UNIVERSITY OF COPENHAGEN

Part 1:

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

Centre for GeoGenetics

Lorlando@snm.ku.dk

Disclaimer

What this talk will not be about


Disclaimer
What this talk will not be about

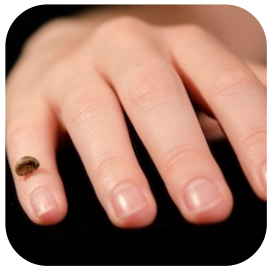
Ancient DNA Research

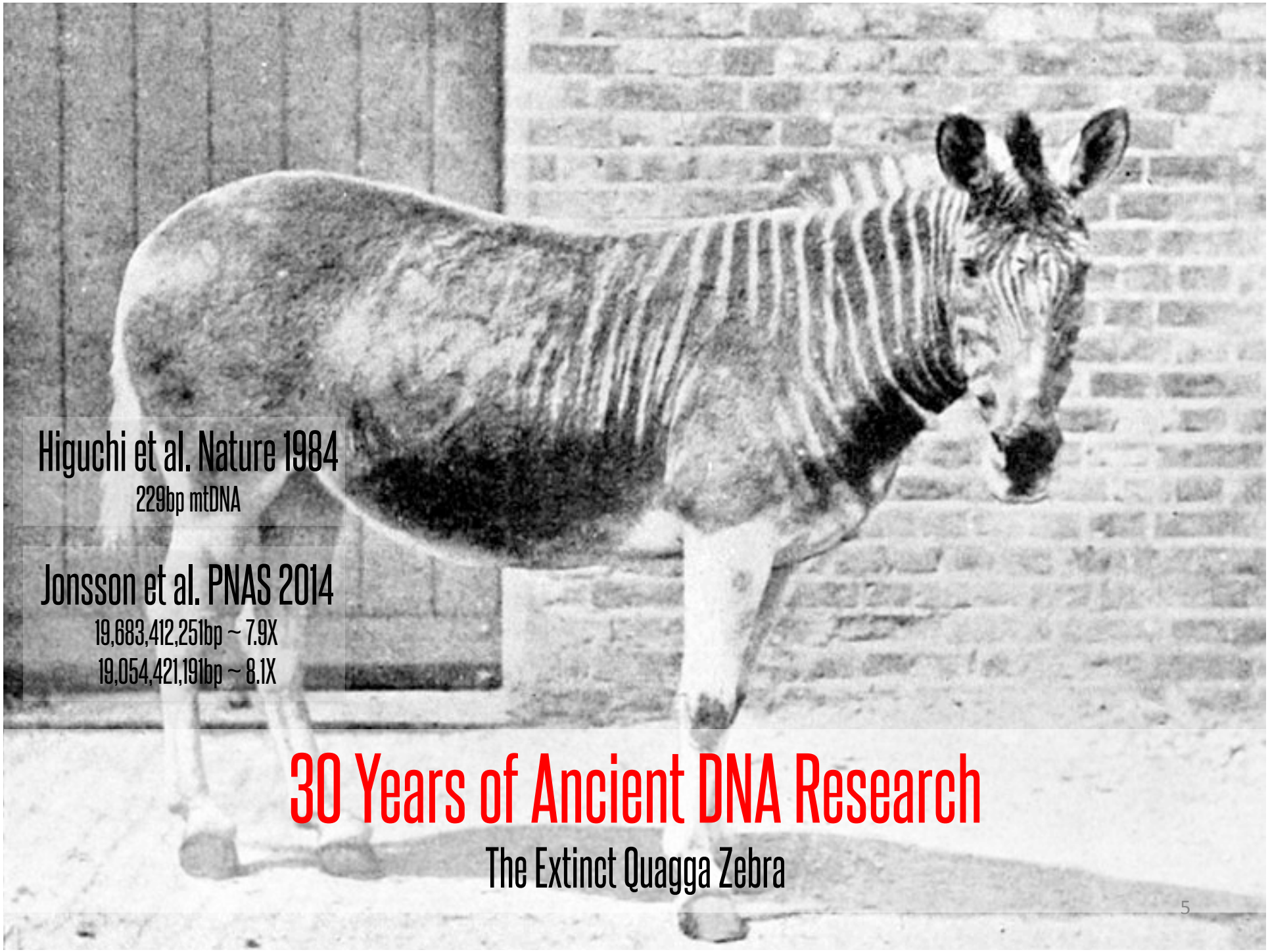
Source Material









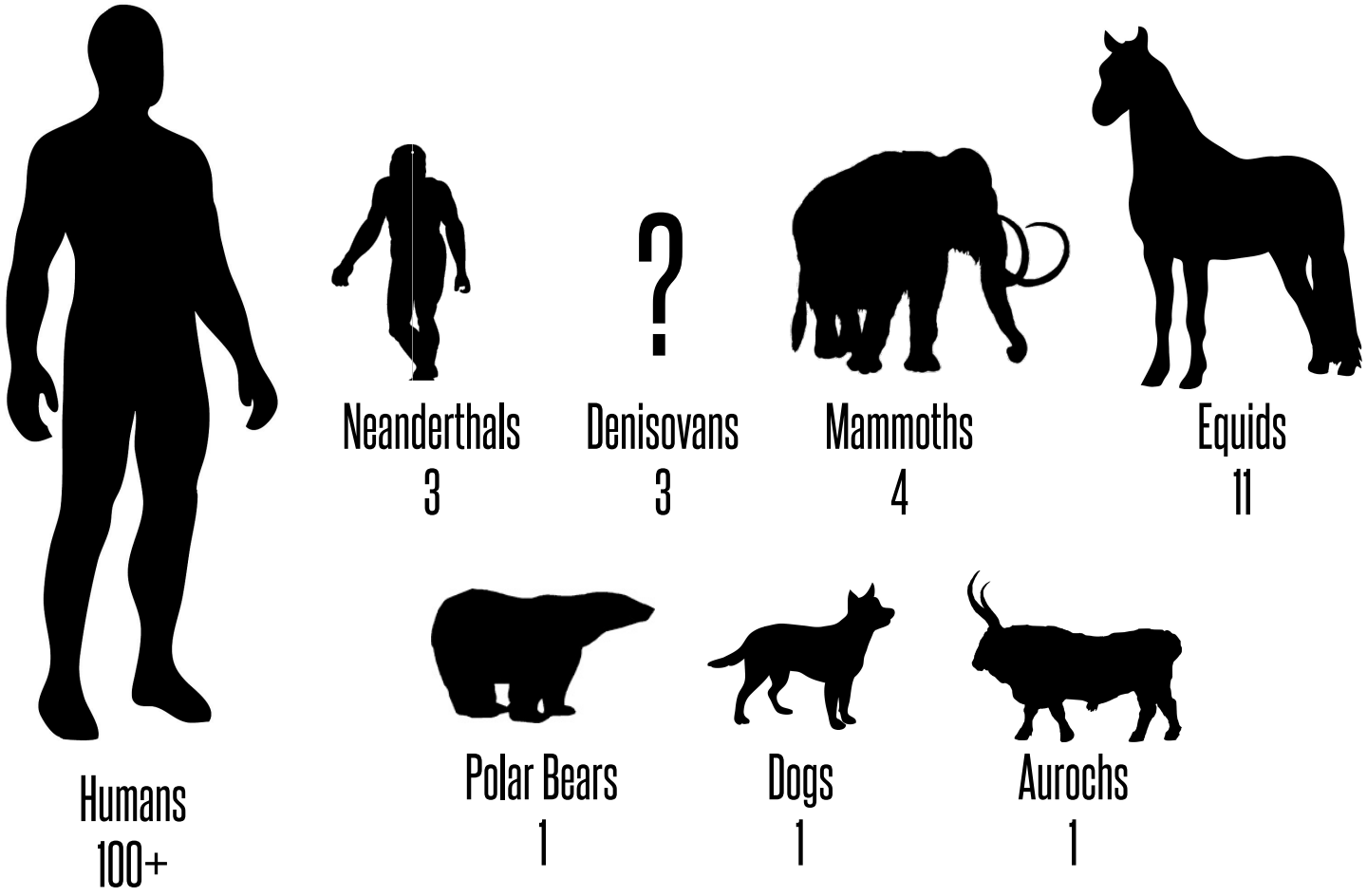



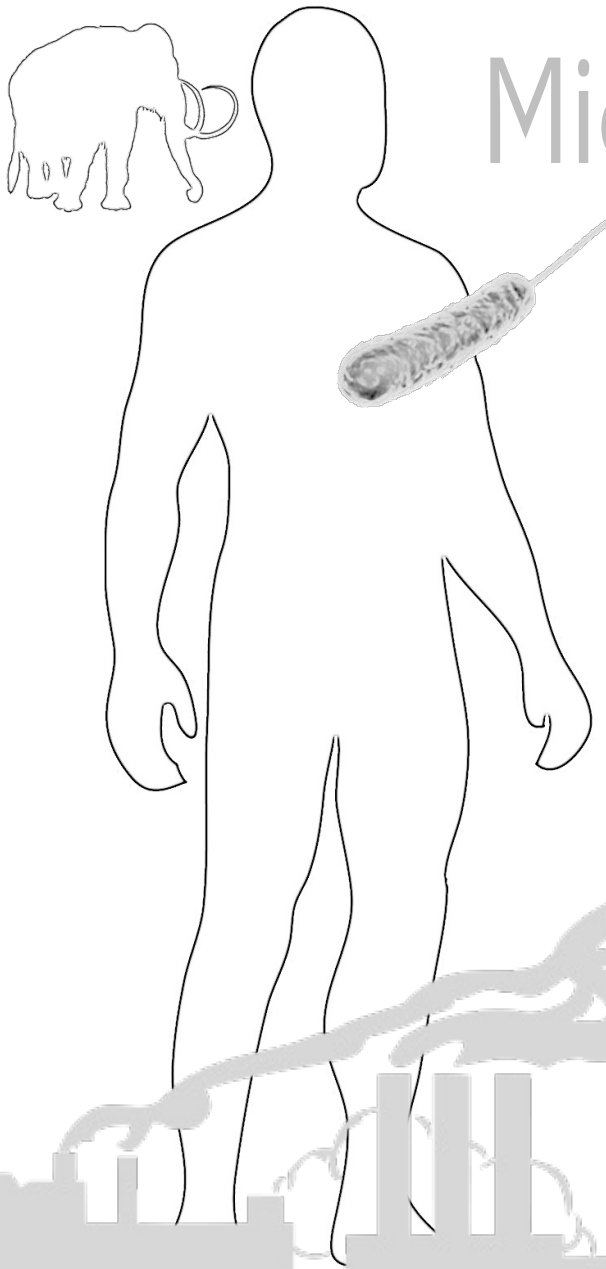
30 Years of Ancient DNA Research

The Extinct Quagga Zebra

Ancient Genomics

>100 Ancient Genomes Have Now Been Characterized





Microbiomes

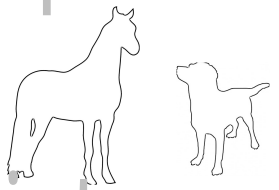
Ecosystems

Epigenomes

Pathogens

Adaptation

Genomes



Origins

Mid-Pleistocene

Migration

Extinction

Admixture

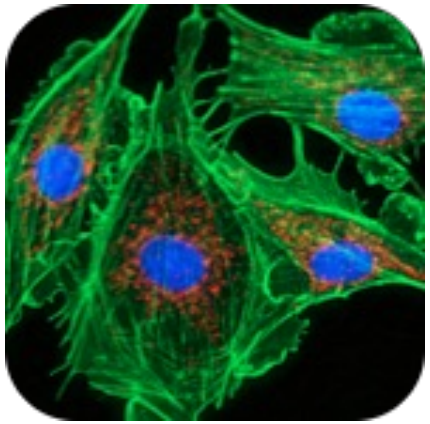
Ancient Genomics

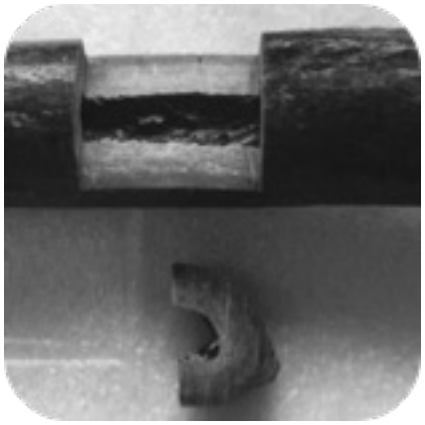
Really?

16,500

3,000,000,000

800 mg - 379 bp





From Krings et al. Cell 1999

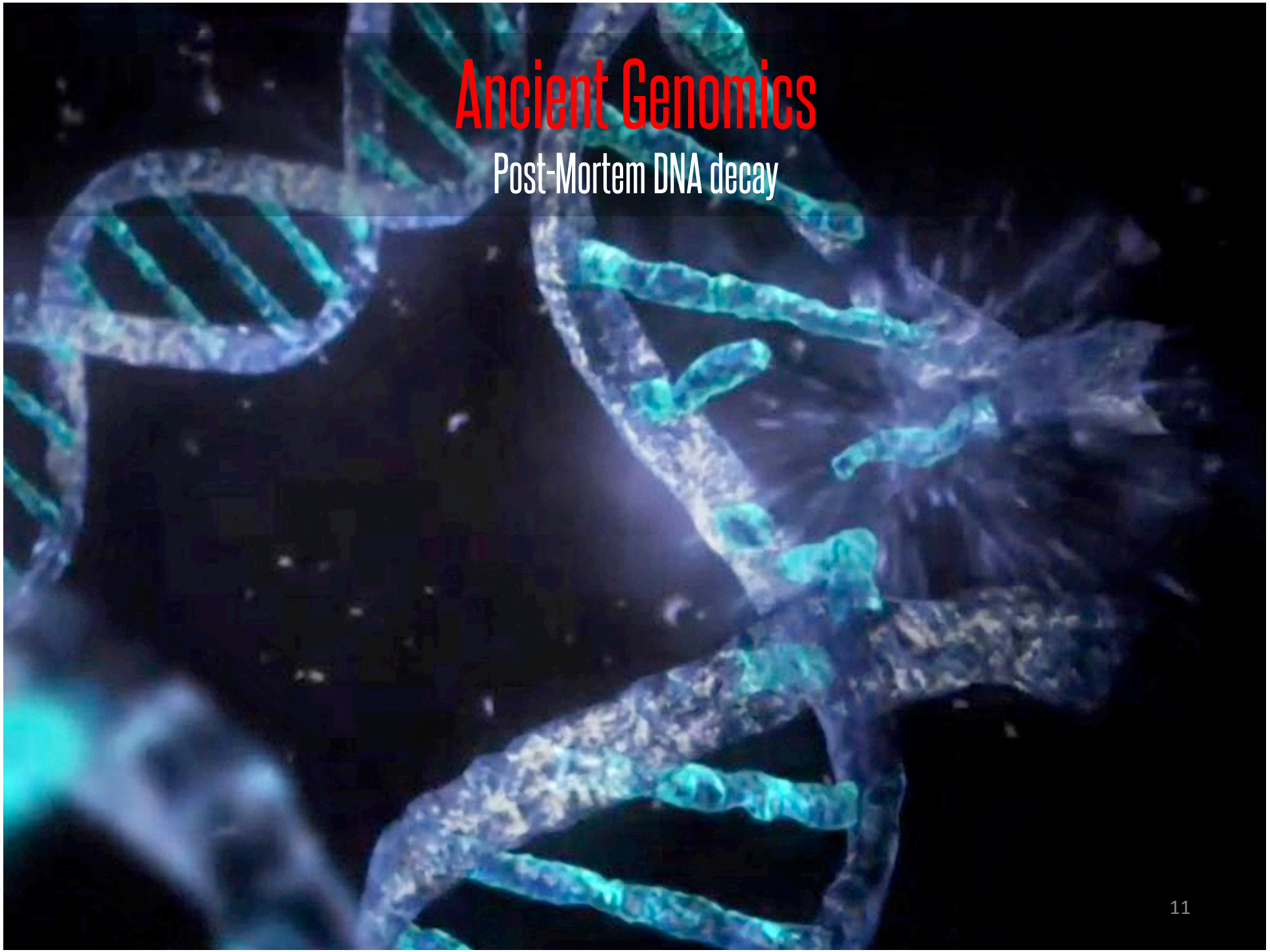
Ancient Genomics

Really?


Ancient Genomics
Really?
6.6 tons
NATURE|Vol 456|20 November 2008

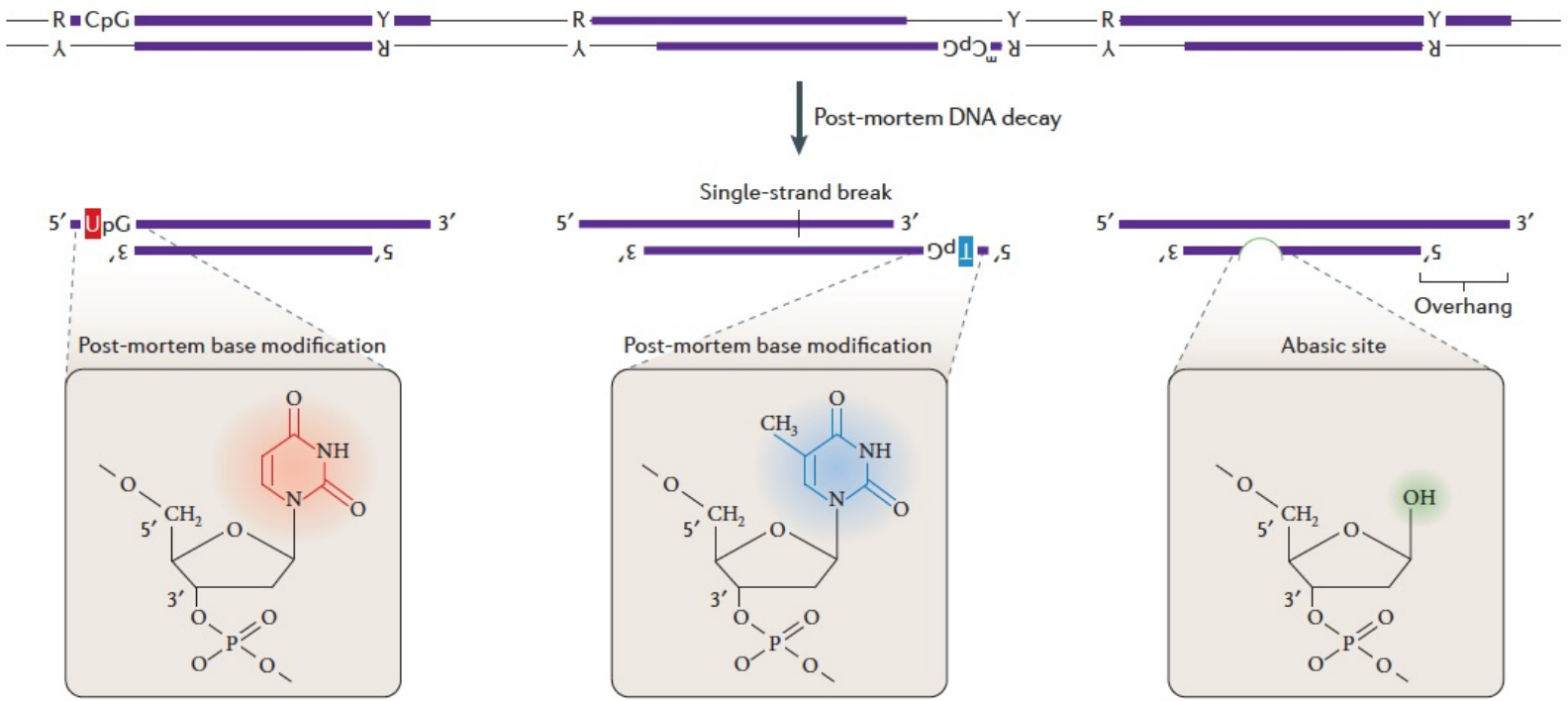
Ancient Genomics

Post-Mortem DNA decay



Ancient Genomics

Post-Mortem DNA decay

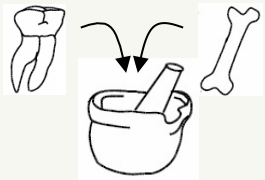


Orlando et al. Nature Rev Genet 2015

Ancient Genomics

Low-Throughtput Approaches

Crushing

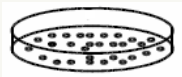


Decalcification
Protein Removal

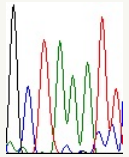
DNA Concentration

INDEPENDENT PCRs / INDEPENDENT LABS

CONSENSUS **AGCGC TGATC**



CLONING



SEQUENCING

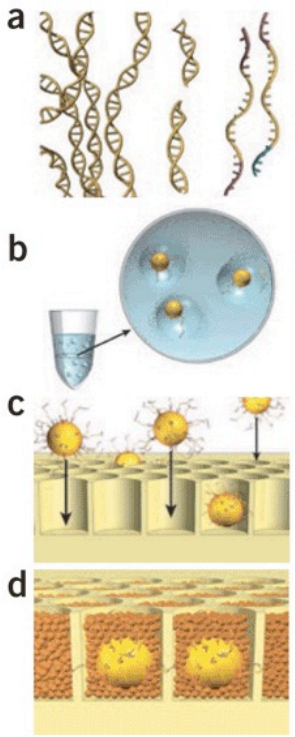
IN SILICO ANALYSIS

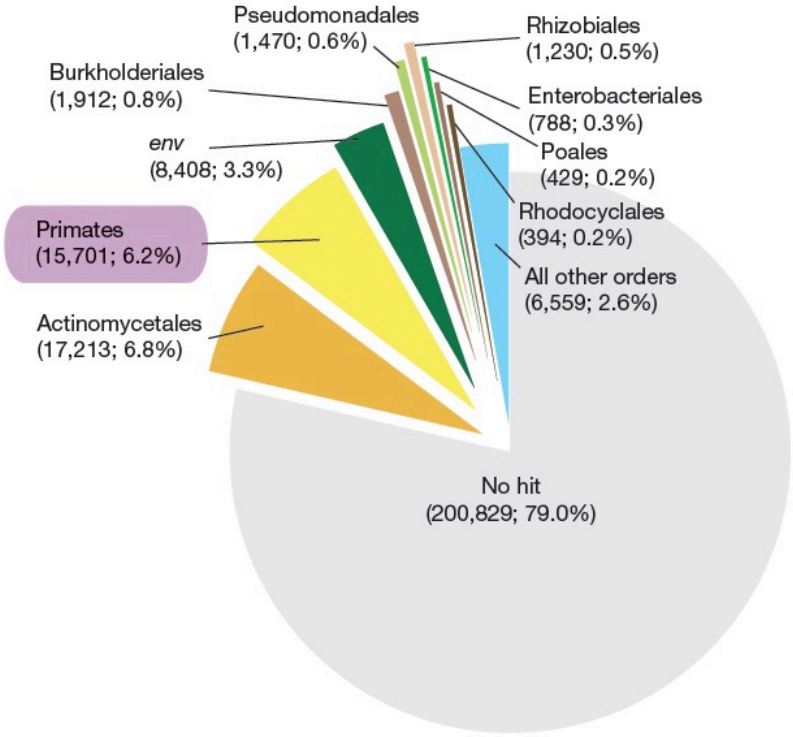
```
..T.. .....
..T.. .A...
..T.. .....
..T.. .....

..... .....
..... ....T
..... ....T
..... .....

..... .....
.A... .....
..... .....
..... .....
```

21,500 years

Ancient Genomics

High-Throughtput Approaches



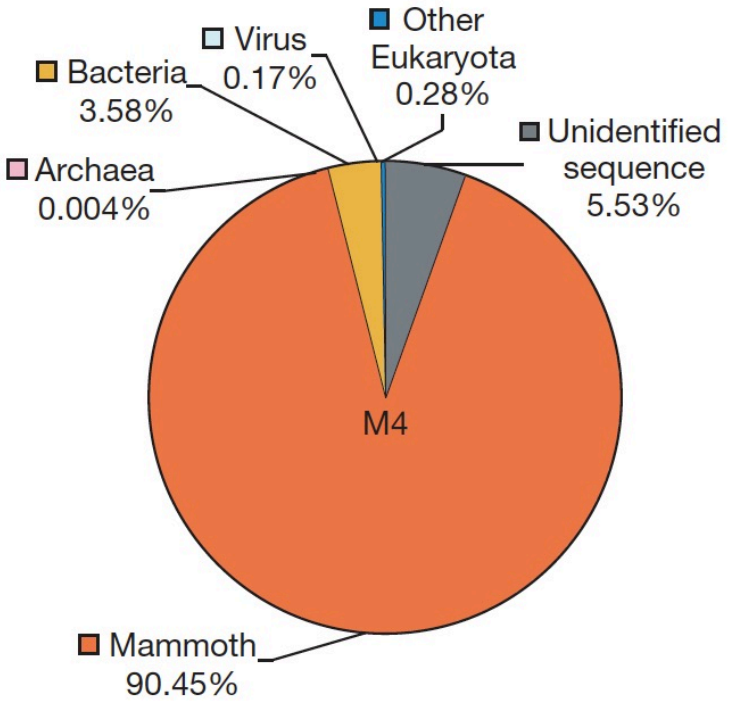


From Green et al. Nature 2006

138.6 yrs

Ancient Genomics

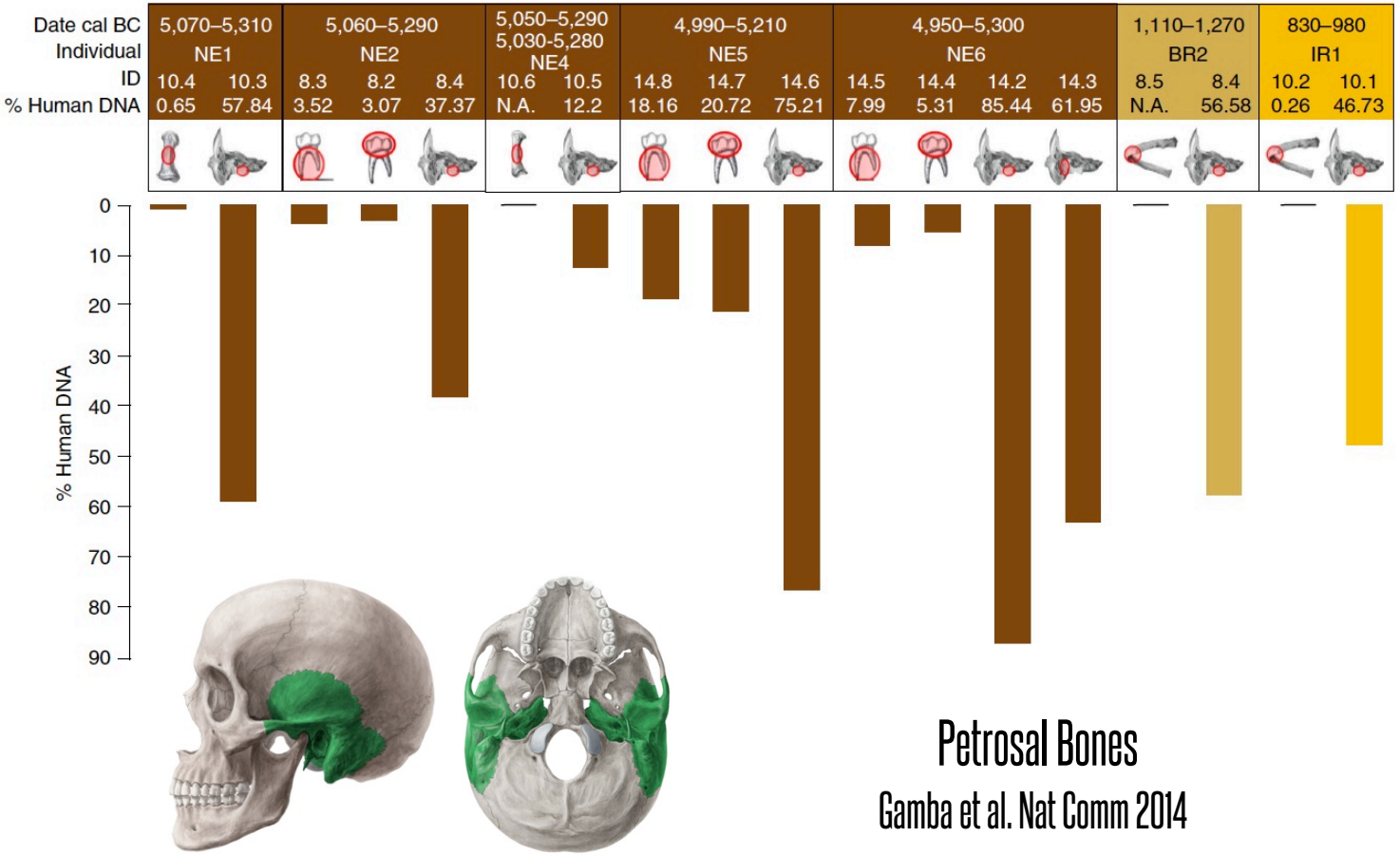
Solution #1: New DNA Reservoirs



From Miller et al. Nature 2008

Ancient Genomics

Solution #1: New DNA Reservoirs

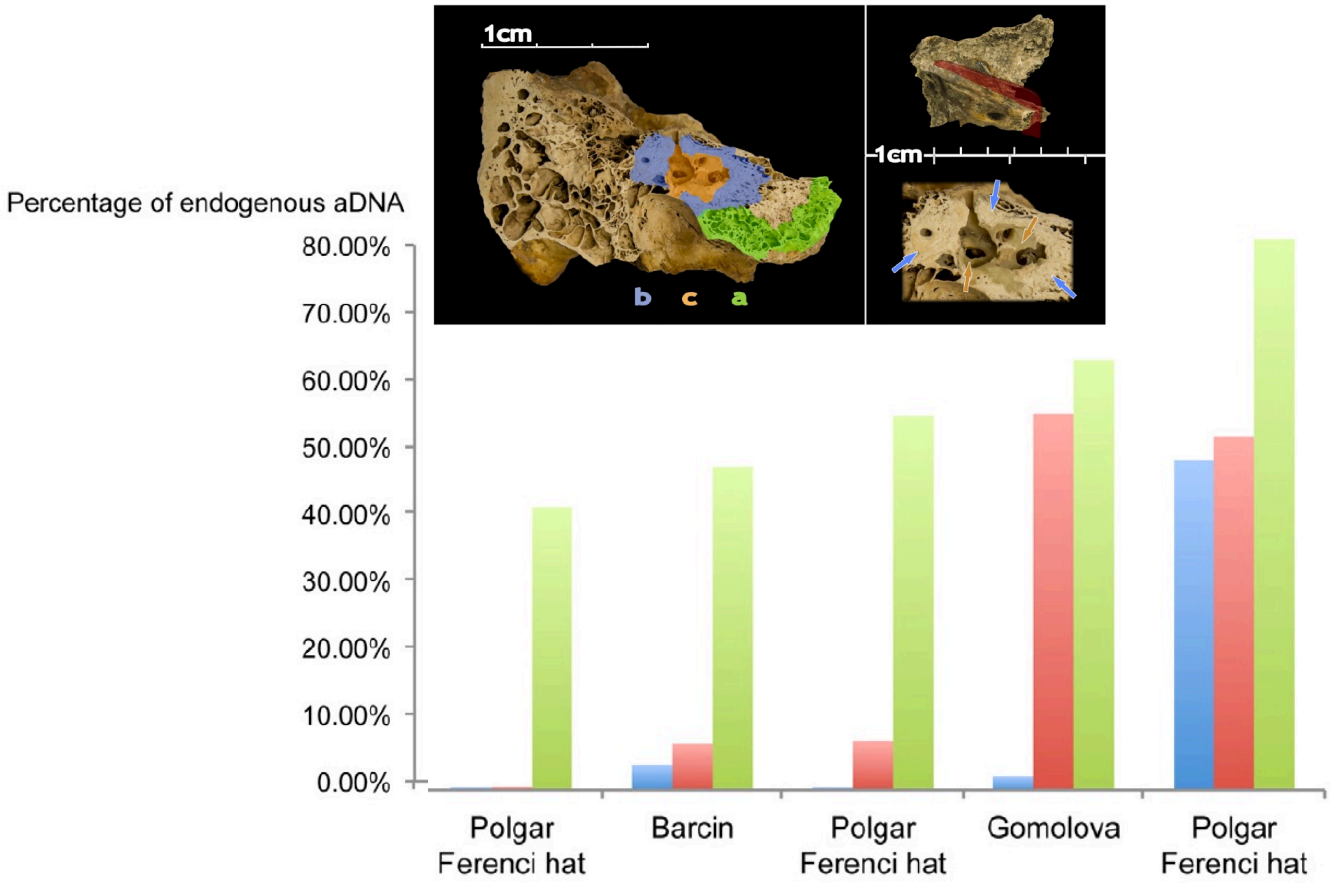


Petrosal Bones

Gamba et al. Nat Comm 2014

Ancient Genomics

Solution #1: New DNA Reservoirs



Petrosal Bones

Pinhasi et al. PLoS One 2015

Ancient Genomics

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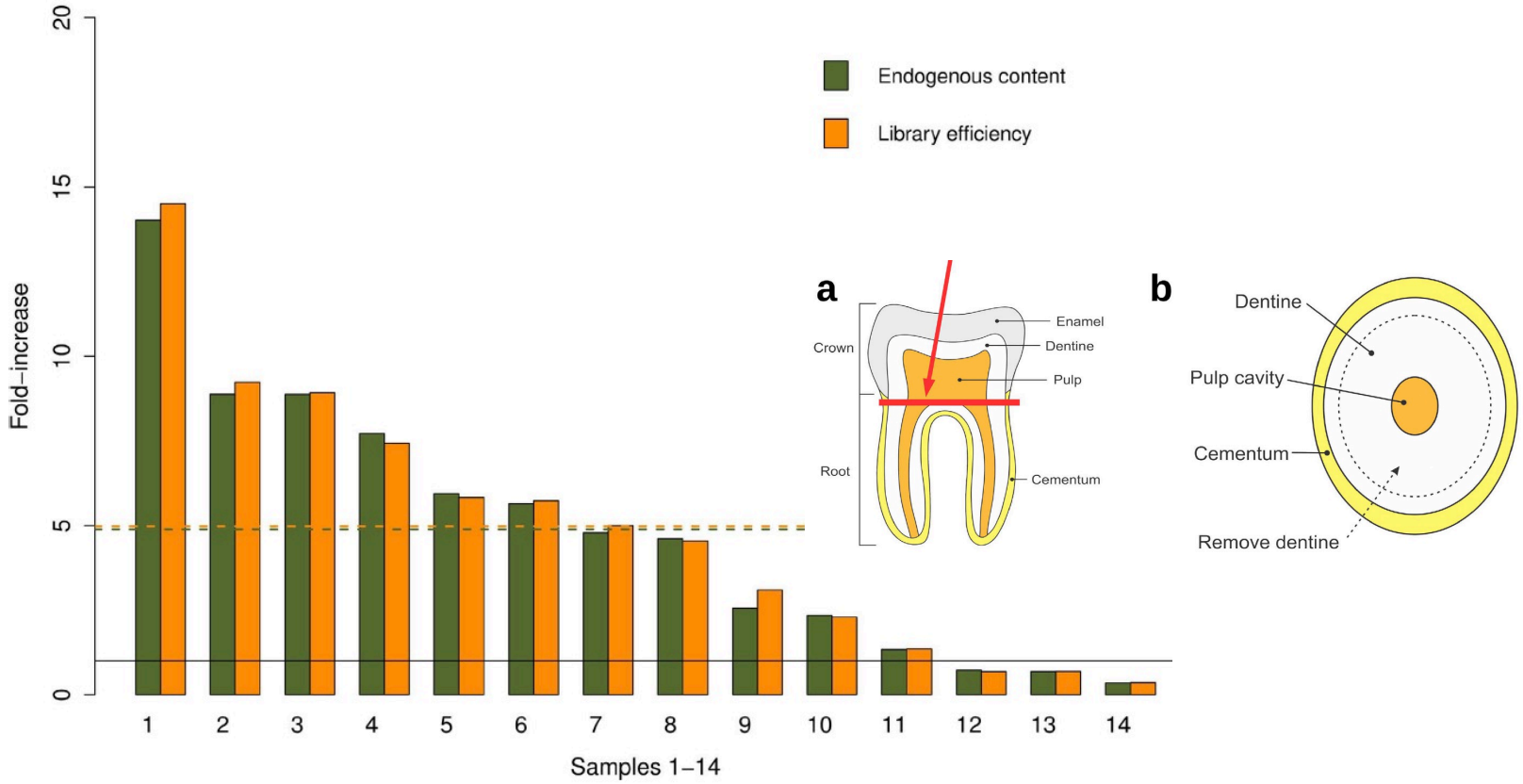
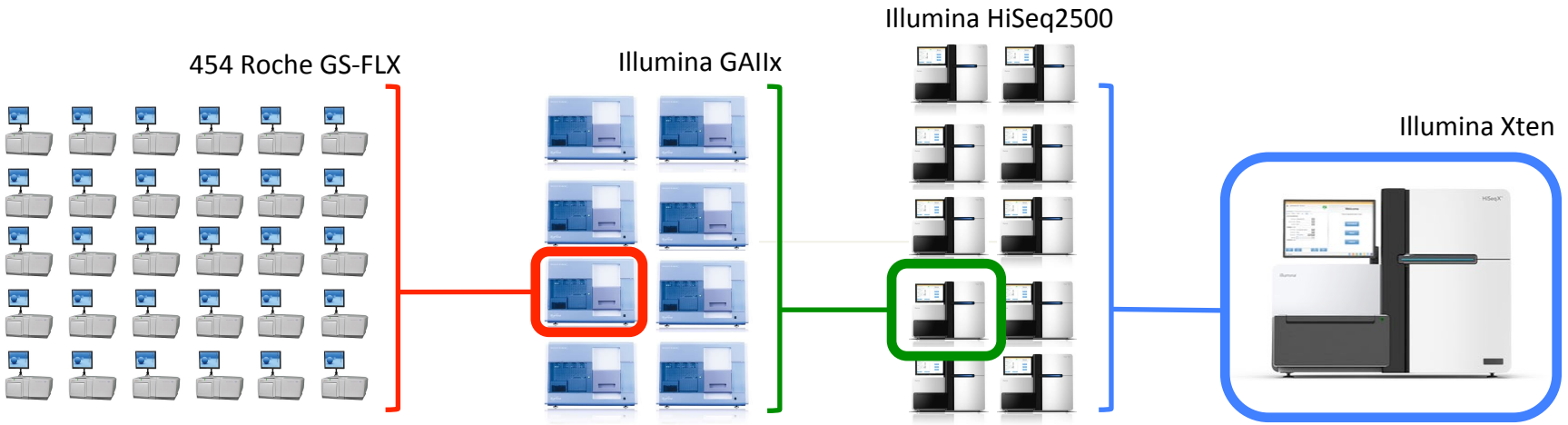


