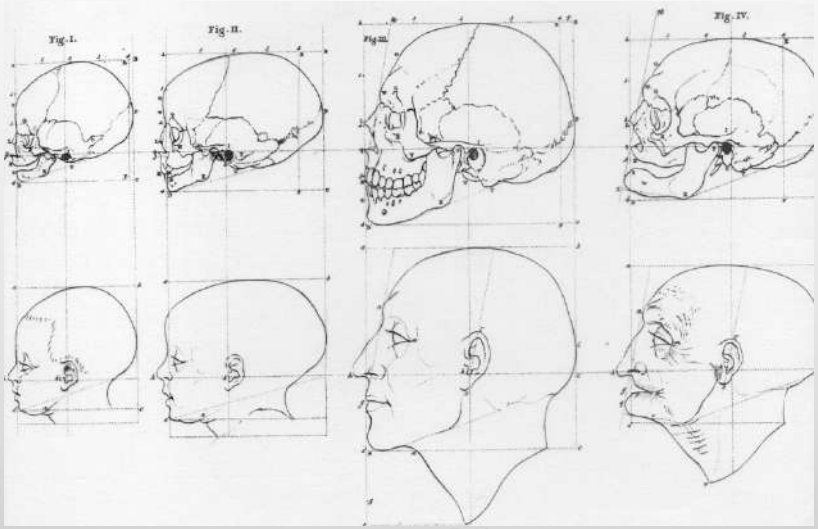
BIOLOGIA DELLO SCHELETRO UMANO

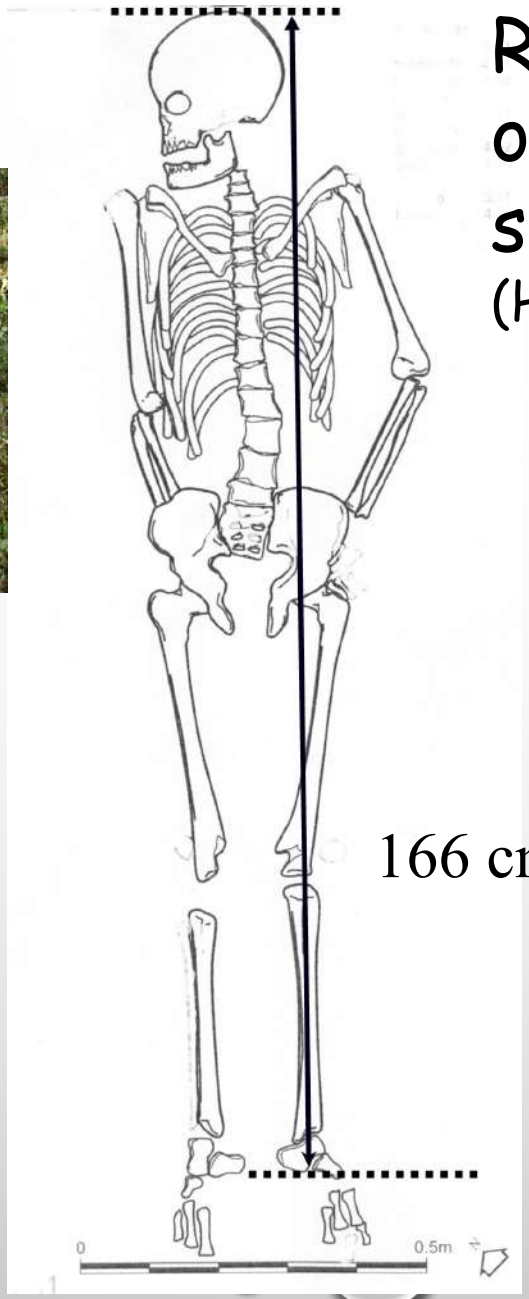
Lezione 4



OTTO DOMANDE PER L'ANTROPOLOGO:

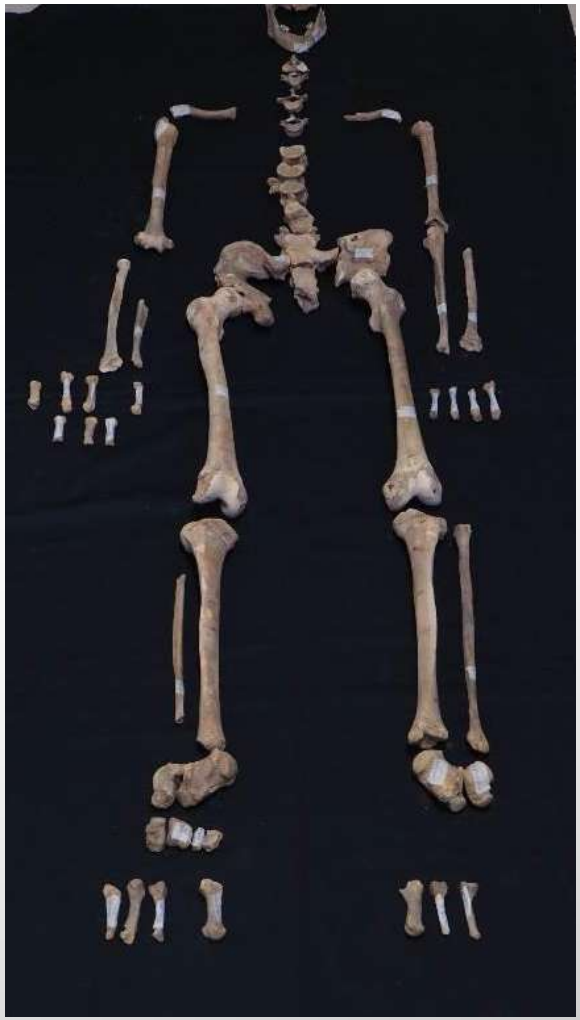
1. è un osso umano?
2. è un reperto recente?
3. sono presenti più persone?
4. di quale origine etnica?
5. di che sesso?
6. di che età ?
7. di quale statura?
8. con quali caratteristiche?

Rilevazione *in situ*, o dal disegno in scala
(Hanson 1992)

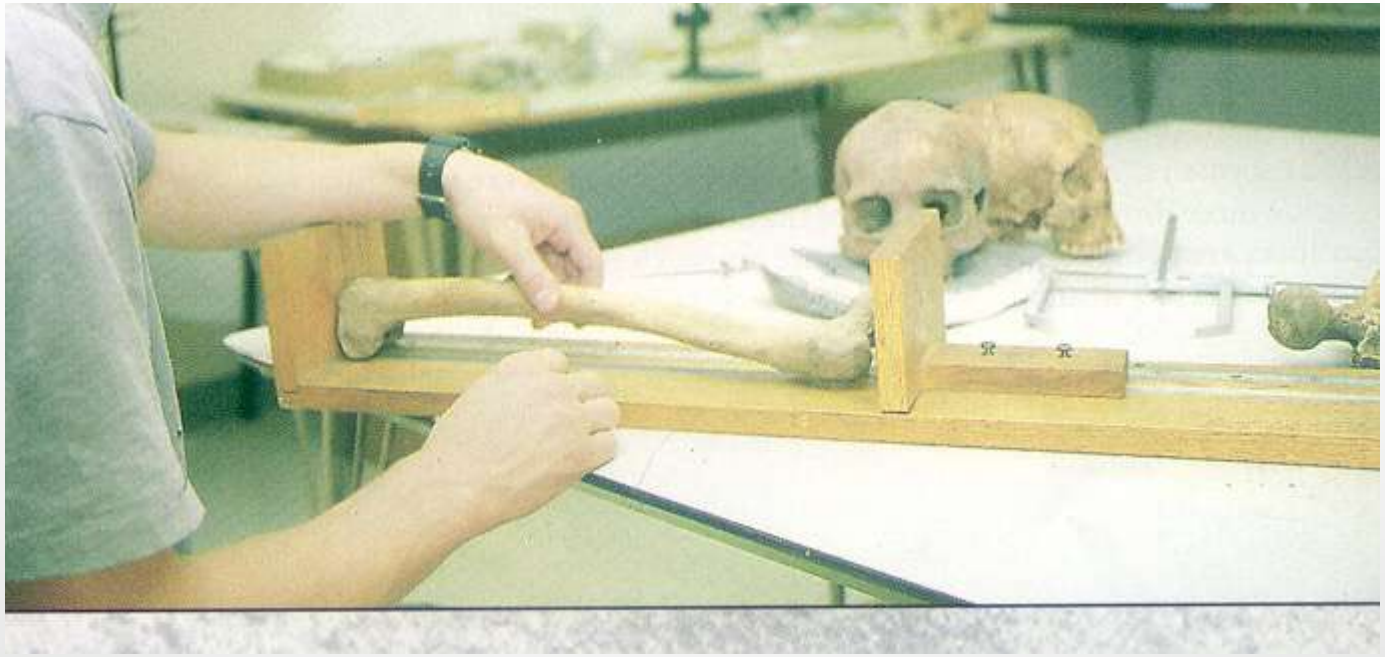


Determinazione della statura in laboratorio

La misura della statura può essere determinata dallo scheletro **sommando tutte le lunghezze** delle ossa che concorrono a determinarla e **stimando lo spessore delle cartilagini.**



Individuo 386C, Necropoli di Spina (VI-III sec.). Foto: A. Vecchi



Dalla misura delle ossa, a seconda del sesso, età e della popolazione, si può det. la statura usando delle **formule** . Anche in questo caso **si ottiene un intervallo**, più che un singolo valore.

TROTTER, 1970

Si utilizzano le lungh. mx (meglio fare una media da diverse ossa)

Equations Used to Estimate Stature, in Centimeters, with Standard Error, from the Long Bones of Various Groups of Individuals between 18 and 30 Years of Age[a]

White Males			**Black Males**		
3.08 Hum	+ 70.45	±4.05	3.26 Hum	+ 62.10	±4.43
3.78 Rad	+ 79.01	±4.32	3.42 Rad	+ 81.56	±4.30
3.70 Uln	+ 74.05	±4.32	3.26 Uln	+ 79.29	±4.42
2.38 Fem	+ 61.41	±3.27	2.11 Fem	+ 70.35	±3.94
2.68 Fib	+ 71.78	±3.29	2.19 Fib	+ 85.65	±4.08
White Females			**Black Females**		
3.36 Hum	+ 57.97	±4.45	3.08 Hum	+ 64.67	±4.25
4.74 Rad	+ 54.93	±4.24	2.75 Rad	+ 94.51	±5.05
4.27 Uln	+ 57.76	±4.30	3.31 Uln	+ 75.38	±4.83
2.47 Fem	+ 54.10	±3.72	2.28 Fem	+ 59.76	±3.41
2.93 Fib	+ 59.61	±3.57	2.49 Fib	+ 70.90	±3.80
East Asian Males			**Mexican Males**		
2.68 Hum	+ 83.19	±4.25	2.92 Hum	+ 73.94	±4.24
3.54 Rad	+ 82	±4.60	3.55 Rad	+ 80.71	±4.04
3.48 Uln	+ 77.45	±4.66	3.56 Uln	+ 74.56	±4.05
2.15 Fem	+ 72.57	±3.80	2.44 Fem	+ 58.67	±2.99
2.40 Fib	+ 80.56	±3.24	2.50 Fib	+ 75.44	±3.52

[a]To estimate stature of older individuals, subtract 0.06 (age in years, 30) cm; to estimate cadaver stature, add 2.5 cm. From Trotter (1970). The tibia is not included; see text for rationale.

