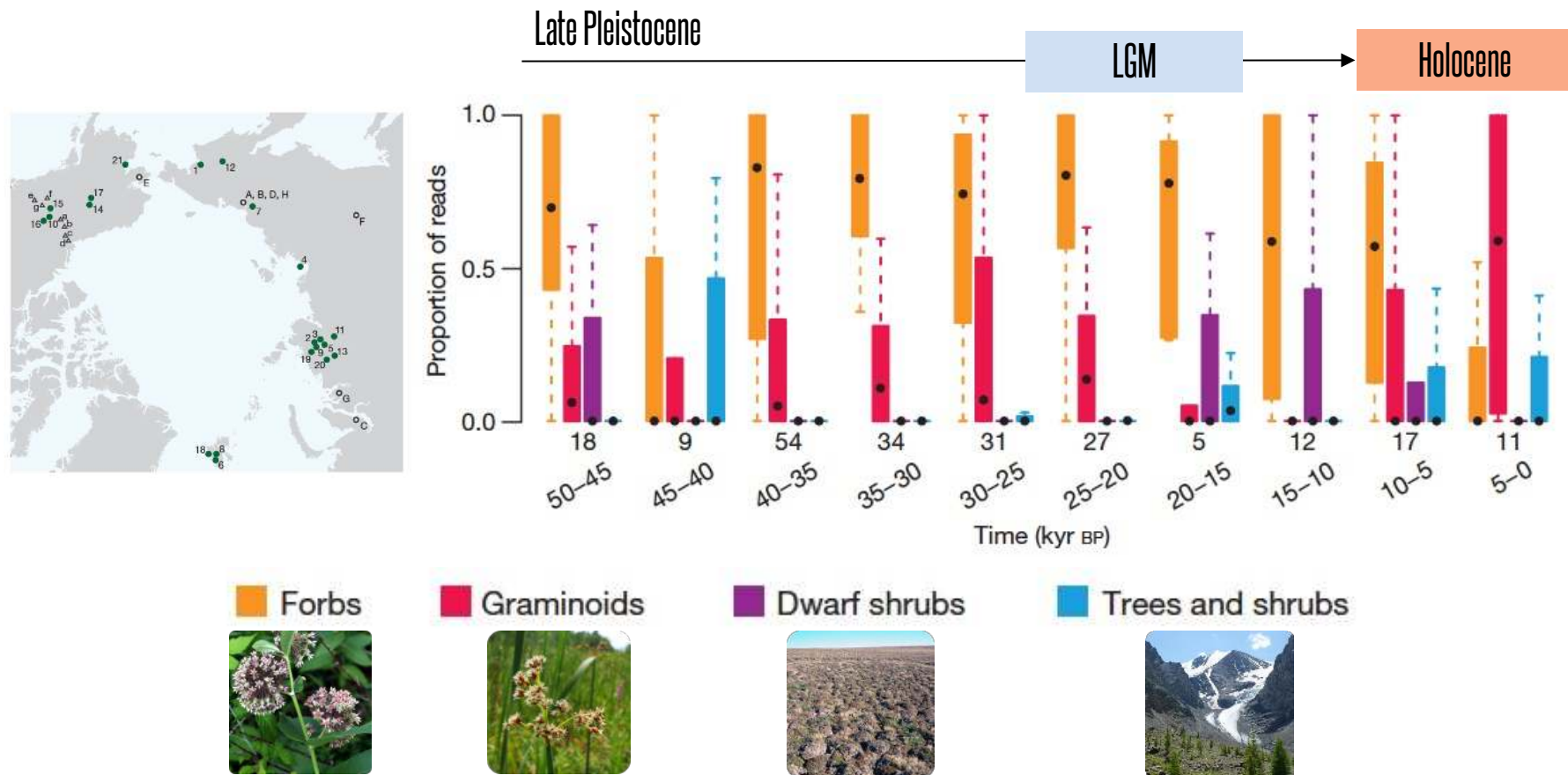


Siberia, 10.4-30 kya

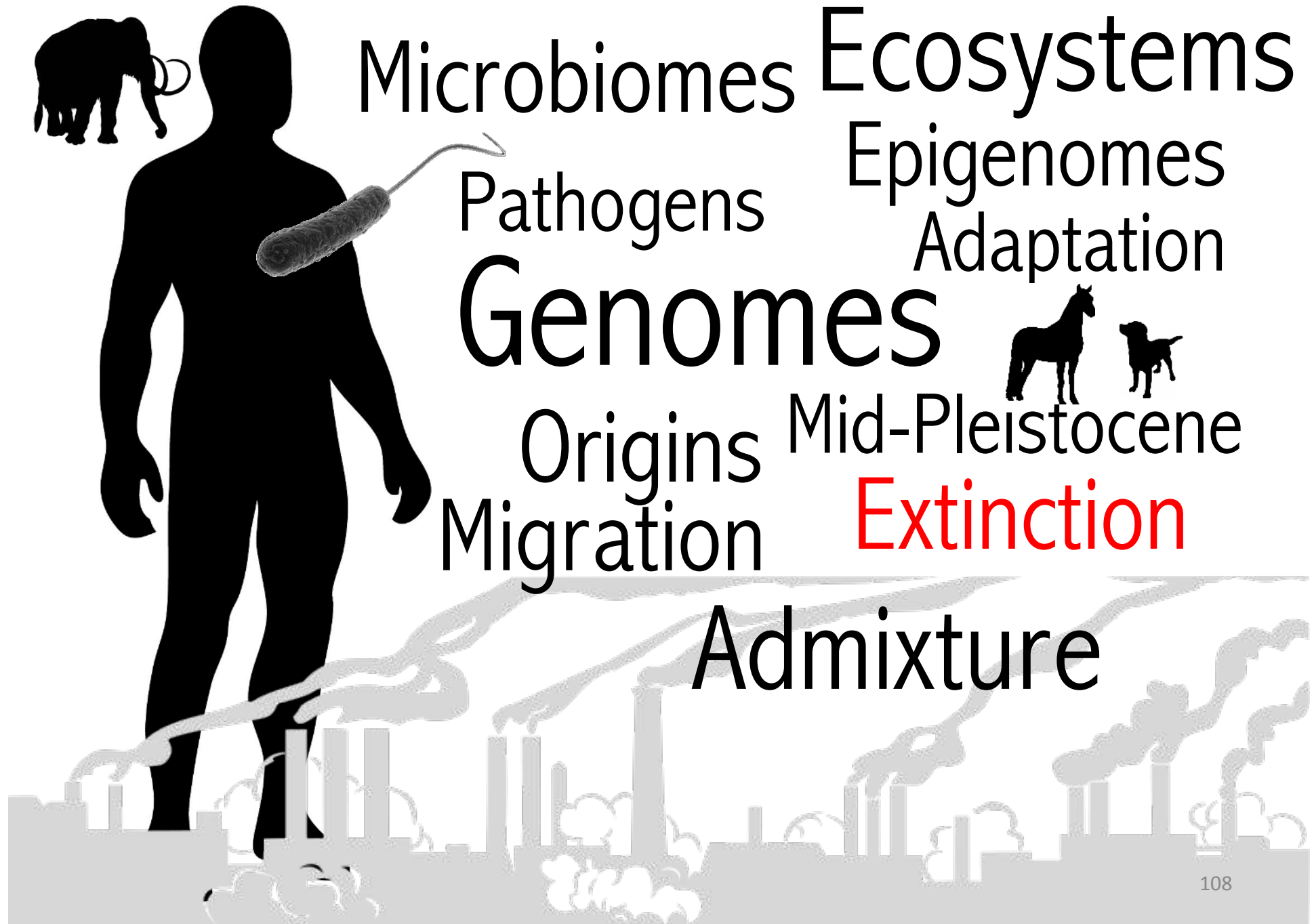
Willerslev et al. Nature 2003

Beyond Genomes, Ecosystems

Mini-metabarcoding