Tooth Cementum

Pinhasi et al. PLoS One 2015

Ancient Genomics

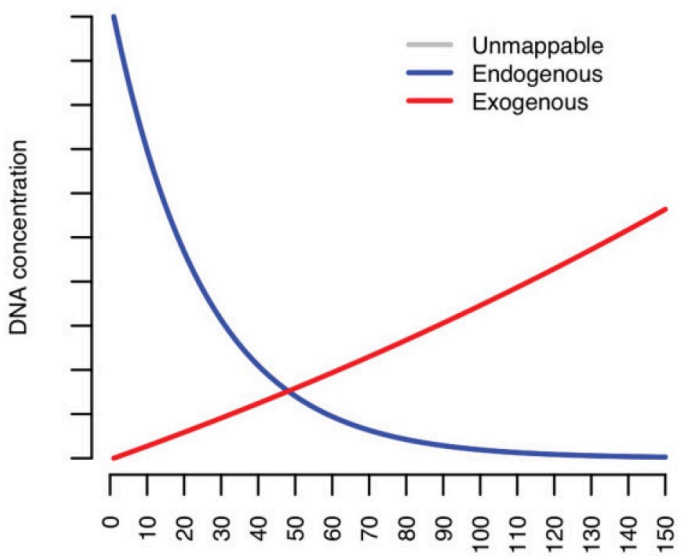
Solution #2: Enhanced Sequencing Power

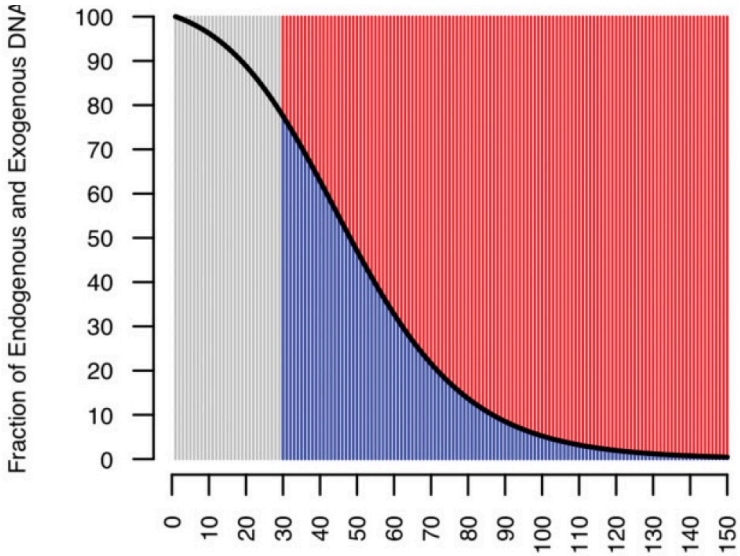


0.5M	250M	4G	30G
0.5d	5d	5d	3d

Ancient Genomics

Solution #3: Develop Ultra-Sensitive Molecular Tools



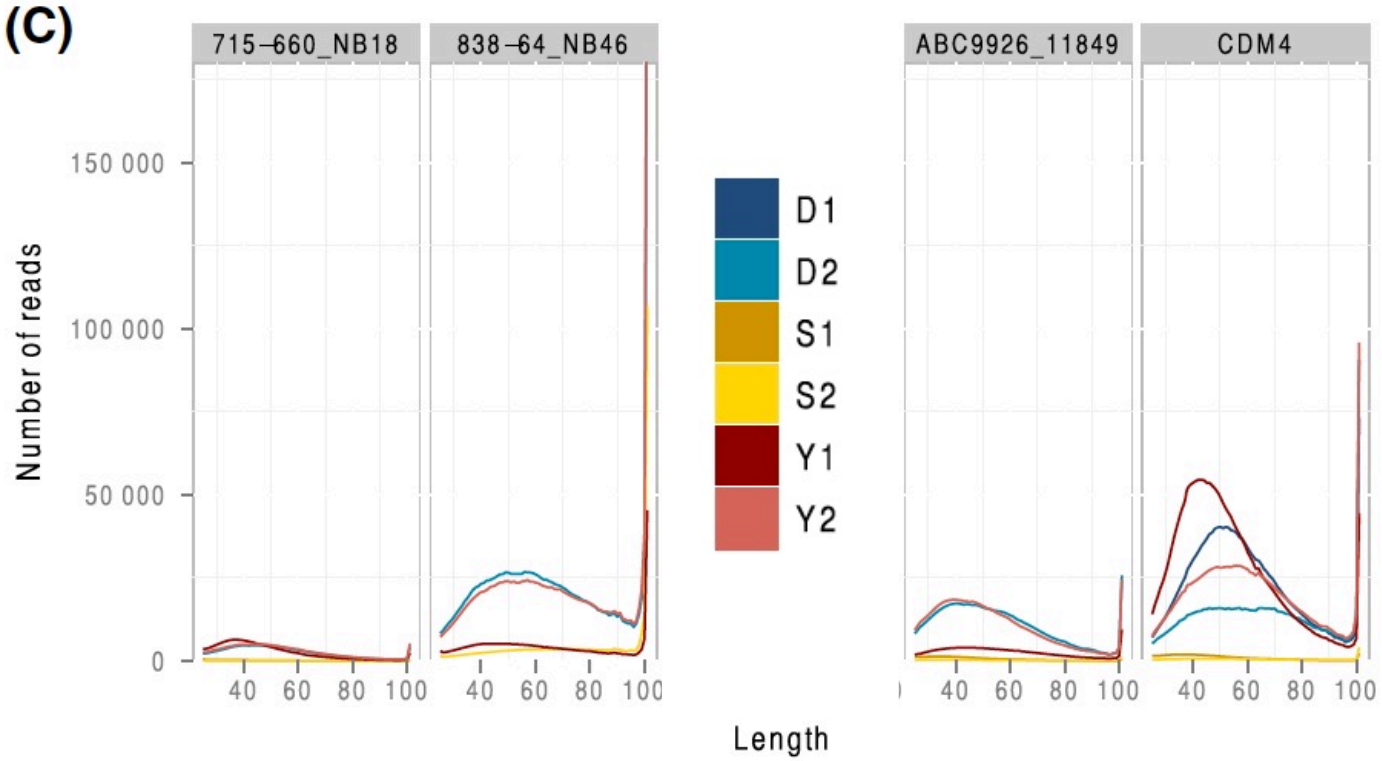


Extraction Methods Tailored to the Ultra-Short

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

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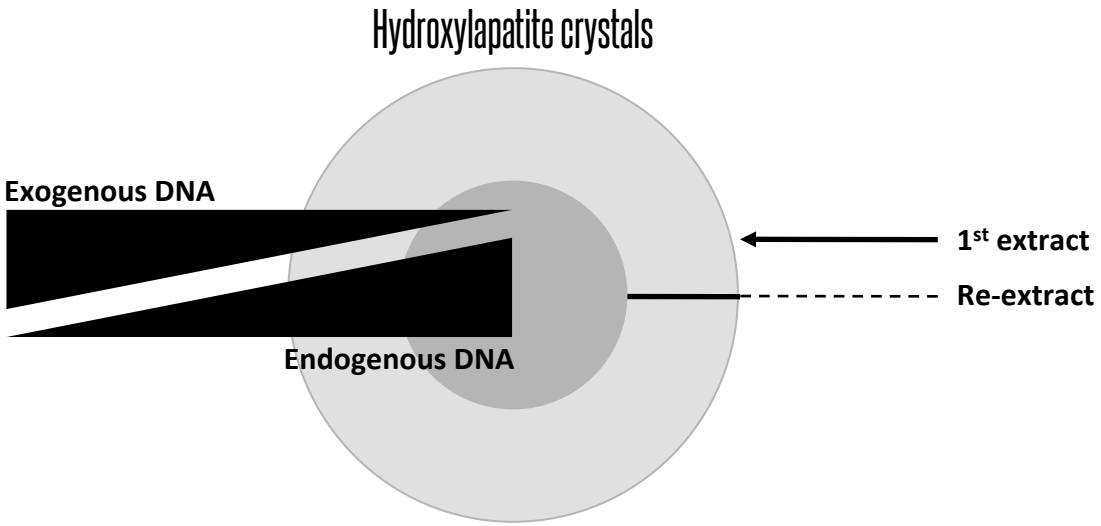
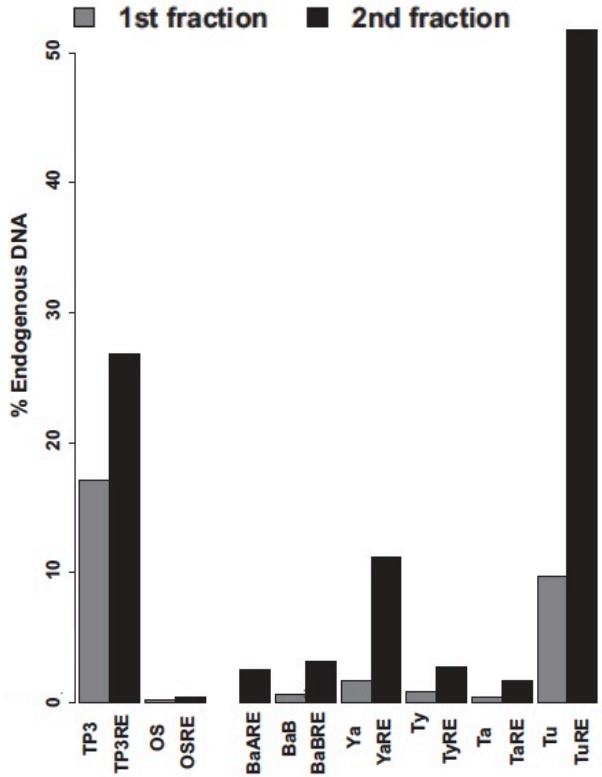


Extraction Procedures Removing Part of the Contaminating Fraction

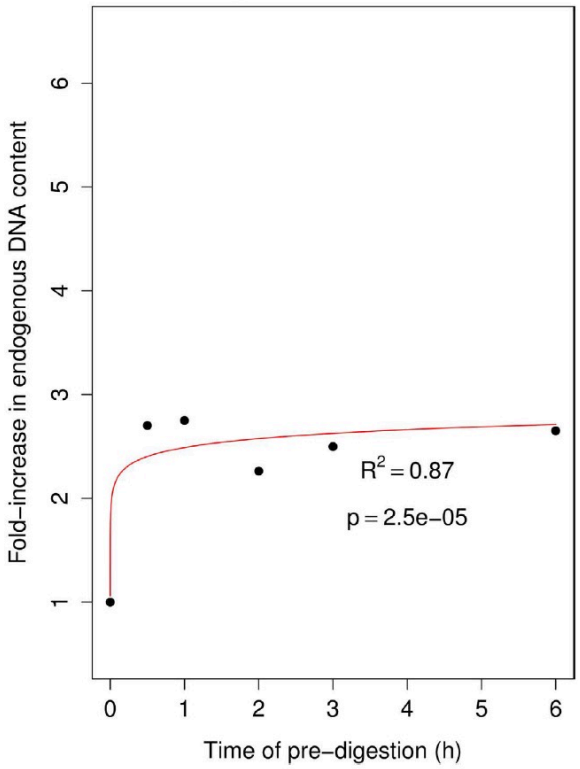
Der Sarkissian et al. Mol Ecol 2014

Ancient Genomics

Solution #3: Develop Ultra-Sensitive Molecular Tools



Der Sarkissian et al. Mol Ecol 2014

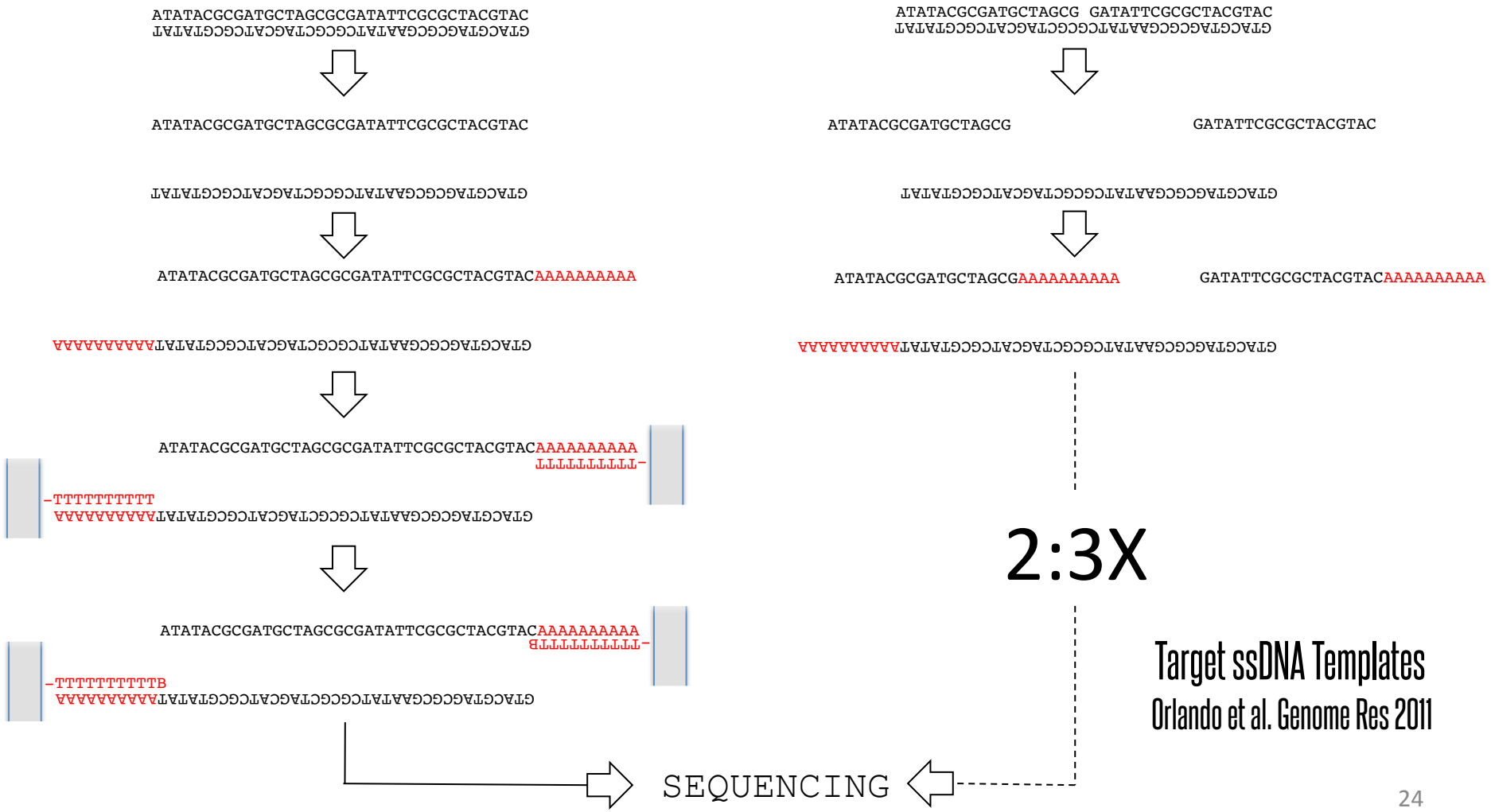


Damgaard et al. Sci Rep 2015

Extraction Procedures Removing Part of the Contaminating Fraction

Ancient Genomics

Solution #3: Develop Ultra-Sensitive Molecular Tools

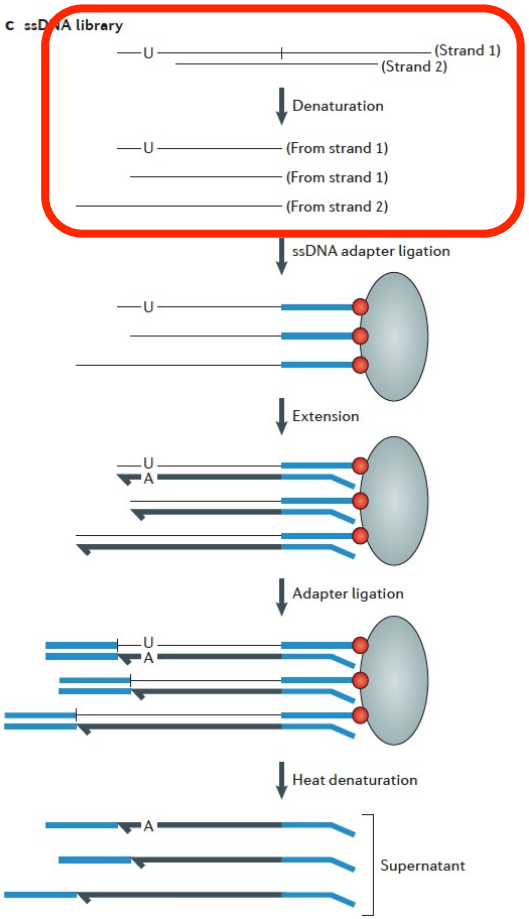


Target ssDNA Templates

Orlando et al. Genome Res 2011

Ancient Genomics

Solution #3: Develop Ultra-Sensitive Molecular Tools

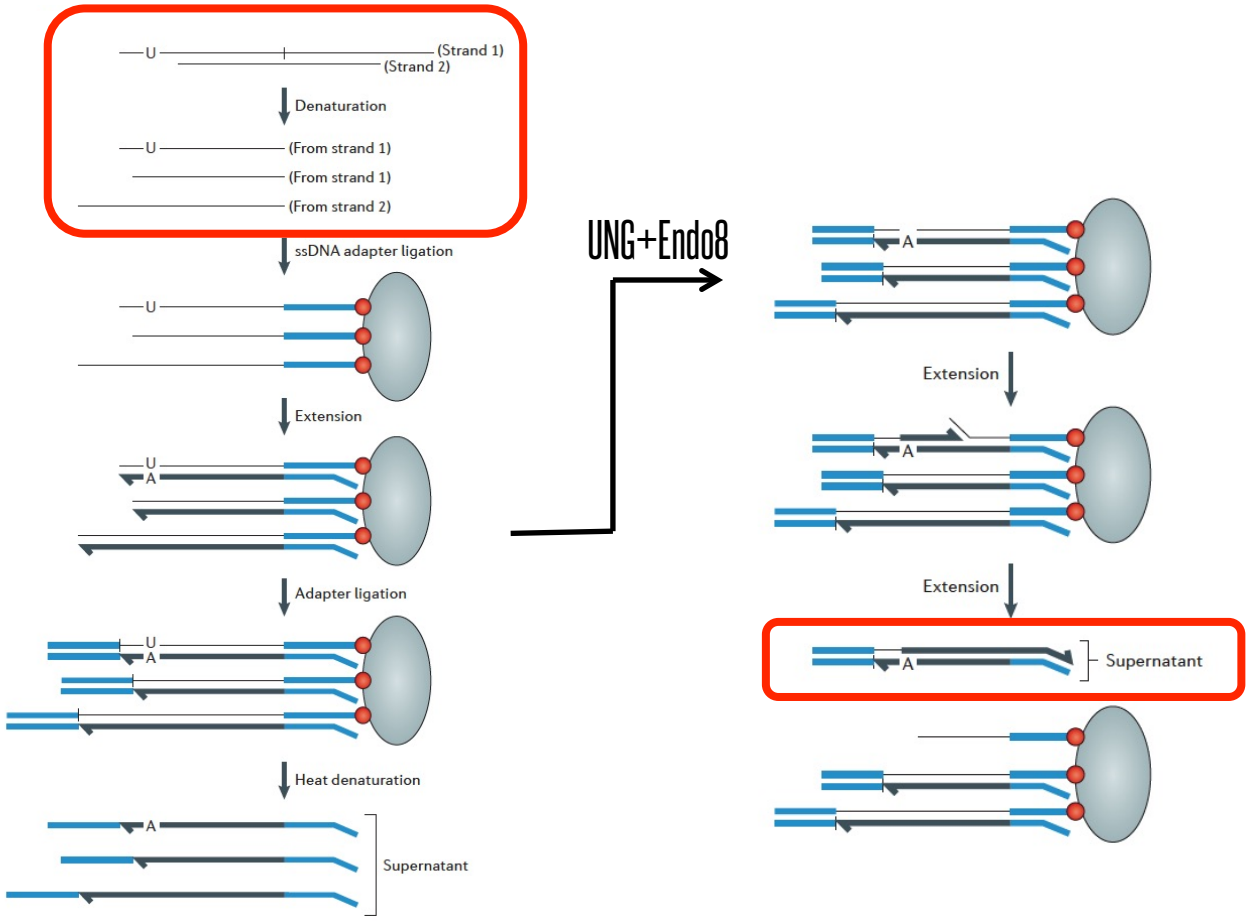


Target ssDNA Templates

Gansauge & Meyer Nat Protoc 2013

Ancient Genomics

Solution #3: Develop Ultra-Sensitive Molecular Tools

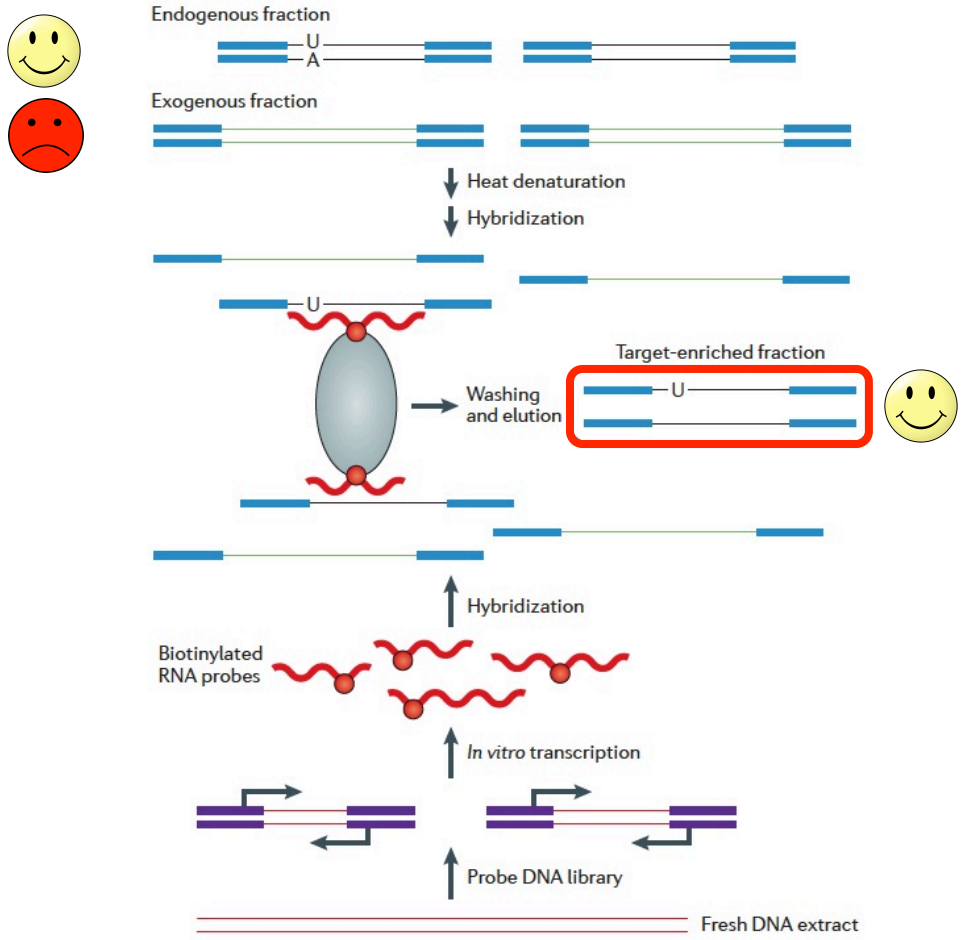


Enrich DNA Libraries for Damaged DNA

Orlando et al. Nat Rev Genetics 2015

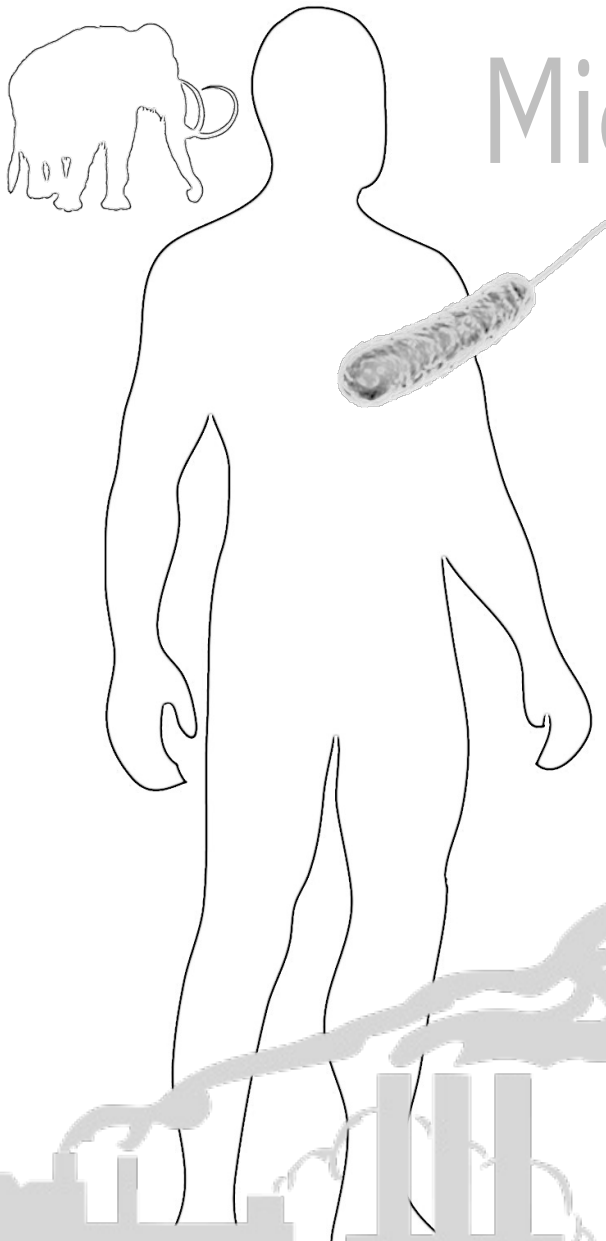
Ancient Genomics

High-Throughtput Approaches: Leveraging on Close Relatives



Enrich DNA Libraries for Target DNA

Orlando et al. Nat Rev Genetics 2015



Microbiomes

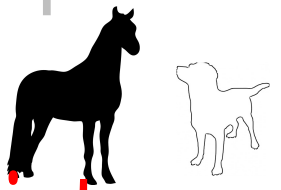
Ecosystems

Epigenomes

Pathogens

Adaptation

Genomes



Mid-Pleistocene

Origins

Migration

Extinction

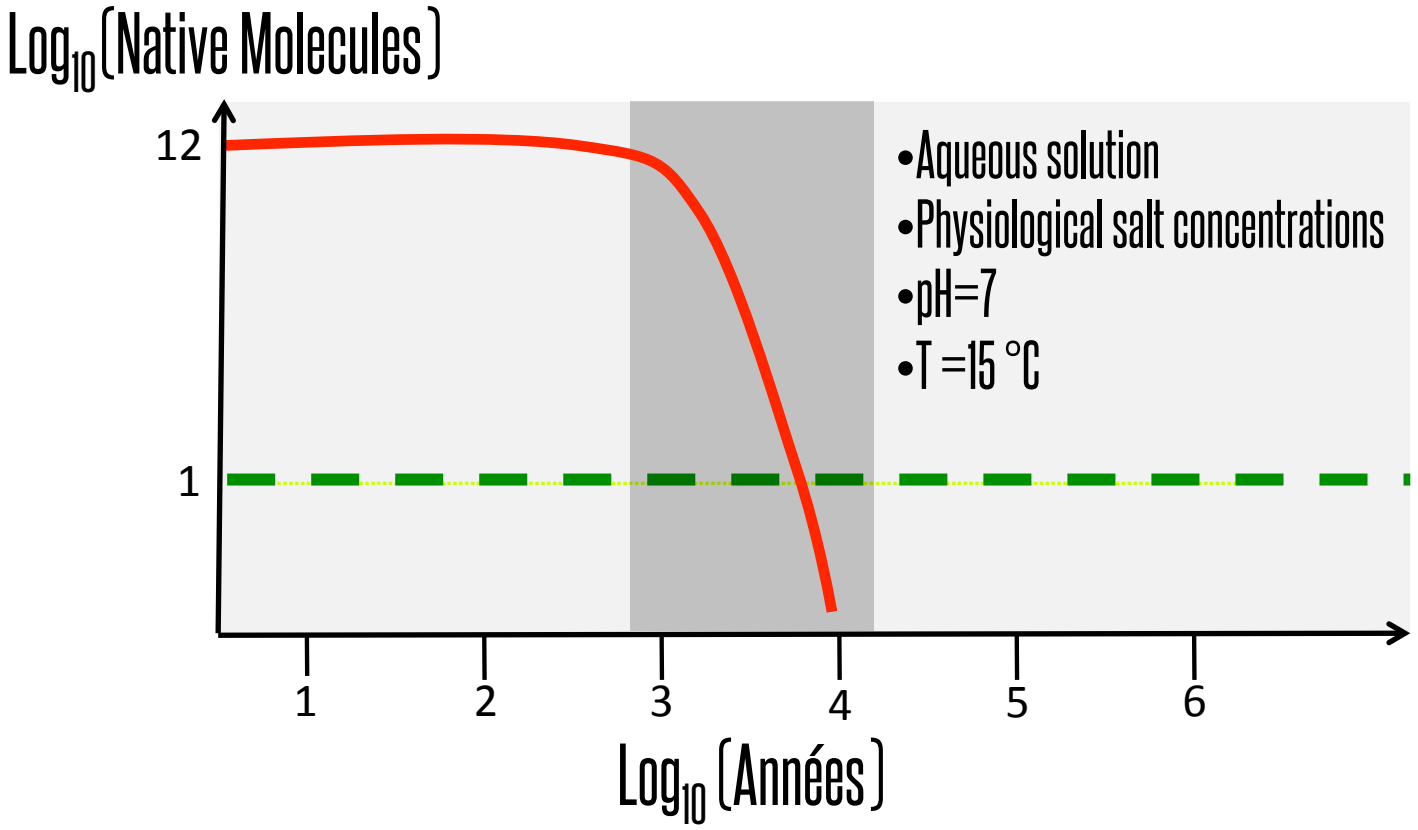
Admixture

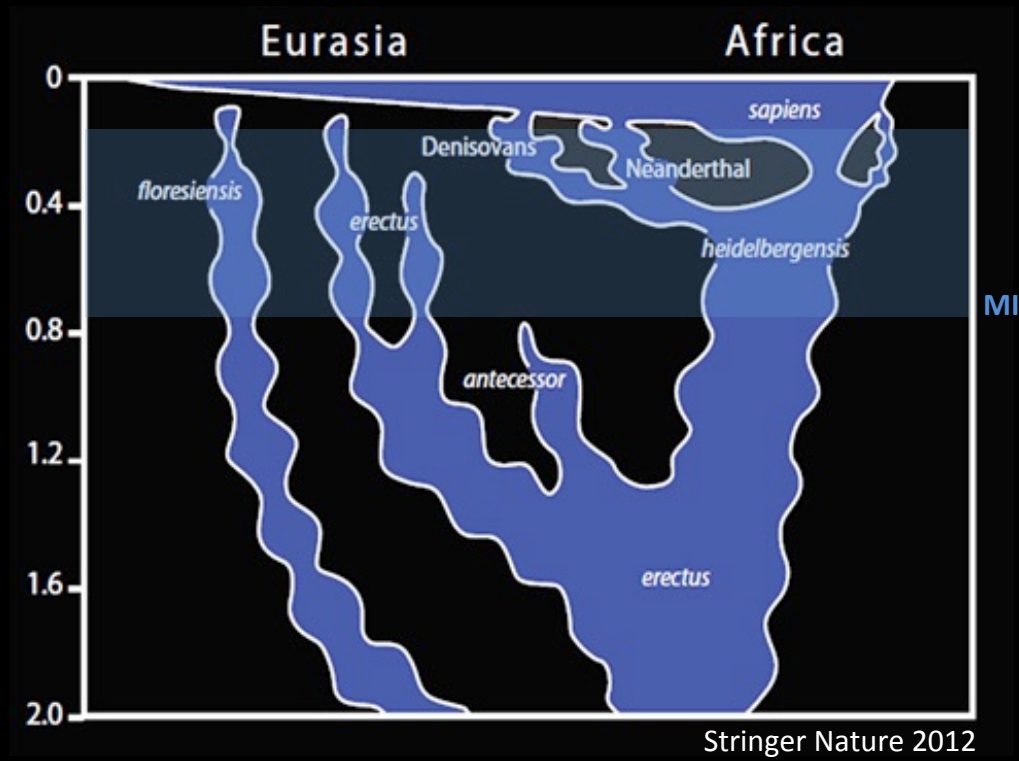
Mr Big-Shot-In-The-Field (2005)