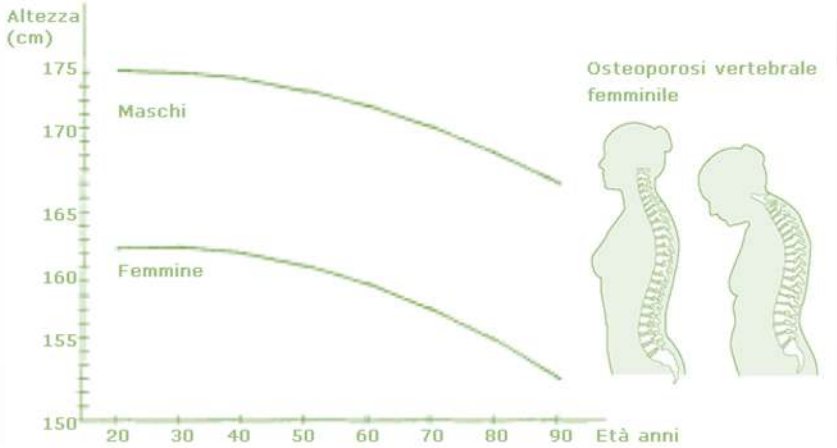
Es.: Qual'era la statura di un individuo M caucasoide, se il suo femore era lungo 45.0 cm?

2.38 X 45.0 + 61.41 = 168.51 ± 3.27 cm

165.2 – 171.8 cm

EFFETTO dell'INVECCHIAMENTO SULLA STATURA

Trotter e Gleser consigliavano semplicemente di ridurre il valore di statura stimato di **0.06 mm** per ogni anno di età sup.ai 30 anni
(in base a studi trasversali)



(studi longitudinali)

Table 4

AMOUNT IN MILLIMETERS THAT SHOULD BE SUBTRACTED FROM TROTTER AND GLESER STATURE ESTIMATIONS TO COMPENSATE FOR AGING IN SUBJECTS OVER AGE 45

Age	*Males*	*Females*
50	4.3	0.4
55	7.4	2.8
60	11.5	7.0
65	16.4	12.9
70	22.2	20.2
75	28.6	28.8
80	35.6	38.5
85	43.2	49.0

Tavole di Manouvrier (1892)

M

Fibula mm	Tibia mm	Femore mm	Statura cm	Omero mm	Radio mm	Ulna mm
			MASCHI			
318	319	392	153,0	295	213	227
323	324	398	155,2	298	216	231
328	330	404	157,1	302	219	235
333	335	410	159,0	306	222	239
338	340	416	160,5	309	225	243
344	346	422	162,5	313	229	246
349	351	428	163,4	316	232	249
353	357	434	164,4	320	236	253
358	362	440	165,4	324	239	257
363	368	446	166,6	328	243	260
368	373	453	167,7	332	246	263
373	378	460	168,6	336	249	266
378	383	467	169,7	340	252	270
383	389	475	171,6	344	255	273
388	394	482	173,0	348	258	276
393	400	490	175,4	352	261	280
398	405	497	176,7	356	264	283
403	410	504	178,5	360	267	287
408	415	512	181,2	364	270	290
413	420	599	183,0	368	273	293

Più usate in Eu. Sottostimano stat. M, sovrastimano stat. F. Manca intervallo.

Es.: Qual'era la statura di un individuo M caucasoide, se il suo femore era lungo 45.0 cm?

MANOUVRIER, 1892

F

Fibula mm	Tibia mm	Femore mm	Statura cm	Omero mm	Radio mm	Ulna mm
288	289	368	142,0	266	195	206
293	294	373	144,0	270	197	209
298	299	378	145,5	273	199	212
303	304	383	147,0	276	201	215
307	309	388	148,8	279	203	217
311	314	393	149,7	282	205	219
316	319	398	151,3	285	207	222
320	324	403	152,8	289	209	225
325	329	408	154,3	292	211	228
330	334	415	155,6	297	214	231
336	340	422	156,8	302	218	235
341	346	429	158,2	307	222	239
346	351	436	159,5	313	226	243
351	358	443	161,2	318	230	247
356	364	450	163,0	324	234	251
361	370	457	168,0	329	238	254
366	376	464	167,0	334	242	258
371	382	471	169,2	339	246	261
376	388	478	171,5	344	250	264

Per omero, radio, ulna: lungh.mx.
Femore: lungh.fisiol.
Tibia: lungh.tot.(senza spina)

Si basa sui dati che il Rollet (1888) aveva rilevato su **100 cadaveri** dell'ospedale di Lione, per ognuno dei quali era stata misurata la statura e la lunghezza delle ossa lunghe degli arti.

Sarebbe opportuno scegliere equazioni sviluppate da popolazioni affini (tenendo conto di origine, età, sesso e possibilmente del periodo)

Stature estimation from tibia percutaneous length: New equations derived from a Mediterranean population
Gualdi-Russo E., Bramanti B., Rinaldo N.
Science & Justice 2018

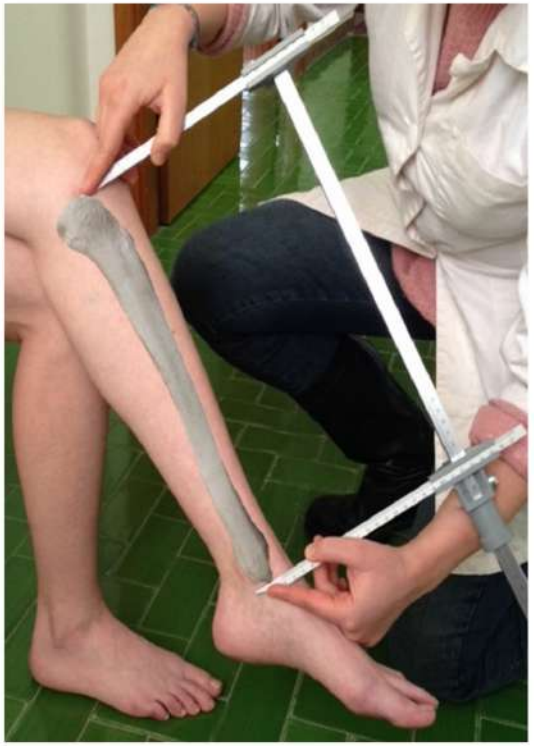


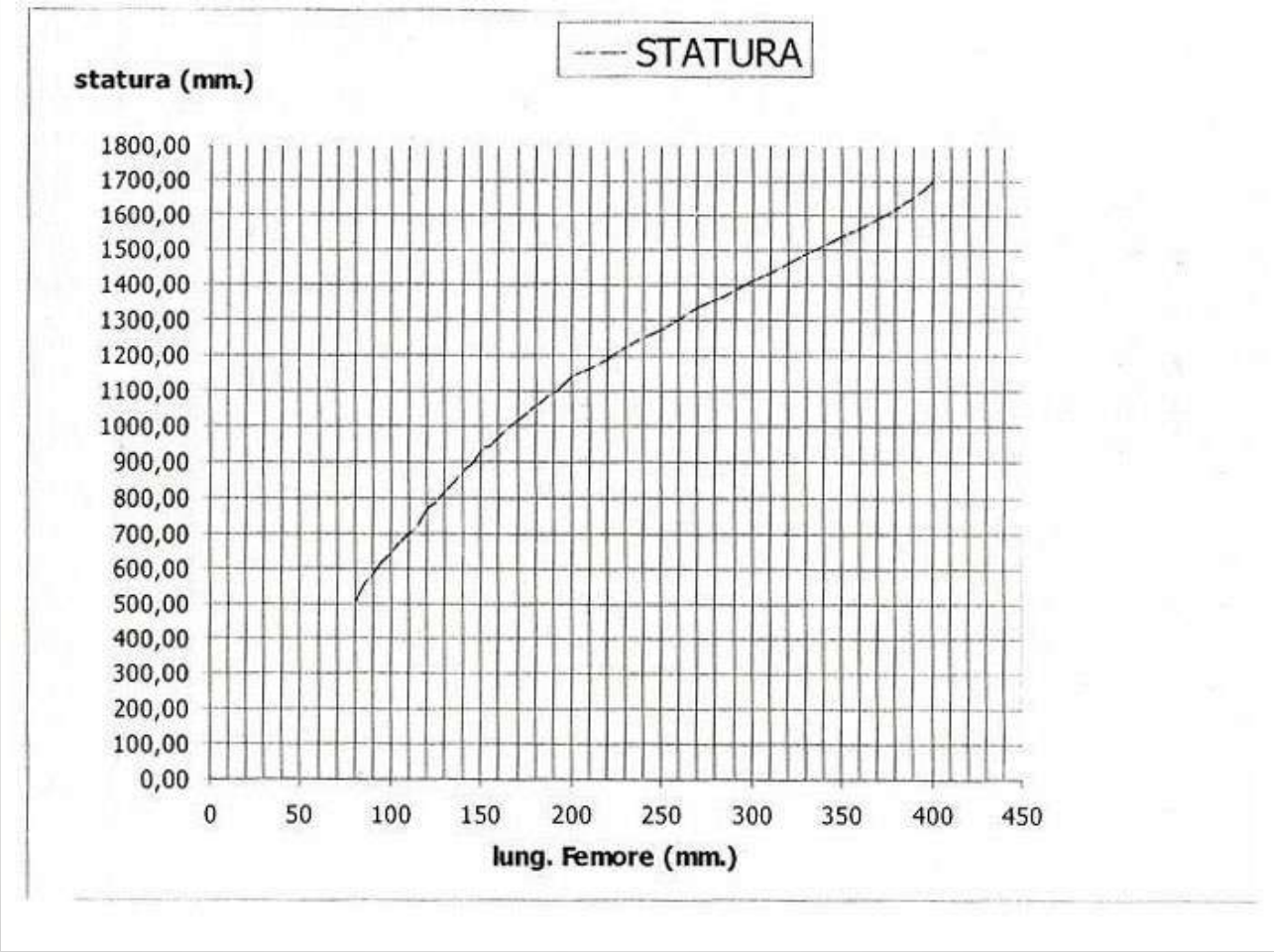
Fig. 1. Anthropometric measurement of percutaneous tibia length.