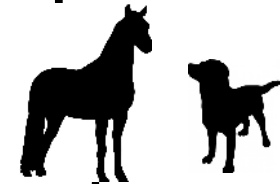
Willerslev et al. Nature 2014



Microbiomes Ecosystems

Pathogens Epigenomes
Adaptation

Genomes

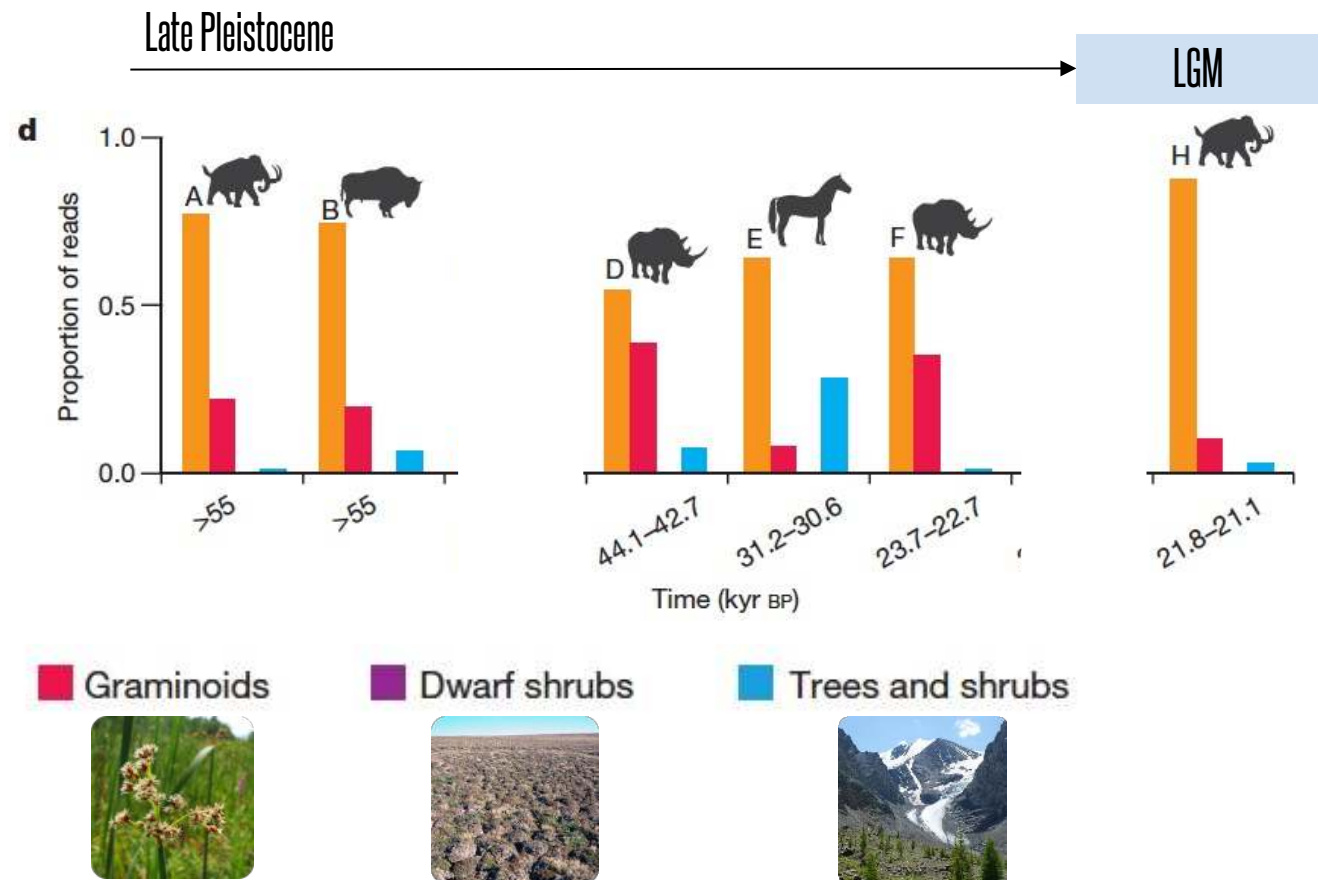


Origins Mid-Pleistocene