"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

Stringer Nature 2012

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

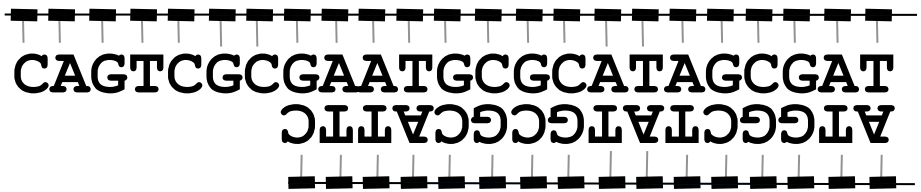
Post-Mortem DNA damage

The First Genome From the Early Middle Pleistocene

Post-Mortem DNA damage

The First Genome From the Early Middle Pleistocene

Post-Mortem DNA damage



The First Genome From the Early Middle Pleistocene

Post-Mortem DNA damage

Reference AAAGTCGCAGTCGCGAATGCGCATAGCGATGCT

X

The First Genome From the Early Middle Pleistocene

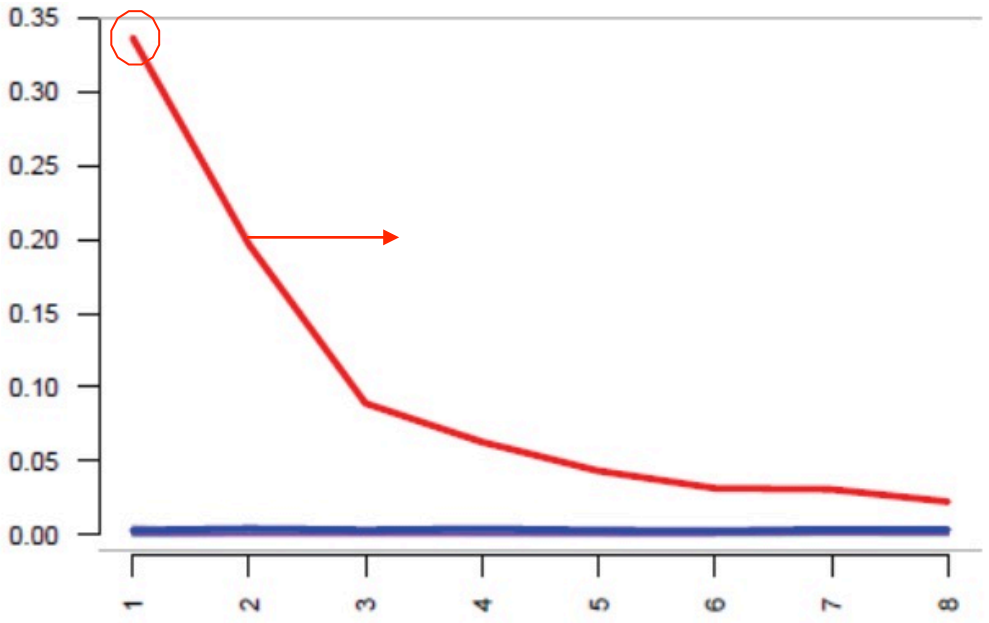
Post-Mortem DNA damage

Reference AAAGTCGCAGTCGCGAATGCGCATAGCGATGCT

The First Genome From the Early Middle Pleistocene

Post-Mortem DNA damage

$P(C_{ref} \rightarrow T_{read})$



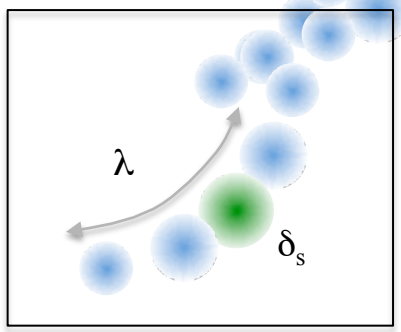
NNNNNNNNNNNNNNNNNNAAGTCGCGAATGCGCATAGCGATNNNNNNNNNNN

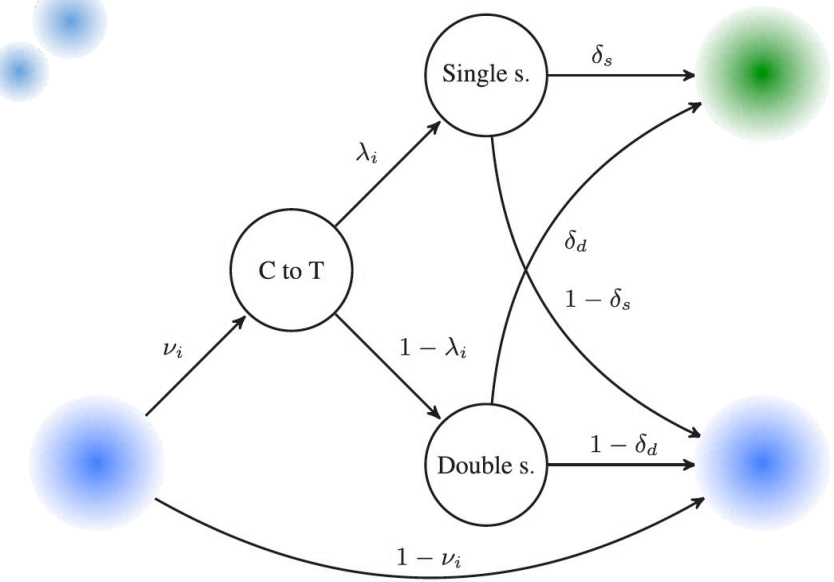
AAGTUGCGAATGCGUATAGCGAT

ATGGCTATGCGCATTC

The First Genome From the Early Middle Pleistocene

Post-Mortem DNA damage





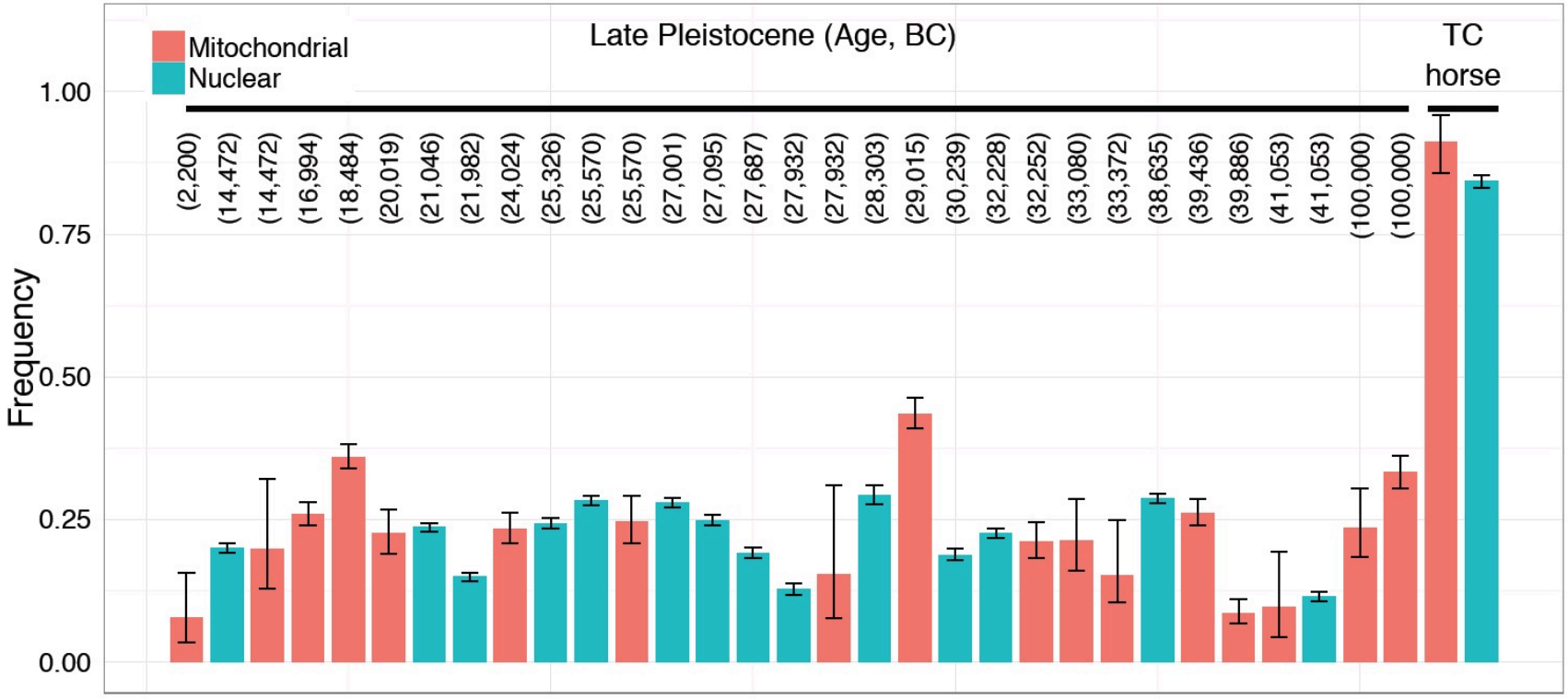
$$S_{A,i} \sim \mathrm{Mul}(D_A, (1,0,0,0) \cdot \Theta(\mu,\rho) \cdot P_{\mathrm{dam}}(\delta_d, \delta_s, \lambda, \nu, i))$$

Jonsson et al. Bioinformatics 2013


Lyakhovsky Island
2,200 BP
New Siberian Islands
16,800 BP
20,100 BP
28,800 BP
34,800 BP
A
Taymir Peninsula
13,389 BP
19,295 BP
20,036 BP
20,366 BP
22,547 BP
22,887 BP
24,149 BP
24,306 BP
24,671 BP
25,019 BP
25,534 BP
27,968 BP
29,643 BP
35,598 BP
38,565 BP
Ural Mountains
13,940 BP
15,200 BP
20,430 BP
23,740 BP
24,200 BP
28,060 BP
28.640 BP
34,800 BP
Yukon Territory
Thistle Creek
~80,000-120,000 BP
560,000-780,000 BP

The First Genome From the Early Middle Pleistocene

Cytosine Deamination Levels



OK but come on, really?


Lyakhovsky Island
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34,800 BP
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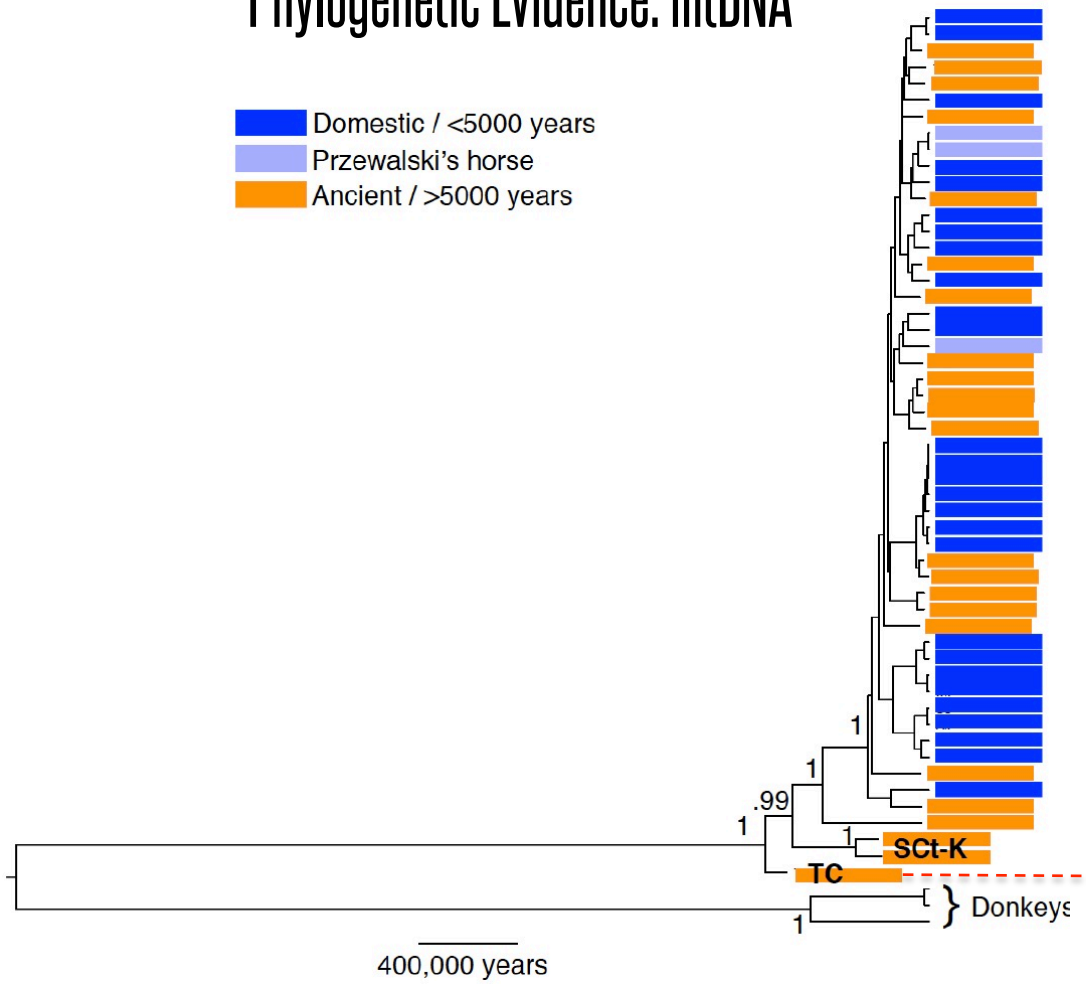
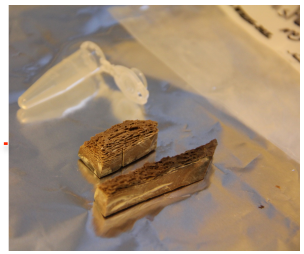
The First Genome From the Early Middle Pleistocene

Phylogenetic Evidence: mtDNA

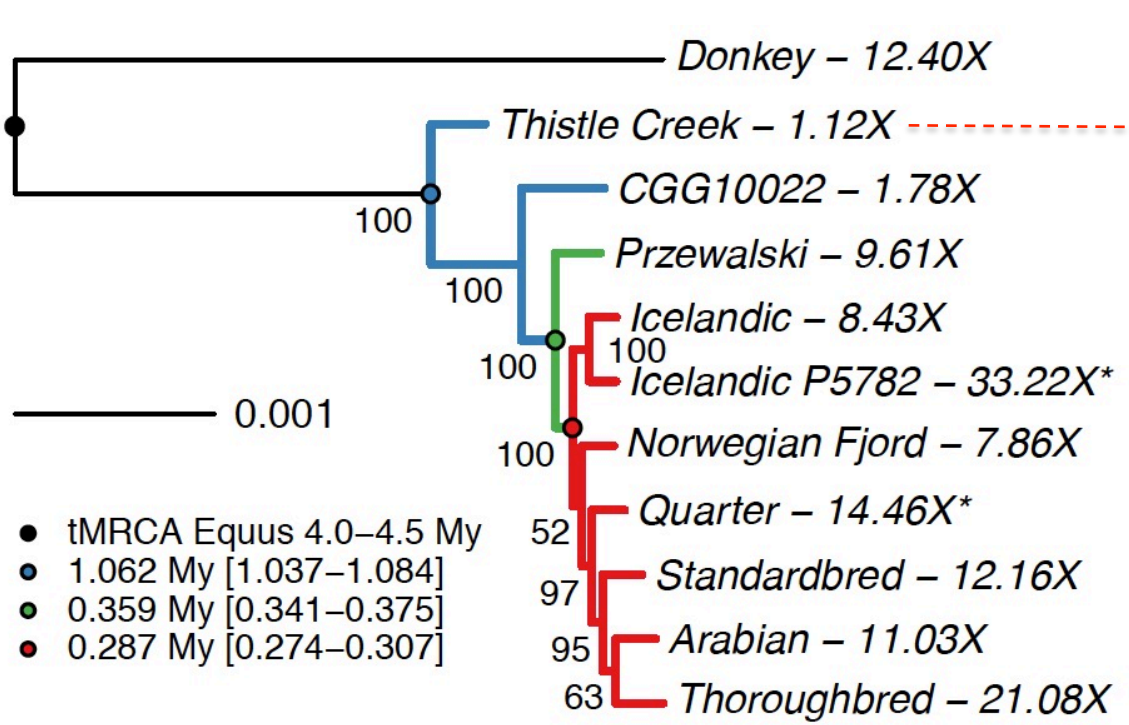




29 novel ancient complete mitochondrial genomes

The First Genome From the Early Middle Pleistocene

Phylogenetic Evidence: nucDNA



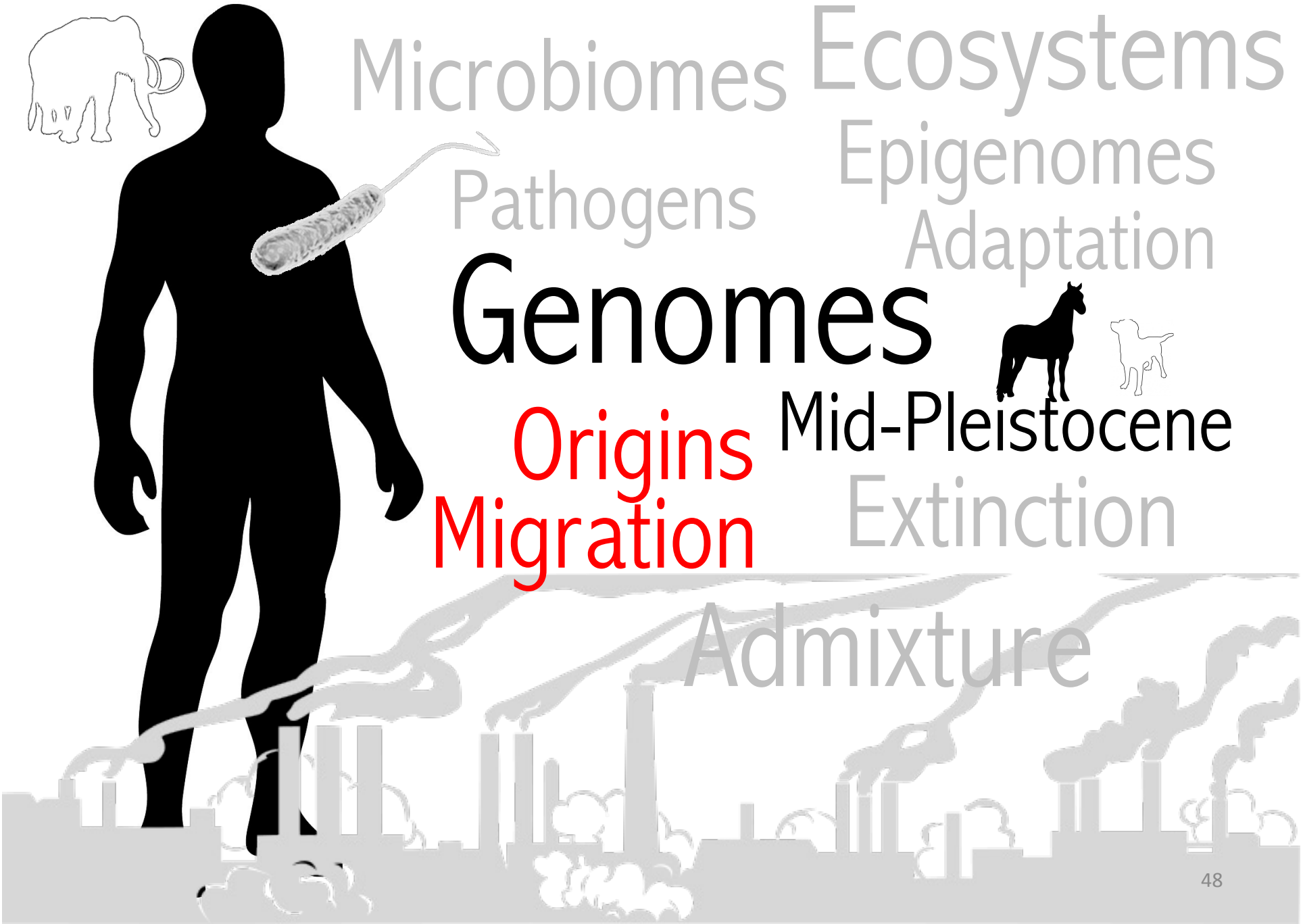
DONKEY

43 KY BP

PRZEWALSKI

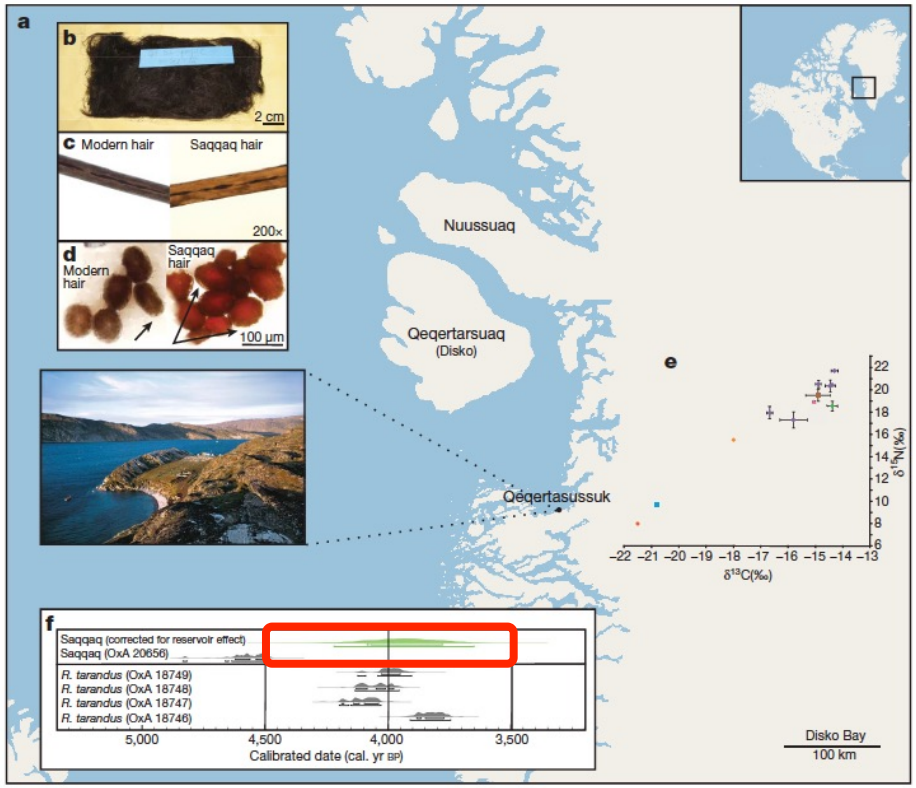
DOMESTIC BREEDS

Really.


Microbiomes
Ecosystems
Epigenomes
Pathogens
Adaptation
Genomes
Origins
Migration
Mid-Pleistocene
Extinction
Admixture

Ancient Genomes Help Refining Our Origins

The First Ancient Human Genome

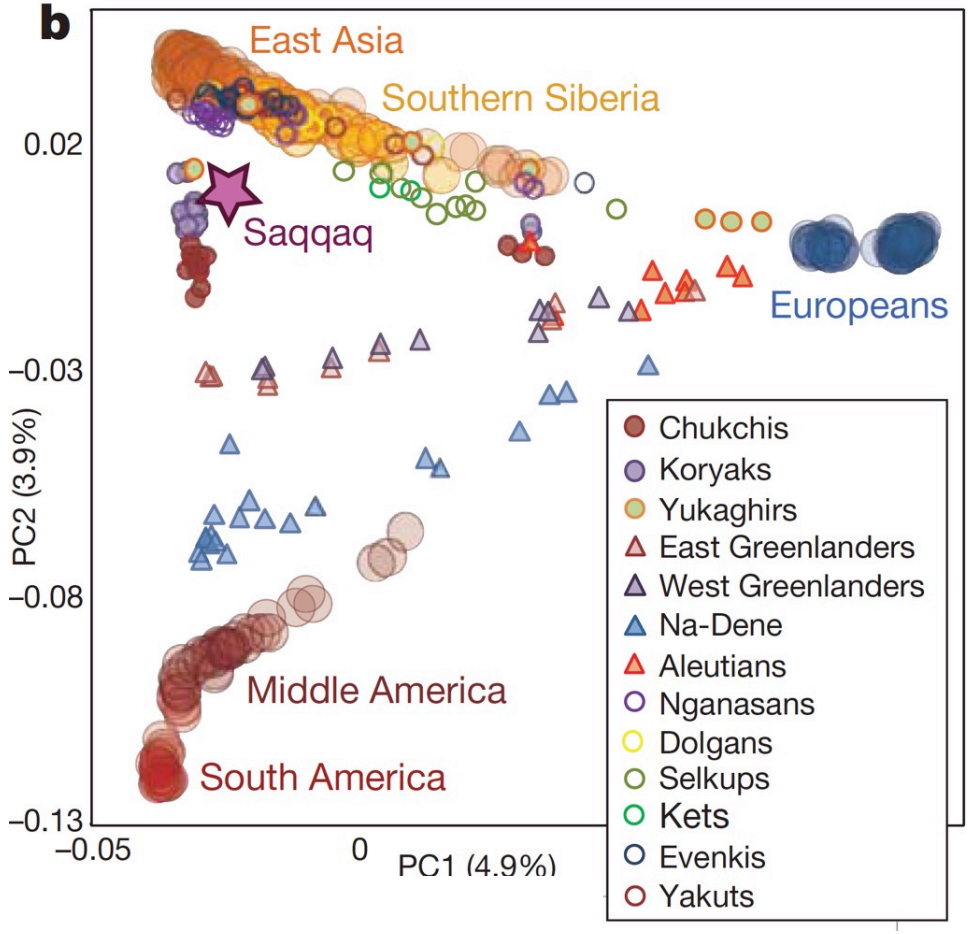


Rasmussen et al. Nature 2010

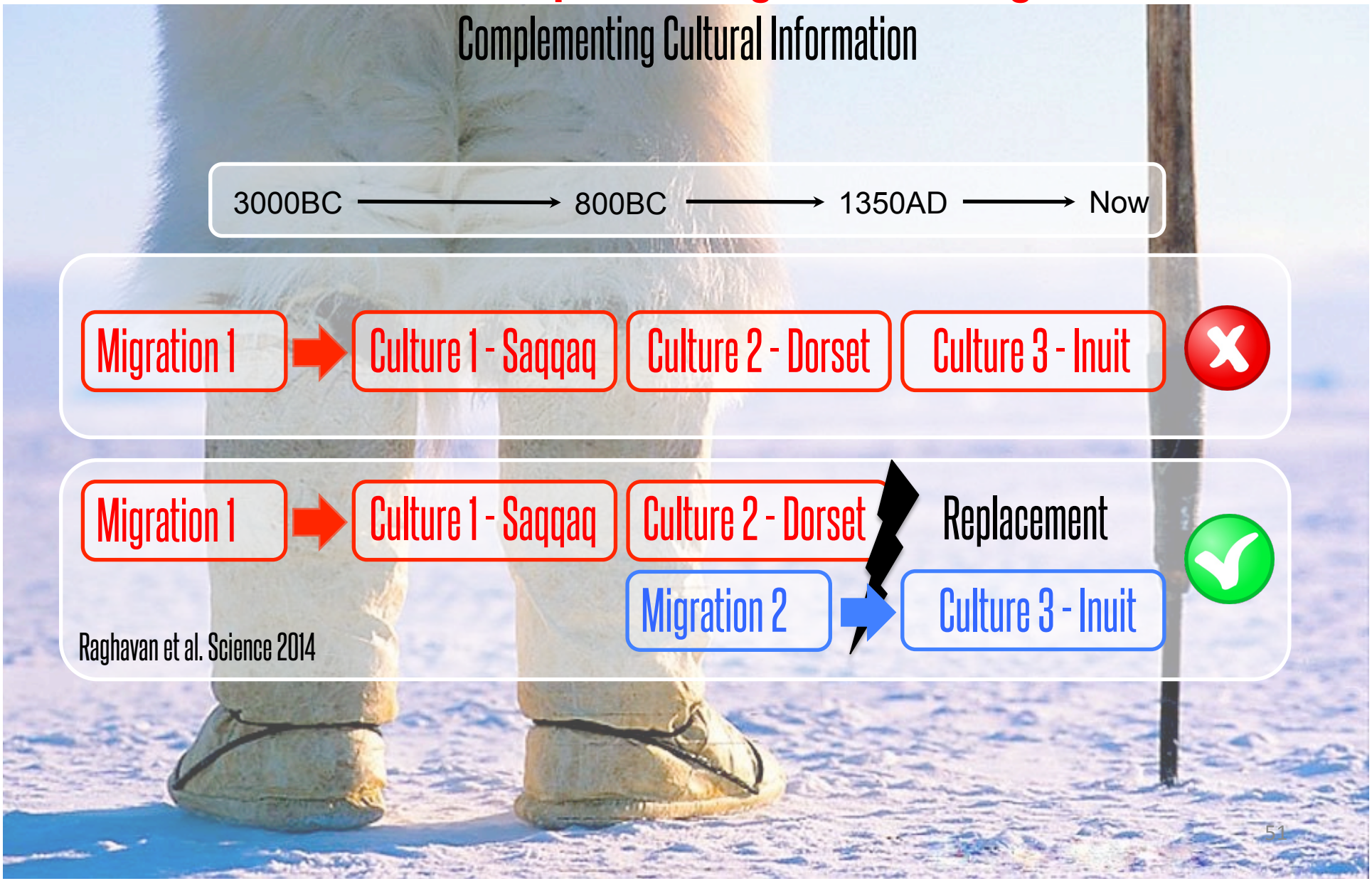
Ancient Genomes Help Refining Our Origins