Table 2
Correlation coefficient and regression equations for the estimation of stature in males, females and combined sexes.

Parameter	Males	Females	Combined
	(n = 204)	(n = 140)	(n = 280)
Linear regression equation	Y = 111.39 + 1.663 * X	Y = 94.45 + 1.899 * X	Y = 80.01 + 2.366 * X
SEE	5.01	4.62	6.01
r	0.686	0.747	0.785
R^2	0.471	0.568	0.616
p value	< 0.001	< 0.001	< 0.001

SEE: standard error of estimate; Y: stature (cm); X: tibia length (cm).

Calcolo statura in individui giovani



Calcolo lunghezza del feto

Metodo Balthazard e Derivieux (1921)

S = 5,6 x lunghezza della diafisi femorale + 8 cm.
S = 6,5 x lunghezza della diafisi omerale + 8 cm.
S = 6,5 x lunghezza della diafisi della tibia + 8 cm.

Metodo Olivier e Pineau (1958)

S = 7,92 x lunghezza della diafisi omerale – 0,32 ± 1,8 cm.
S = 8,73 x lunghezza della diafisi dell'ulna – 1,07 ± 1,59 cm.
S = 6,29 x lunghezza della diafisi del femore +4,42 ± 1,82 cm.
S = 7,85 x lunghezza della diafisi della fibula + 2,78 ± 1,55 cm.
S = 7,39 x lunghezza della diafisi della tibia + 3,55 ± 1,8 cm

S= lunghezza corpo

LUNGH. FETALE	ETA' IN MESI LUNARI	LUNGH. FETALE	ETA' IN MESI LUNARI
17,65	4 ¼	37,85	7 ¼
19,81	4 ½	39,13	7 ½
21,88	4 ¾	40,37	7 ¾
23,80	5	41,58	8
25,64	5 ¼	42,74	8 ¼
27,40	5 ½	43,84	8 ½
29,08	5 ¾	44,97	8 ¾
30,69	6	46,03	9
32,23	6 ¼	47,07	9 ¼
33,72	6 ½	48,08	9 ½
35,15	6 ¾	49,06	9 ¾
36,52	7	50,02	10 NASCITA

Stima dell'Età del feto da S

Oppure: ETA' = 5,6 x lunghezza fetale.