Migration Extinction

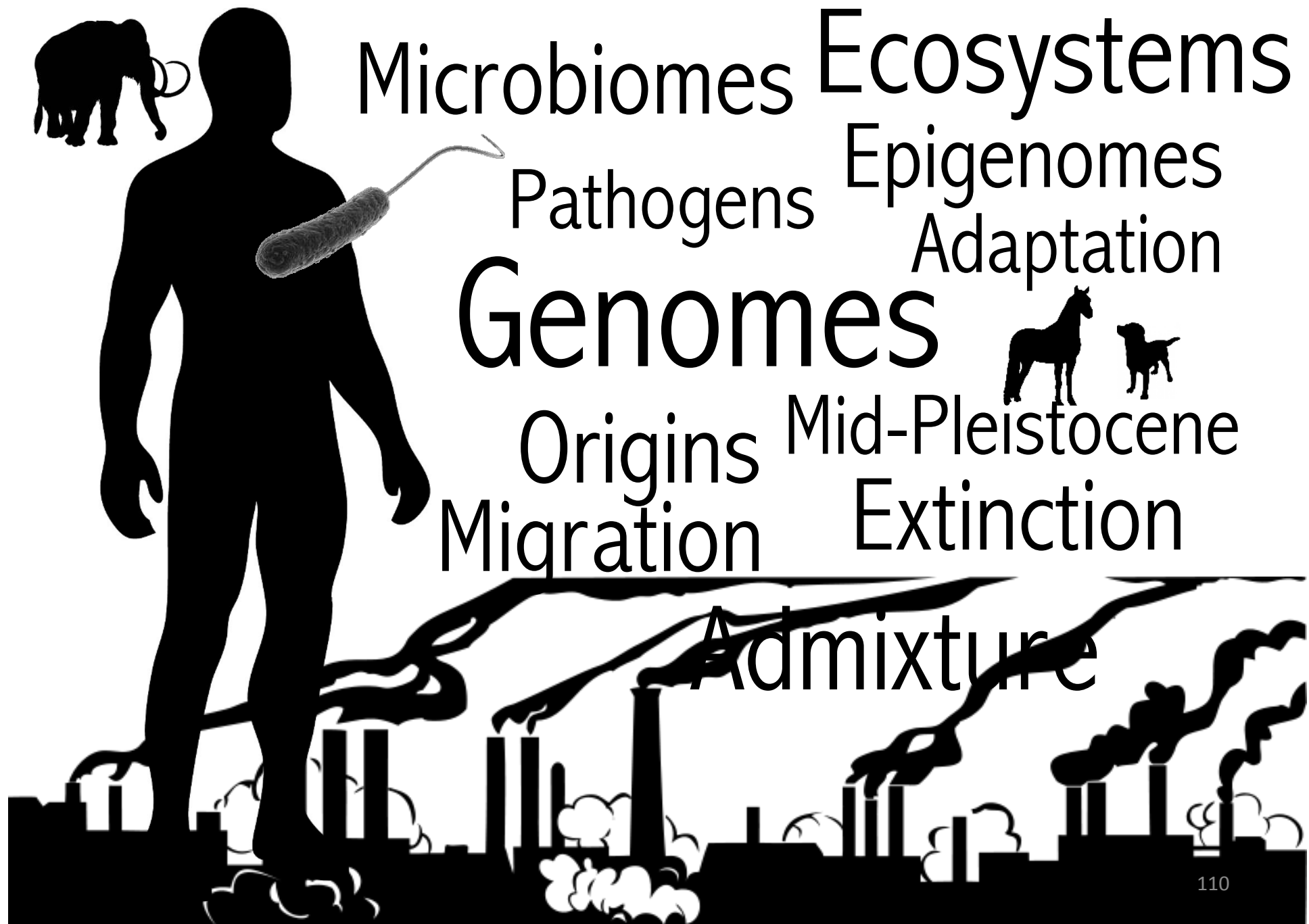
Admixture

Beyond Genomes, Ecosystems

Mini-metabarcoding



Willerslev et al. Nature 2014



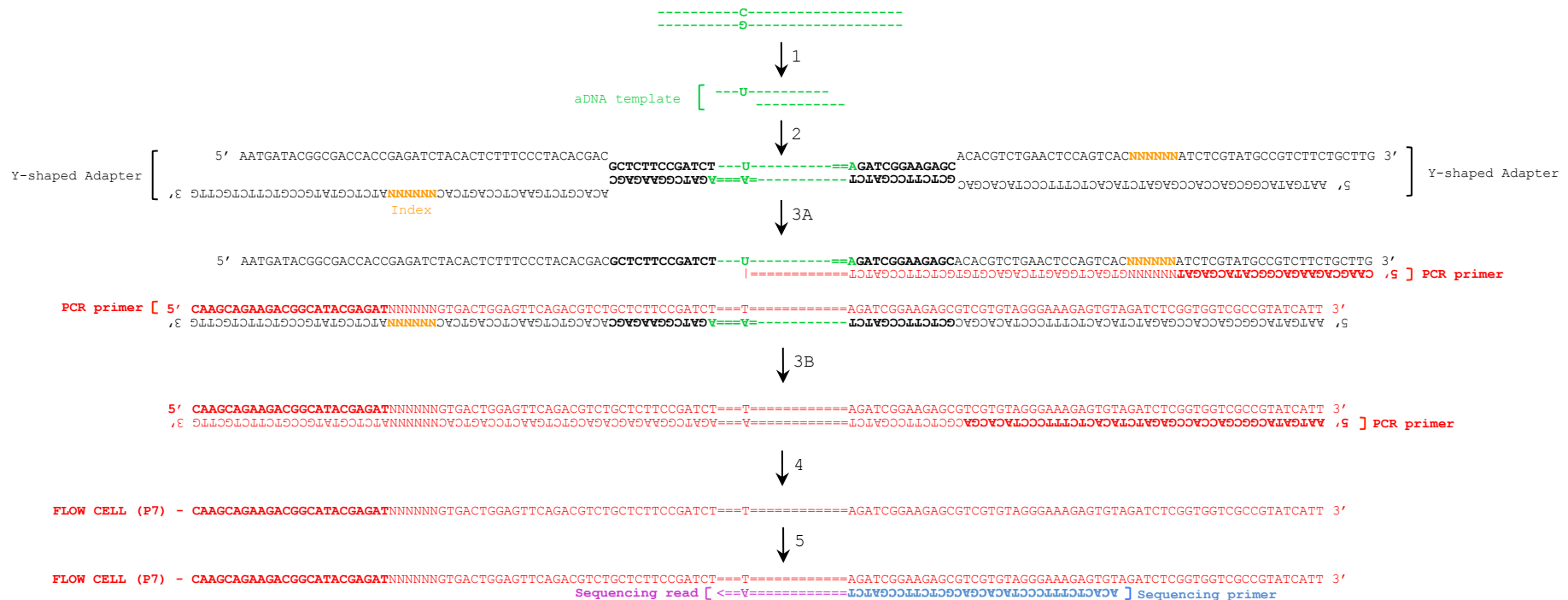