The Peopling of the New World Arctic

Rasmussen et al. Nature 2010

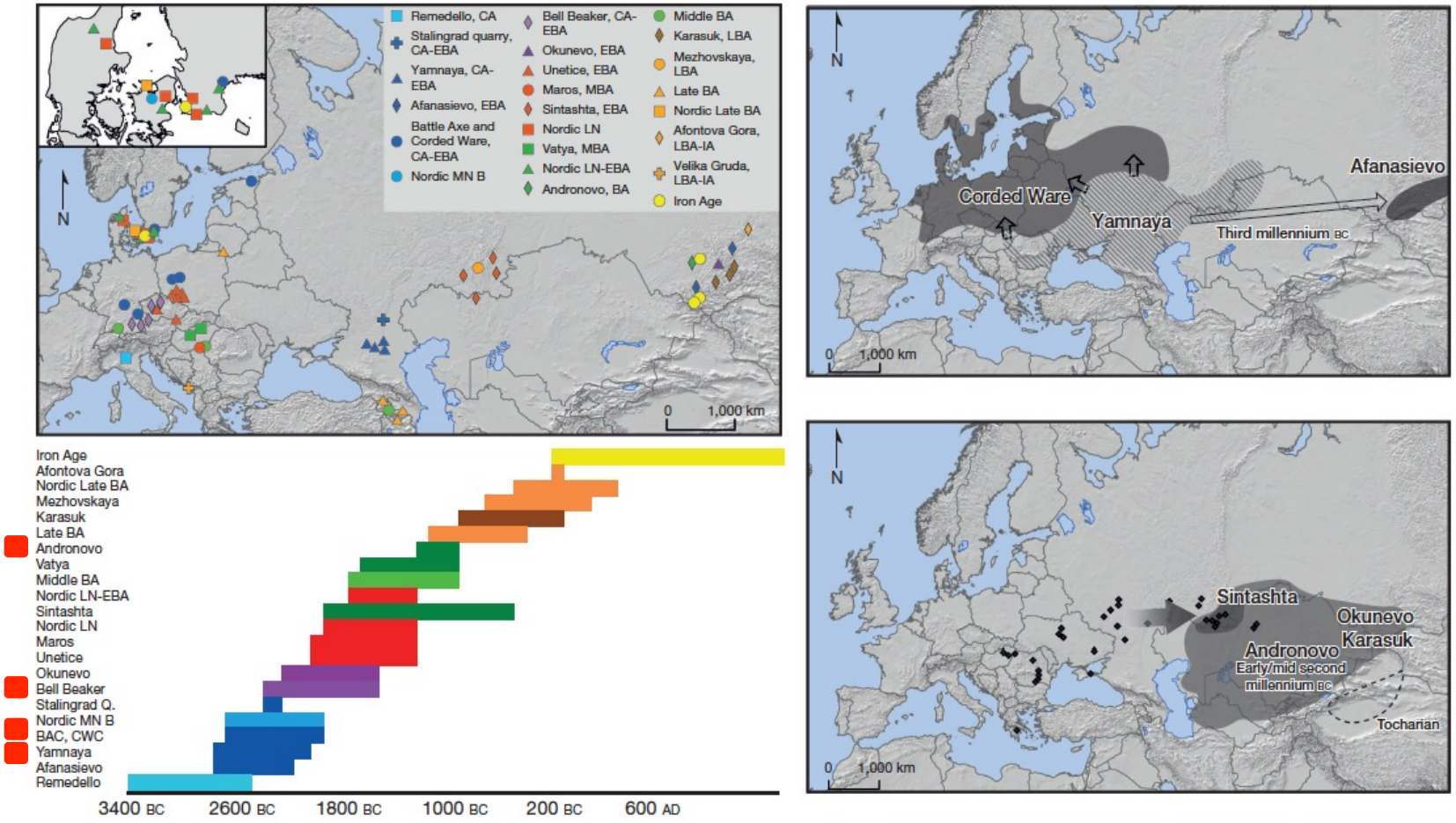


Ancient Genomes Help Refining Archaeological Models



Bronze Age Genomics

>100 Ancient Human Genomes

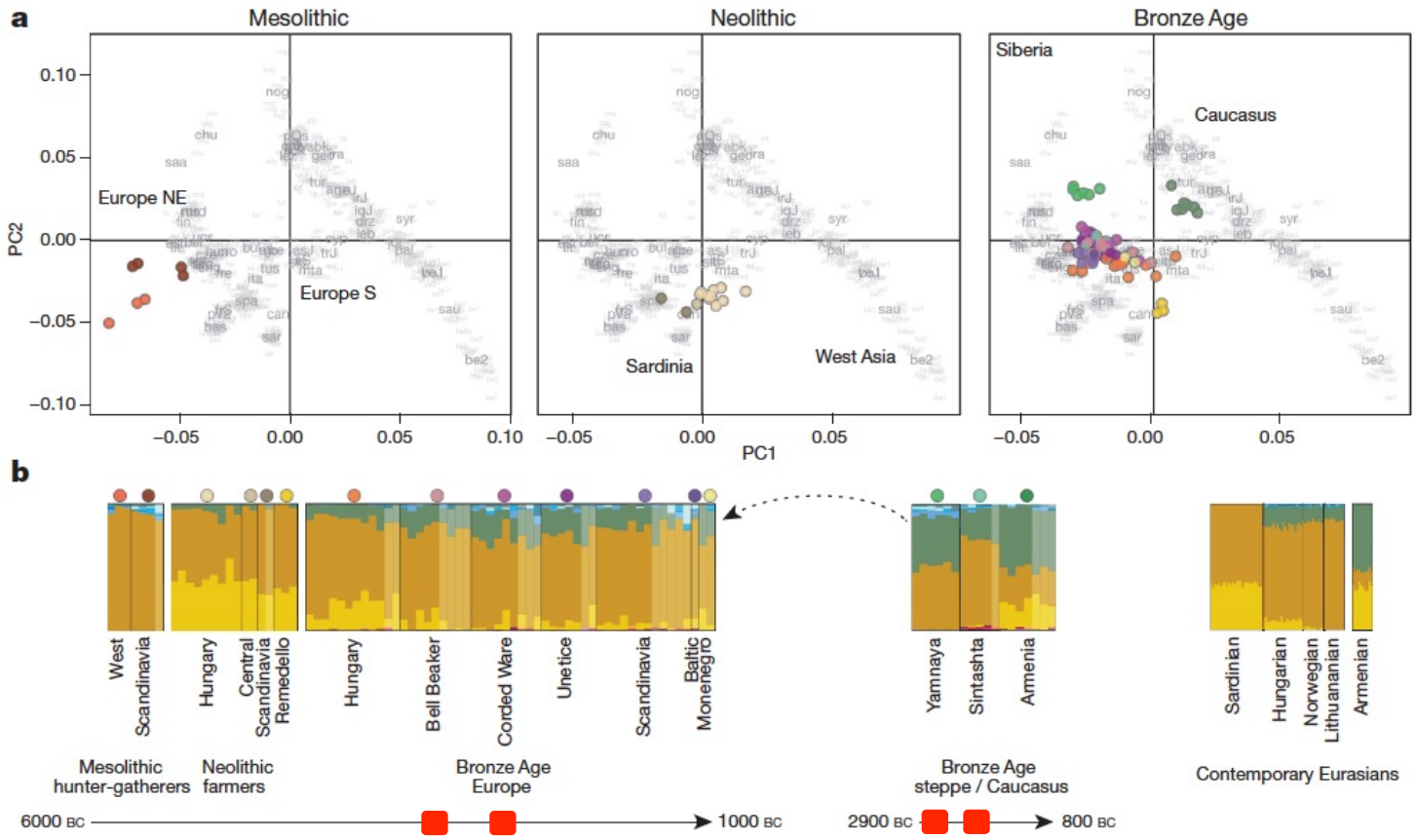


Allentoft et al. Nature 2015

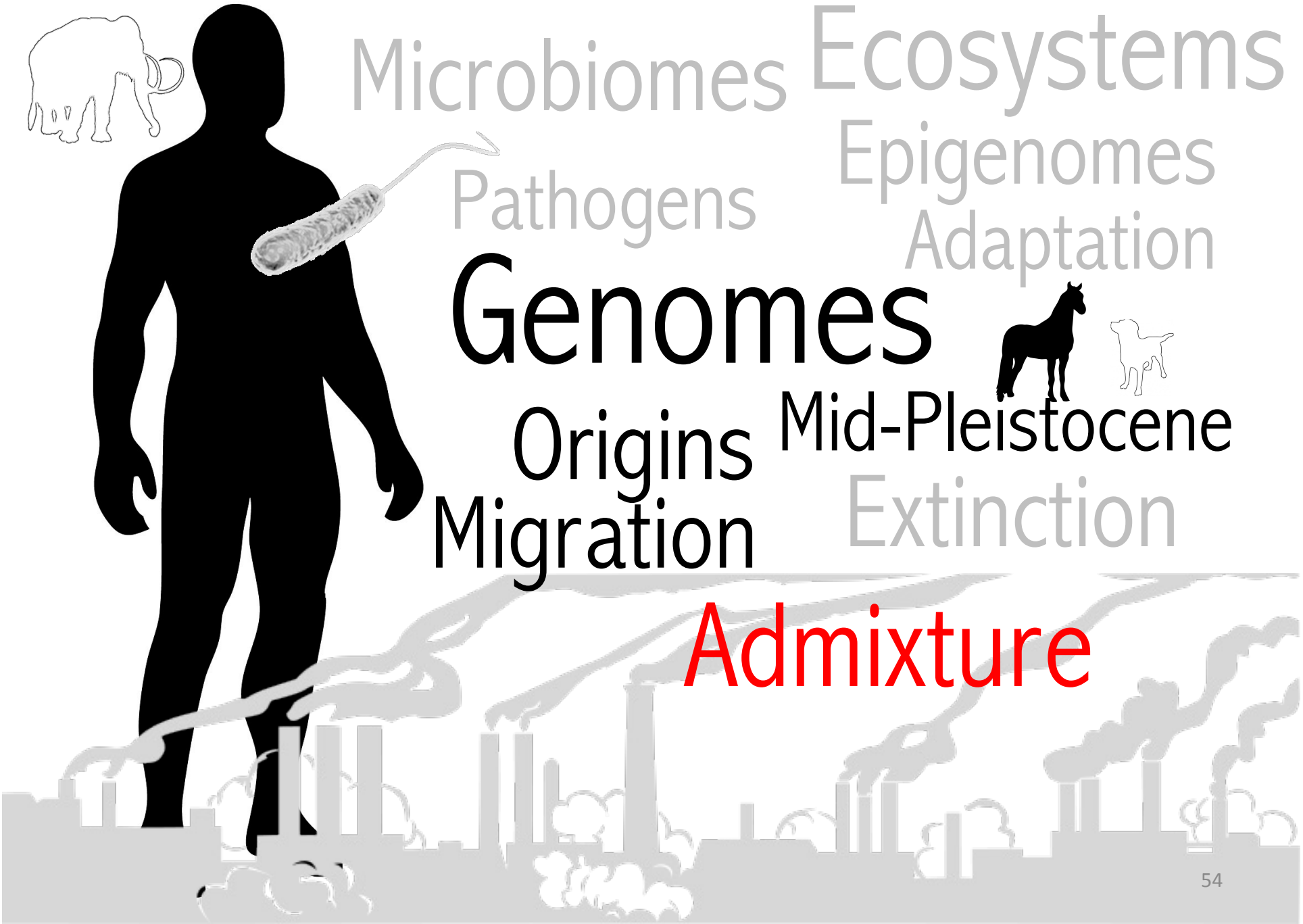
Bronze Age Genomics

>100 Ancient Human Genomes

Europe

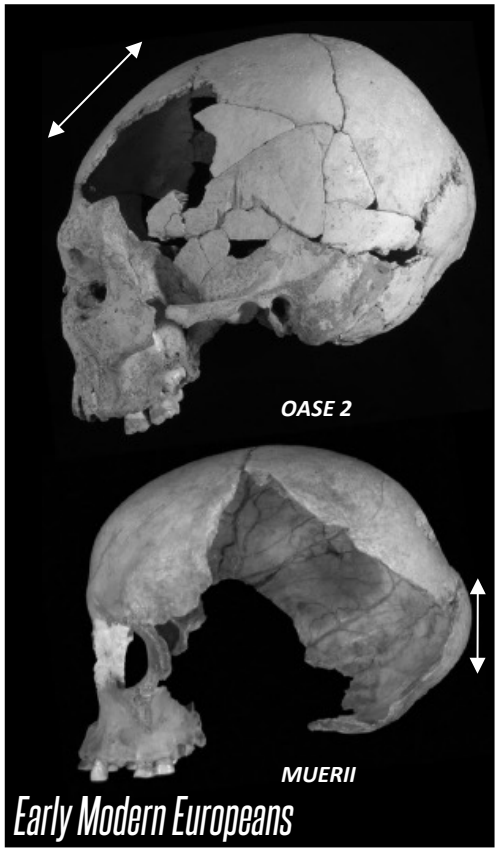


Allentoft et al. Nature 2015


Microbiomes
Ecosystems
Epigenomes
Pathogens
Adaptation
Genomes
Origins
Mid-Pleistocene
Migration
Extinction
Admixture

Archaic hominins

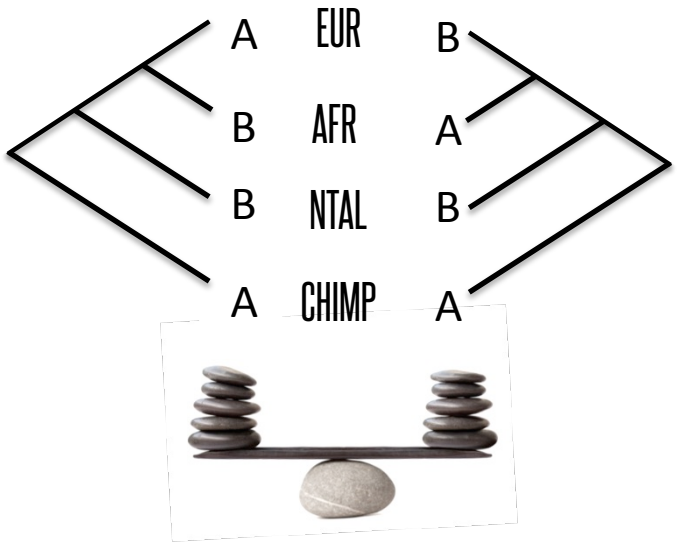
Neandertal Genomics



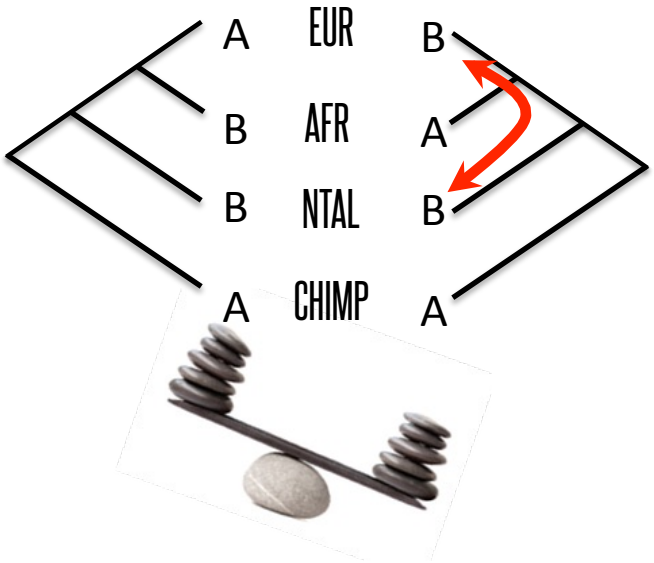
Archaic hominins

Neandertal Genomics

D-Statistics



NO Admixture

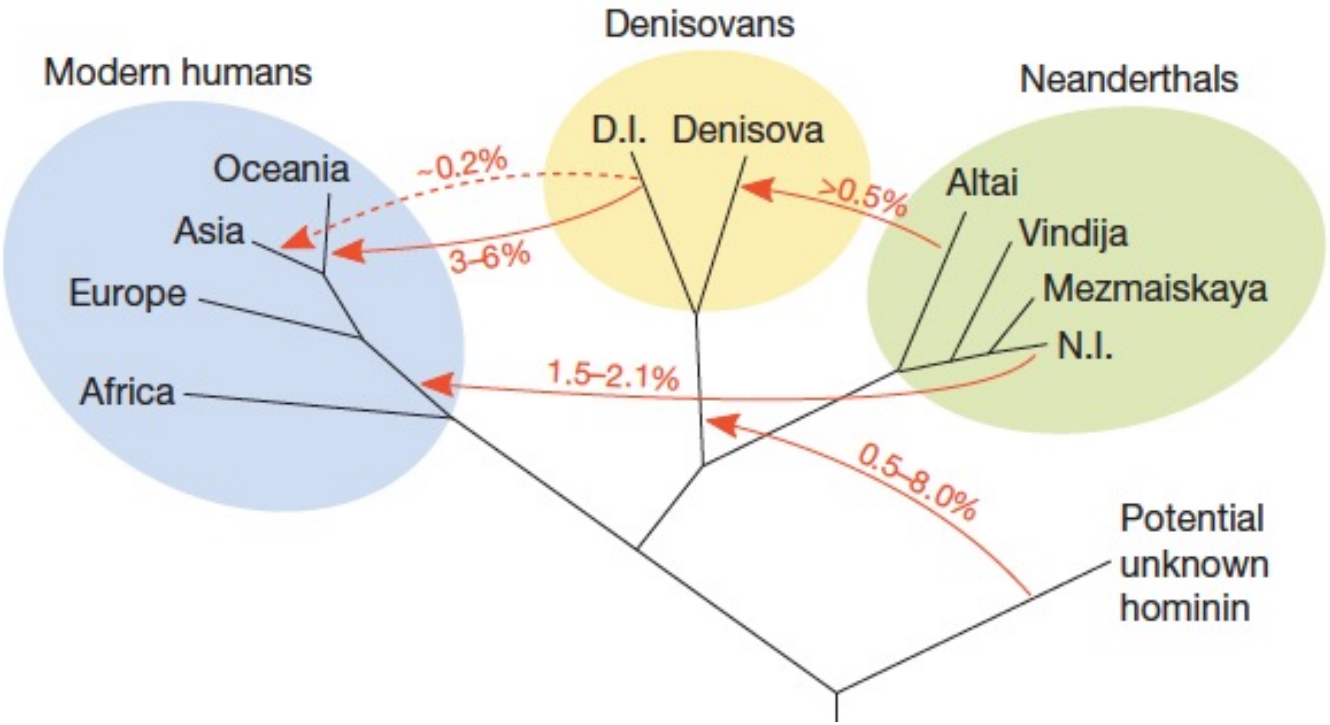


Admixture

Archaic hominins

Neandertals, Denisovans, Ghosts

Admixture

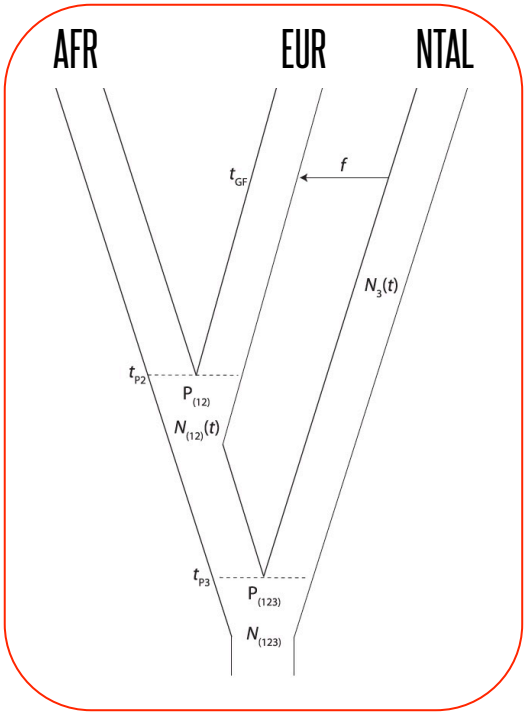


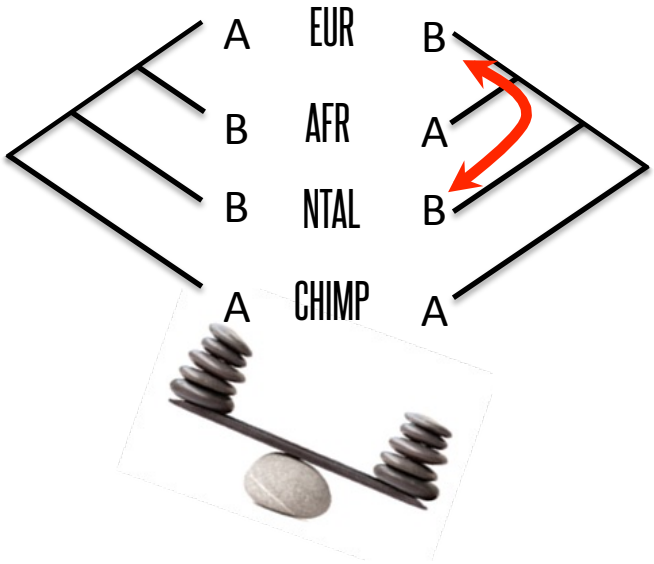
From Prufer et al. Nature 2014

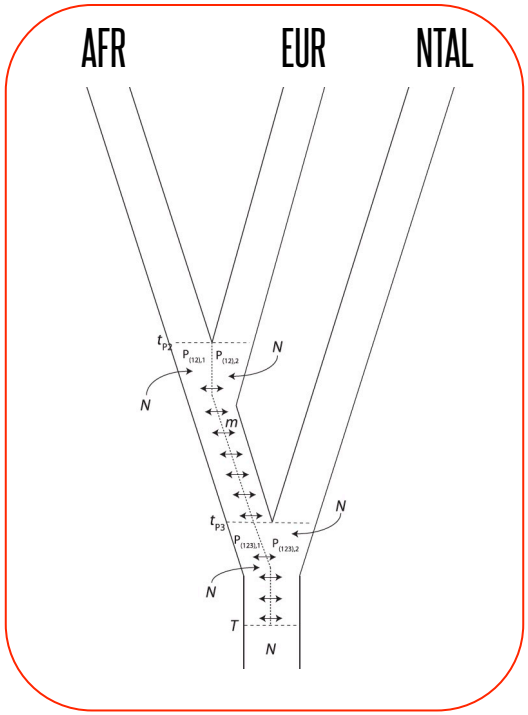
Archaic hominins

Neandertals, Denisovans, Ghosts

Admixture







Admixture

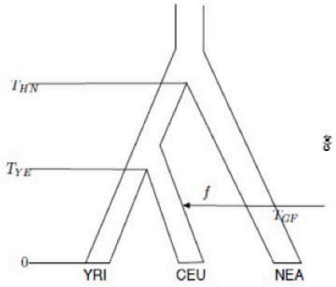
From Durand et al. Mol Biol Evol 2011

Archaic hominins

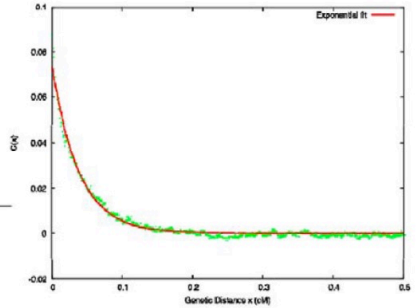
Neandertals, Denisovans, Ghosts

Admixture

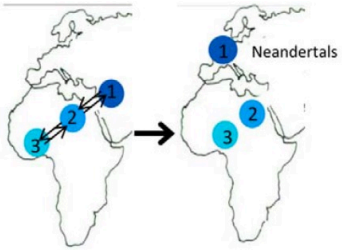
(a) Gene flow <100,000 years ago



Long-Range LD expectation | Neand_Der

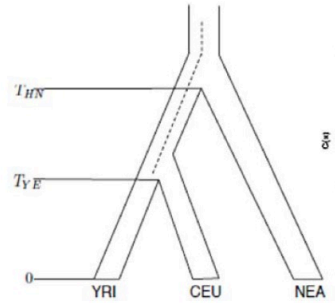


(b) Ancient structure >230,000 years ago

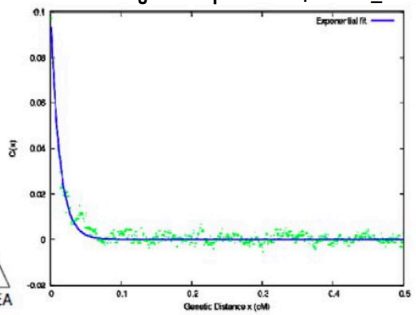


Human structure to Neandertal-modern human divergence

Northeast Africans retain extra proximity to Neandertals



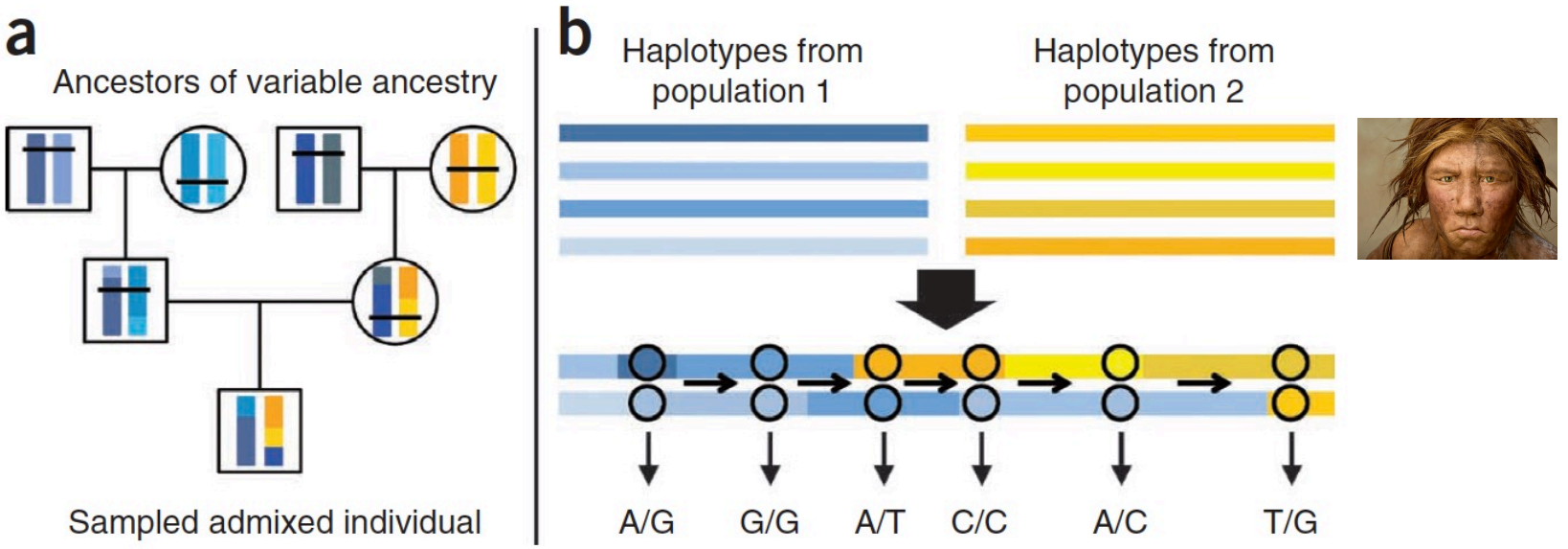
Short-Range LD expectation | Neand_Der



Sankararaman et al. PLoS Genet 2012

Archaic hominins

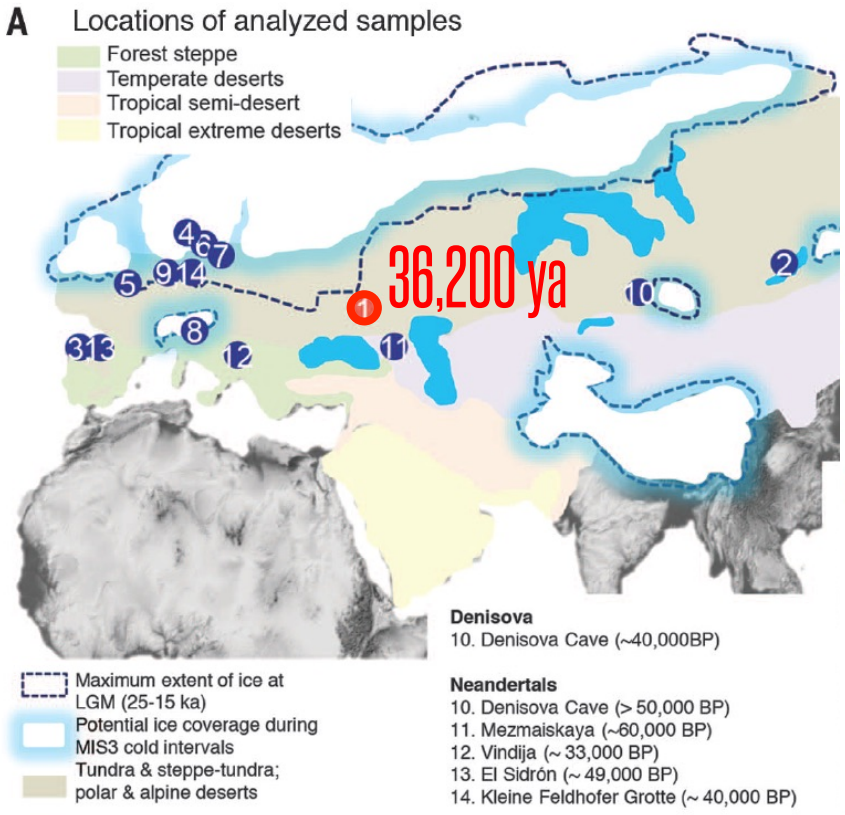
Dating the Admixture with Neanderthals (~55 kyr ago)

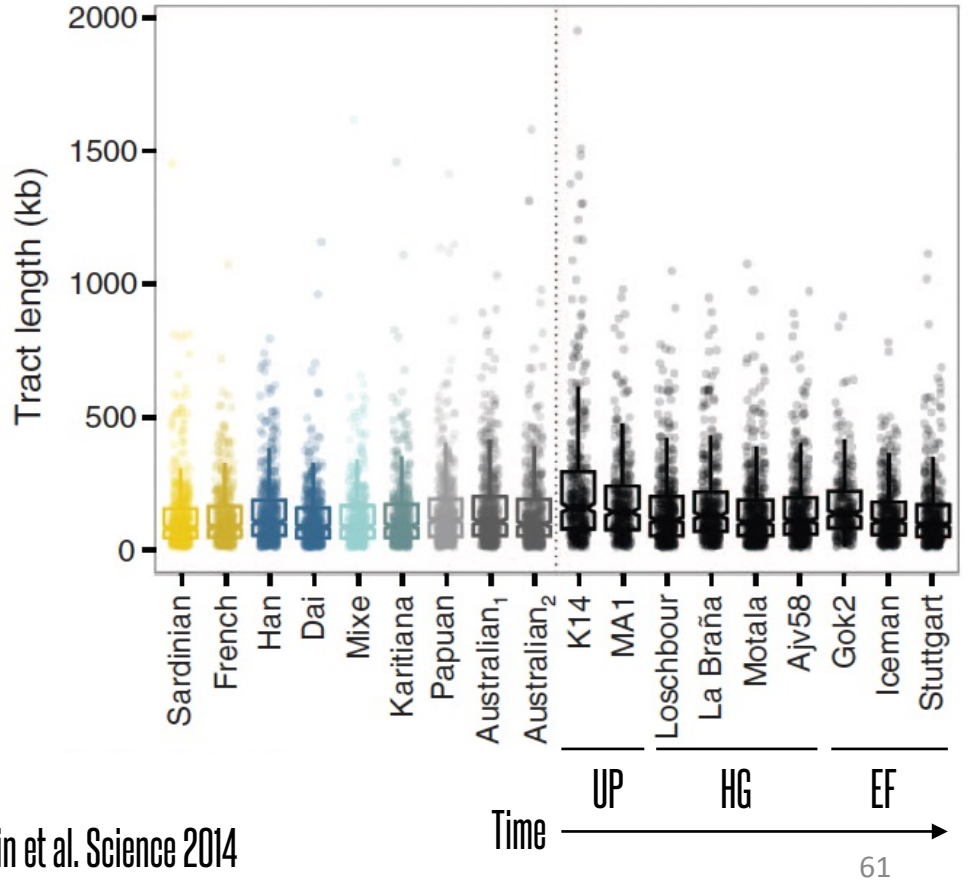


Wegmann et al. Nat Genet 2011

Archaic hominins

Dating the Admixture with Neanderthals

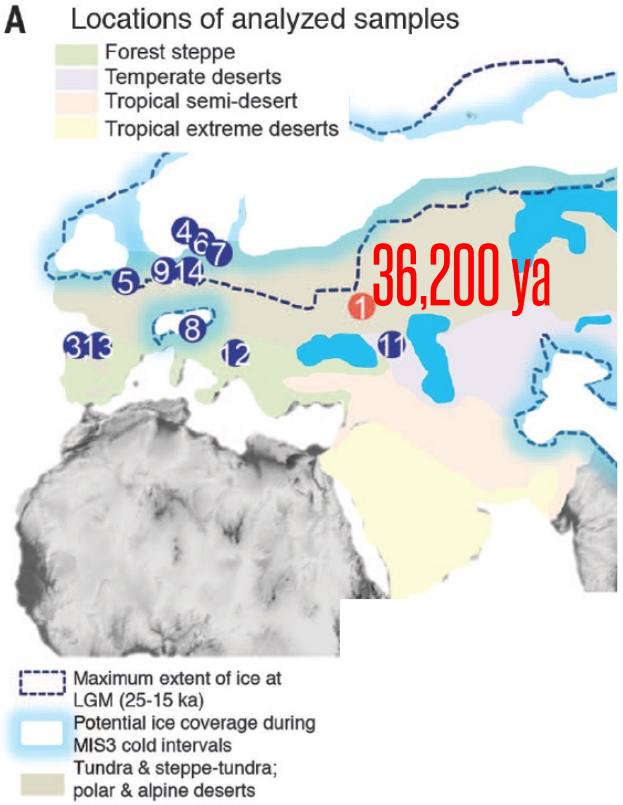


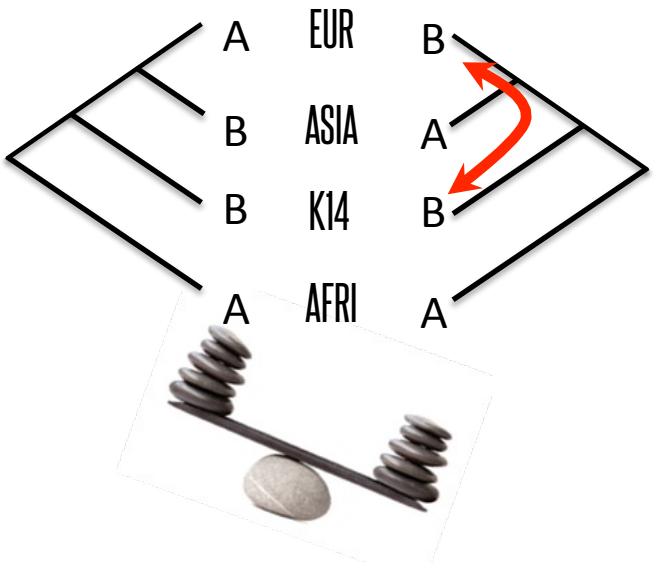


Orlando-Seguin et al. Science 2014

Upper Paleolithic Humans

Kostenki K14 (f3- and D-statistics)