Esempio: se la lungh. Calcolata (S) è 24 cm

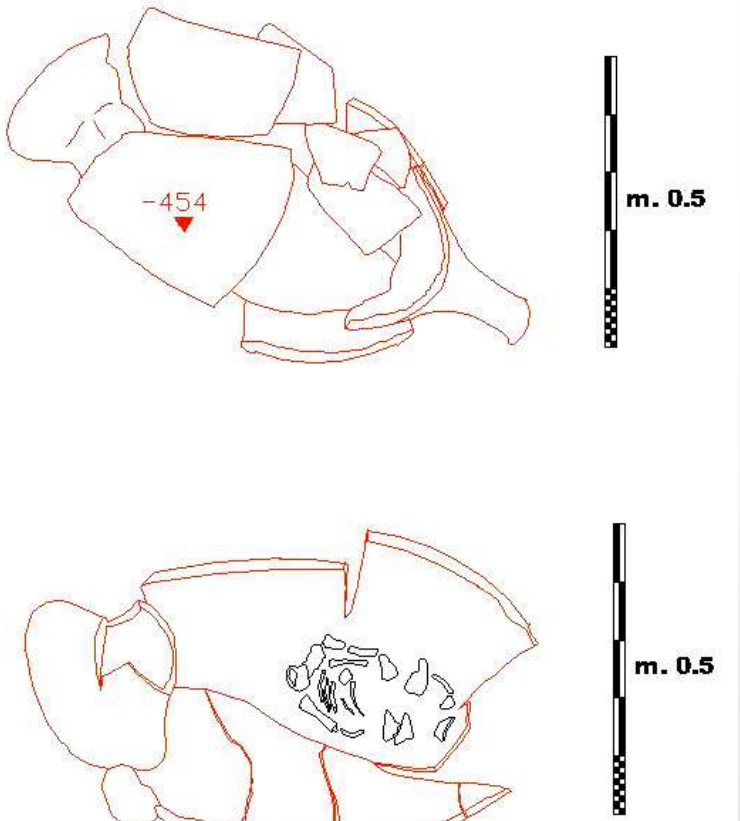
Età = 5.6 x 24=134.4 gg

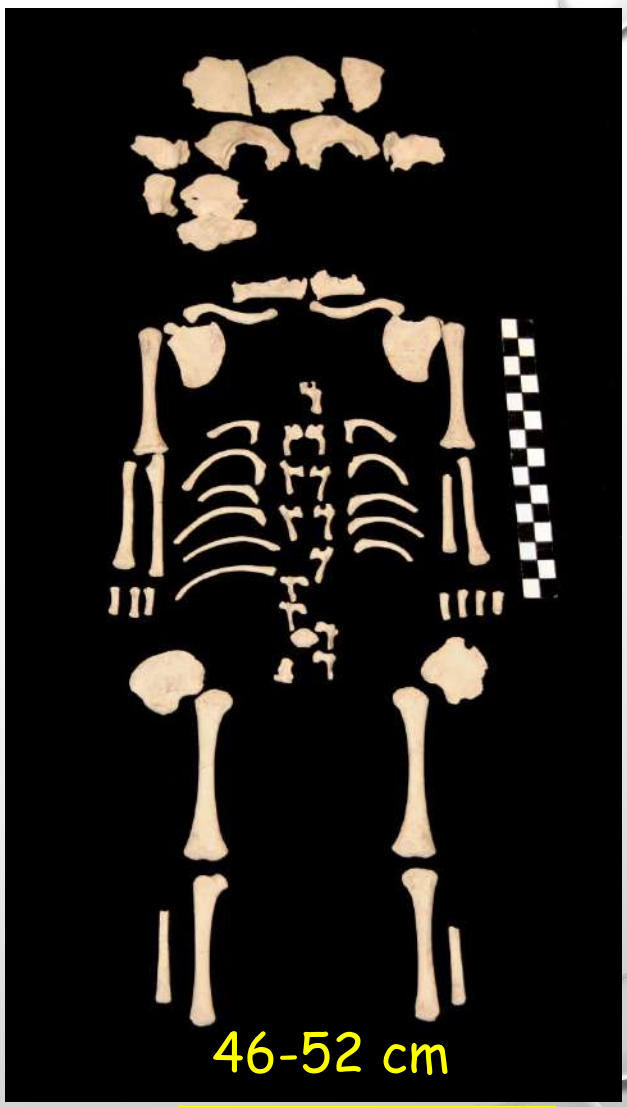
Mese lunare = 27 giorni 7 ore 43 minuti

4.98 mesi lunari

necropoli di epoca romana

Tb 110





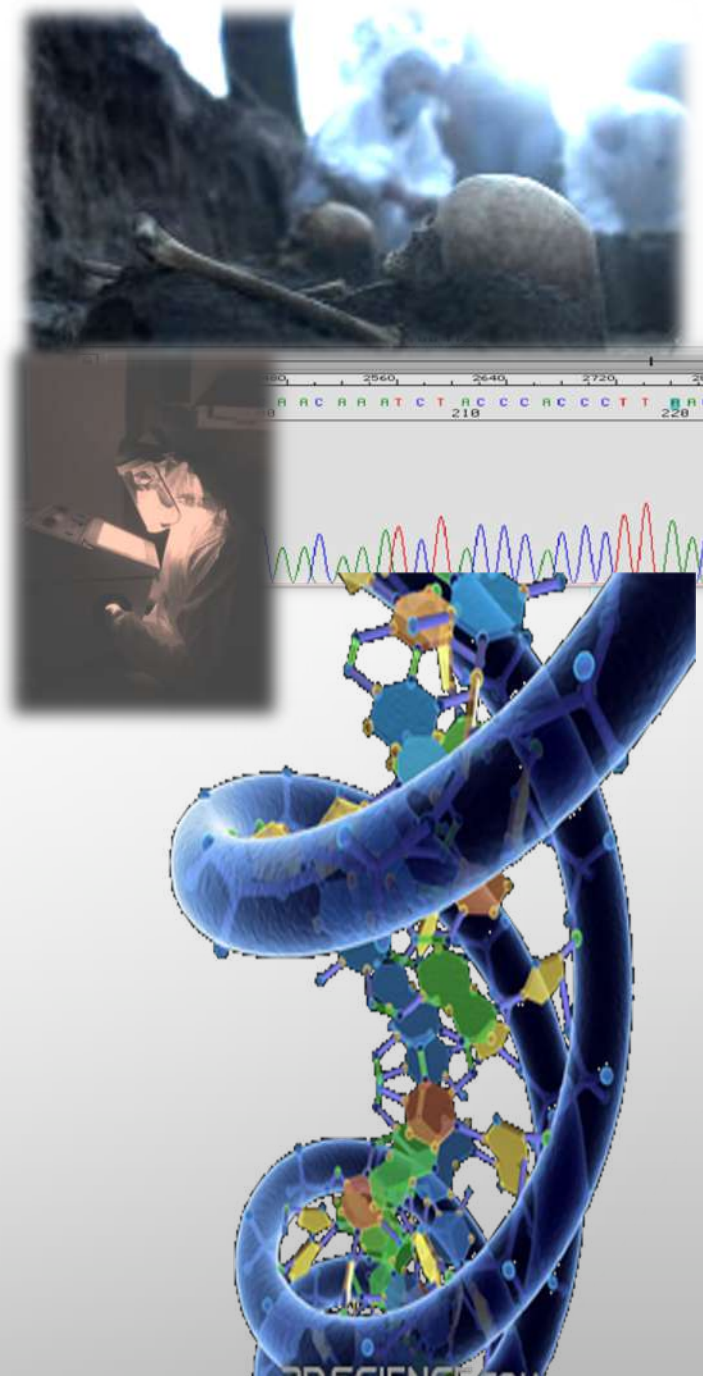
Con il metodo di Olivier e Pineau

9.2 mesi lunari

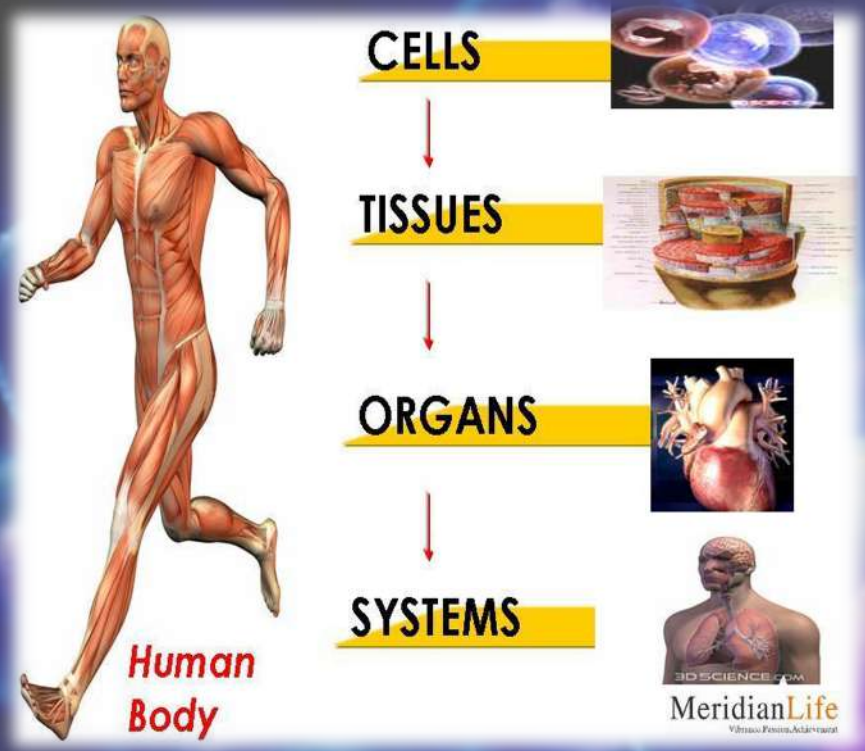
Ancient DNA (aDNA) Analyses of Human remains:

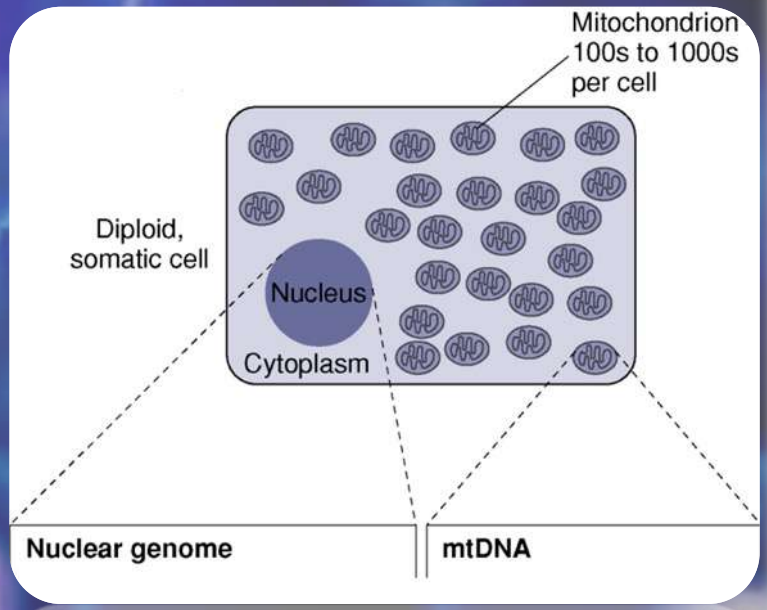
37 years of evolution of a scientific discipline

Barbara Bramanti



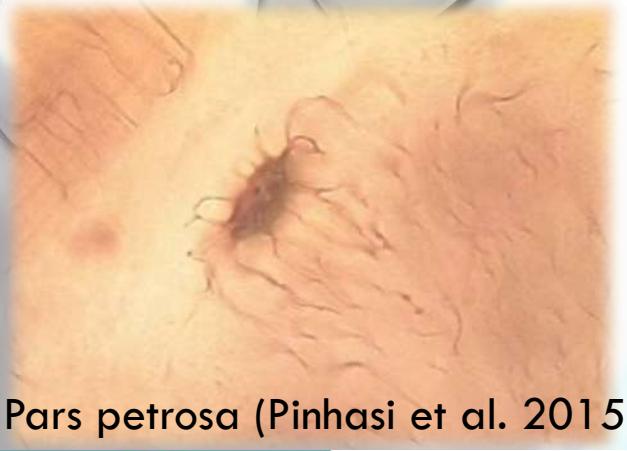
What is ancient DNA (aDNA)?


CELLS
TISSUES
ORGANS
SYSTEMS
Human Body
MeridianLife


Mitochondrion
100s to 1000s
per cell
Diploid,
somatic cell
Nucleus
Cytoplasm
Nuclear genome
mtDNA

The principal source of aDNA

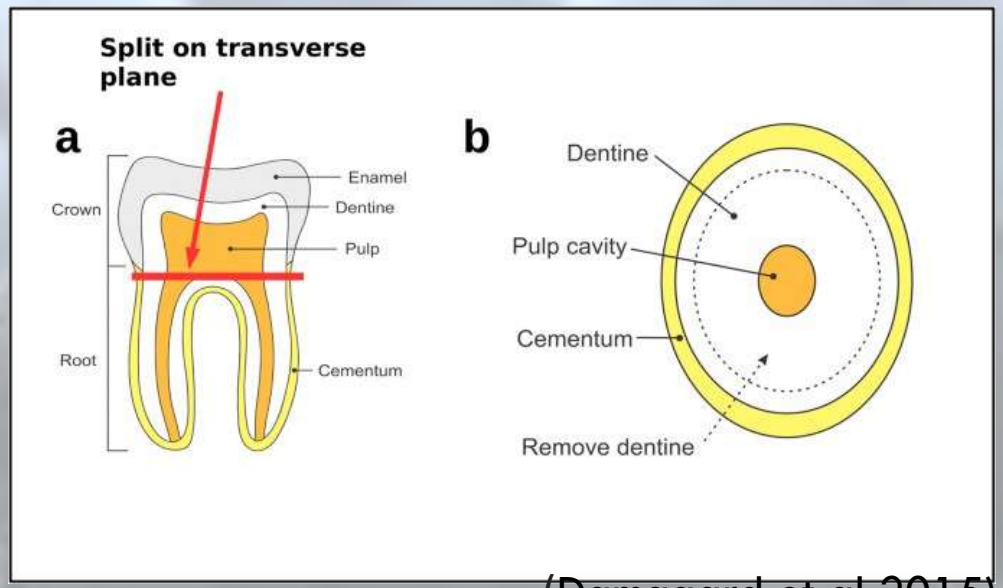
Skeleton (bones and teeth)



Pars petrosa (Pinhasi et al. 2015)

(Pilli et al. 2018)

(Orfanou et al. 2020)
dx.doi.org/10.17504/protocols.io.bqd8ms9w



(Damgaard et al 2015).
https://doi.org/10.1038/srep11184

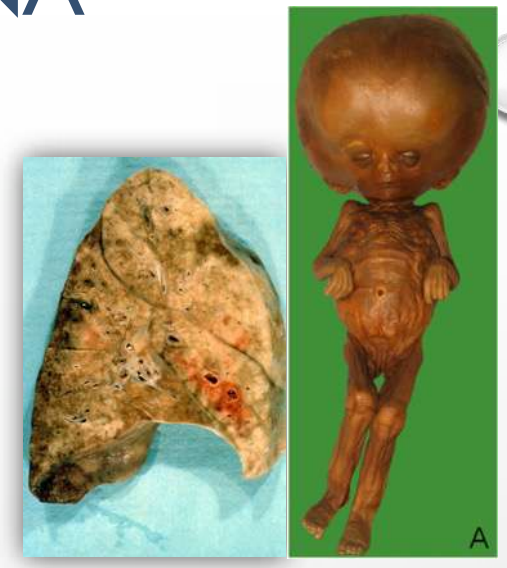
Other sources of aDNA

Corpi imbalsamati

Mummie naturali

Capelli

Tartaro



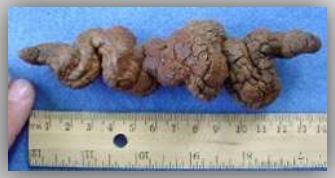
Preparati anatomici

Insetti

Artefatti

Sedimenti e
sporcizia di grotta

Pece di betulla
(di 5000 anni fa)



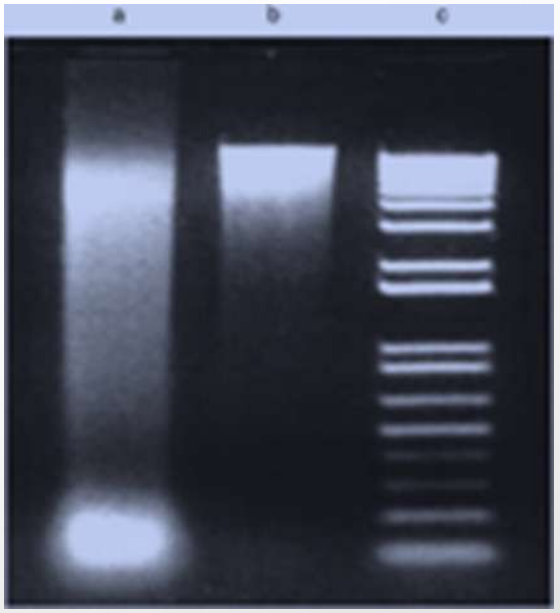
Coproliti

Piante, frutti

- DNA umano
- DNA animale
- DNA vegetale
- DNA batterico
- DNA fungino
- …

Ancient DNA (aDNA)

- Degraded, demaged fragmented DNA
- Low amount
- *Postmortem* base modifications
- Prone to environmental contamination