Post-Mortem Cytosine Deamination



The First Ancient Epigenome

Cytosine Methylation and Gene Expression

Post-Mortem Cytosine Deamination

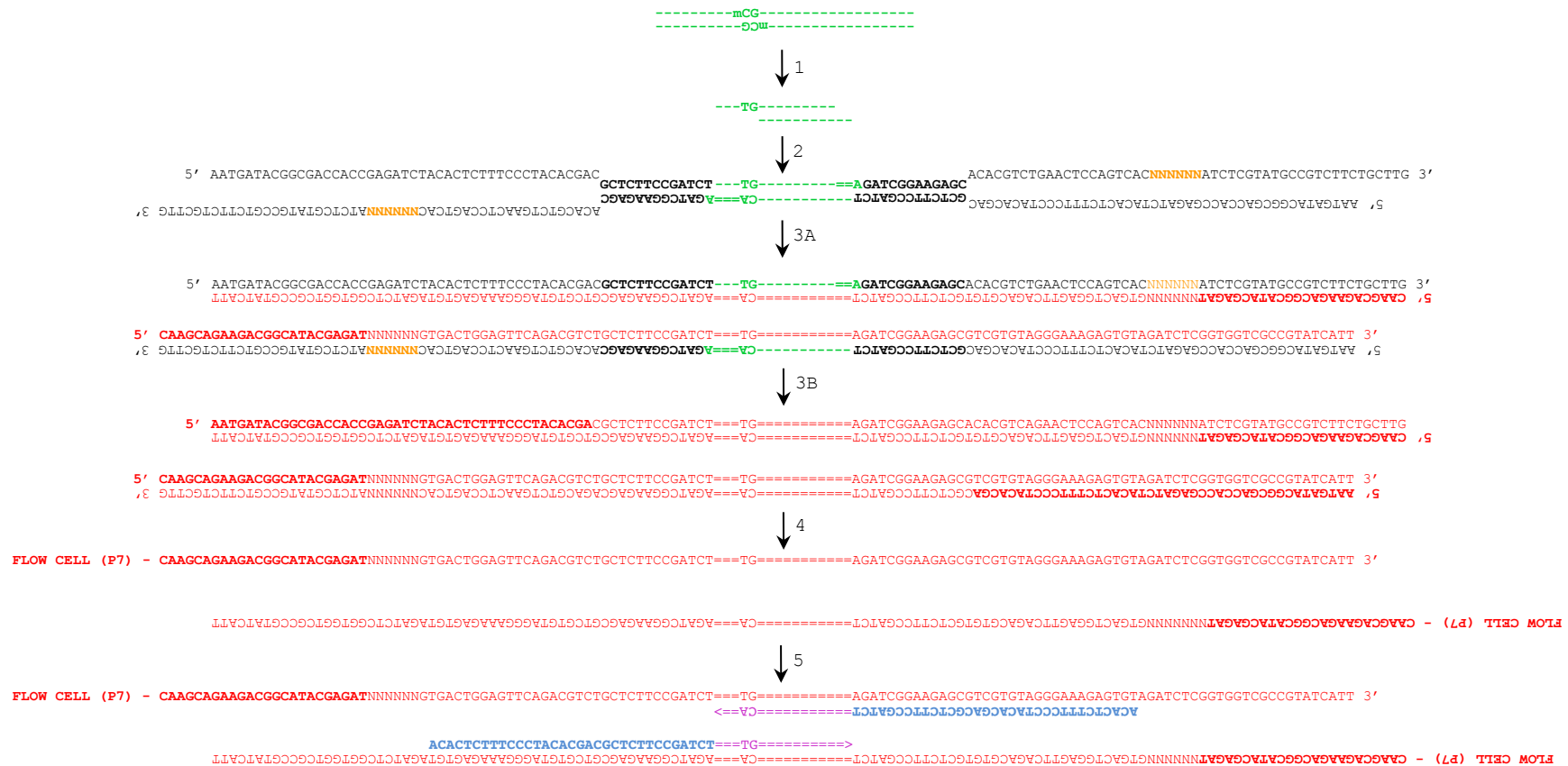


