

Ancient DNA



Two parts

1. Background and problems

2. Examples

Part 1: What it is not about



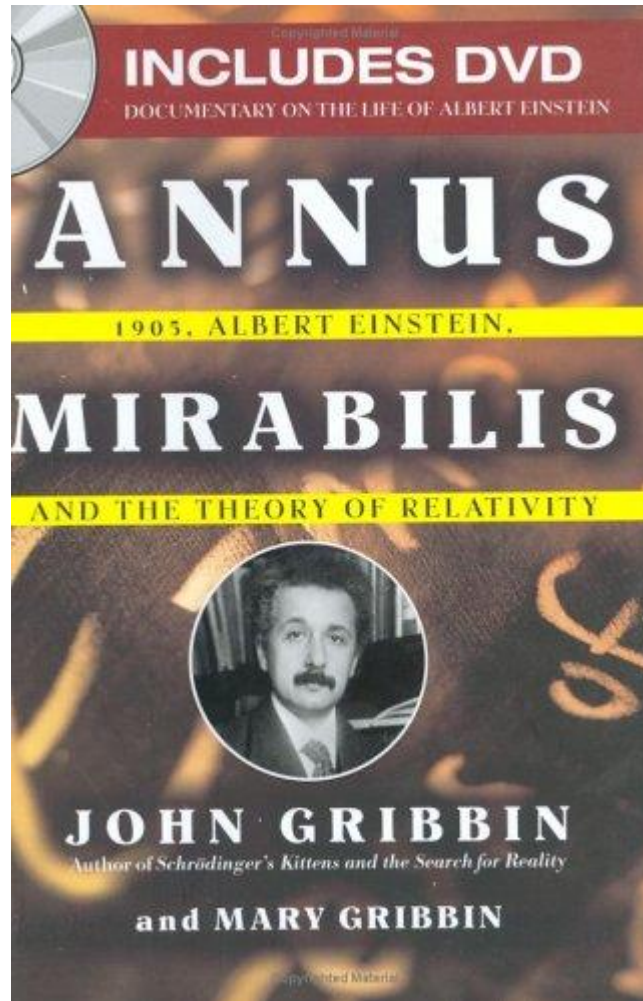
What it is about: Genome sequencing



Before 2005: small data sets, limited scope

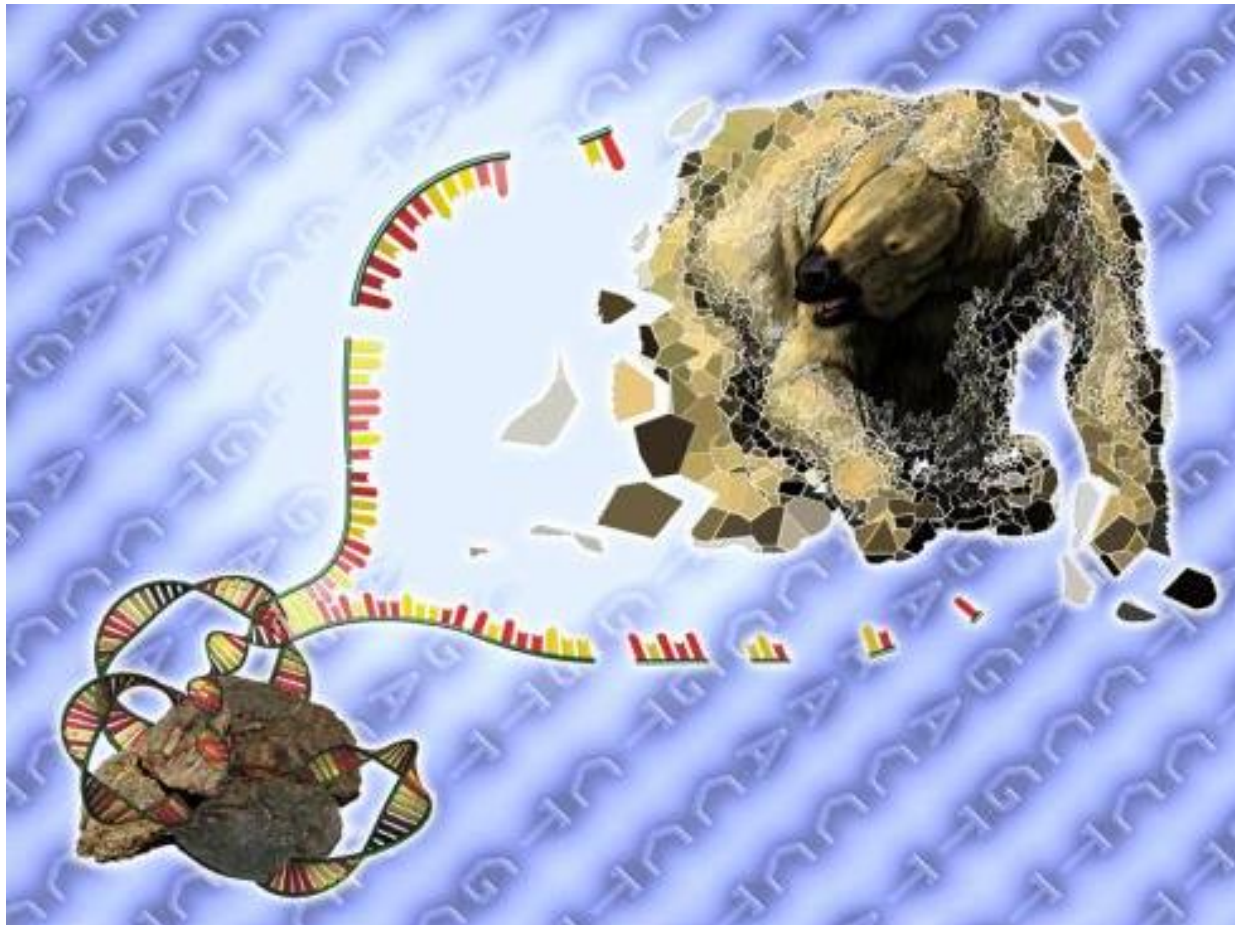


Annus mirabilis 1905



Annus mirabilis 2005: slightly less important

Birth of paleogenomics



Ancient DNA's annus mirabilis 2005

2nd June 2005

Genomic Sequencing of Pleistocene Cave Bears

**James P. Noonan,^{1,2} Michael Hofreiter,³ Doug Smith,¹
James R. Priest,² Nadin Rohland,³ Gernot Rabeder,⁴
Johannes Krause,³ J. Chris Detter,^{1,5} Svante Pääbo,³
Edward M. Rubin^{1,2*}**

Despite the greater information content of genomic DNA, ancient DNA studies have largely been limited to the amplification of mitochondrial sequences. Here we describe metagenomic libraries constructed with unamplified DNA extracted from skeletal remains of two 40,000-year-old extinct cave bears. Analysis of ~1 megabase of sequence from each library showed that despite significant microbial contamination, 5.8 and 1.1% of clones contained cave bear inserts, yielding 26,861 base pairs of cave bear genome sequence. Comparison of cave bear and modern bear sequences revealed the evolutionary relationship of these lineages. The metagenomic approach used here establishes the feasibility of ancient DNA genome sequencing programs.

Ancient DNA's annus mirabilis 2005

21st July 2005



Opinion

TRENDS in Ecology and Evolution Vol.20 No.10 October 2005

Full text provided by www.sciencedirect.com



Assessing ancient DNA studies

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Ancient DNA's annus mirabilis 2005

31st July 2005

nature

Vol 437 | 15 September 2005 | doi:10.1038/nature03959

ARTICLES

Genome sequencing in microfabricated high-density picolitre reactors

Marcel Margulies^{1*}, Michael Egholm^{1*}, William E. Altman¹, Said Attiya¹, Joel S. Bader¹, Lisa A. Bemben¹, Jan Berka¹, Michael S. Braverman¹, Yi-Ju Chen¹, Zhoutao Chen¹, Scott B. Dewell¹, Lei Du¹, Joseph M. Fierro¹, Xavier V. Gomes¹, Brian C. Godwin¹, Wen He¹, Scott Helgesen¹, Chun He Ho¹, Gerard P. Irzyk¹, Szilveszter C. Jando¹, Maria L. I. Alenquer¹, Thomas P. Jarvie¹, Kshama B. Jirage¹, Jong-Bum Kim¹, James R. Knight¹, Janna R. Lanza¹, John H. Leamon¹, Steven M. Lefkowitz¹, Ming Lei¹, Jing Li¹, Kenton L. Lohman¹, Hong Lu¹, Vinod B. Makhijani¹, Keith E. McDade¹, Michael P. McKenna¹, Eugene W. Myers², Elizabeth Nickerson¹, John R. Nobile¹, Ramona Plant¹, Bernard P. Puc¹, Michael T. Ronan¹, George T. Roth¹, Gary J. Sarkis¹, Jan Fredrik Simons¹, John W. Simpson¹, Maithreyan Srinivasan¹, Karrie R. Tartaro¹, Alexander Tomasz³, Kari A. Vogt¹, Greg A. Volkmer¹, Shally H. Wang¹, Yong Wang¹, Michael P. Weiner⁴, Pengguang Yu¹, Richard F. Begley¹ & Jonathan M. Rothberg¹

The proliferation of large-scale DNA-sequencing projects in recent years has driven a search for alternative methods to reduce time and cost. Here we describe a scalable, highly parallel sequencing system with raw throughput significantly greater than that of state-of-the-art capillary electrophoresis instruments. The apparatus uses a novel fibre-optic slide of individual wells and is able to sequence 25 million bases, at 99% or better accuracy, in one four-hour run. To achieve an approximately 100-fold increase in throughput over current Sanger sequencing technology, we have developed an emulsion method for DNA amplification and an instrument for sequencing by synthesis using a pyrosequencing protocol optimized for solid support and picolitre-scale volumes. Here we show the utility, throughput, accuracy and robustness of this system by shotgun sequencing and *de novo* assembly of the *Mycoplasma genitalium* genome with 96% coverage at 99.96% accuracy in one run of the machine.

454 Life Sciences: 20 Mb sequence data



Ancient DNA's annus mirabilis 2005

11th November 2005

Ancient DNA from the First European Farmers in 7500-Year-Old Neolithic Sites

**Wolfgang Haak,^{1*} Peter Forster,² Barbara Bramanti,¹
Shuichi Matsumura,² Guido Brandt,¹ Marc Tänzler,¹
Richard Villems,³ Colin Renfrew,² Detlef Gronenborn,⁴
Kurt Werner Alt,¹ Joachim Burger¹**

The ancestry of modern Europeans is a subject of debate among geneticists, archaeologists, and anthropologists. A crucial question is the extent to which Europeans are descended from the first European farmers in the Neolithic Age 7500 years ago or from Paleolithic hunter-gatherers who were present in Europe since 40,000 years ago. Here we present an analysis of ancient DNA from early European farmers. We successfully extracted and sequenced intact stretches of maternally inherited mitochondrial DNA (mtDNA) from 24 out of 57 Neolithic skeletons from various locations in Germany, Austria, and Hungary. We found that 25% of the Neolithic farmers had one characteristic mtDNA type and that this type formerly was widespread among Neolithic farmers in Central Europe. Europeans today have a 150-times lower frequency (0.2%) of this mtDNA type, revealing that these first Neolithic farmers did not have a strong genetic influence on modern European female lineages. Our finding lends weight to a proposed Paleolithic ancestry for modern Europeans.

Ancient DNA's annus mirabilis 2005

18th December 2005



Ancient DNA's annus mirabilis 2005

20th December 2005



REPORTS

Metagenomics to Paleogenomics: Large-Scale Sequencing of Mammoth DNA

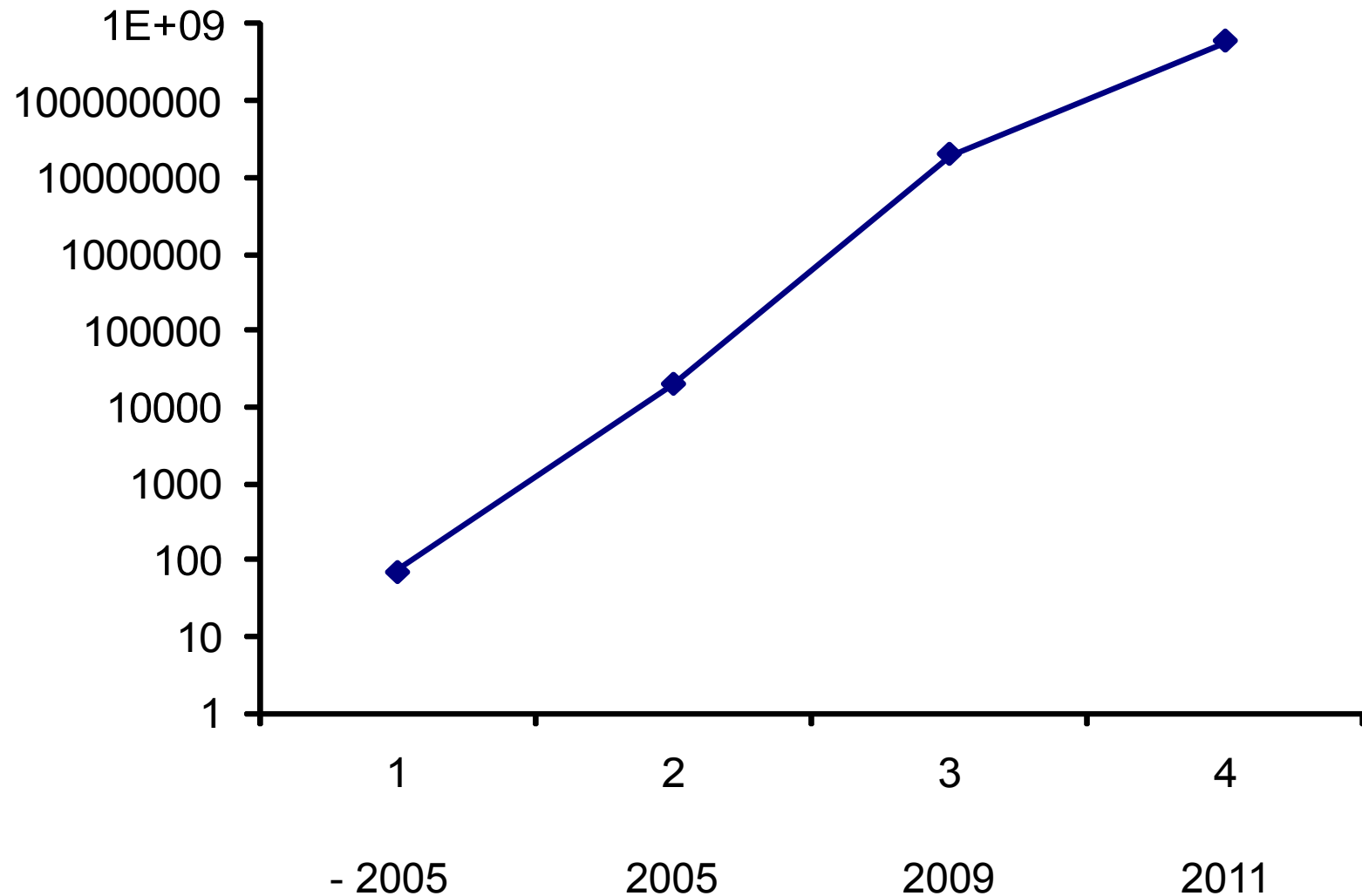
Hendrik N. Poinar,^{1,2,3*} Carsten Schwarz,^{1,2} Ji Qi,⁴ Beth Shapiro,⁵ Ross D. E. MacPhee,⁶ Bernard Buigues,⁷ Alexei Tikhonov,⁸ Daniel H. Huson,⁹ Lynn P. Tomsho,⁴ Alexander Auch,⁹ Markus Rampp,¹⁰ Webb Miller,⁴ Stephan C. Schuster^{4*}

We sequenced 28 million base pairs of DNA in a metagenomics approach, using a woolly mammoth (*Mammuthus primigenius*) sample from Siberia. As a result of exceptional sample preservation and the use of a recently developed emulsion polymerase chain reaction and pyrosequencing technique, 13 million base pairs (45.4%) of the sequencing reads were identified as mammoth DNA. Sequence identity between our data and African elephant (*Loxodonta africana*) was 98.55%, consistent with a paleontologically based divergence date of 5 to 6 million years. The sample includes a surprisingly small diversity of environmental DNAs. The high percentage of endogenous DNA recoverable from this single mammoth would allow for completion of its genome, unleashing the field of paleogenomics.

From 454 to Illumina sequencing



Sequencing yield per machine run in kilobases



Sequencing yield per machine run in kilobases

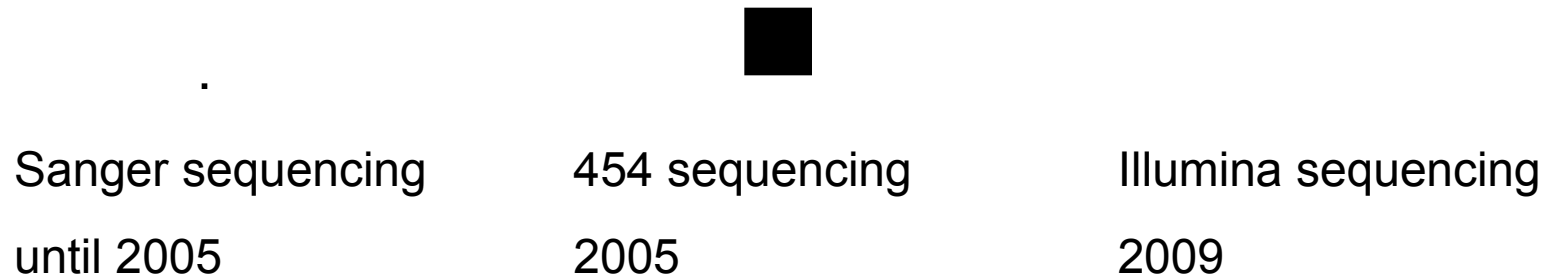
.

Sanger sequencing
until 2005

454 sequencing
2005

Illumina sequencing
2009

Sequencing yield per machine run in kilobases



Paleogenomics: Neanderthals and human evolution



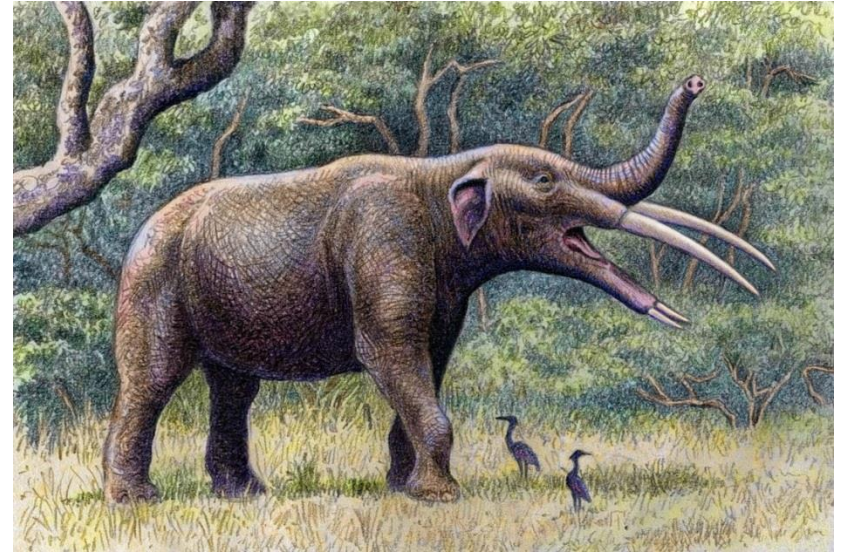
But I won't talk about this



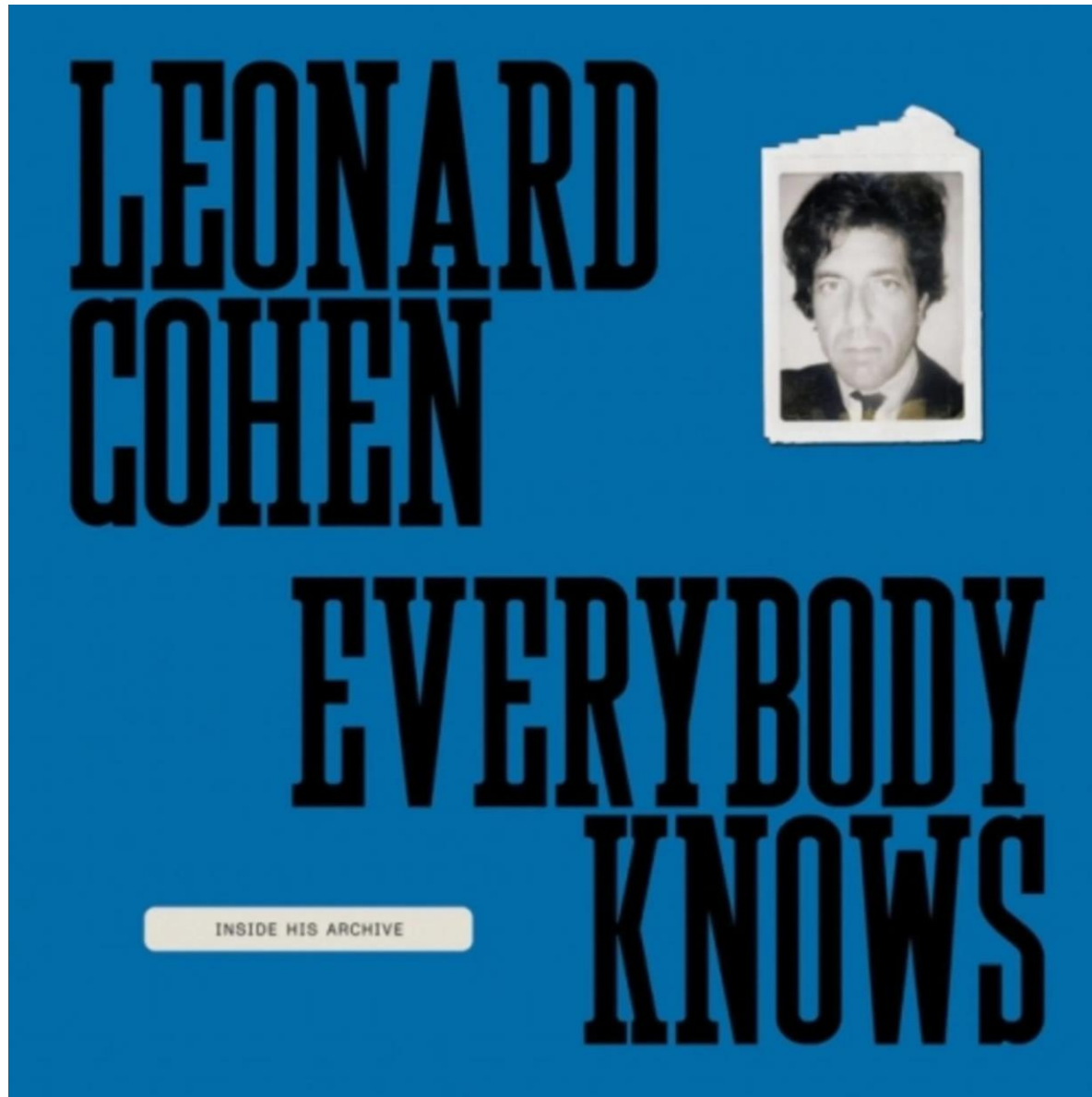
It's not my job



There are much more exciting species!



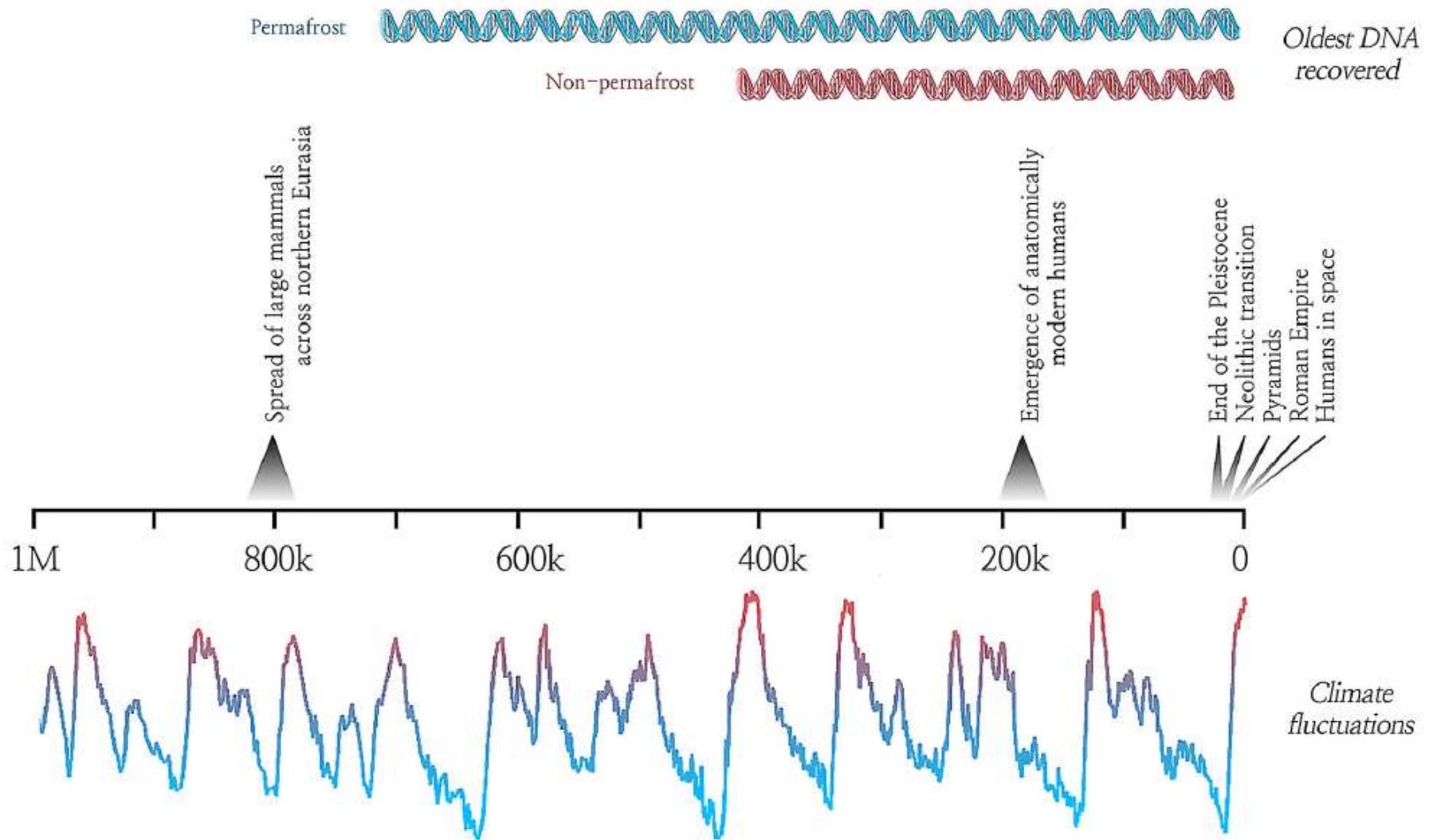
And anyway



So, what is ancient DNA?

1. No clear definition or age limit.
2. Broadly speaking, DNA from samples that have not been stored with the explicit aim of preserving DNA.
3. Sometimes distinction between museum (< 200 years), historical (< 500 years) and “true” ancient DNA (> 500 years).

And how old does it get??



Article

Million-year-old DNA sheds light on the genomic history of mammoths

<https://doi.org/10.1038/s41586-021-03224-9>

Received: 3 July 2020

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Check for updates

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Article

A 2-million-year-old ecosystem in Greenland uncovered by environmental DNA

<https://doi.org/10.1038/s41586-022-05453-y>

Received: 30 September 2021

Accepted: 18 October 2022

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Open access



Check for updates

Kurt H. Kjær^{1,27}, Mikkel Winther Pedersen^{1,27}, Bianca De Sanctis^{2,3}, Binia De Cahsan⁴, Thorfinn S. Korneliussen¹, Christian S. Michelsen^{1,5}, Karina K. Sand¹, Stanislav Jelavić^{1,6}, Anthony H. Ruter¹, Astrid M. A. Schmidt^{7,8}, Kristian K. Kjeldsen⁹, Alexey S. Tesakov¹⁰, Ian Snowball¹¹, John C. Gosse¹², Inger G. Alsos¹³, Yucheng Wang^{1,2}, Christoph Dockter¹⁴, Magnus Rasmussen¹⁴, Morten E. Jørgensen¹⁴, Birgitte Skadhauge¹⁴, Ana Prohaska^{1,2}, Jeppe Å. Kristensen^{15,16}, Morten Bjerager¹⁷, Morten E. Allentoft^{1,18}, Eric Coissac^{13,19}, PhyloNorway Consortium^{*,**}, Alexandra Rouillard^{1,21}, Alexandra Simakova¹⁰, Antonio Fernandez-Guerra¹, Chris Bowler²⁰, Marc Macias-Fauria²², Lasse Vinner¹, John J. Welch³, Alan J. Hidy²³, Martin Sikora¹, Matthew J. Collins^{24,25}, Richard Durbin³, Nicolaj K. Larsen¹ & Eske Willerslev^{1,2,26}

Late Pliocene and Early Pleistocene epochs 3.6 to 0.8 million years ago¹ had climates resembling those forecasted under future warming². Palaeoclimatic records show strong polar amplification with mean annual temperatures of 11–19 °C above contemporary values^{3,4}. The biological communities inhabiting the Arctic during this time remain poorly known because fossils are rare⁵. Here we report an ancient environmental DNA⁶ (eDNA) record describing the rich plant and animal assemblages of the Kap København Formation in North Greenland, dated to around two million years ago. The record shows an open boreal forest ecosystem with mixed vegetation

What characterizes ancient DNA?

1. Generally small amounts of DNA – kind of
2. Contamination – sometimes
3. Short fragments – largely true
4. Miscoding lesions – generally true

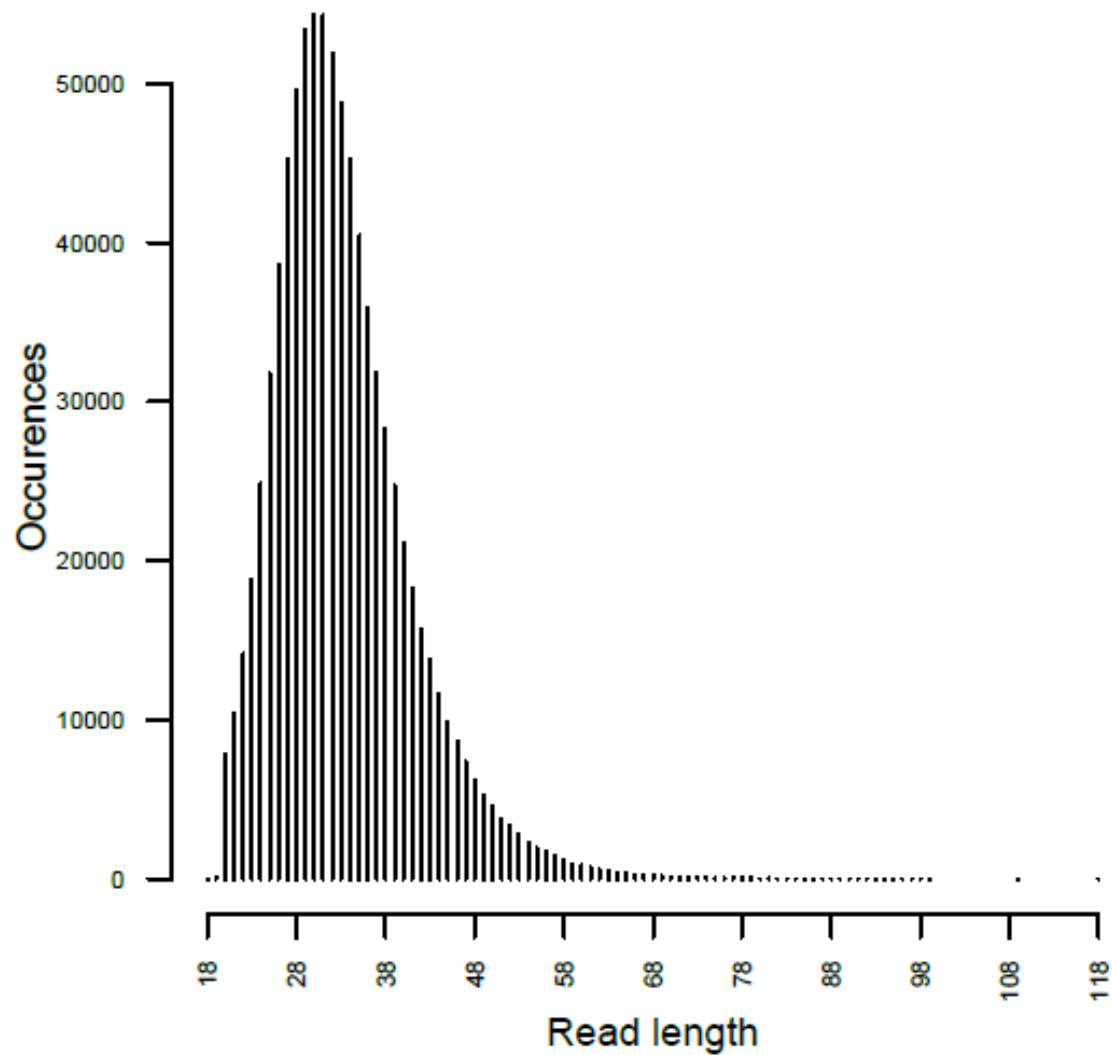
Properties of DNA



Properties of ancient DNA



Thus, ancient DNA is short



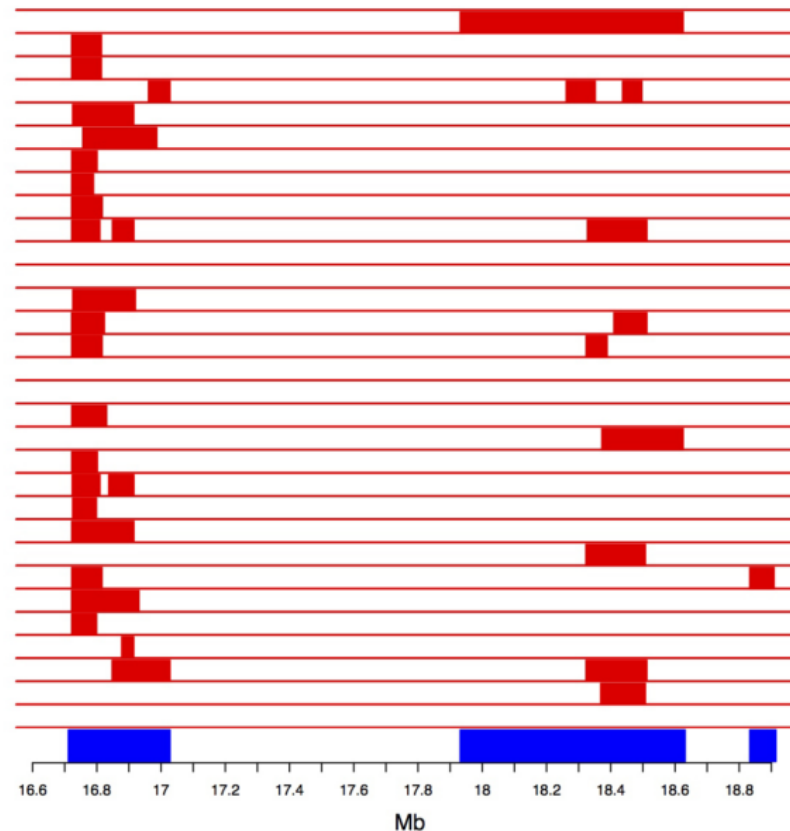
Unless it's found in modern species

LETTER

doi:10.1038/nature12961

The genomic landscape of Neanderthal ancestry in present-day humans

Sriram Sankararaman^{1,2}, Swapan Mallick^{1,2}, Michael Dannemann³, Kay Prüfer³, Janet Kelso³, Svante Pääbo³, Nick Patterson^{1,2} & David Reich^{1,2,4}



1. Present in low amounts?

material was digested (2 h at 50 °C), the fine powder only yielded **trace amounts** of DNA. In contrast, the coarse powder

Even in a well-preserved sample, aDNA is generally only present in **small amounts**, and the number of samples

Rohland & Hofreiter 2007a

Hofreiter et al 2013

of ancient DNA. It accounts for the fact that ancient DNA is usually **highly fragmented and present only in trace amounts**.

Gansauge & Meyer 2013

DNA sequences to be determined (3). Third, because ancient DNA is **present in low amounts** or absent in many specimens,

Briggs et al. 2007

General considerations about handling The samples used for extraction will usually contain only **trace amounts** of DNA. To avoid cross-contamination

Stiller et al. 2009

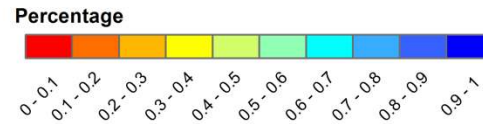
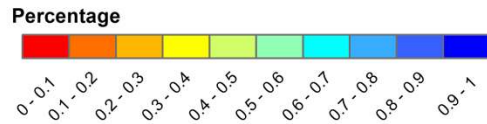
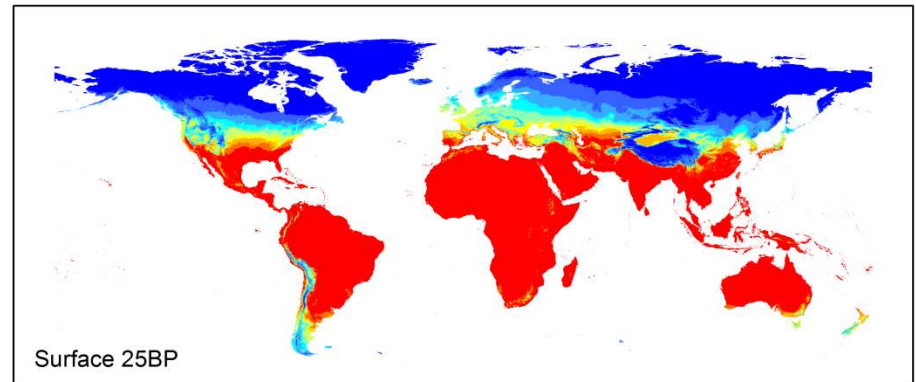
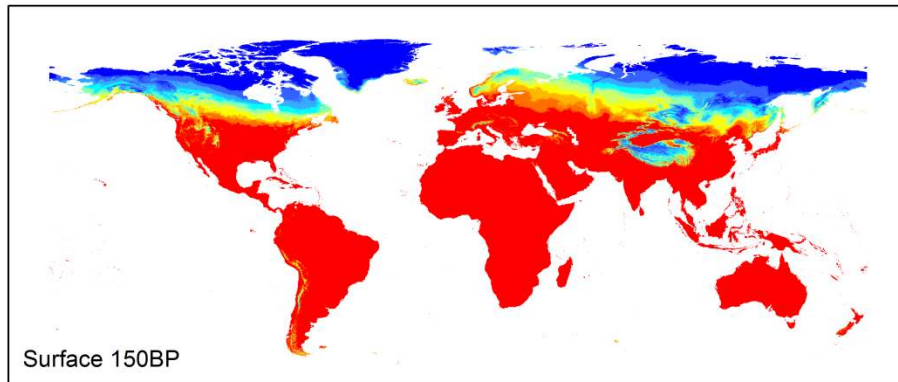
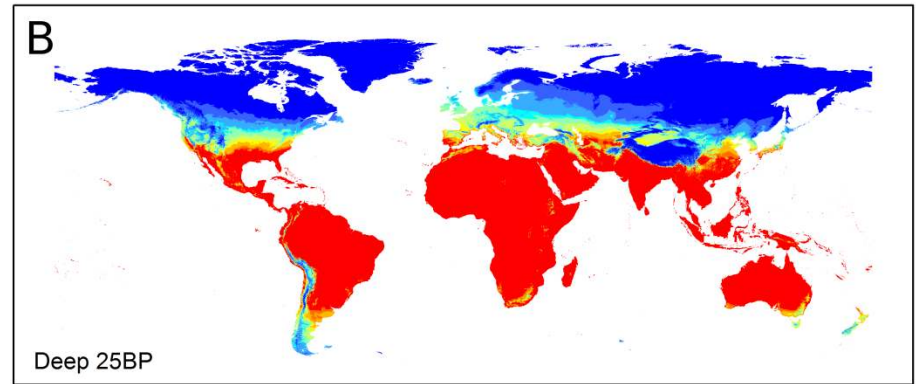
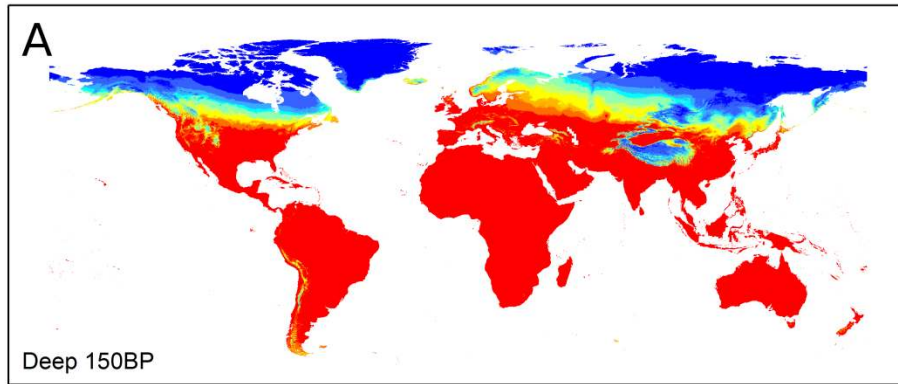
to observe evolution in “real time.” However, the DNA isolated from such material, commonly referred to as “ancient DNA,” is typically present in **low amounts**, heavily fragmented, chemically

Rohland & Hofreiter 2007b

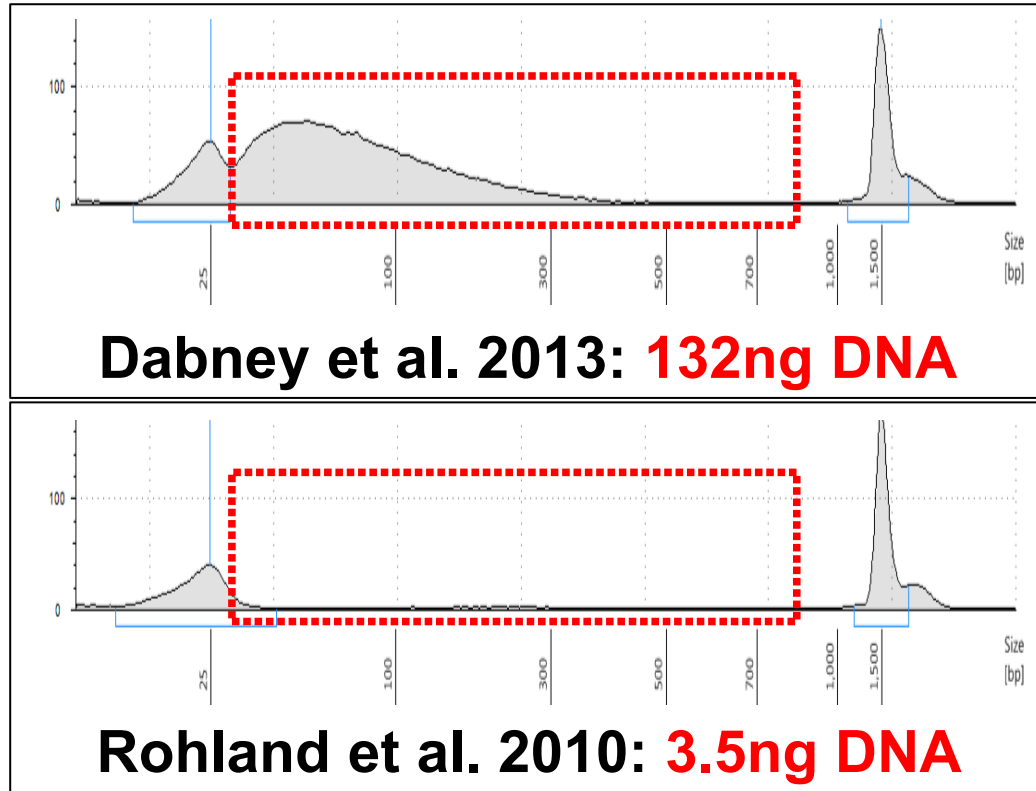
Despite these advances, sequencing ancient human DNA continues to be challenging for several reasons. First, only **trace amounts** of highly fragmented DNA are usually preserved in an-

Gansauge & Meyer 2014

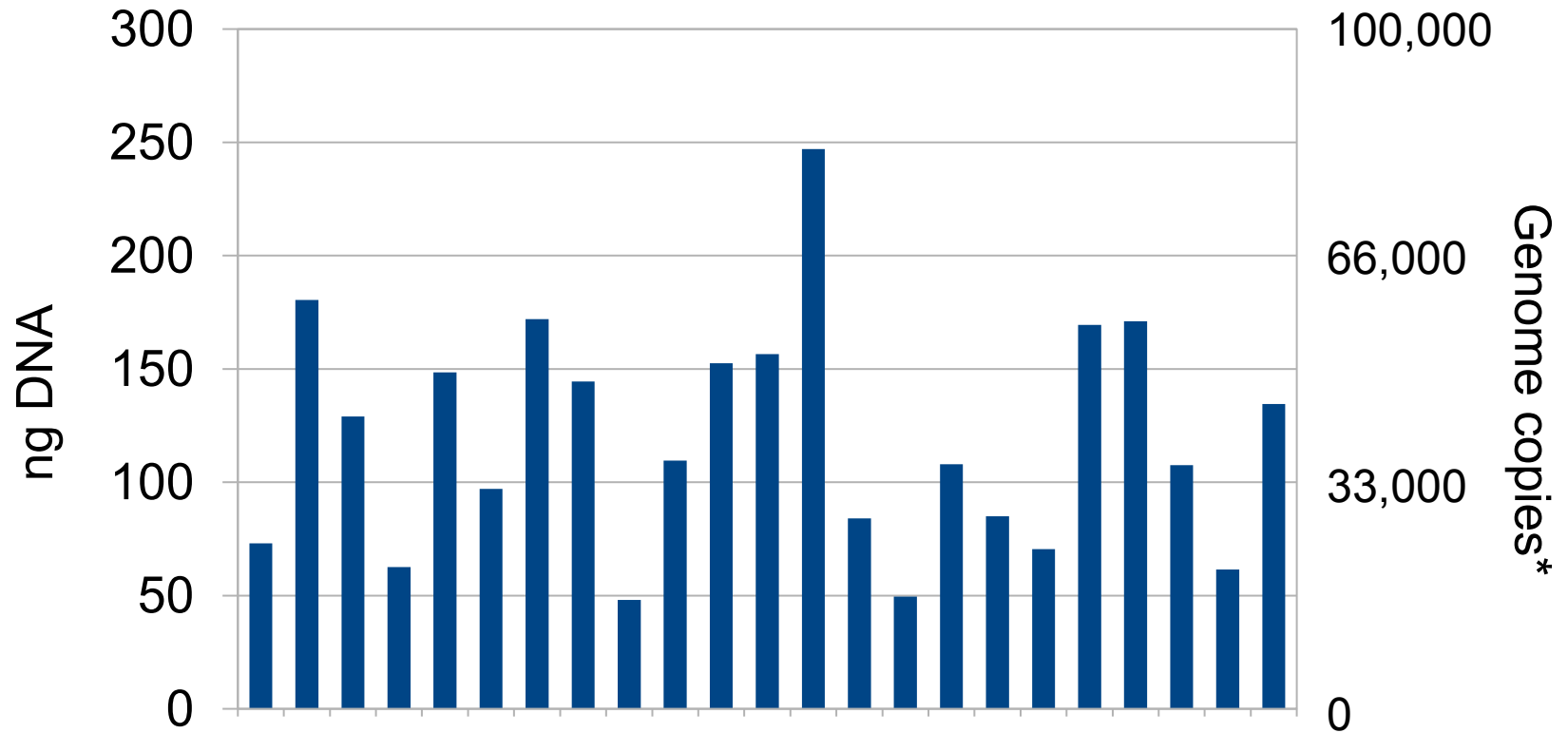
Temperature and DNA preservation



Partially a problem of DNA extraction

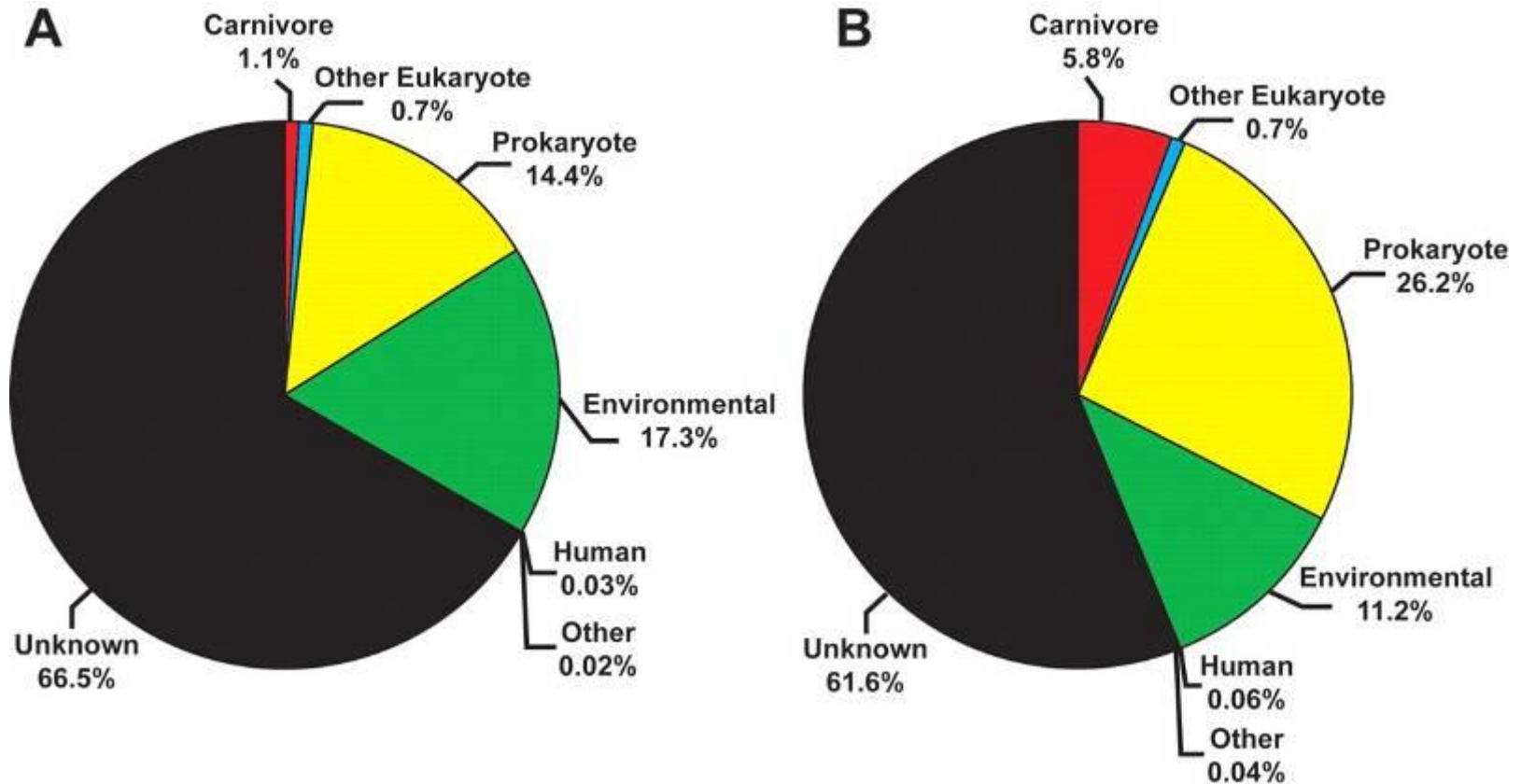


DNA from 50 mg cave bear bone powder



*** assuming 100% cave bear DNA**

But often little endogenous DNA



What
Do I Do
Now



Solution 1: Get rich



Solution 2: Bleach your samples



Solution 3: Target your DNA



Solution 4: Luck

RESEARCH ARTICLES

A High-Coverage Genome Sequence from an Archaic Denisovan Individual

Matthias Meyer,^{1*†} Martin Kircher,^{1*†} Marie-Theres Gansauge,¹ Heng Li,² Fernando Racimo,¹ Swapan Mallick,^{2,3} Joshua G. Schraiber,⁴ Flora Jay,⁴ Kay Prüfer,¹ Cesare de Filippo,¹ Peter H. Sudmant,⁶ Can Alkan,^{5,6} Qiaomei Fu,^{1,7} Ron Do,² Nadin Rohland,^{2,3} Arti Tandon,^{2,3} Michael Siebauer,¹ Richard E. Green,⁸ Katarzyna Bryc,³ Adrian W. Briggs,³ Udo Stenzel,¹ Jesse Dabney,¹ Jay Shendure,⁶ Jacob Kitzman,⁶ Michael F. Hammer,⁹ Michael V. Shunkov,¹⁰ Anatoli P. Derevianko,¹⁰ Nick Patterson,² Aida M. Andrés,¹ Evan E. Eichler,^{6,11} Montgomery Slatkin,⁴ David Reich,^{2,3†} Janet Kelso,¹ Svante Pääbo^{1†}



contamination. The fraction of hominin endogenous DNA is commonly smaller than 1% and rarely approaches 5% (1, 7), which makes shotgun sequencing of the entire genome economically and logistically impractical. The only known exception is the Denisovan phalanx, which contains ~70% endogenous DNA. However, an

Solution 5:



Solution 5: choose wisely

ARTICLE

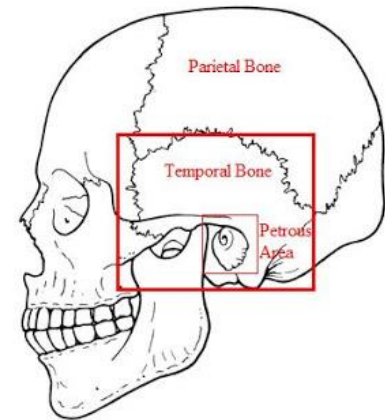
Received 4 Apr 2014 | Accepted 11 Sep 2014 | Published 21 Oct 2014