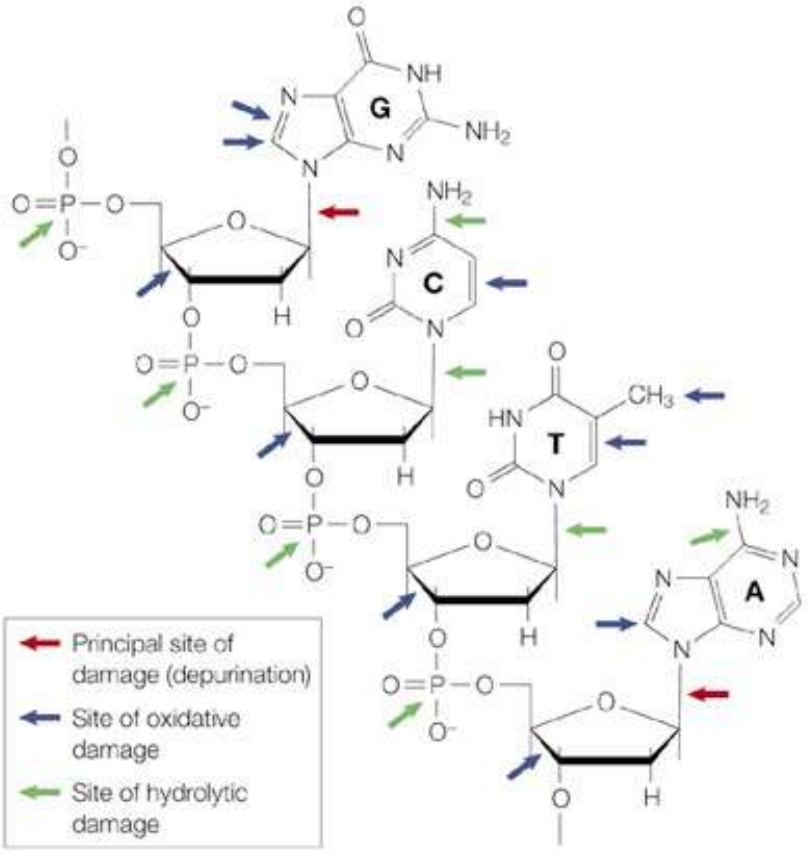
aDNA

Modern DNA

Features of aDNA

FRAGMENTATION due to:

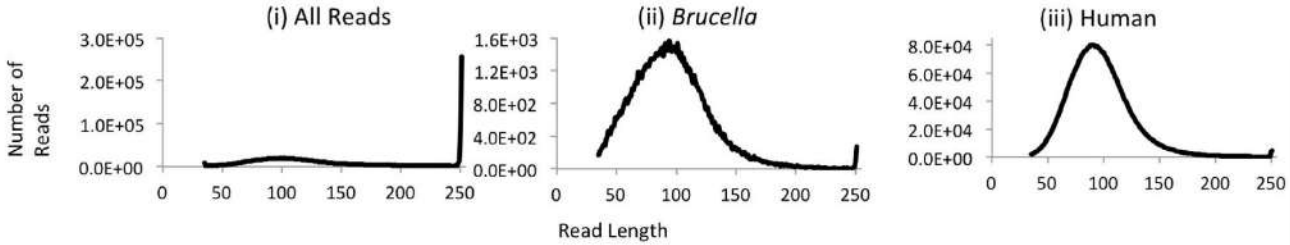
- **Oxidative lesions**
- **Depurination (abasic sites)**
- **Idrolitic reactions**



Hofreiter et al. 2001 https://doi.org/10.1038/35072071

Nature Reviews | Genetics

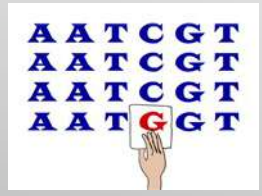
Lunghezza media dei frammenti (reads) di aDNA

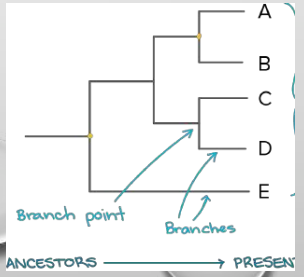


Kay et al. 2014

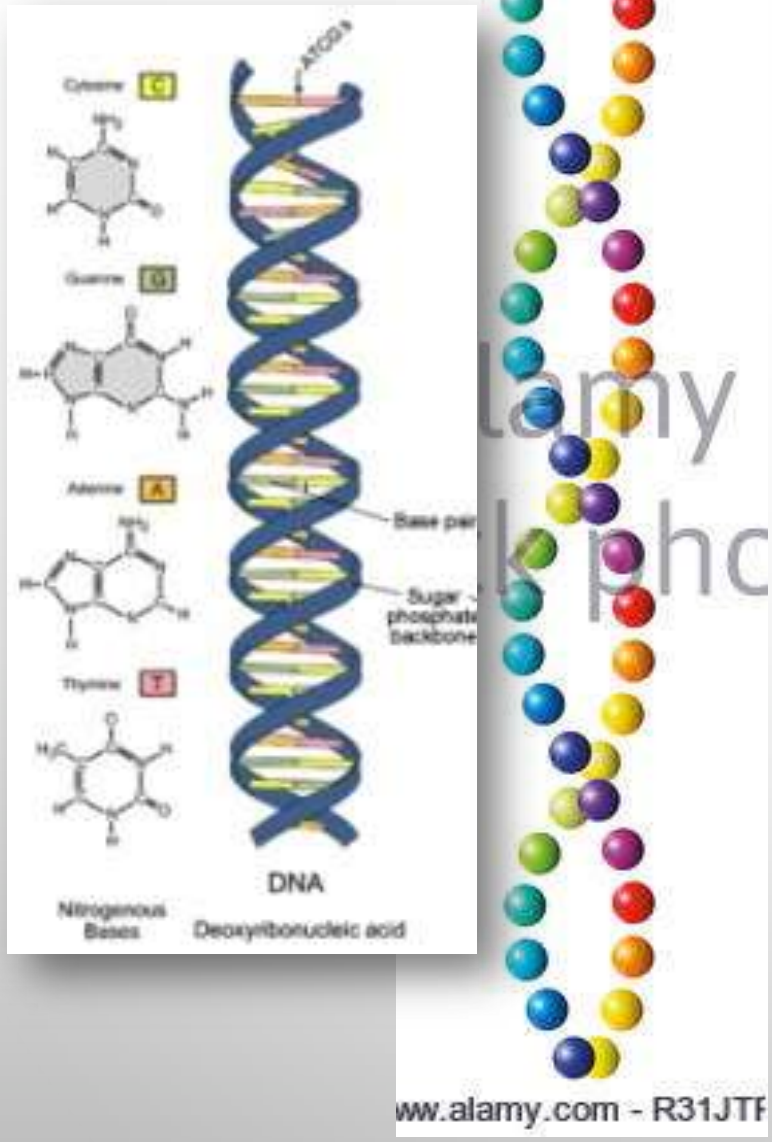
Allignment of fragmented DNA, sequenced by NGS (Next Generation Sequencing)

Confronto con altri genomi (moderni & antichi)

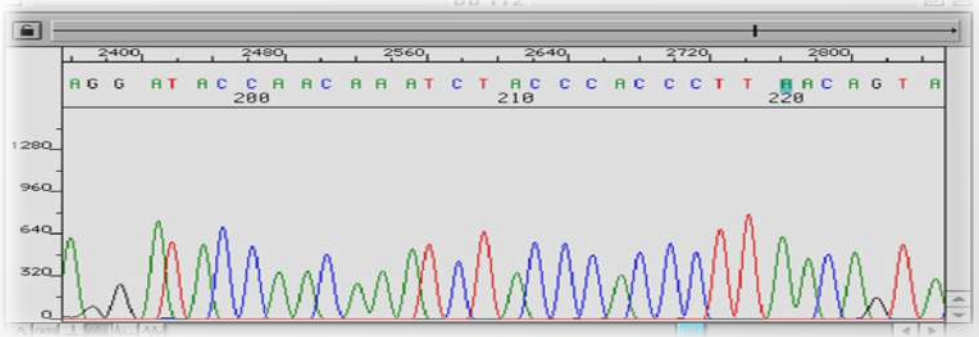




The genetic information is coded in the DNA molecules (4 nucleotides, A, G, C, T)



SEQUENCING



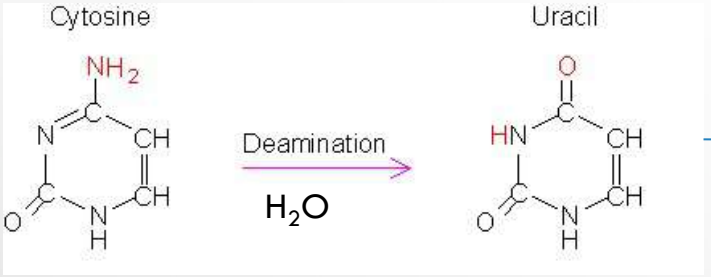
Variability: SNP = Single Nucleotide Polymorphism

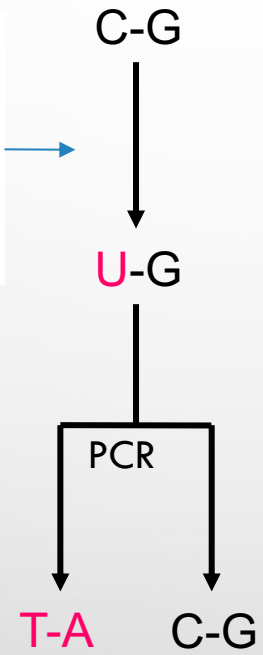
	16037	16067	16071	16081	16126	16129	16135	16145	16160	16166	16170	16172	16182	16183	16185	16189	16223	16224	16255	16260	16265	16278	16292	16298	16311	16319	16360	16362	16391	PO (n)	PK (n)	PS (n)	PL (n)	Total
CRS	A	C	C	C	T	G	T	G	A	A	A	T	A	A	C	T	C	T	G	C	A	C	C	T	T	G	C	T	G					
Wht1																	T						T							7	10	6	13	36
Wht2																	T													1	0	0	0	1
Iht1						A											T												A	0	1	0	0	1
Iht2						A					C						T								C				A	0	1	7	0	8
Iht3						A		A									T												A	0	0	0	1	1
Iht4						A				C							T												A	0	0	0	4	4
Xht1													C		C	T	T		A			T								0	0	0	1	1
Xht2				T									C		C	T	T		A			T								0	0	0	2	2
Xht3													C		C	T	T					T								1	0	0	0	1
Mht1						A								T		T	C		T				C							2	5	1	0	8
Mht2						A				G				T		T	C		T				C							0	1	0	0	1
Mht3								G															C				C			1	0	0	0	1
Other	G		T								C																			0	0	1	0	1
Total																														12	18	15	21	66

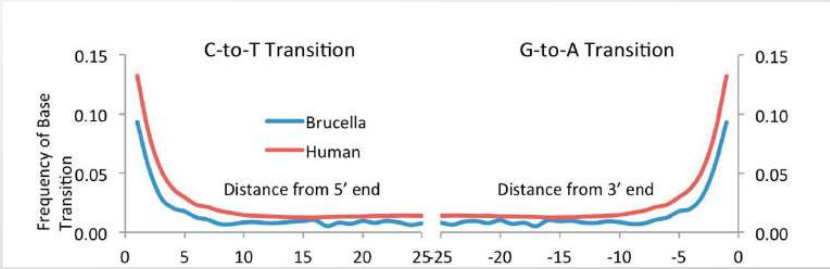
Features of aDNA

SOSTITUZIONI DI BASI

Deaminazione della Citosina







Kay et al. 2014

E' così tipica, soprattutto alla fine dei frammenti, che permette il riconoscimento del aDNA!!