Orlando-Sequin et al. STAR 2015

The First Ancient Epigenome

Cytosine Methylation and Gene Expression

Post-Mortem Cytosine Deamination

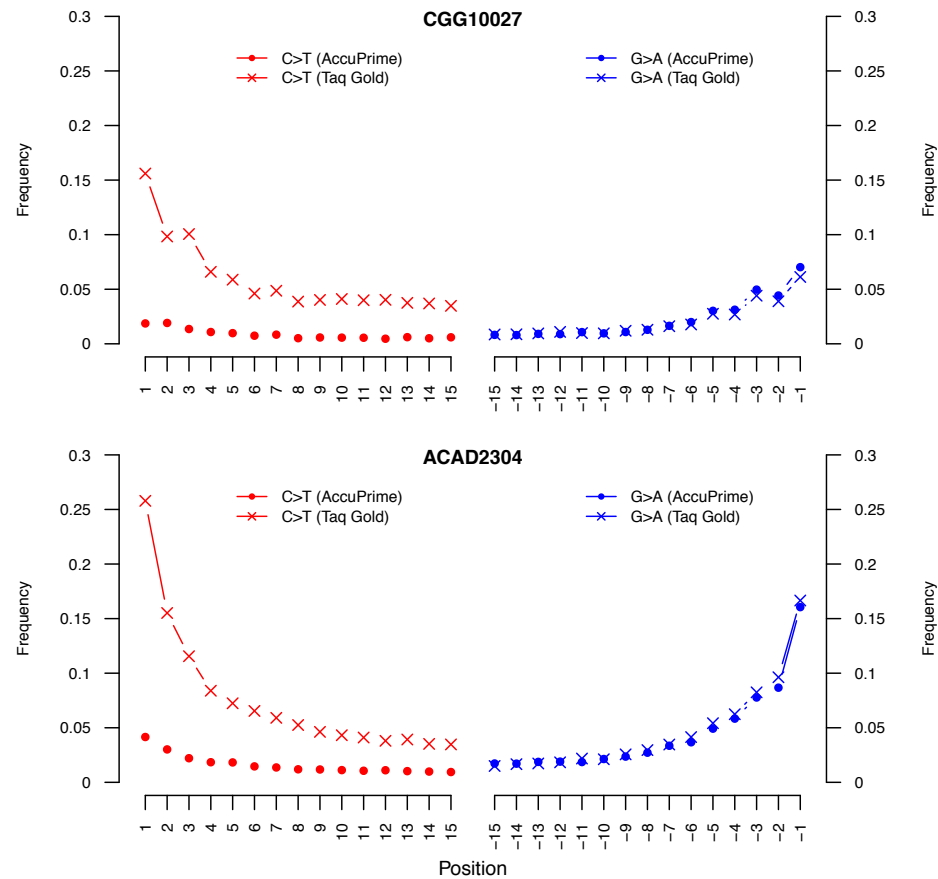


Orlando-Seguín et al. STAR 2015

The First Ancient Epigenome

Cytosine Methylation and Gene Expression

Post-Mortem Cytosine Deamination



Orlando-Seguin et al. STAR 2015

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