Seguin-Orlando et al. Science 2014

Upper Paleolithic Humans

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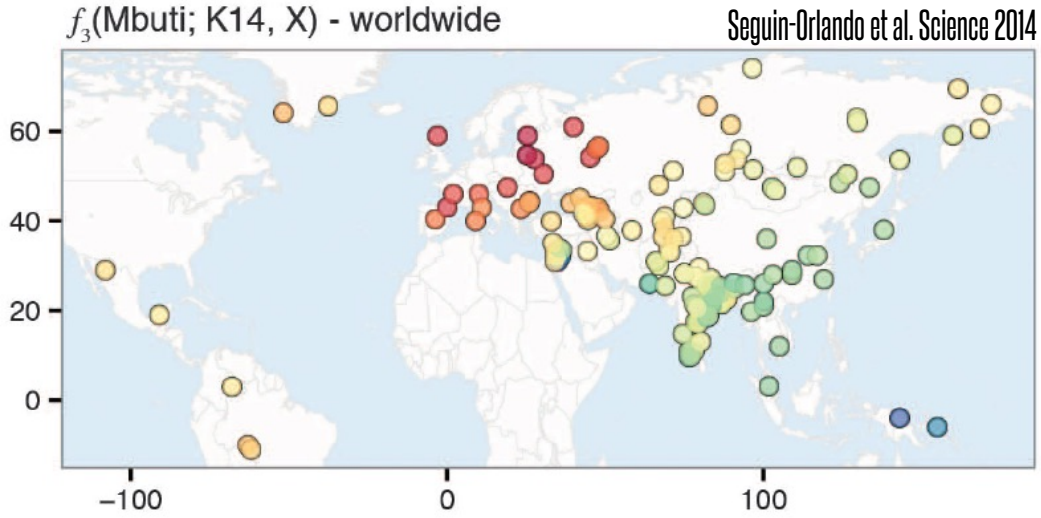
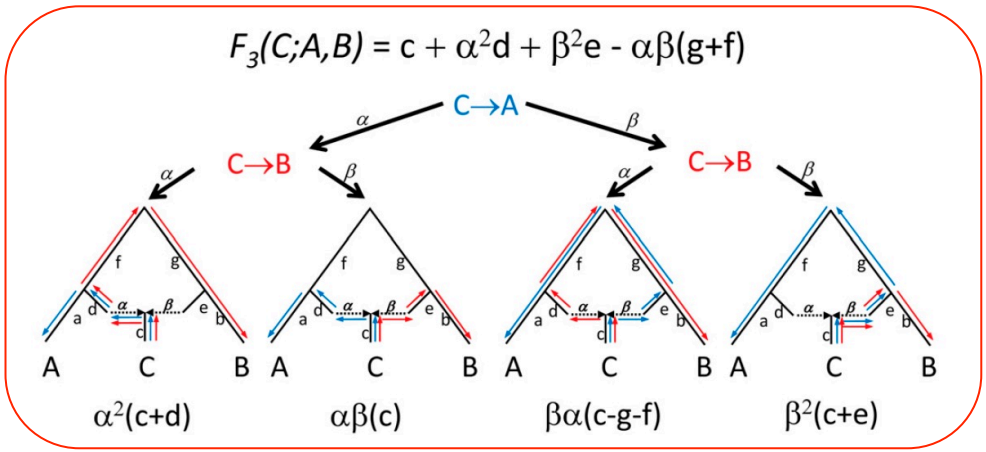
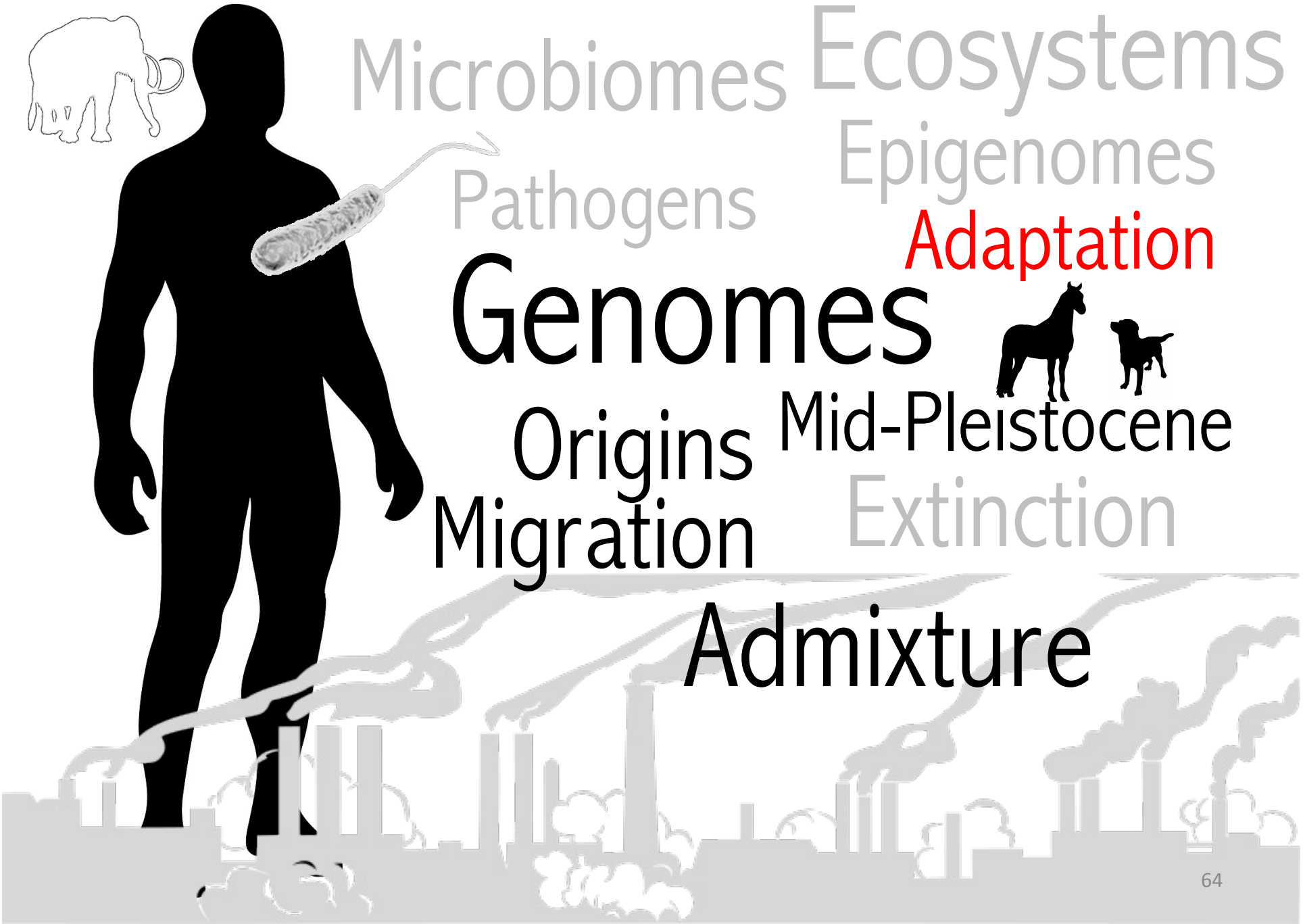




Patterson et al. Genetics 2012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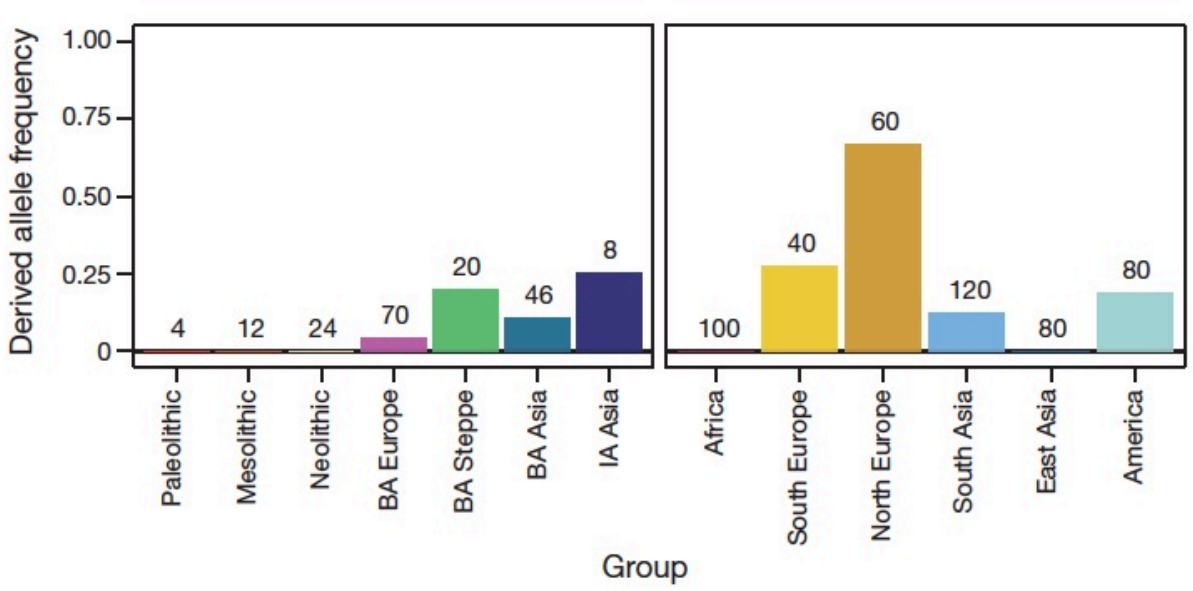
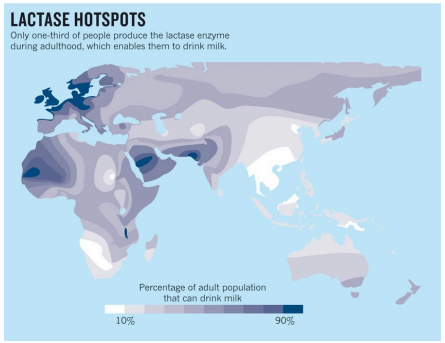
Lactose Tolerance

$f_{\%}(LCT)$



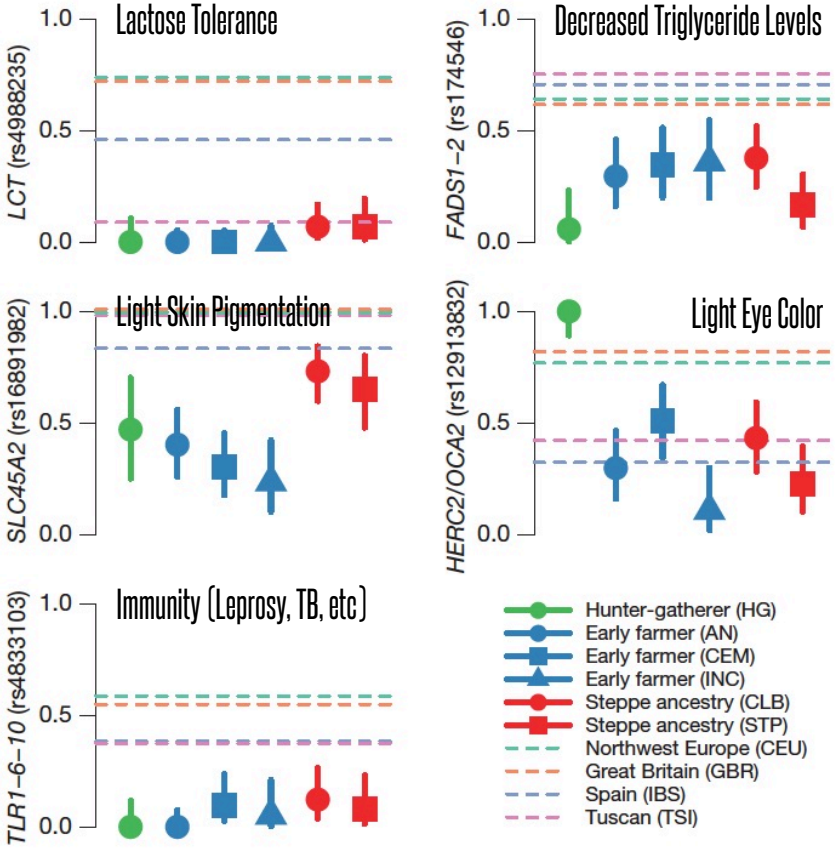


Allentoft et al. Nature 2015

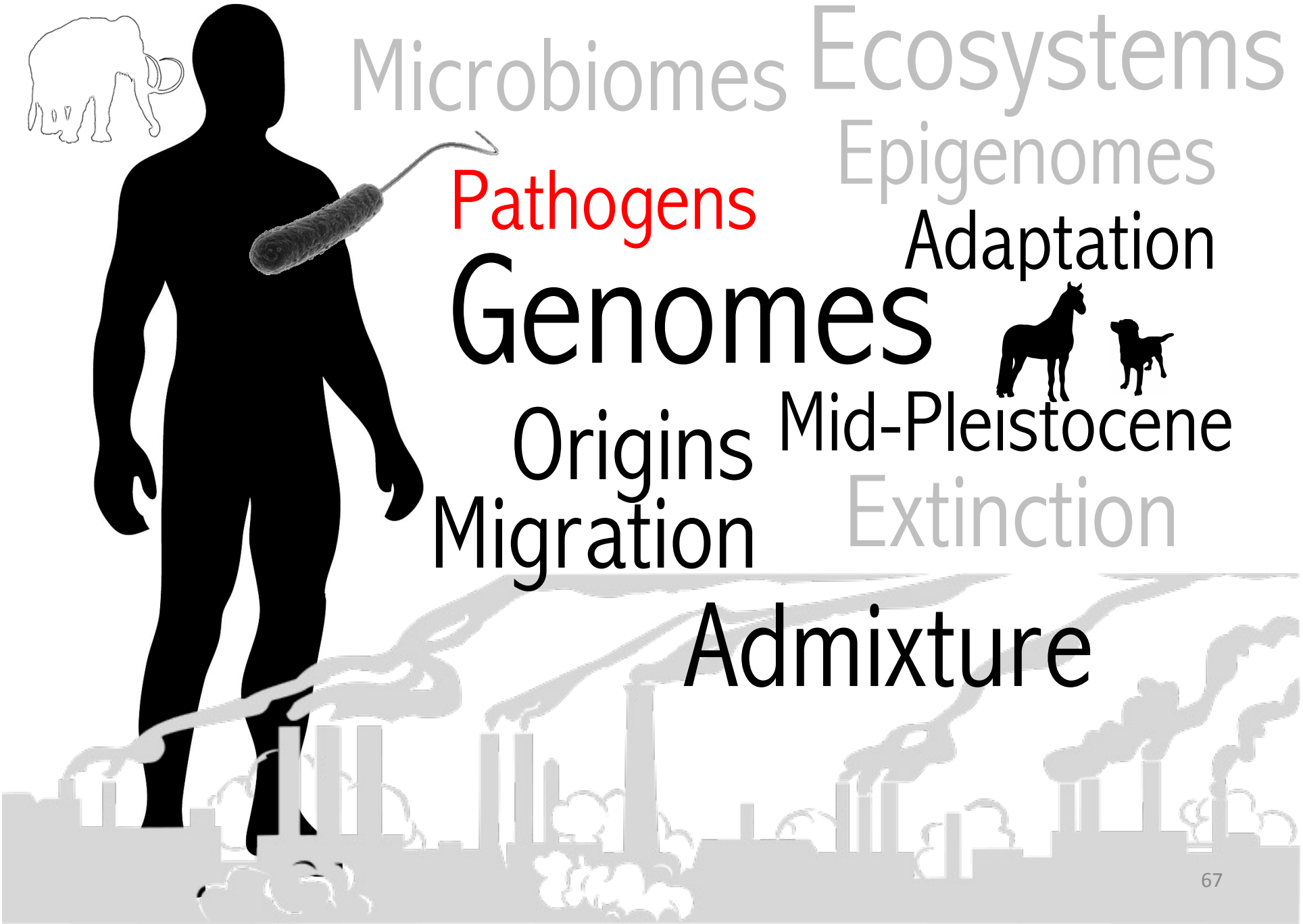
Bronze Age Genomics

>100 Ancient Human Genomes

Selection Patterns



Mathieson et al. Nature 2015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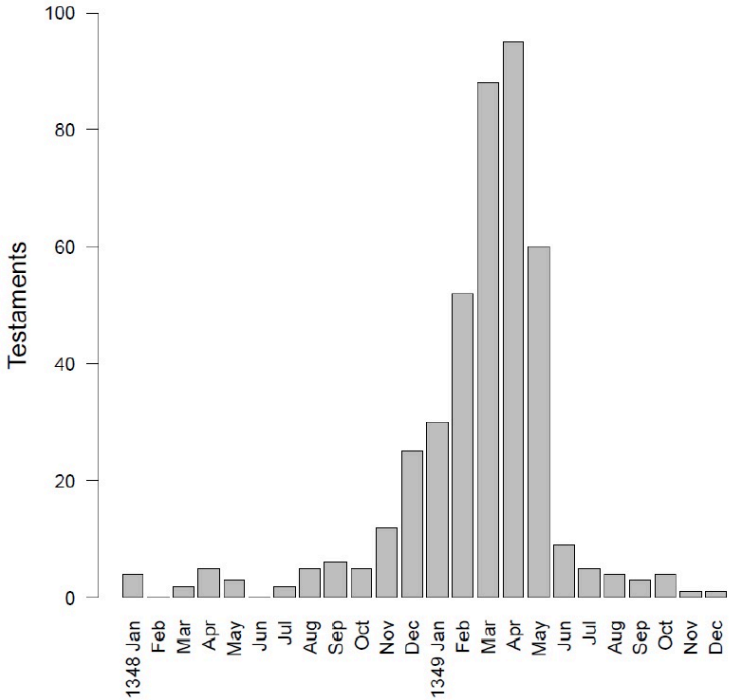
Beyond Genomes, Microbiomes

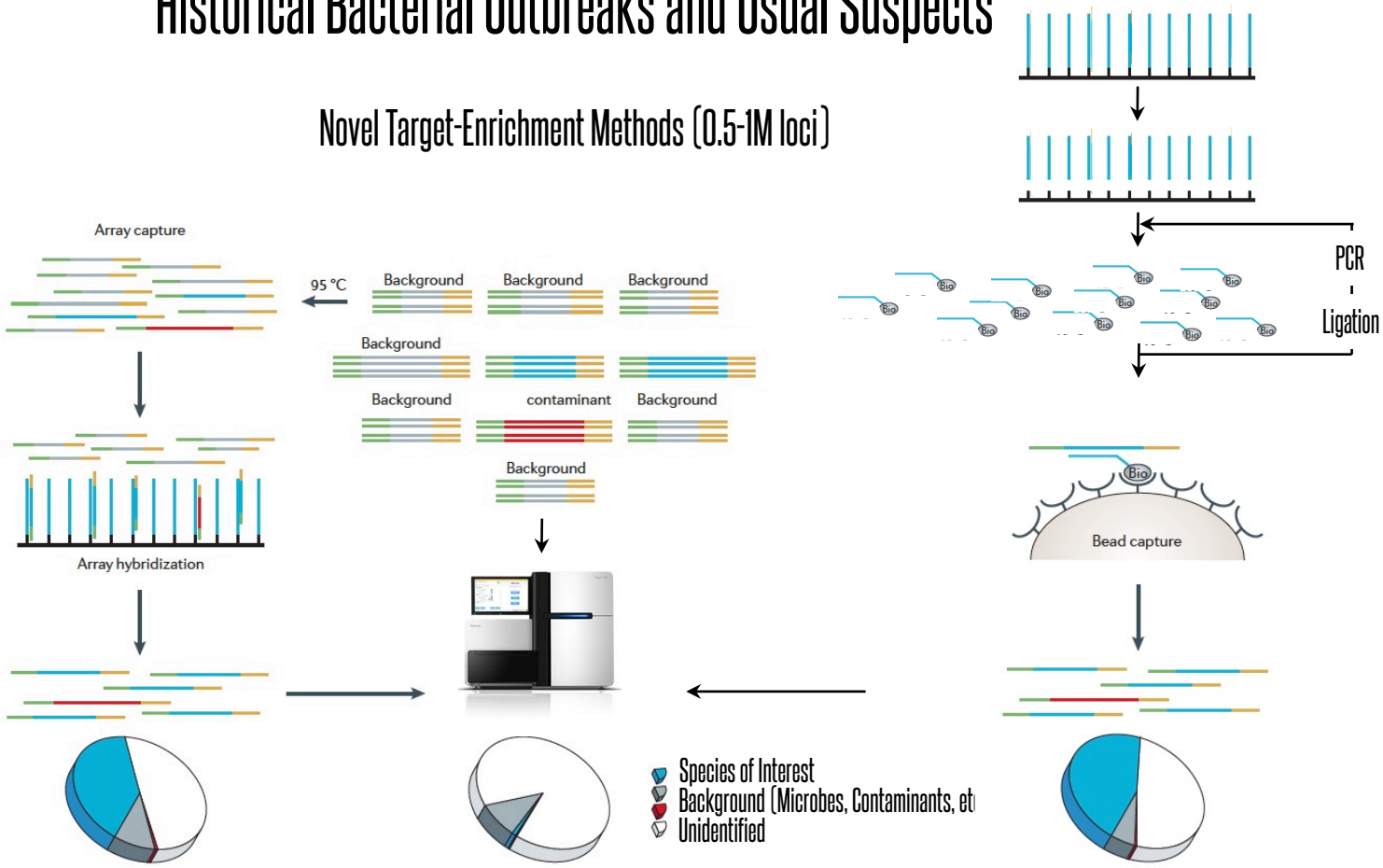
Historical Bacterial Outbreaks and Usual Suspects



Beyond Genomes, Microbiomes

Historical Bacterial Outbreaks and Usual Suspects

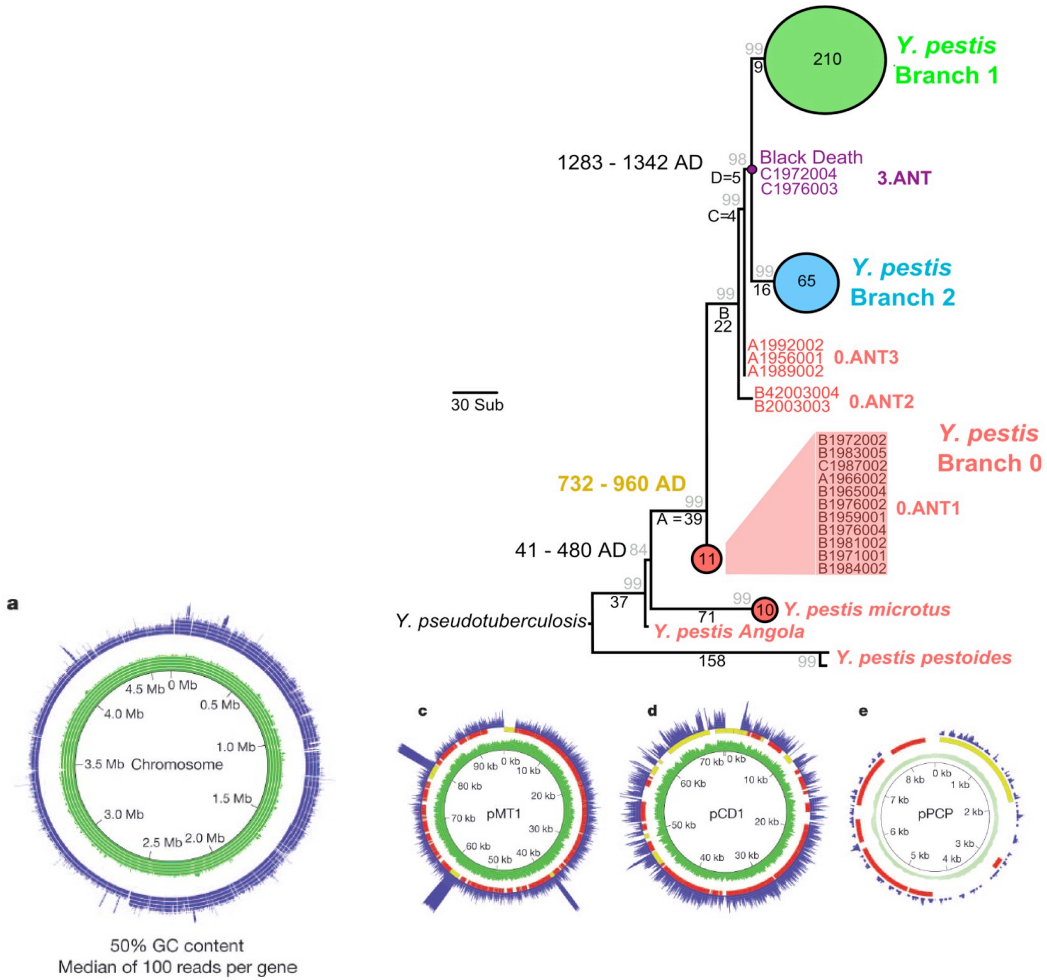
Novel Target-Enrichment Methods (0.5-1M loci)



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

Beyond Genomes, Microbiomes

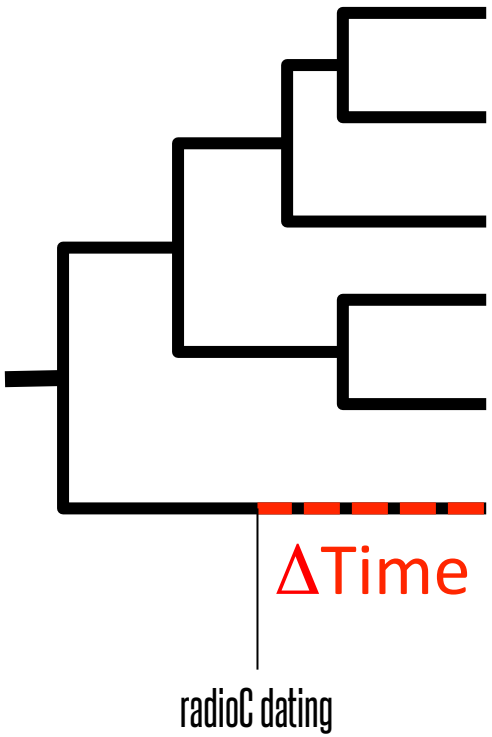
Historical Bacterial Outbreaks and Usual Suspects



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

Beyond Genomes, Microbiomes

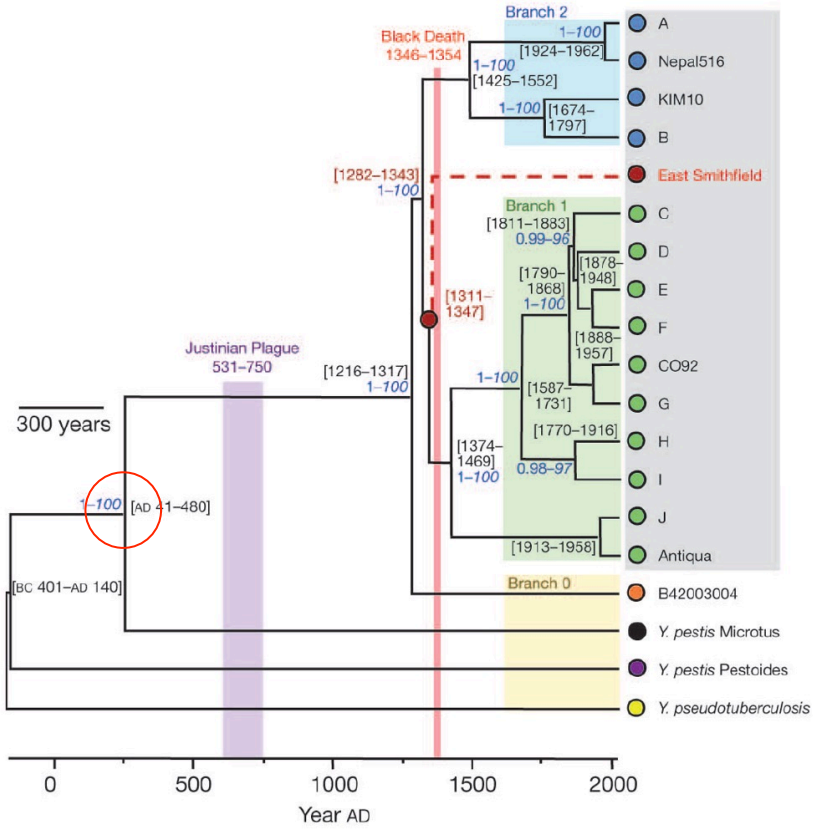
Historical Bacterial Outbreaks and Usual Suspects



Branch-Shortening based Calibration of Molecular Clocks

Beyond Genomes, Microbiomes

Historical Bacterial Outbreaks and Usual Suspects

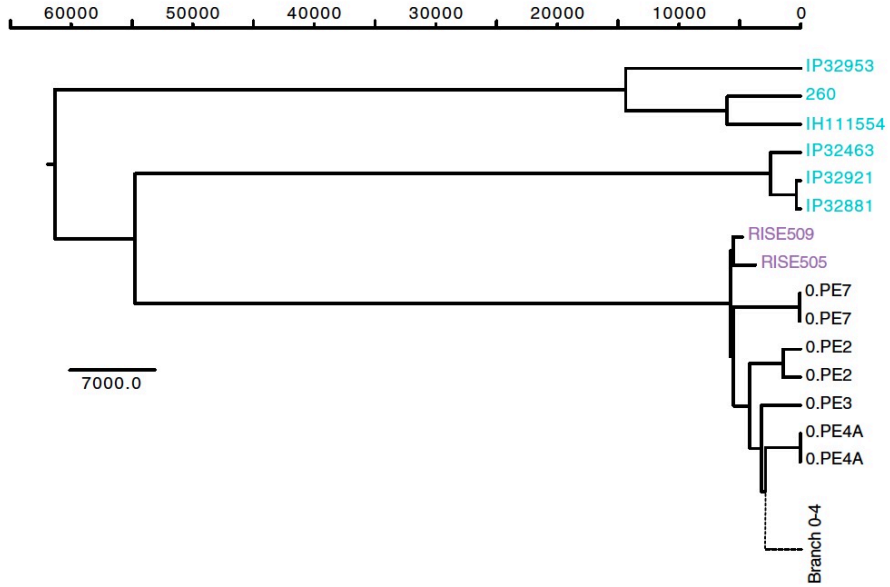


Branch-Shortening based Calibration of Molecular Clocks

Beyond Genomes, Microbiomes

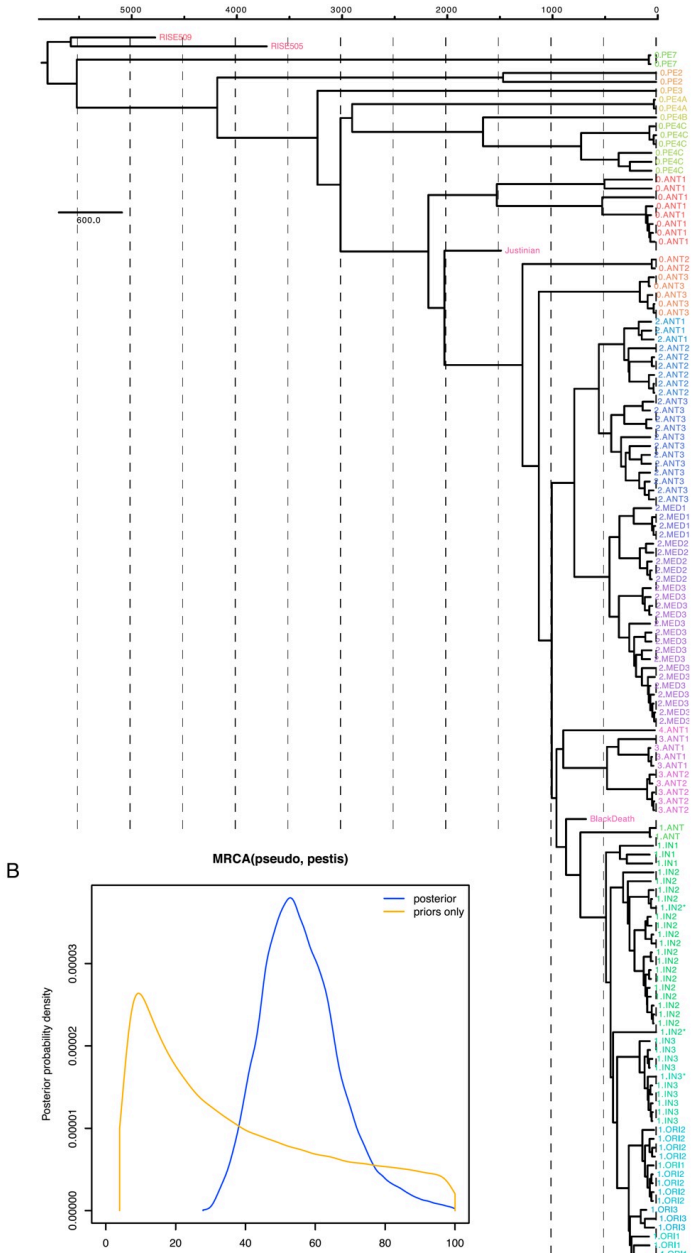
Historical Bacterial Outbreaks and Usual Suspects