HOW LONG CAN aDNA BE PRESERVED?

Secondo uno studio di Allentof (2012) il tempo di decadimento del DNA/2 sarebbe 521 anni…

Ambienti ideali

Fattori ambientali che inducono degradazione dell'aDNA:

- Umidità
- Microrganismi, insetti, muffe
- Temperature elevate
- Acidità (-pH)

2016: 430,000-year-old DNA of an early-Neanderthal found in Spain's Sima de los Huesos.

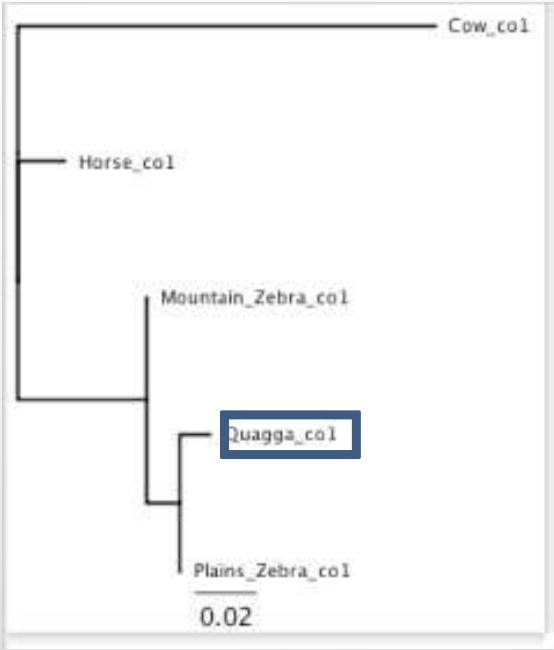
2013: full genome of an ancestral horse species (permafrost of North America more than 700,000 years ago) - the oldest complete genome sequenced thus far.

2021: Oldest Modern Human Genome Reconstructed Using DNA From 45,000-Year-Old Skull

A bit of History…

1984 Russell G. Higuchi and colleagues carried out the first complete ancient DNA study

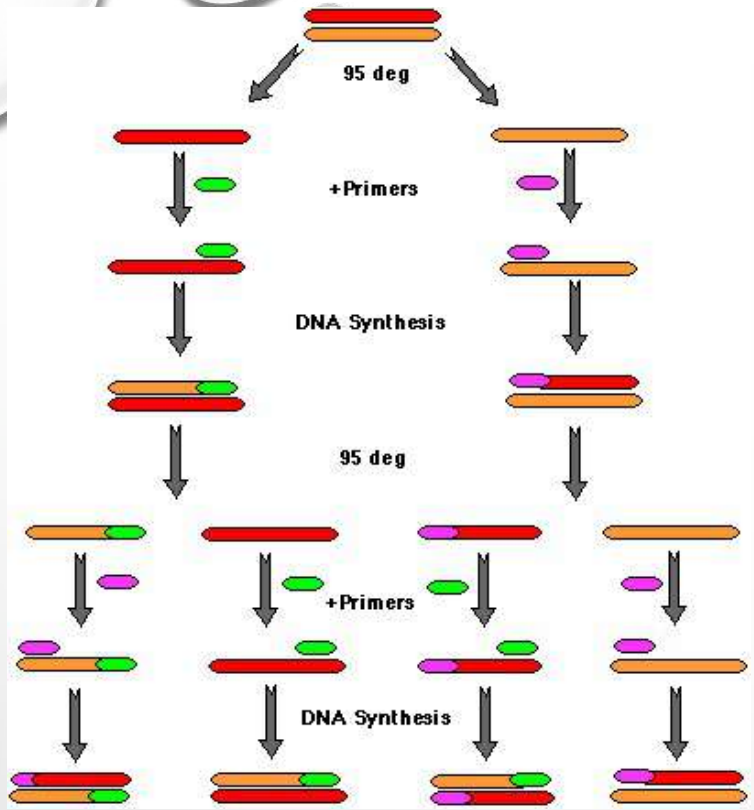
Higuchi R, Bowman B, Freiberger M, Ryder OA, Wilson AC, *DNA sequences from the quagga, an extinct member of the horse family*, in *Nature*, vol. 312, n° 5991, 1984, pp. 282–4



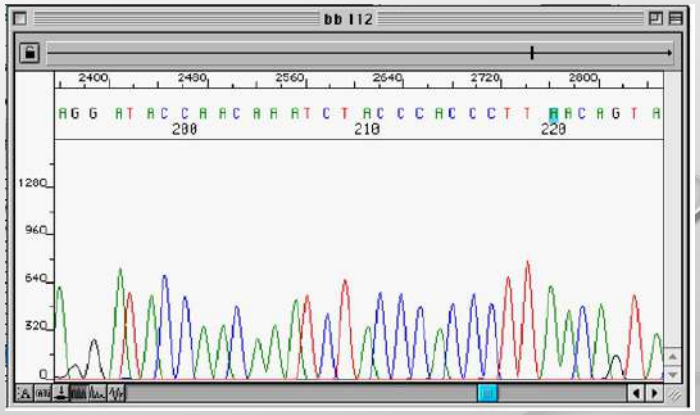
Family of quaggas (*Equus quagga quagga*), 150 years old, at the Naturhistorische Museum in Mainz

Pääbo, S. Molecular cloning of Ancient Egyptian mummy DNA, *Nature* **314**, 644-645 (1985)

The first ancient human sequence (ca. 2,400 YBP) contained only two sequencing errors (1989).



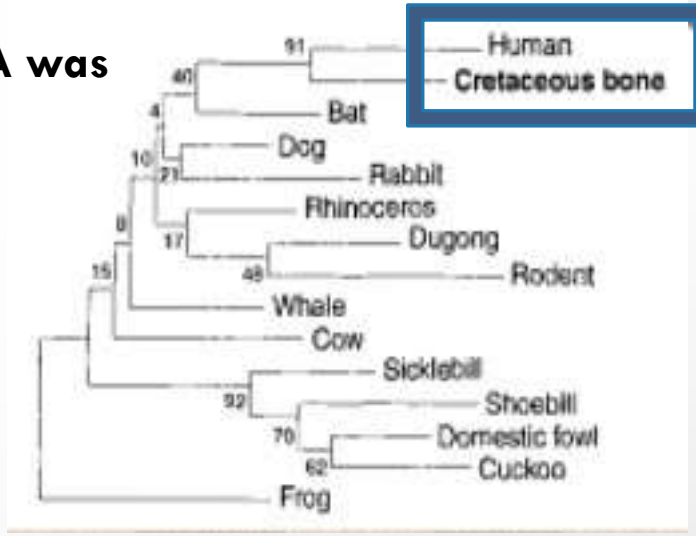
1984 K. Mullis invented the PCR



1994. Scott Woodward claimed to have sequenced aDNA from an 80 million years old Dinosaur bone

CANO,R. J., H. N. POINAR,D. W. ROUBIK,and G. 0. POINAR JR. 1992. Enzymatic amplification and nucleotide sequencing of portions of the 18s rRNA gene of the bee Proplebeia dominicana (Apidae: Hymenoptera) isolated from 25-40 million year old Dominican amber. Med. Sci. Res. 20:619- 622.

1995. S.B. Hedges, S. Paabo and M. Allard demonstrated that **Woodward's dinosaur DNA was instead (male) human DNA**



Poly professor brews beer with 45-million-year-old yeast (January, 18th, 2011)

Continuing concerns about the rigor of research on ancient DNA and that "high-profile journals continue to publish studies that do not meet the necessary controls" prompt a list summarizing "criteria of authenticity" required for work published in this area. The role of the polio vaccination program carried out in Central Africa in the late 1950s in the origin of HIV and AIDS (as posited in the book *The River*) is hotly debated. And "the myth...that efficient use of nuclear resources is a proliferation threat" is challenged, and it is suggested that "electricity produced from existing nuclear by-products would be equivalent to that needed by the United States, at present use rates, for hundreds of years."

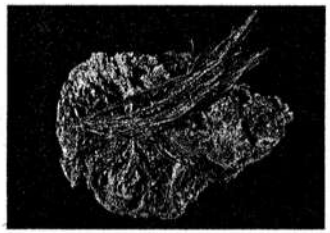
Ancient DNA: Do It Right or Not at All

At the recent 5th International Ancient DNA Conference in Manchester, U.K., reported by Erik Stokstad in his News Focus article "Divining diet and disease from DNA" (28 Jul., p. 530), one presentation boldly opened with the claim that the field was now mature and could move ahead with confidence. This optimism is unfounded, as demonstrated by the notable absence of "criteria of authenticity" from many presentations at the conference. Ancient DNA research presents extreme technical difficulties because of the minute amounts and degraded nature of surviving DNA and the exceptional risk of contamination. The need to authenticate results became obvious in the mid-1990s when a series of high-profile studies were shown to be unrepeatable (*1*). For example, DNA reputed to come from a dinosaur (*2*) was actually contamination by a human mitochondrial gene insertion in the nucleus (numt) (*3*). Over the ensuing years, criteria have been developed and put into practice by some practitioners in the field. Regrettably, despite the recommendation that such criteria be routinely applied (*4*–*6*), high-profile journals continue to publish studies that do not meet the necessary controls (*7*), and many new researchers fail to utilize them. To publicize these standards, we summarize the key criteria below.

Physically isolated work area. To avoid contamination, it is essential that, prior to the amplification stage, all ancient DNA research is carried out in a dedicated, isolated environment. A building in which large amounts of the target DNA are routinely amplified is obviously undesirable (*8*).

Control amplifications. Multiple extraction and PCR controls must be performed to detect sporadic or low-copy number contamination, although carrier effects do limit their efficacy (*4, 9*). All contaminated results should be reported, and positive controls should generally be avoided, as they provide a contamination risk.

Appropriate molecular behavior. PCR amplification strength should be inversely related to product size (large 500– to 1000–base pair products are unusual). Reproducible mitochondrial DNA (mtDNA) results should be obtainable if single-copy nuclear or pathogen DNA is detected. Deviations from these expectations should be justified; e.g., with biochemical data. Sequences should make phylogenetic sense.