DOI: 10.1038/ncomms6257

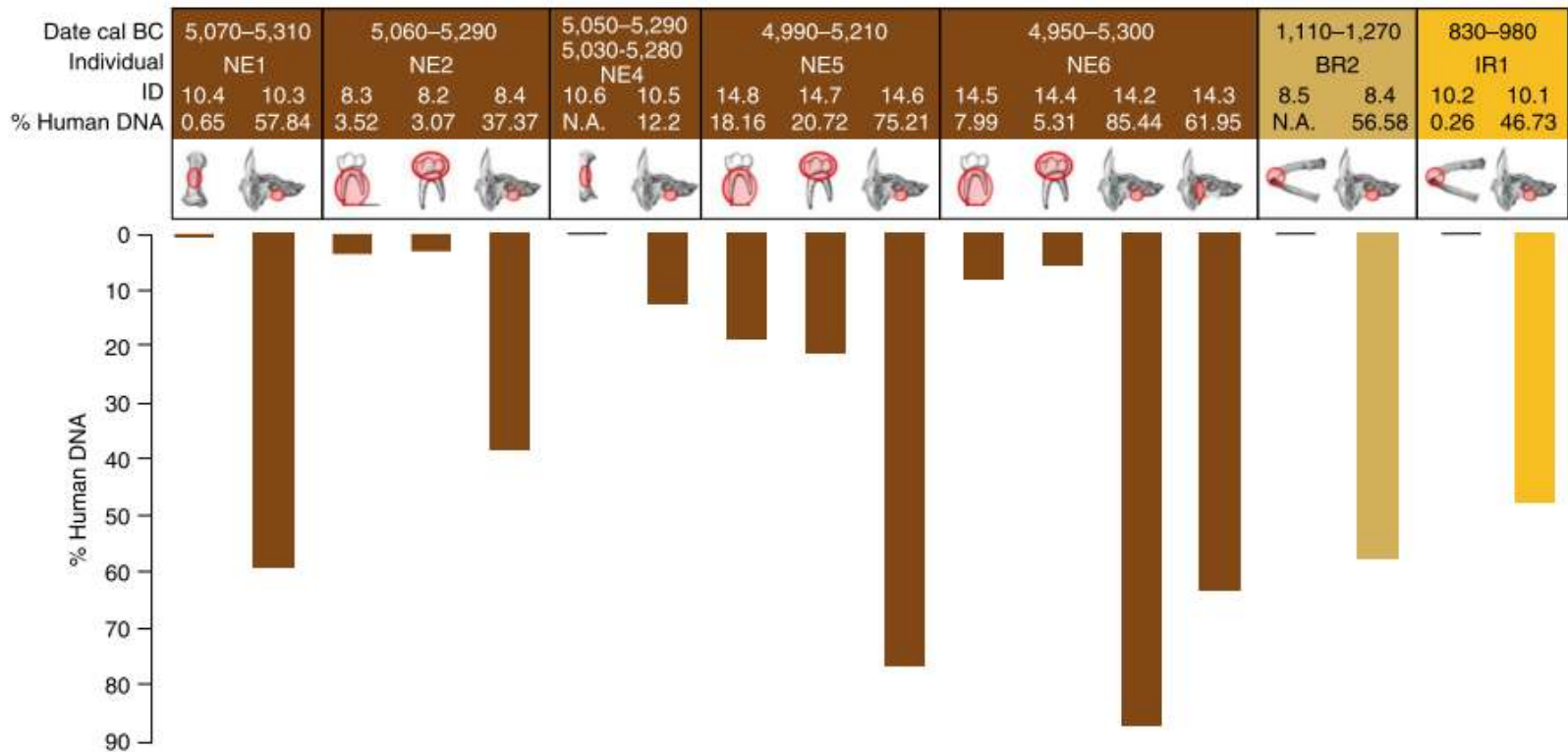
OPEN

Genome flux and stasis in a five millennium transect of European prehistory

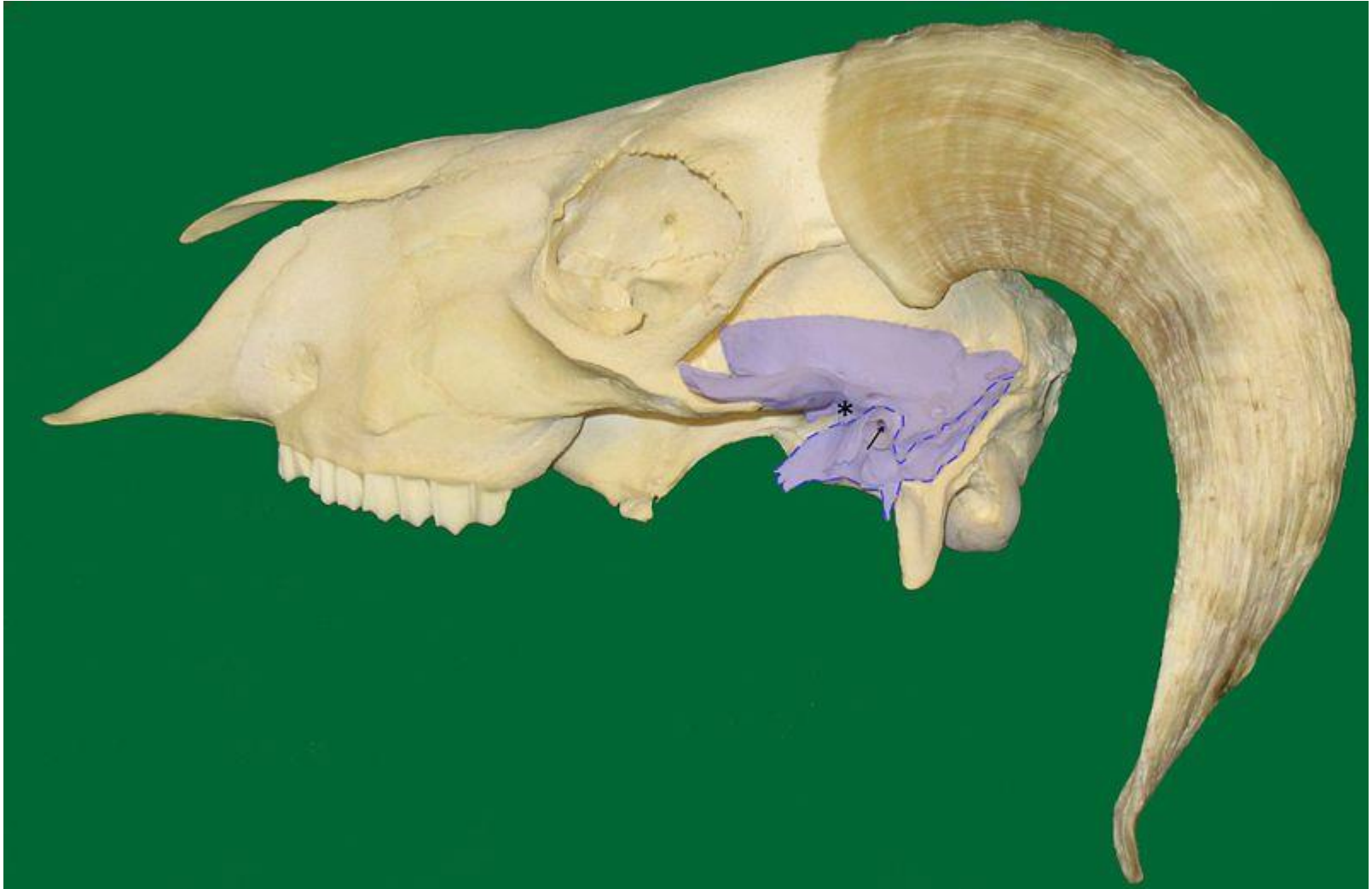
Cristina Gamba^{1,2,3}, Eppie R. Jones³, Matthew D. Teasdale³, Russell L. McLaughlin³, Gloria Gonzalez-Fortes⁴, Valeria Mattiangeli³, László Domboróczy⁵, Ivett Kővári⁶, Ildikó Pap⁷, Alexandra Anders⁸, Alasdair Whittle⁹, János Dani¹⁰, Pál Raczky⁸, Thomas F.G. Higham¹¹, Michael Hofreiter⁴, Daniel G. Bradley^{3,*} & Ron Pinhasi^{1,2,3,12,*}



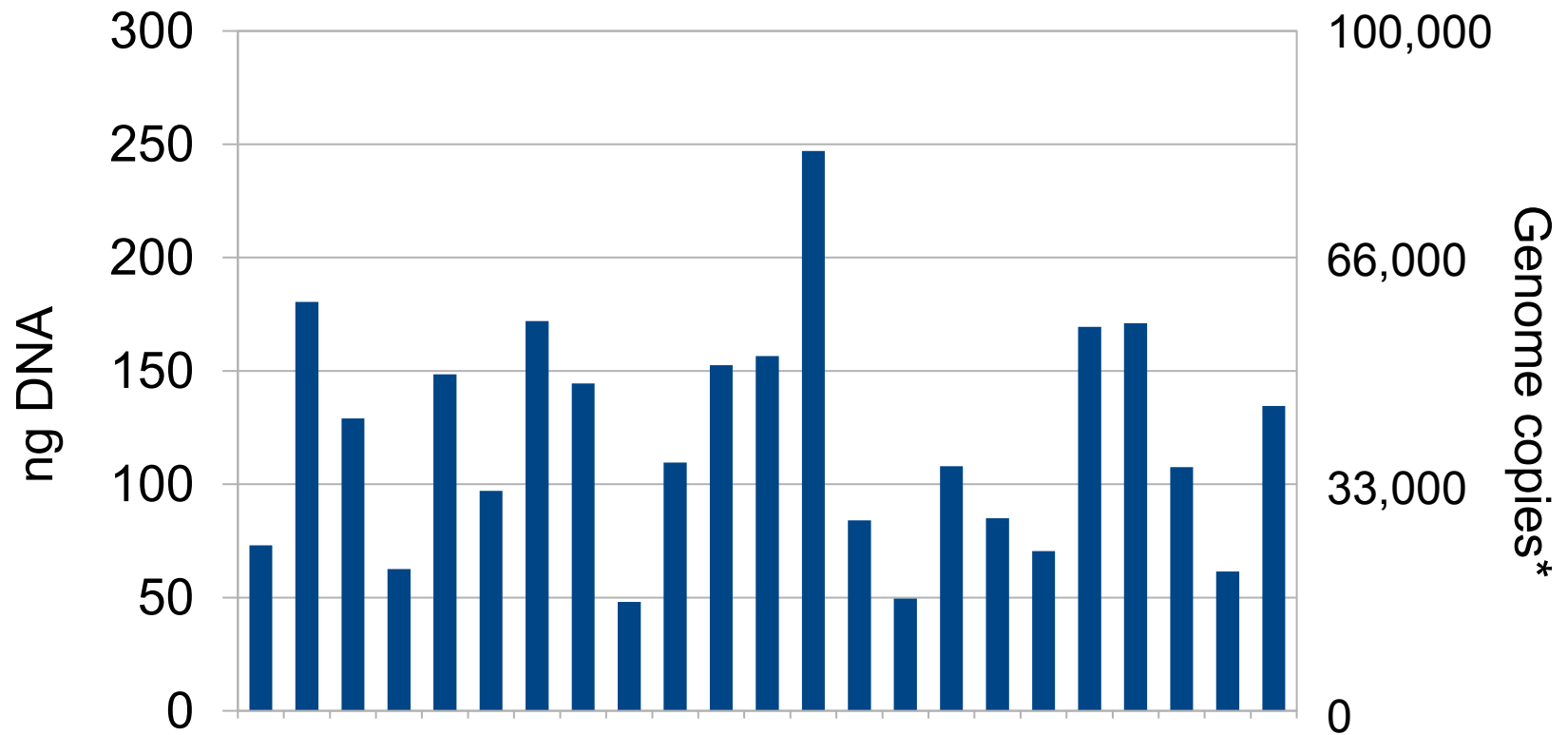
Choose the right bones



The petrous bone

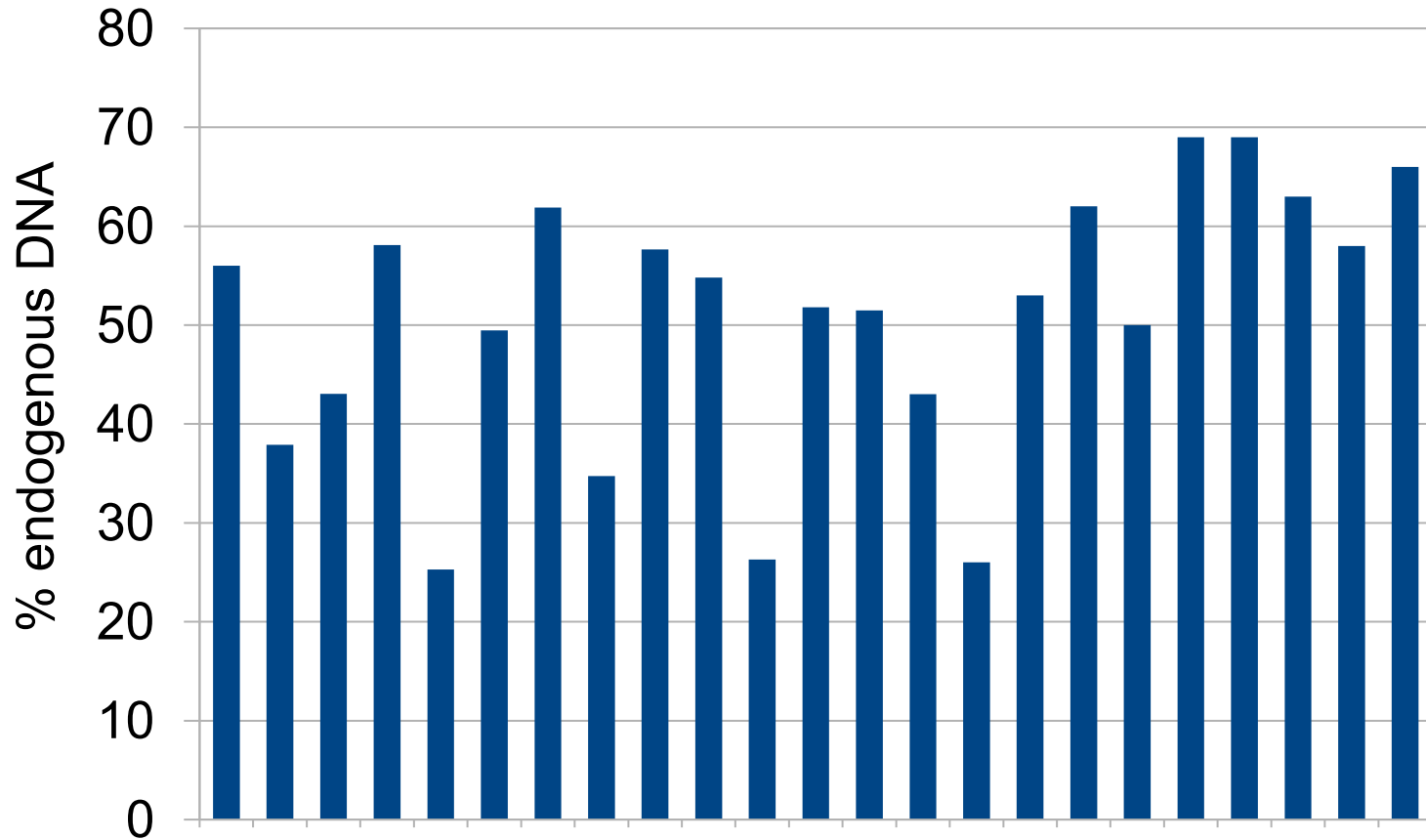


DNA from 50 mg cave bear **petrous** bone powder

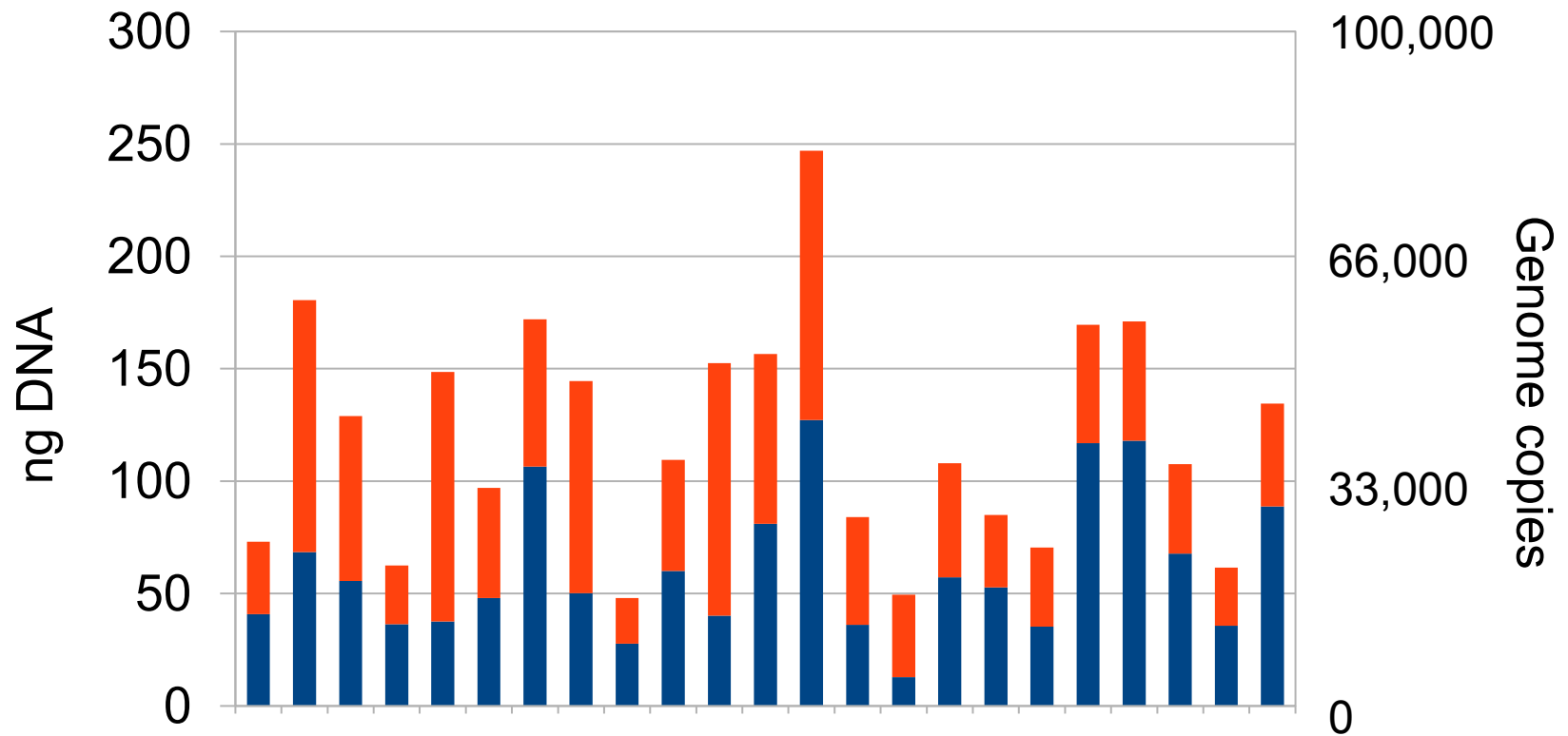


* assuming 100% cave bear DNA

Endogenous content



Real genome copies from 50 mg bone powder

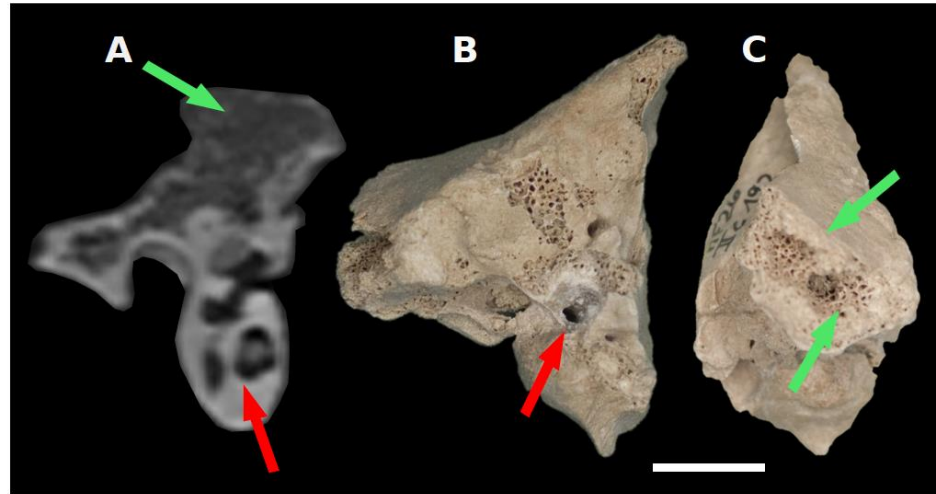


Low amounts of DNA?

Often correct.....

But you can obtain large amounts of DNA from some ancient samples.

Improve your choice



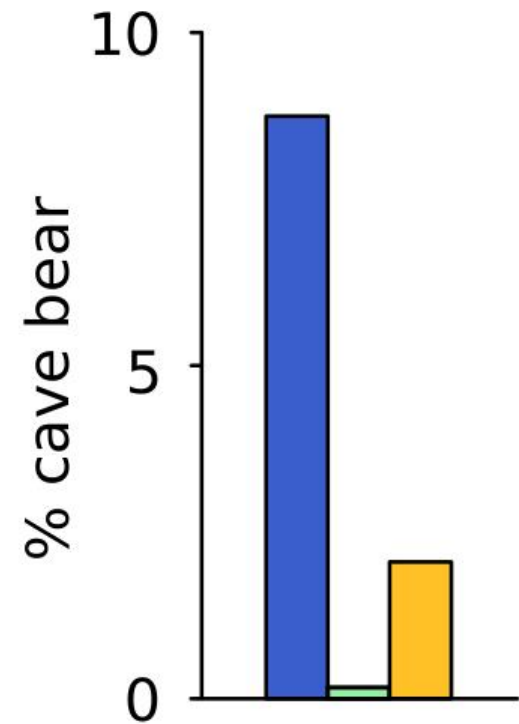
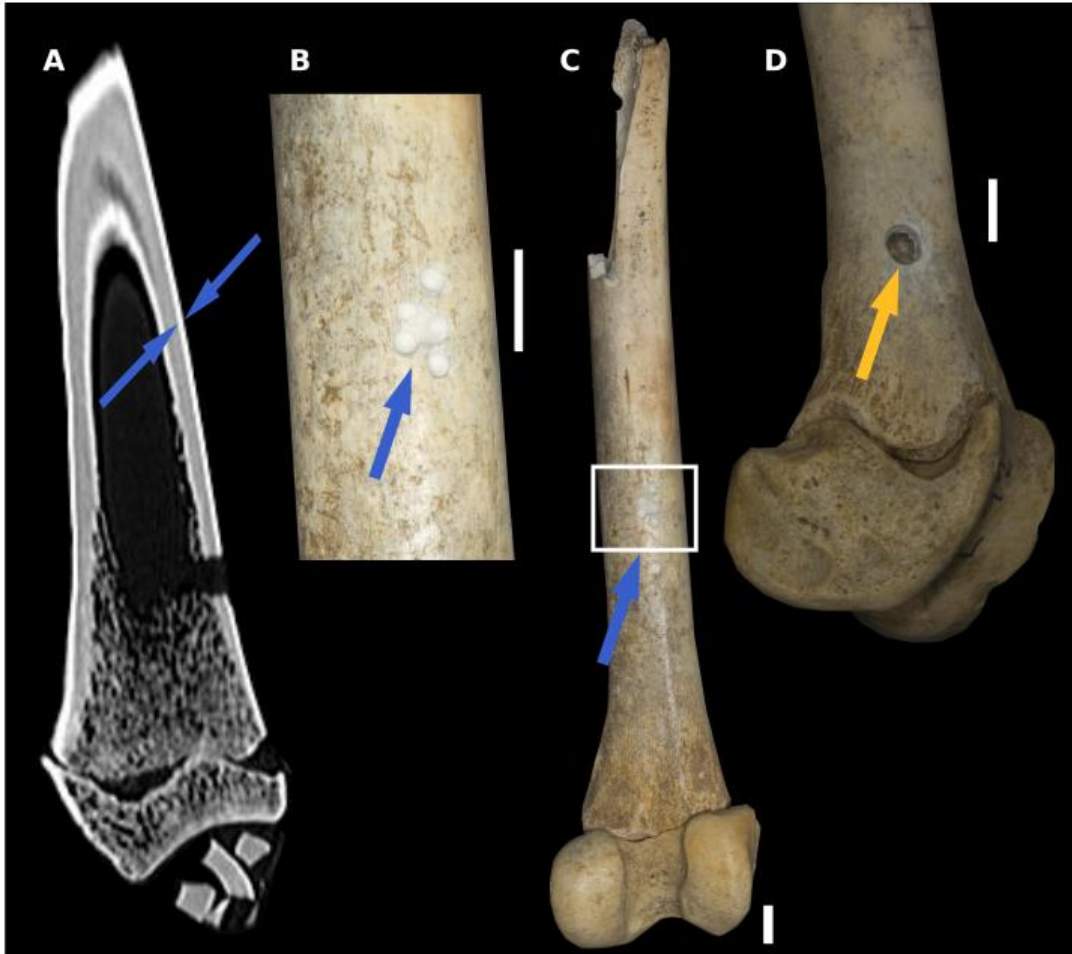
FROM THE COVER

WILEY **MOLECULAR ECOLOGY
RESOURCES**

Optimized DNA sampling of ancient bones using Computed Tomography scans

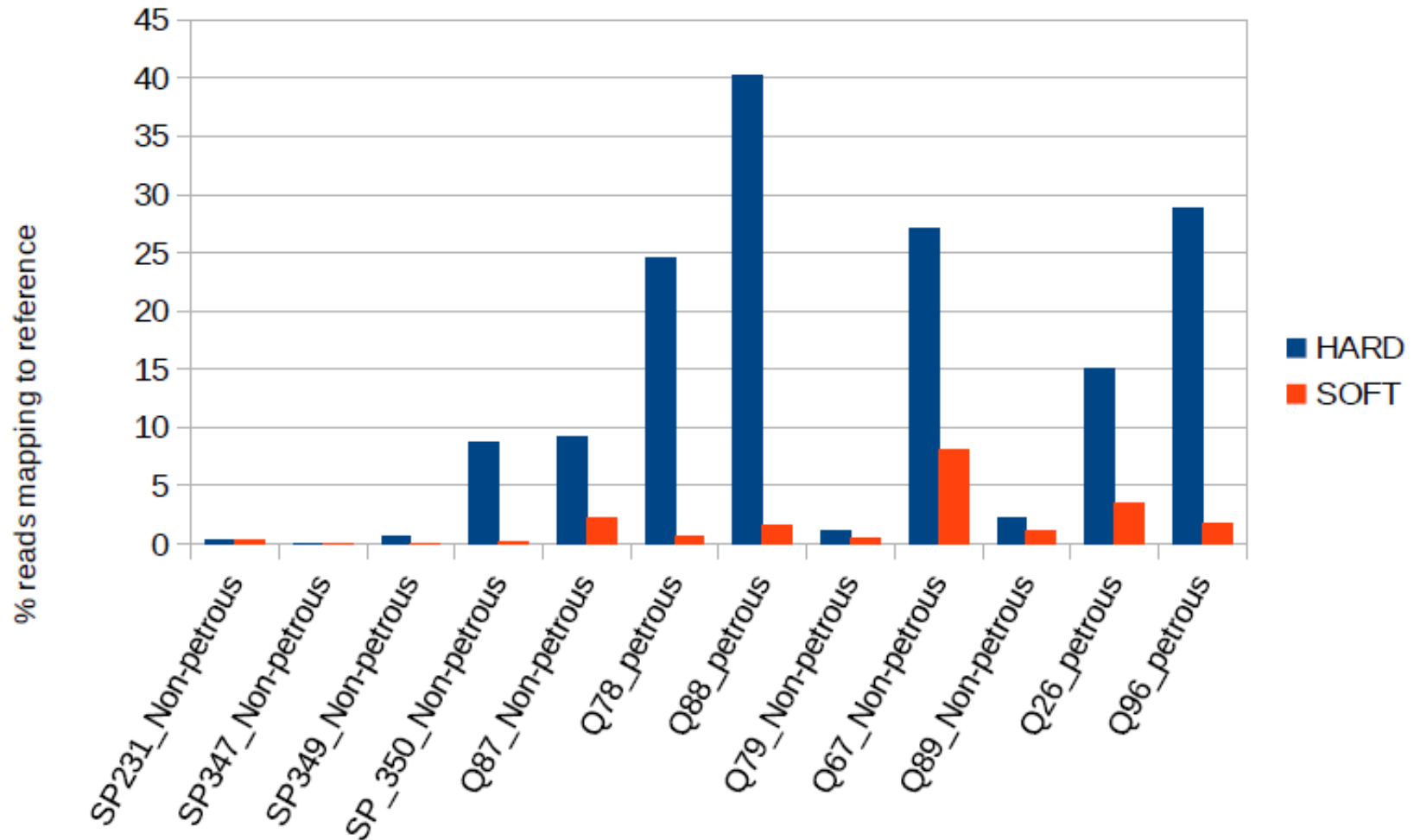
Federica Alberti¹  | Javier Gonzalez¹ | Johanna L. A. Paijmans¹ | Nikolas Basler¹ |
Michaela Preick¹ | Kirstin Henneberger¹ | Alexandra Trinks^{1,2} | Gernot Rabeder³ |
Nicholas J. Conard⁴ | Susanne C. Münzel⁴ | Ulrich Joger⁵ | Guido Fritsch⁶ |
Thomas Hildebrandt⁶ | Michael Hofreiter¹ | Axel Barlow¹

Works also with other bones



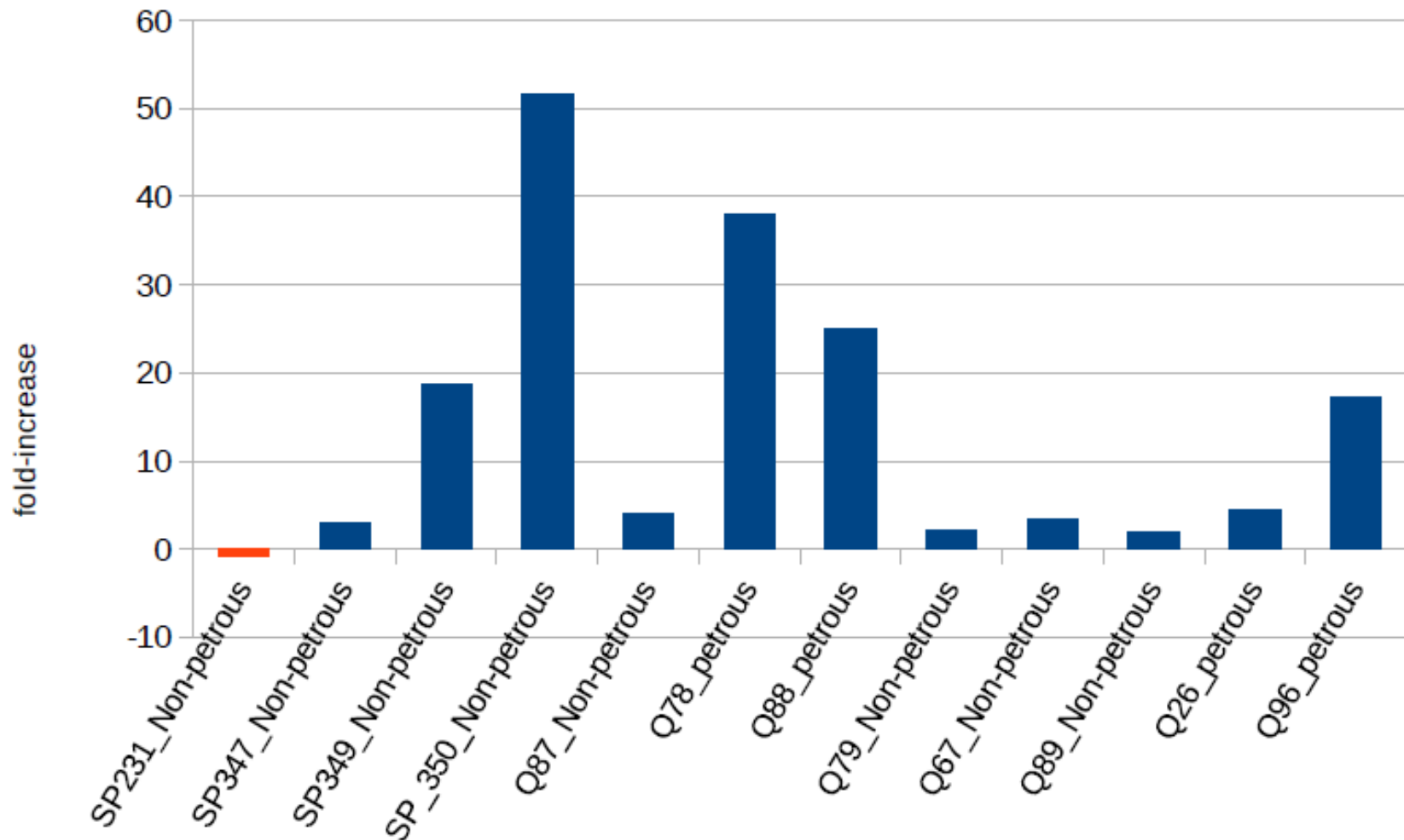
So, if you do not have a petrous – use a CT-scan

Endogenous contents for hard and soft parts of 12 cave bear bones



Relative increase in endogenous DNA

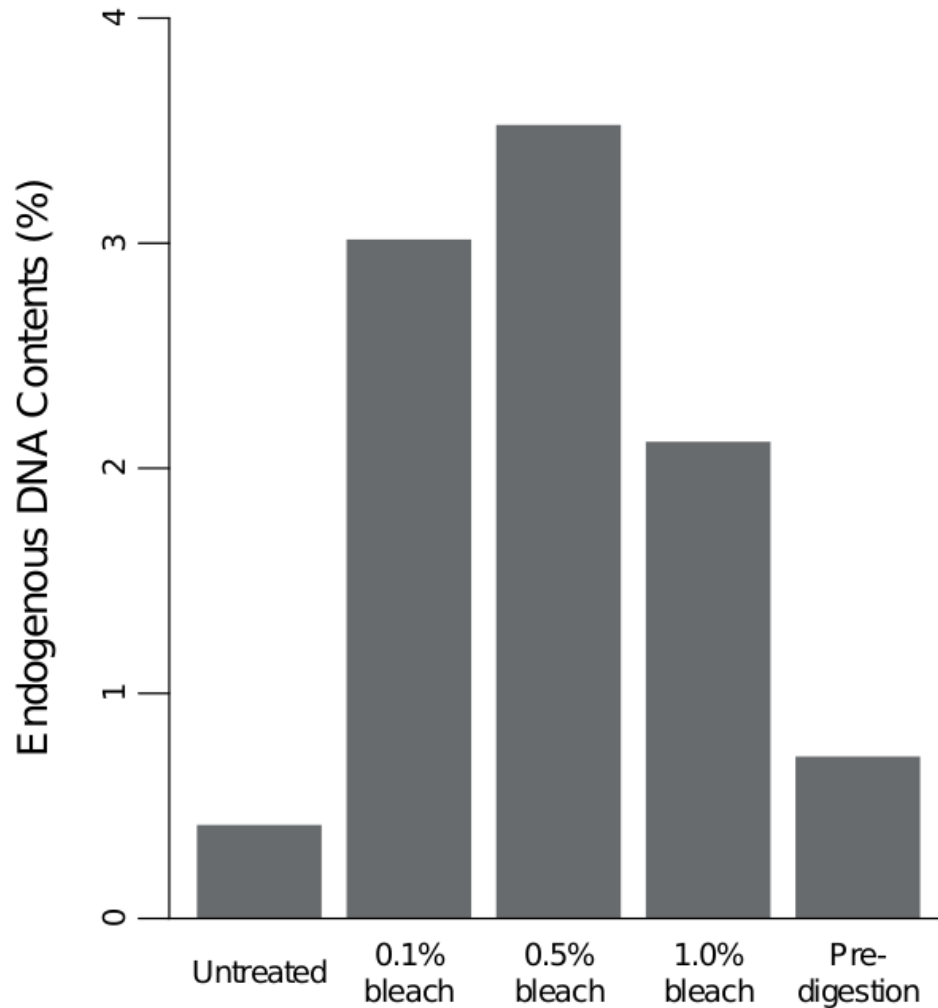
Fold increases in endogenous content for hard part relative to soft
(note first sample gave a reduction)



Solution 2: Bleach your samples



At least it helps to some extent



Basler et al. *BMC Res Notes* (2017) 10:754
<https://doi.org/10.1186/s13104-017-3061-3>

BMC Research Notes

RESEARCH NOTE

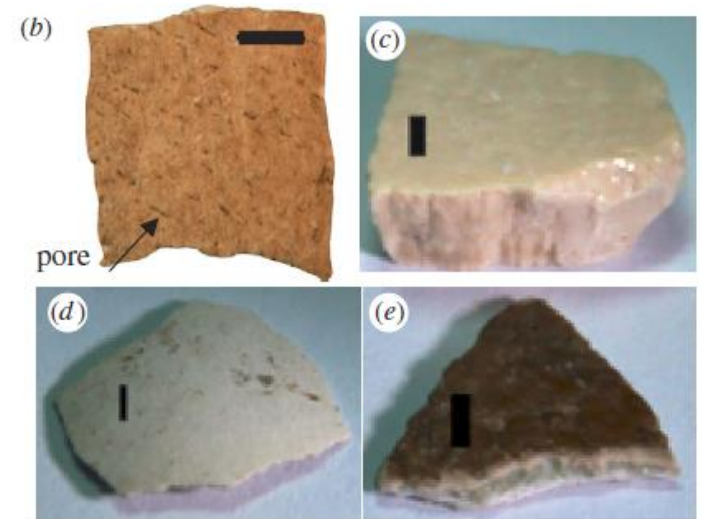
Open Access

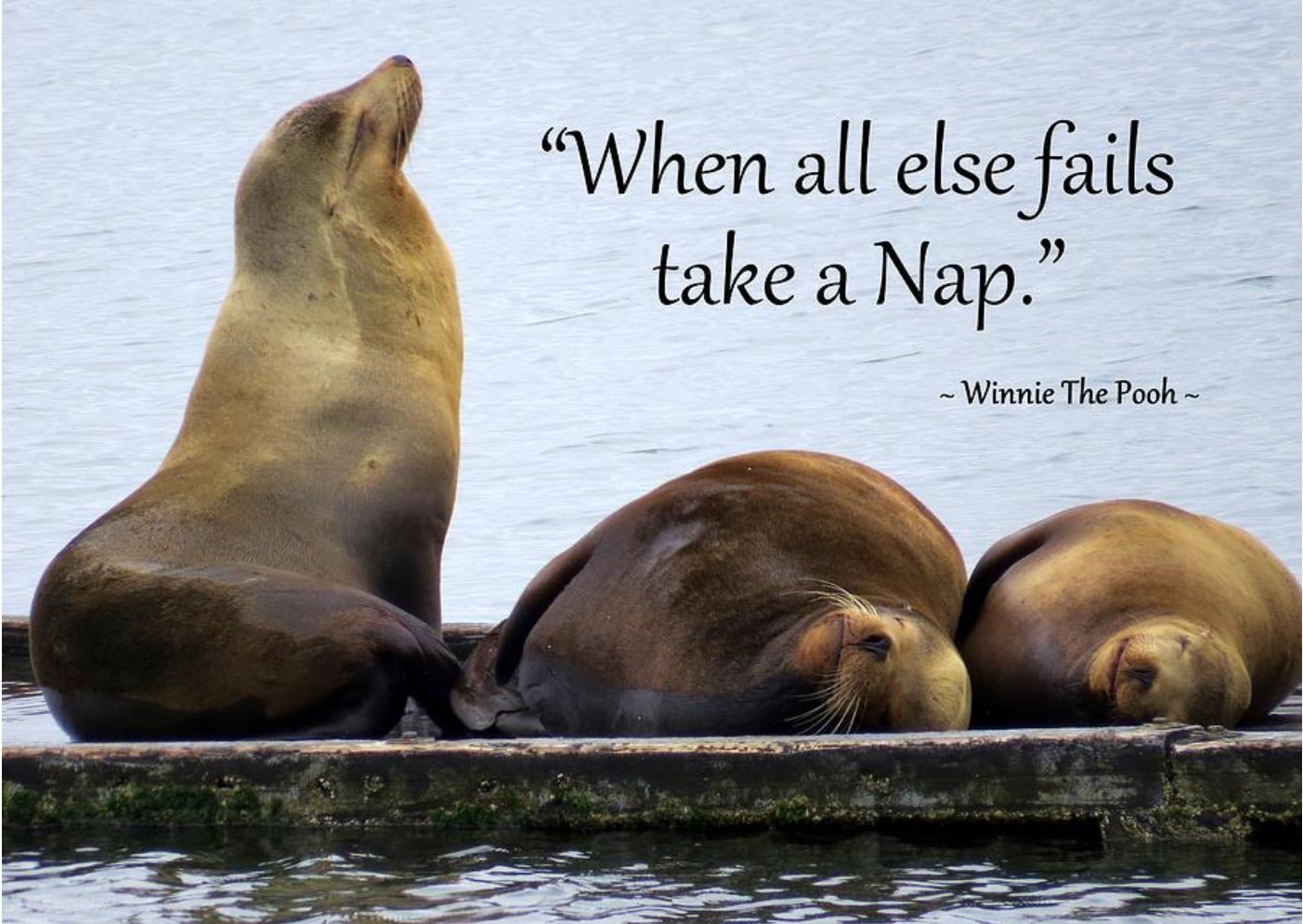


Reduction of the contaminant fraction of DNA obtained from an ancient giant panda bone

Nikolas Basler¹, Georgios Xenikoudakis¹, Michael V. Westbury¹, Lingfeng Song², Guilian Sheng^{2*} and Axel Barlow^{1*}

How about other aDNA substrates?



A photograph of three seals on a wooden pier. One seal is sitting upright on the left, looking towards the sky. Two other seals are lying down on the right, appearing to be asleep. The background is a body of water.

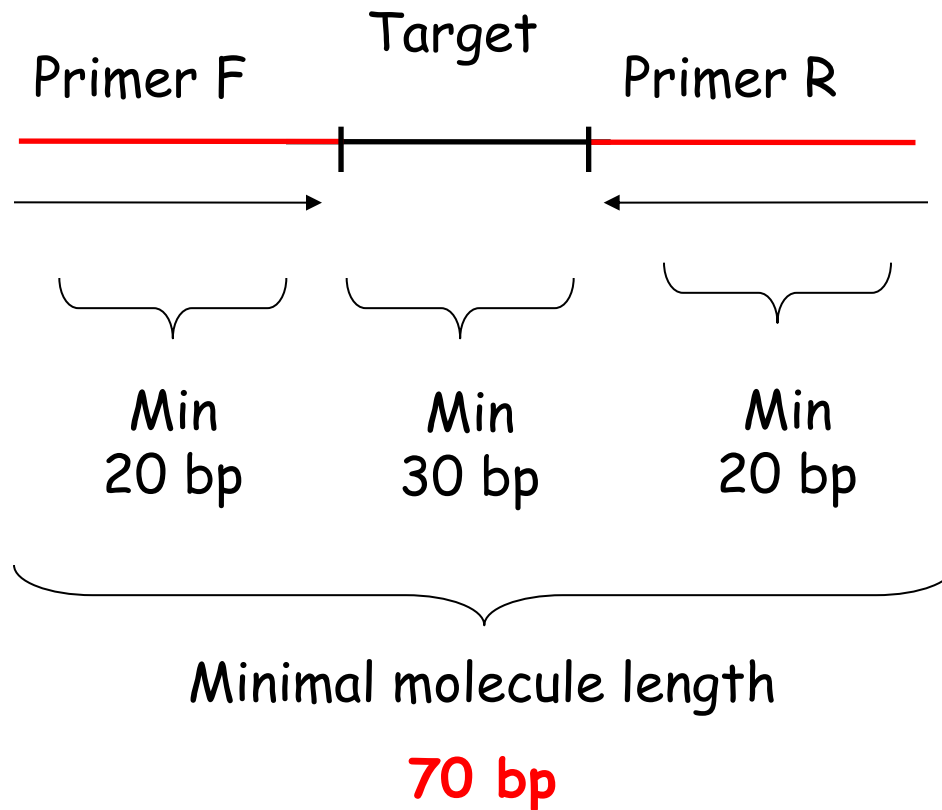
“When all else fails
take a Nap.”

~ Winnie The Pooh ~

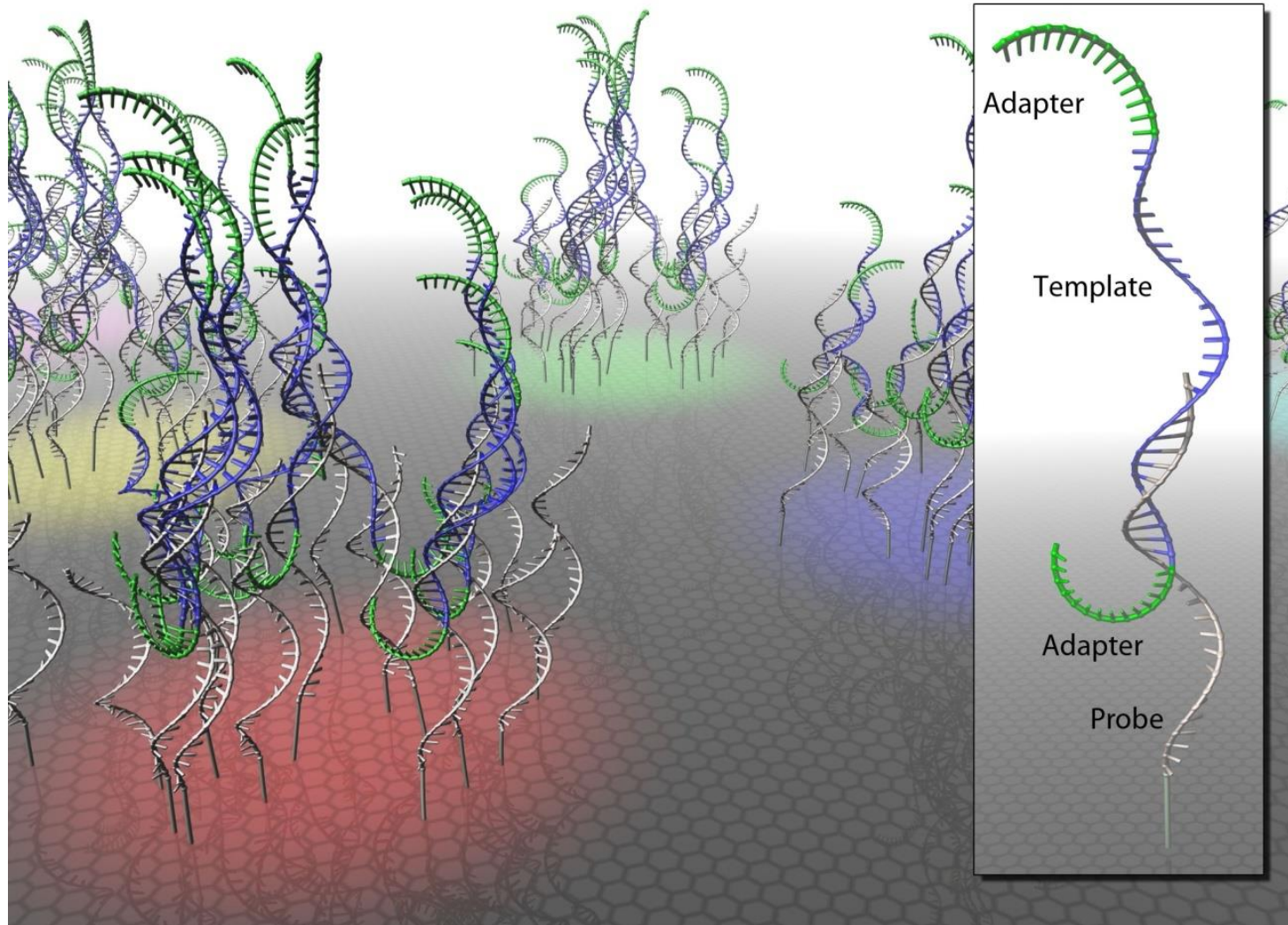
Solution 3: Target your DNA



Limitations inherent to PCR



DNA hybridization capture



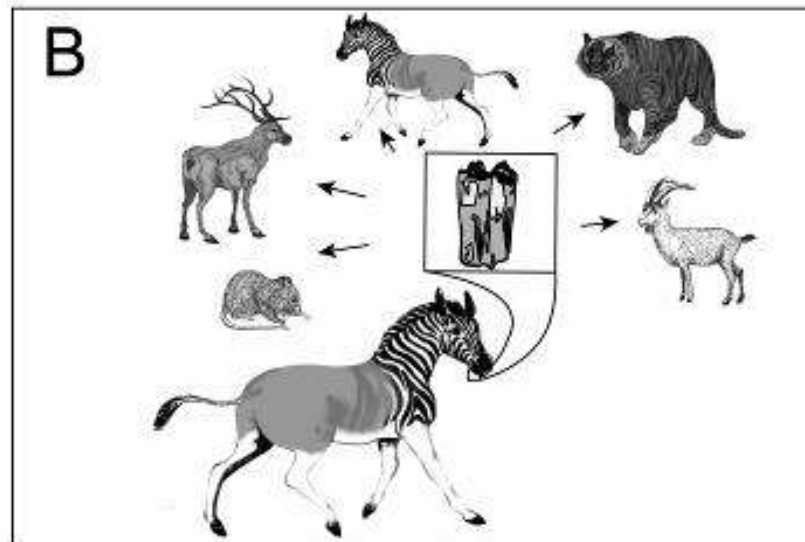
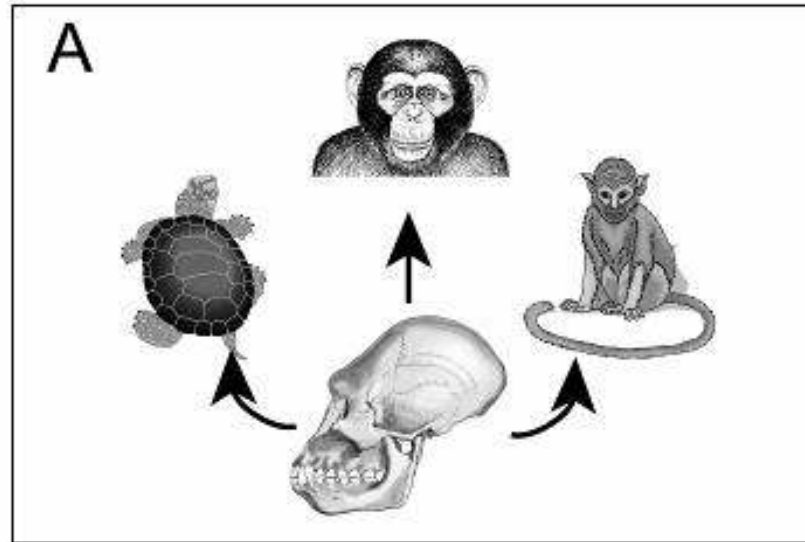
Nice but.....

Sample ID	Location	Mitochondrial reference					
		Mappable reads	Mapped reads	unique mapped read	Mapped bp	Depth	Read length
510	NE China(Zhaodong)	9073046	1054870	26968	1883592	114.21	16492
1017	NE China(SonghuaRiver)	3066021	1413536	26647	1779650	107.9	16499
696	NE China(Qinggang)	2209143	1137192	24730	1587582	96.6	16443
564	NE China(Qinggang)	2693221	761104	24049	1454268	88.2	16489
508	NE China(Zhaodong)	2767554	616556	18687	1088019	66.79	16290
539	NE China(Daqing)	3454017	811496	19121	989439	60.5	16367
1016	NE China(Qinggang)	2799561	515682	13214	720338	44.1	16327
1011	NE China(Qinggang)	3072086	555920	12465	676867	41.6	16275
565	NE China(SonghuaRiver)	3160689	215725	5260	261276	16.5	15855
1099	S China(Fuyuan, Yunan)	2313878	29494	224	11487	7.1	1625
1093	S China(Fuyuan, Yunan)	2553691	1263	57	2957	4.2	708
15	N China(Qinhuangdao, Linxian cave)	2042719	10585	33	1937	3.65	531
8	N China(Liaoning Pigeon cave)	2915876	6715	56	3211	2.53	1269
7	N China(Liaoning Pigeon cave)	1375153	349	30	1740	2.3	747
1092	S China(Fuyuan, Yunan)	1827333	740	13	499	1.4	365
22	N China(Qinhuangdao, Linxian cave)	2375921	222	14	550	1.11	495
19	N China(Qinhuangdao, Linxian cave)	2118585	518	21	741	1.08	686

2. Contamination, after all?

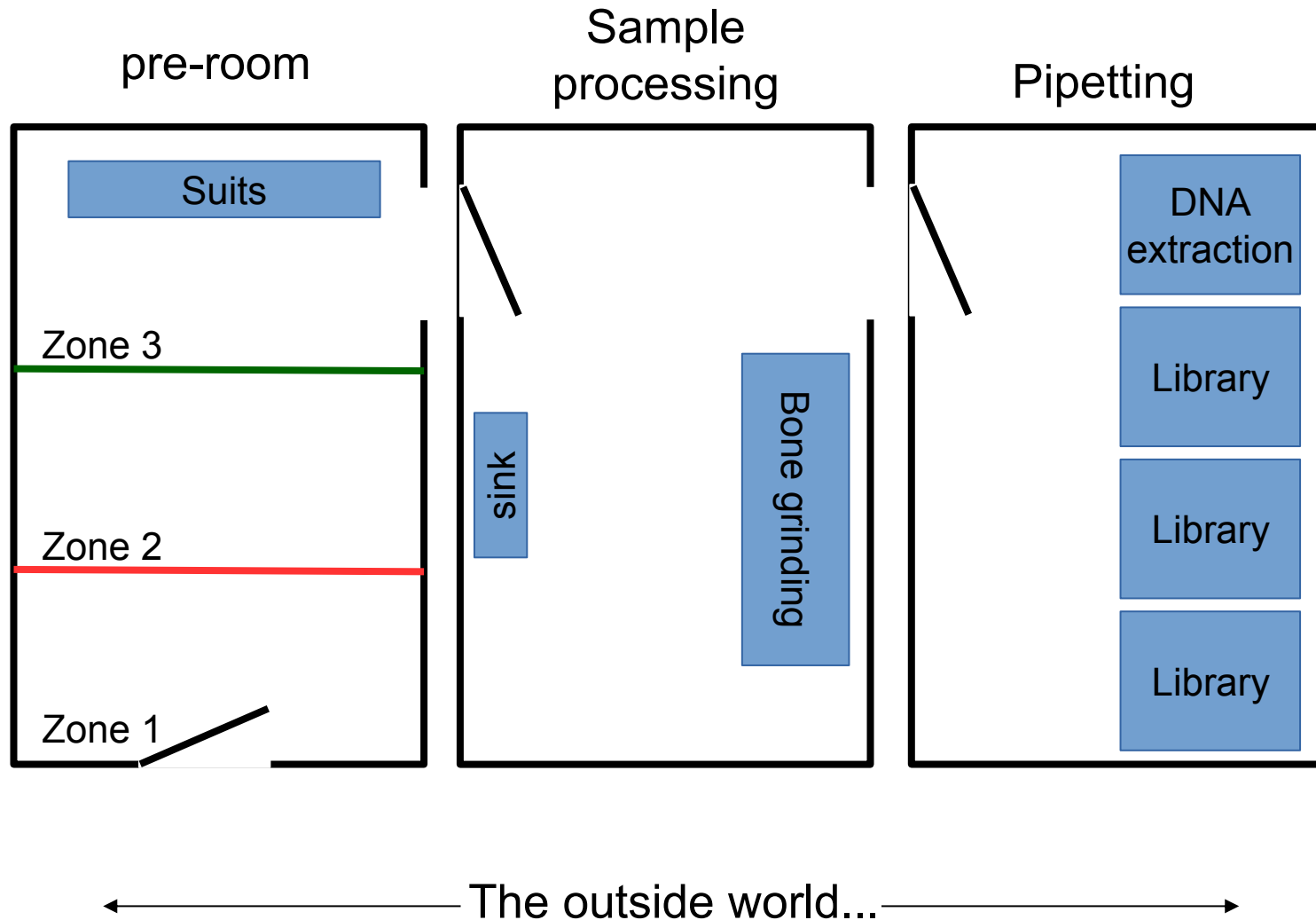


Well, it does exist

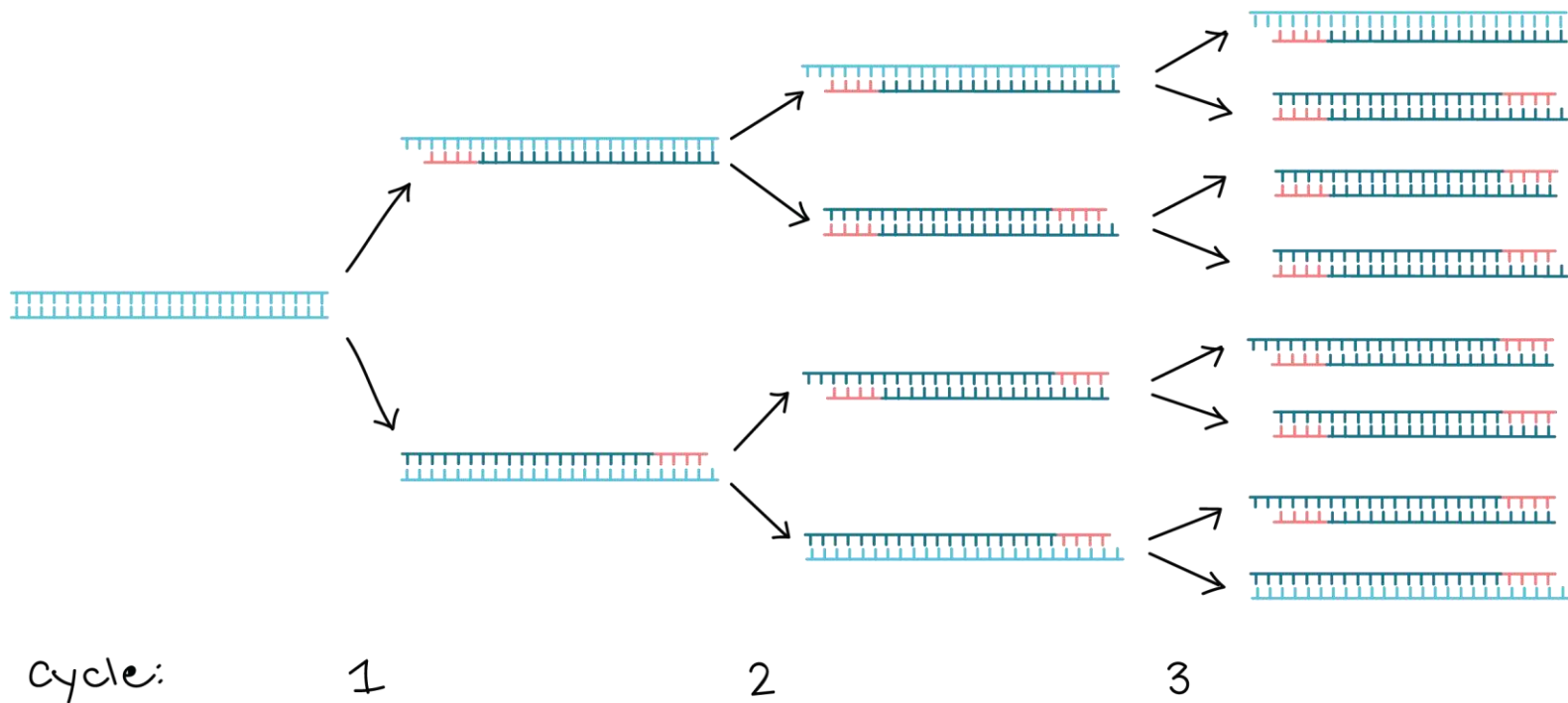




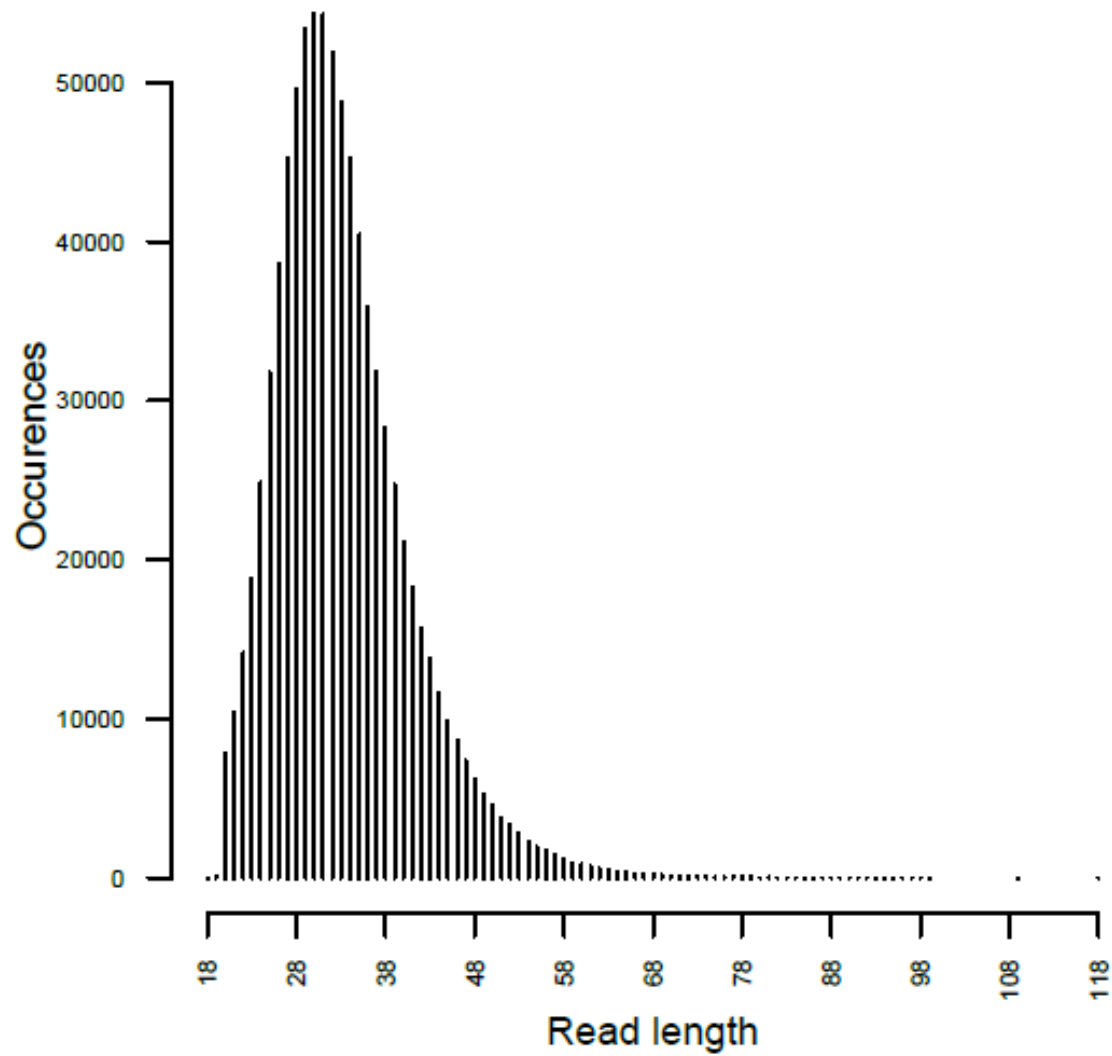
Ancient DNA lab



The real problem: amplified DNA



3. Ancient DNA is short



Ancient DNA fragment length: Rollo et al. 1988

Short but faithful pieces of ancient DNA

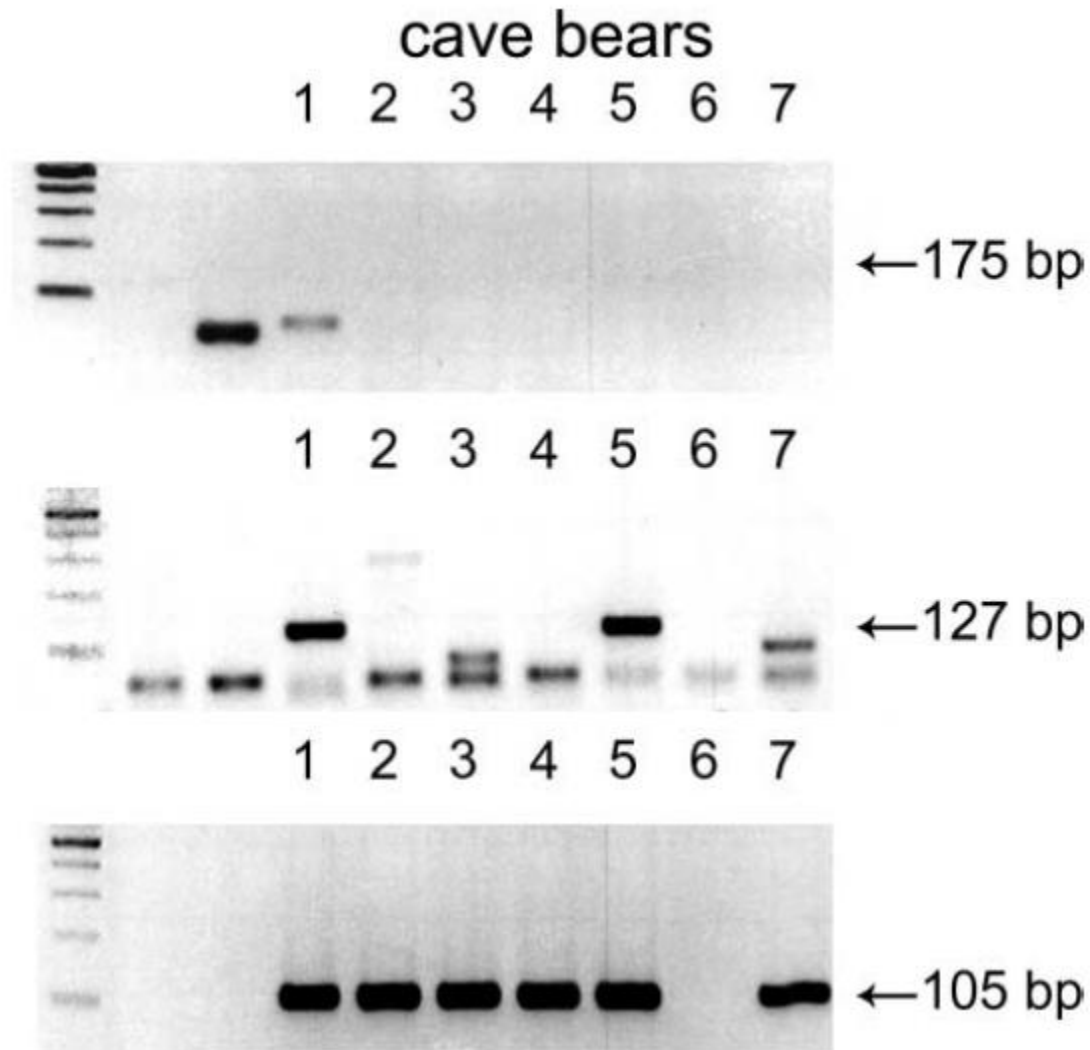
FRANCO ROLLO
AUGUSTO AMICI
ROBERTO SALVI