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

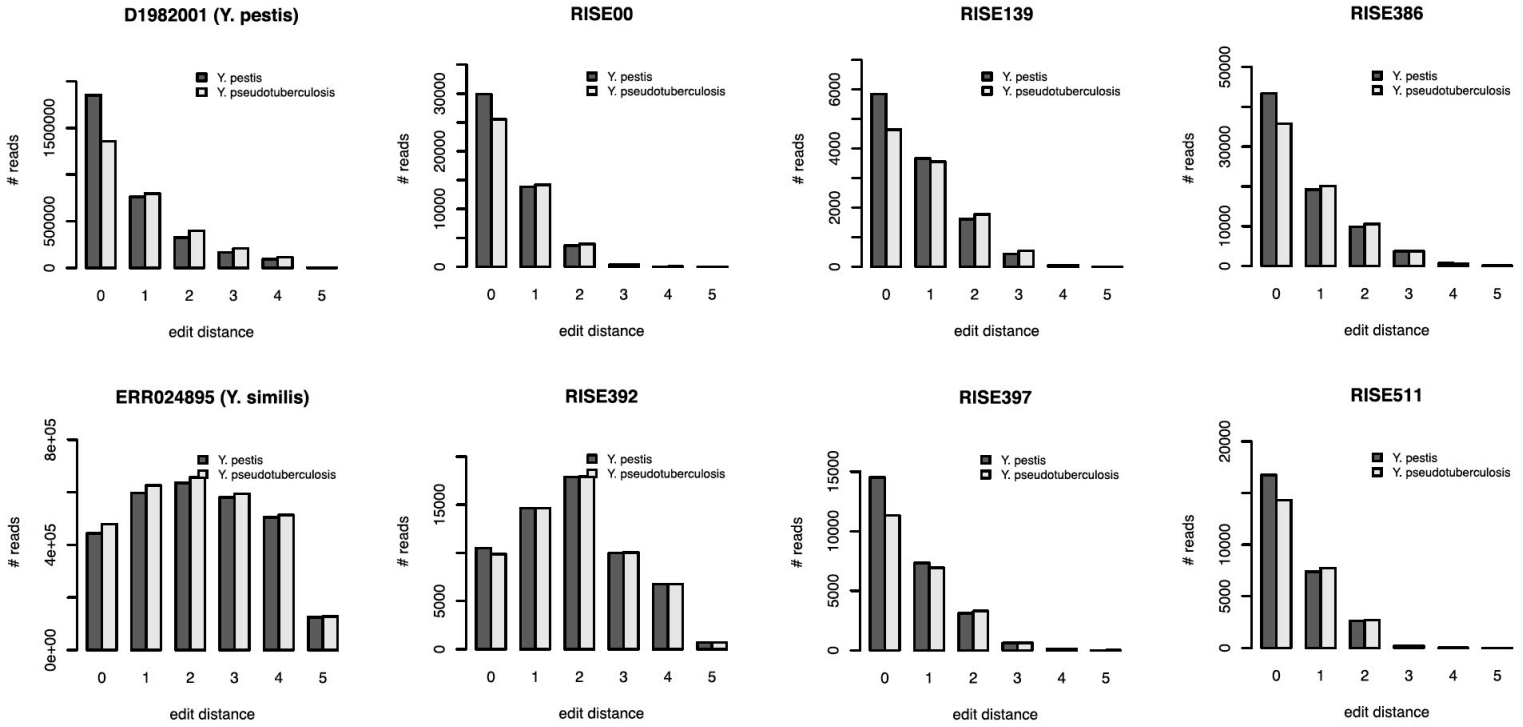
Rasmussen et al. Cell 2015



Beyond Genomes, Microbiomes

Historical Bacterial Outbreaks and Usual Suspects

Edit-Distance Distribution

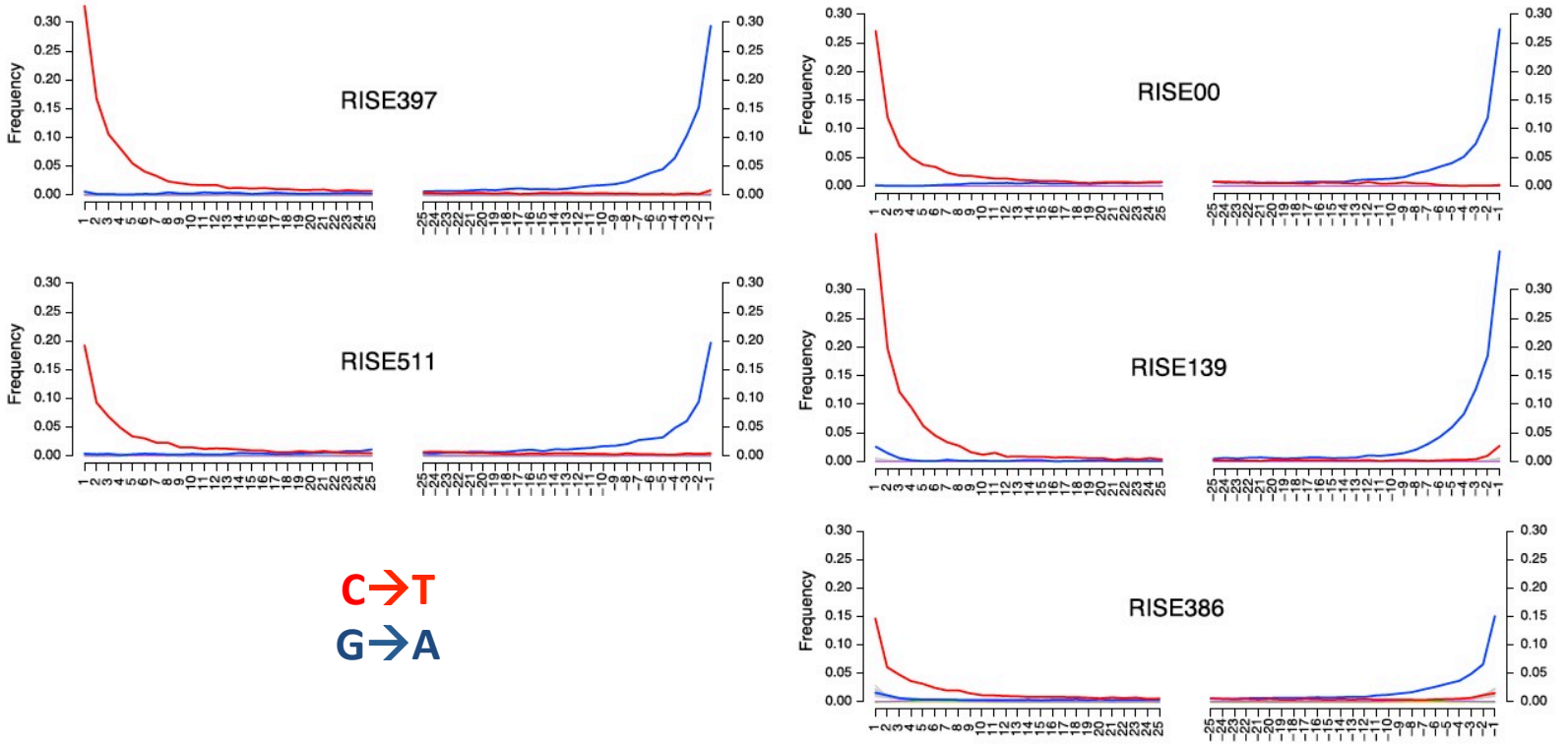


Rasmussen et al. Cell 2015

Beyond Genomes, Microbiomes

Historical Bacterial Outbreaks and Usual Suspects

Nucleotide Mis-incorporation Patterns

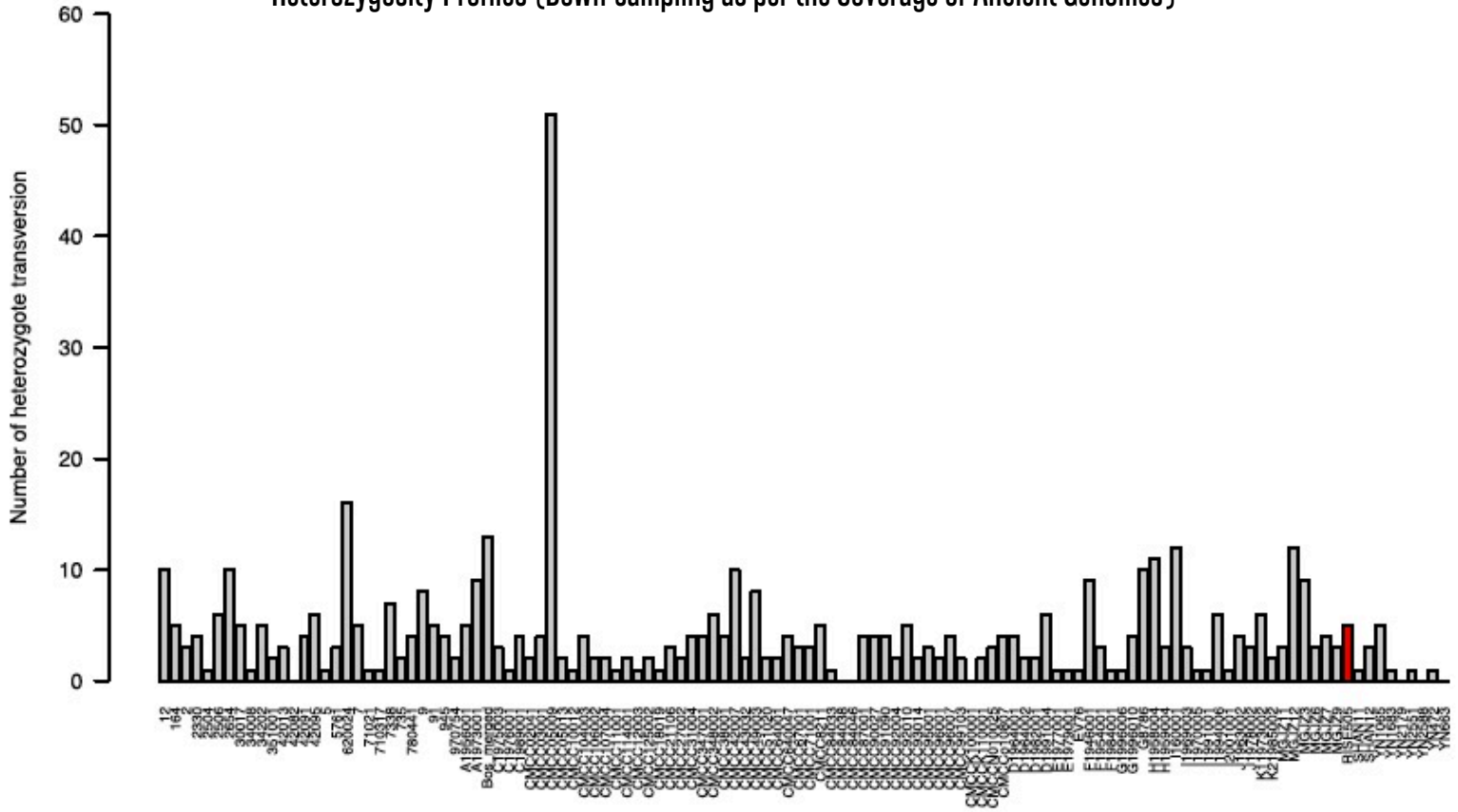


Rasmussen et al. Cell 2015

Beyond Genomes, Microbiomes

Historical Bacterial Outbreaks and Usual Suspects

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

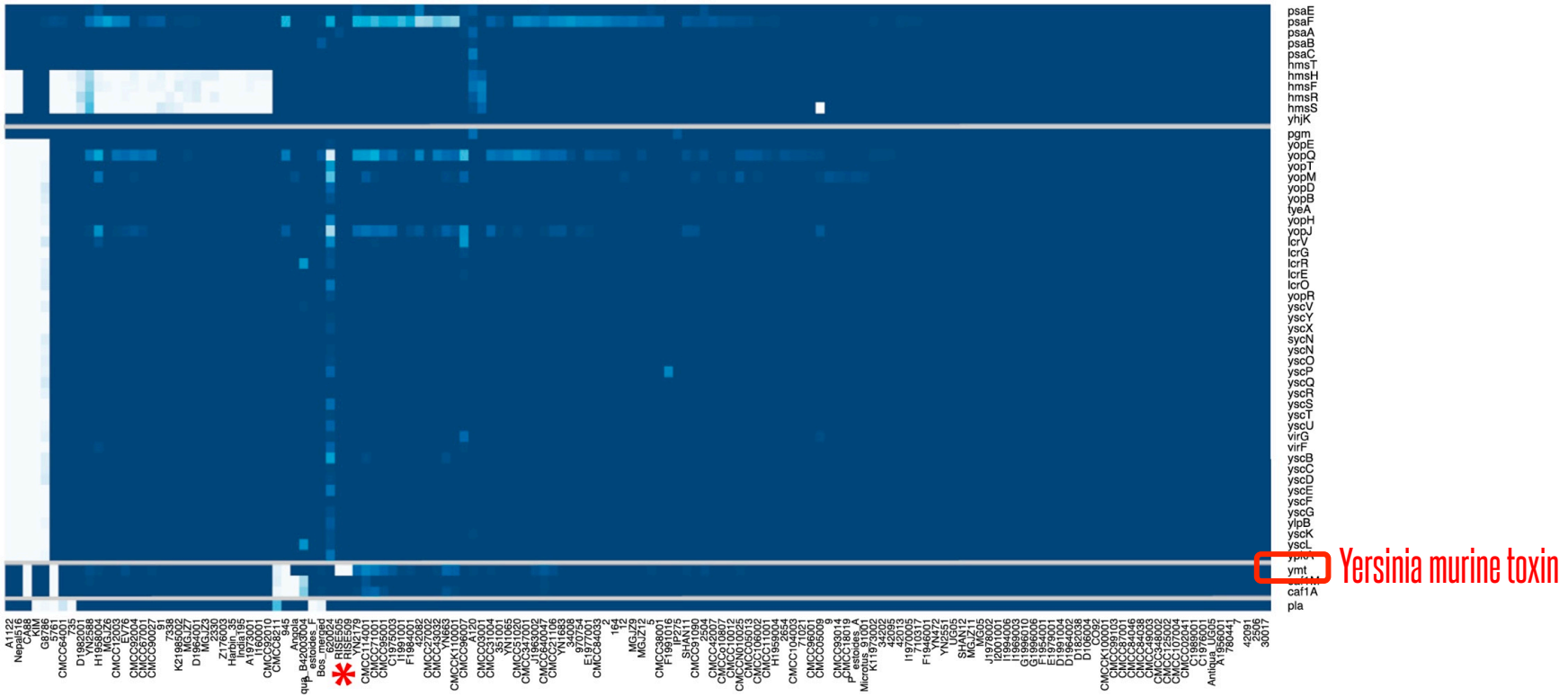


Rasmussen et al. Cell 2015

Beyond Genomes, Microbiomes

Historical Bacterial Outbreaks and Usual Suspects

Average coverage at 55 Virulence Genes



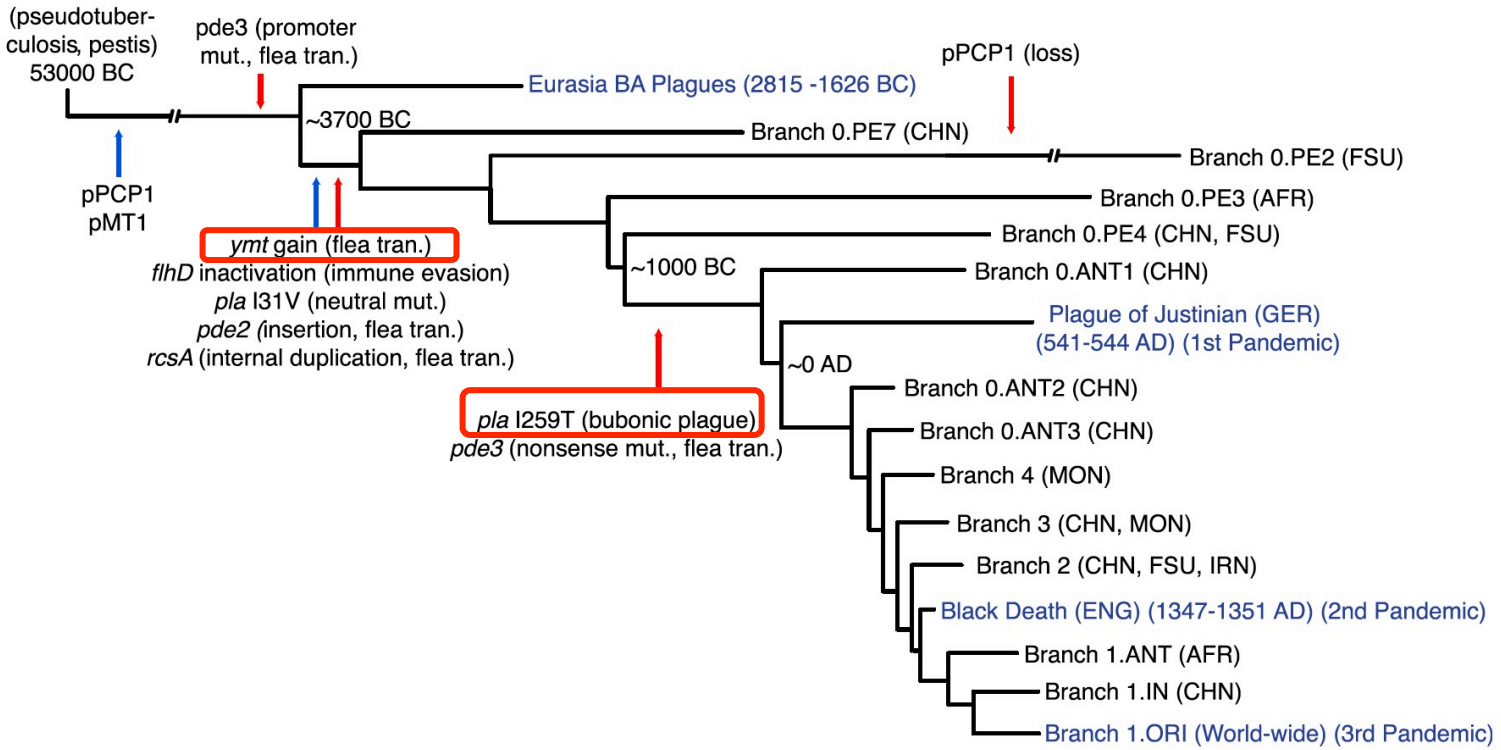
Yersinia murine toxin

Rasmussen et al. Cell 2015

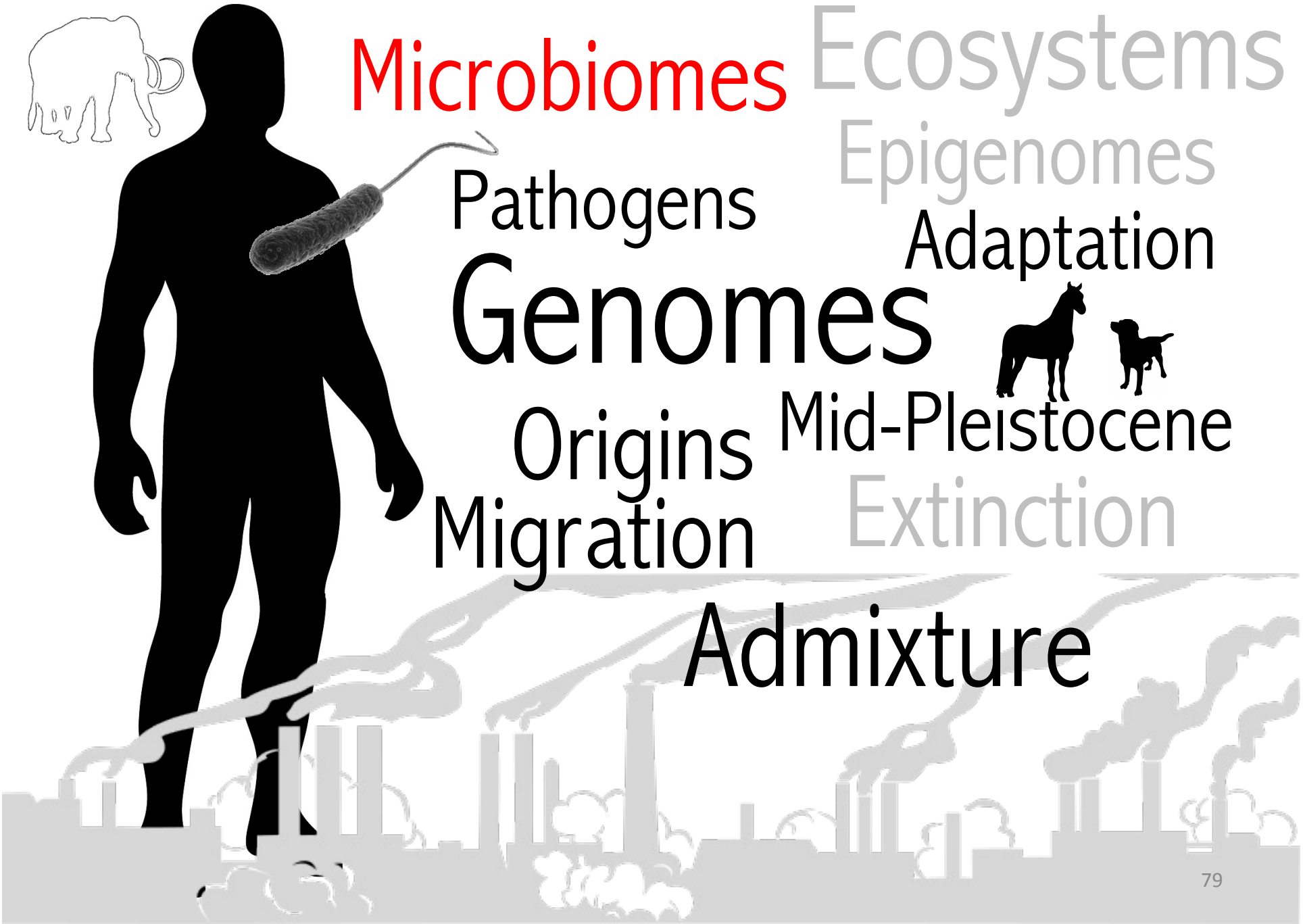
Beyond Genomes, Microbiomes

Historical Bacterial Outbreaks and Usual Suspects

The Genetic Makeup of a Deadly Human Pathogen



Rasmussen et al. Cell 2015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Beyond Genomes, Epigenomes

Ancient Microbiomes



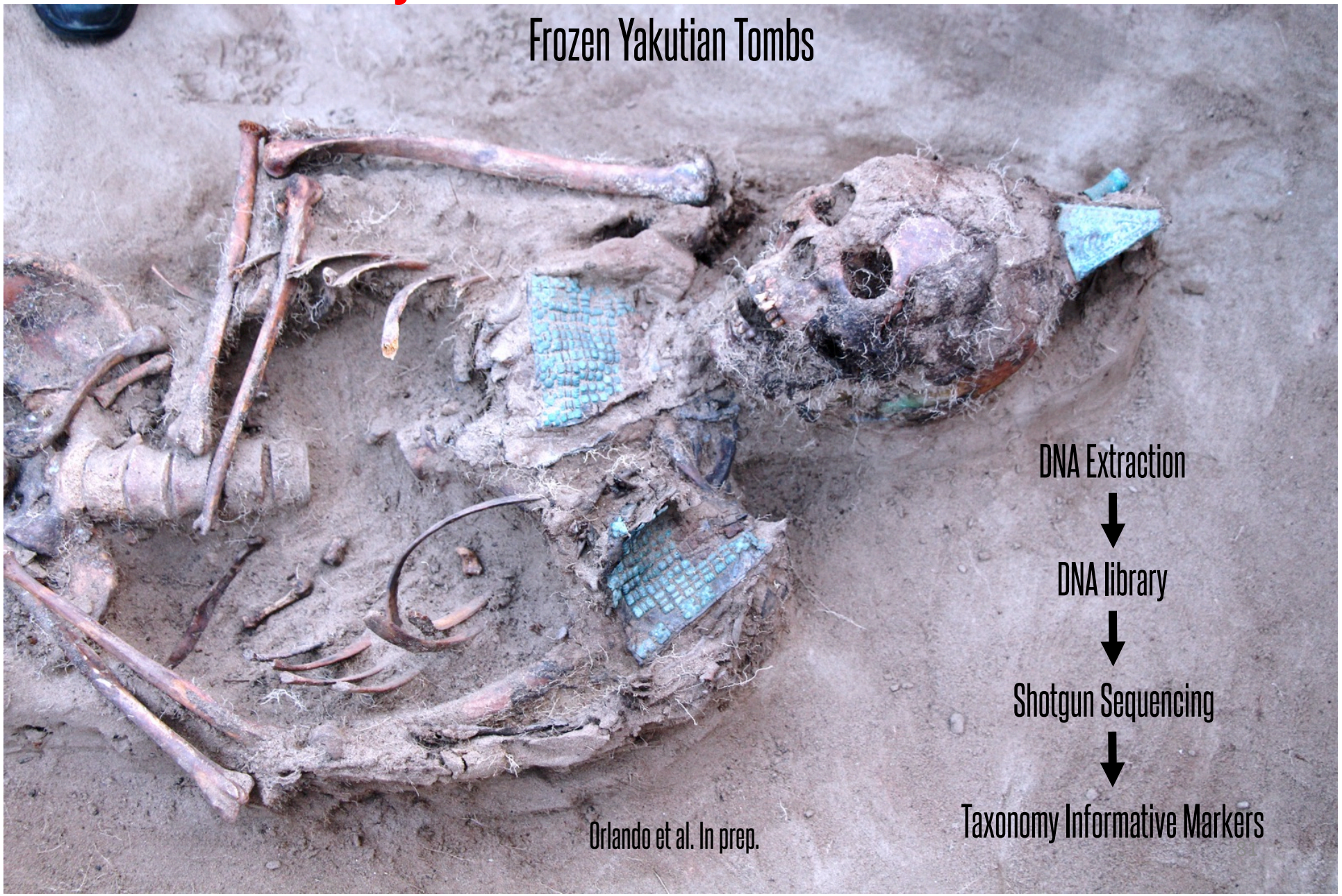



Warriner et al. Nature Genetics 2013

The Dental Plaque Metagenome Is Extremely Rich in Ancient DNA

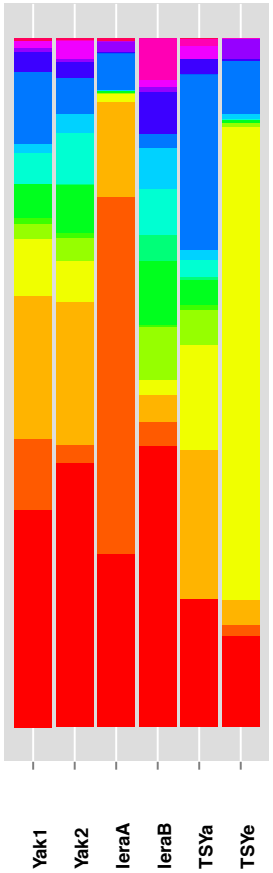
Past Diet, Diseases, And Behaviour

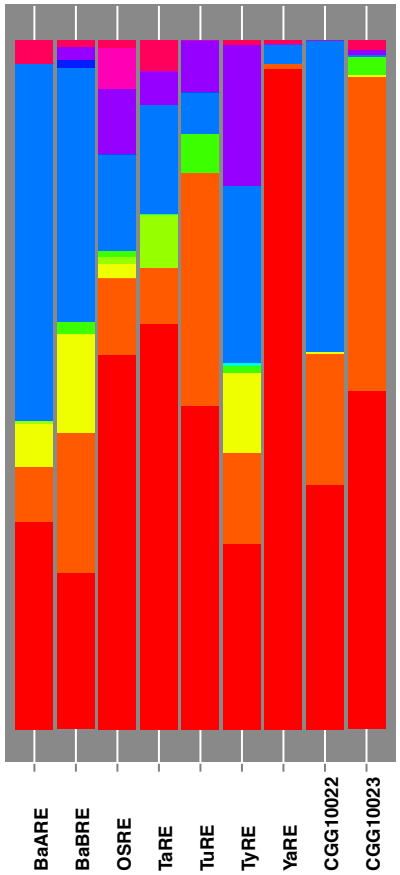
Beyond Genomes, Microbiomes

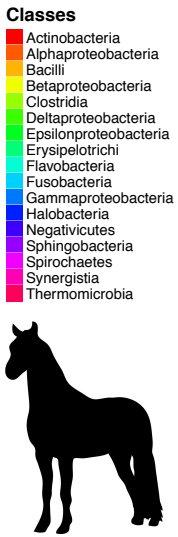


Beyond Genomes, Microbiomes

Frozen Yakutian Tombs



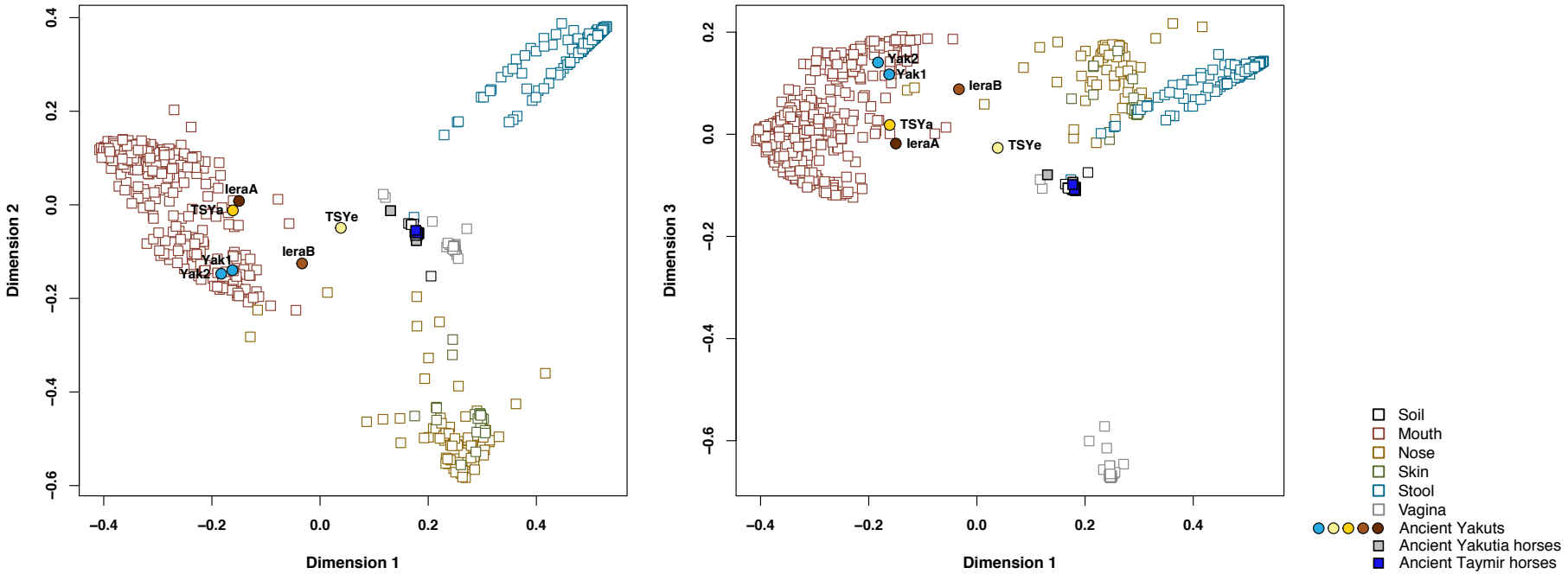




Orlando et al. In prep.

Beyond Genomes, Microbiomes

Frozen Yakutian Tombs



Orlando et al. In prep.

Beyond Genomes, Microbiomes

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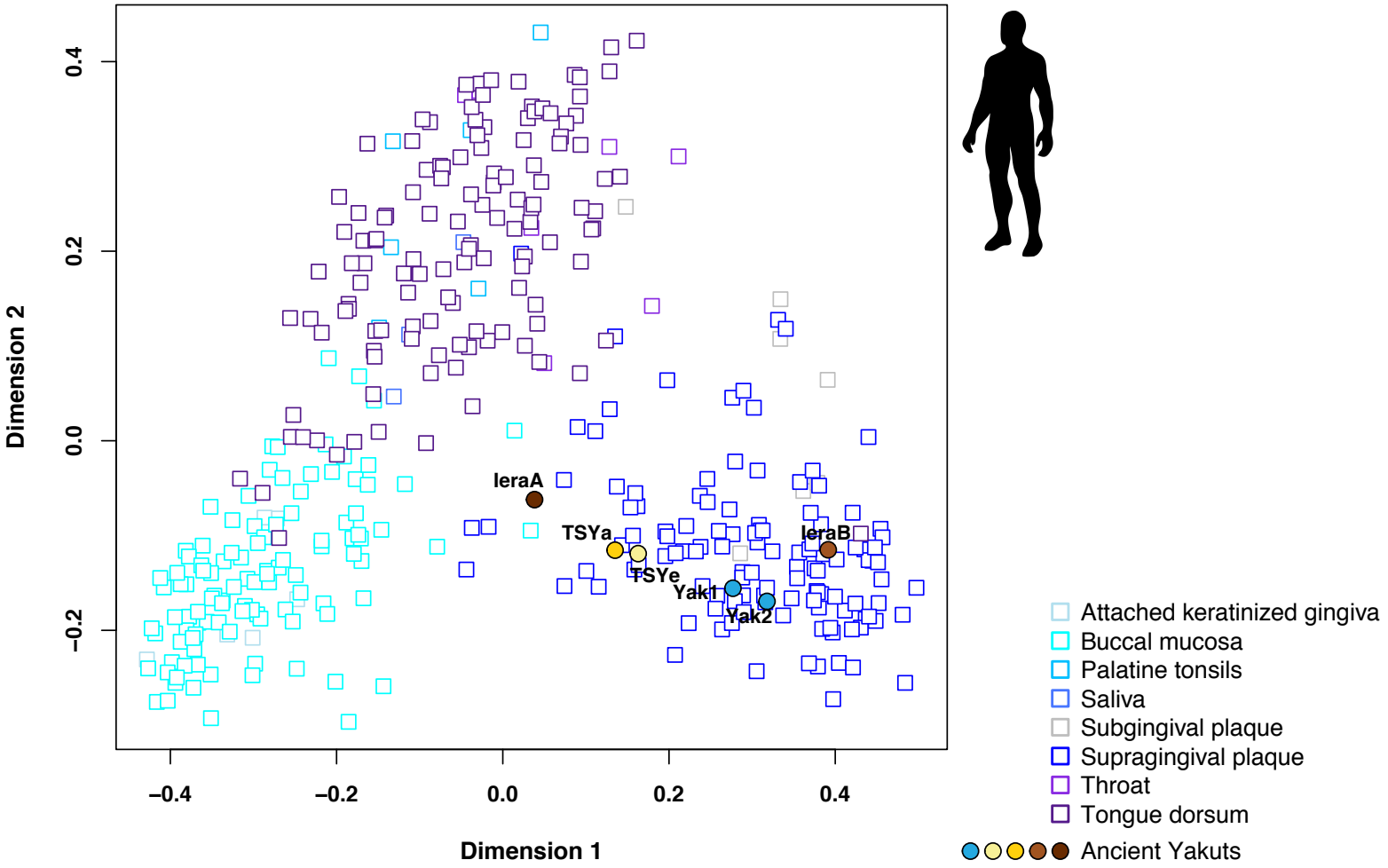
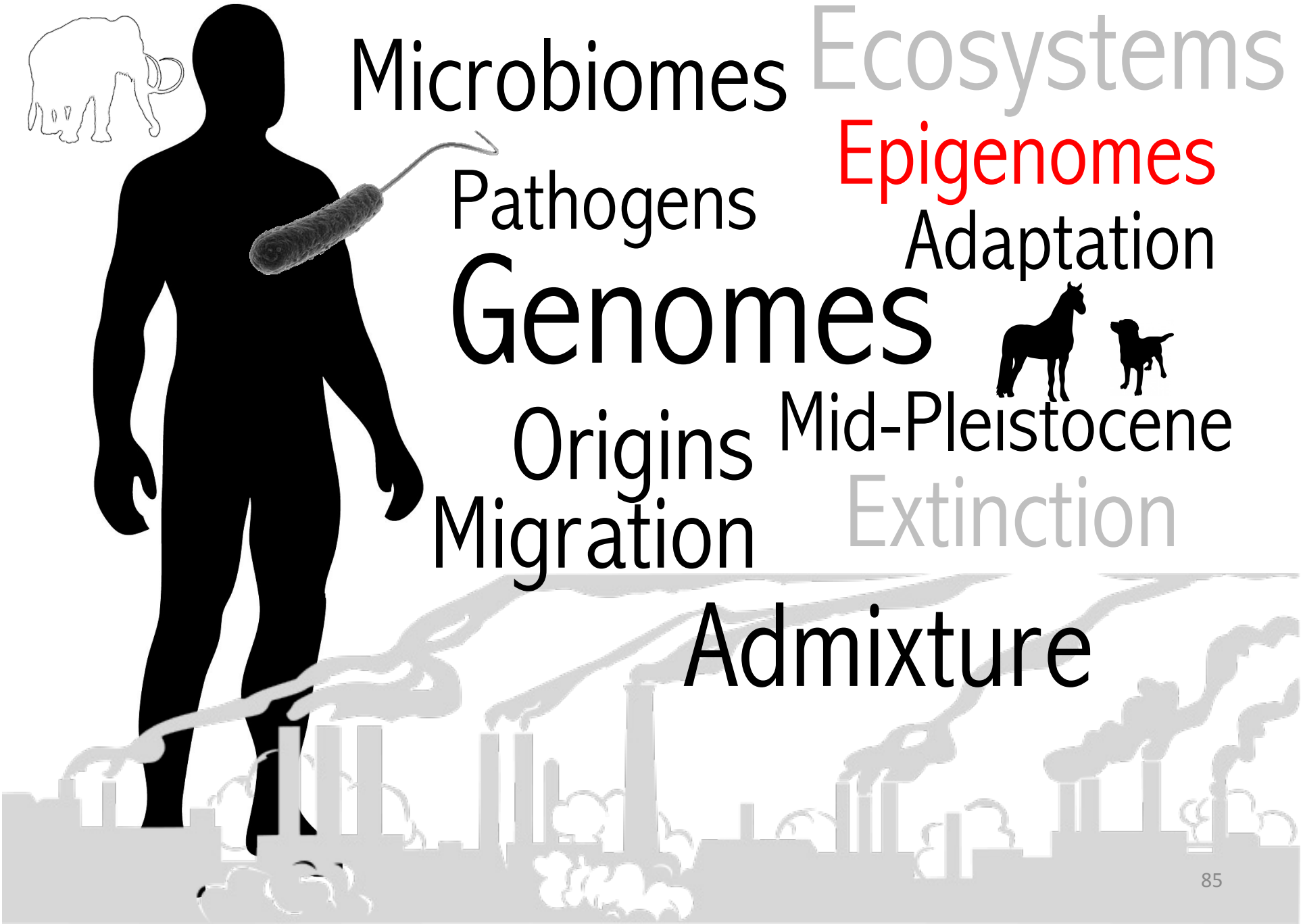