Human paleofeces, 8000 to 500 years old, from Hinds Cave, Texas, USA, is a good source of DNA for both humans and the food they ate.

Reproducibility. Results should be repeatable from the same, and different, DNA extracts of a specimen. Different, overlapping primer pairs should be used to increase the chance of detecting numts (*10*) or contamination by a PCR product.

Cloning. Direct PCR sequences must be verified by cloning amplified products to determine the ratio of endogenous to exogenous sequences, damage-induced errors, and to detect the presence of numts. Overlapping fragments are desirable to confirm that sequence variation is authentic and not the product of errors introduced when PCR amplification starts from a small number of damaged templates (*11*).

Independent replication. Intra-laboratory contamination can only be discounted when separate samples of a specimen are extracted and sequenced in independent laboratories. This is particularly important with human remains or novel, unexpected results.

Biochemical preservation. Indirect evidence for DNA survival in a specimen can be provided by assessing the total amount, composition, and relative extent of diagenetic change in amino acids and other residues (*12, 13*).

Quantitation.* The copy number of the DNA target should be assessed using competitive PCR (*4, 11*). When the number of starting templates is low (<1,000), it may be impossible to exclude the possibility of sporadic contamination, especially for human DNA studies.

Associated remains.* In studies of human remains where contamination is especially problematic, evidence that similar DNA targets survive in associated faunal material is critical supporting evidence. Faunal remains also make good negative controls for human PCR amplifications.

We recognize that adherence to these criteria as part of routine good practice is both expensive and time-consuming. However, failure to do so can only lead to an increasing number of dubious claims, which will bring the entire field into further disrepute. If ancient DNA research is to progress and fulfill its potential as a fully-fledged area of evolutionary research, then it is essential that journal editors, reviewers, granting agencies, and researchers alike subscribe to criteria such as these for all ancient DNA research.

Alan Cooper
Departments of Zoology and Biological Anthropology, University of Oxford, Oxford OX2 6UE, UK. E-mail: alan.cooper@zoo.ox.ac.uk

Hendrik N. Poinar
Max Planck Institute for Evolutionary Anthropology, Inselstrasse 22, D-04103 Leipzig, Germany. E-mail: poinar@eva.mpg.de

*For important discoveries, additional criteria are also essential.

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2. S. R. Woodward, N. J. Weyand, M. Bunnell, *Science* **266**, 1229 (1994).
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4. O. Handt, M. Krings, R. H. Ward, S. Pääbo, *Am. J. Hum. Genet.* **59**, 368 (1996).
5. A. Cooper, *Am. J. Hum. Genet.* **60**, 1001 (1997).
6. R. Ward and C. Stringer, *Nature* **388**, 225 (1997).
7. M. Scholz *et al.*, *Am. J. Hum. Genet.* **66**, 1927 (2000).
8. T. Lindahl, *Nature* **365**, 700 (1993).
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11. M. Krings *et al.*, *Cell* **90**, 19 (1997).
12. H. N. Poinar, M. Höss, J. L. Bada, S. Pääbo, *Science* **272**, 864 (1996).
13. H. N. Poinar and B. A. Stankiewicz, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 8426 (1999).

5 years later…

- Physically isolated work area
- Multiple analyses (Reproducibility)
- [Independent replication]
- Criteria for authenticity (signals of decay, phylogeny, …)

The aDNA Laboratory

The aDNA lab at CEES in Oslo

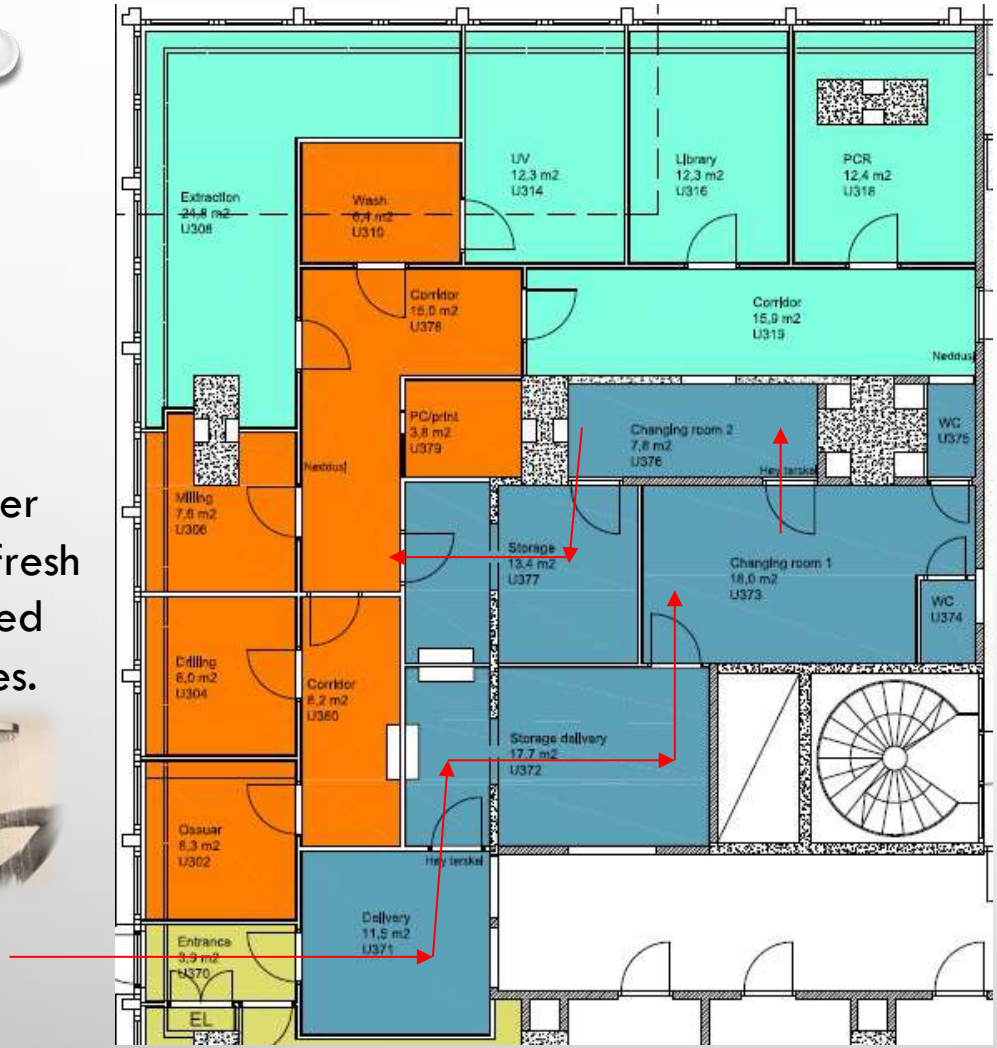
Entrance with Special Kay

Emergency exit

- Only authorised workers are allowed to enter the lab after a special training.
- Independent entrance
- Separate ventilation system with positive pressure.

The aDNA lab at CEES in Oslo

Shower and fresh washed clothes.



Wear protective clothes.

Leave your clothes in the lockers.

Leave your pieces of external clothes in the lockers.

Inside the lab

aDNA worker's outfit and behaviour:

1. One-way rule, freshly showered and freshly washed clothes, direct way, never entering building with offices and other labs prior to aDNA lab

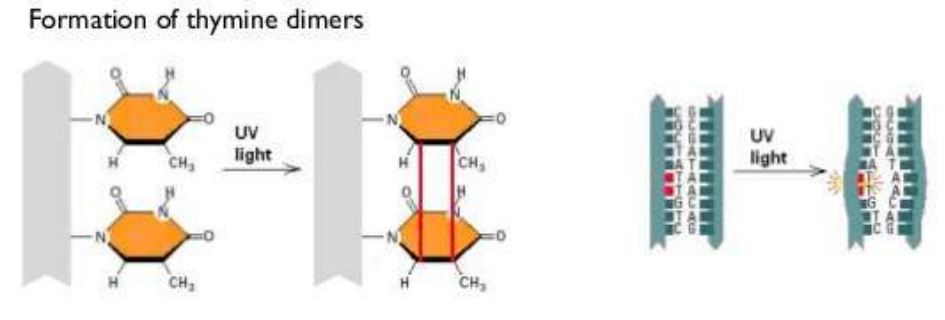
2. Cover skin, prevent loss of eyelashes and hair in the lab to protect aDNA-lab environment from worker's DNA:

- Caps/medical head wear
- Surgical facemask
- Helmet and visor
- Overall
- 2-3 pairs of gloves
- Overshoes

3. Keep Clean!

UV-irradiation

- Produce dimers between two consecutives pyrimidines (especially between two thymines)
- Results in **inhibition of the PCR-reaction**



UV-irradiation of all disposables and working area

UV-irradiation of samples

Even water for cleaning is UV-irradiated!

Experimental procedures

Advices for Sampling

- Wear protective clothes by handling even in the repository (at least gloves and face mask)
- Don't wash the samples for aDNA analyses!!!
- Don't use glue or other chemicals!!!
- Don't write on the specimens!!! Use bags.
- **If possible, isolate two samples of each individual for aDNA analyses during the excavation**
- **Take contact with an accredited aDNA expert for advices asap**

Work-flow

Introduction in the aDNA-Lab

Cataloging & UV

Sandblasting

Milling

Extraction

Preparation of PCRs and libraries for **sequencing**

Una **Library** contiene gli inserti di DNA, uniti ad adapters per NGS e un bar-code

Shotgun Sequencing (Metagenomic analysis)

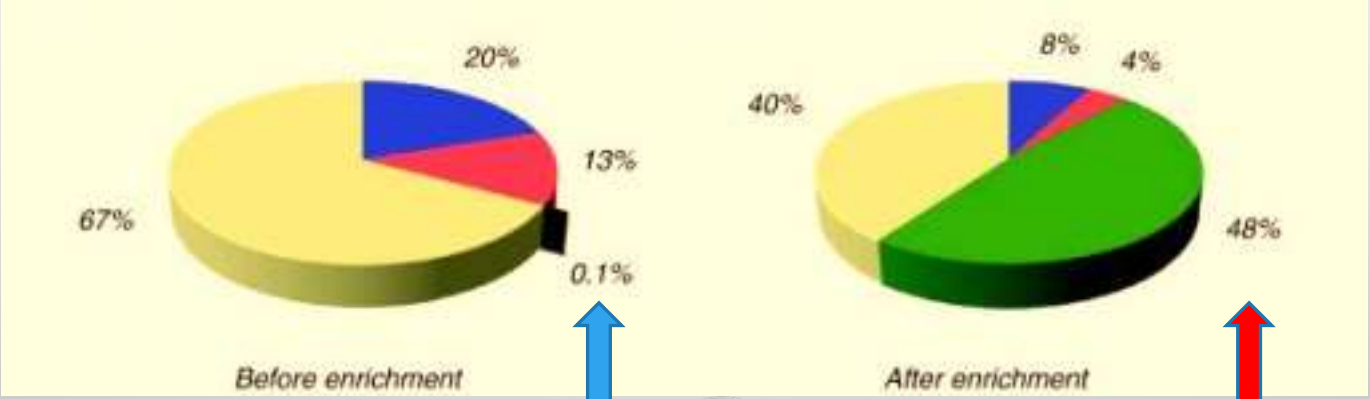
(outside the aDNA)

Whole collection of genomes isolated from a sample.