ANNAROSA GARBUGLIA

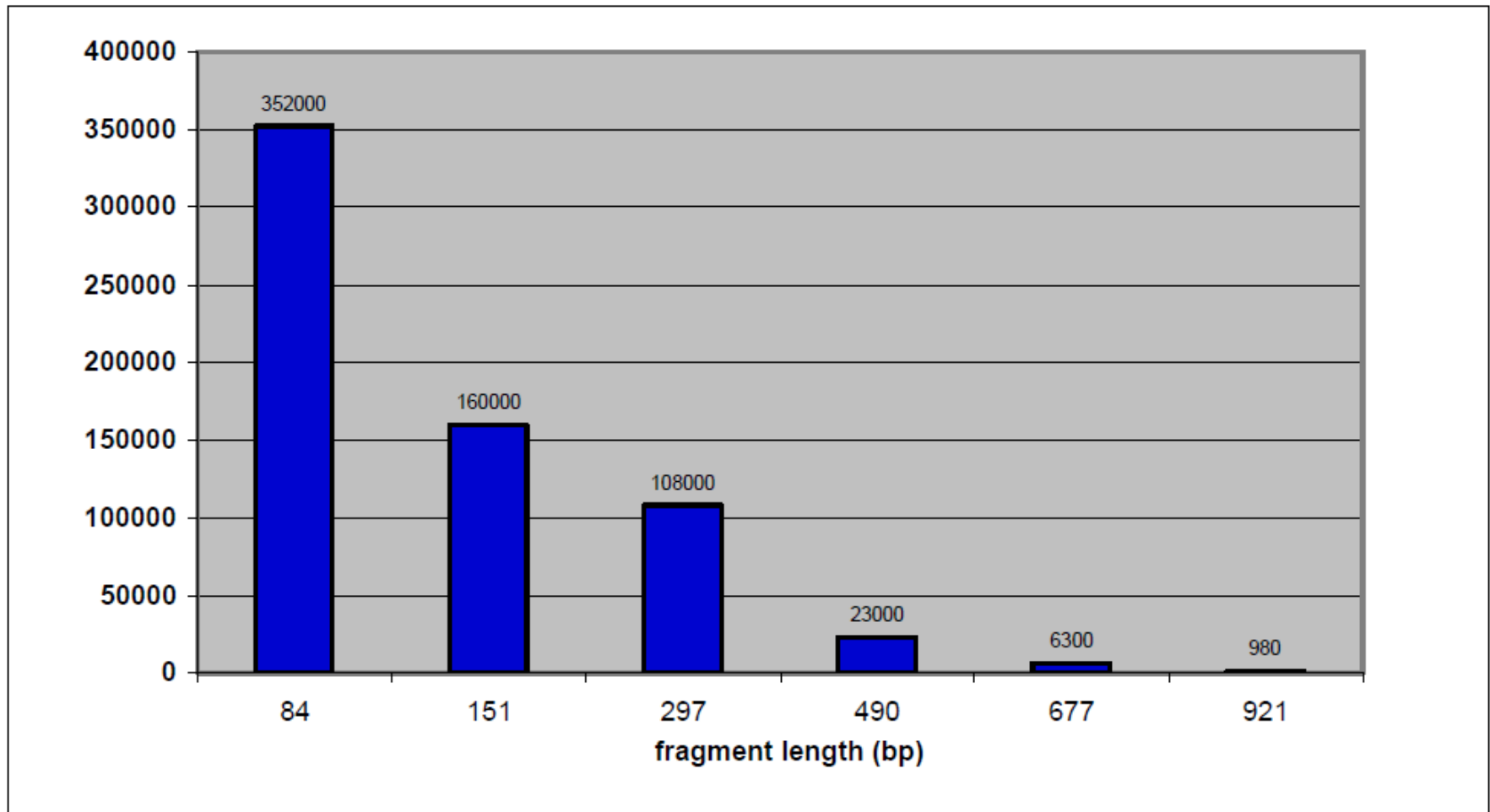
*Dipartimento di Biologia Cellulare,
via F. Camerini 2,
I-62032 Camerino,
Italy*

NATURE VOL. 335 27 OCTOBER 1988

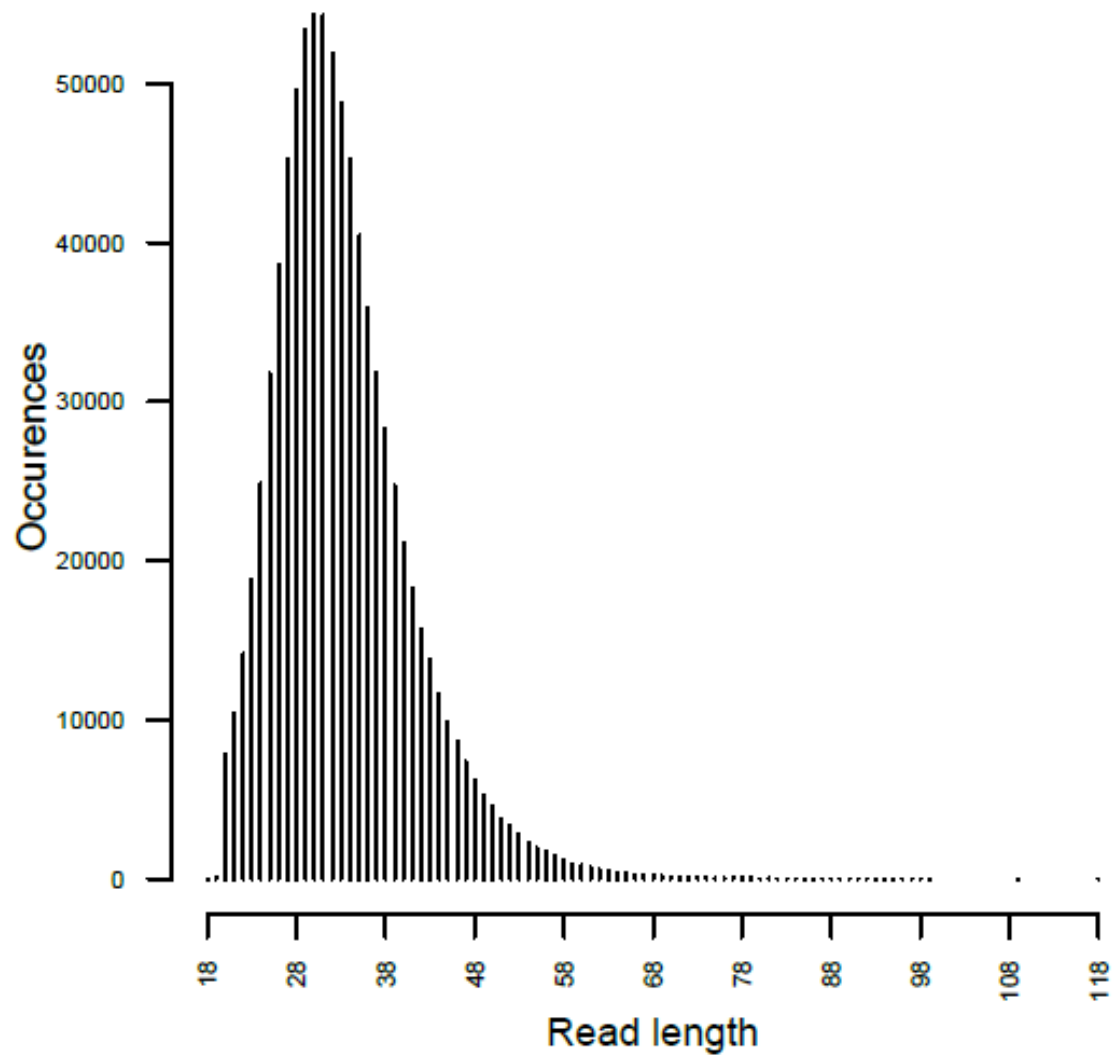
Ancient DNA fragment length: Pääbo et al. 2004



Ancient DNA fragment length: Poinar et al. 2006



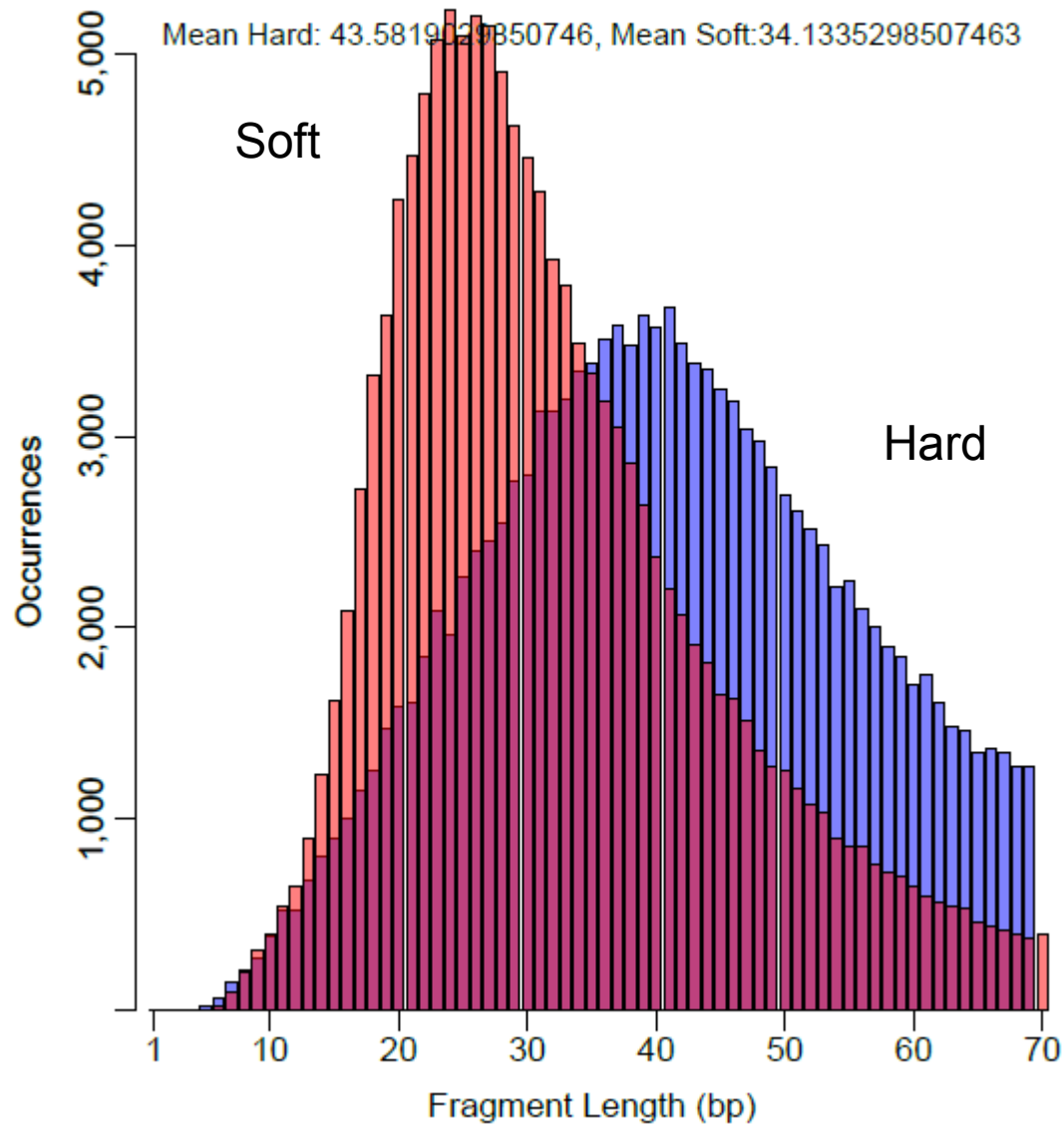
Very old DNA: $\sim 120,000$ years



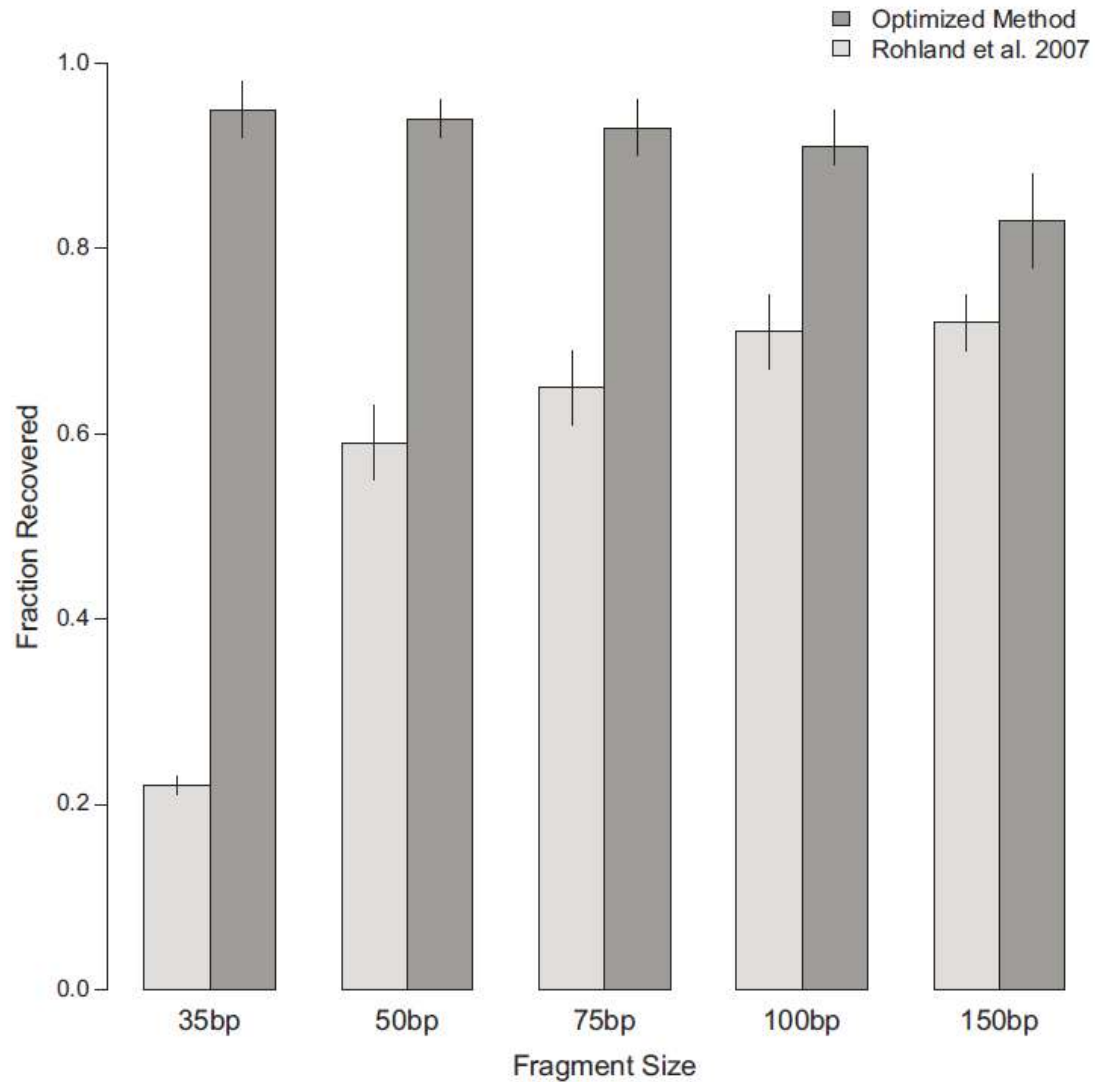
But to be fair.....



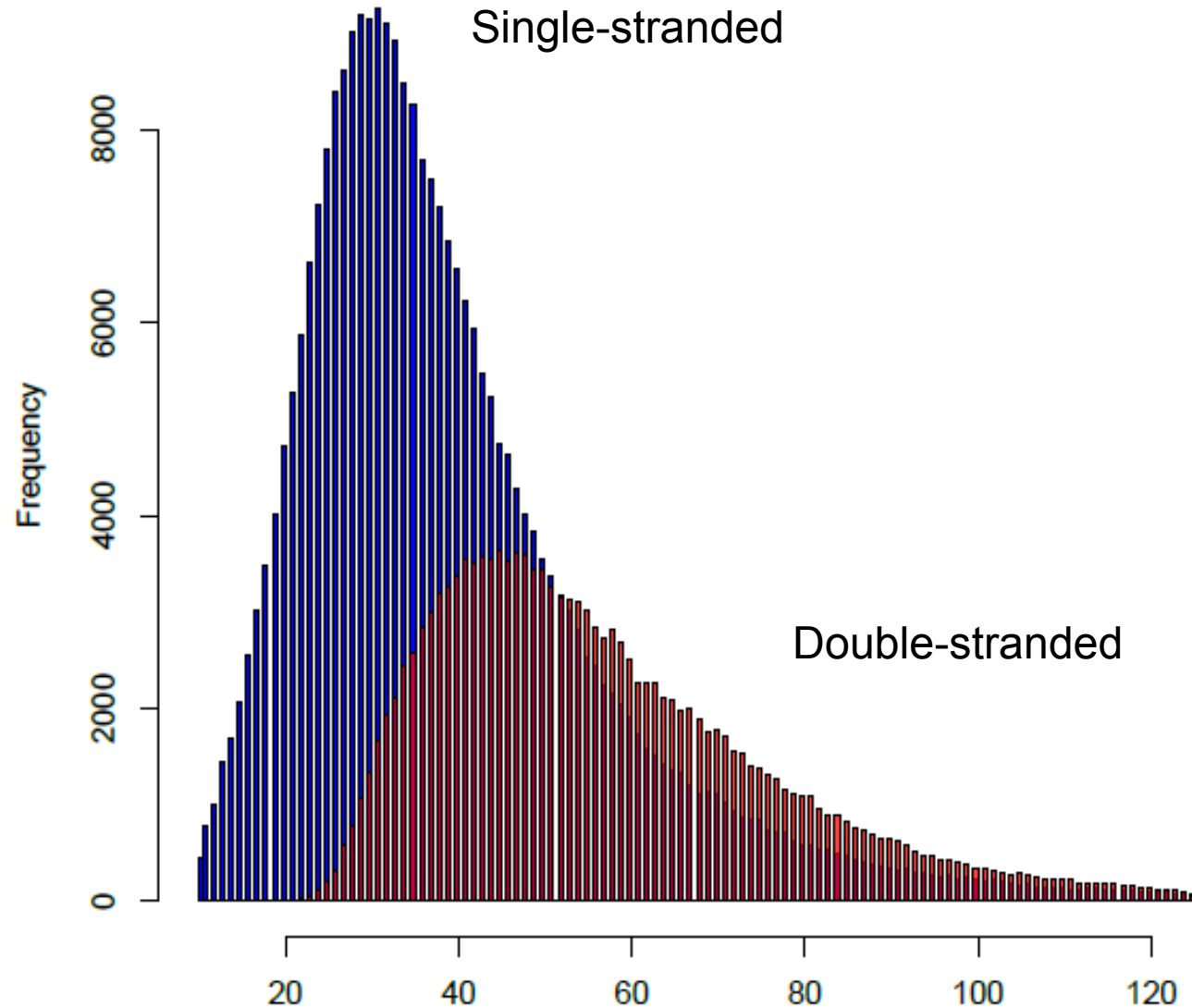
Because it depends a bit – on the bone chosen



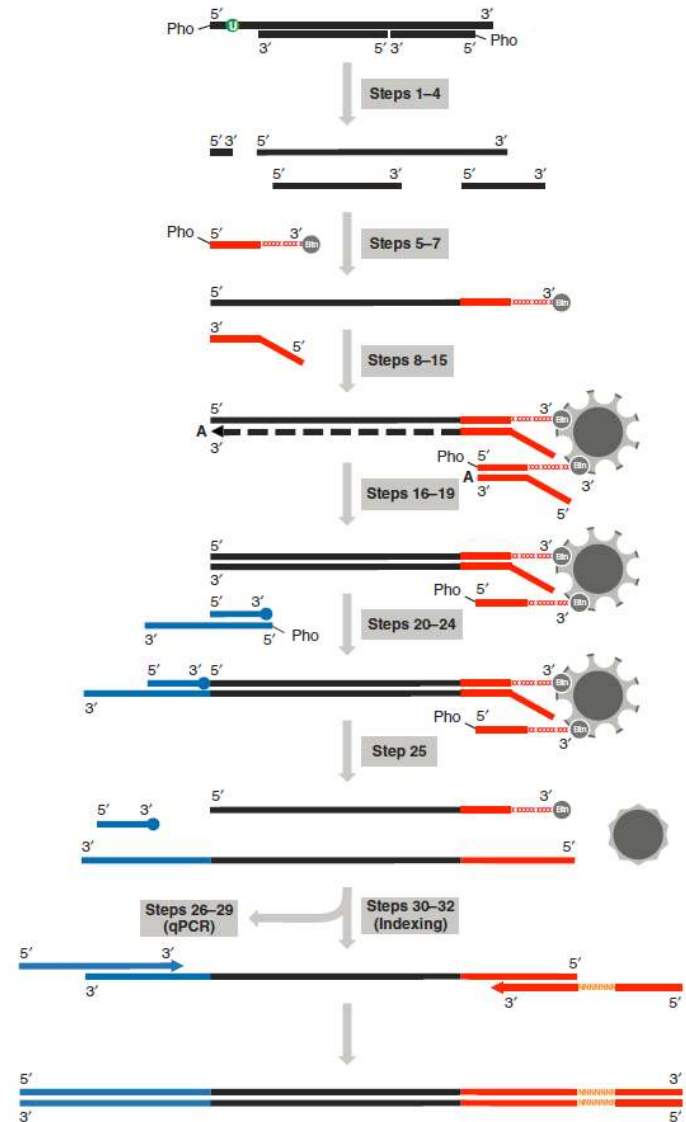
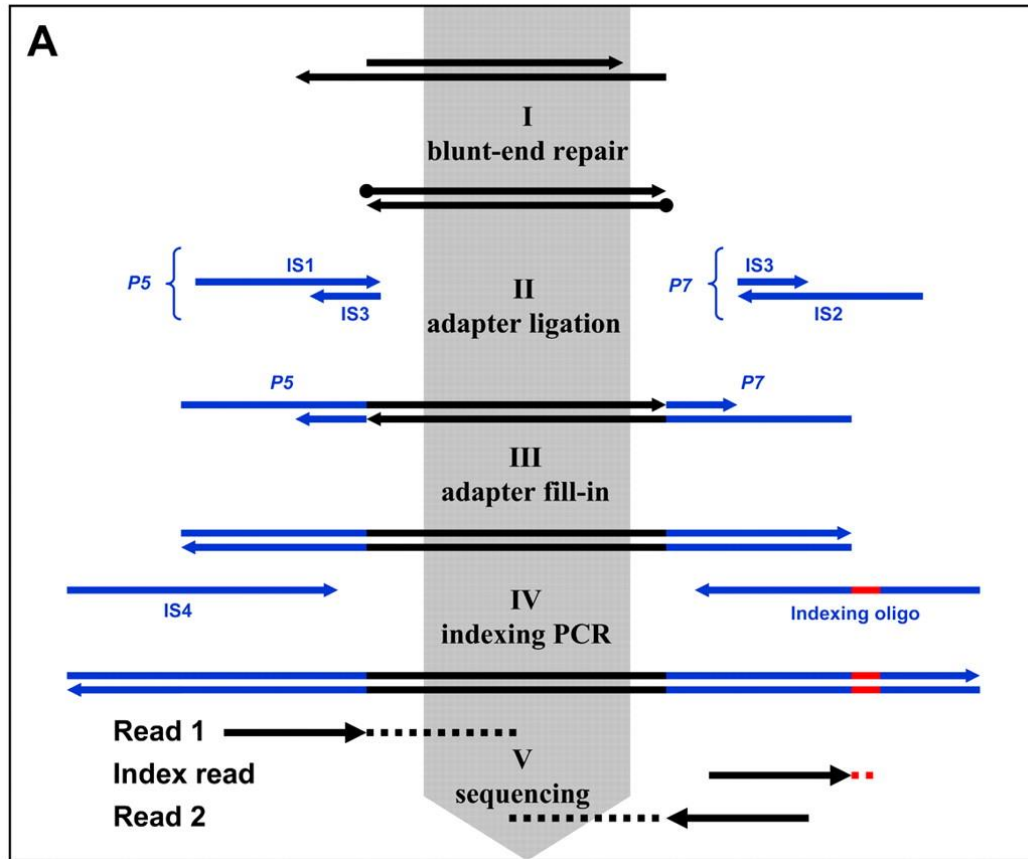
On the DNA extraction used



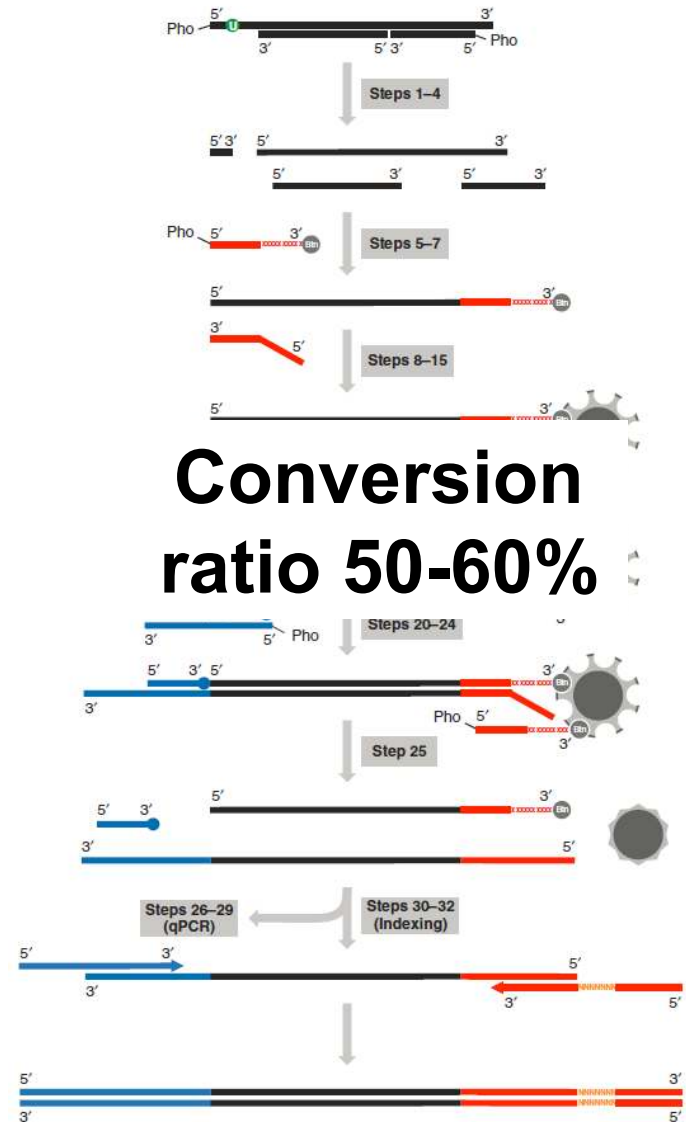
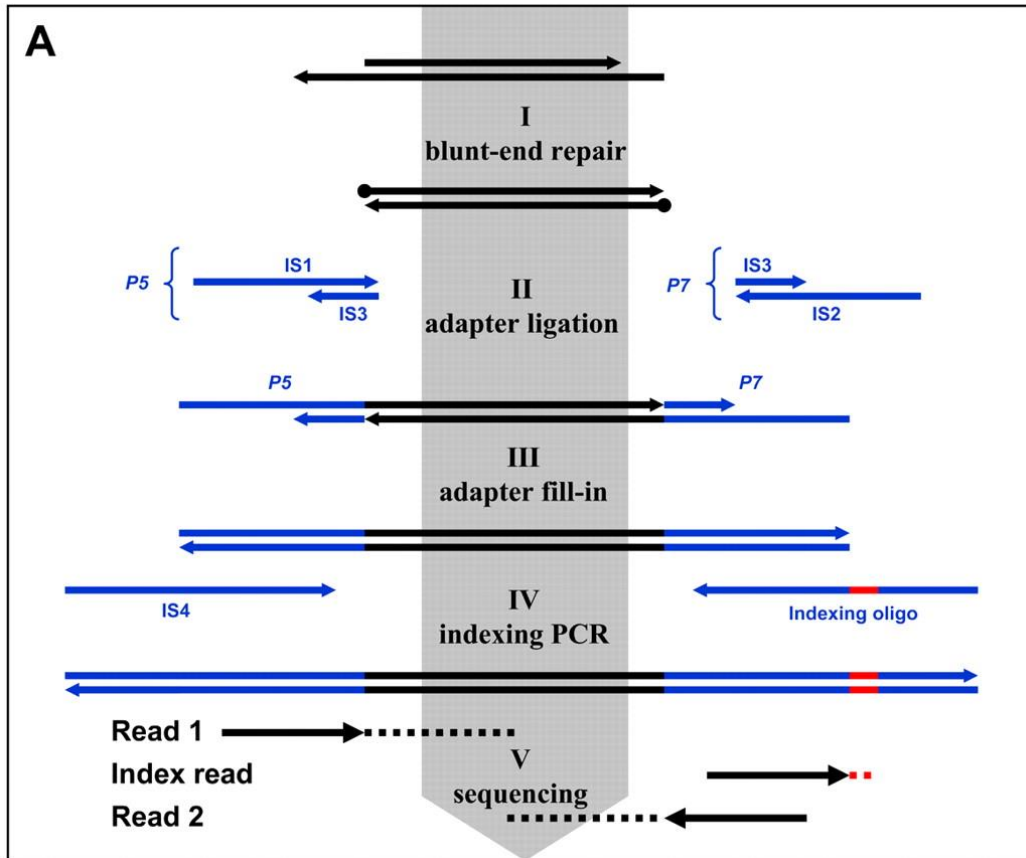
And on the library construction method used



Double-stranded vs. single-stranded



Double-stranded vs. single-stranded



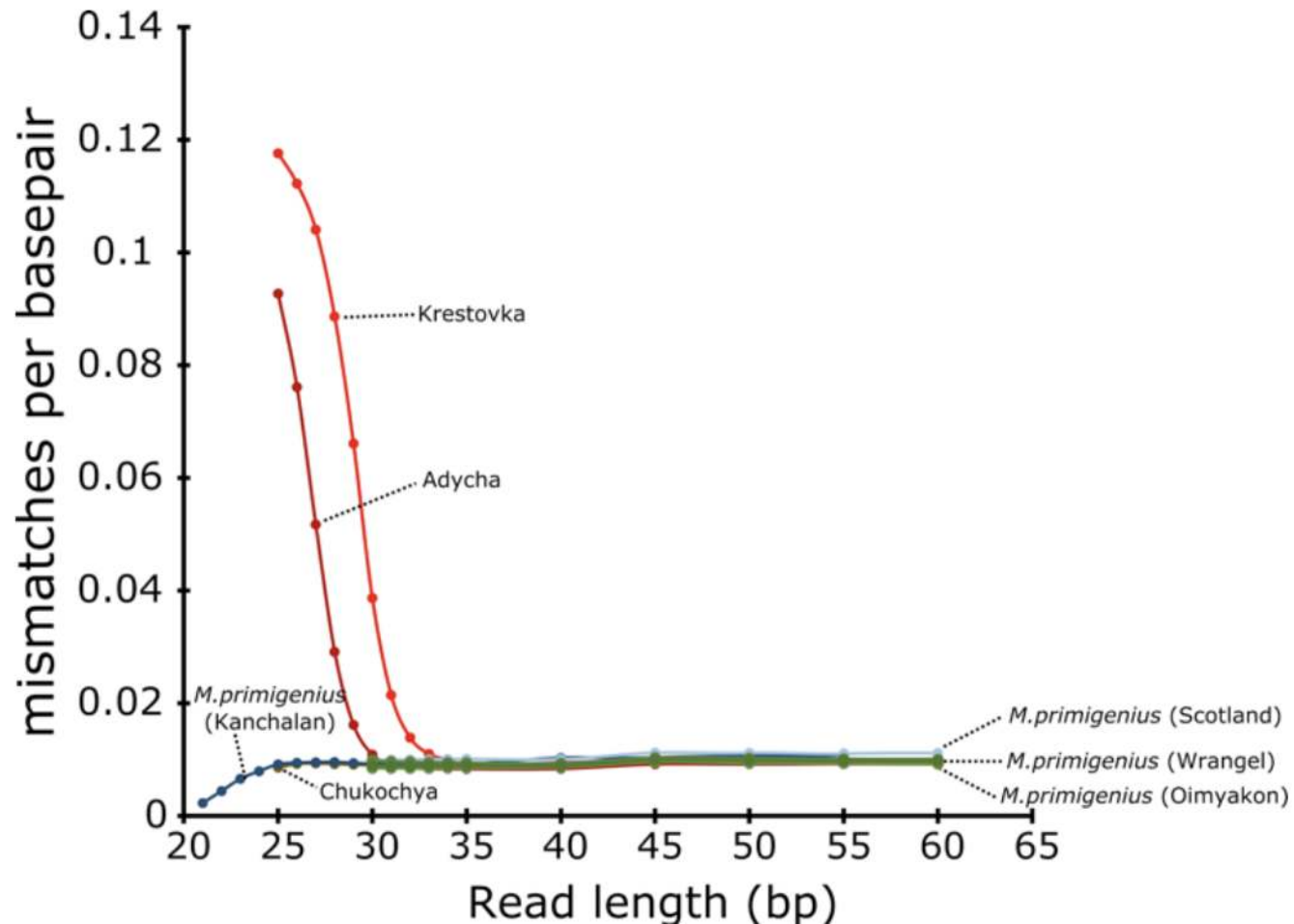
Problems of short fragments: mismapping

Sample	L=10 bp	L=15 bp	L=20 bp	L=30 bp
Ancient Samples				
KG5	22	9	9	9
874/11	5			
874/6	45	26	24	24
752/63	25			
1137/16	5			
1427/41	47	26	26	26
1427/59	1			
MAY10	7			
Modern Reference Horses				
M3160 (<i>LP/LP</i>)	375	370	370	293
M3252 (<i>LP/lp</i>)	44	44	44	44

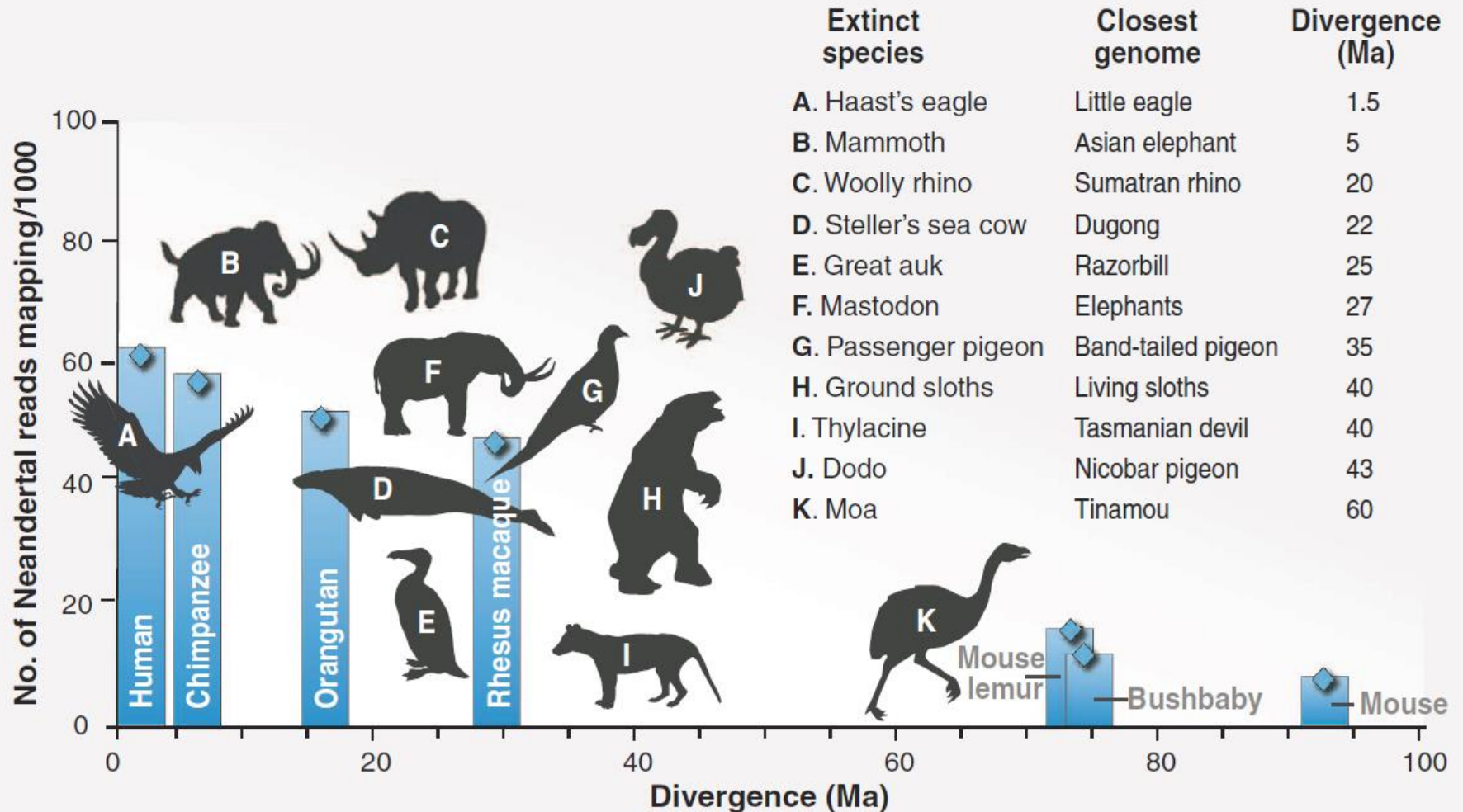
Problems of short fragments: mismapping

```
r110 0 0
r111 0 0
r112 0 0
r113 0 0
r114 0 0
r115 195 10
r116 208 0
r117 1156 2
r118 2610 61
r119 14573 1108
r120 25000 2165
r121 25011 2090
r122 25014 1901
r123 25001 1677
r124 25008 1277
r125 25000 758
r126 25012 411
r127 25002 215
r128 25004 142
r129 25027 124
r130 25020 109
r131 25017 106
r132 25024 103
r133 25014 115
r134 25024 109
r135 25025 111
r140 25000 118
r145 25020 102
r150 25000 110
r155 25025 110
r160 25020 91
```

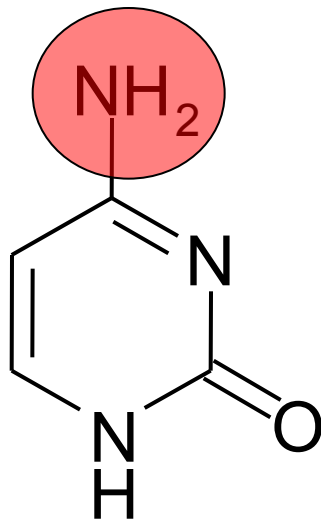
Problems of short fragments: mismapping



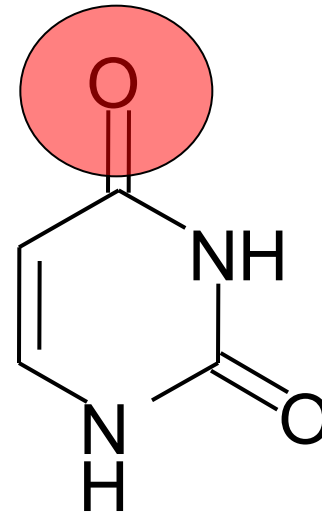
Problems of short fragments: no mapping at all



4. Miscoding lesions

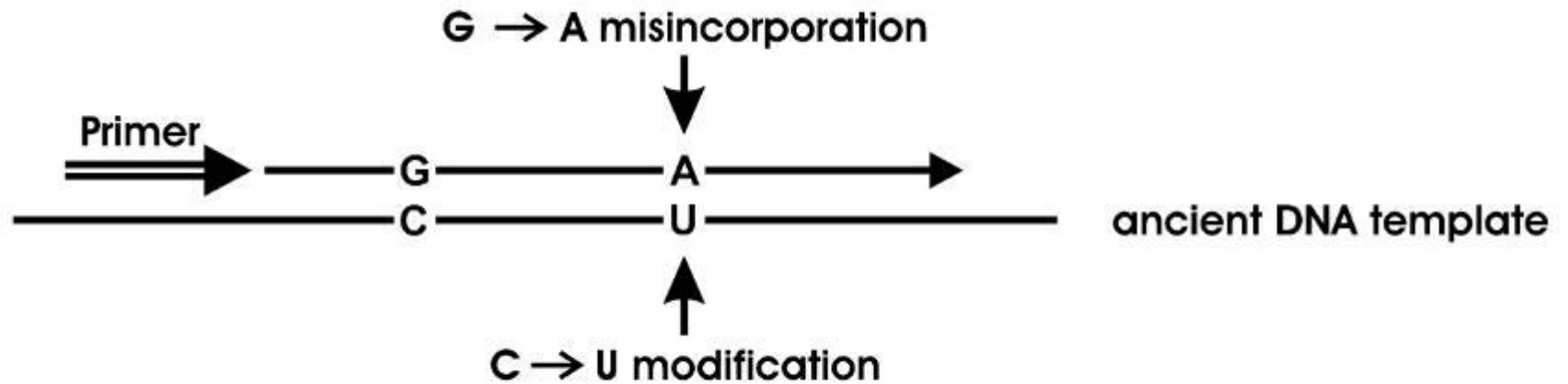


Cytosine

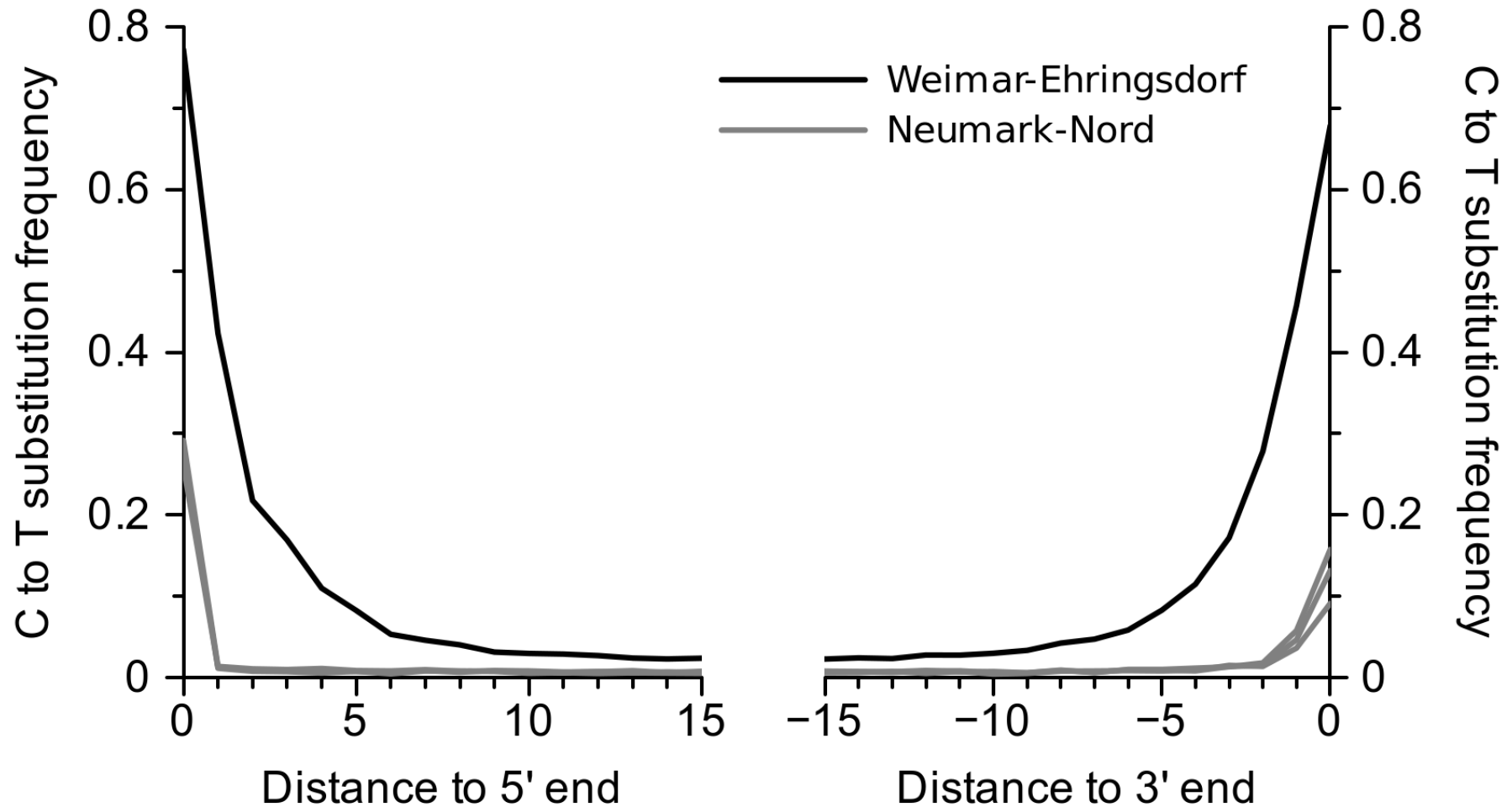


Uracil

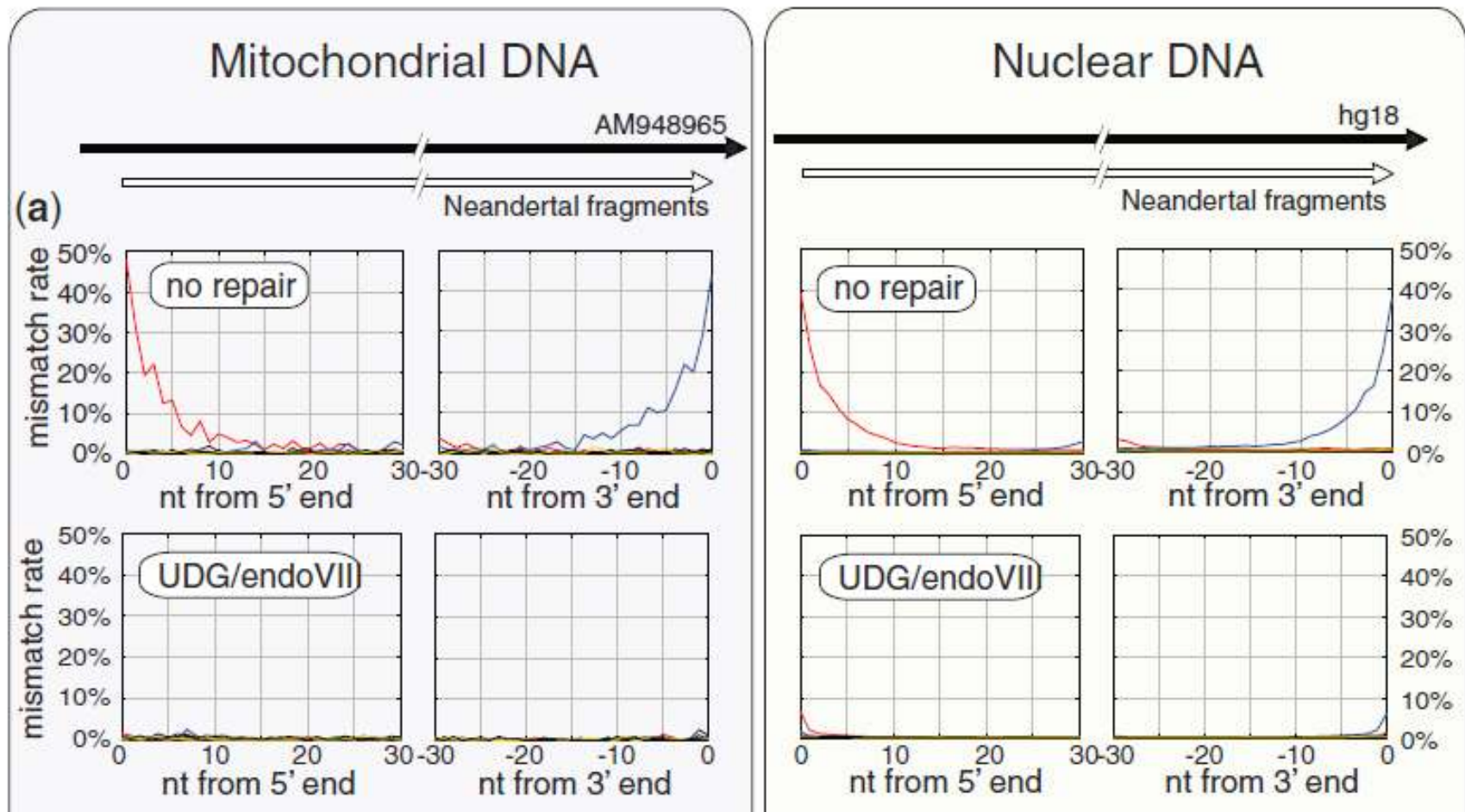
Miscoding lesions



High frequency at fragment ends



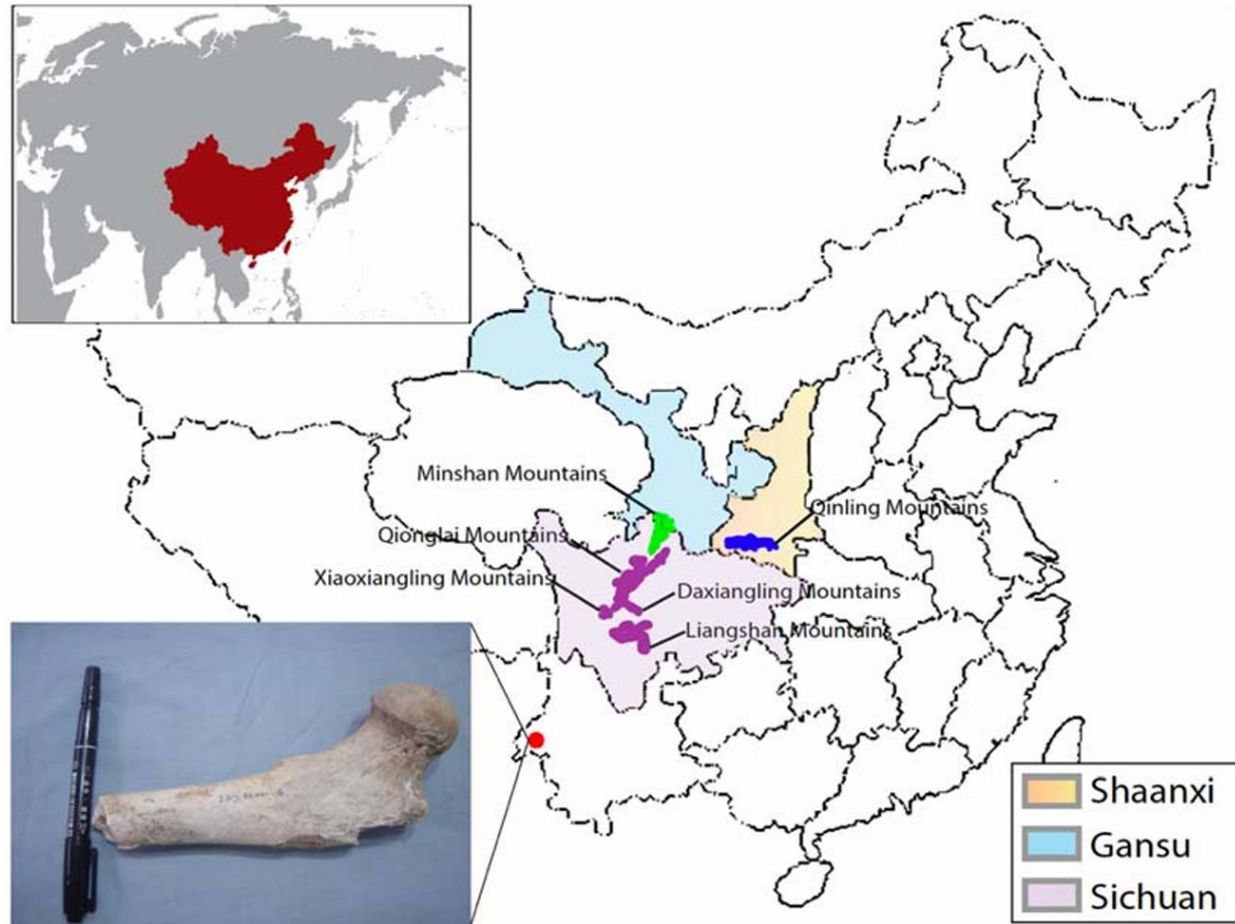
And enzymatic removal



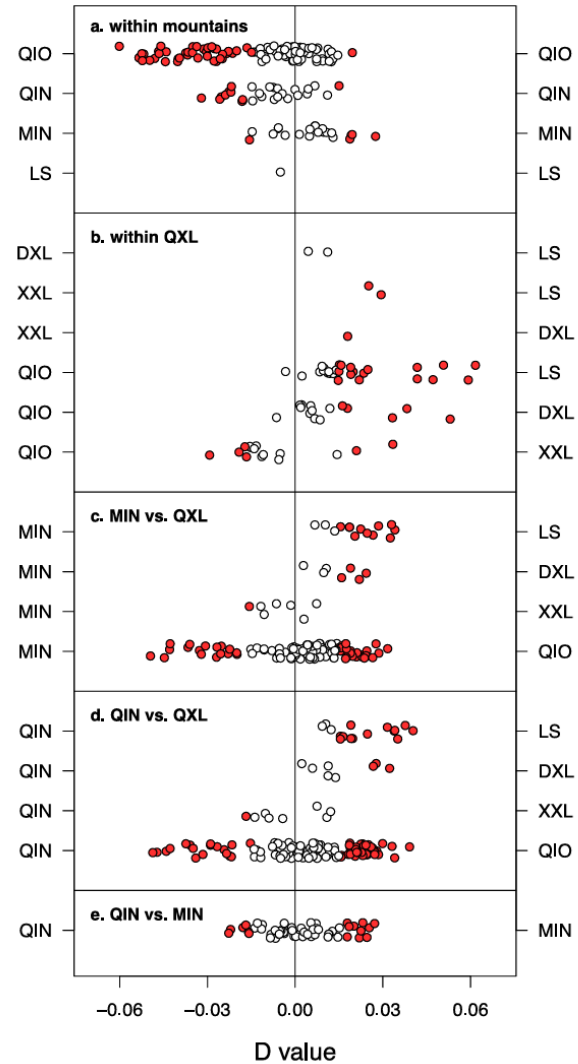
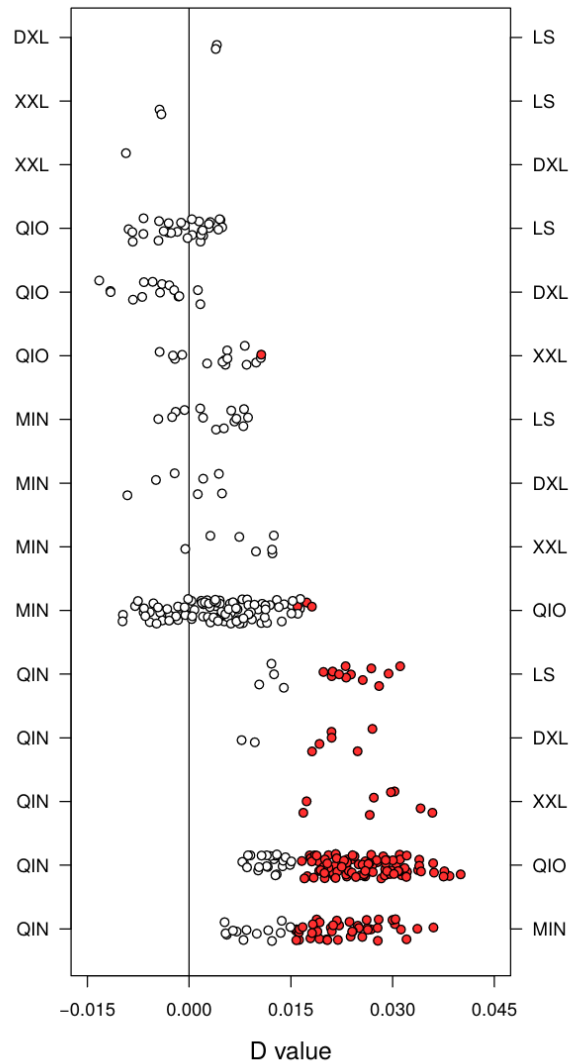
Mapping bias