Orlando et al. In prep.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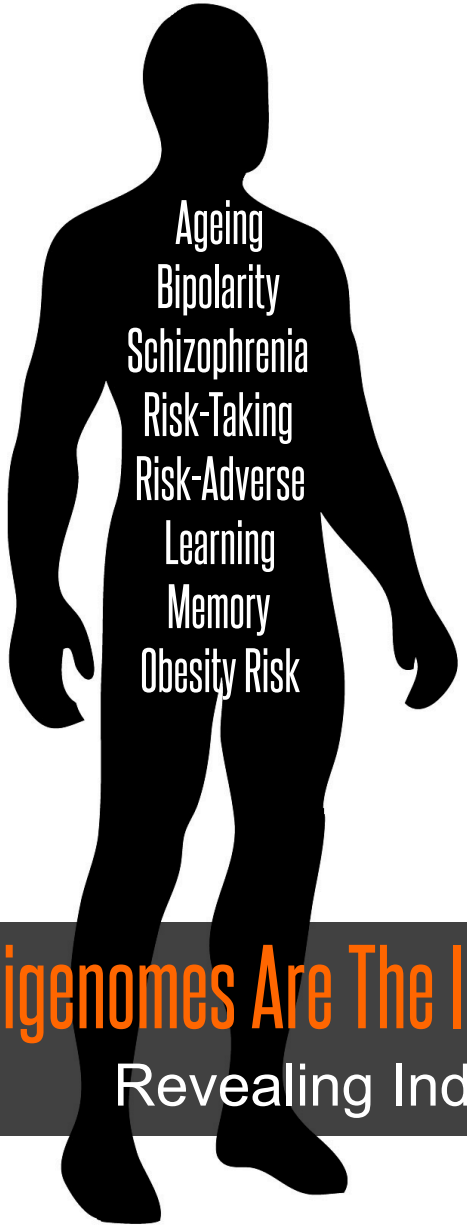
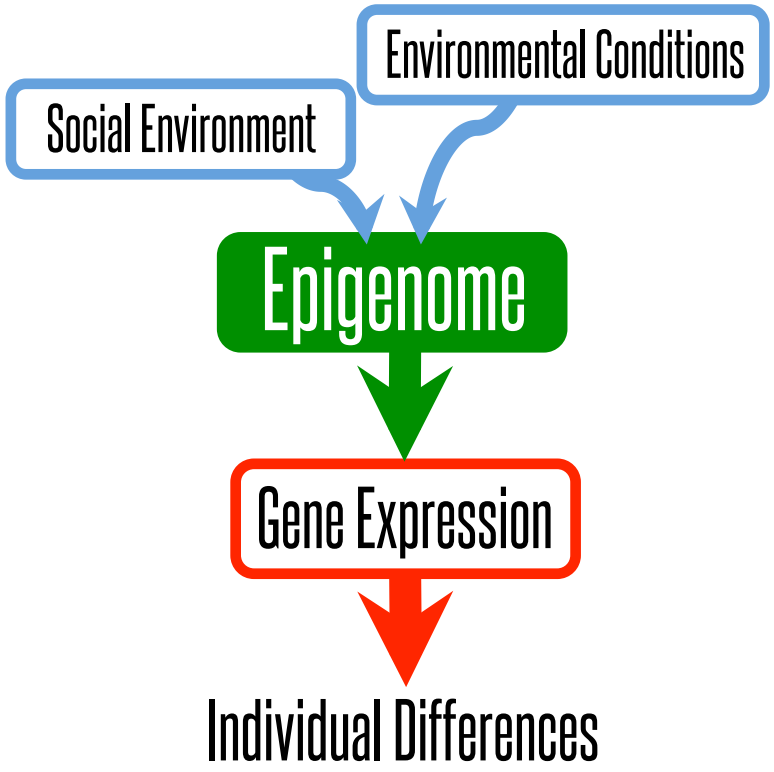
Beyond Genomes, Epigenomes



Ancient EpiGenomics

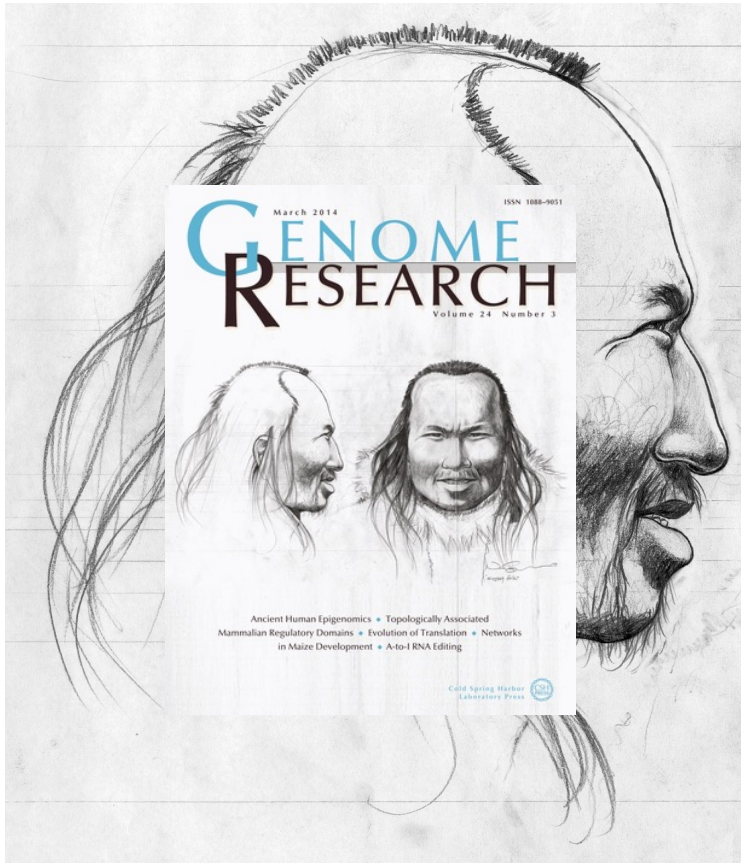


Epigenomes Are The Interface Between Genes And The Environment

Revealing Individual Differences Even In The Past

Beyond Genomes, Epigenomes

Ancient EpiGenomics



EpiGenome

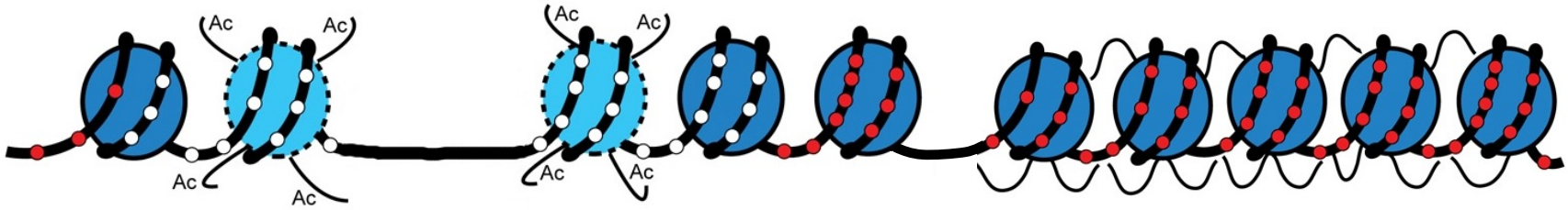
Pedersen et al. Genome Res 2014

Genome

Rasmussen et al. Nature 2010

The First Ancient Epigenome

Rationale: Nucleosome Protection After Death



The First Ancient Epigenome

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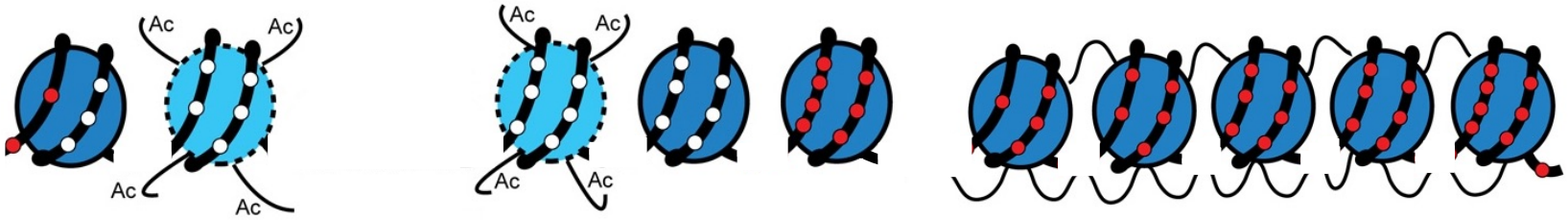

The First Ancient Epigenome

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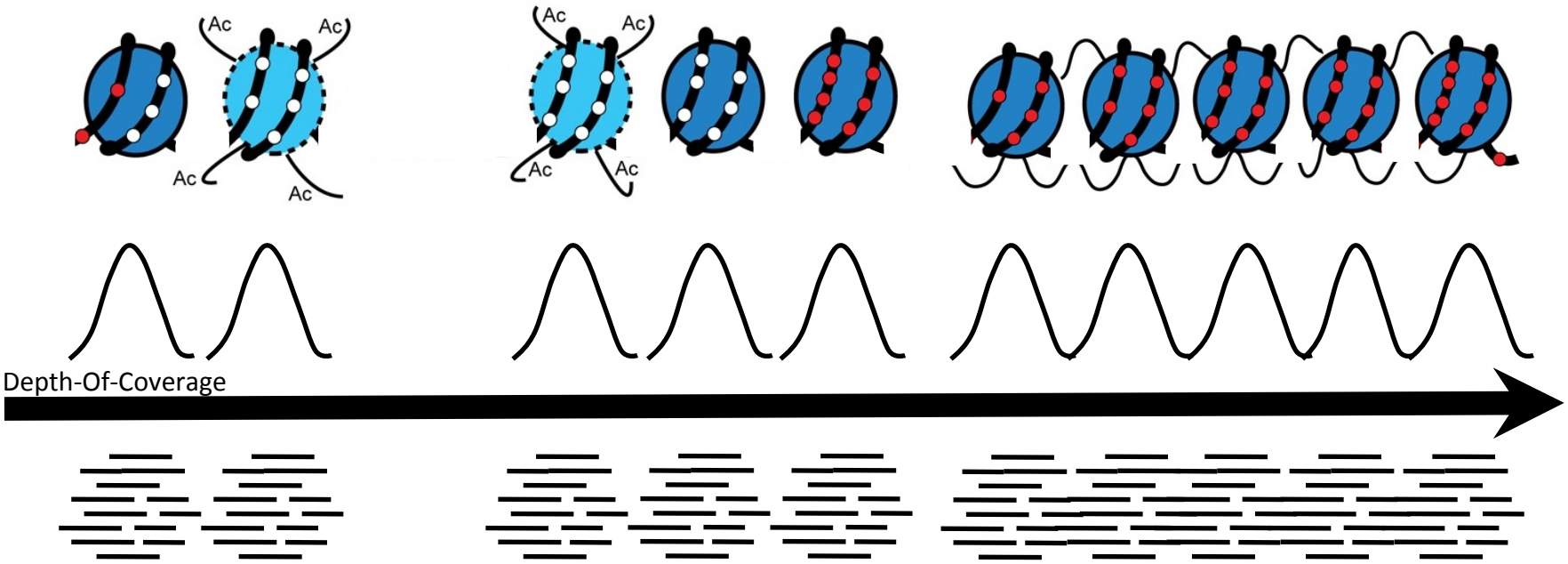
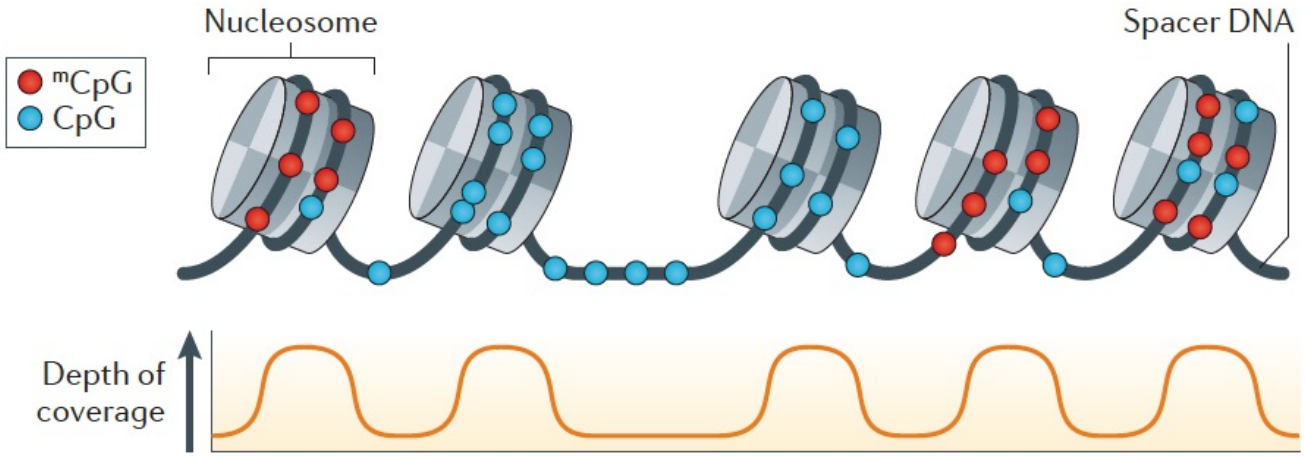


The First Ancient Epigenome

Prediction Summary

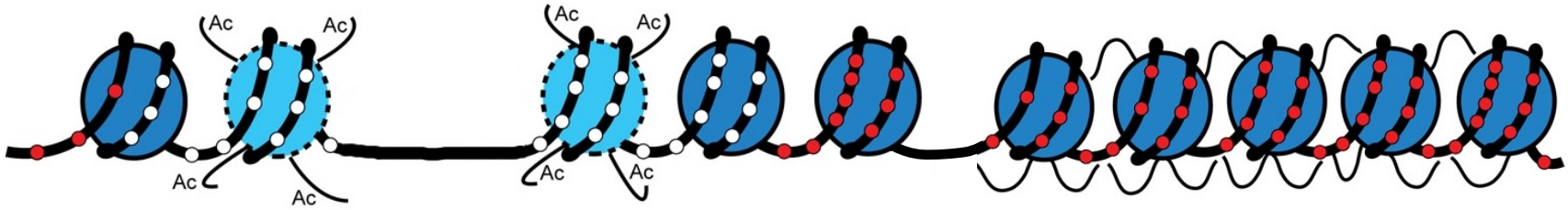
Genome-Wide Nucleosome and Methylation Maps



Orlando et al. Nat Rev Genetics 2015

The First Ancient Epigenome

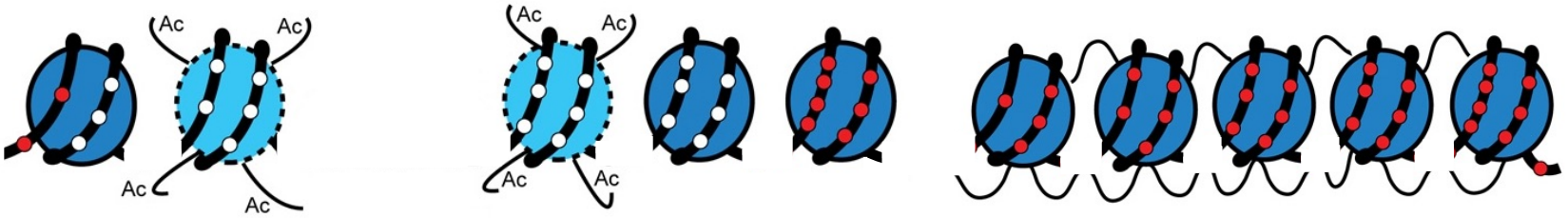
Rationale: Cytosine Deamination After Death



CpG ○ **^{m}CpG** ●

The First Ancient Epigenome

Rationale: Cytosine Deamination After Death



CpG

NH_2

UpG ○

^{m}CpG

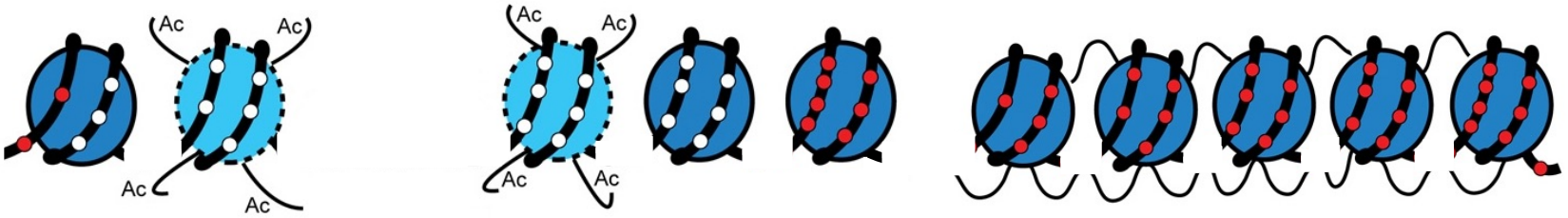
NH_2

TpG ●

The First Ancient Epigenome

Cytosine Methylation and Gene Expression

Post-Mortem Cytosine Deamination



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The First Ancient Epigenome

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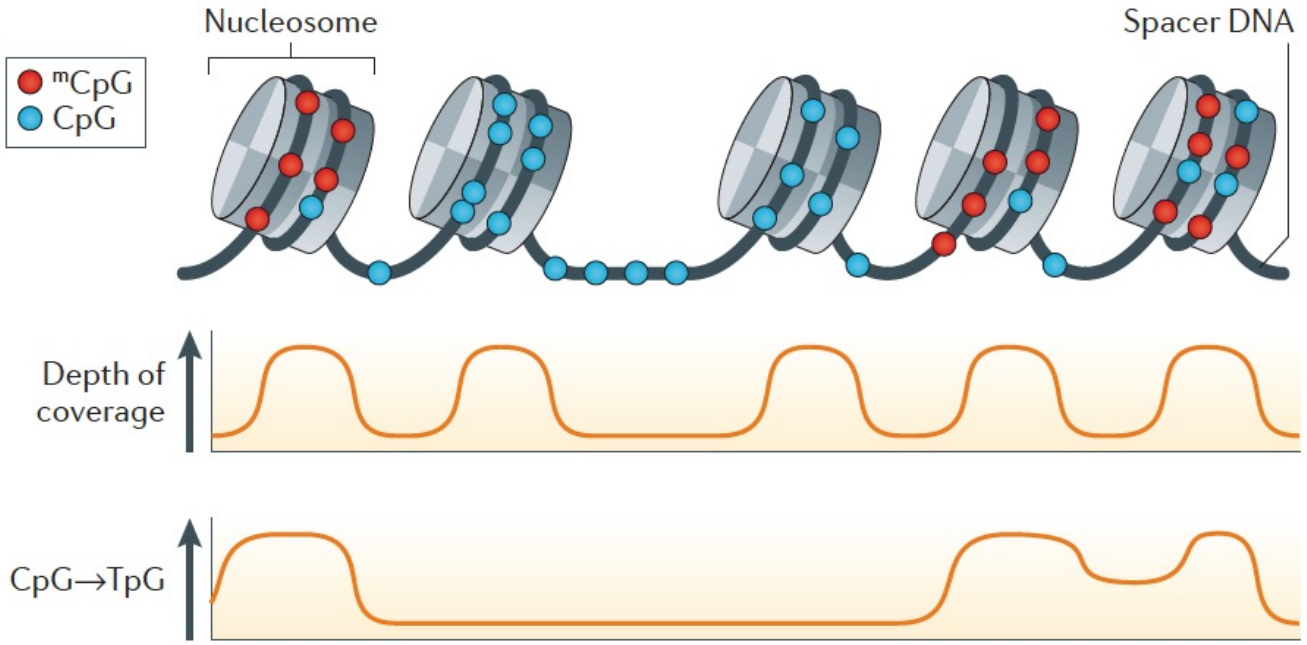
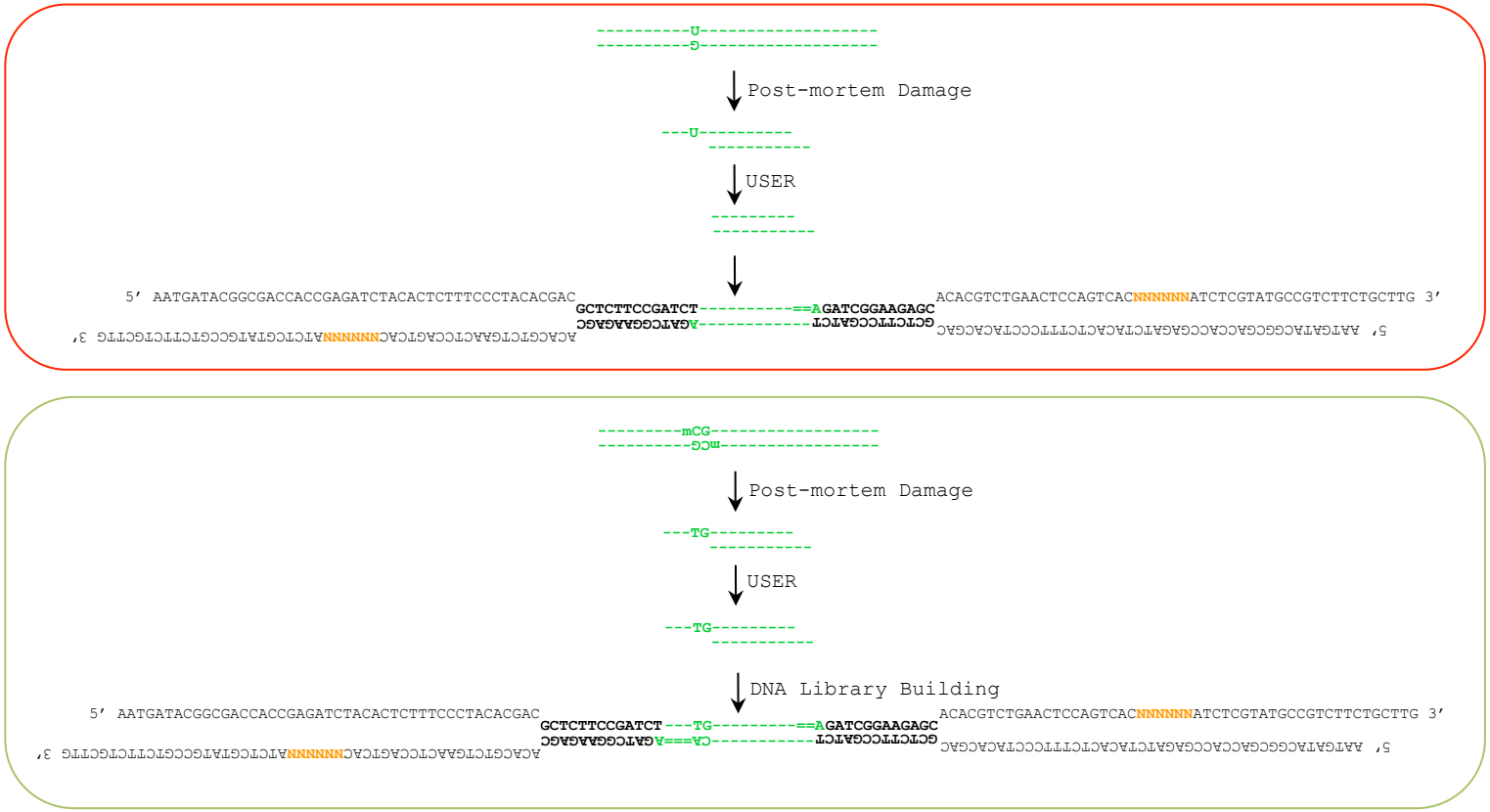


Orlando et al. Nat Rev Genetics 2015

The First Ancient Epigenome

Cytosine Methylation and Gene Expression

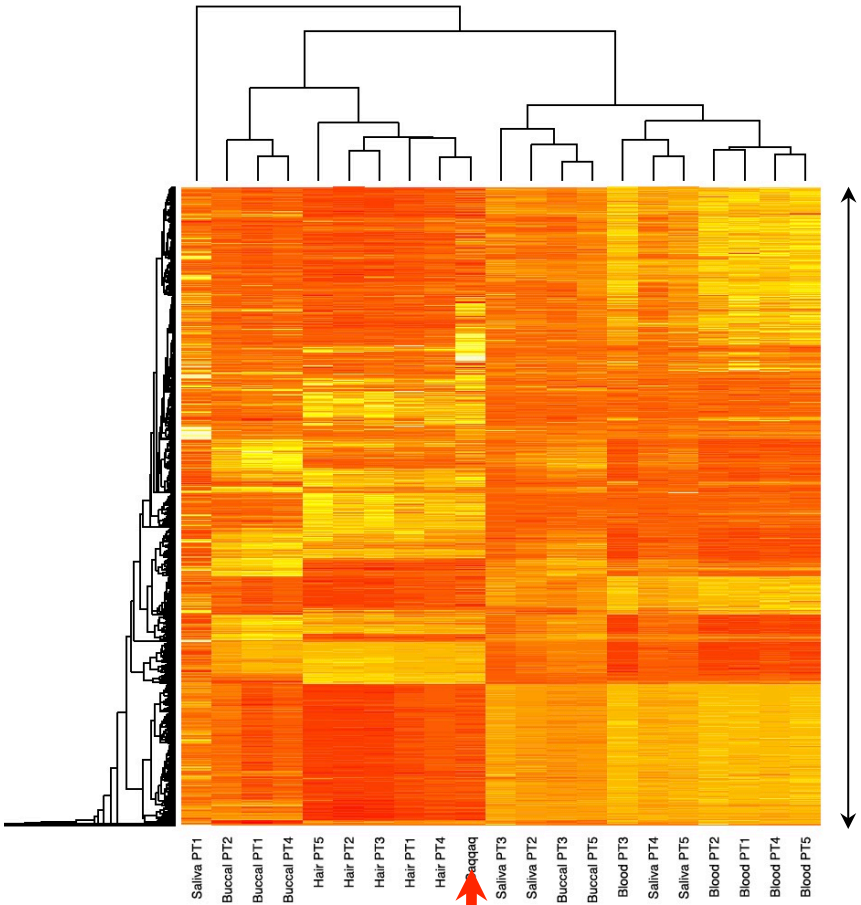
Post-Mortem Cytosine Deamination



From Briggs et al. Nuc Acids Res 2010

Ancient EpiGenomics

The First Genome-Wide Methylation Map

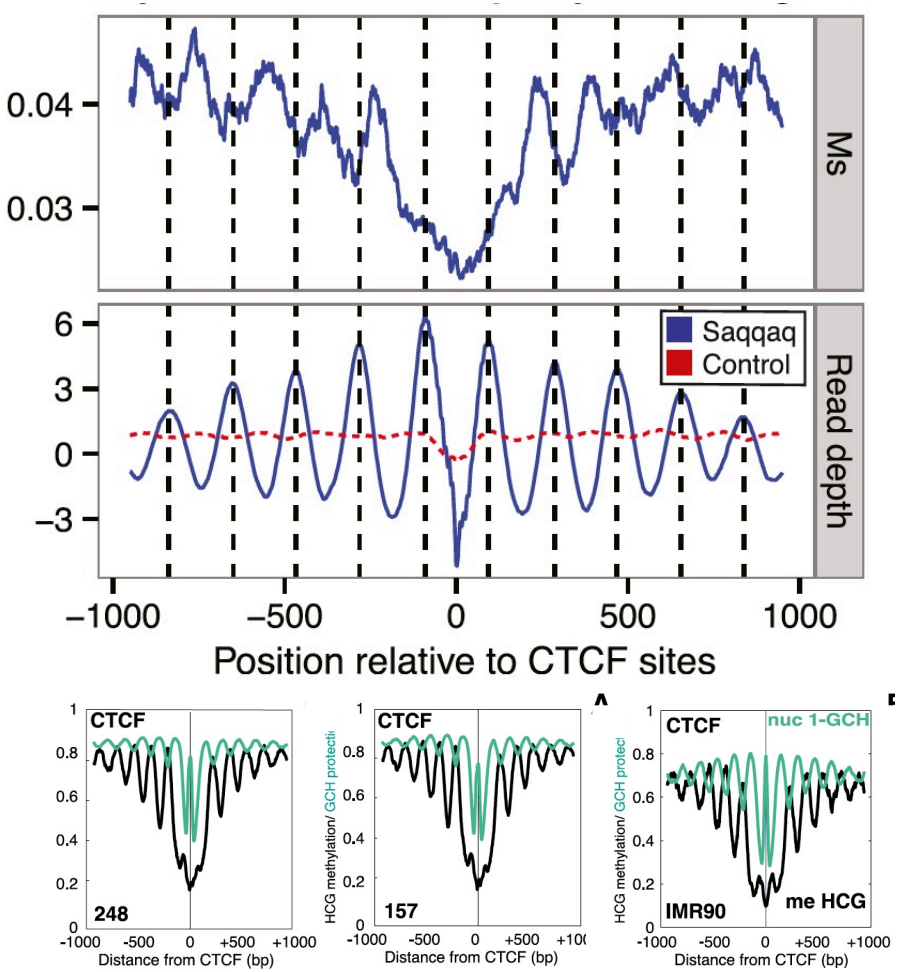


Illumina 450K

Pedersen et al. Genome Res 2014

Ancient EpiGenomics

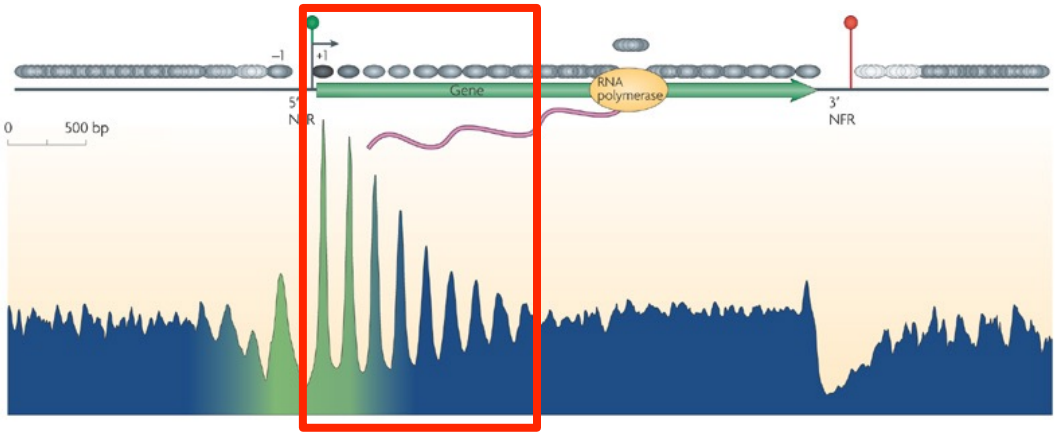
Confronting Nucleosome & Methylation Maps



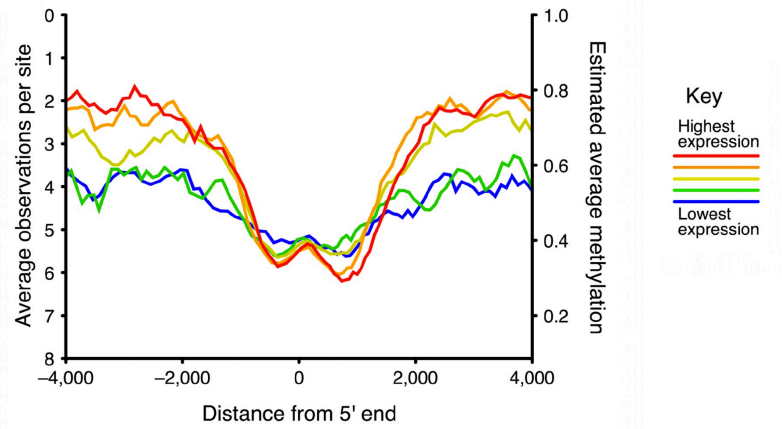
Pedersen et al. Genome Res 2014

Ancient EpiGenomics

Predicting Ancient Gene Expression Levels



From Jiang &Pugh Nature Rev Genetics 2009

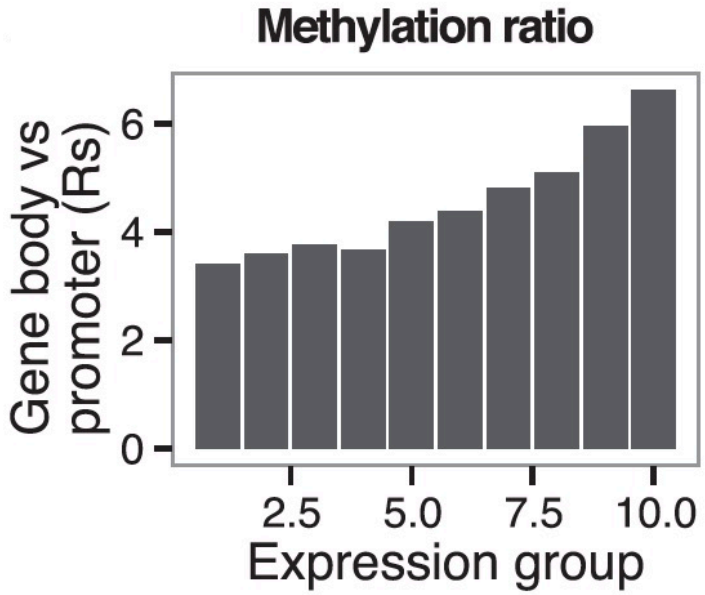


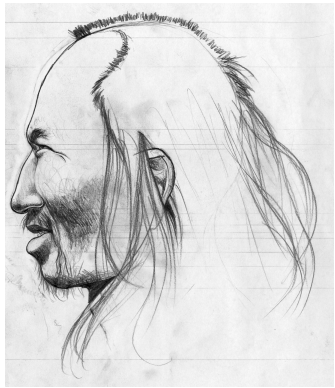
From Ball et al. Nature Biotech 2009

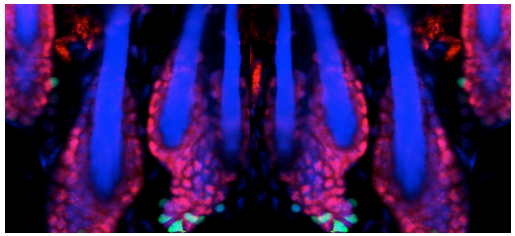
Ancient EpiGenomics

Predicting Ancient Gene Expression Levels (Methylation-based)