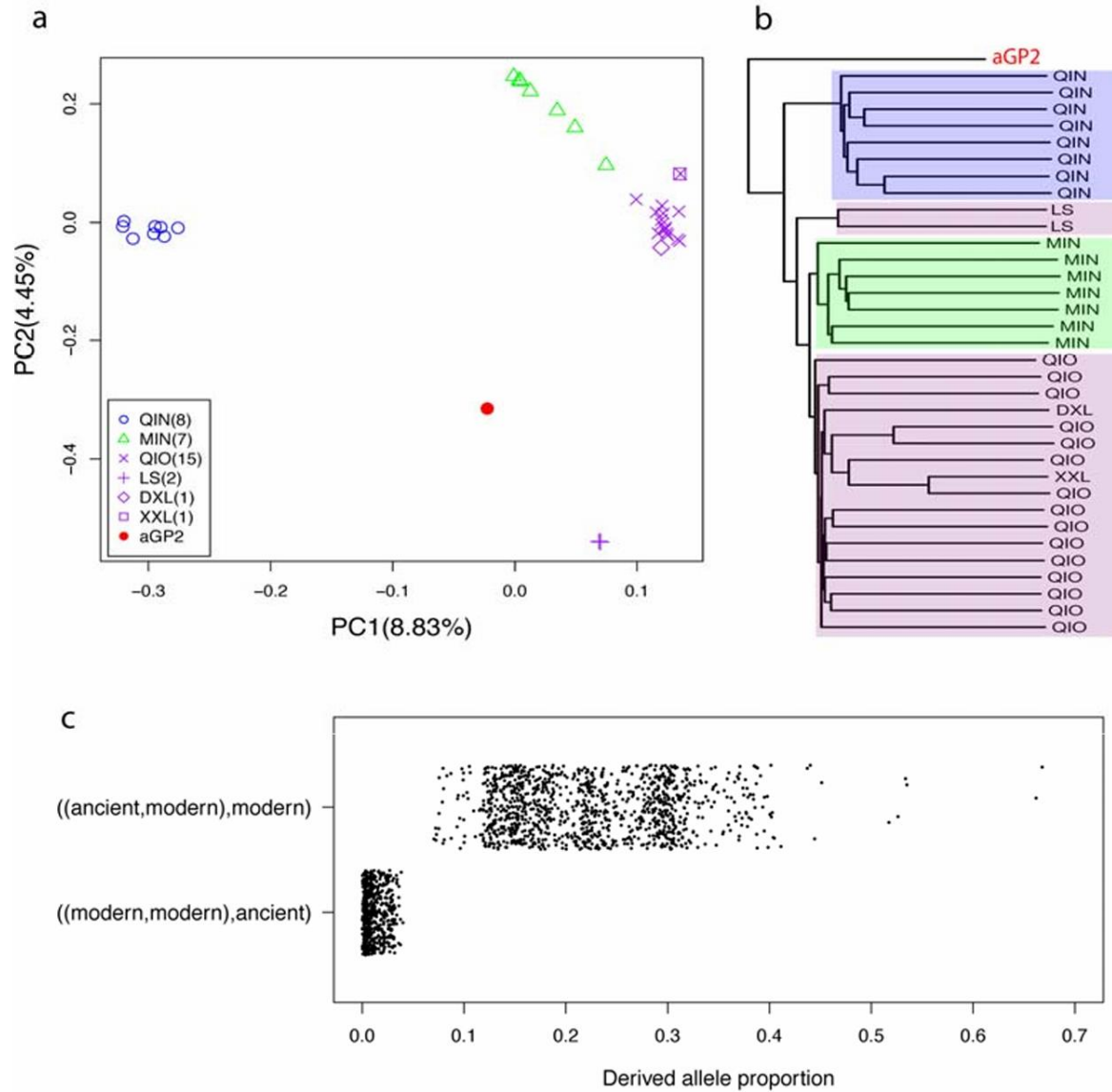
Fossil giant panda



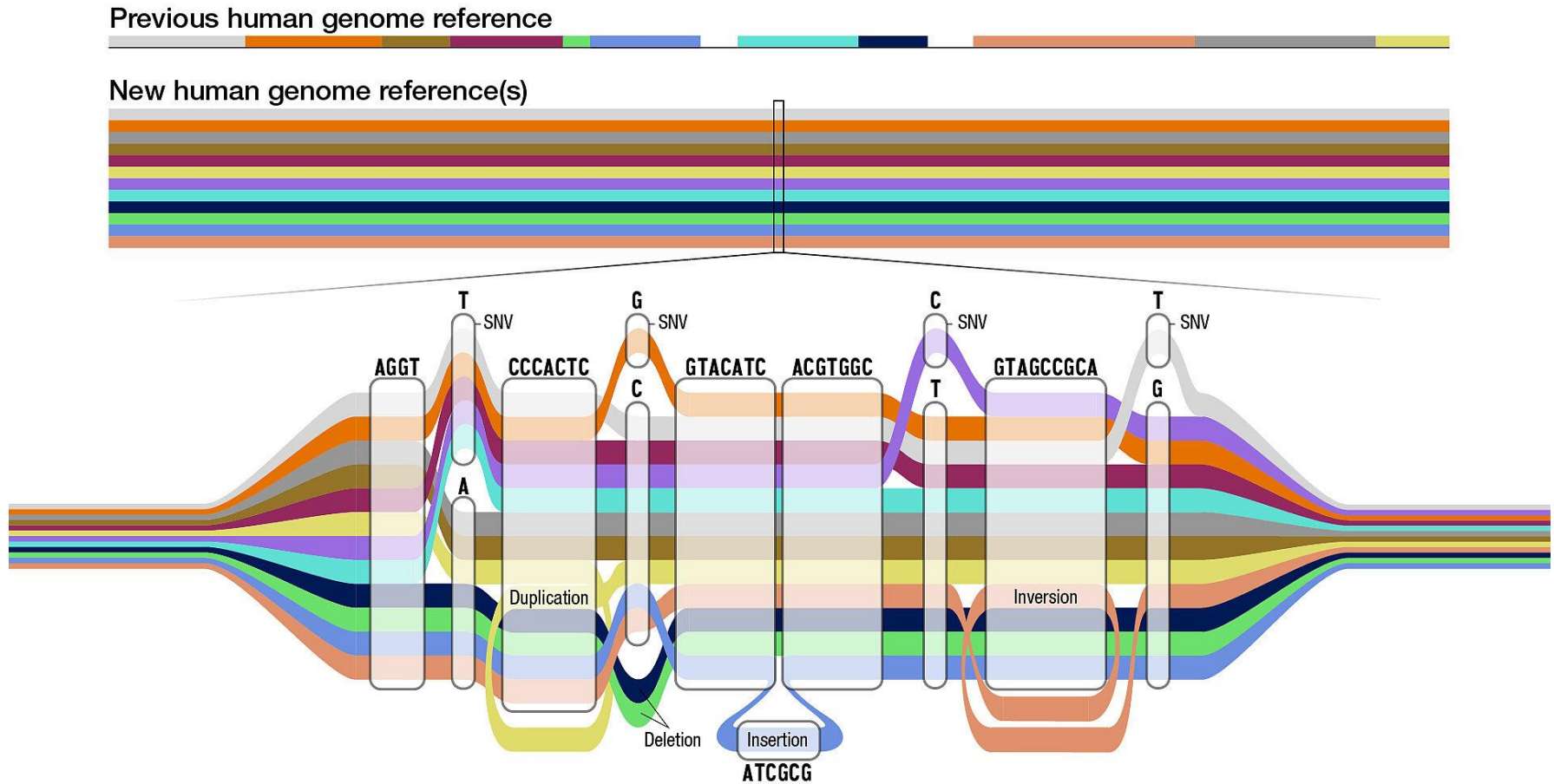
Ingroup bias



Outgroup bias?

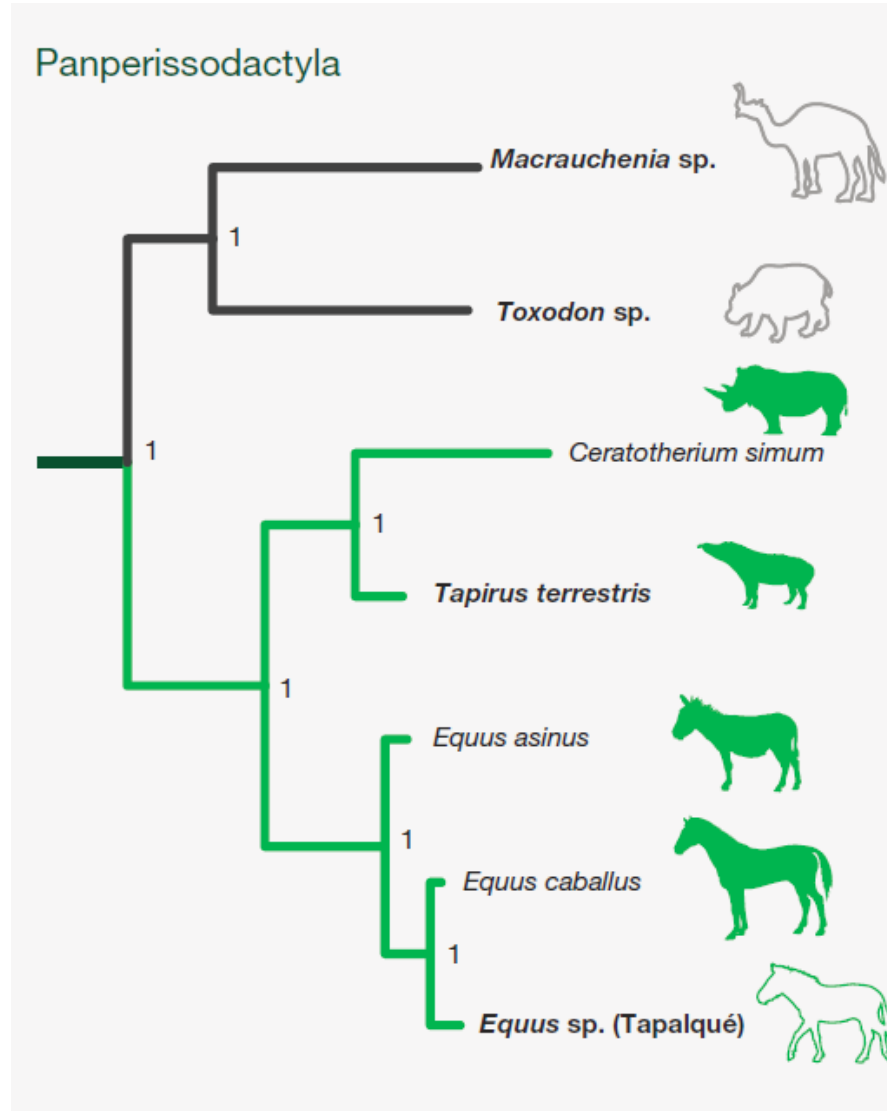


Solution: pangenome mapping?



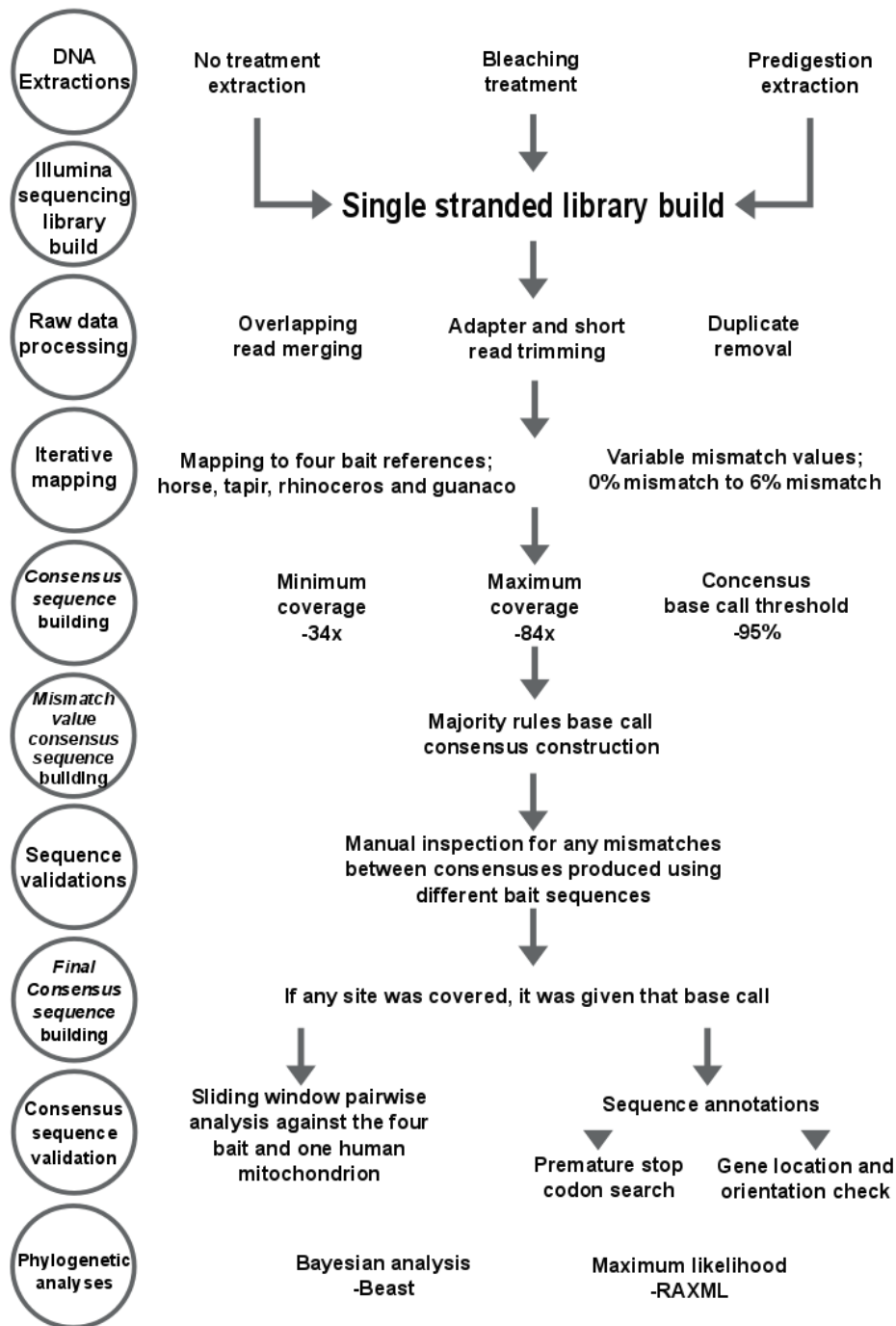


Phylogenetic distance

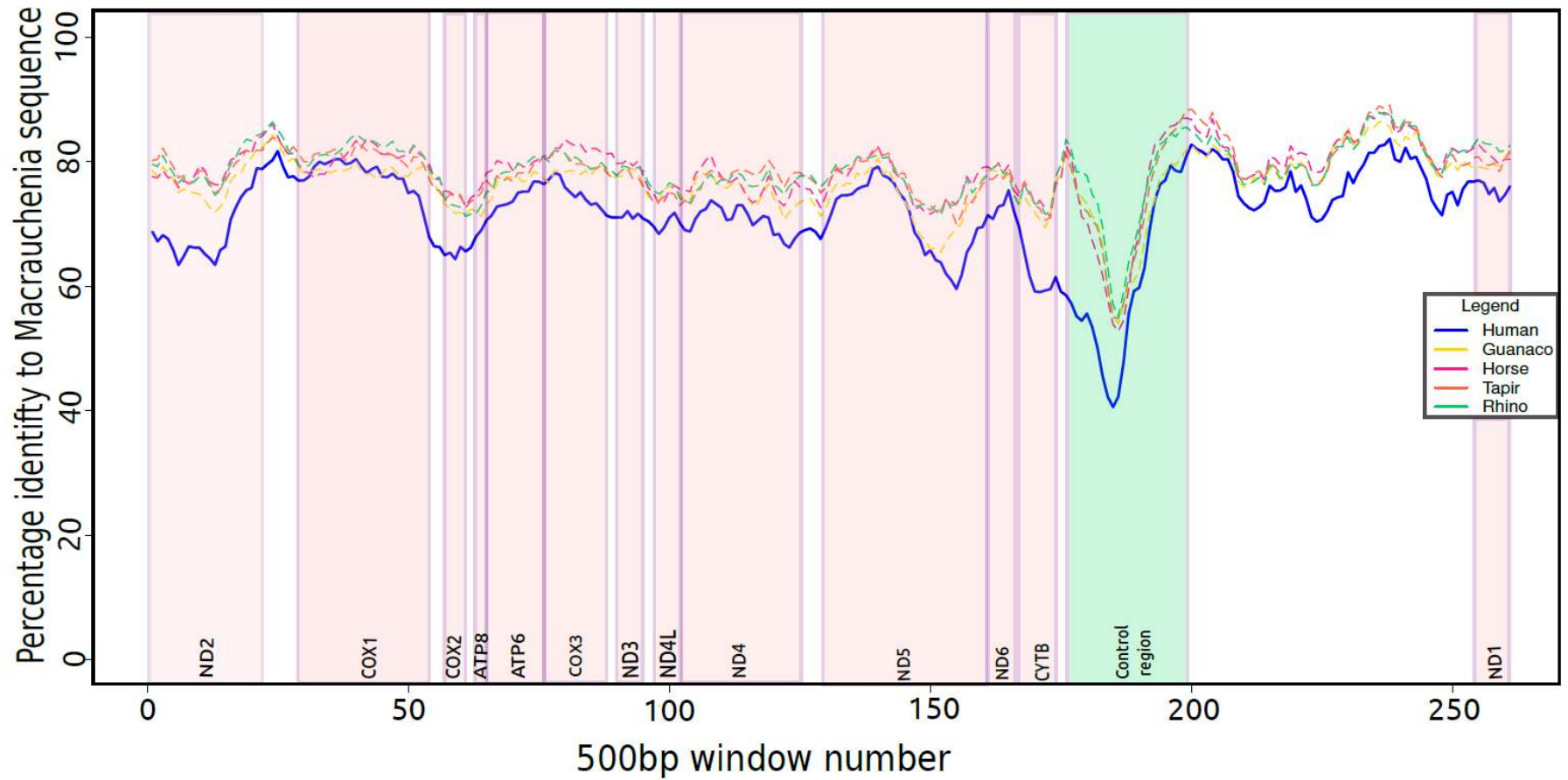


Mostly from warm to hot climate

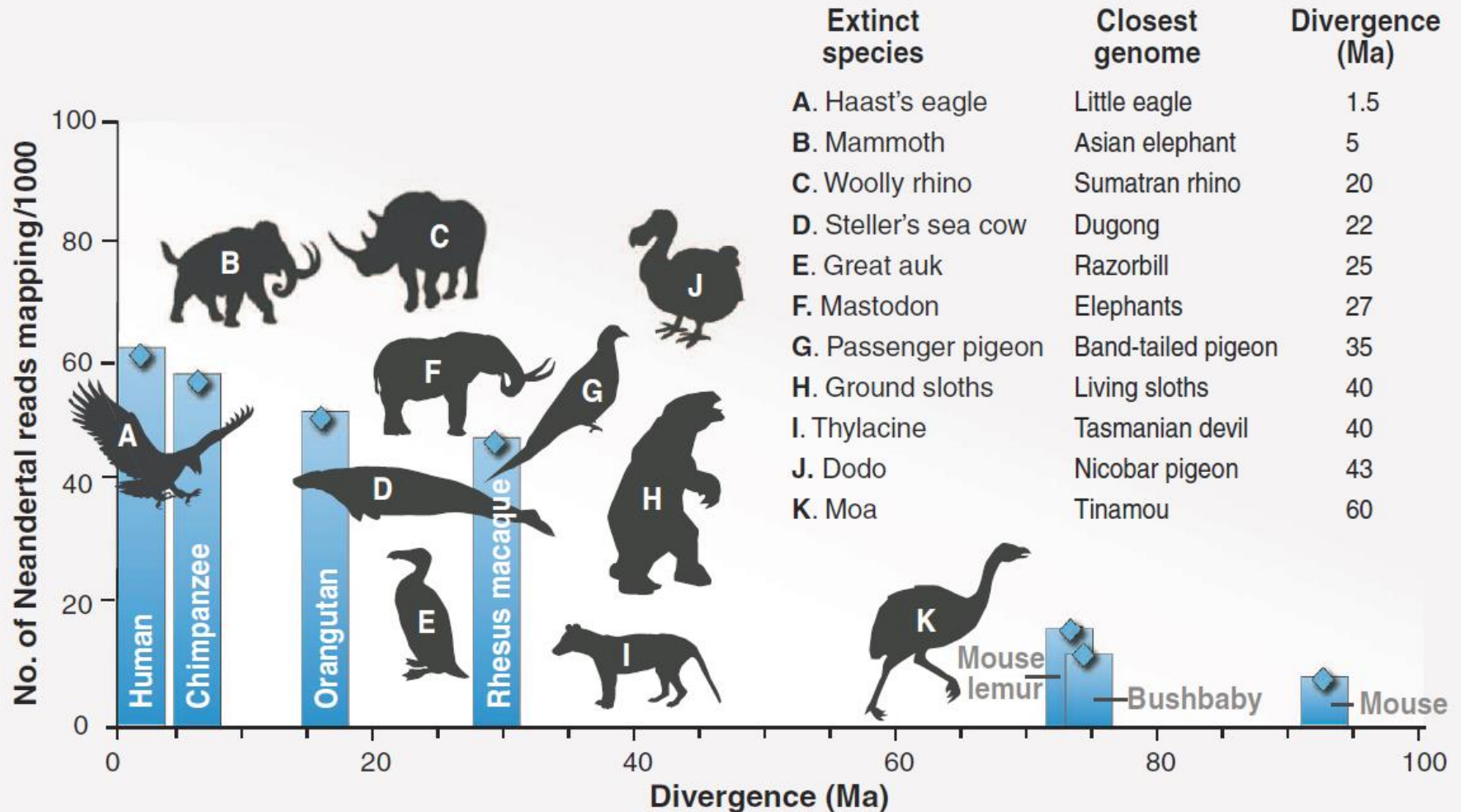




Quality check



Would a nuclear genome be possible?



Thanks.....

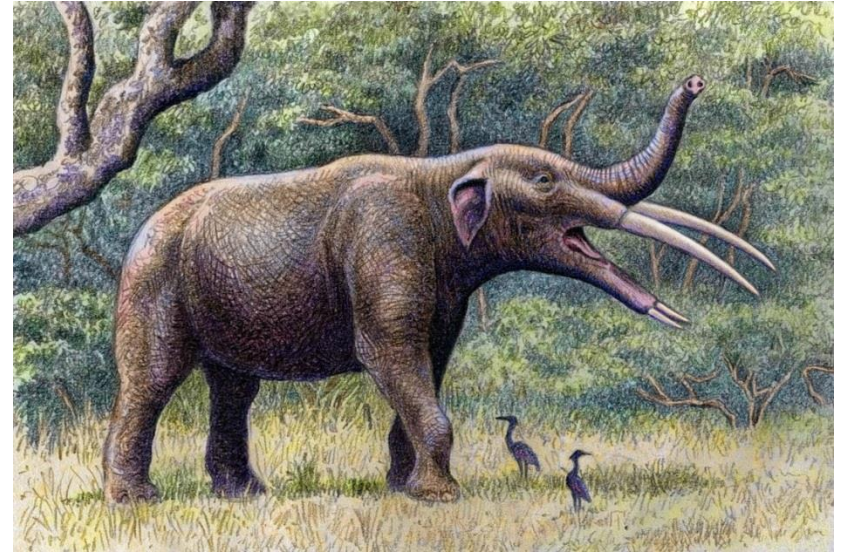
.....for listening so far



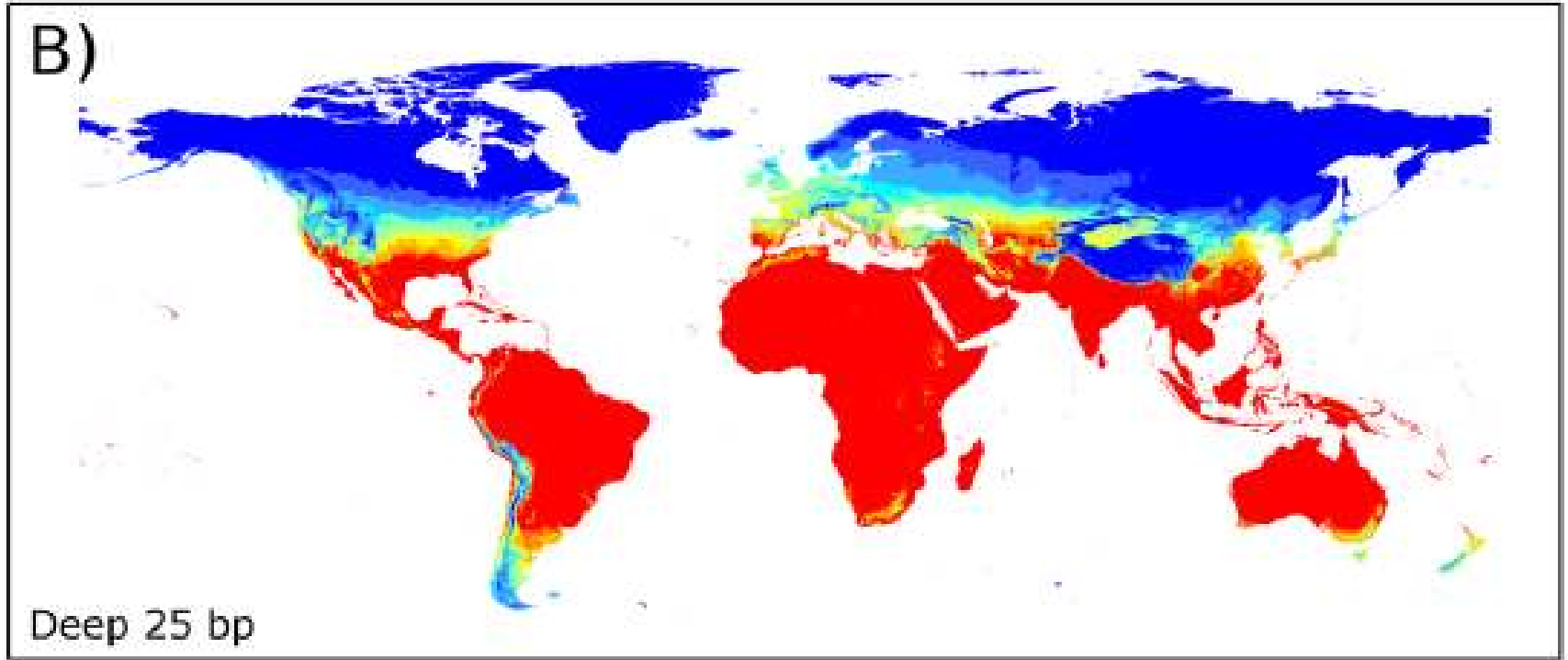
@ondseba



Part 2: What to do with ancient DNA



Choose samples from colder regions



Cave bears



Bear evolution



Bear evolution and gene flow



Gene flow among species



↔
Geneflow

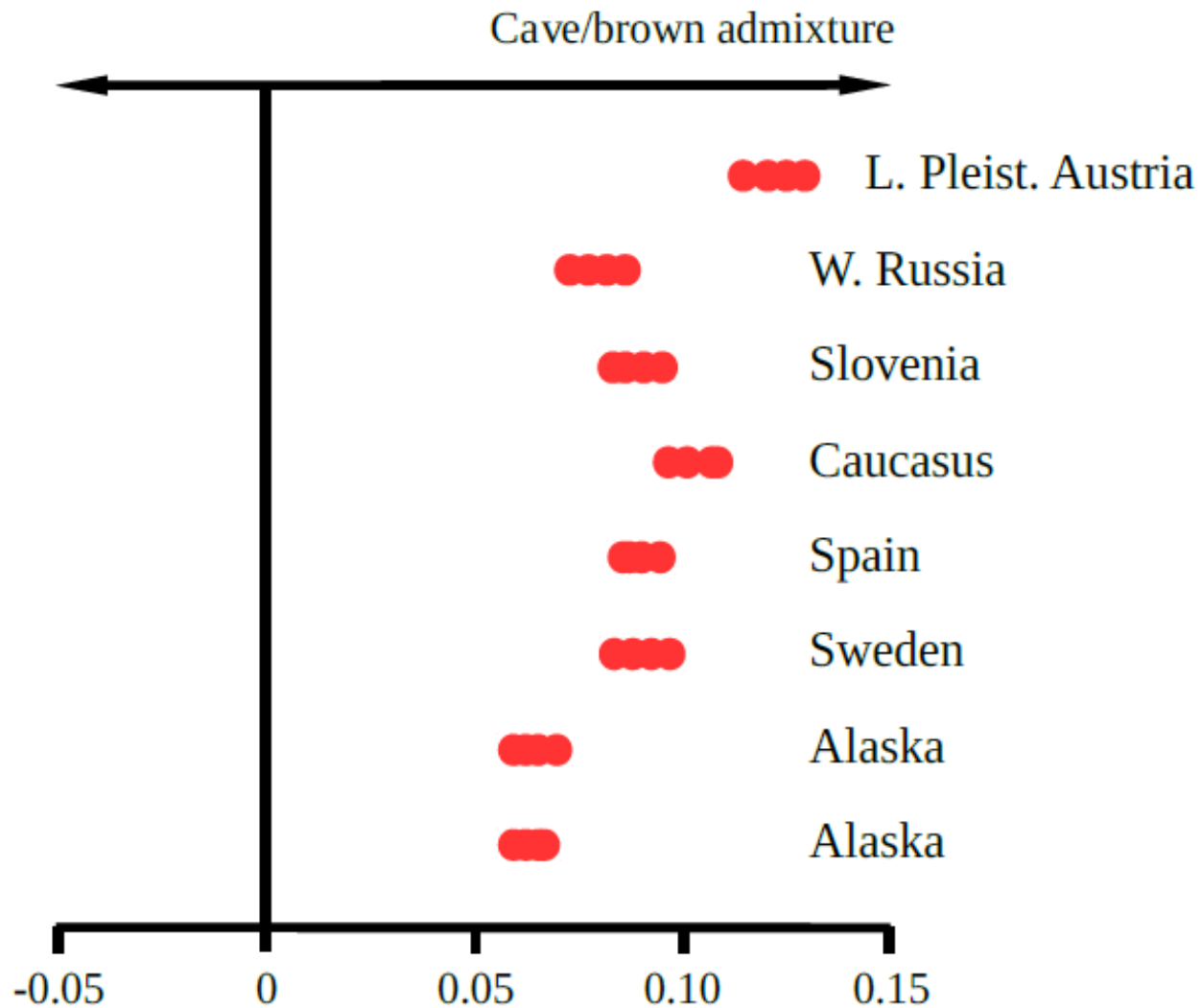


↔
Geneflow?

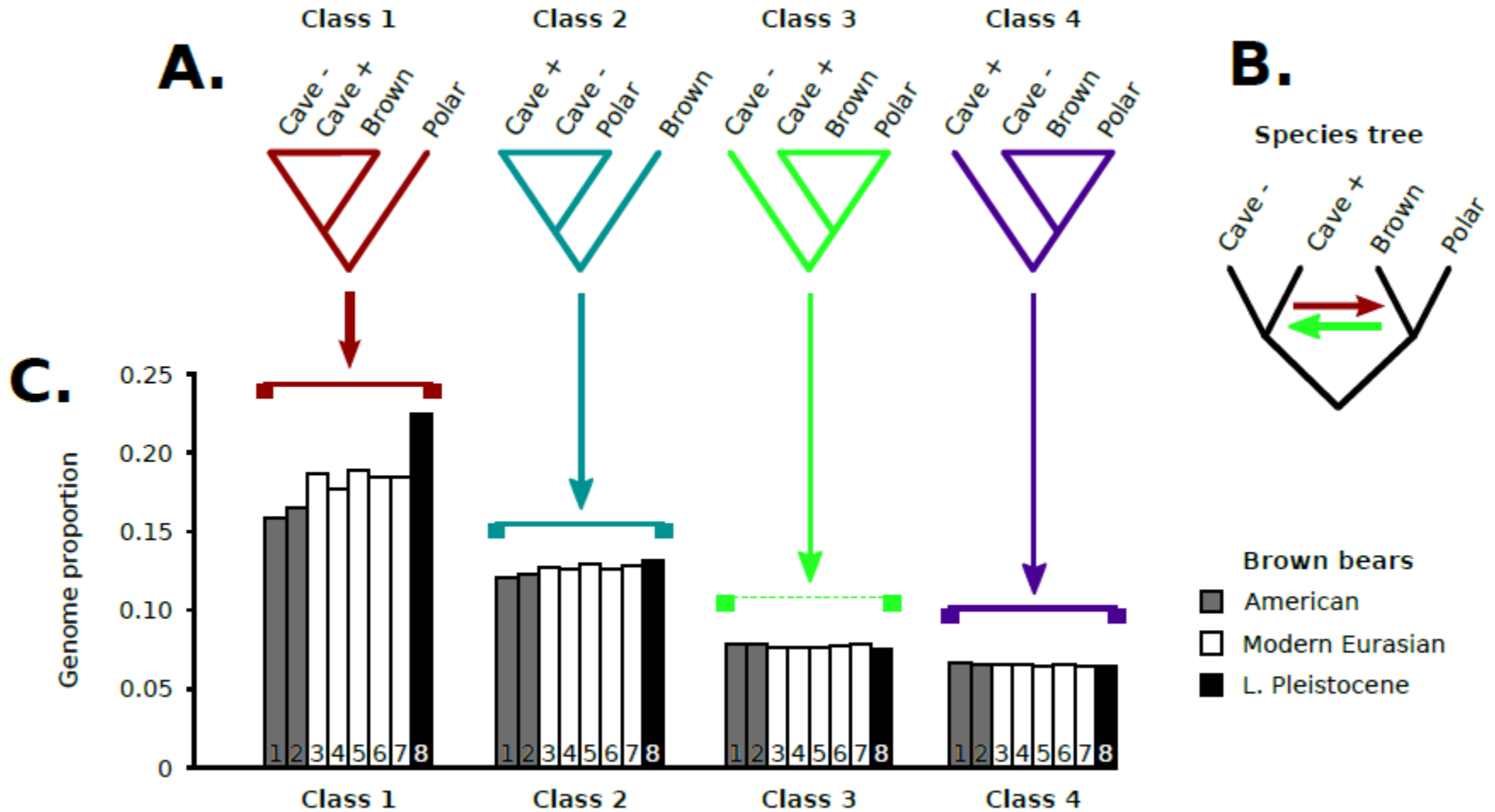


↔
Geneflow?

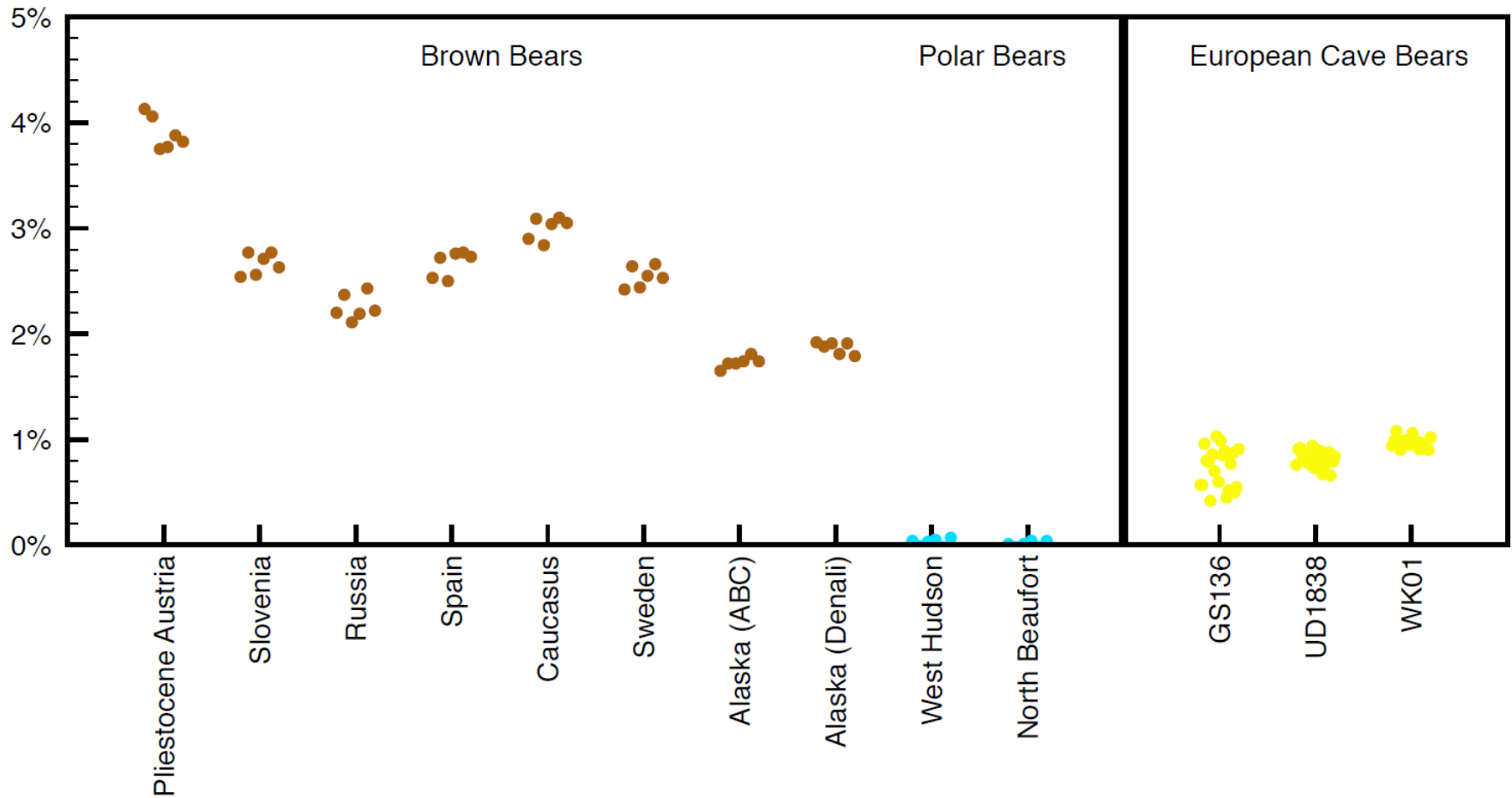
D-statistics



Phylogenetic admixture analysis



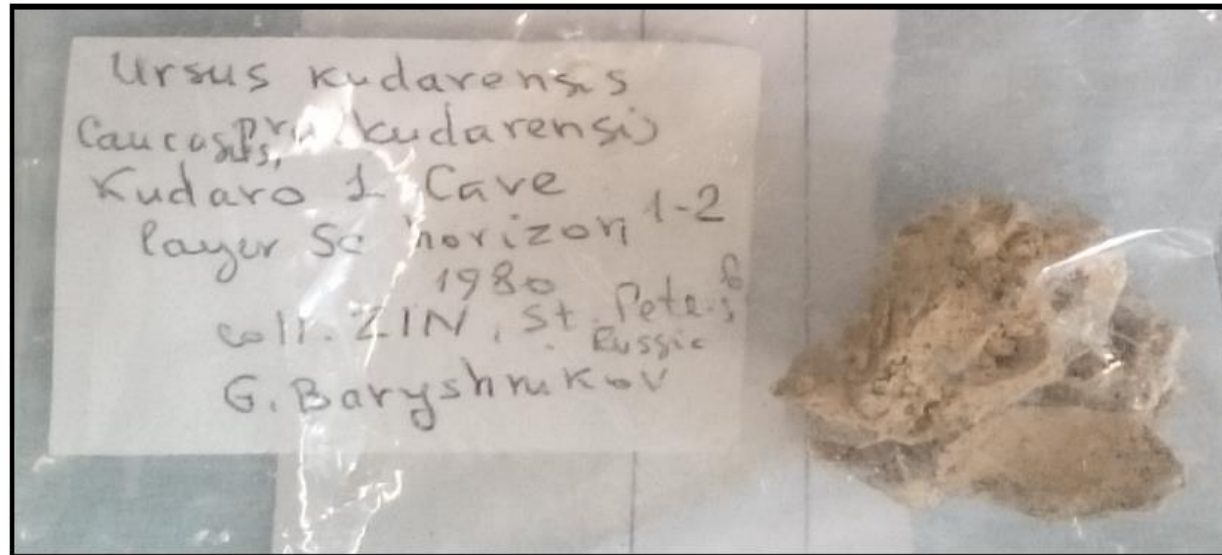
Percentage of admixture



Caucasus



Caucasus cave bears



U. kudarensis praekudarensis

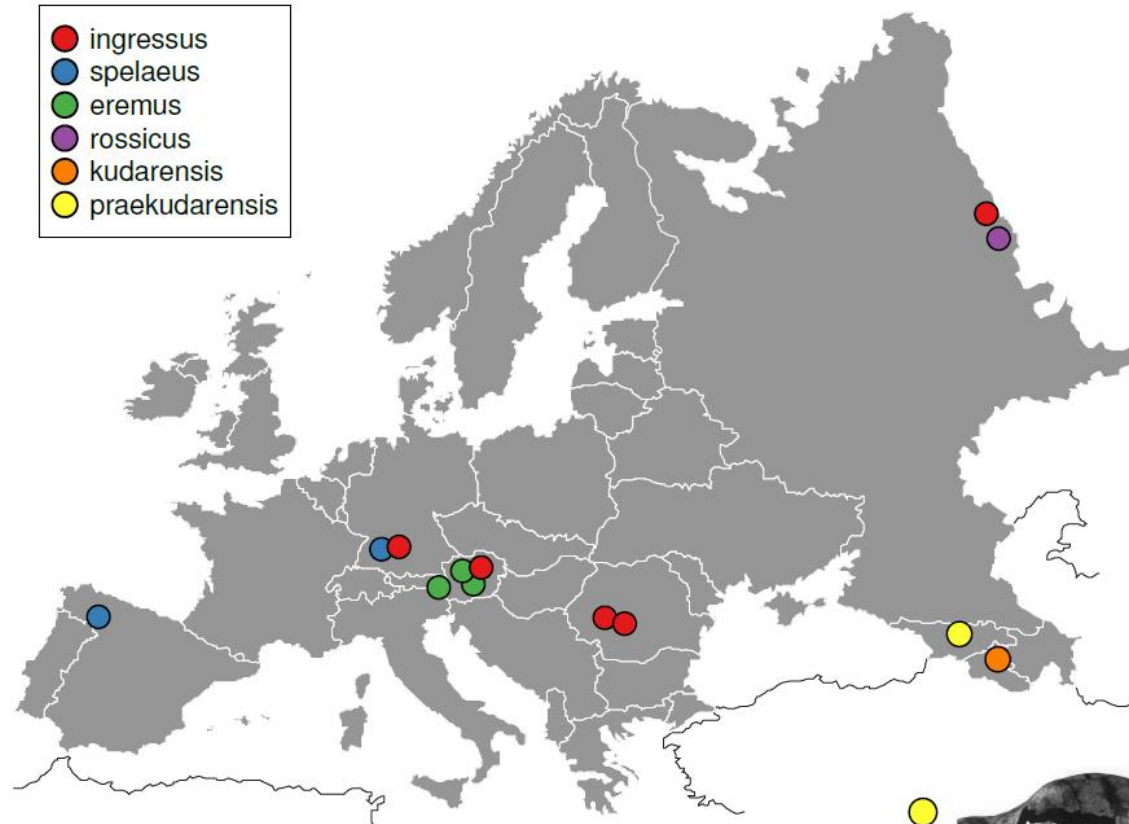
Kudaro 1 cave, Caucasus

Layer 5c, horizon 1-2

Oxygen-Isotope Stage 10

360,000 ± 90,000 years

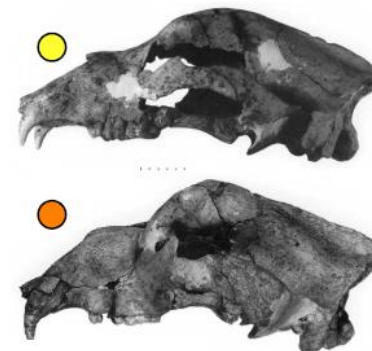
European cave bears



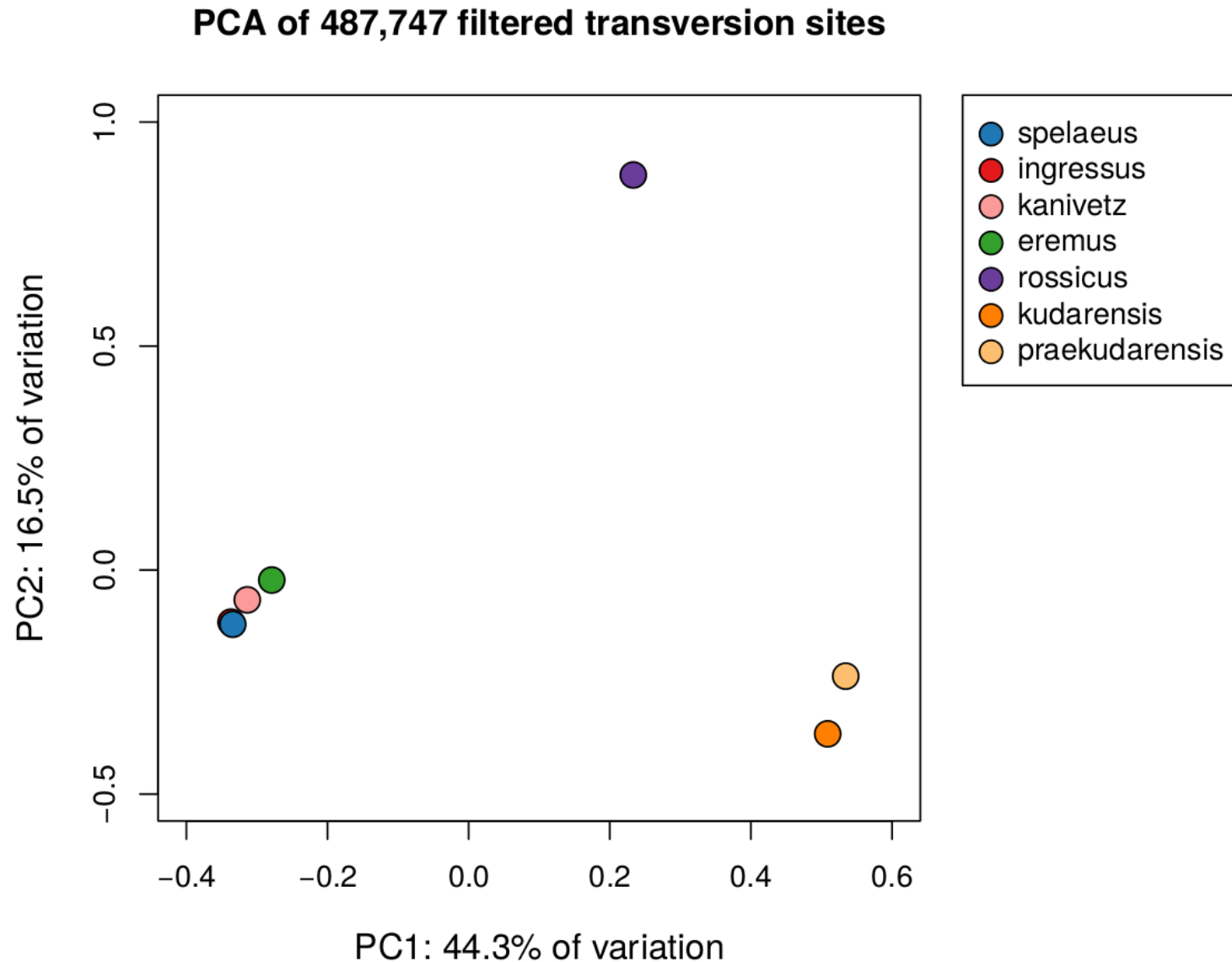
Cave Bears from the Paleolithic of the Greater Caucasus

Gennady F. Baryshnikov

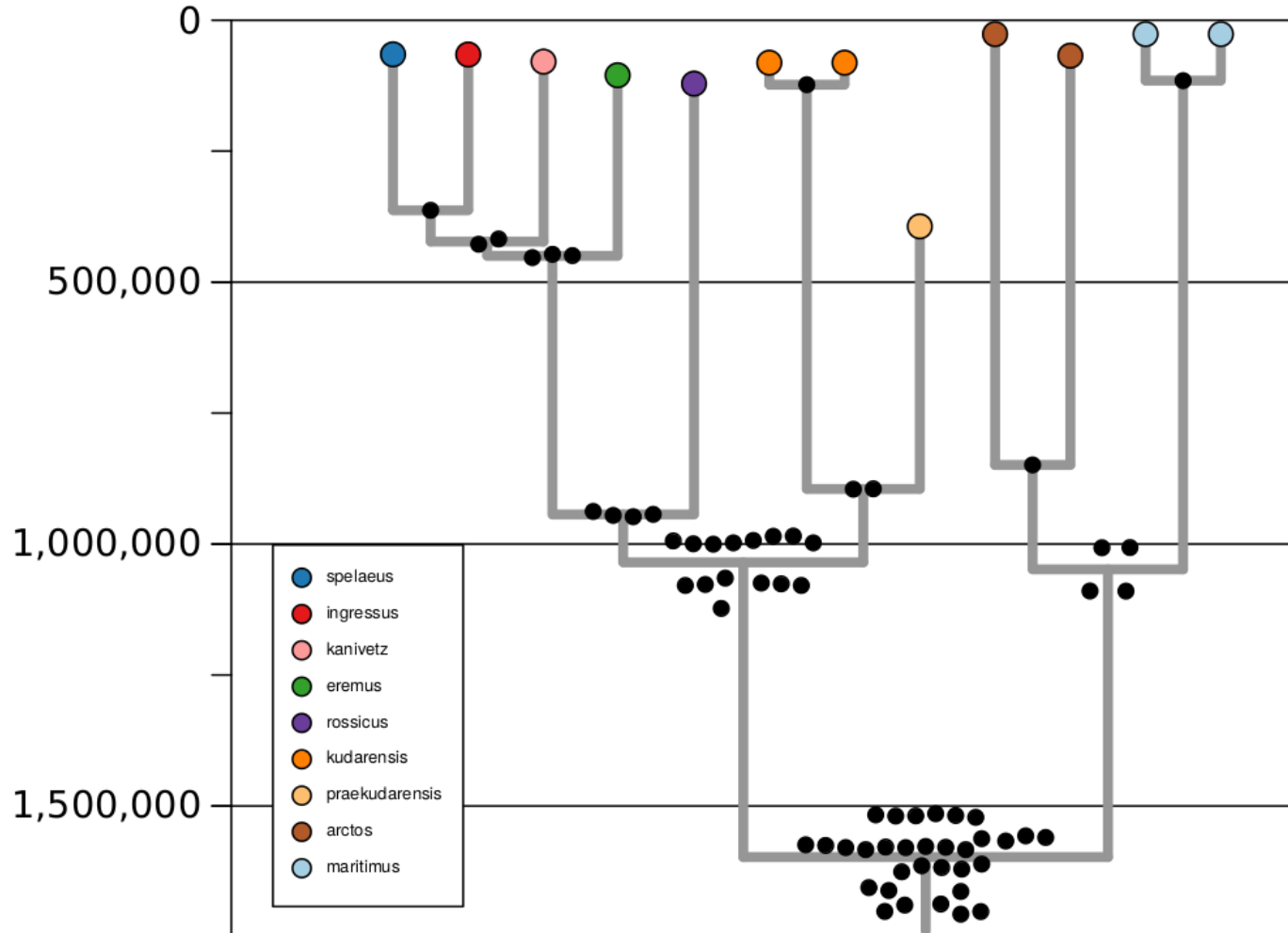
Zoological Institute, Russian Academy of Sciences, St. Petersburg



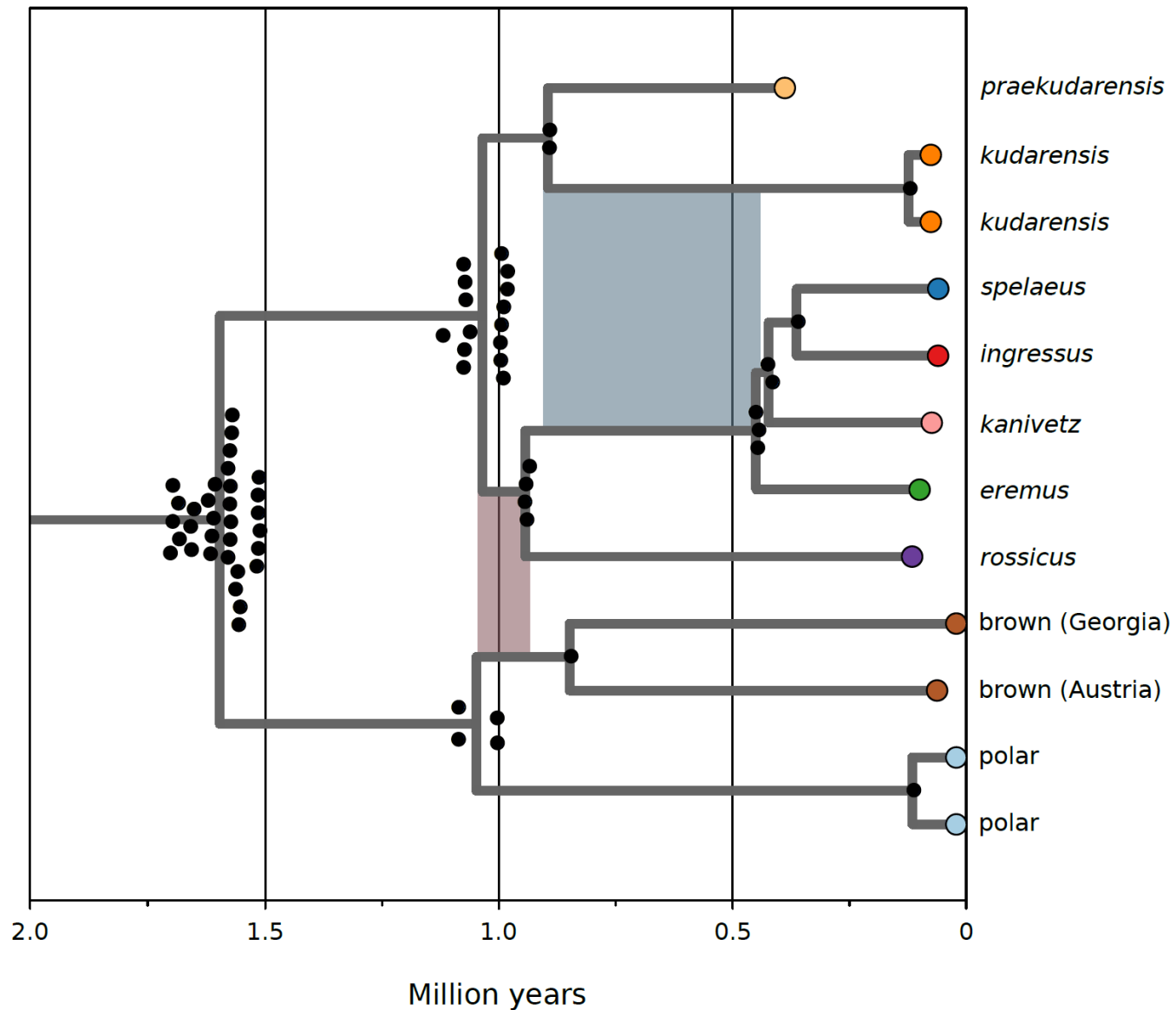
Cave bear nuclear relationships.....



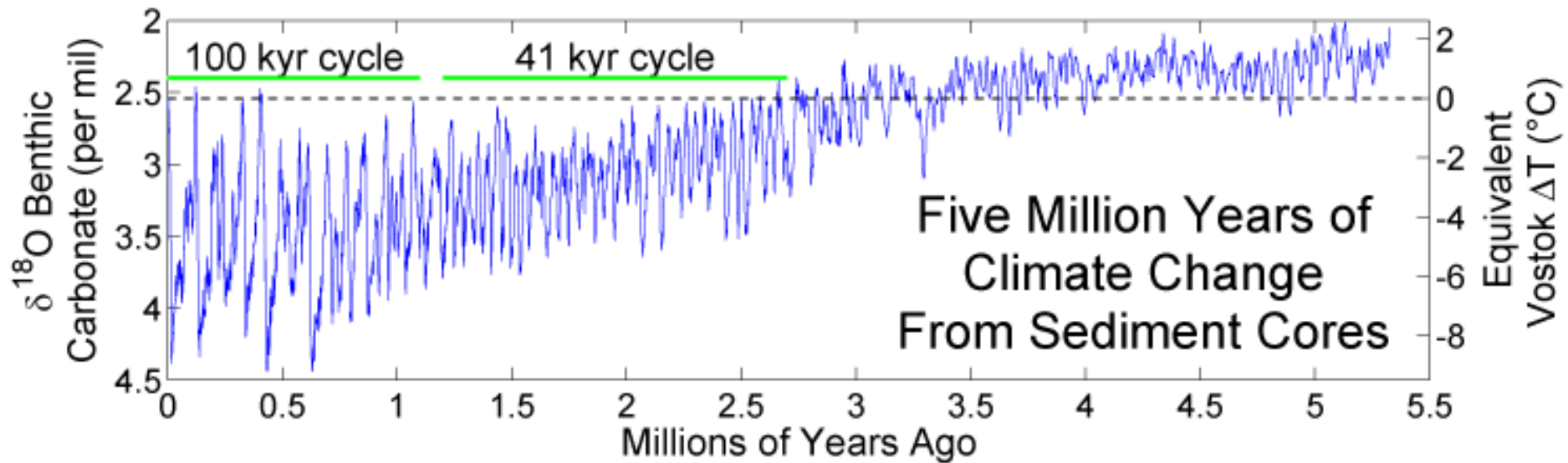
.....and bear evolution from a temporal perspective



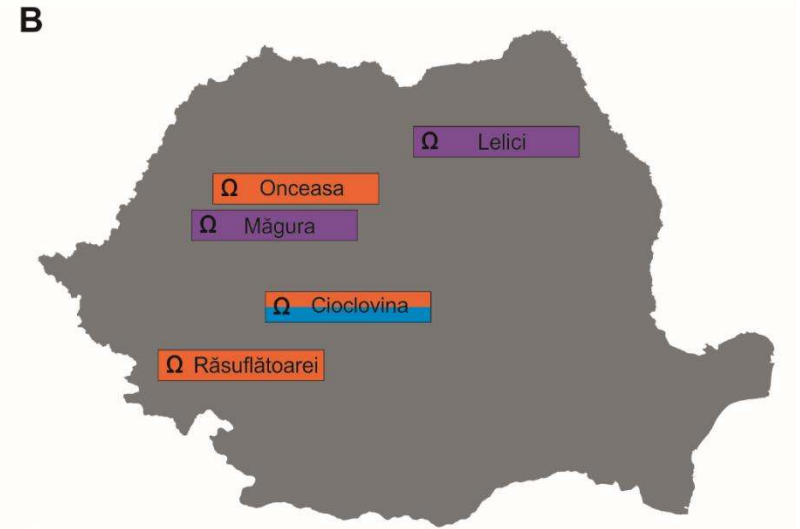
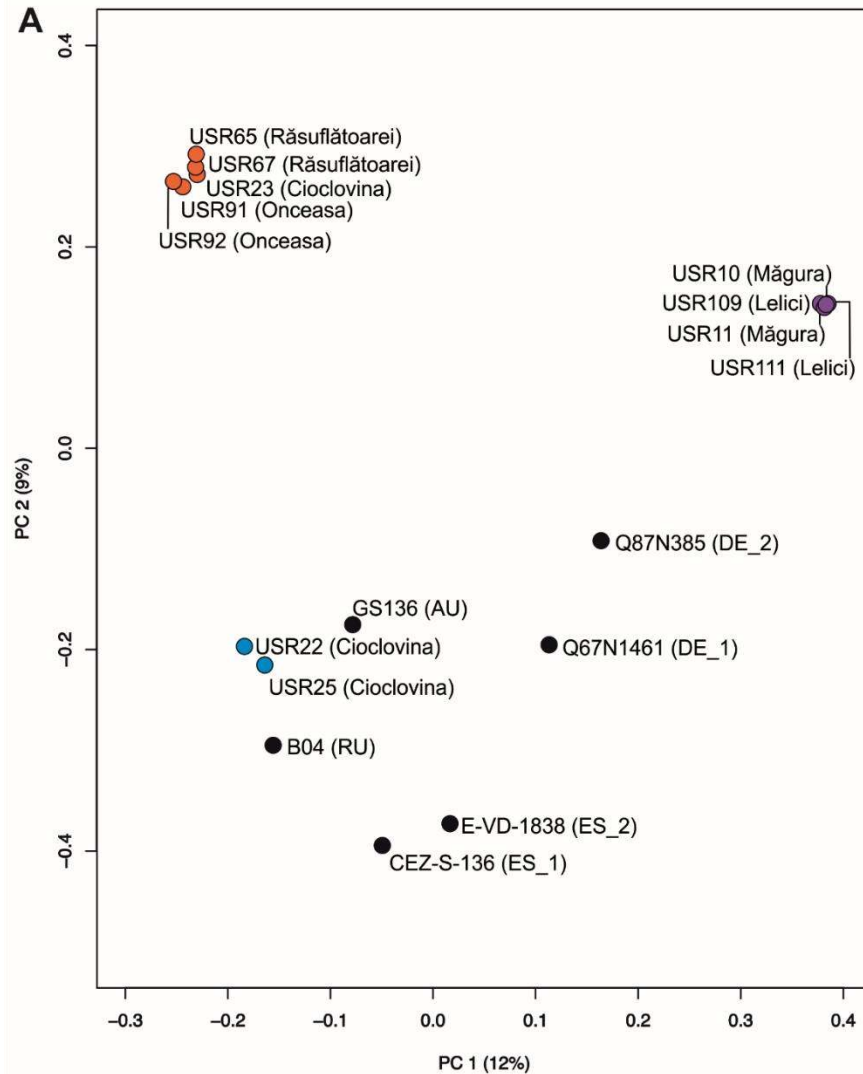
Dating admixture – 500,000 years after divergence



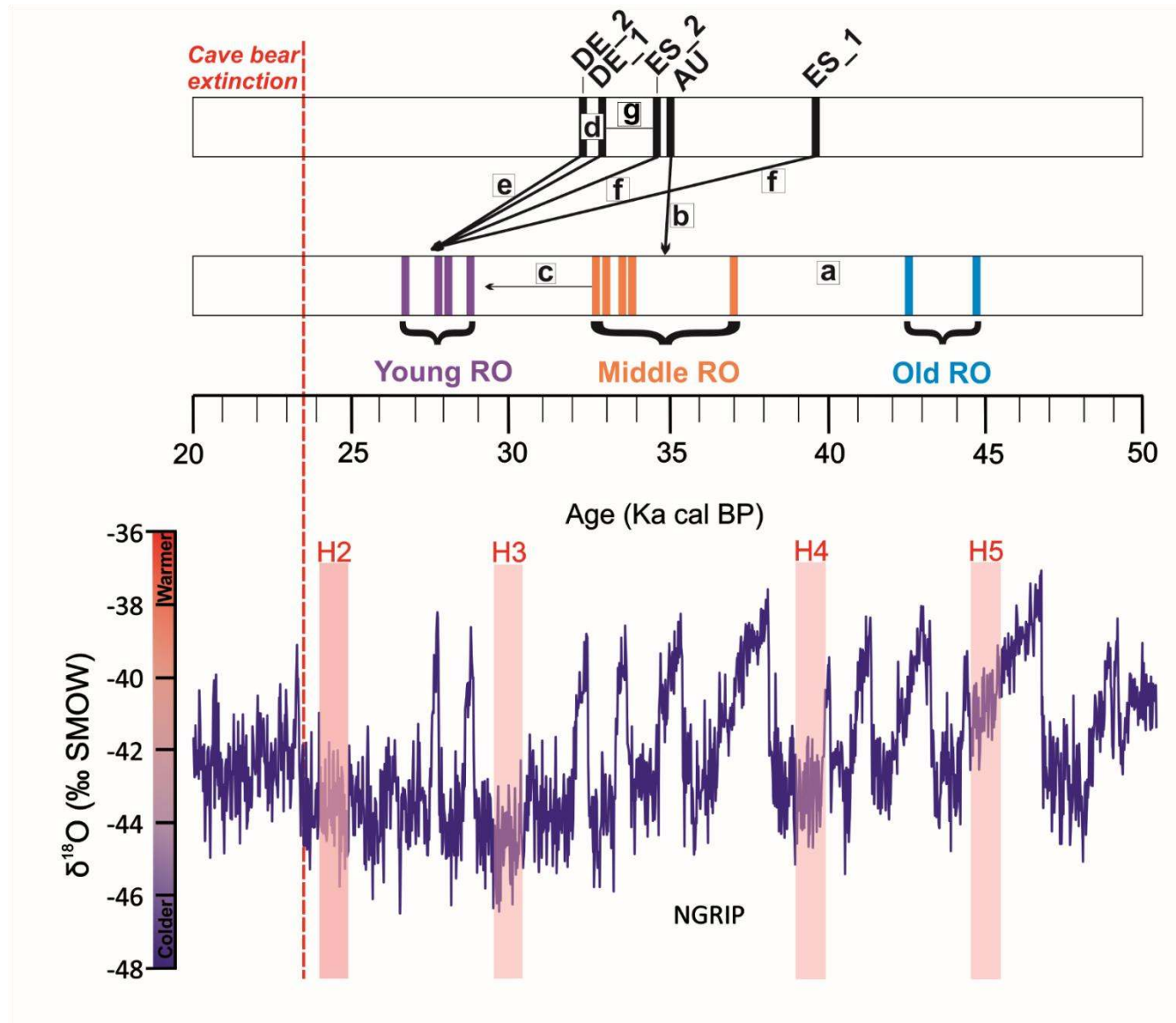
Natural climate change causing divergence?



Temporal genomic differentiation in Romania



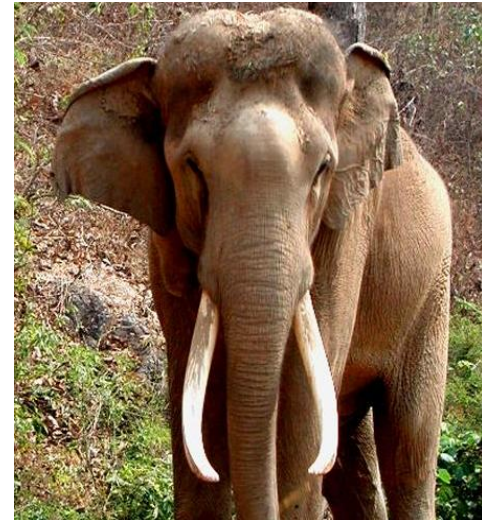
And cave bear population dynamics

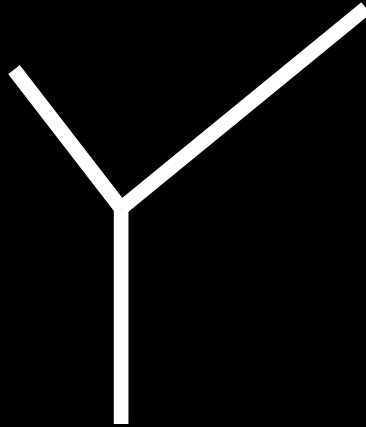


Summary cave bears

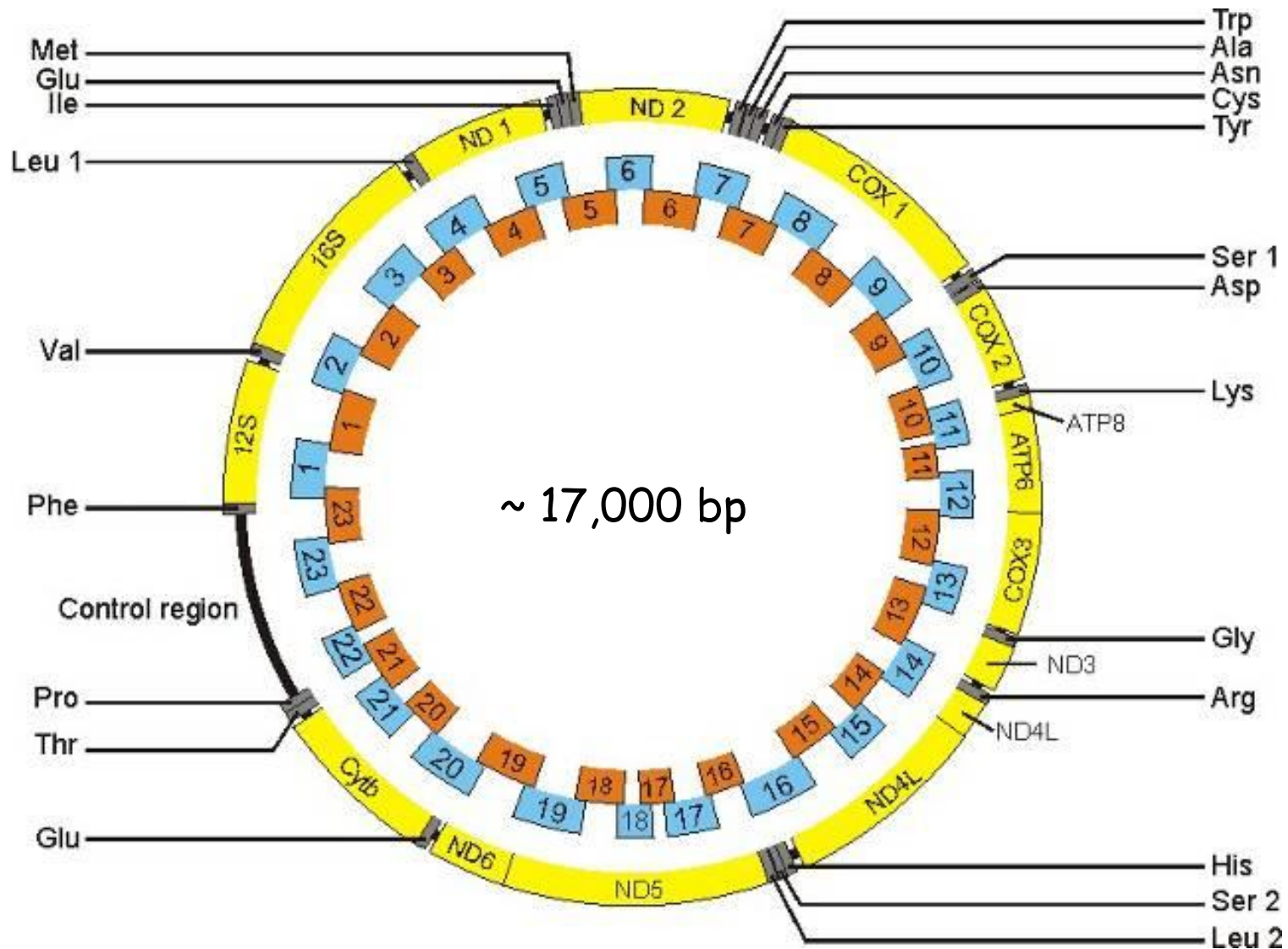
- Gene flow within and between species is an important factor in bear evolution
- The European cave bear has partially survived in the brown bear, at least on the genomic level
- Genomes from very old fossils can be used to calibrate phylogenetic trees
- Cave bears did not like cold climate phases

Elephant evolution





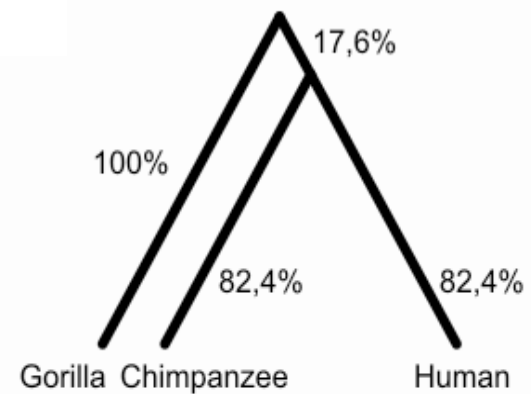
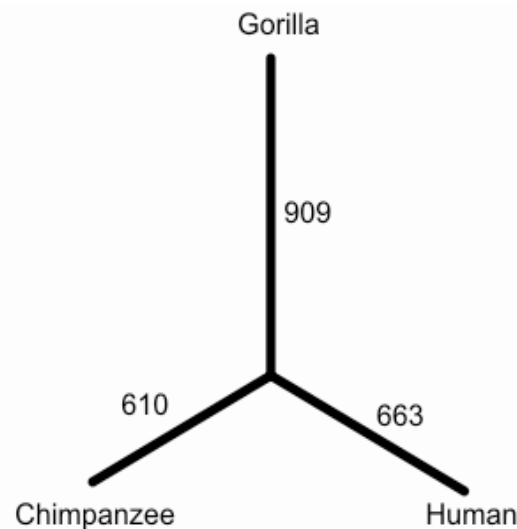
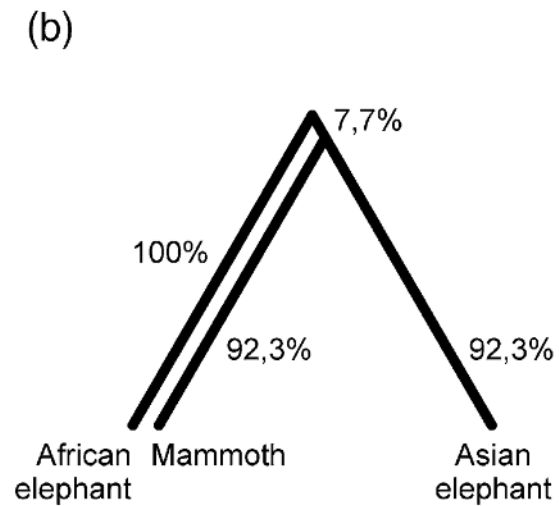
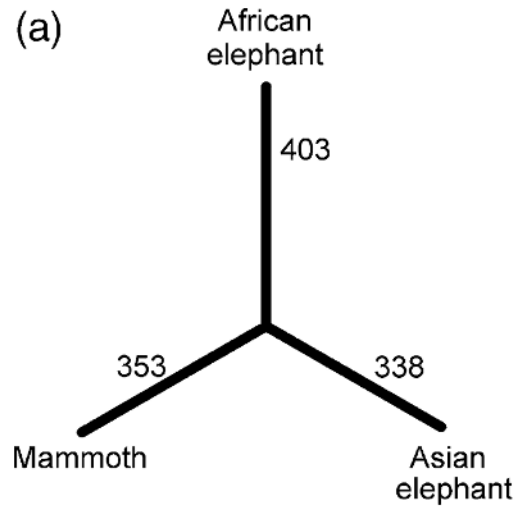
The mitochondrial genome



The choice of outgroup - living relatives



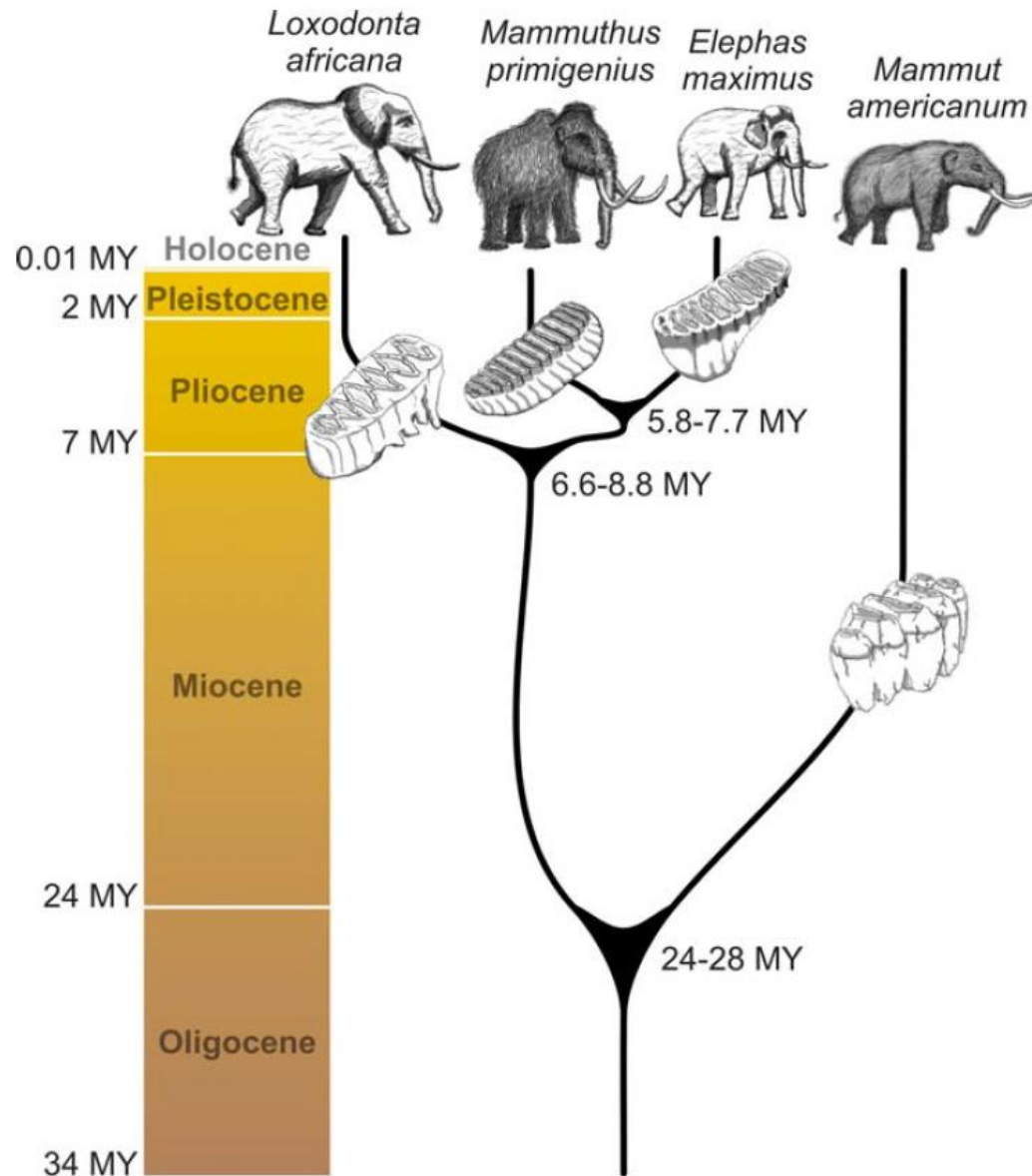
Phylogeny without outgroup



The choice of outgroup – extinct relatives



Phylogenetics

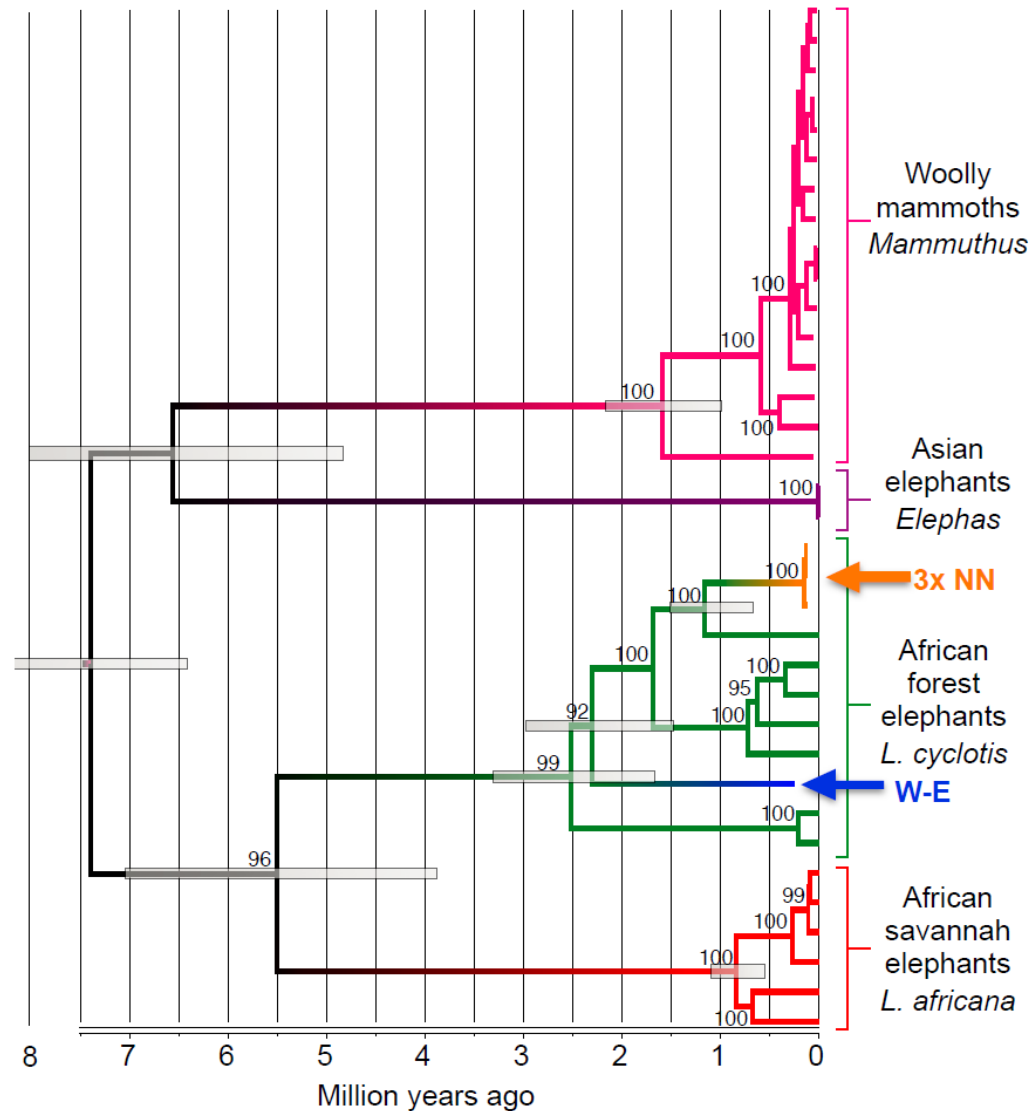


Palaeoloxodon antiquus



~120,000 years old

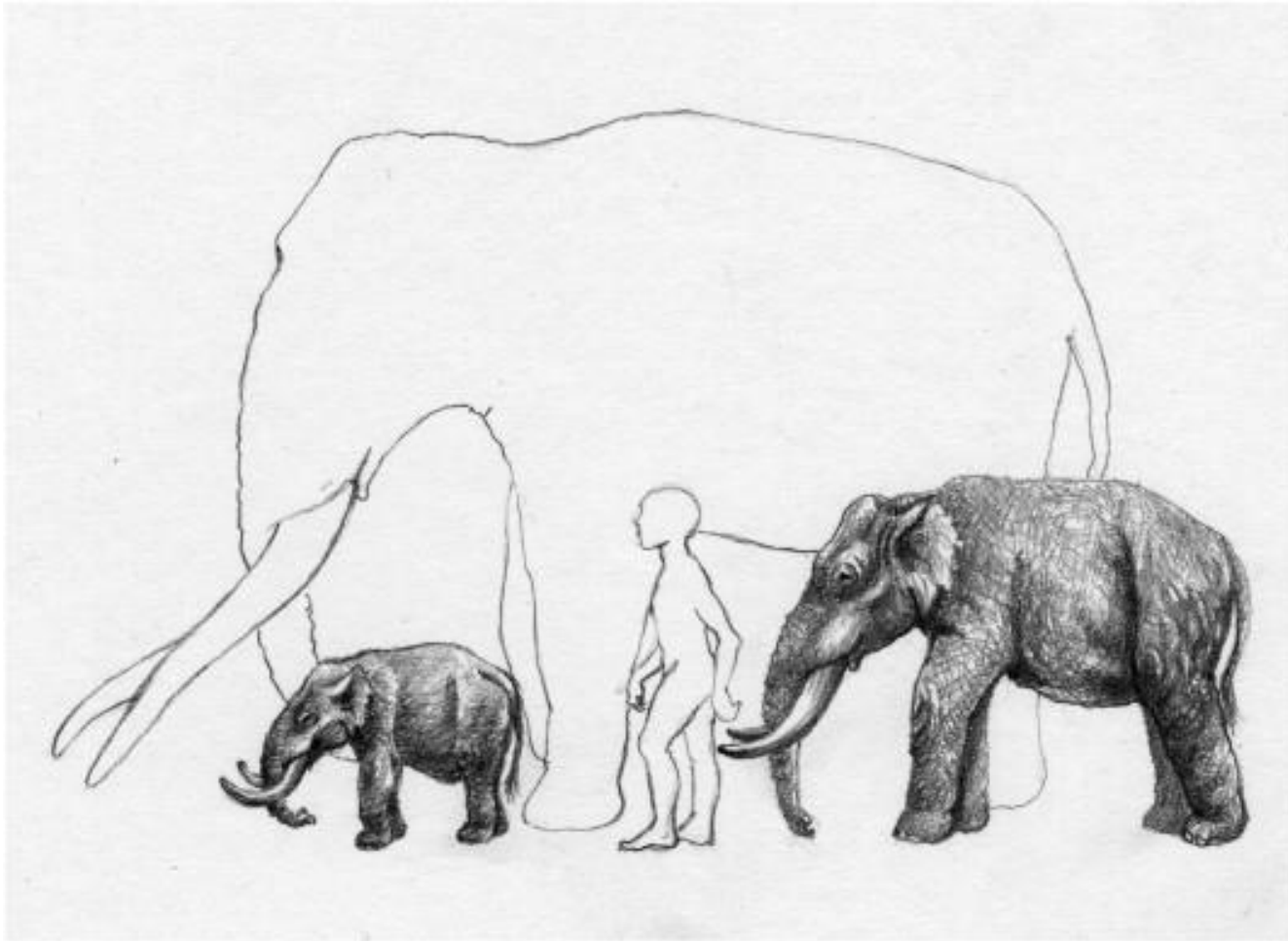
Mitogenome tree



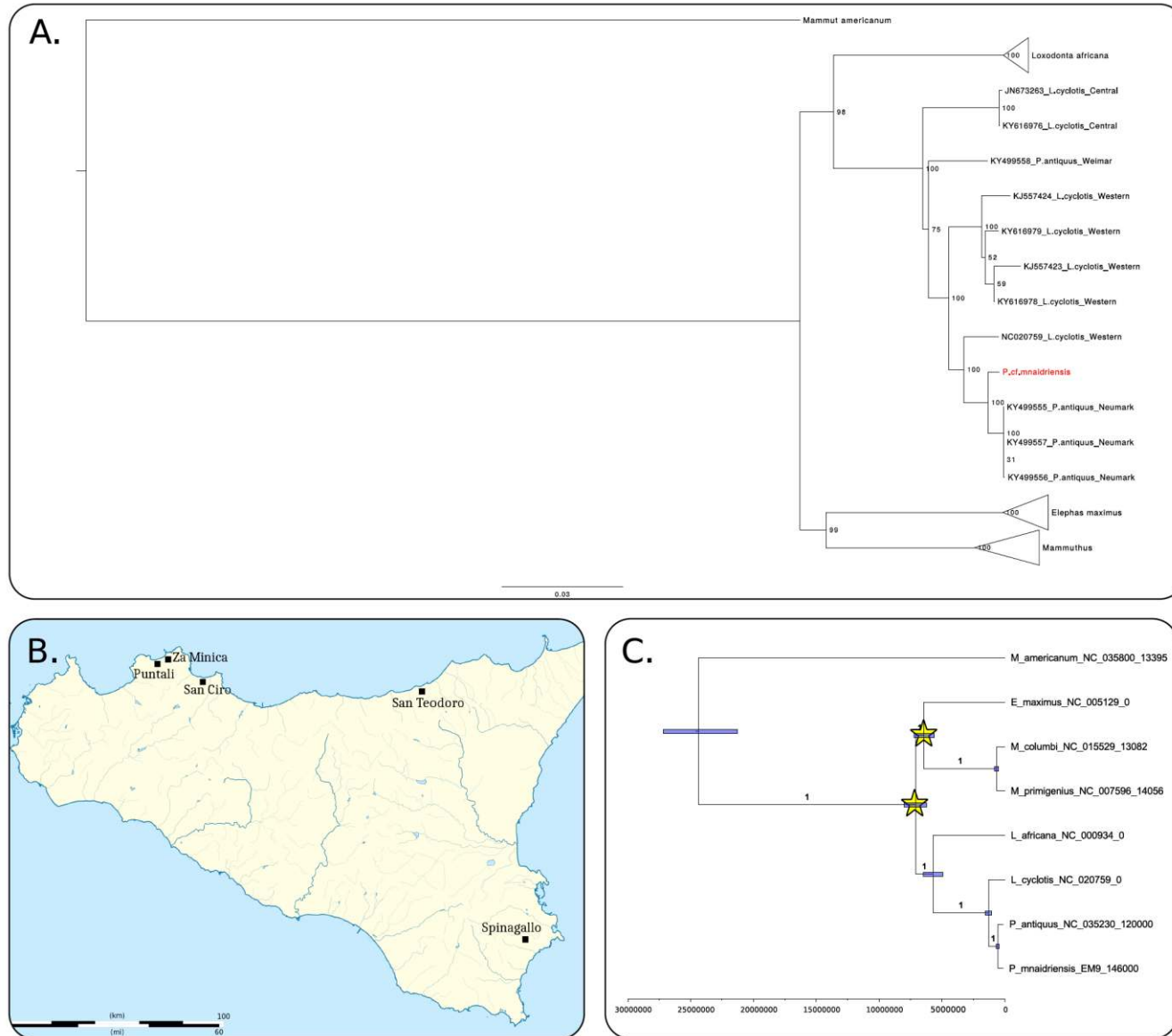
Sicily



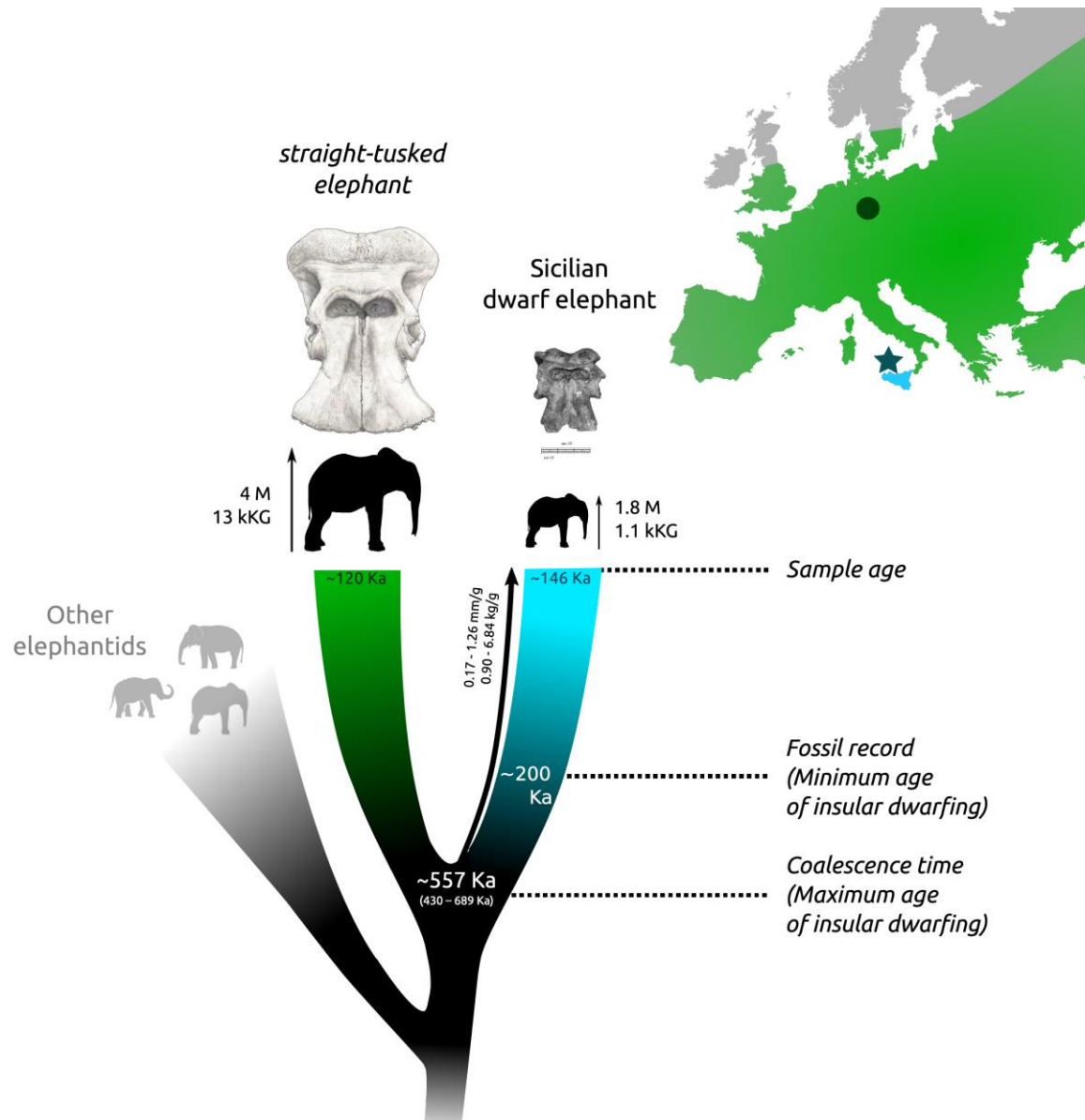
Palaeoloxodon mnaidriensis



Geographical origin and mitogenome phylogeny



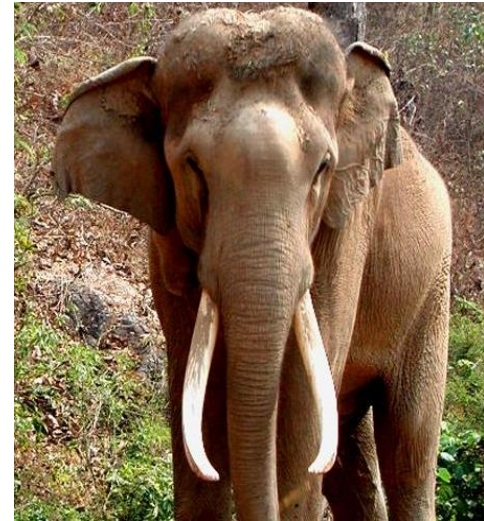
Insular dwarfing



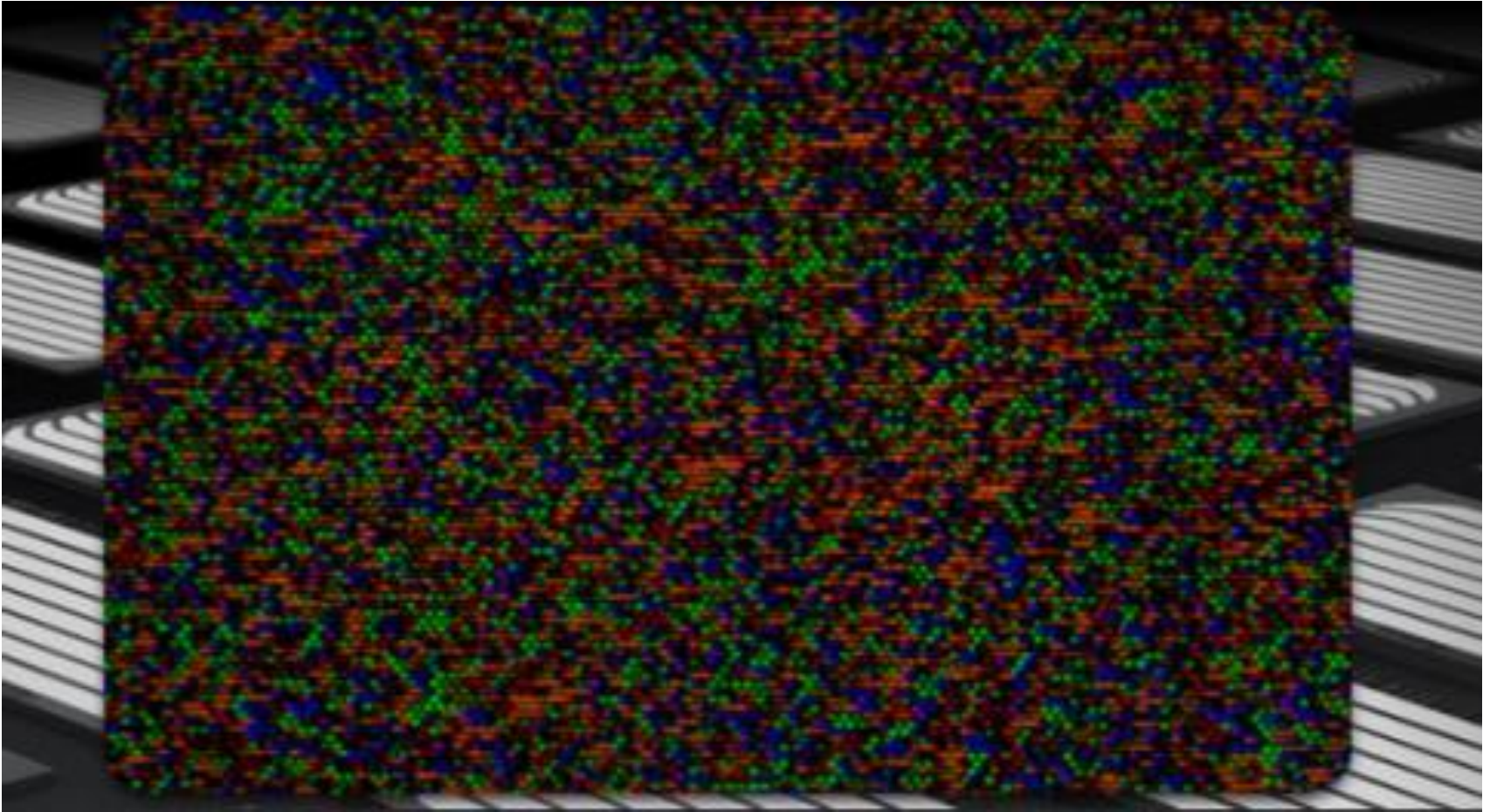
African forest elephant



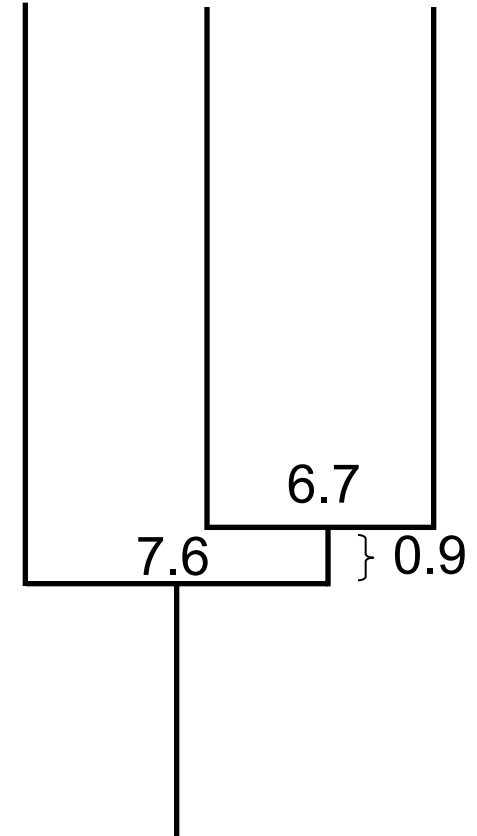
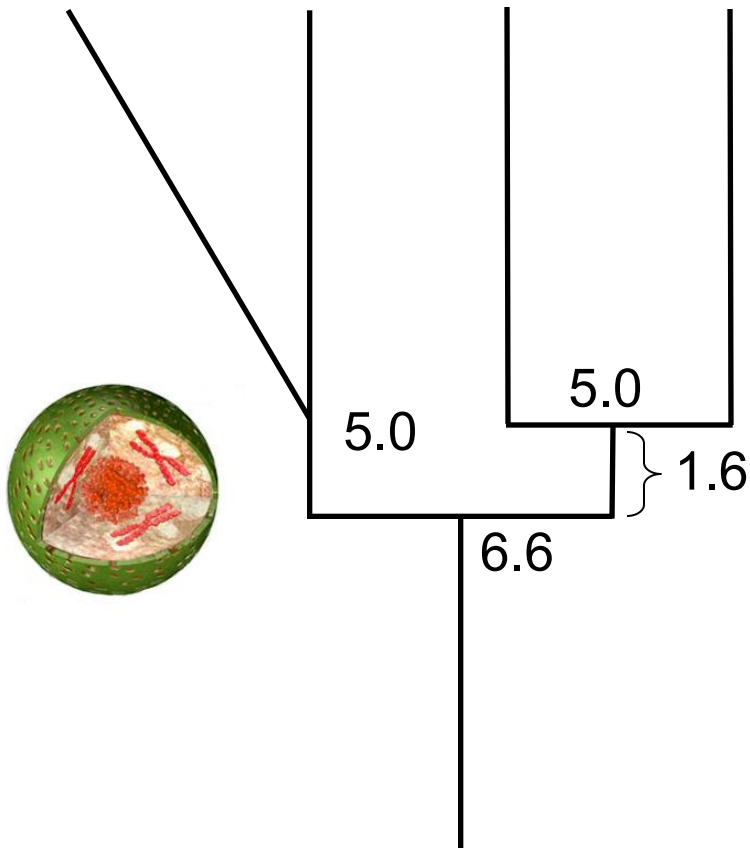
How are these related?



Nuclear DNA sequencing



Mitochondrial vs. nuclear phylogenies

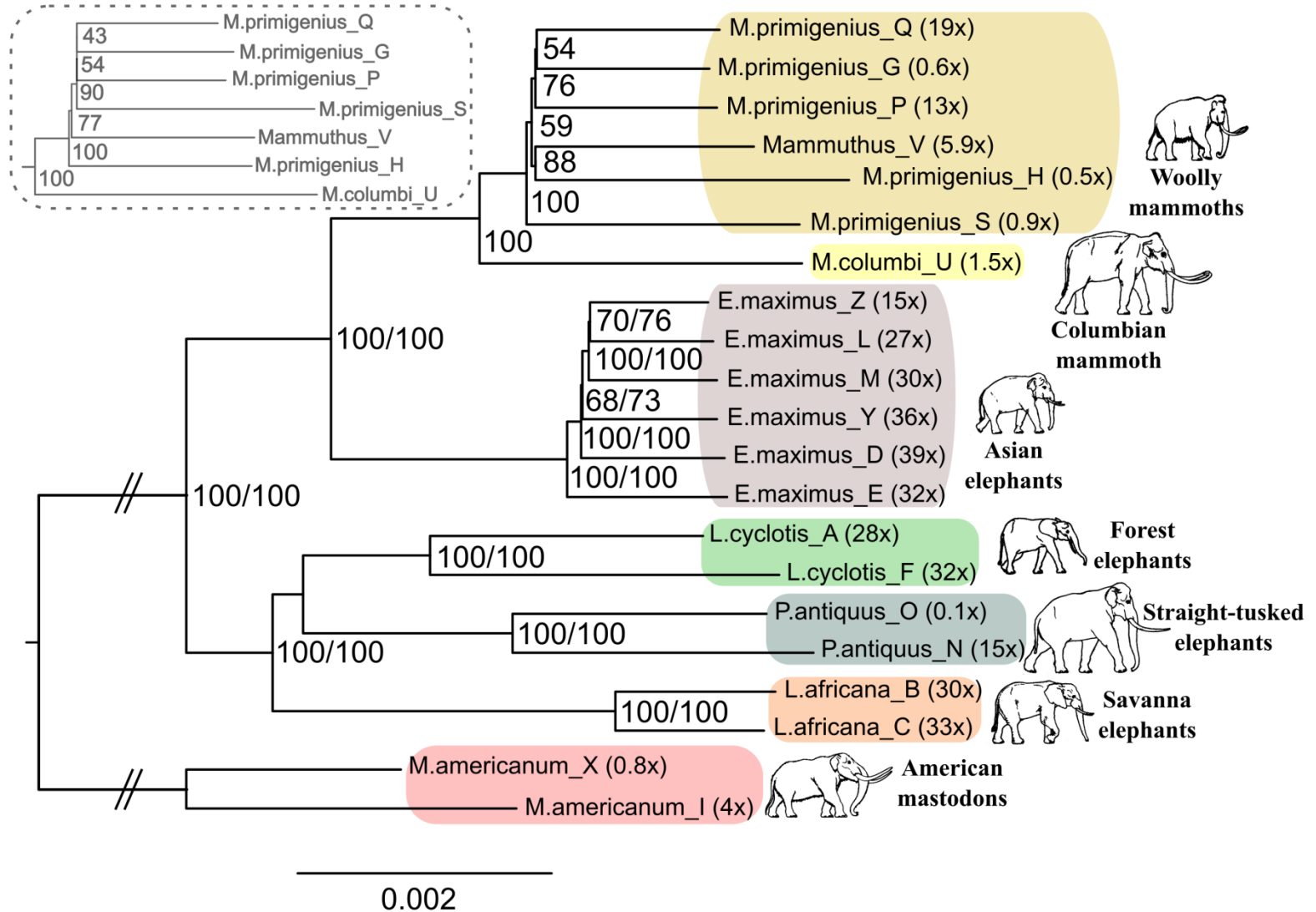


Palaeoloxodon antiquus

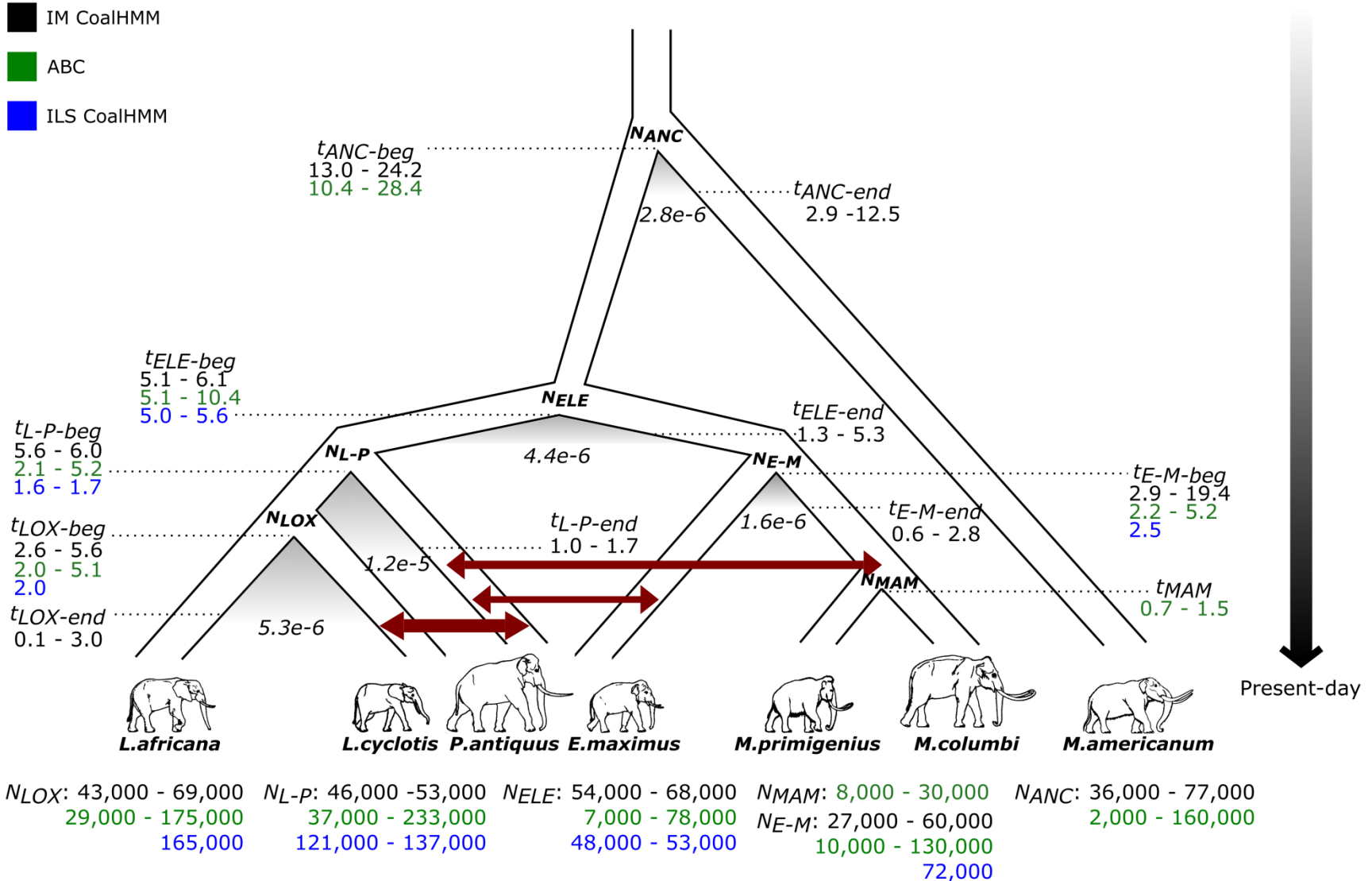


~120,000 years old

Palaeoloxodon antiquus – nuclear tree



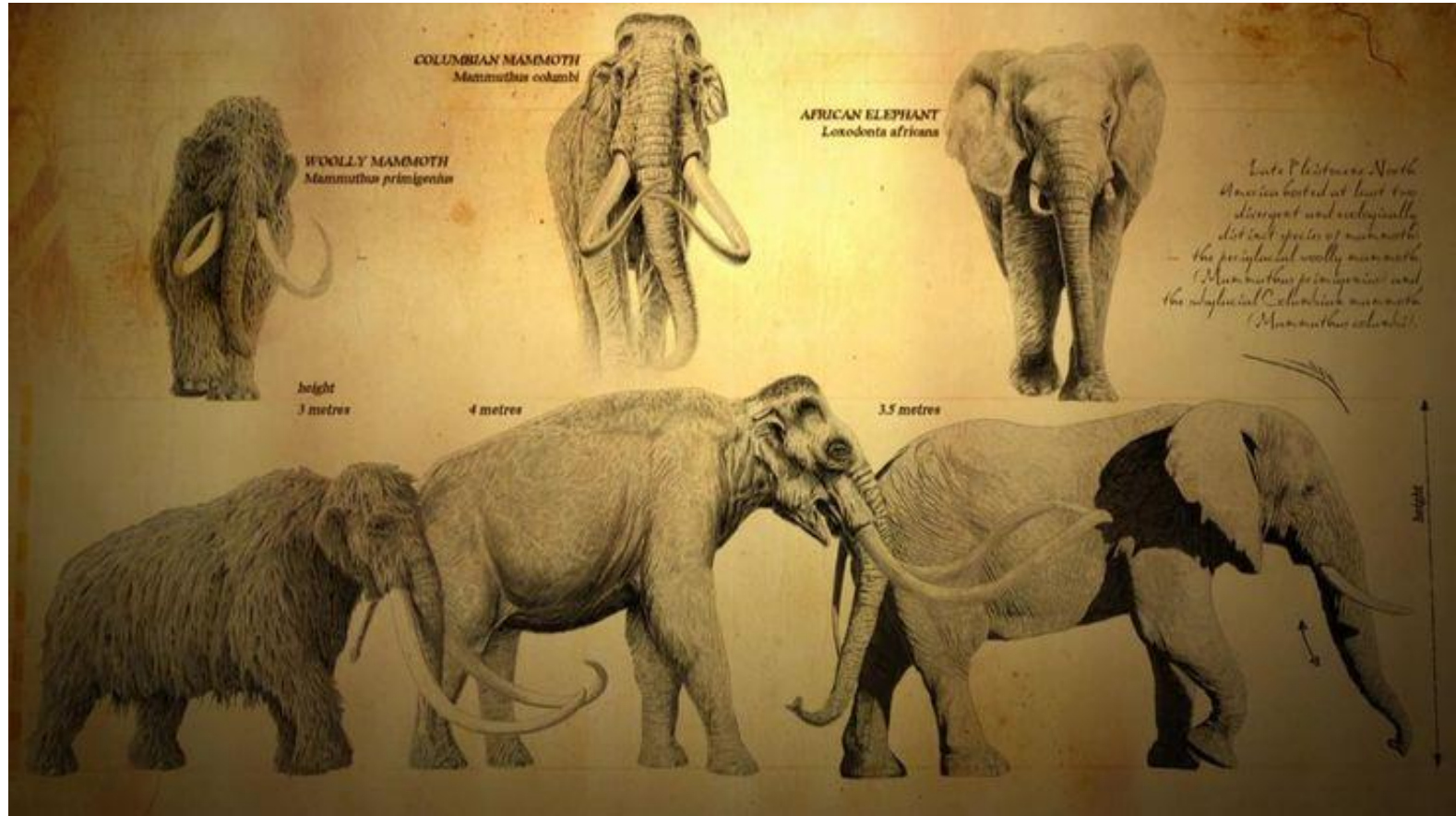
Palaeoloxodon antiquus – an extinct hybrid species