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





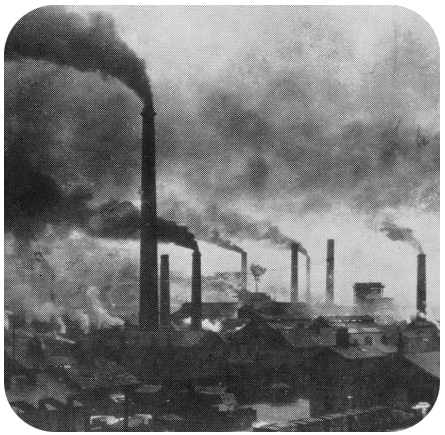


Pedersen et al. Genome Res 2014

Beyond Genomes, Epigenomes

What Ancient EpiGenomics Could Reveal

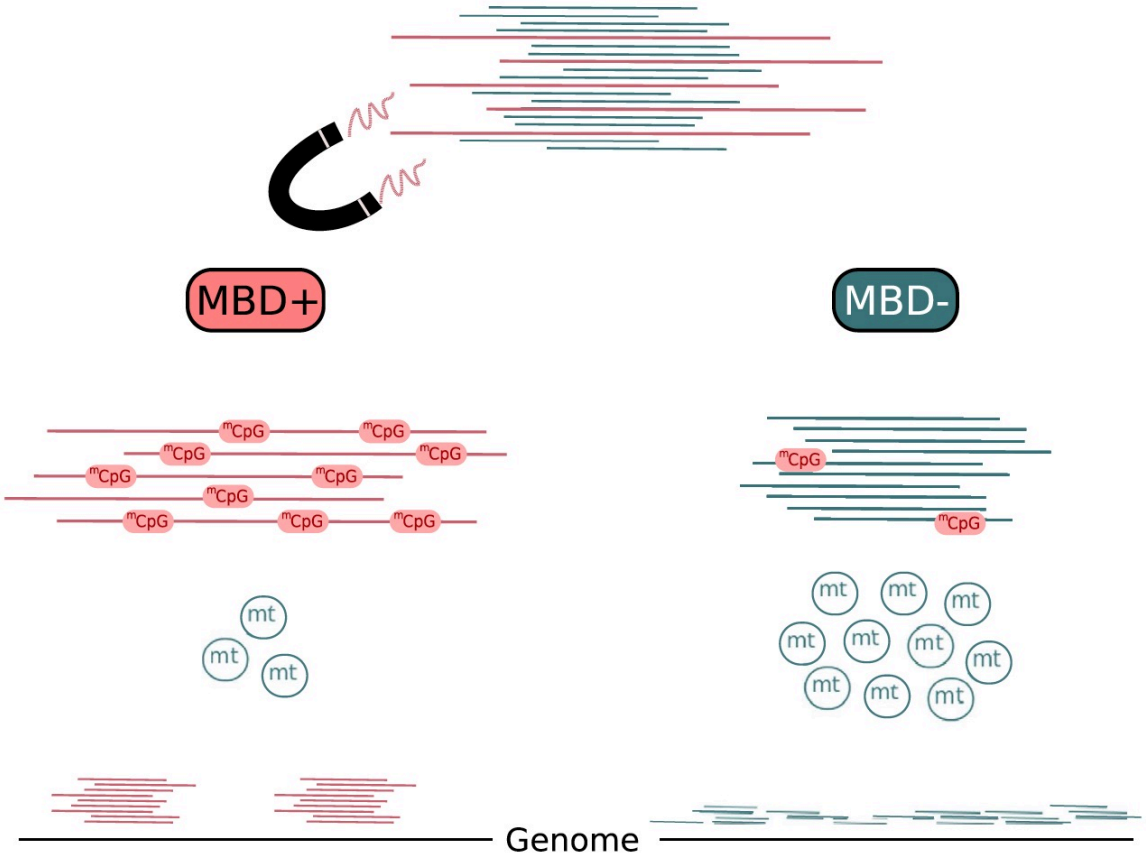


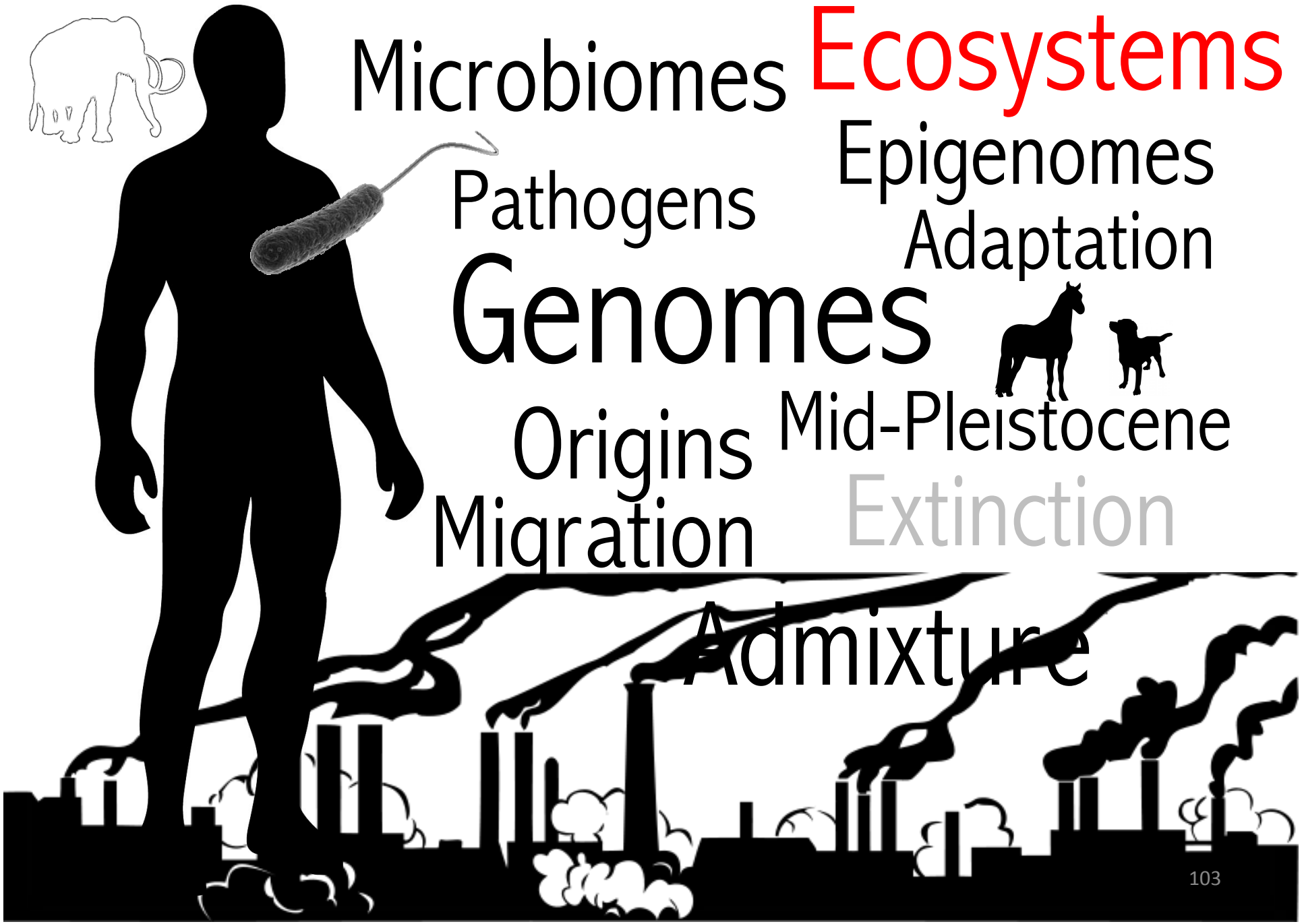
- Extinction
- Domestication
- Change in Life Style

Beyond Genomes, Epigenomes

Ancient Methylomes



Seguin-Orlando et al. Sci Rep 2015

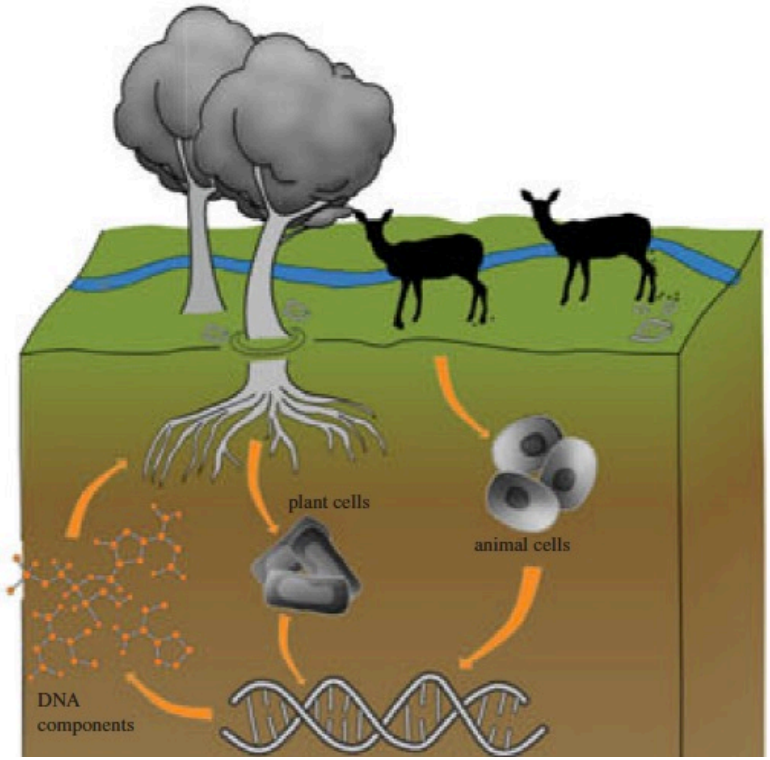

Microbiomes
Ecosystems
Epigenomes
Pathogens
Adaptation
Genomes
Origins
Mid-Pleistocene
Migration
Extinction
Admixture

Beyond Genomes, Ecosystems

Mini-metabarcoding

Beyond Genomes, Ecosystems

Mini-metabarcoding



Winther-Pedersen et al. Proc B 2015

DNA Extraction

↓

Minibarcode PCR amplification

↓

Deep Amplicon-Sequencing

↓

Molecular Taxonomic Assignment

Beyond Genomes, Ecosystems

Mini-metabarcoding

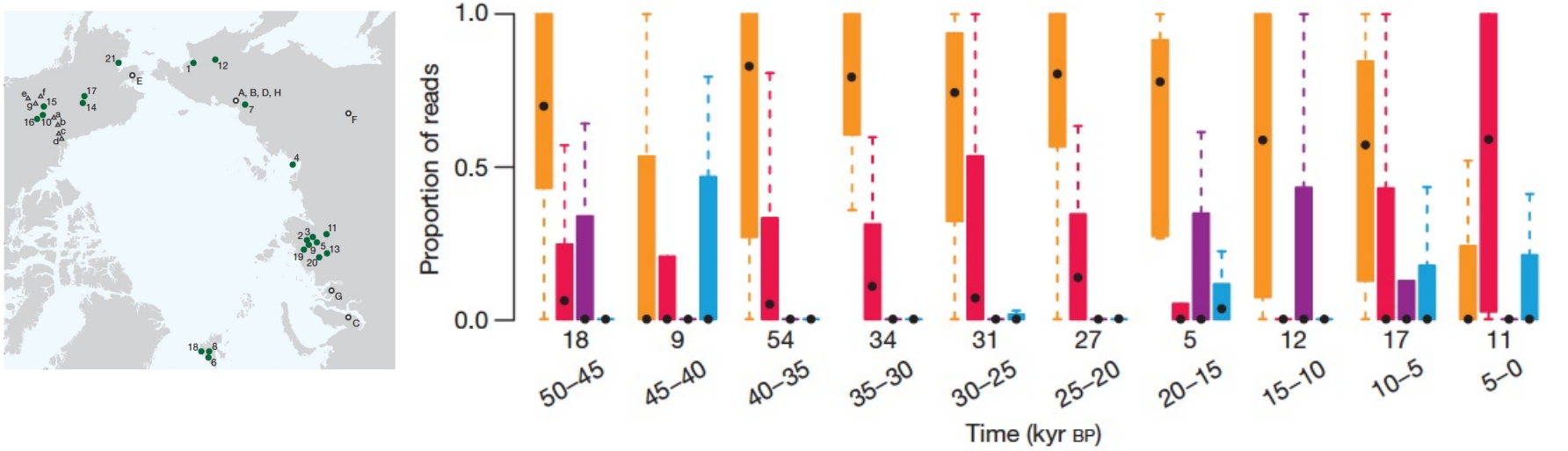
New Zealand, 600 ya

Siberia, 10.4-30 kya

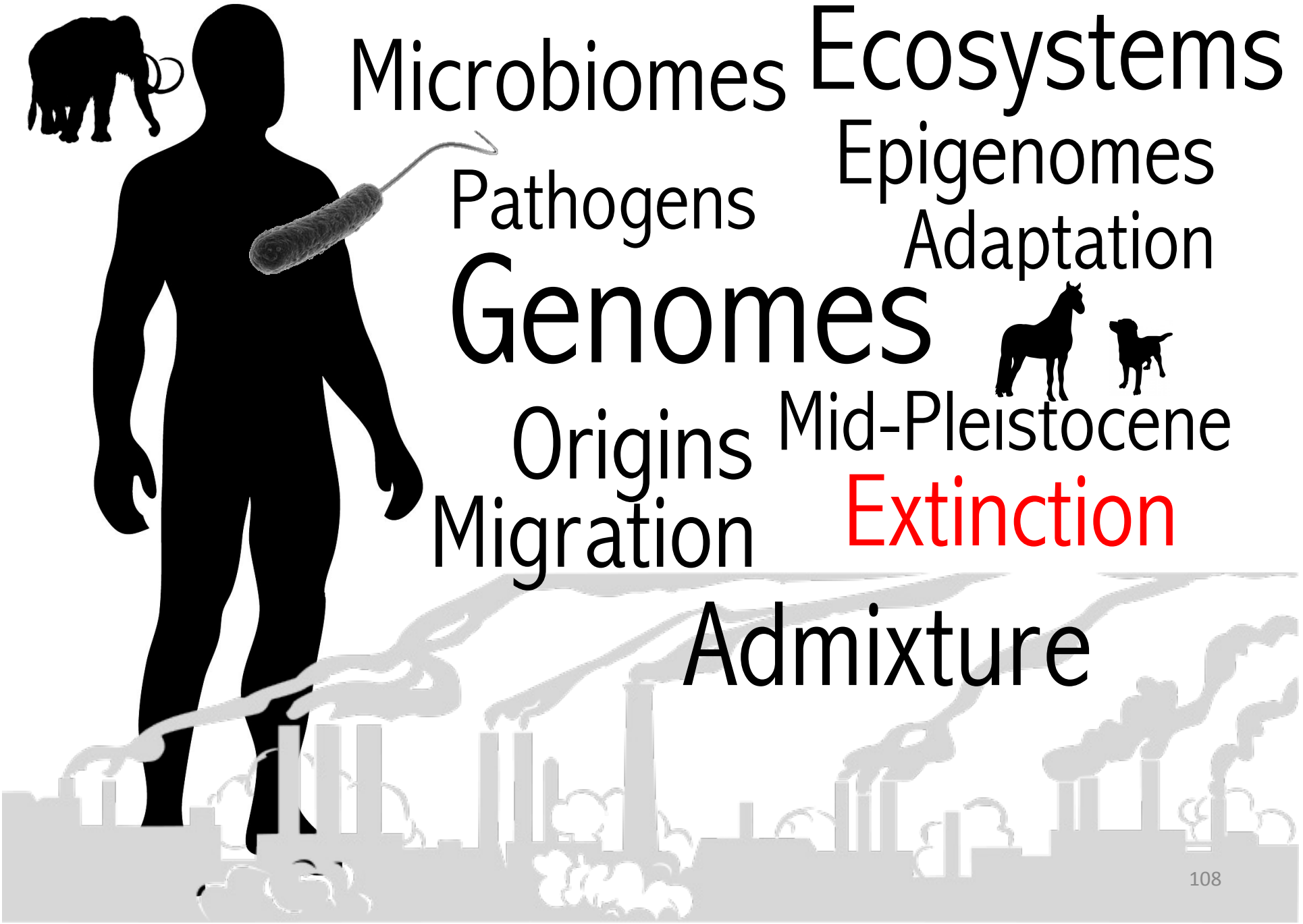
Willerslev et al. Nature 2003

Beyond Genomes, Ecosystems

Mini-metabarcoding



Willerslev et al. Nature 2014


Microbiomes
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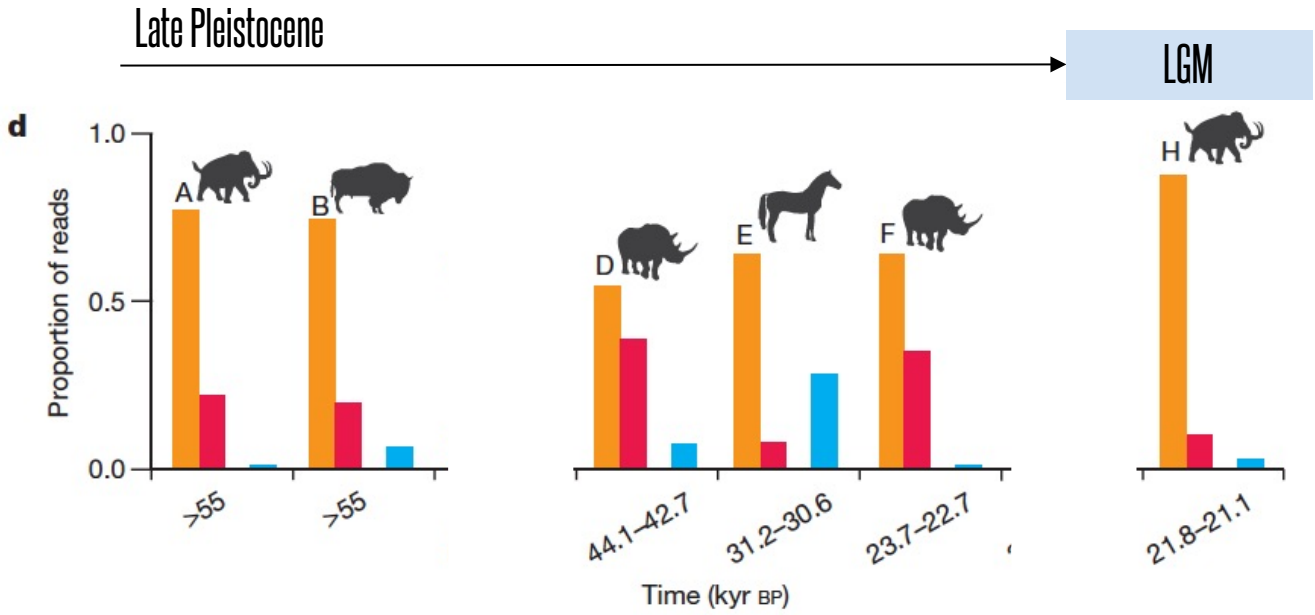
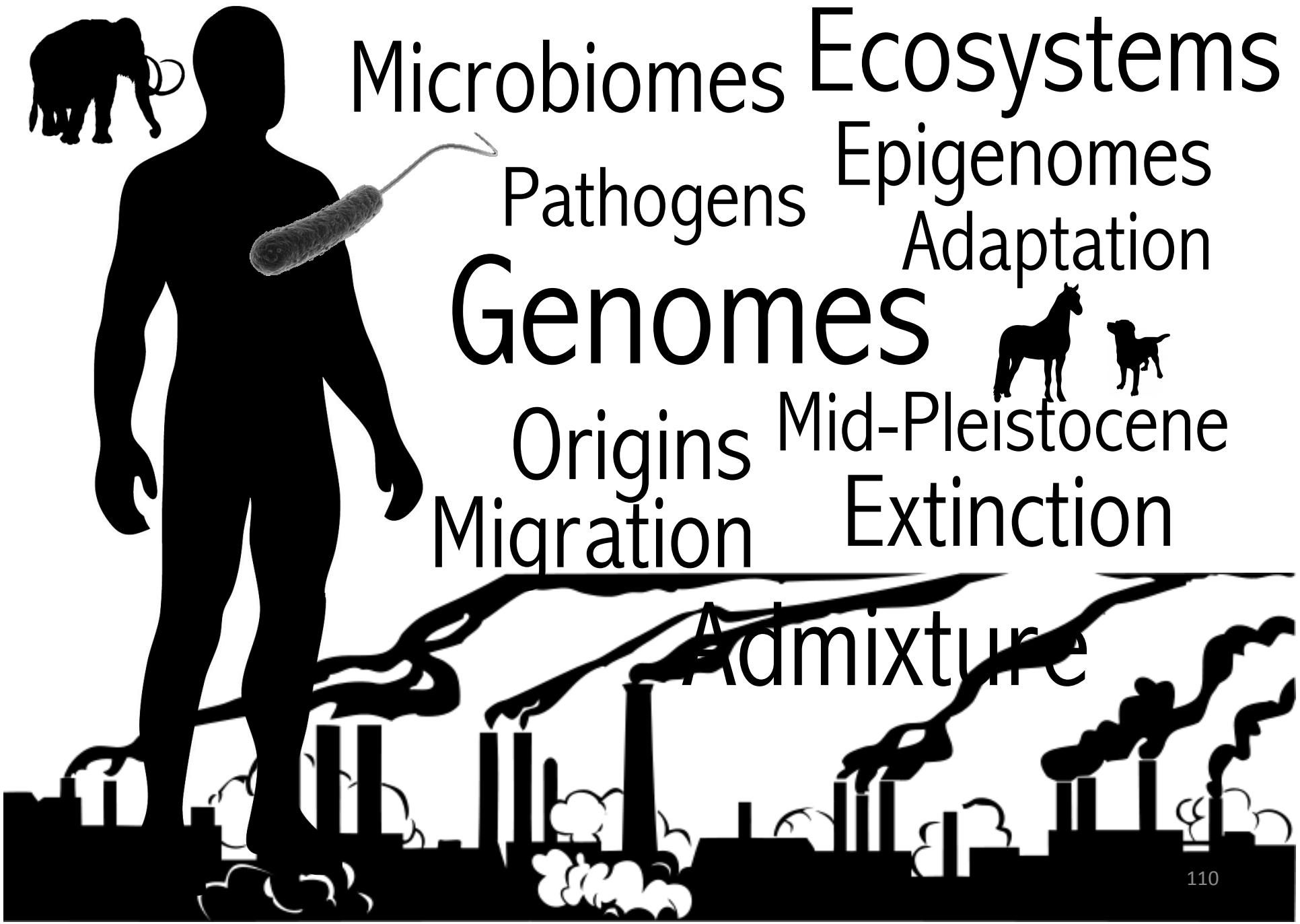


Willerslev et al. Nature 2014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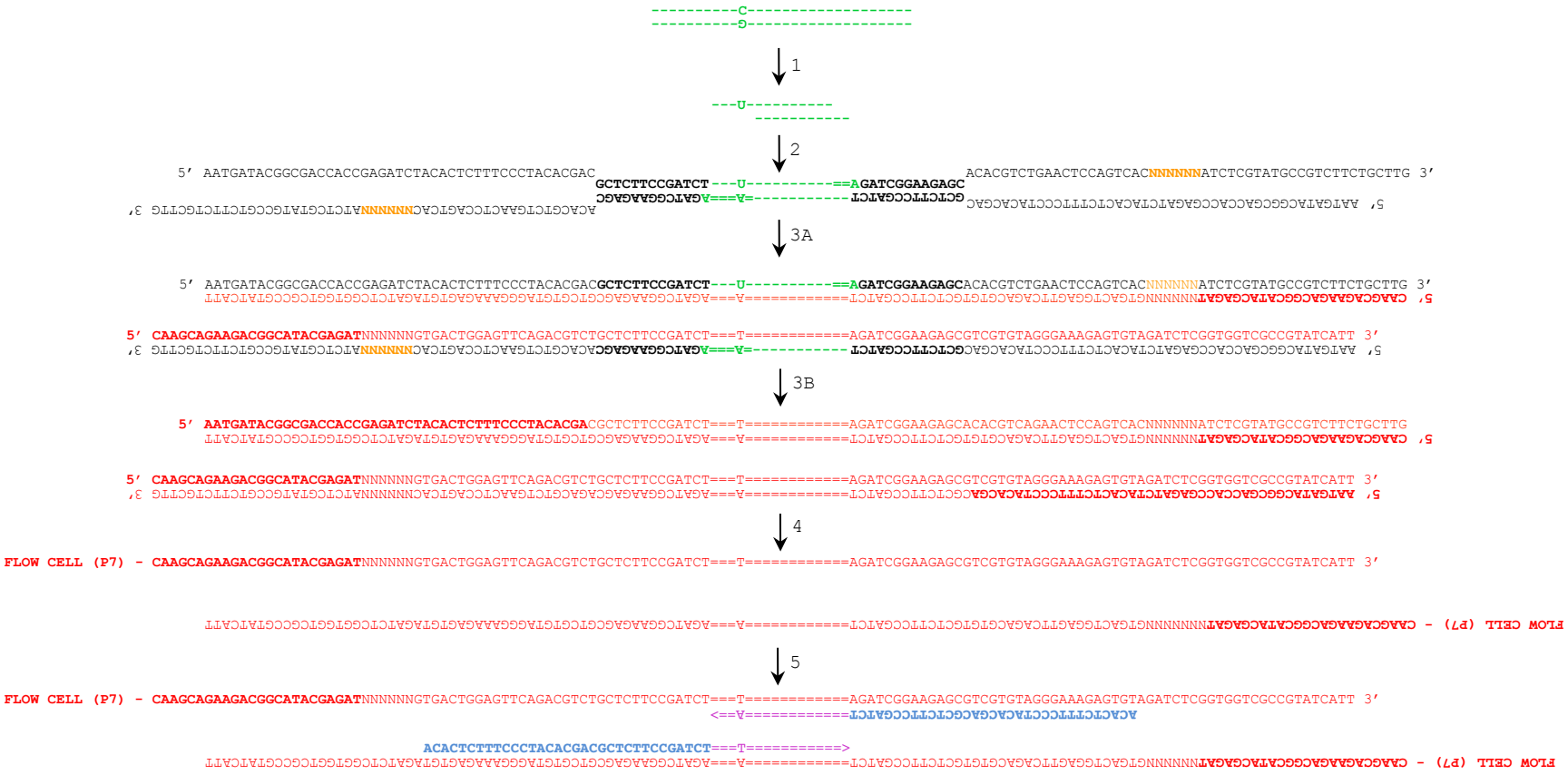
The First Ancient Epigenome

Cytosine Methylation and Gene Expression

Post-Mortem Cytosine Deamination



Orlando-Seguin et al. STAR 2015

The First Ancient Epigenome

Cytosine Methylation and Gene Expression

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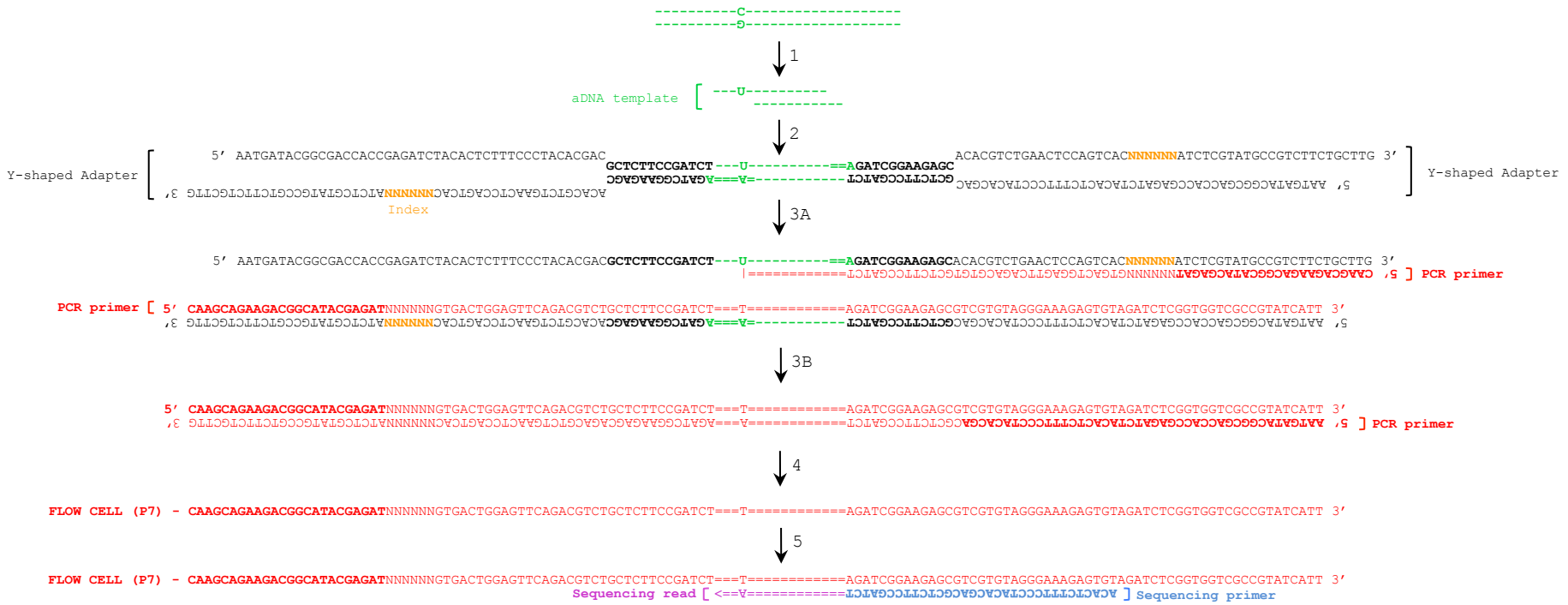

Orlando-Seguin et al. STAR 2015

The First Ancient Epigenome

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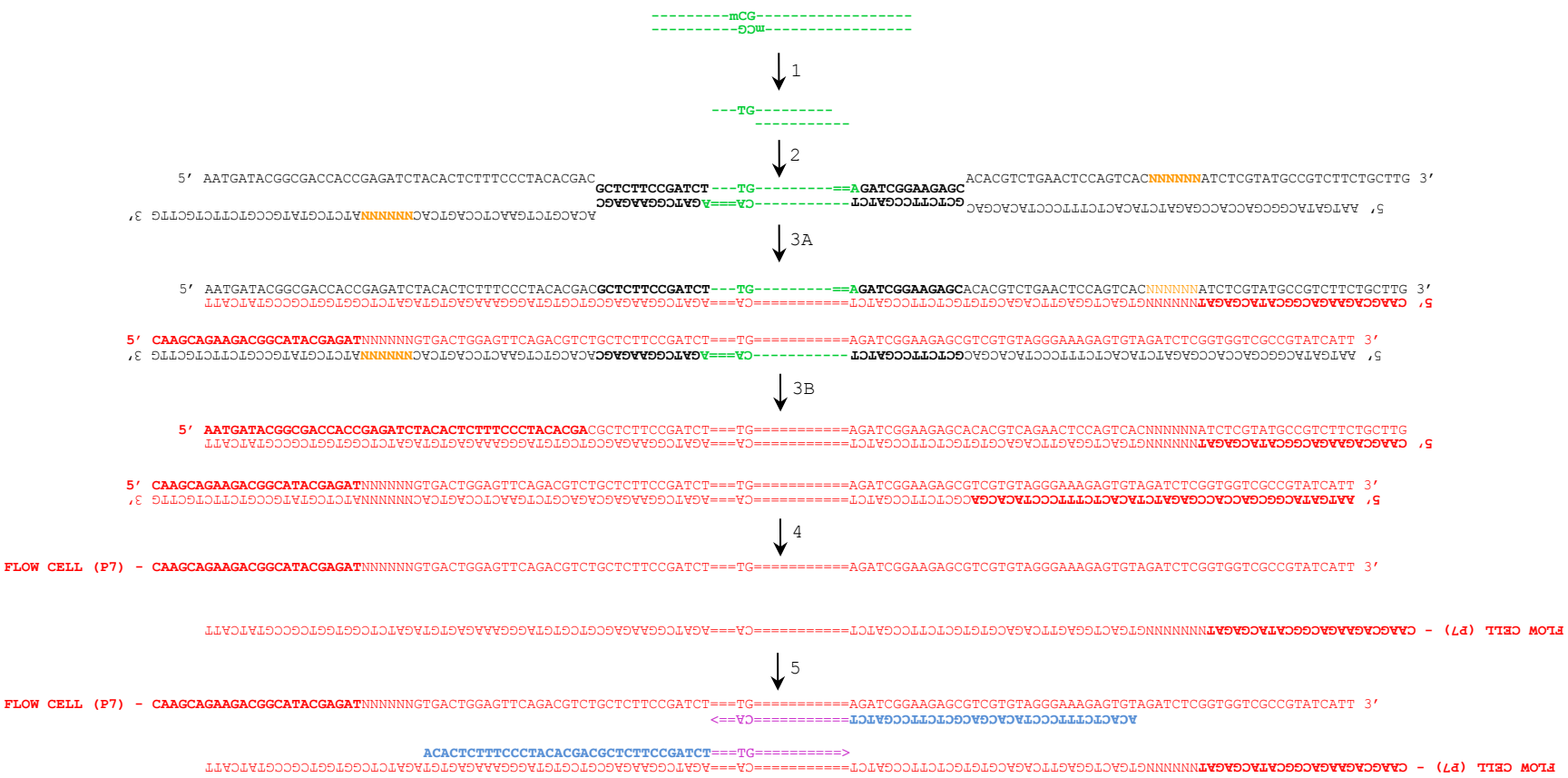

Orlando-Seguin et al. STAR 2015

The First Ancient Epigenome

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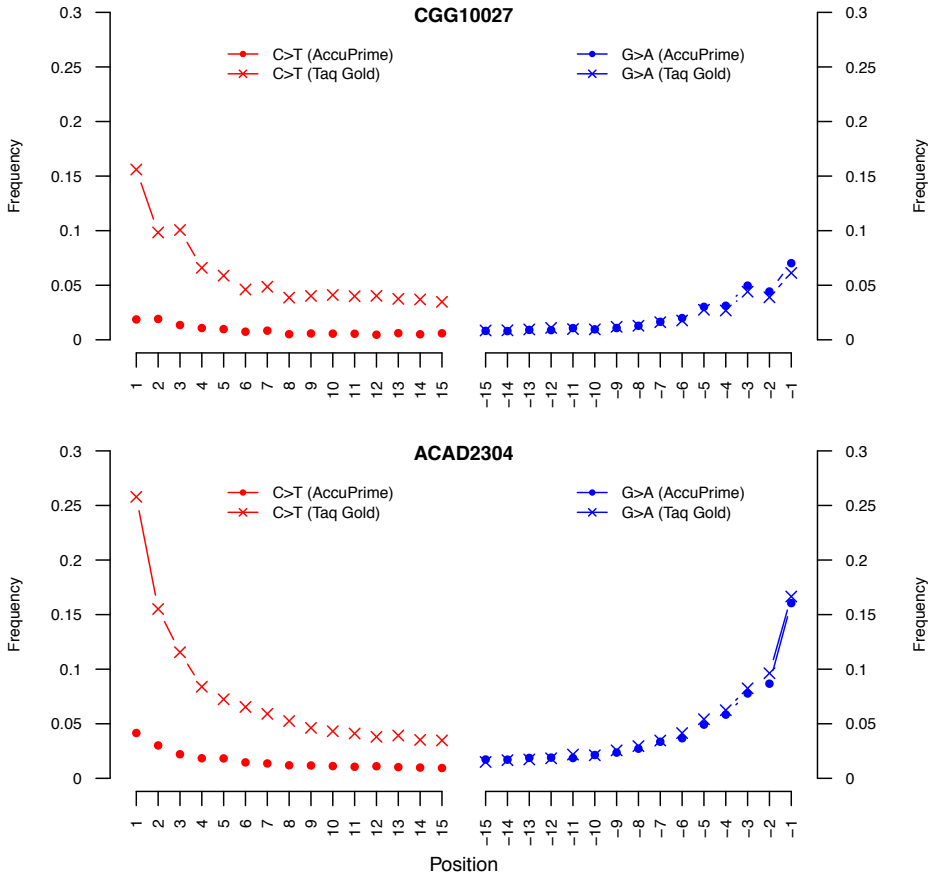

Orlando-Seguin et al. STAR 2015

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