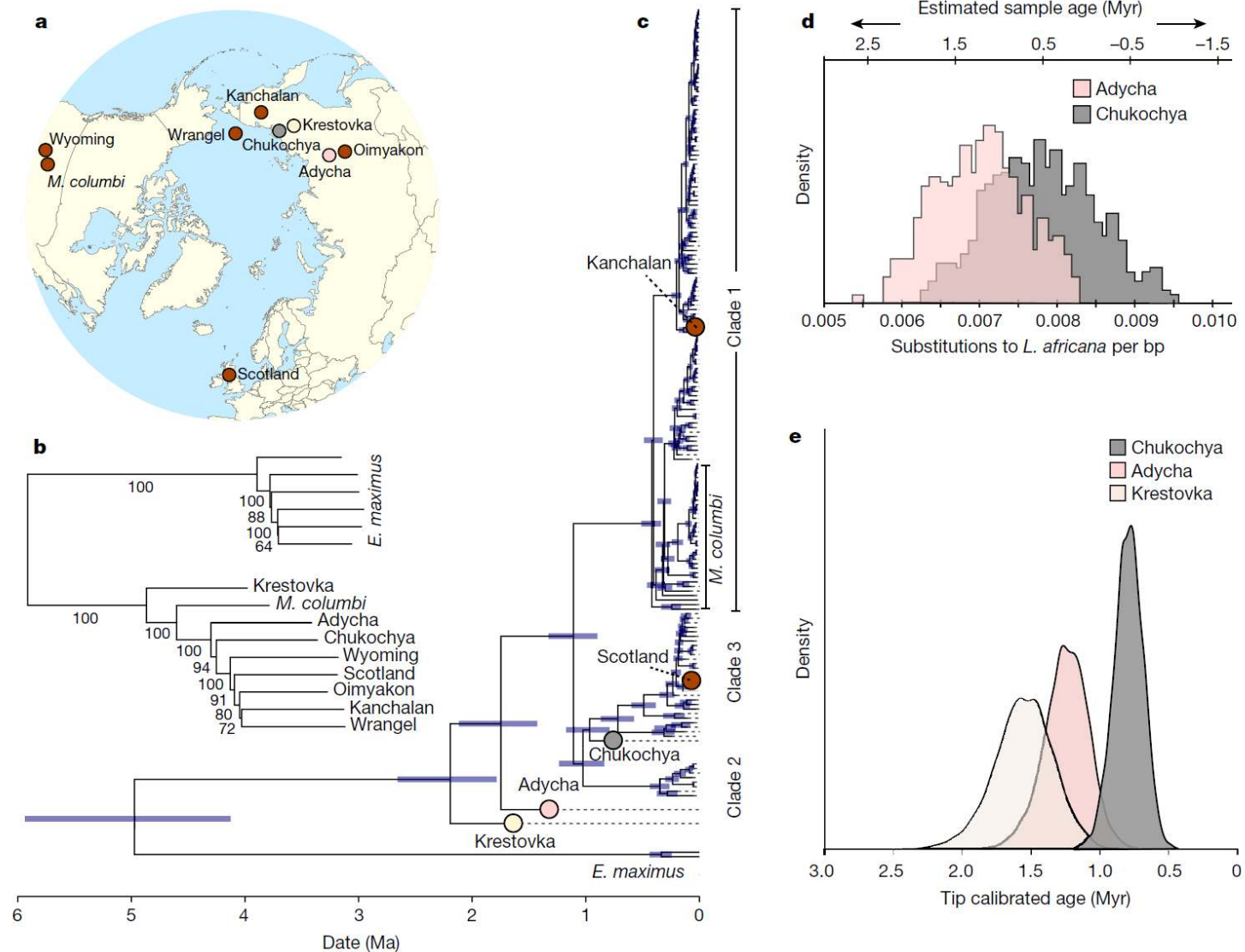
Mammoths



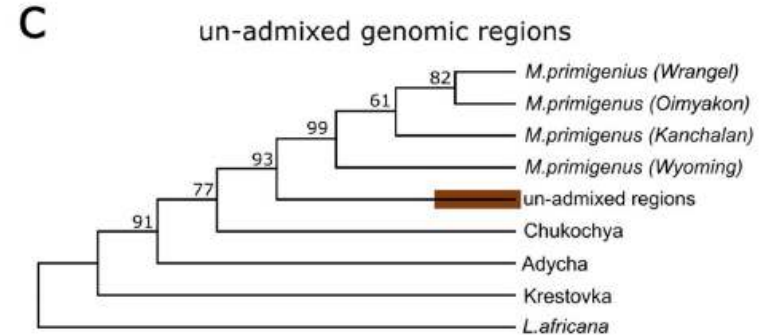
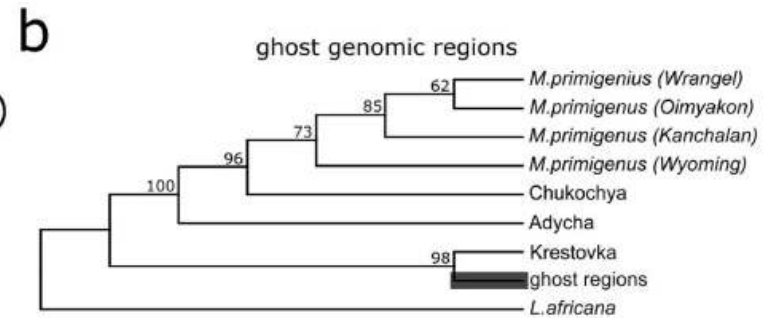
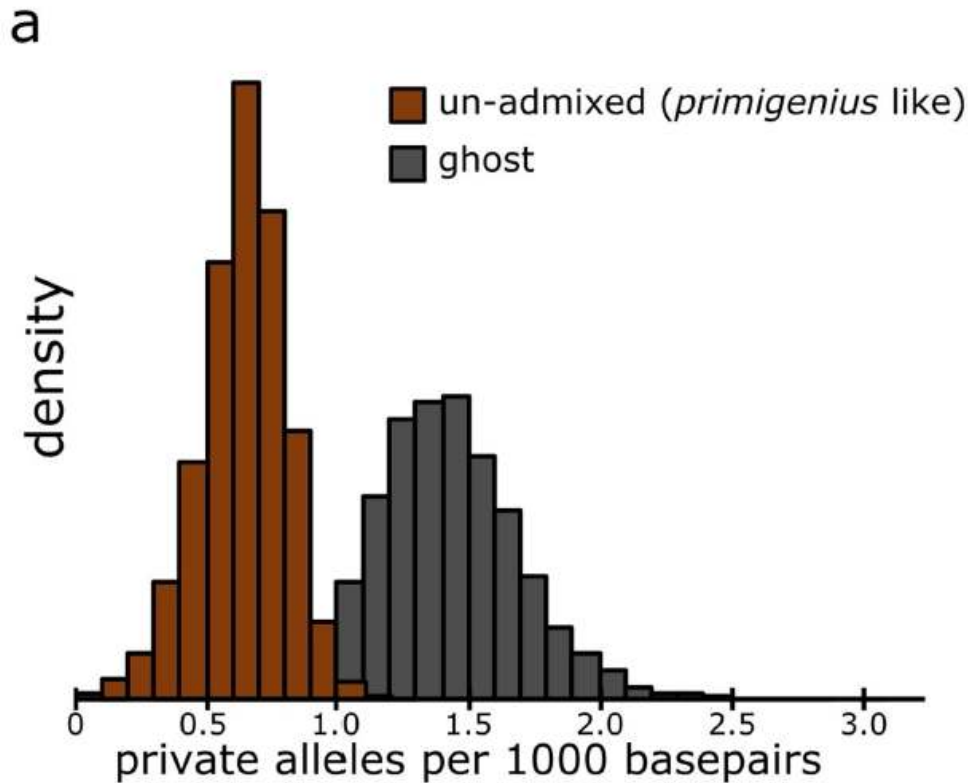
Mammoths



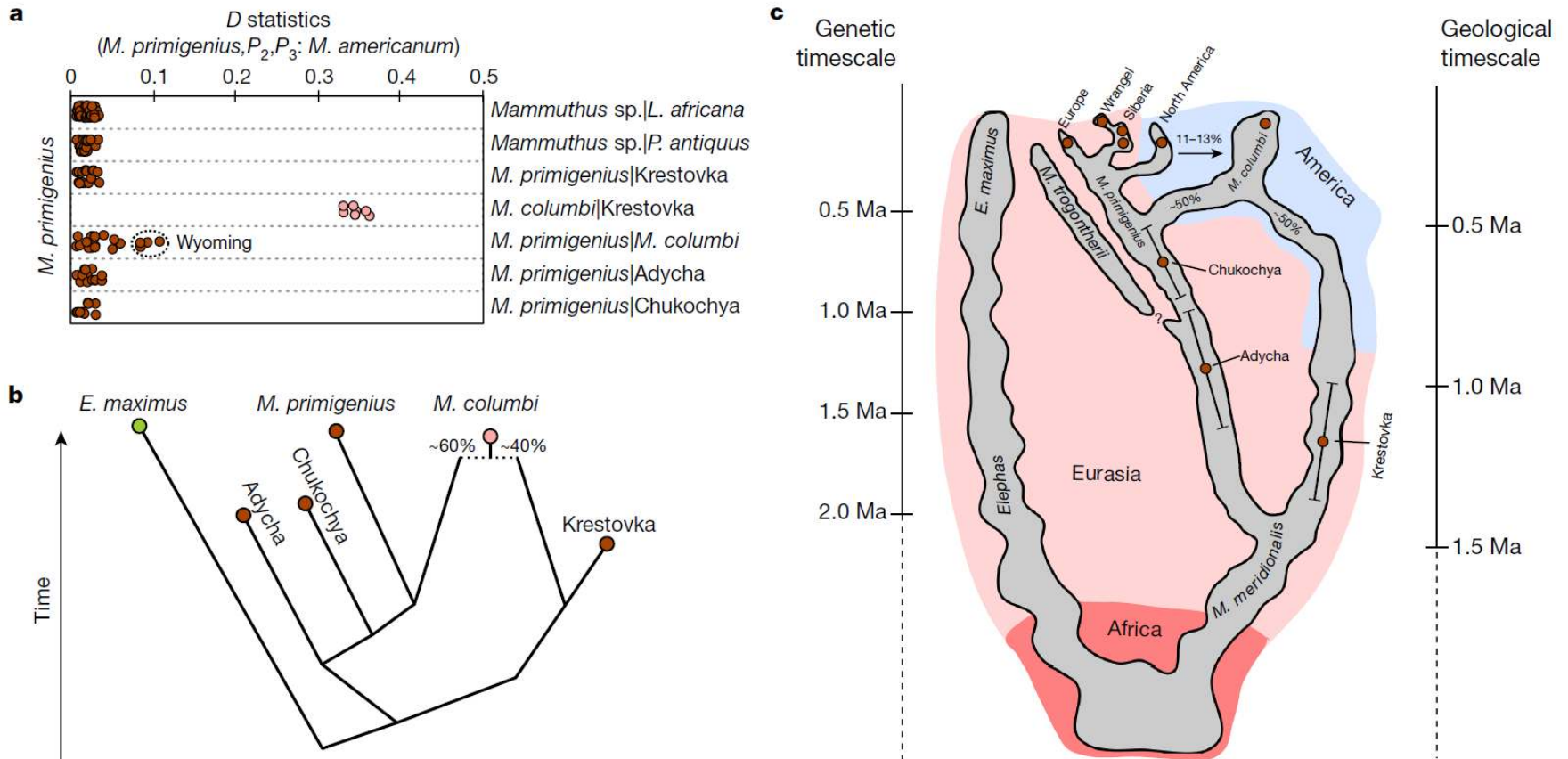
Mammoths – samples, age and phylogeny



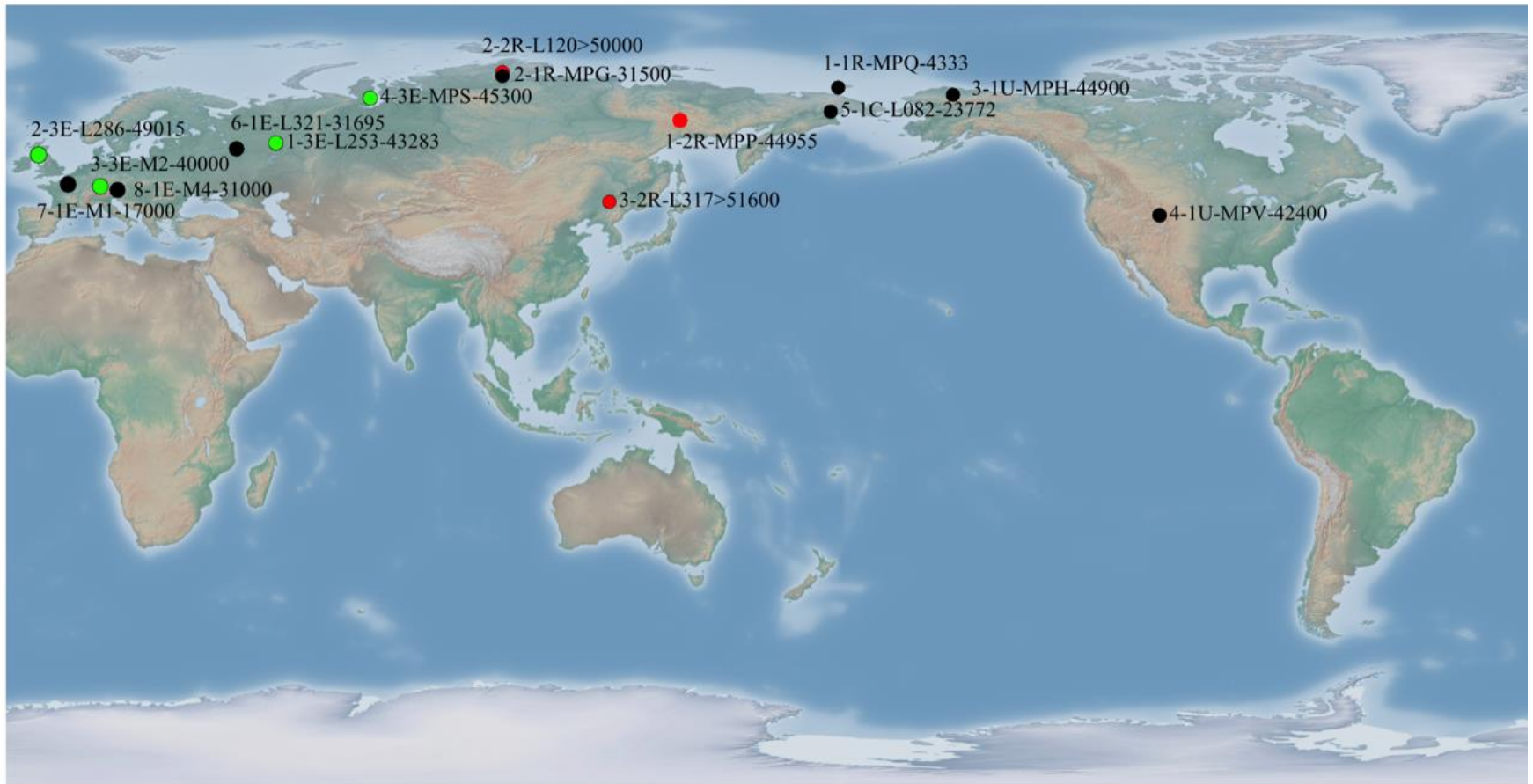
Columbian mammoth – ghost ancestry



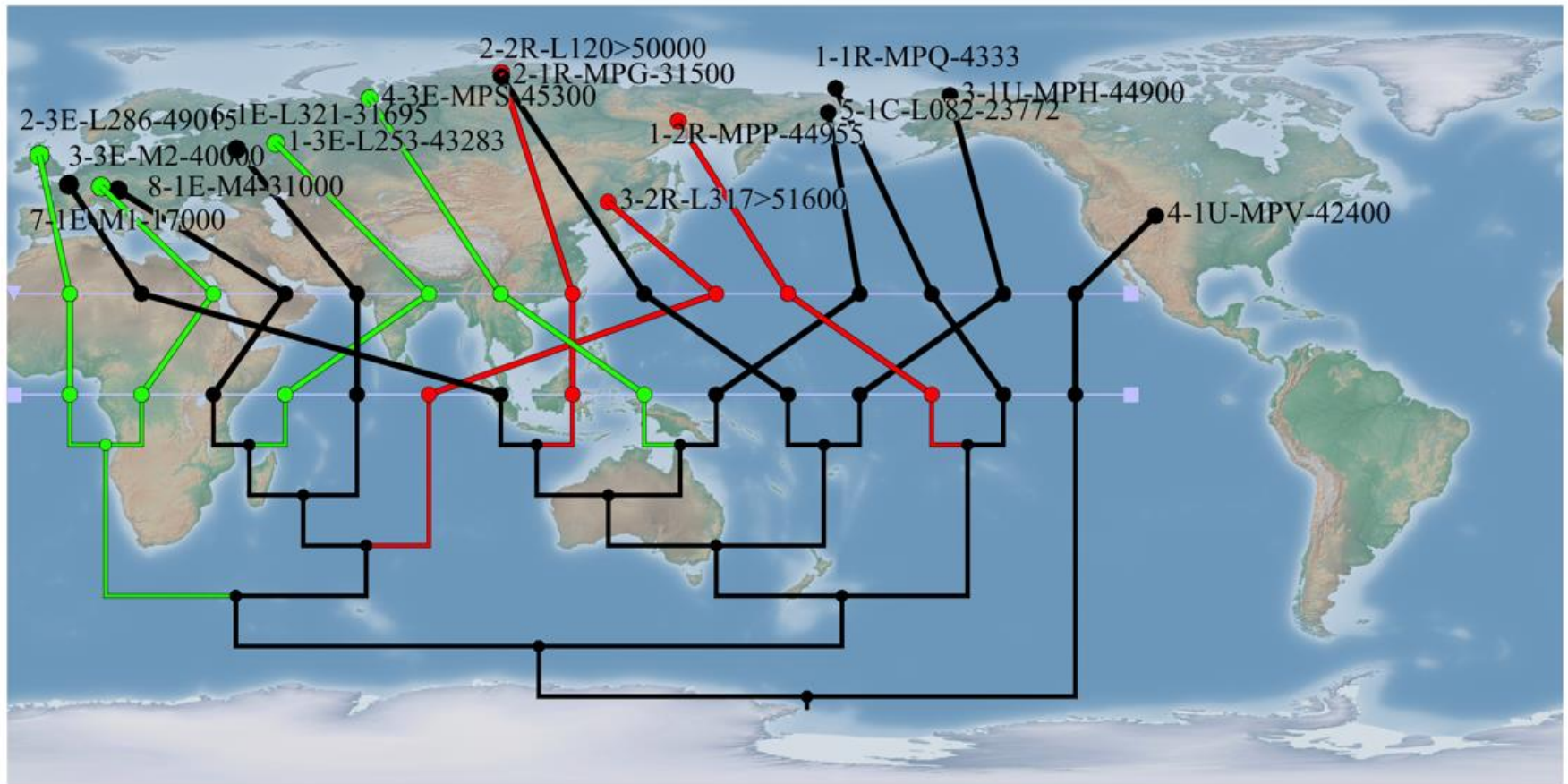
Mammoths – population history



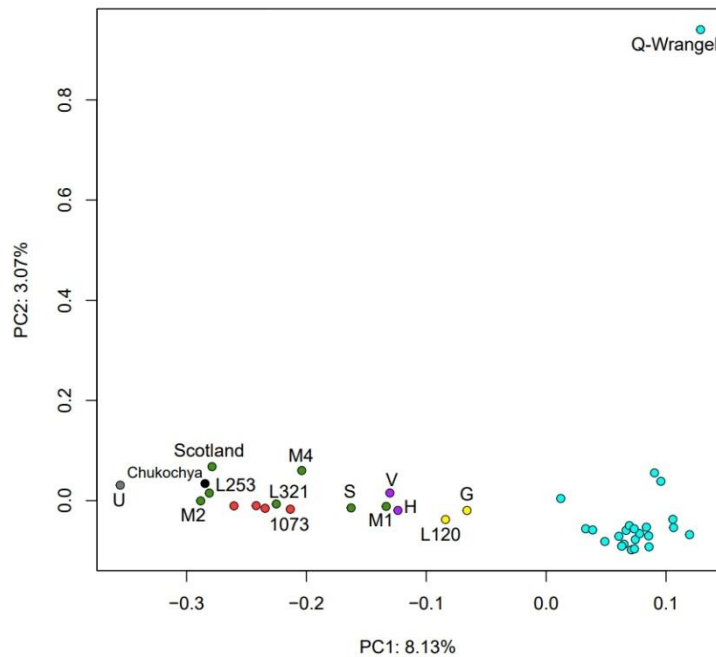
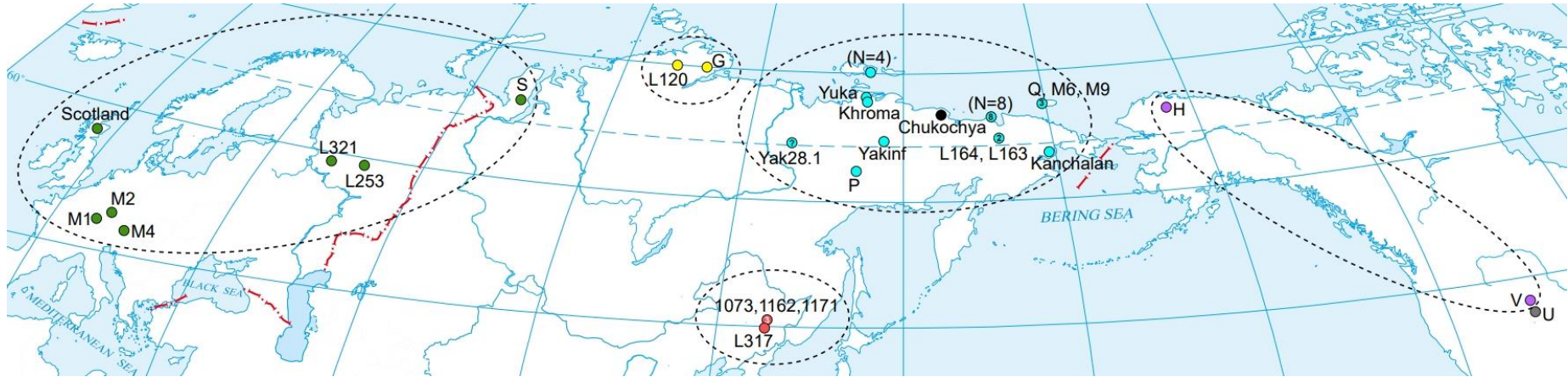
More mammoths



Mitochondrial haplogroups vs. nuclear tree

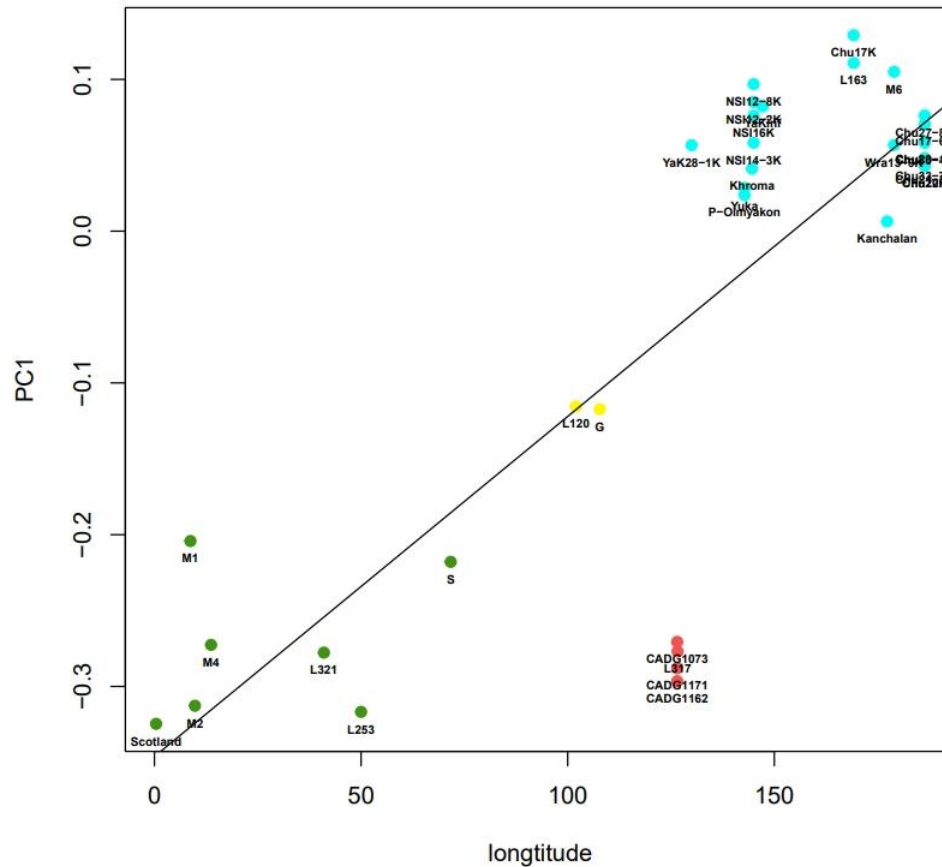


Even more mammoths



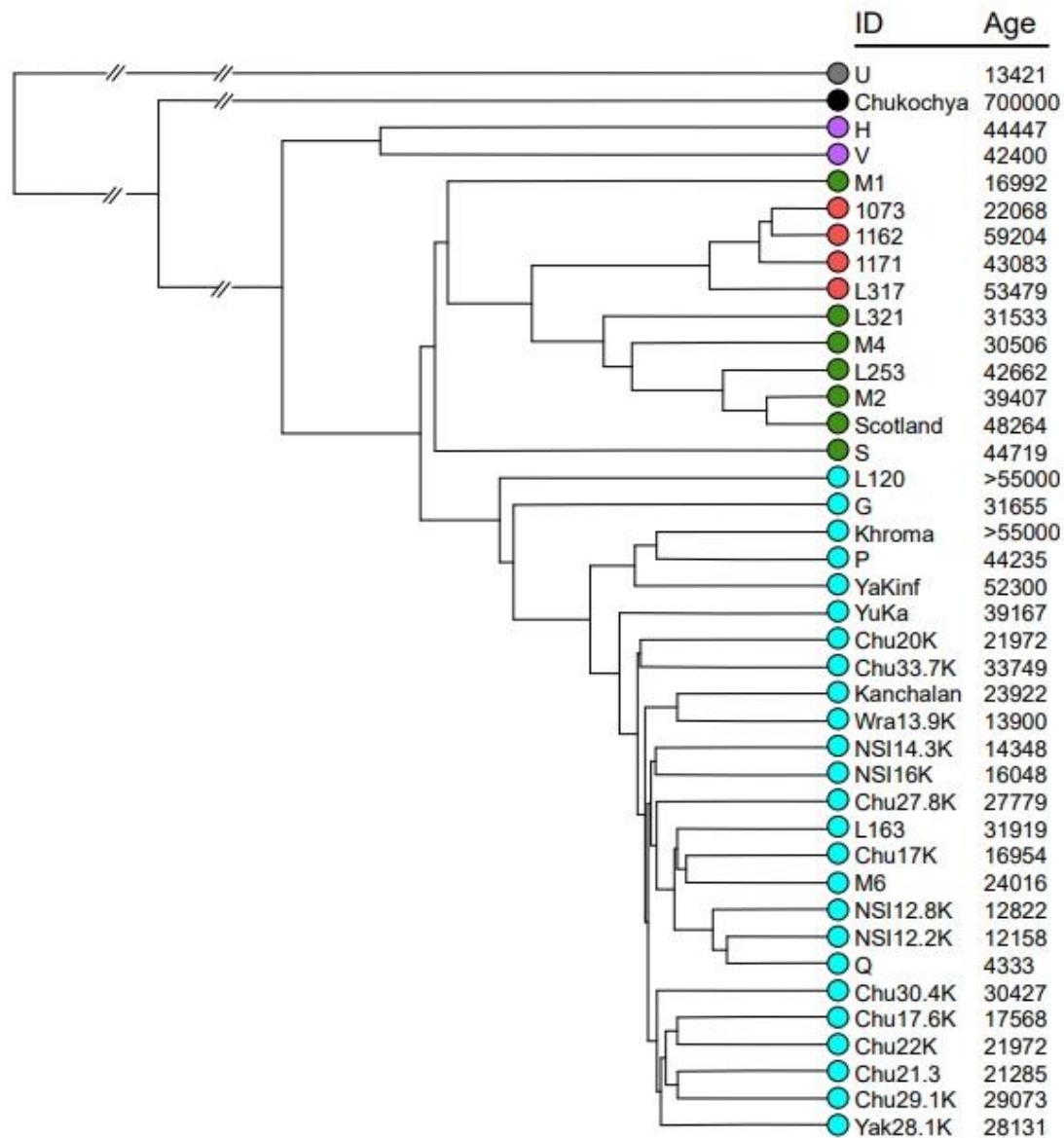
Geographical vs. genetic structure

PC1 vs longitude

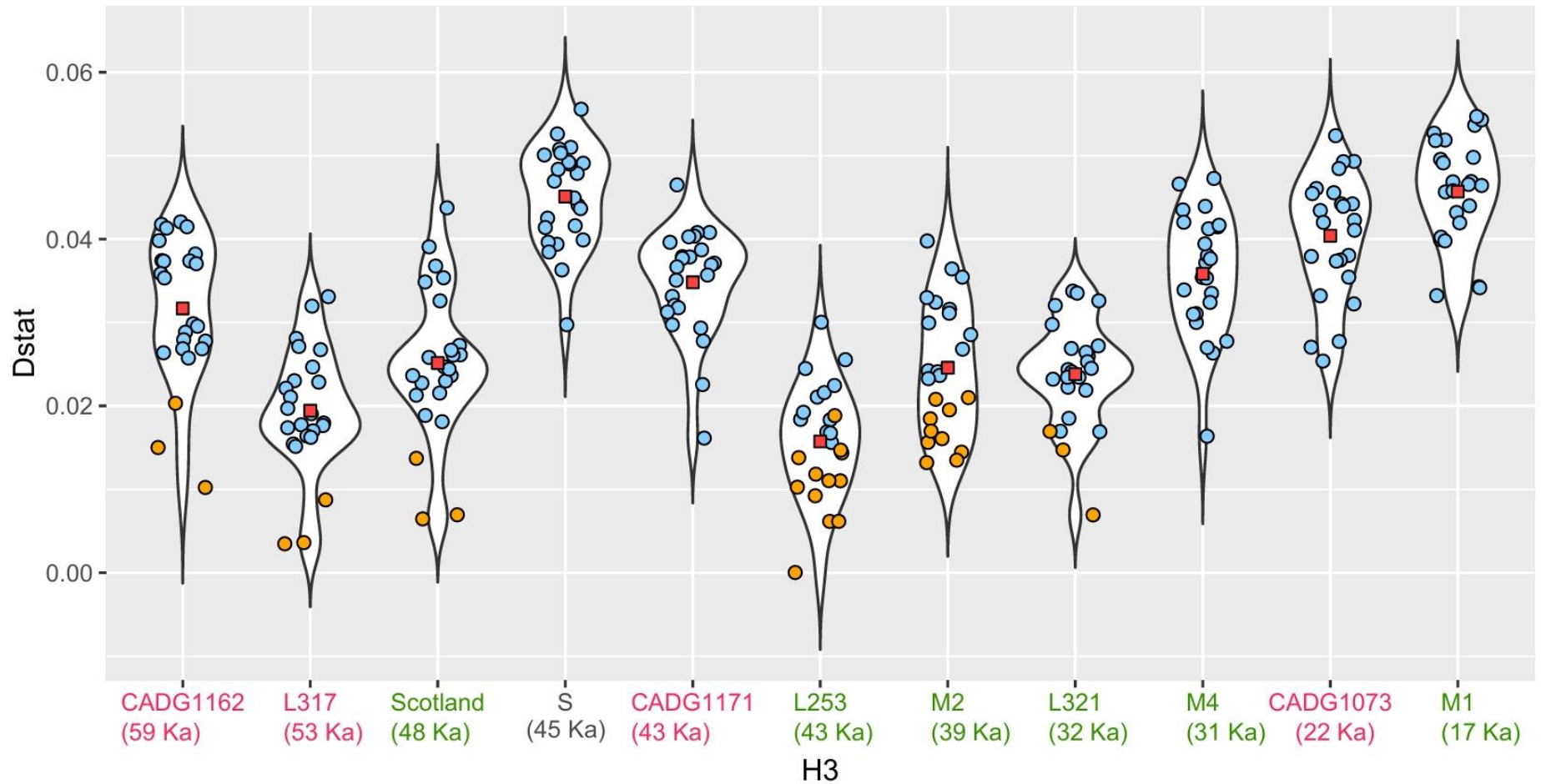


P value: 5.65e-09 Adjusted R-squared: 0.637

NJ-tree using all samples



Siberian admixture over time and space



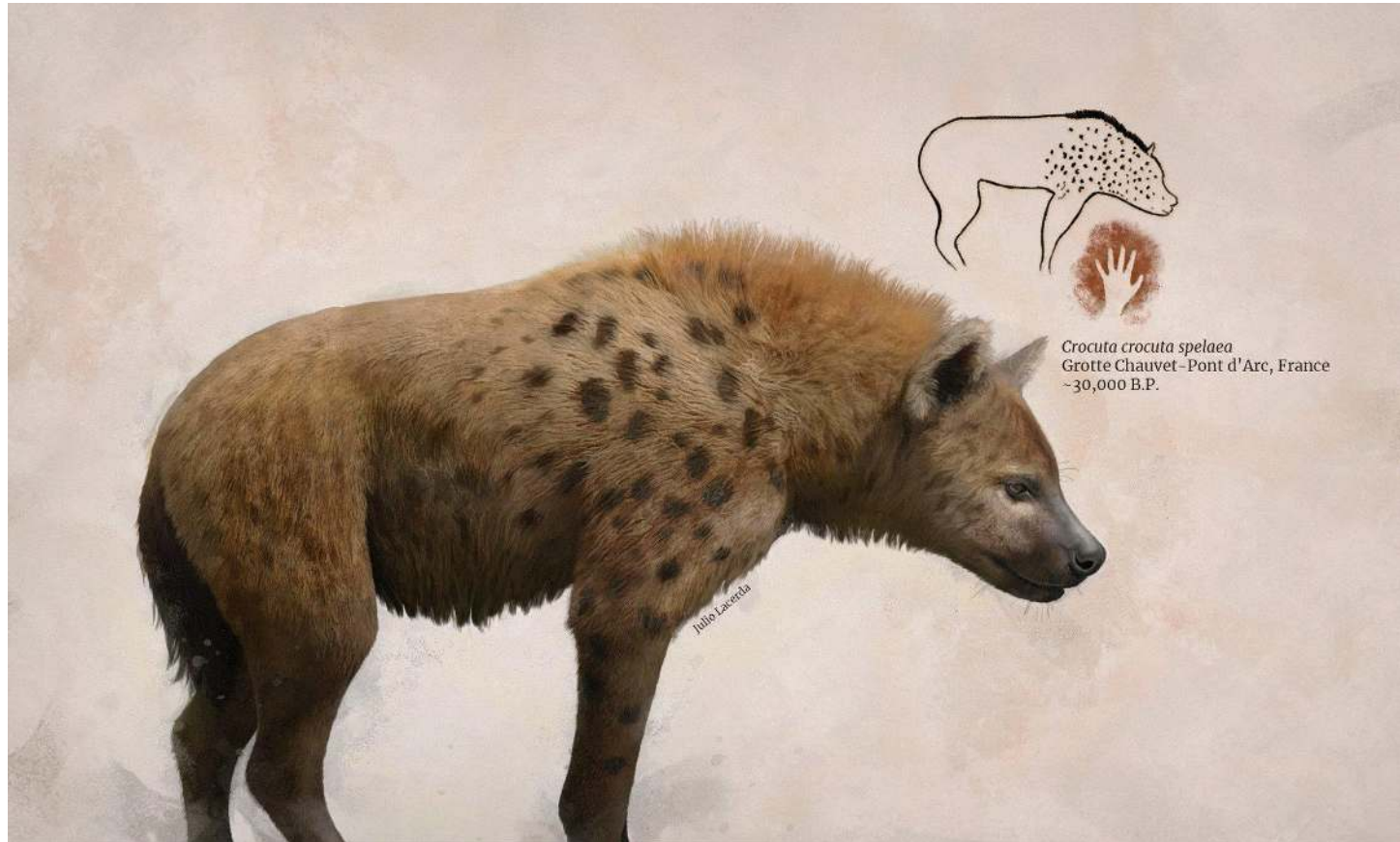
Summary elephants

- Gene flow within and between species is an important factor in elephant evolution
- The European straight-tusked elephant was a three-way hybrid
- Chinese and European mammoth are more closely related than either to Siberian ones
- Siberian influence increases over time

Cave hyenas



„Real“ cave hyena



As seen by stone age artists



Crocota - distribution

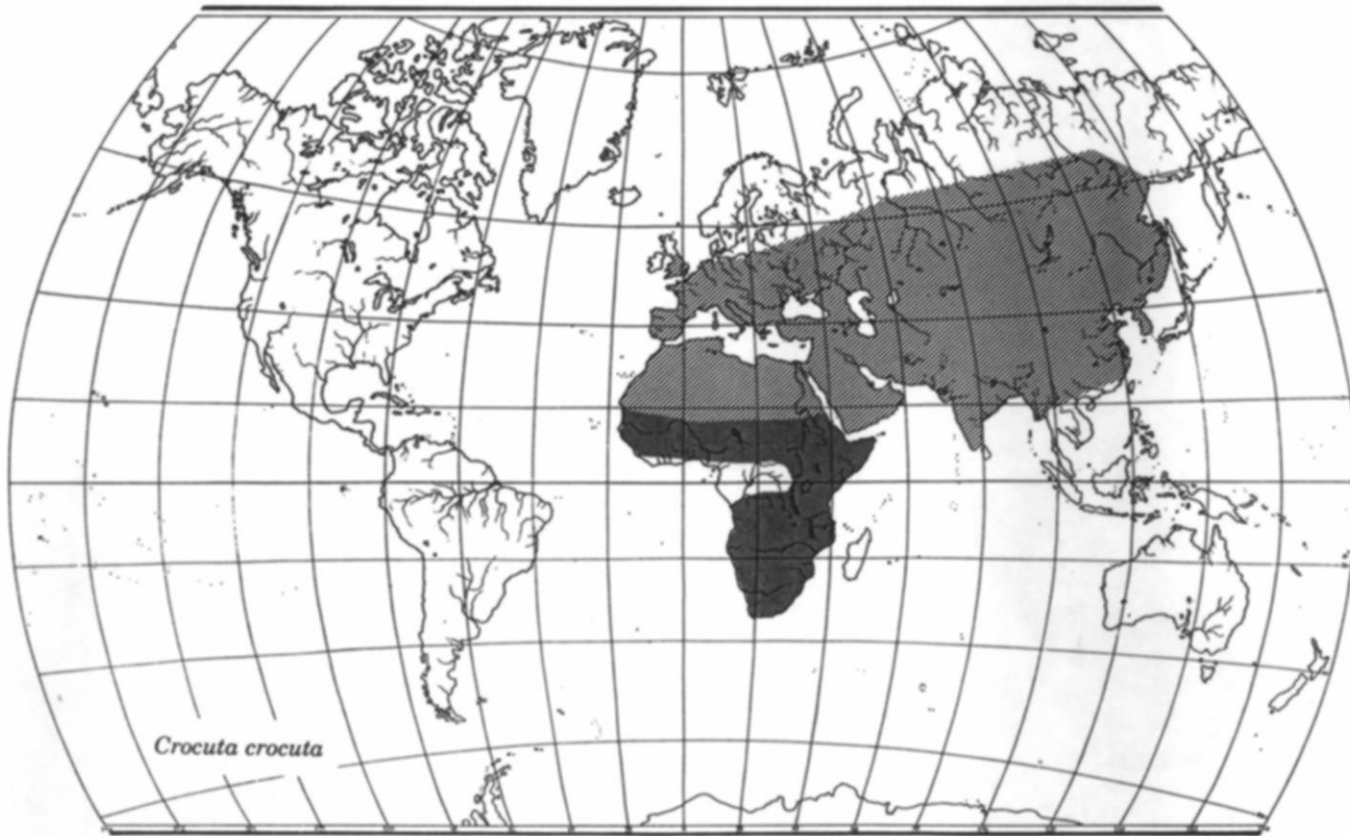
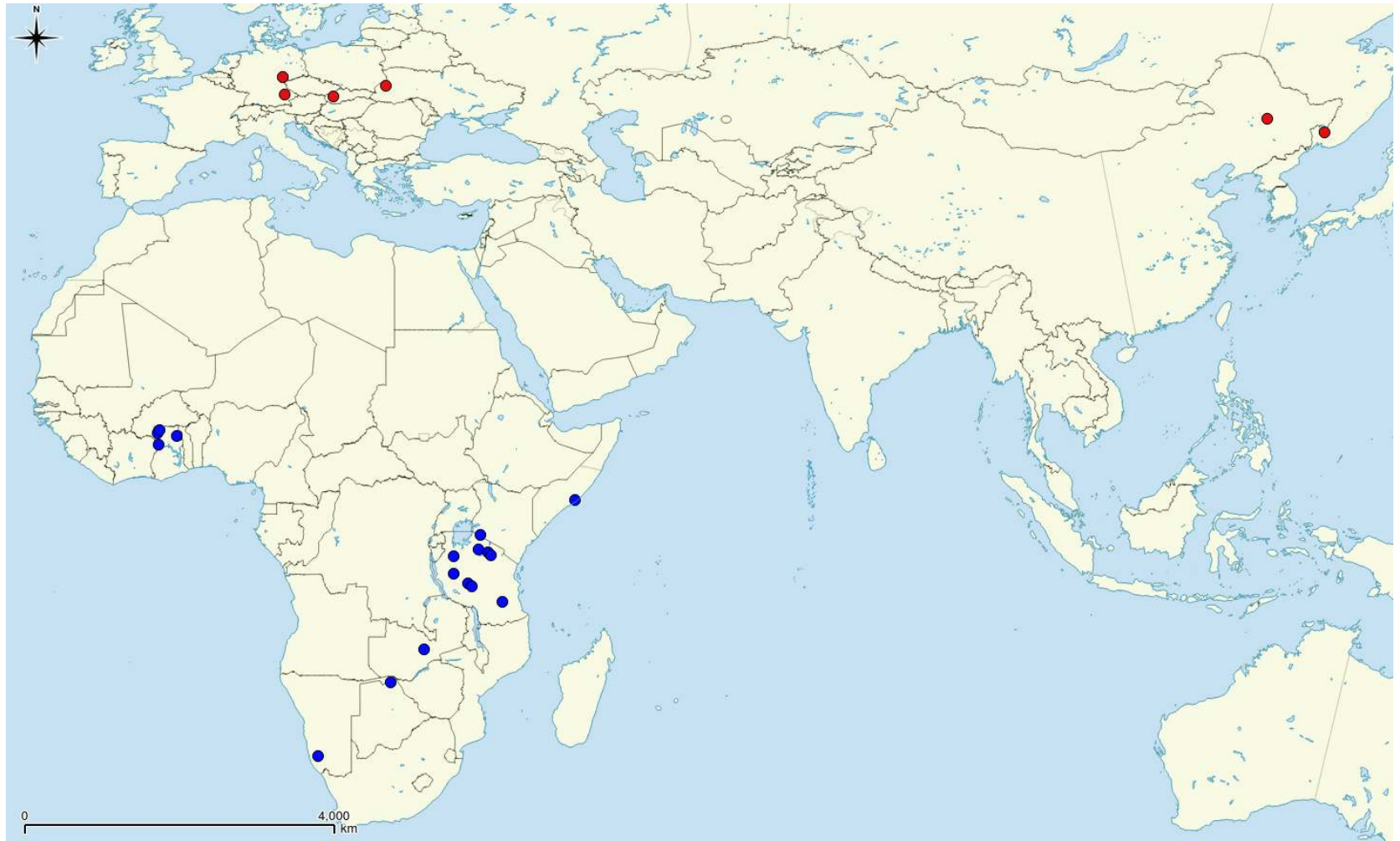
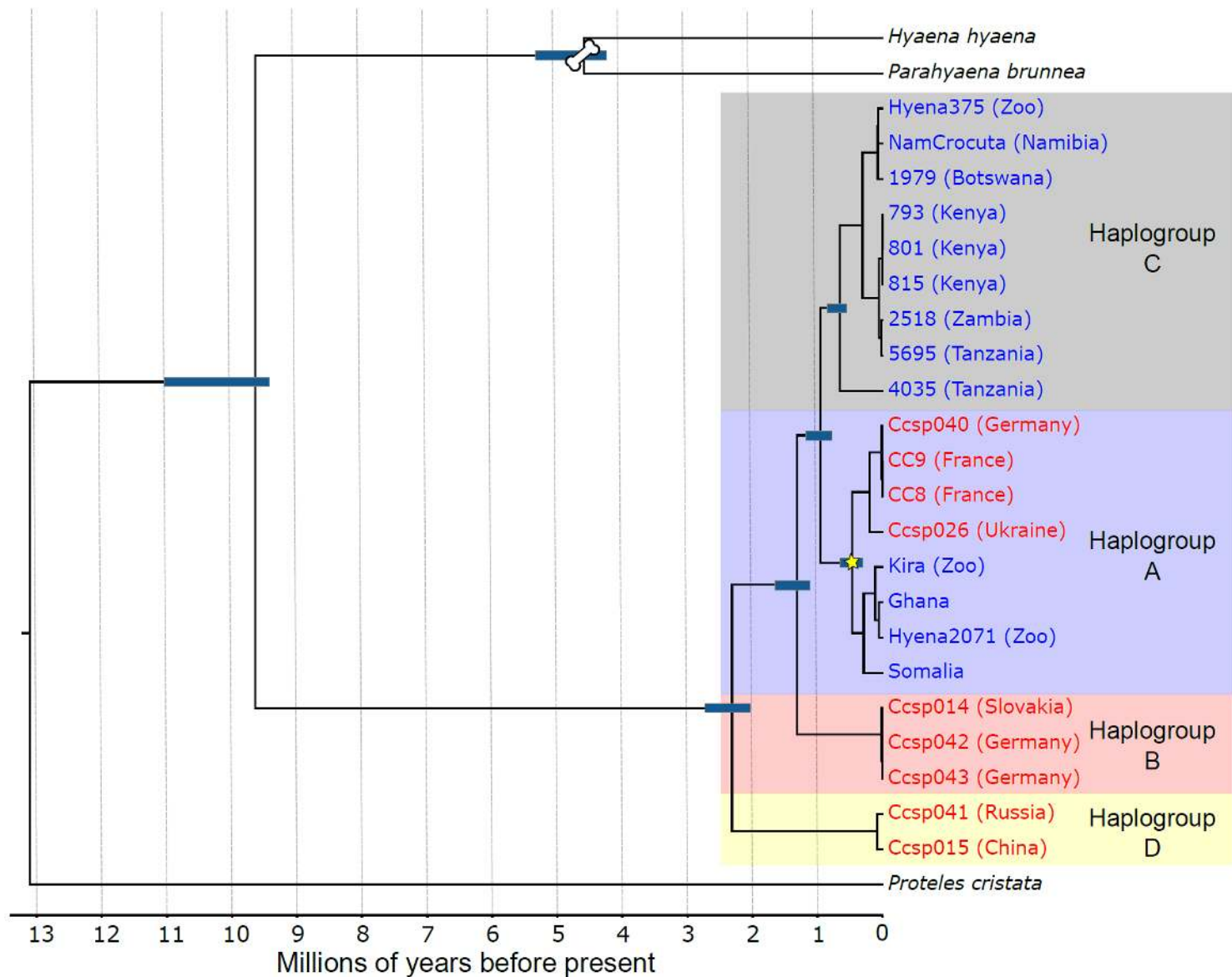


Fig. 8. Map showing (stippled) current range of *C. crocuta* and (shaded) maximum range in the Pleistocene.

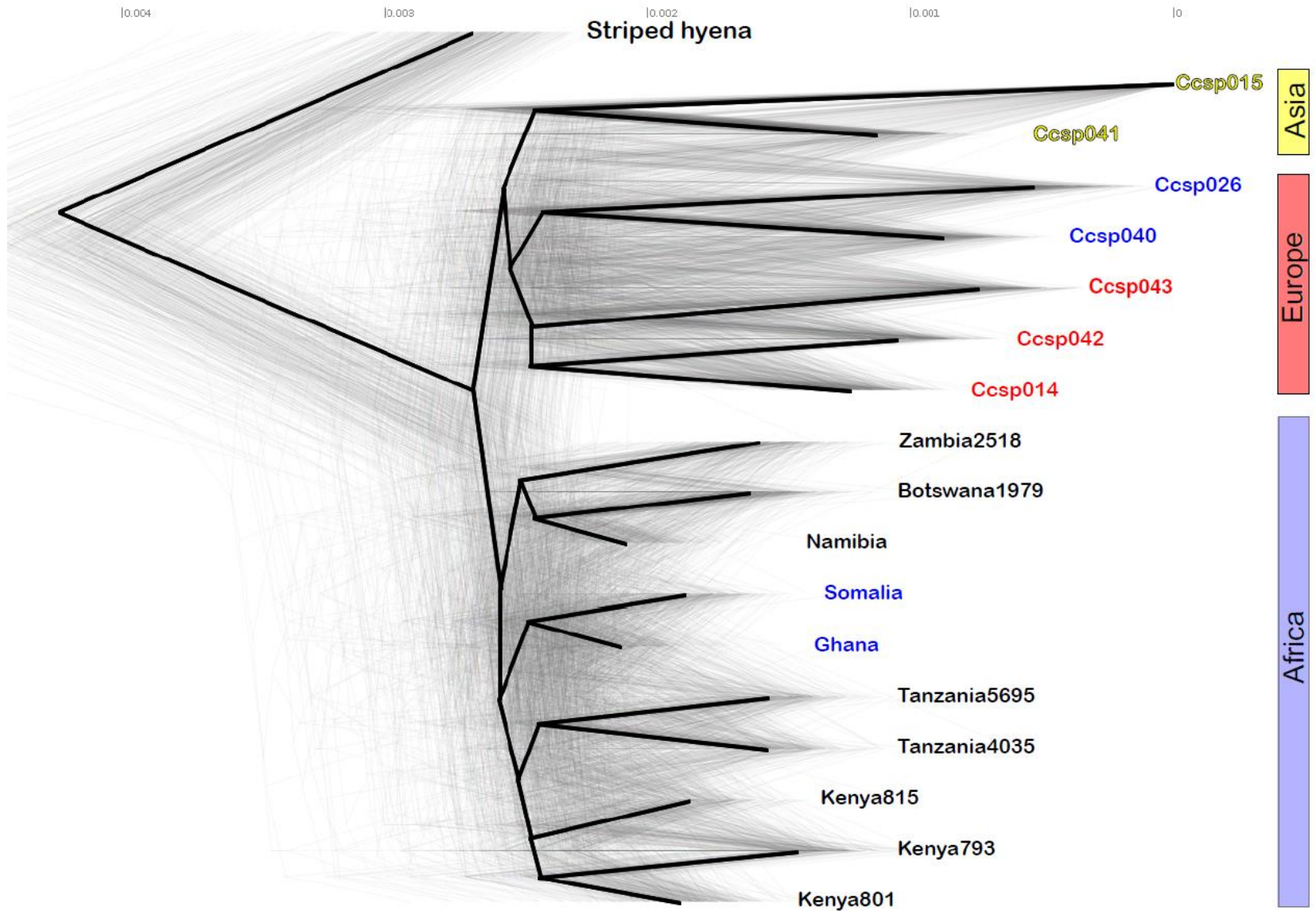
Sampling



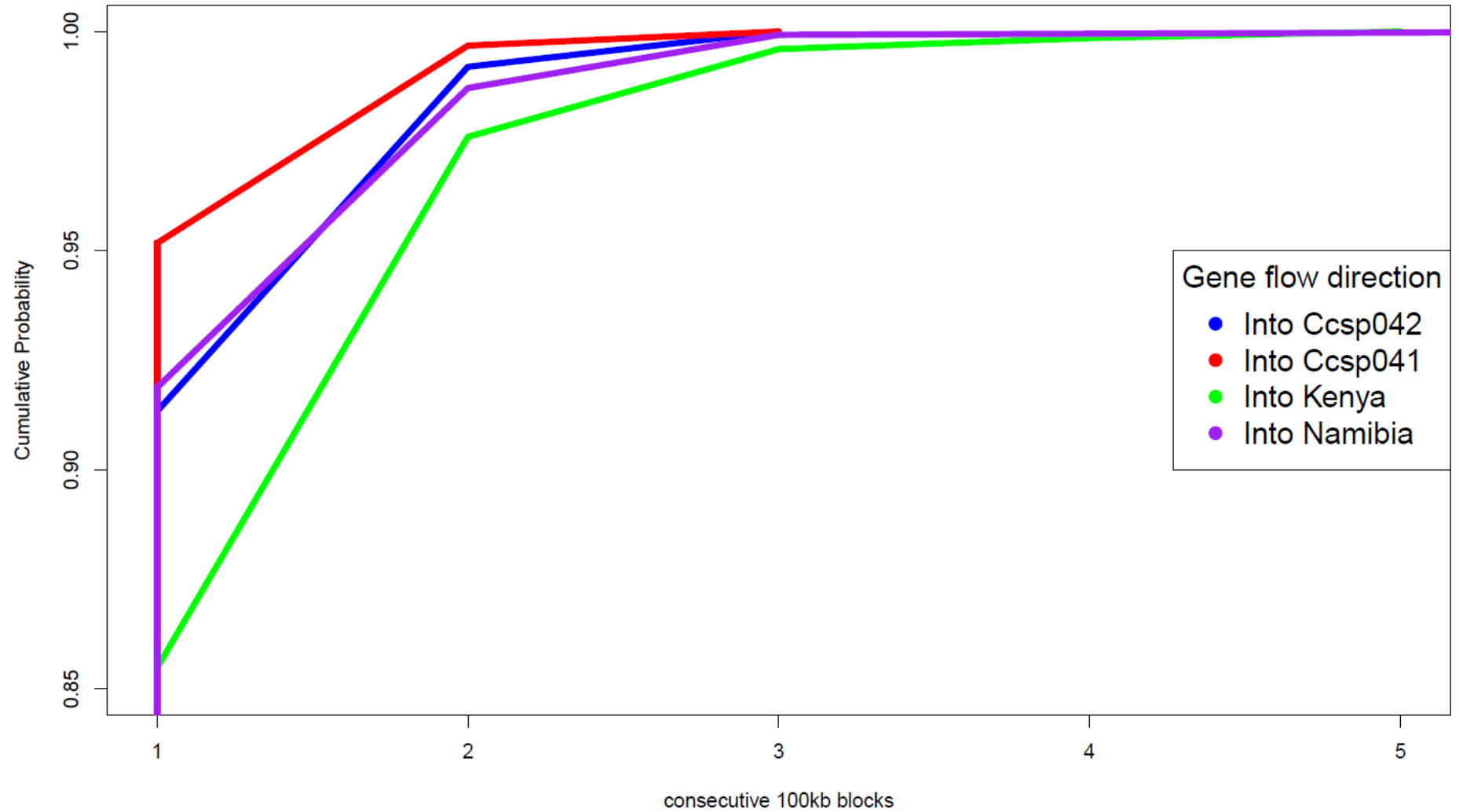
Mitogenome tree



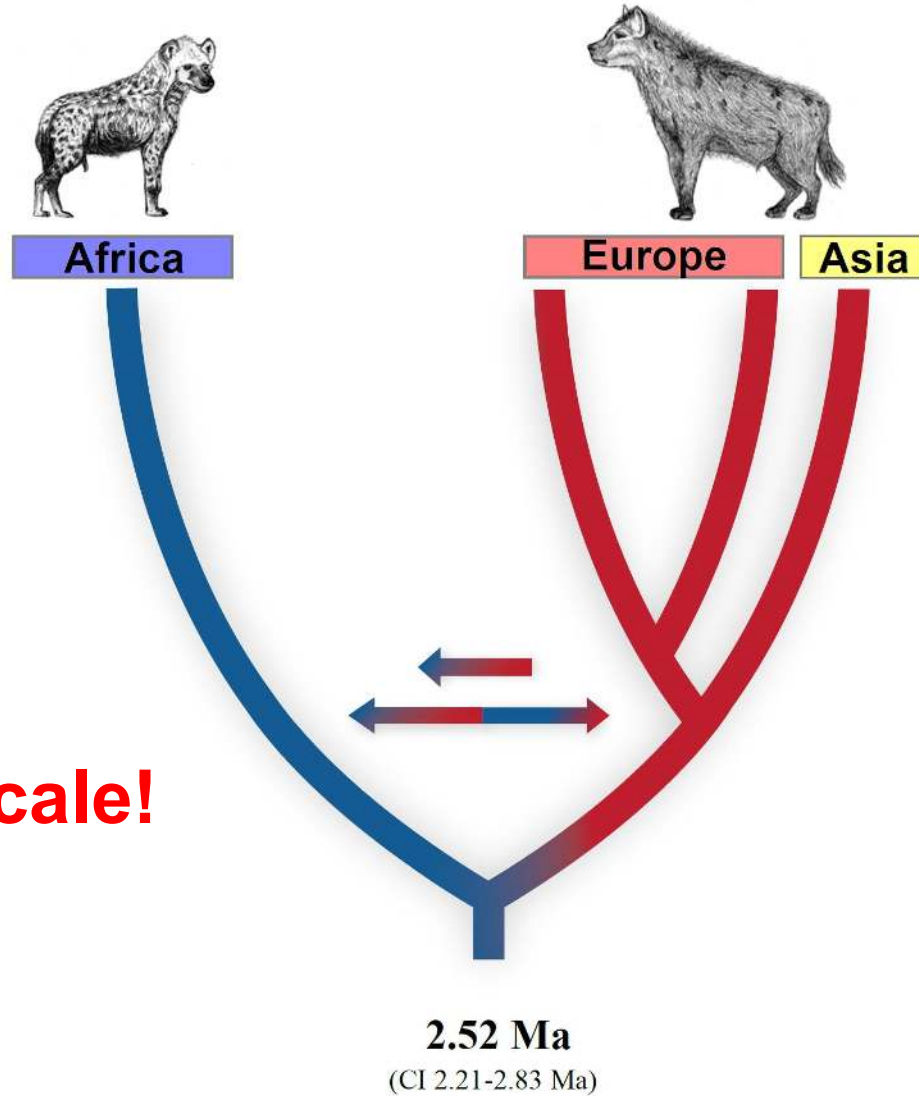
Nuclear tree



Crocota gene flow



Hyena dated tree and admixture 2 my after divergence



Tree not to scale!

Spotted hyenas are different



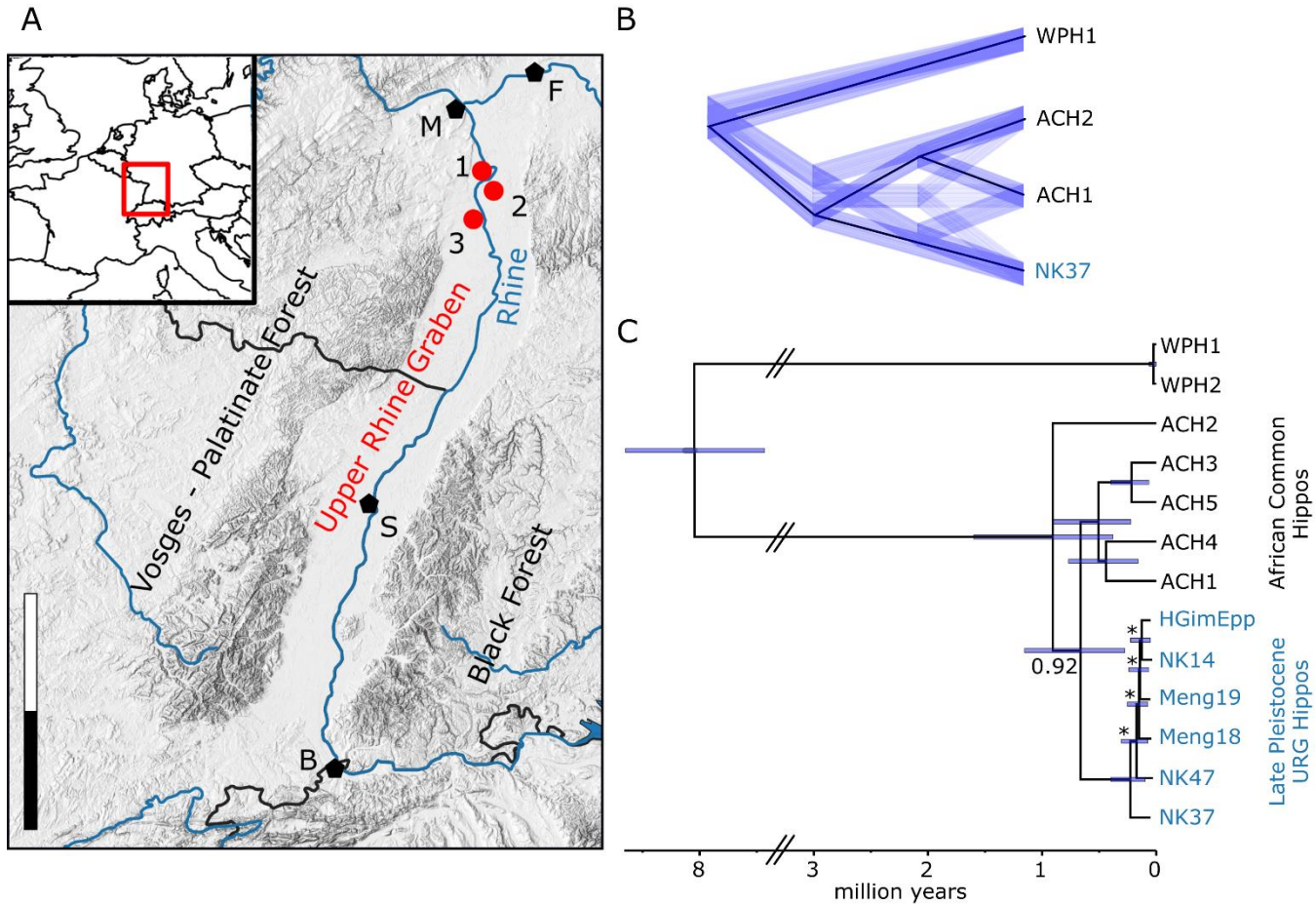
Conclusions

- Spotted and cave hyenas diverged a long time (2.5 my) ago
- There is a discordance between mitochondrial and nuclear tree similar to humans, Neanderthals and Denisovans
- We find evidence for multiple gene flow events between Africa and Eurasia, until almost 2 my after the initial divergence

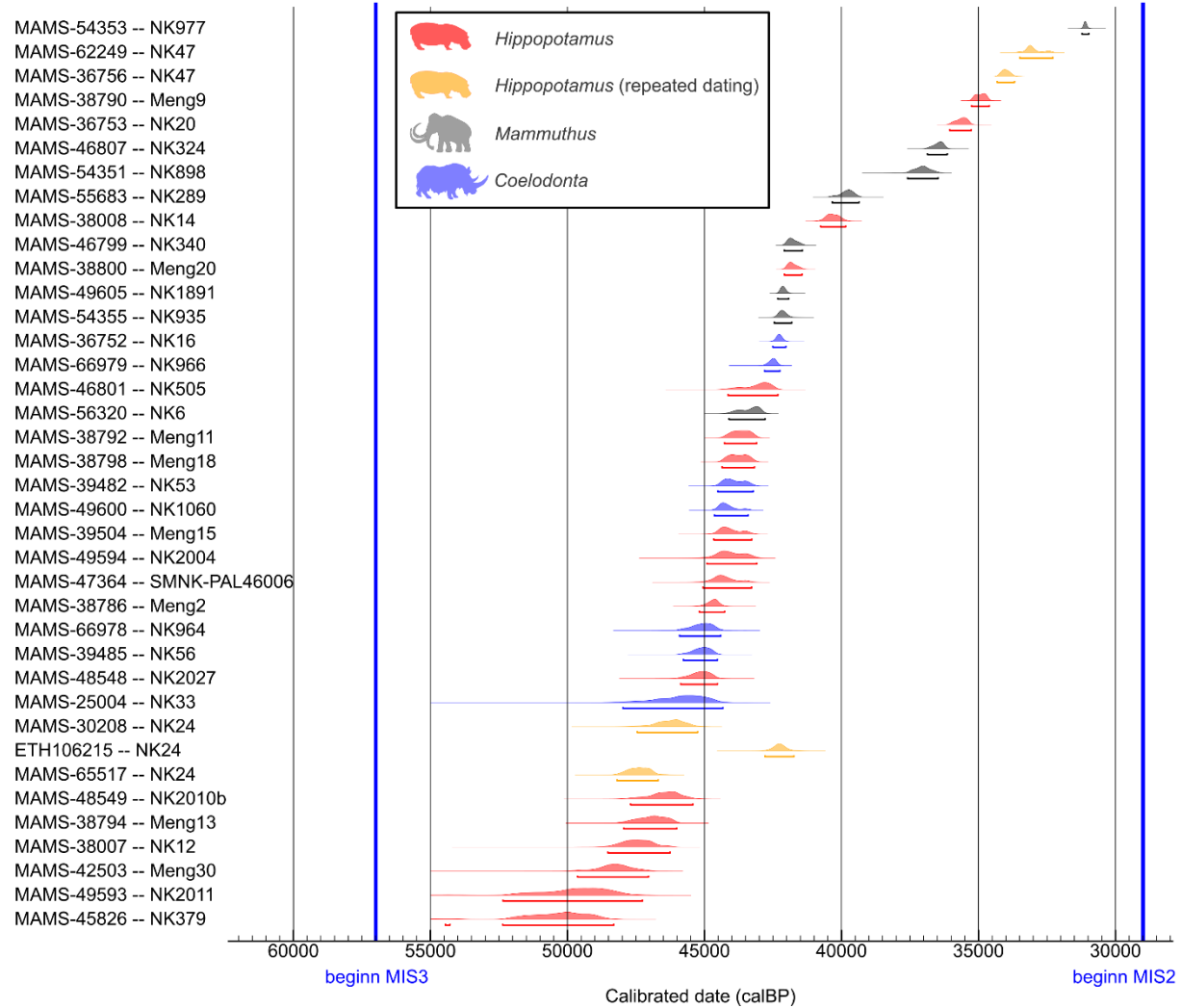
Pleistocene hippos



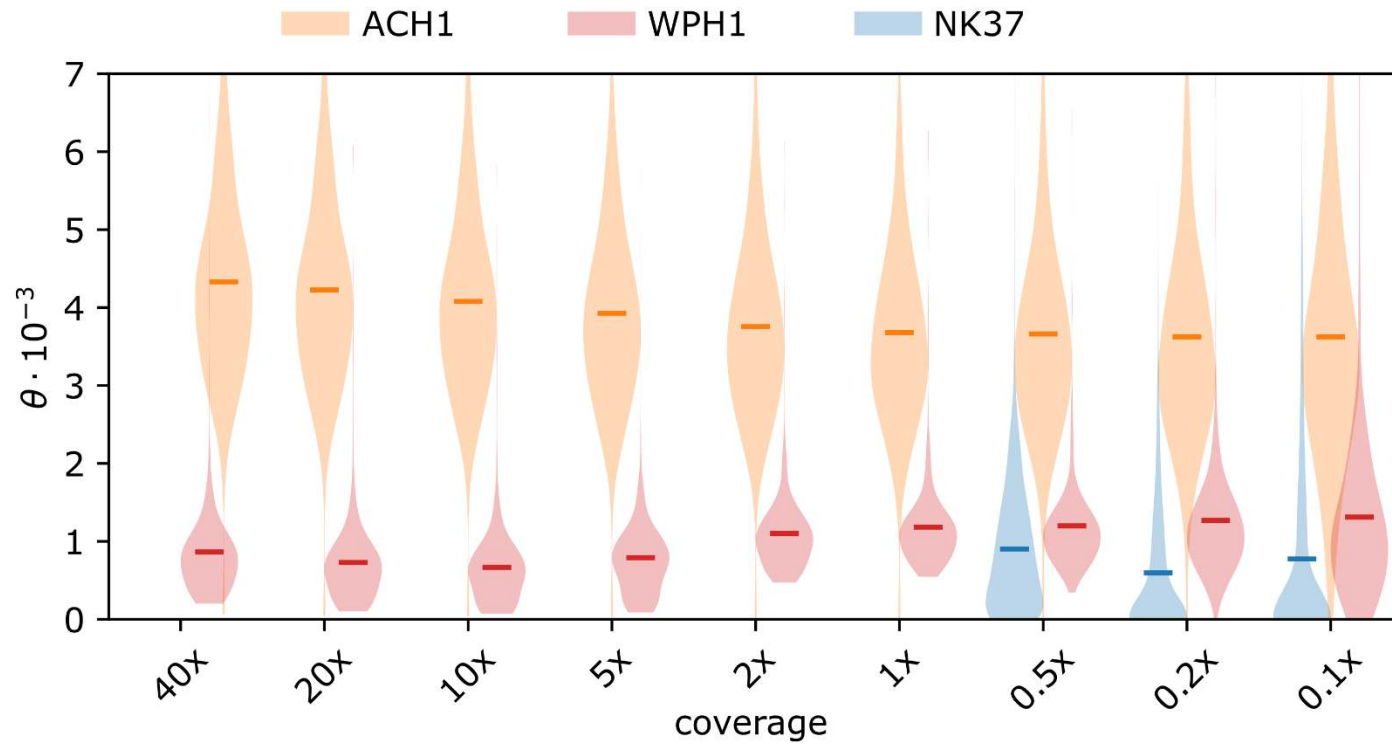
Sampling and phylogenetic trees



Sample ages



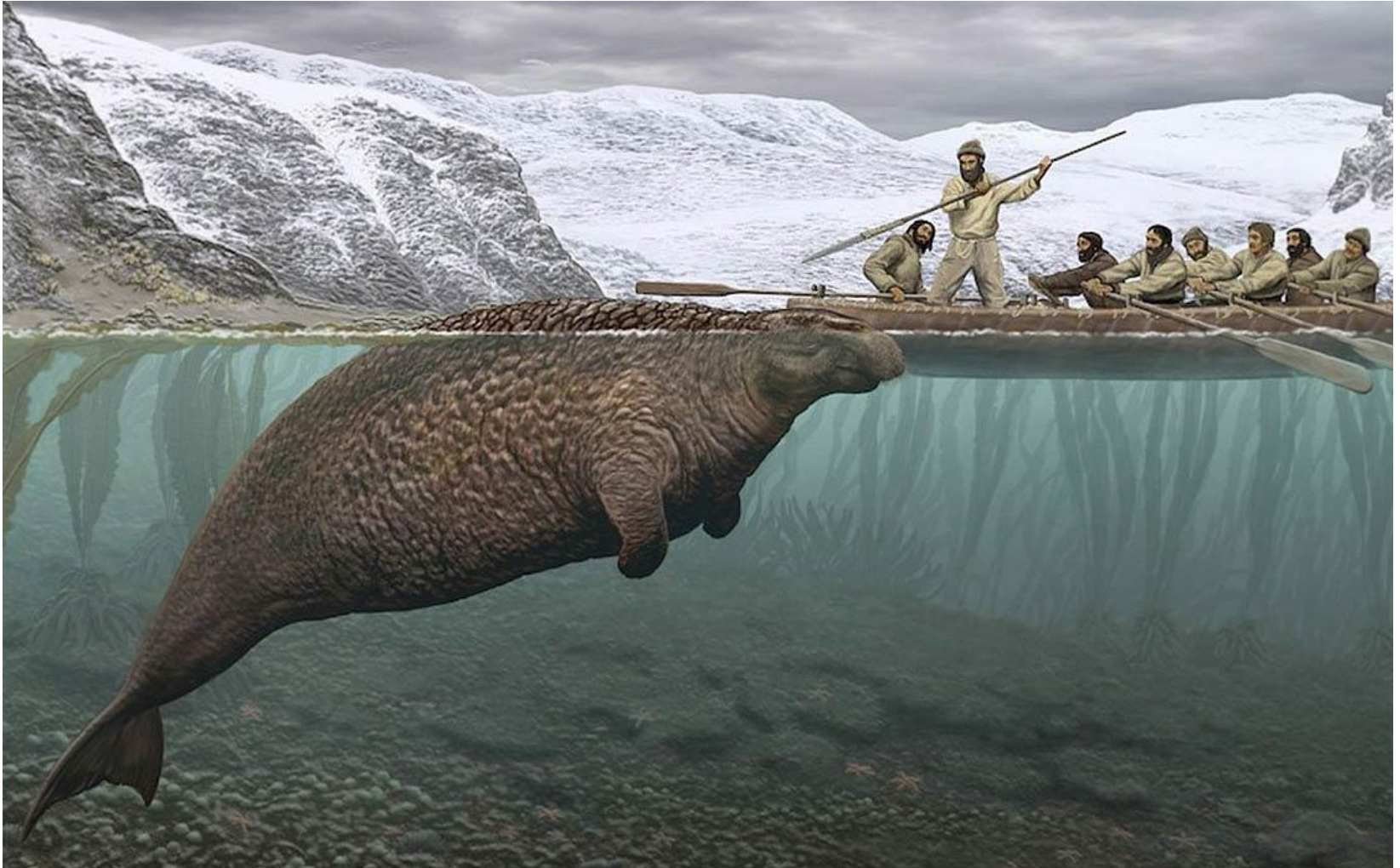
Low genetic diversity



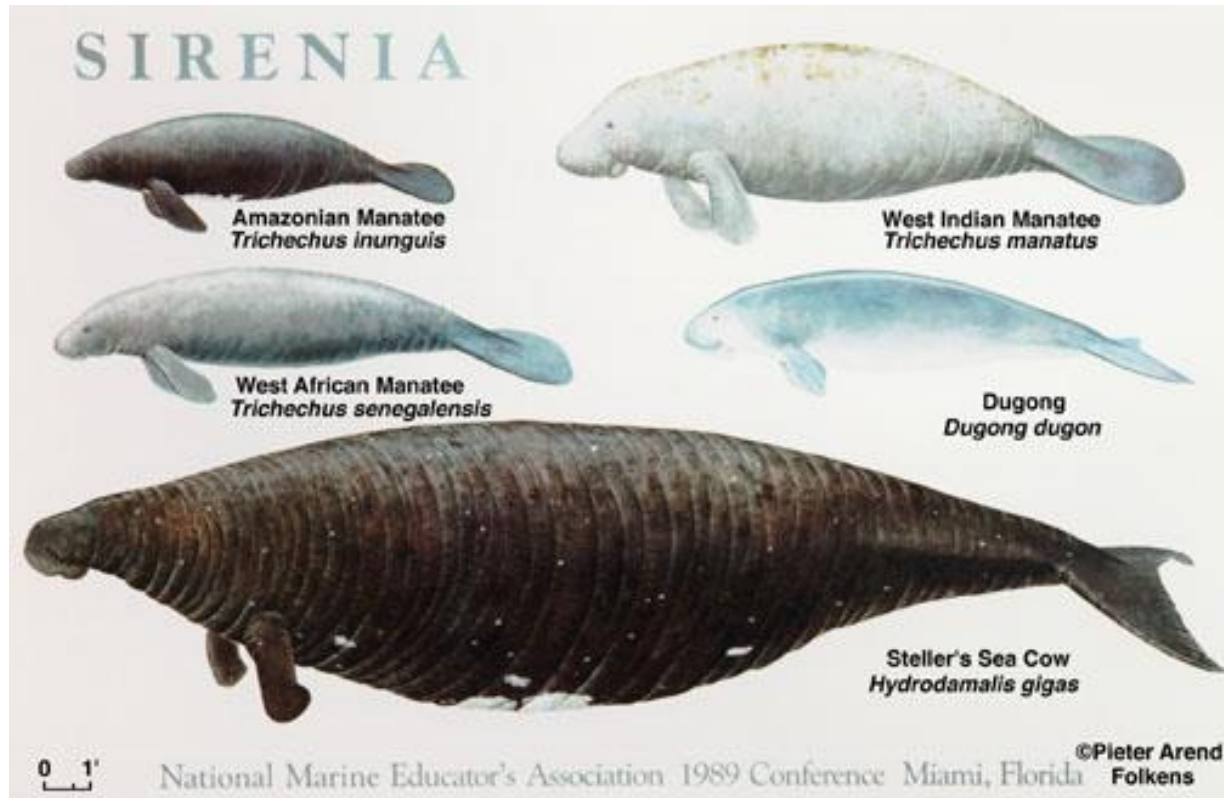
Summary hippos

- Late Pleistocene European hippos were closely related to extant African ones
- The Rhine valley hippos are not Eemian in age
- Pleistocene ecosystems were not analogous to modern ones
- The Rhine valley hippos were a relict population

Steller's sea cow: † 1768

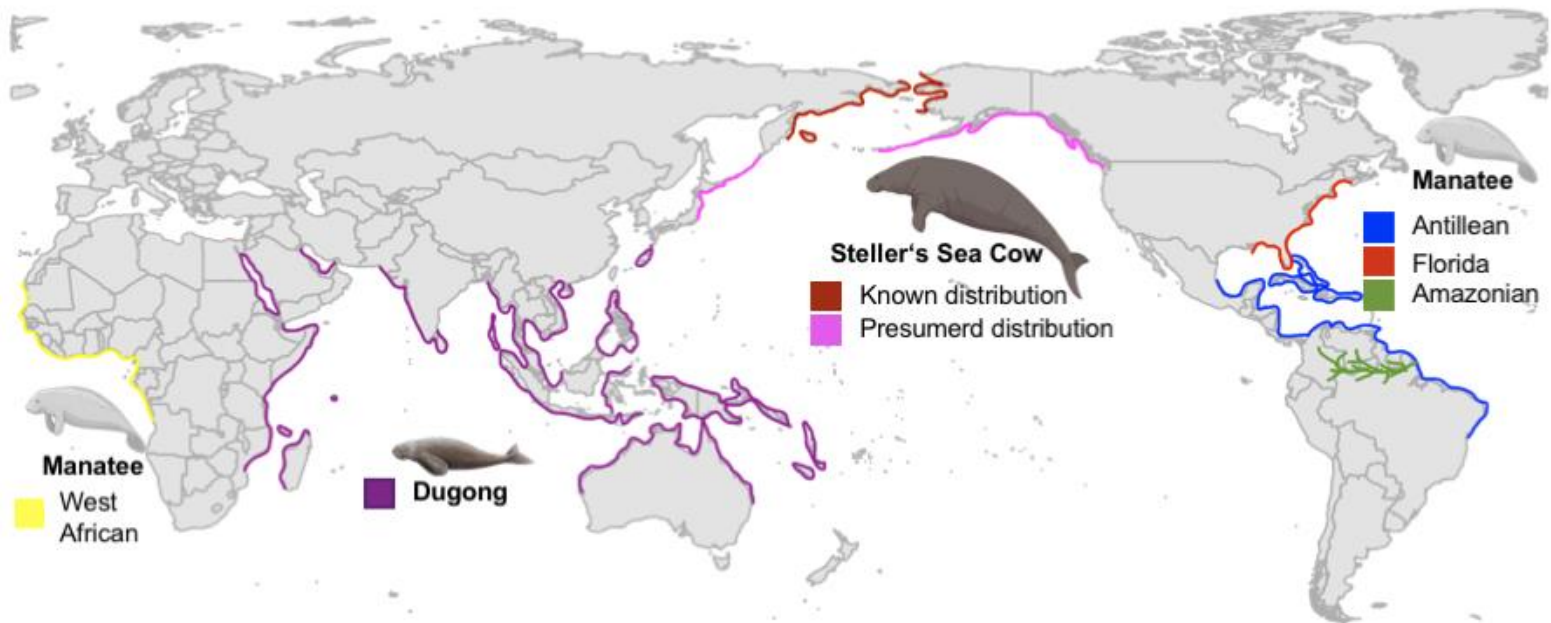


Size comparison



Distribution

A



Steller's sea cow skin

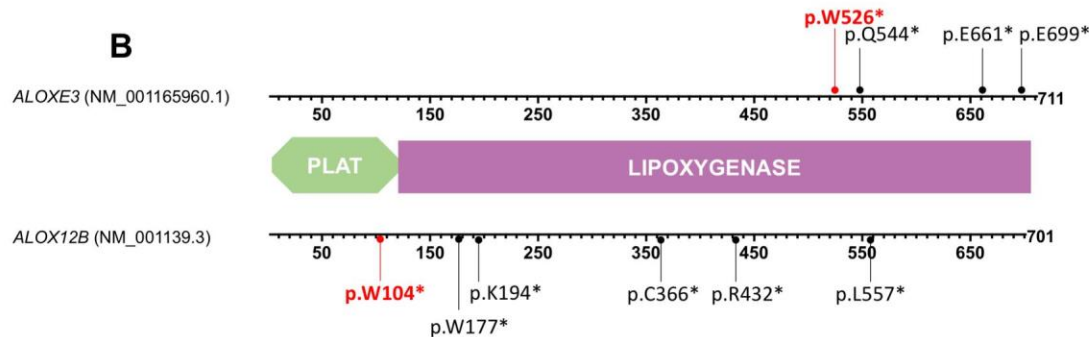


Functional analysis

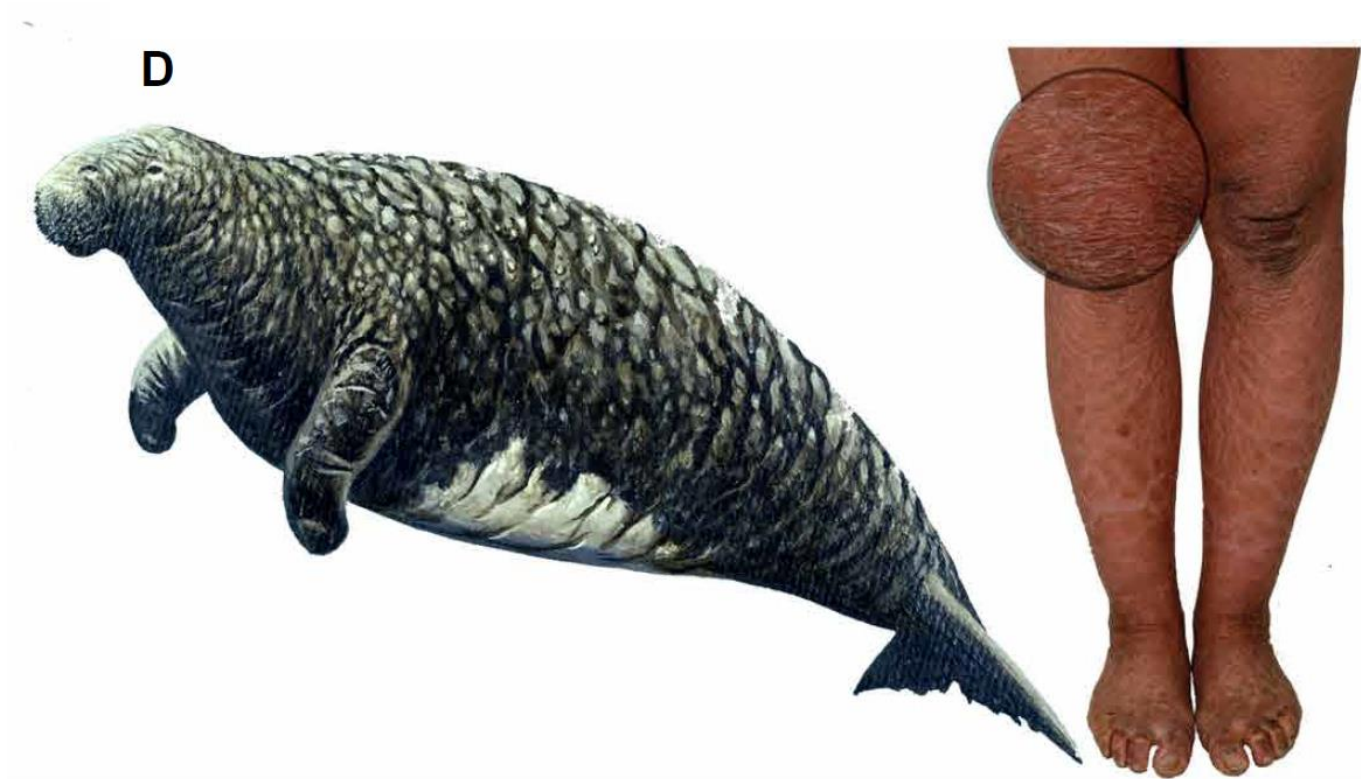
A

	ALOXE3 (NM_001165960.1)	ALOX12B (NM_001139.3)
Human	EWDWLLAKT WVRNSEF	R I YHFPAYQWMDGYET
Chimp	EWDWLLAKT WVRNSEF	R I YHFPAYQWMDGYET
Rat	EL DWLLAKT WVRNSEF	RVYHFPAYQWMDGYET
Mouse	EL DWLLAKT WVRNSEF	RVYHFPAYQWMDGYET
Dog	YWDWLLAKT WVRNSEF	R I YHFPAYRWMDGYET
Dugong	YWDWLLAKT WVRNAEF	R I YHFPAYQWMDGYKT
Steller's sea cow	YWDWLLAKT * VRNAEF	R I YHFPAYQ * MDGYKT
Manatee	YWDWLLAKT WVRNAEF	R I YHFPAYQWMDGYKT
Elephant	DWDWLLAKT WVRNAEF	RTYHFPAYQWMDGYET
Frog	EWDWTLAKL WVRSEF	ETAQFPLFLW I SDYGT
Tetraodon	ET DWLLAKF Y LKNAYA	DTYHFP I YRW I DDTKV
Zebrafish	PP DWLLAKMWVRNSDF	EMEVFPCNKW I AADGH

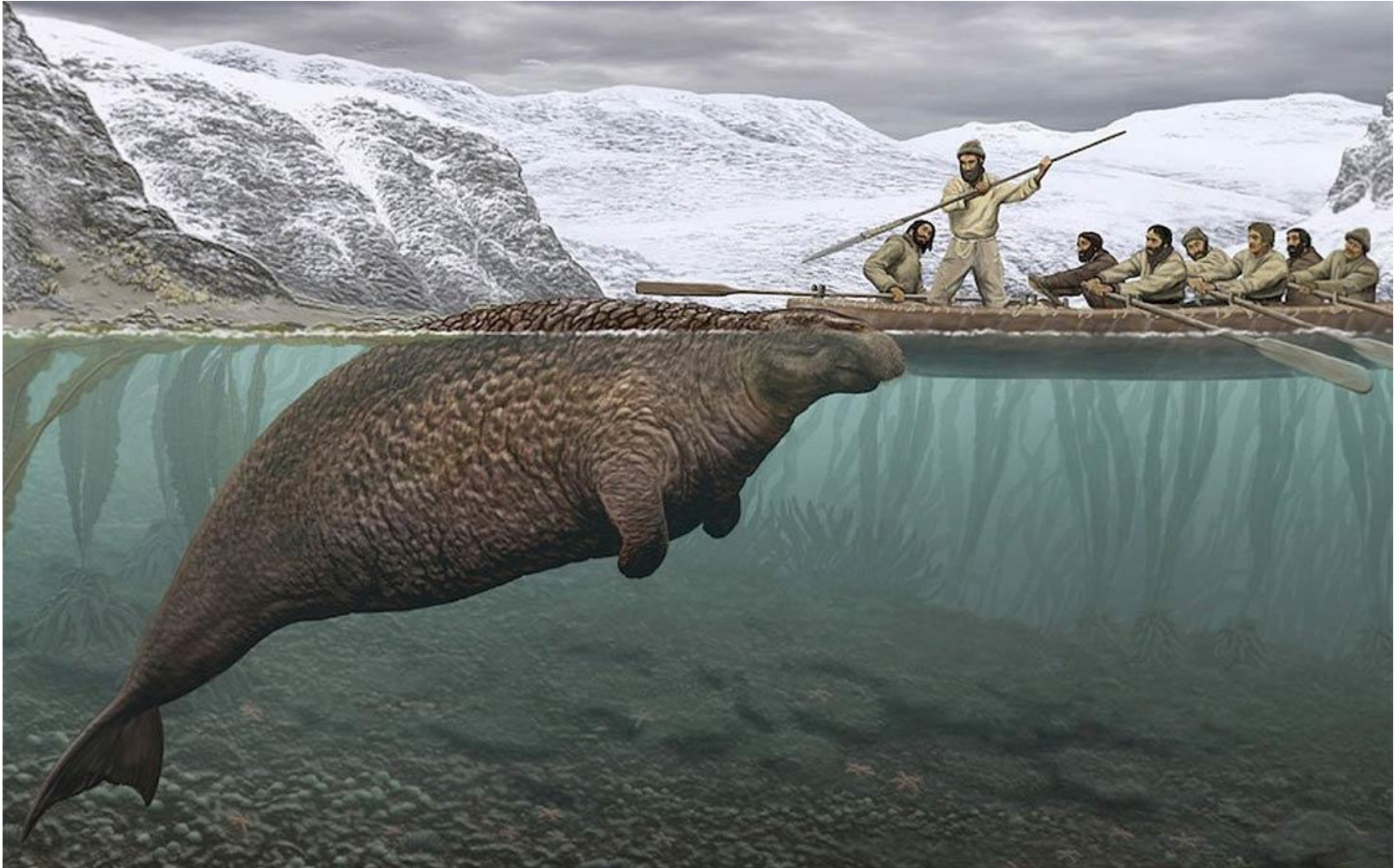
B



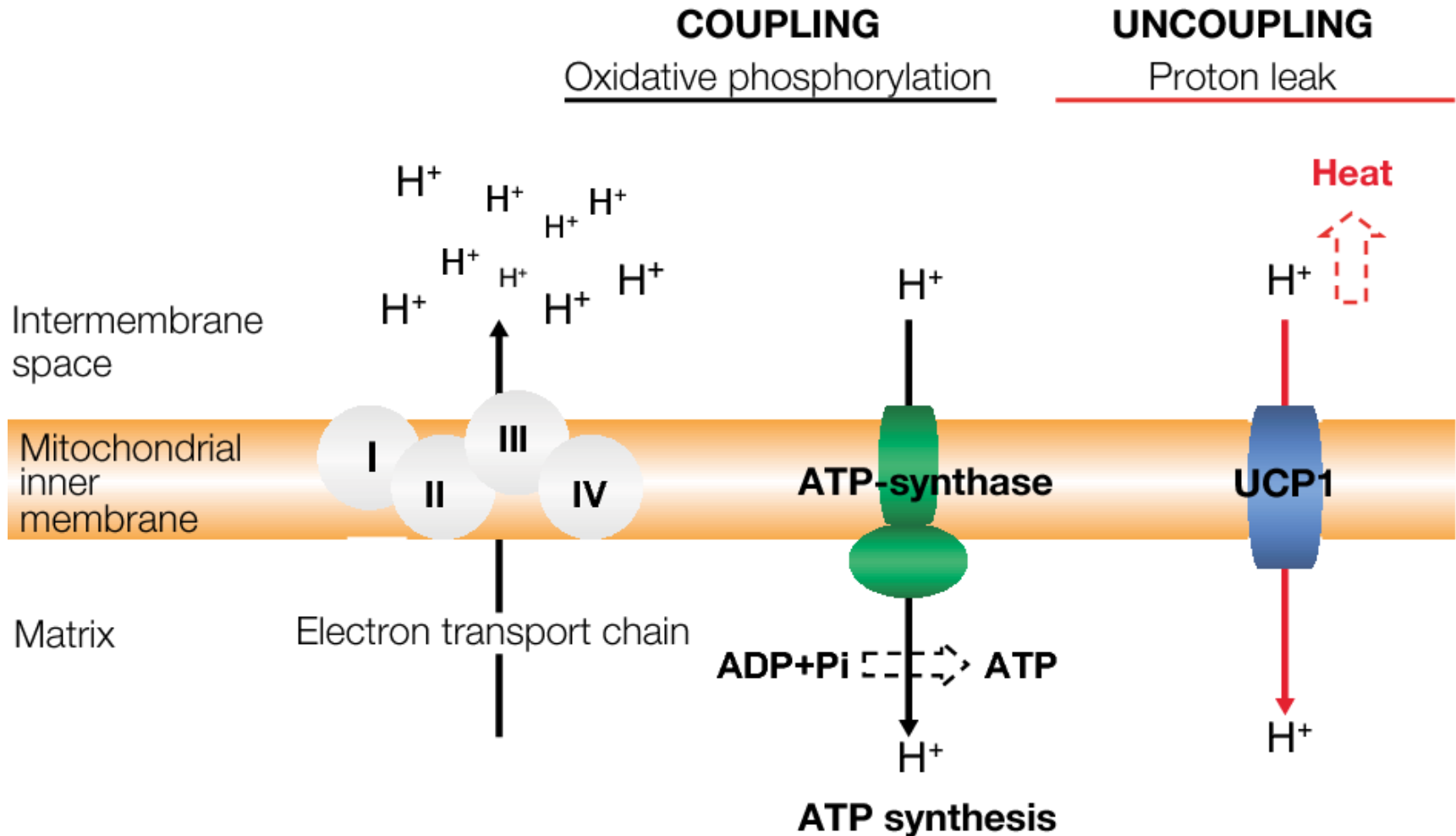
Explaining the phenotype



Steller's sea cow: habitat

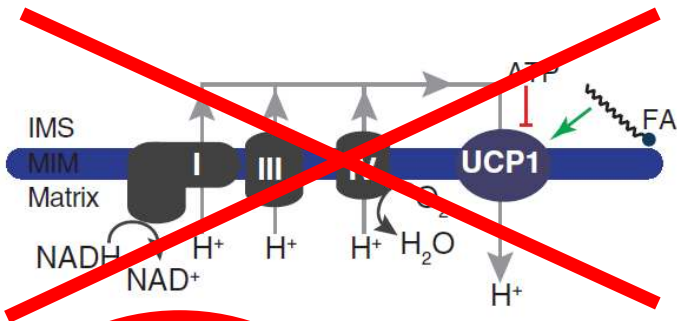


Non-shivering thermogenesis: UCP1

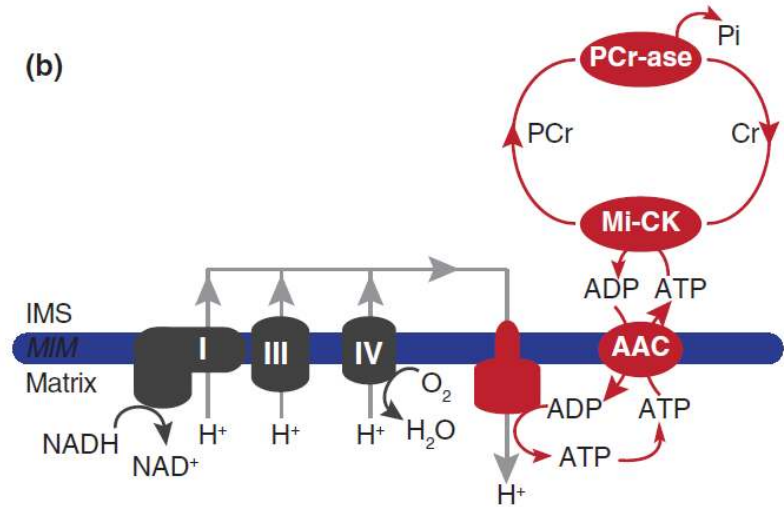


Thermogenic futile cycles

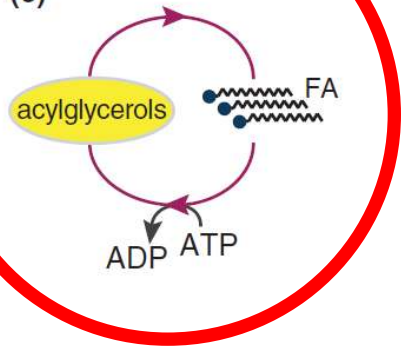
(a)



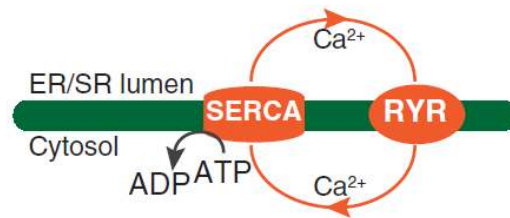
(b)



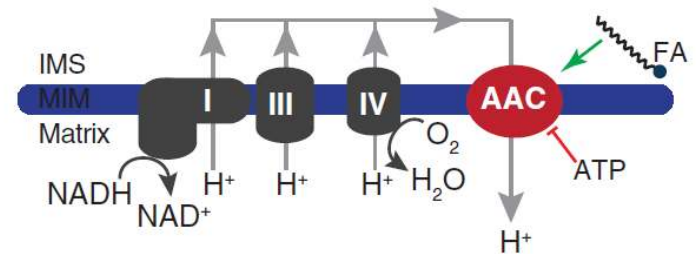
(c)



(d)



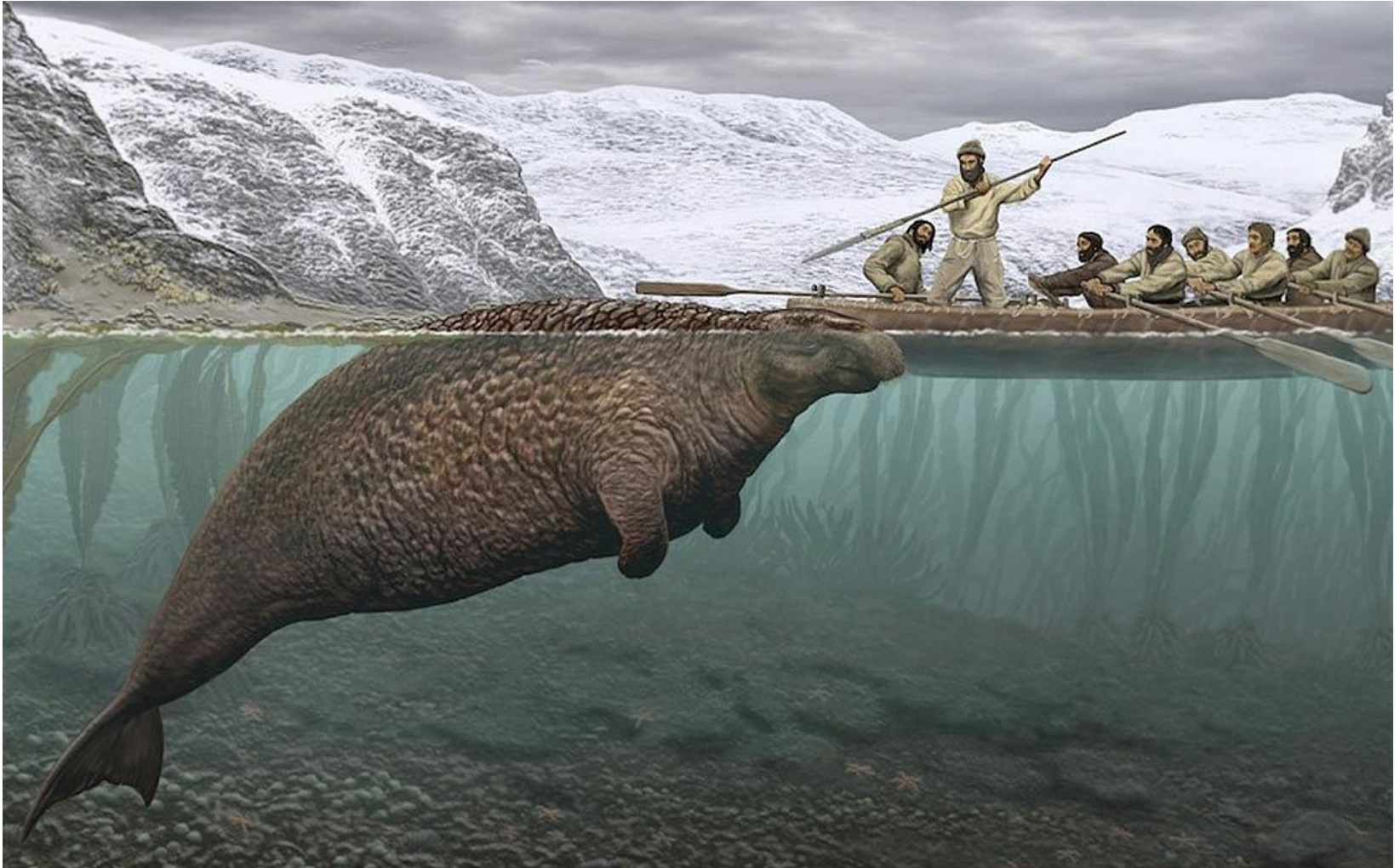
(e)



Steller's sea cow fat deposits

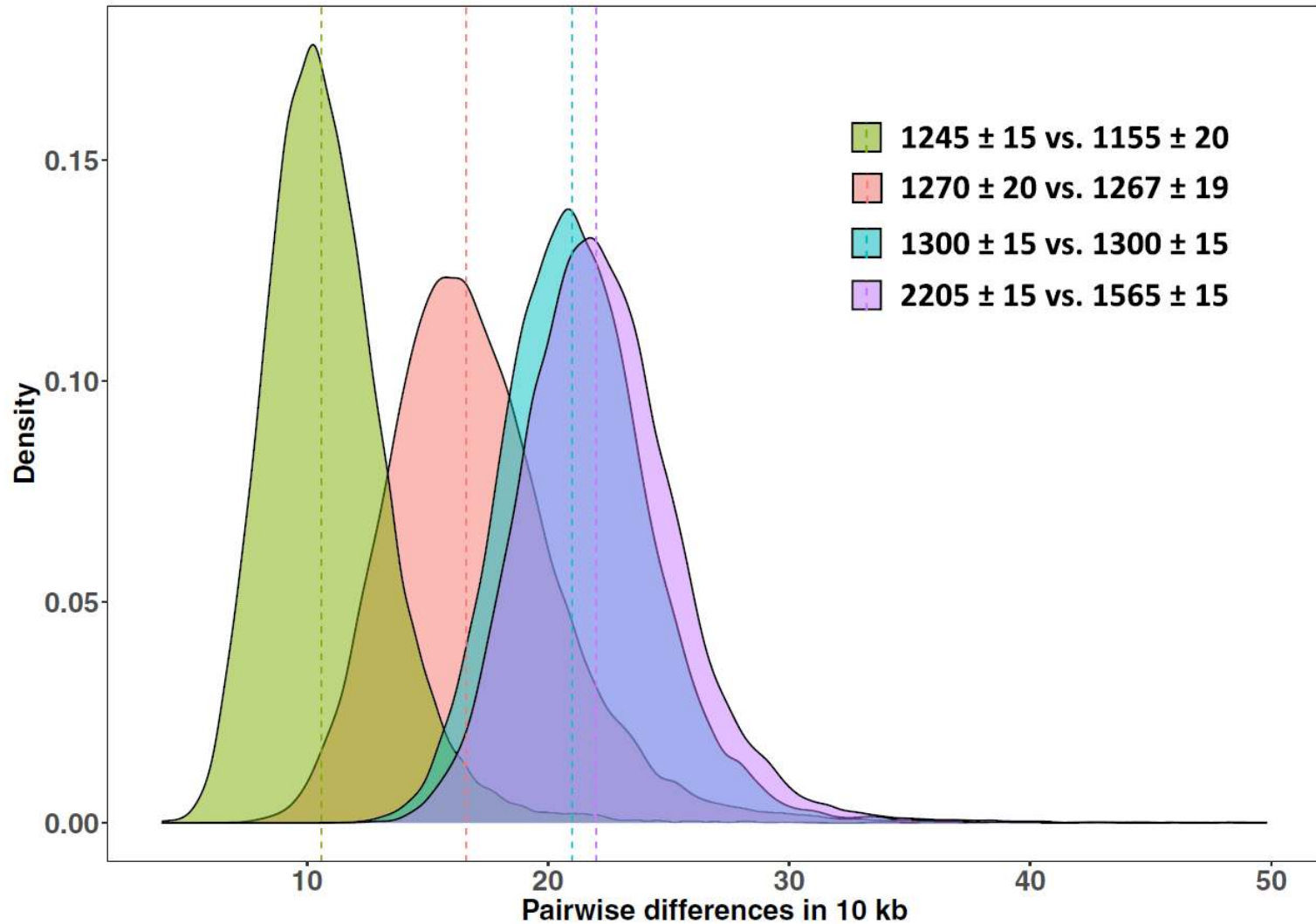


Steller's sea cow: † 1768

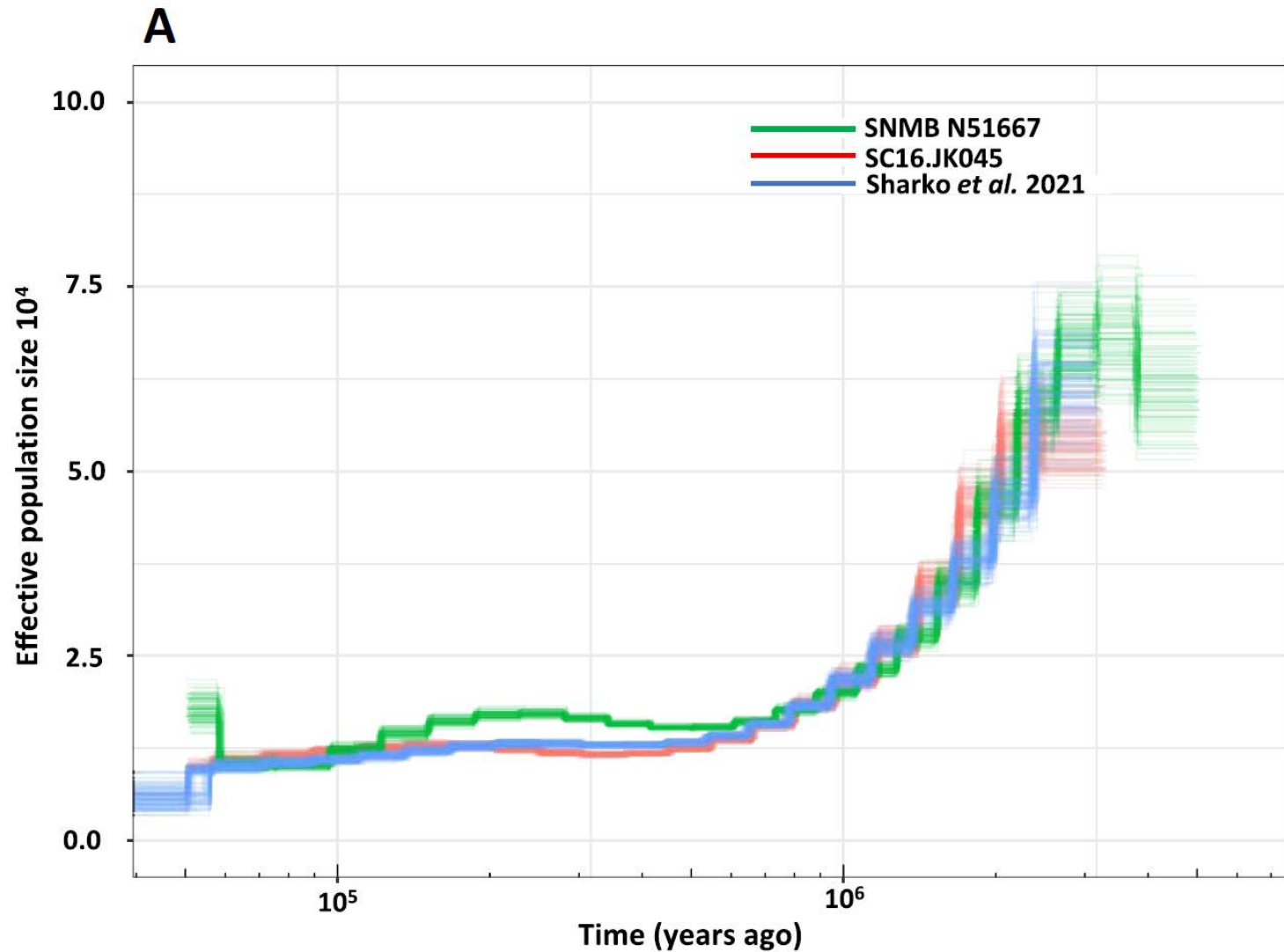


Genetic diversity over time

B



Population size over time



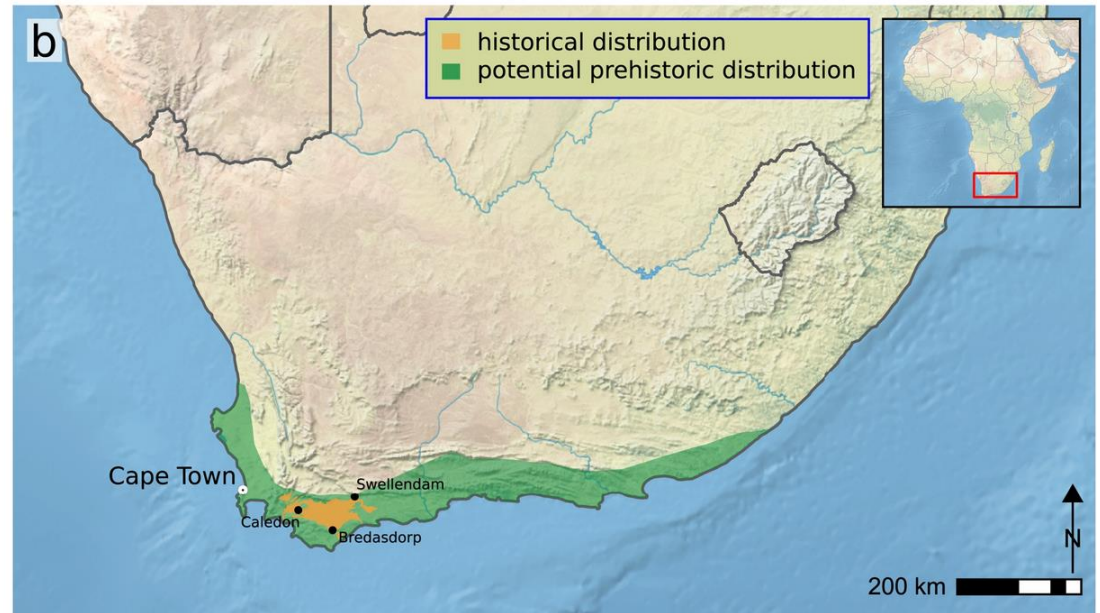
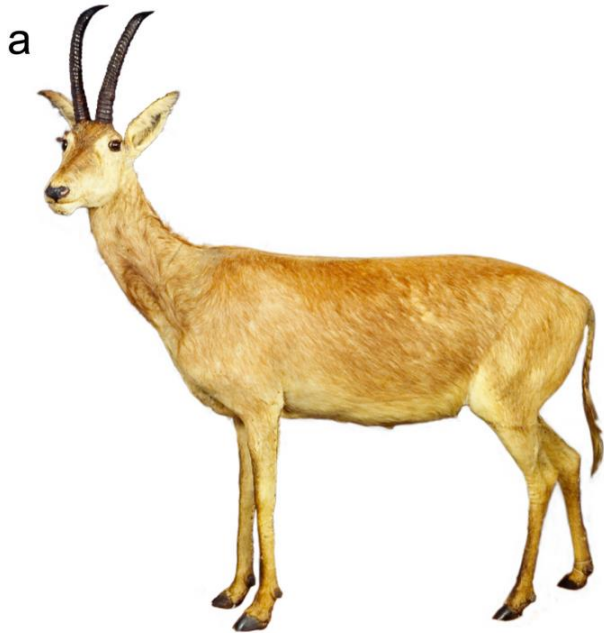
Summary Steller's sea cow

- Low population size for the last 1,000,000 years
- Further loss of genetic diversity between 2,000 and 1,000 years ago
- Genetic cause for skin phenotype and probably cold-adaptation revealed

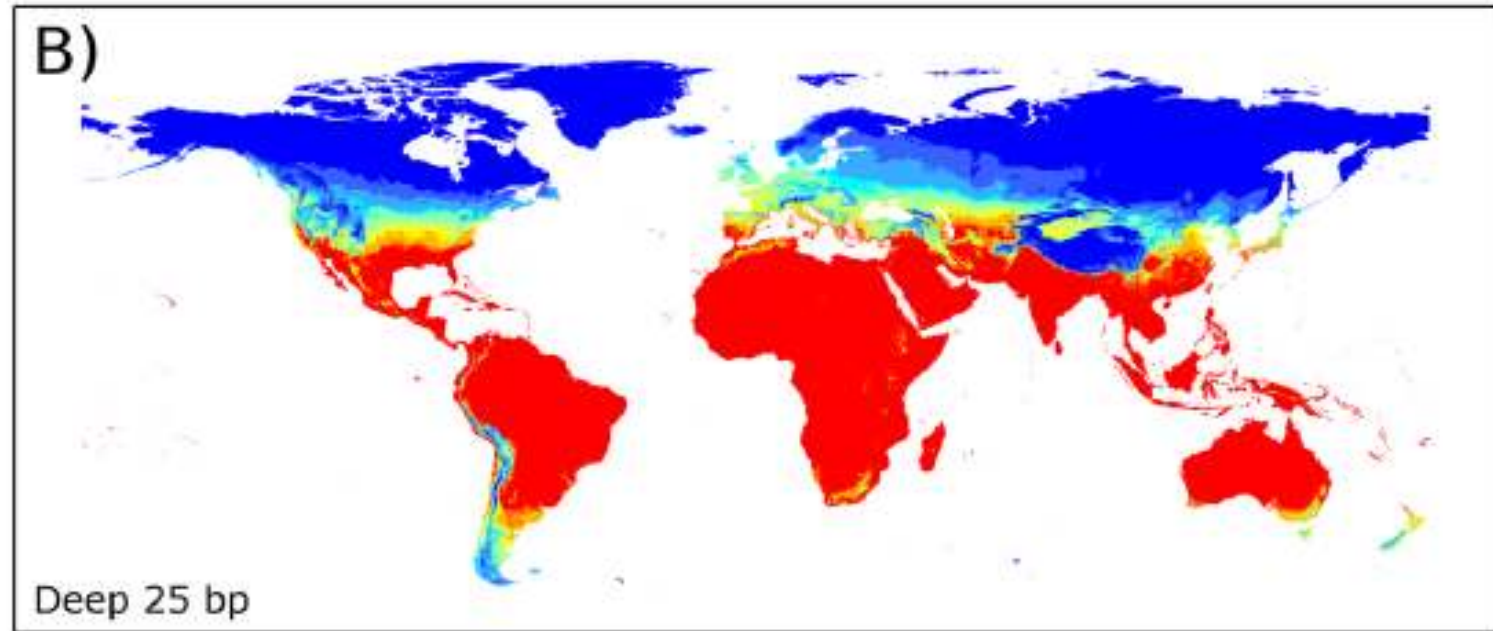
South-African bluebuck † ~1800



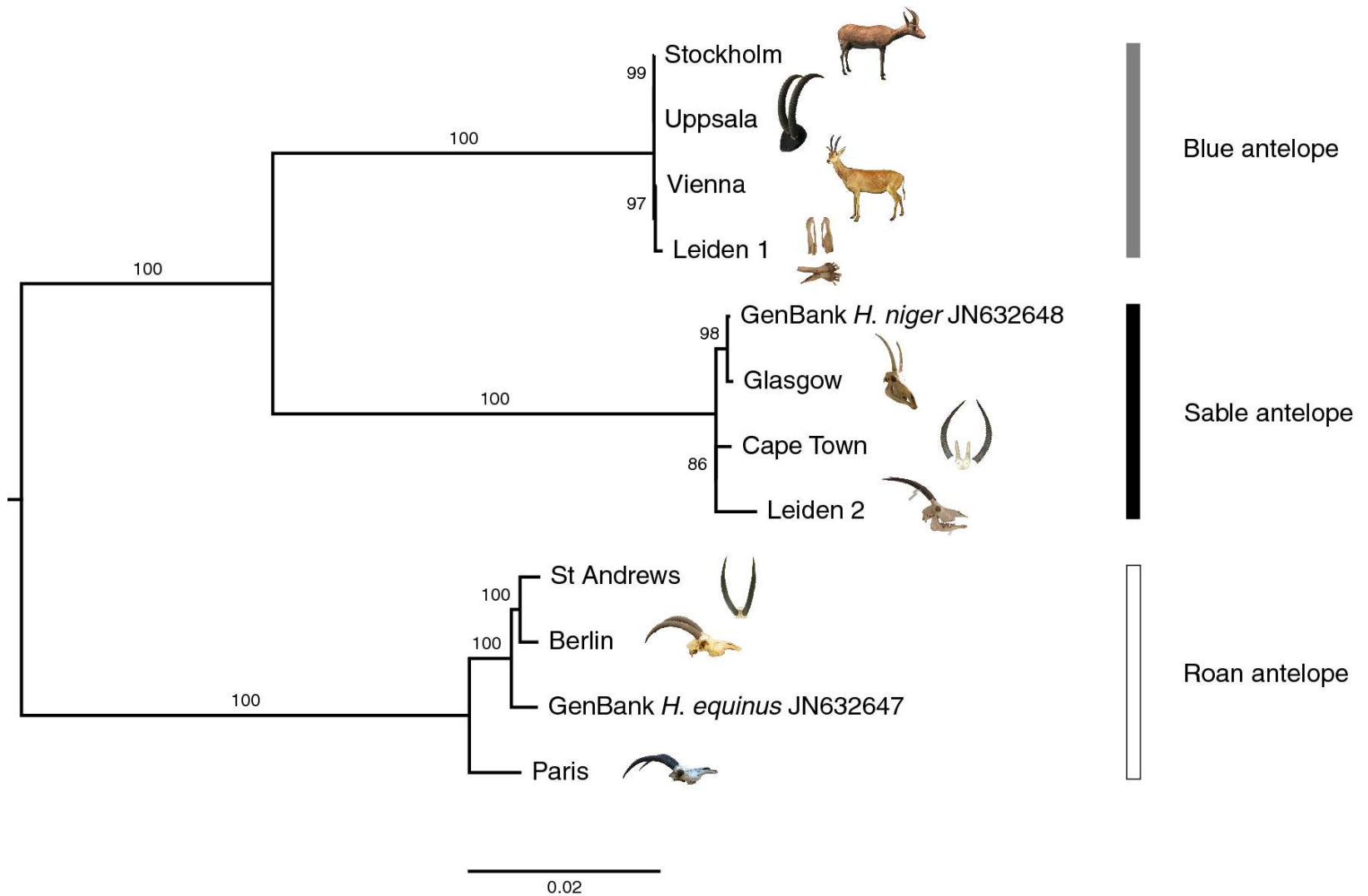
Distribution



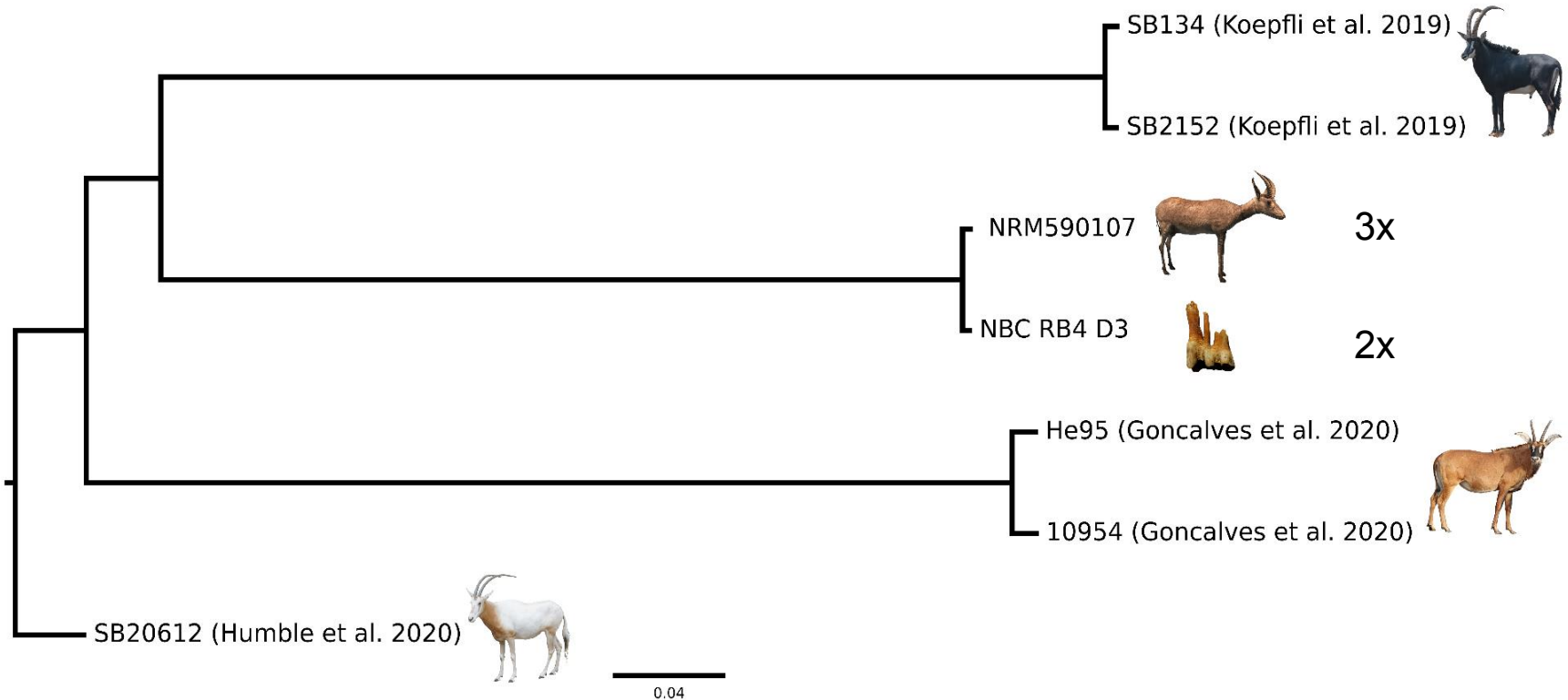
Problem 1: Poor DNA preservation



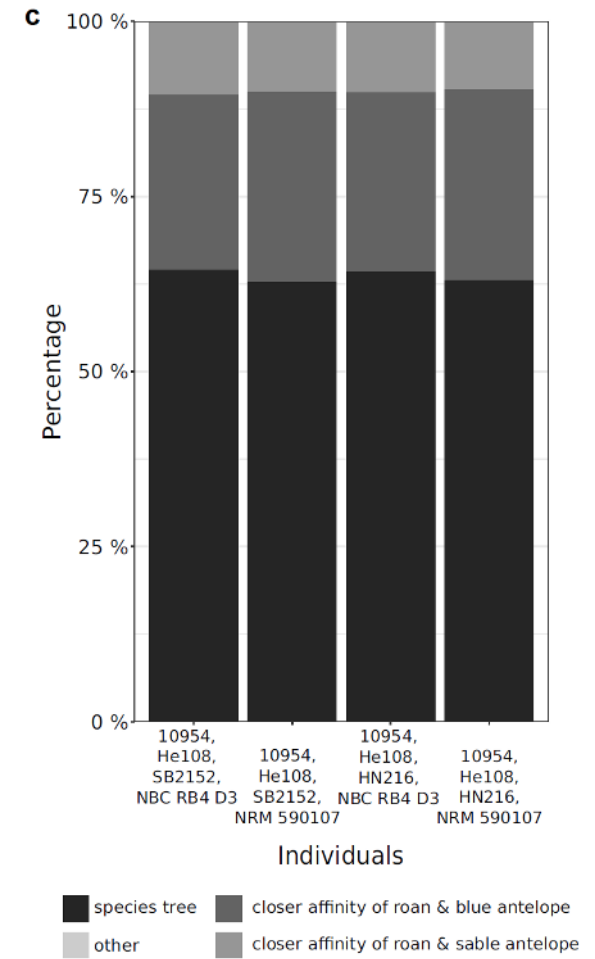
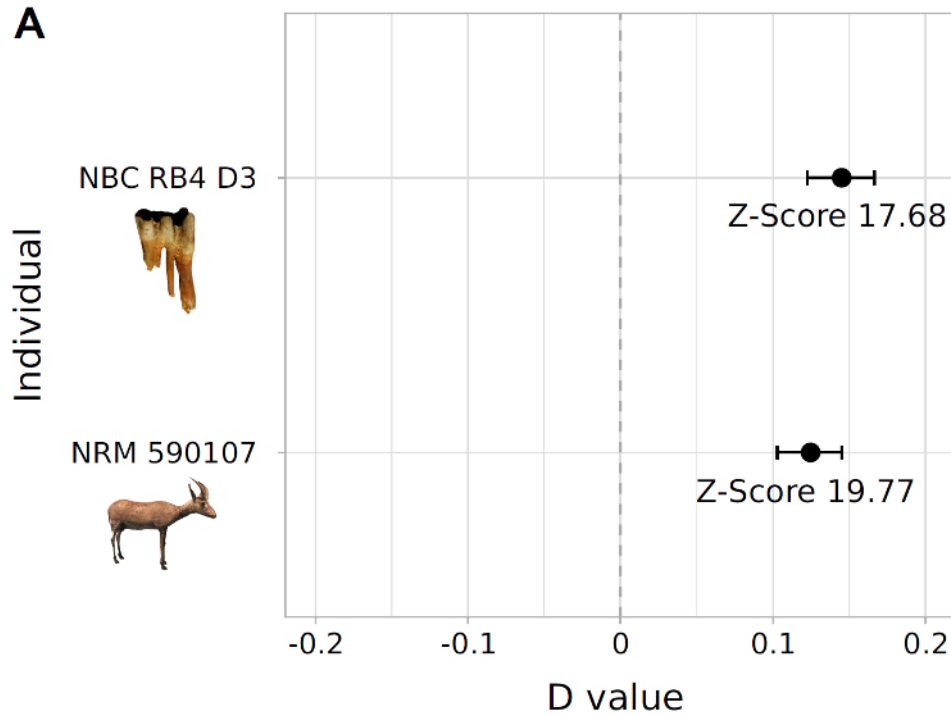
Problem 2: Few museum samples



Initial nuclear sequencing at low coverage

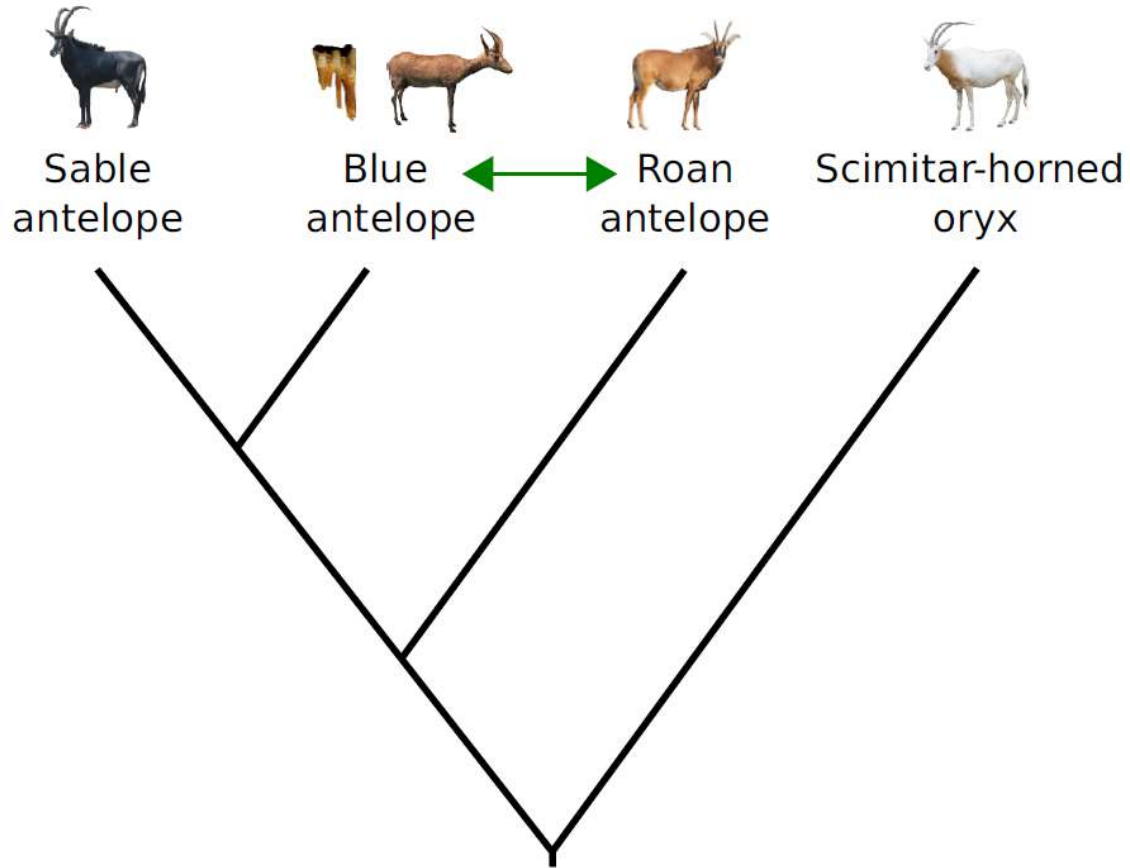


Evidence for post-divergence gene flow

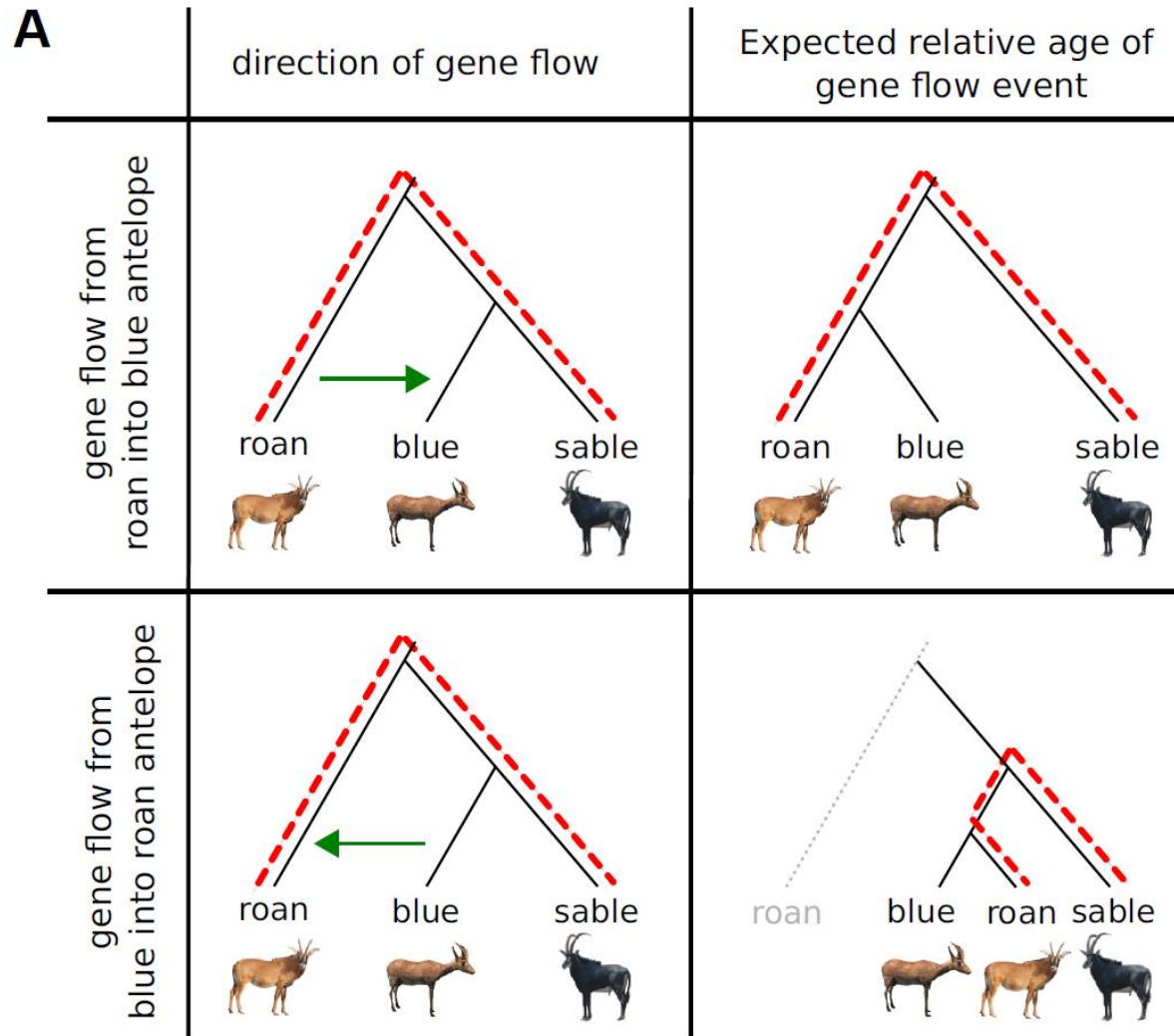


How to determine its direction?

B



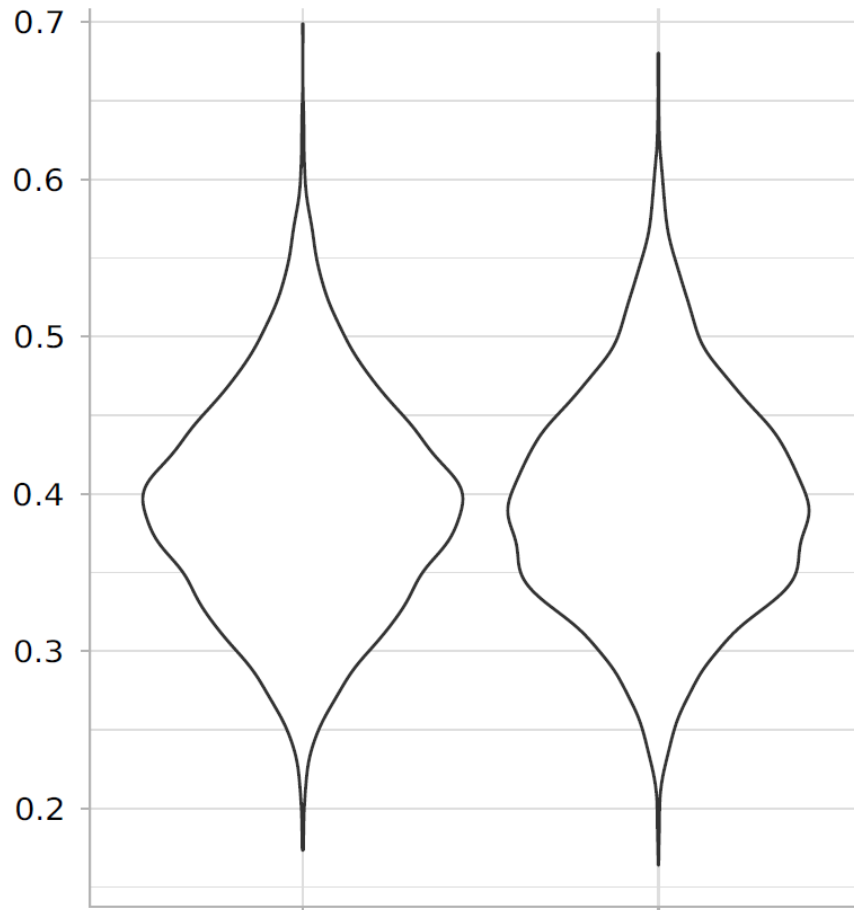
How to determine its direction?



How to determine its direction?

B

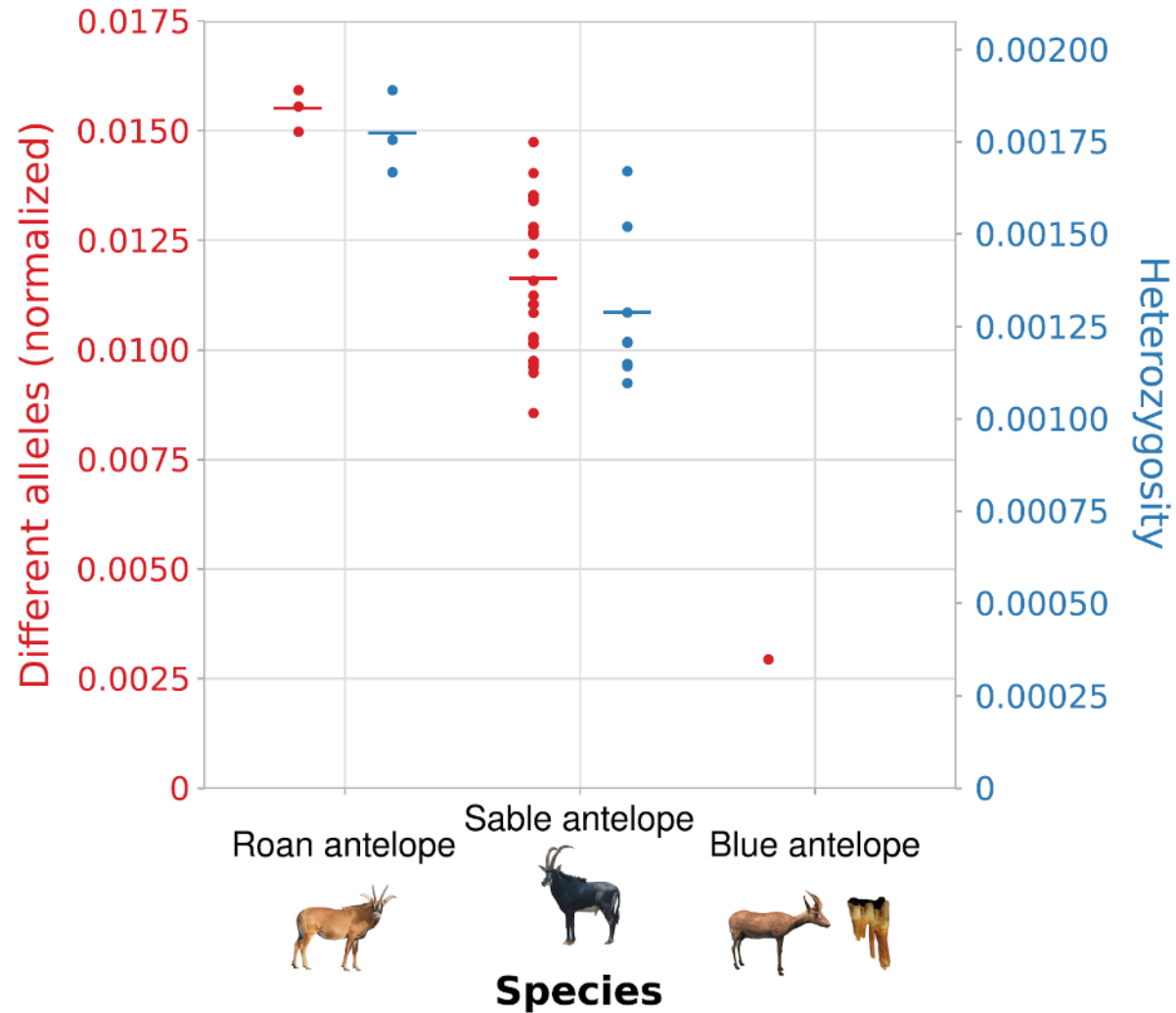
Observed distance between roan and sable
antelope as a proportion of tree length



((blue, roan), sable); ((blue, sable), roan);
species tree

Tree topology

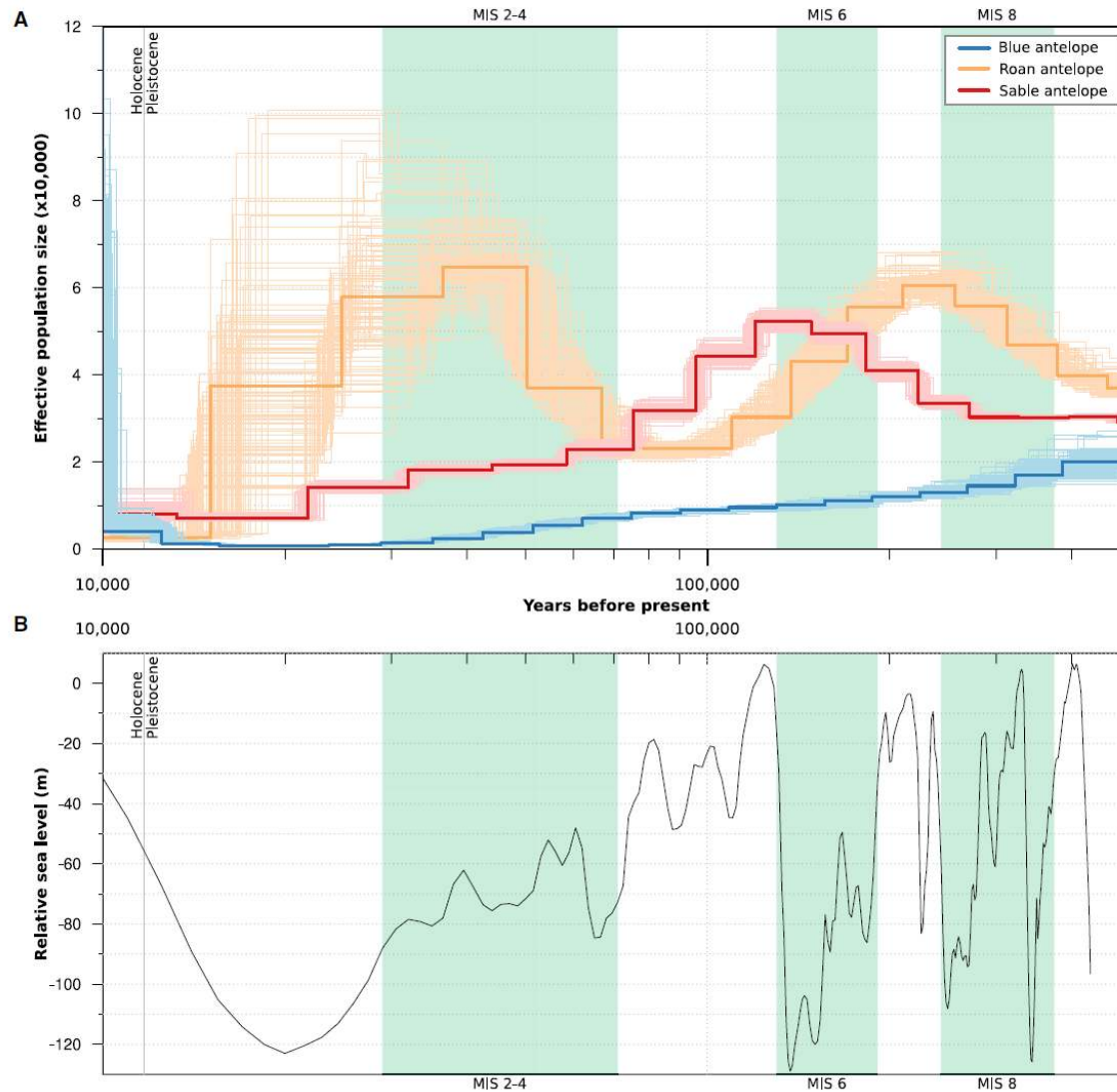
Low genetic diversity



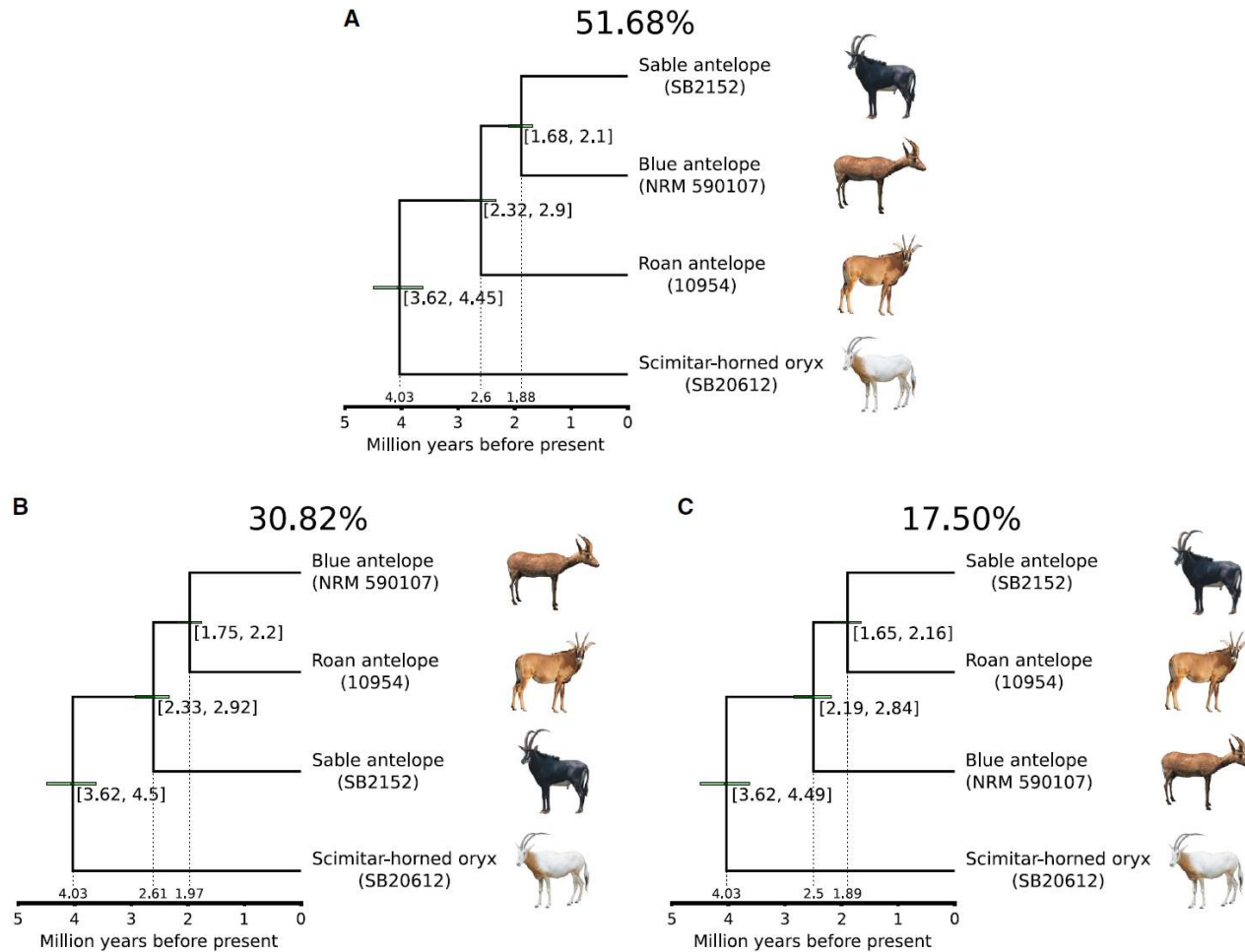
Coverage improved to 40x



Long-term low population size



Dating alternative topologies



Summary bluebuck

- Low population size for the last 500,000 years
- No connection between population size and climatic fluctuations
- Asymmetric gene flow happened during second speciation

=> not really secondary gene flow



STAR TREK VI

THE UNDISCOVERED COUNTRY

Can de-extinction become real?



Phenotype reconstruction based on mammoth noDNA (possibly contaminated)

Jurassic Park? No



Mammoth safari?

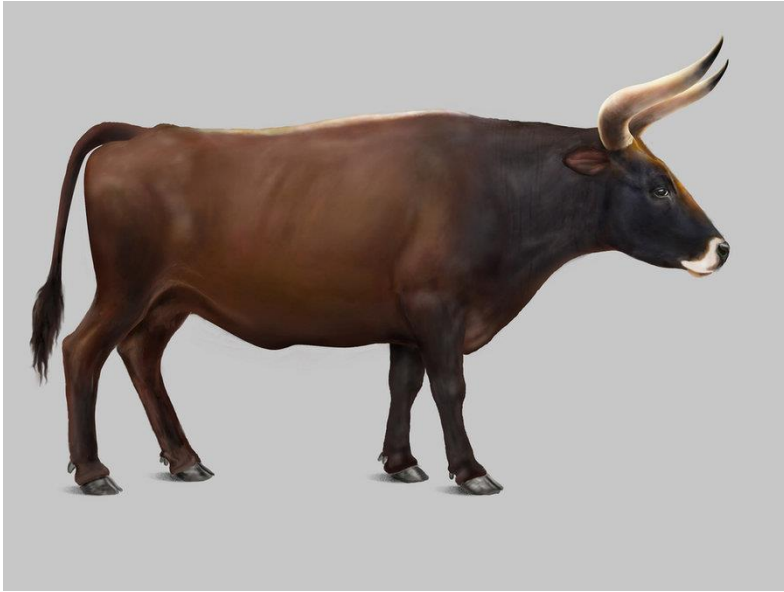
Maybe....



De-extinction: 3 possibilities



1. Backbreeding



2. Cloning



3. Genome-modification

Creating a “mammophant”

1 Most mammoth DNA is shared by elephants

2 Gene editing allows the addition of mammoth-like traits into the genome of an elephant

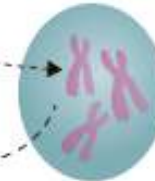
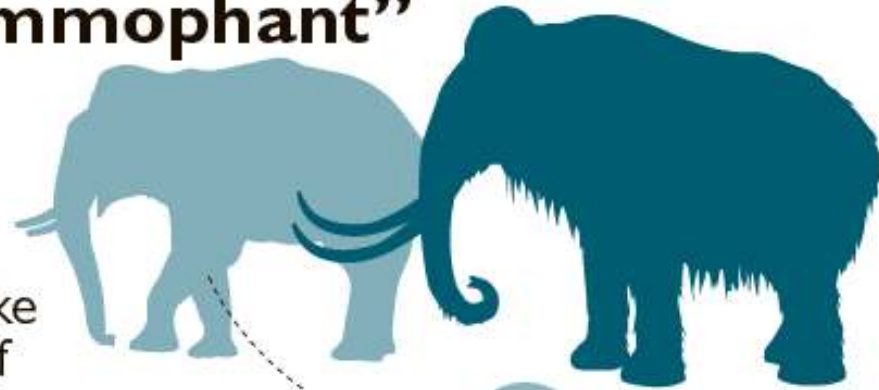


3 An elephant cell is reprogrammed to become an embryonic cell



4 The modified genome is introduced into the cell

5 The embryo is then cultivated in an artificial womb



Cell from elephant

Colossal Biosciences



Colossal Laboratories
& Biosciences

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BACK THE WOOLLY MAMMOTH

In the minds of many, this creature is gone forever. But not in the minds of our scientists, nor the labs of our company. We're already in the process of the de-extinction of the Woolly Mammoth. Our teams have collected viable DNA samples, and are editing the genes that will allow this wonderful megafauna to once again thunder through the Arctic.

Discover the Science &
Technologies that will revive
the Mammoth



Mammoths are iconic



Cloned animals



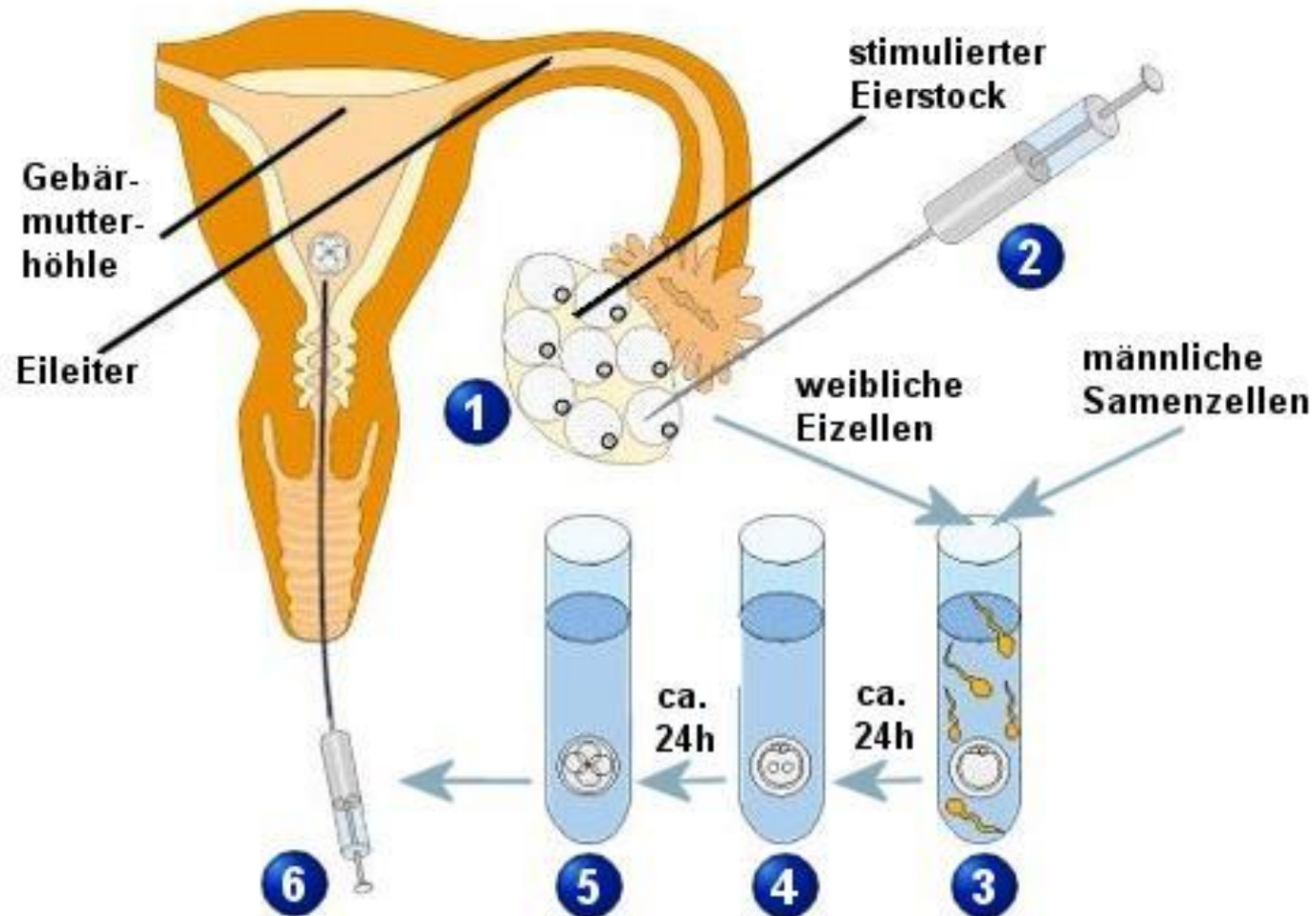
No elephants.....



Problem 1: breeding elephants



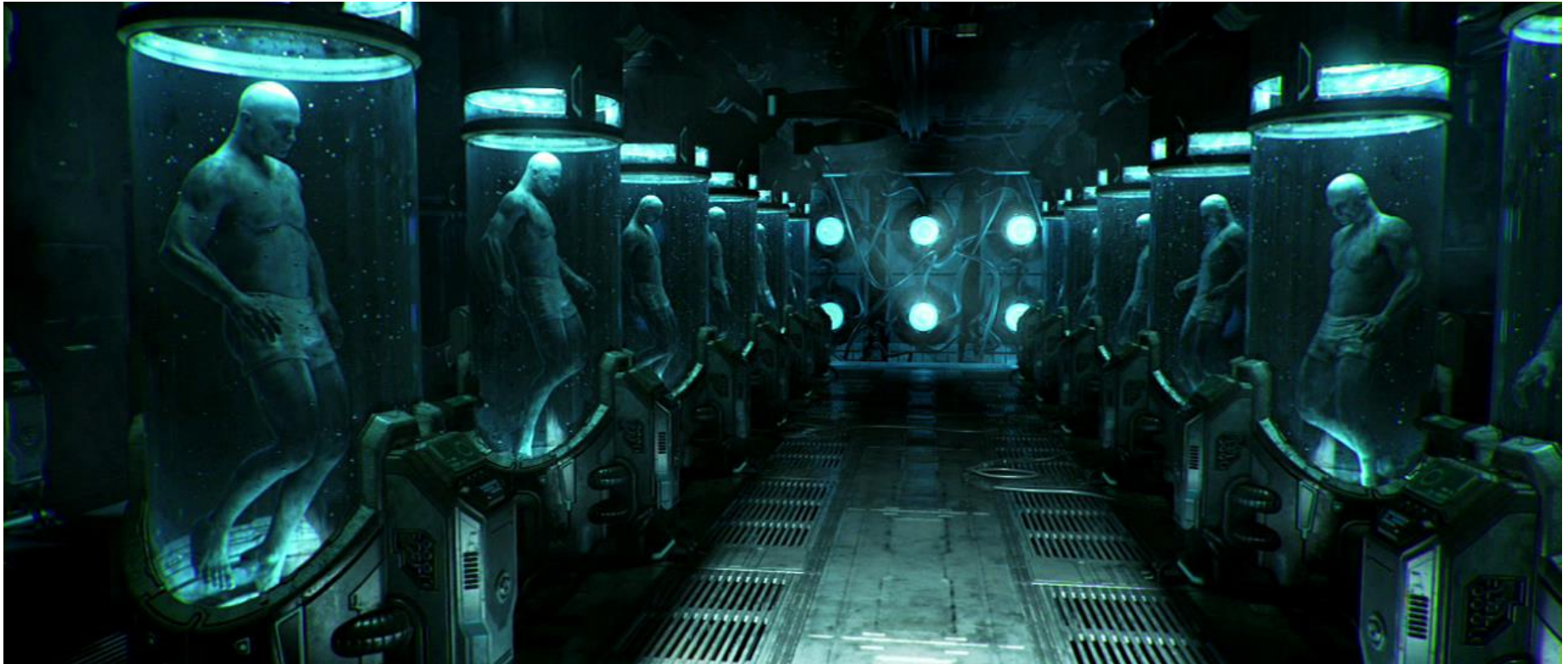
Problem 2: reproductive biology



Problem 3: generation time



Artificial womb?



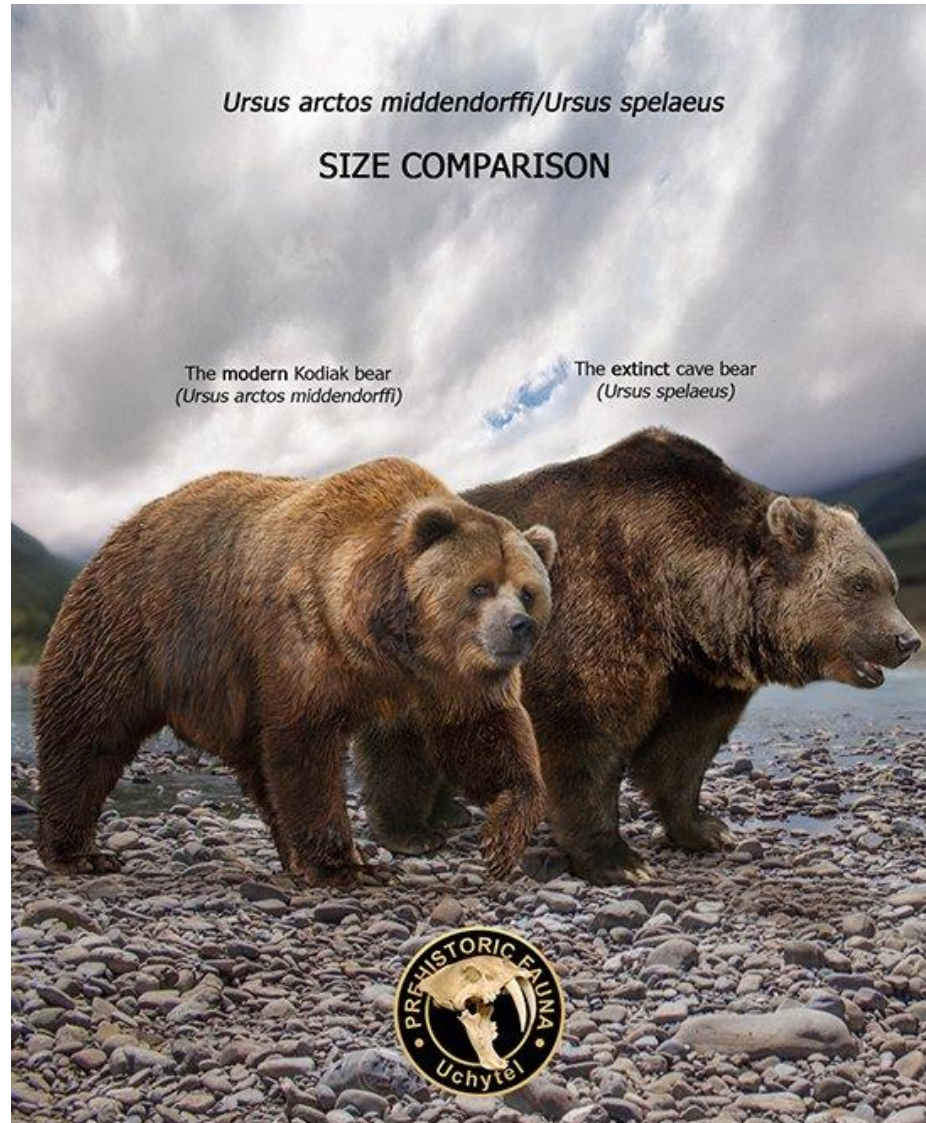
Better candidates: lava mouse



Better candidates: cave bear



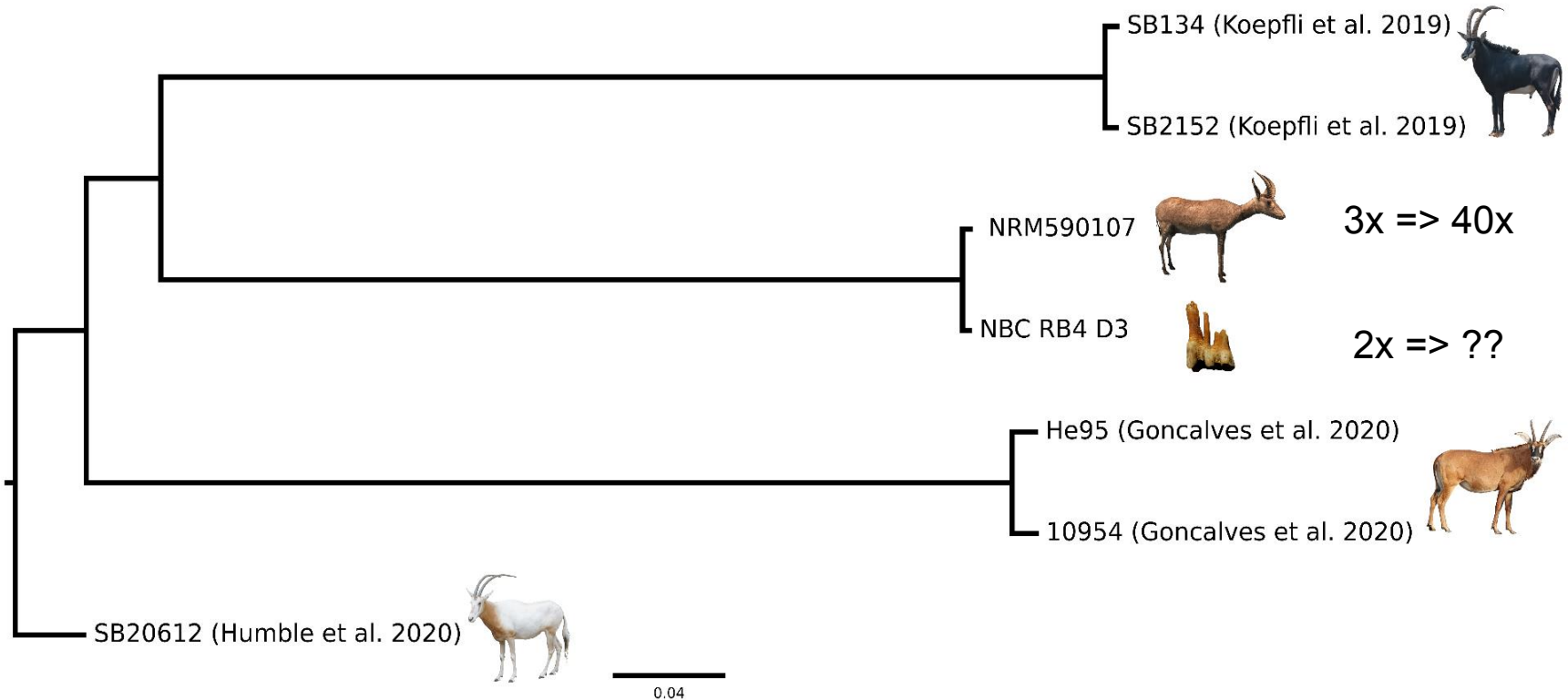
In theory.....



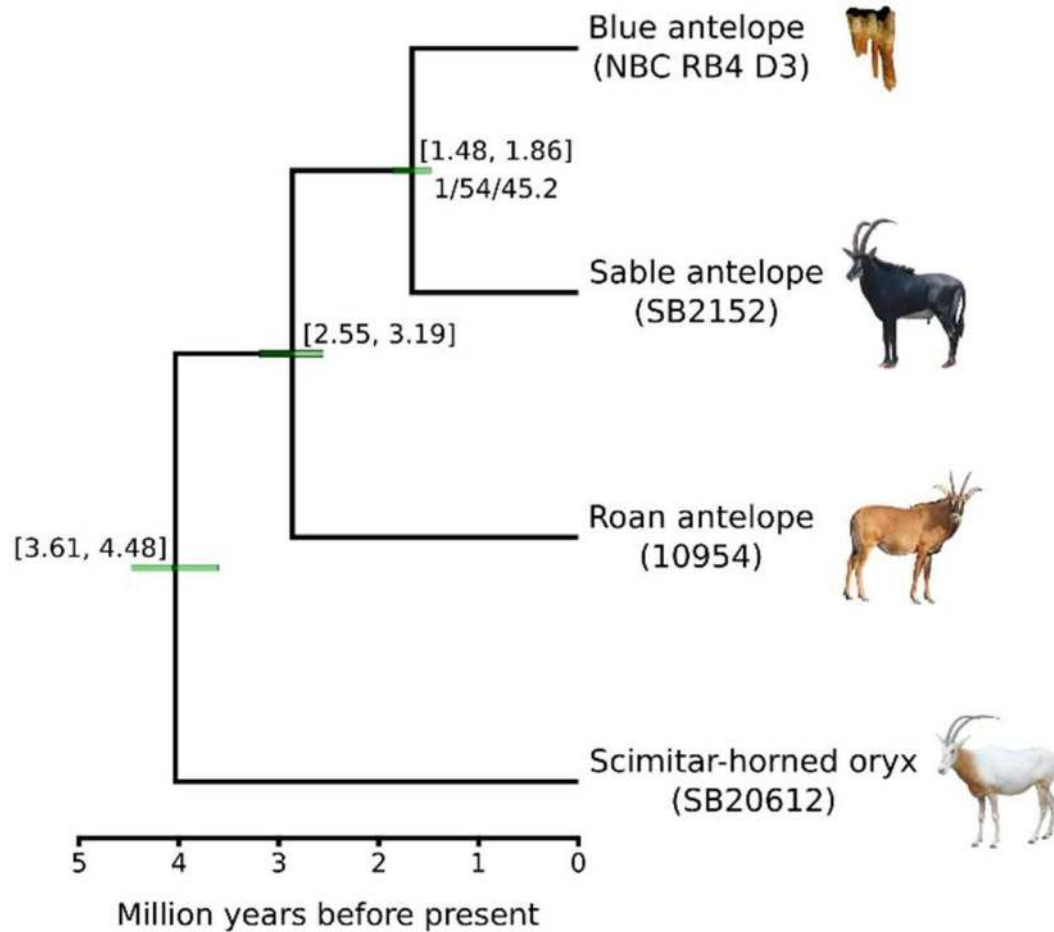
In practice: South-African bluebuck ~1800 †



Advantages: 1) first genomes available



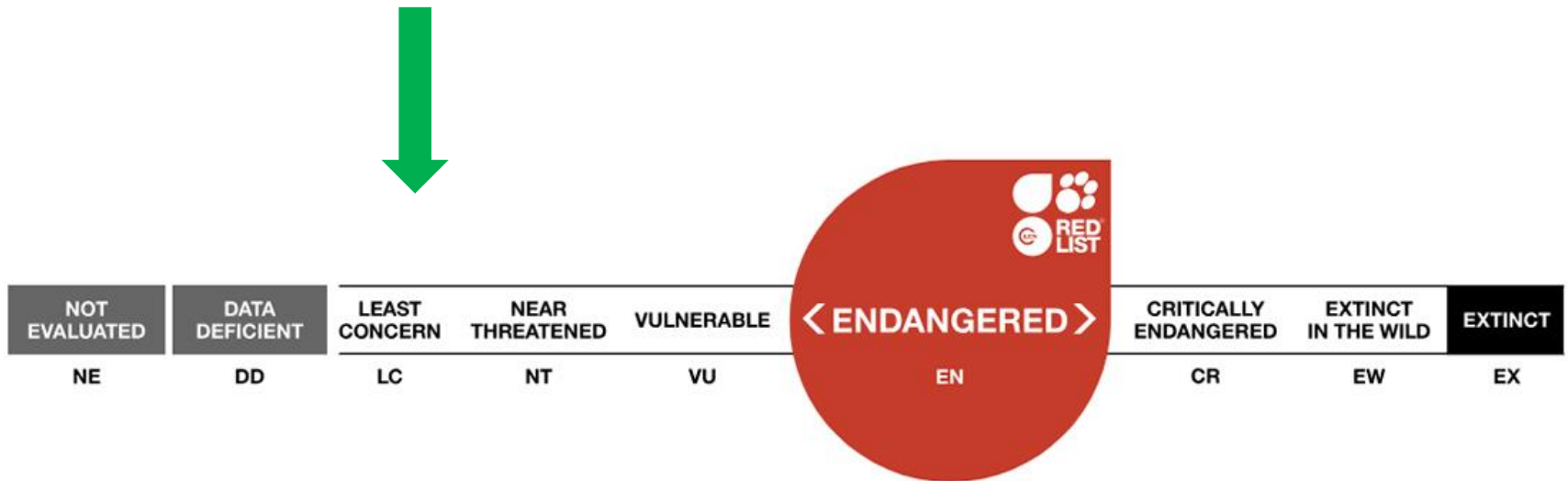
Advantages: 2) Very close extant relative



Advantages: 3) Also easy to breed



Advantages: 4) No conservation concern



Advantages: 5) It's almost a cow (or a goat)



De-extinction? Maybe.....







Ende