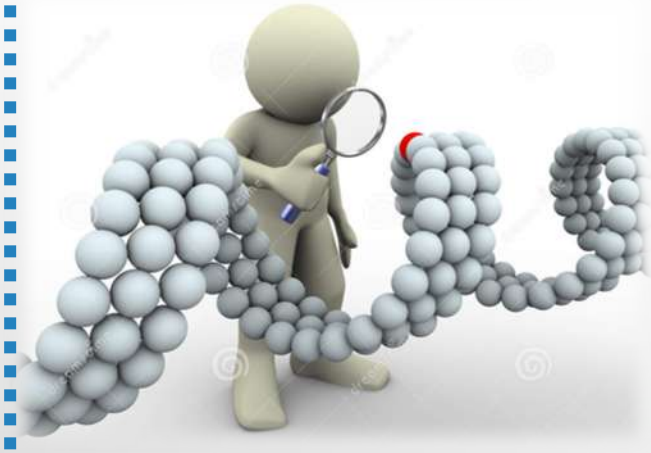
Endogen DNA 1% !!

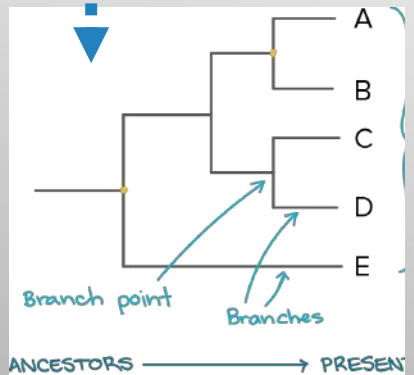
Target enrichment (Capture) & Sequencing

(outside the aDNA)



(Bioinformatic work



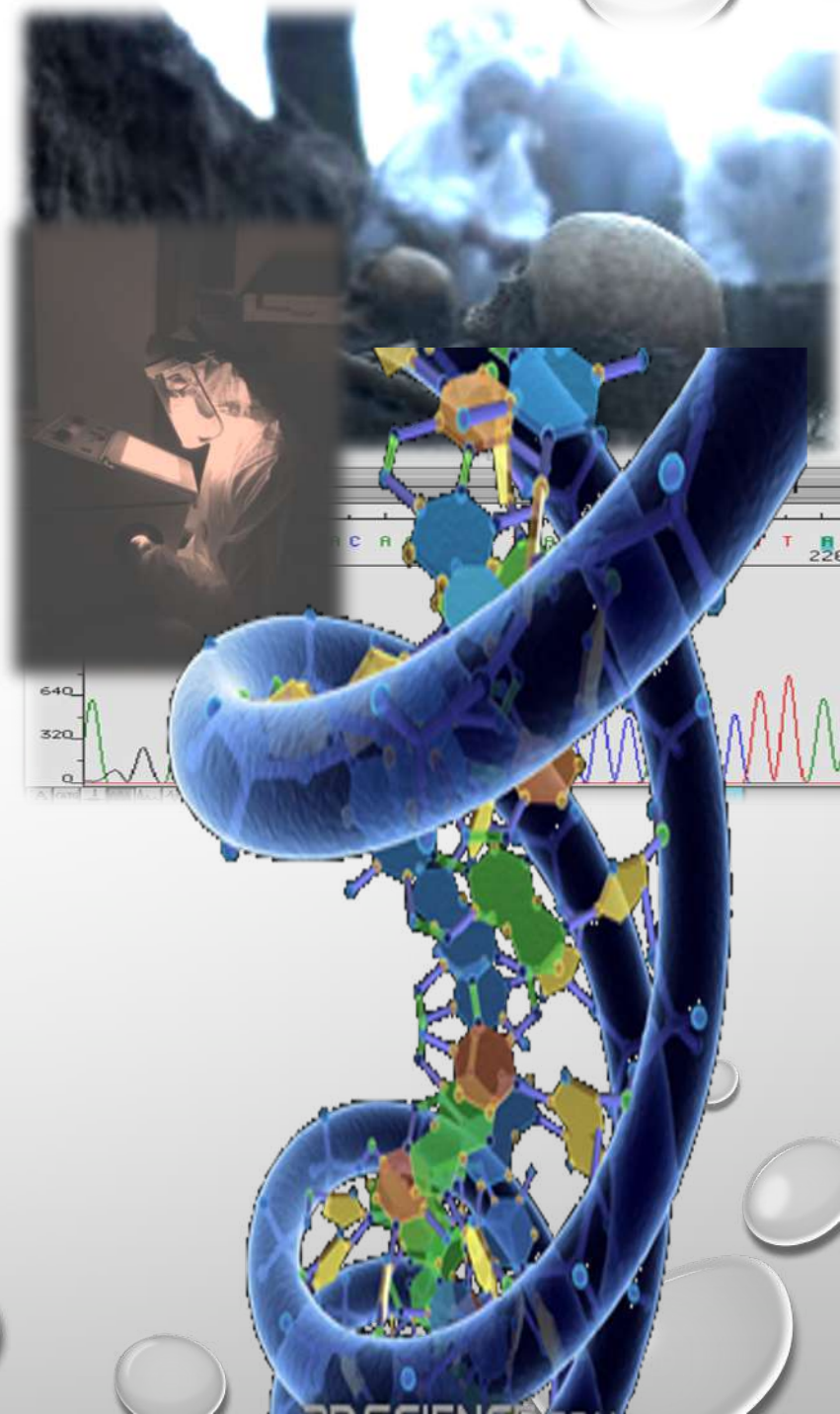


- Loading reads (+ quality info).
- Loading reference sequence(s).
- Demultiplexing (sorting the reads into different files according to their indexes).
- Paired end splitting (sorting for reads sequenced in two directions).
- Trimming (adapters) and filtering of reads according to various quality criteria (for instance length).
- Calculating global statistics on the project.
- Aligning the reads against the reference sequence(s).
- SNPs (or SNVs) calling and
- BLASTing
- …)

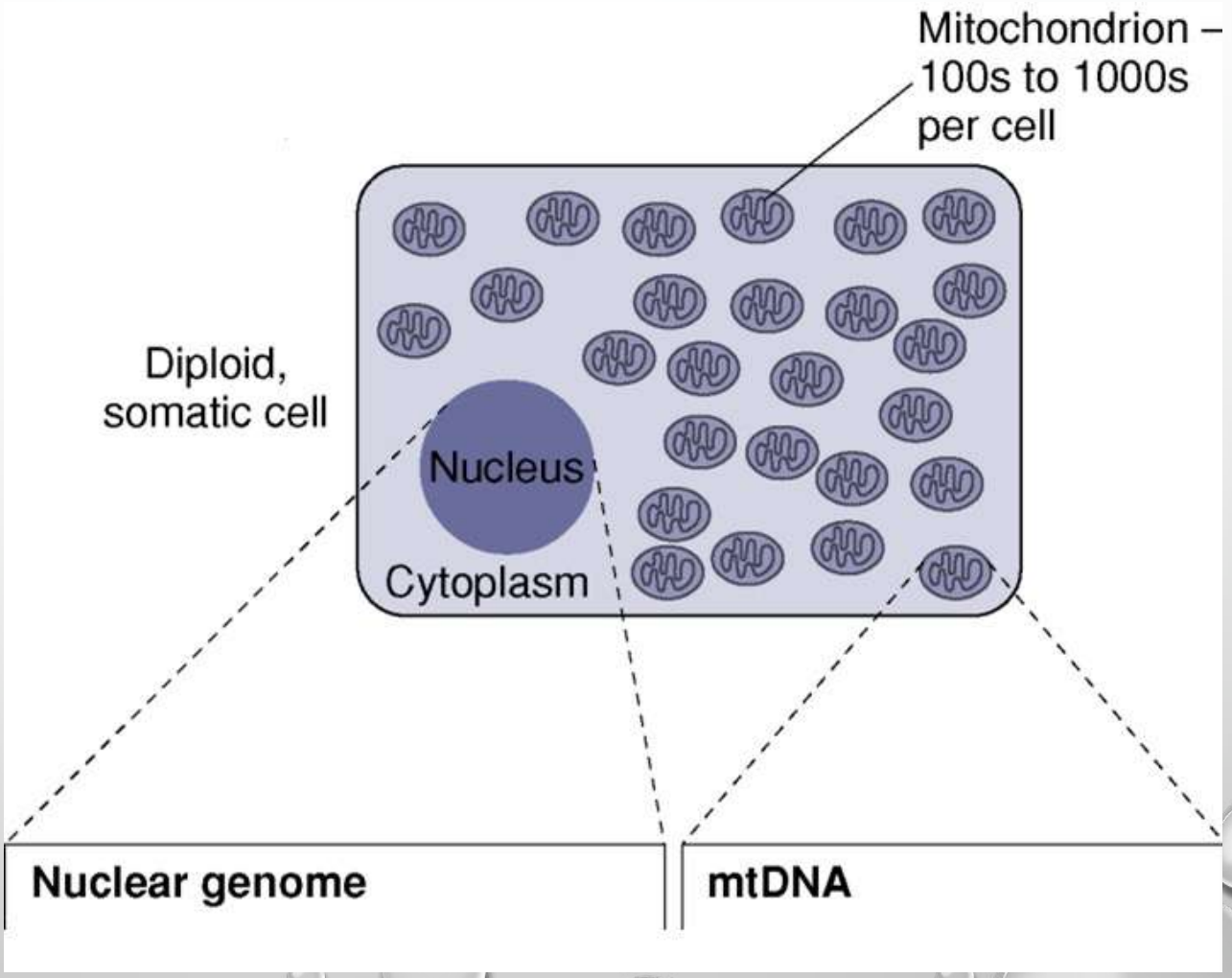
SNP & coverage

Short fragments, *post mortem* bases Substitutions and loss of bp
@ Position 263 A/G = actual SNP (replicated in different fragments)

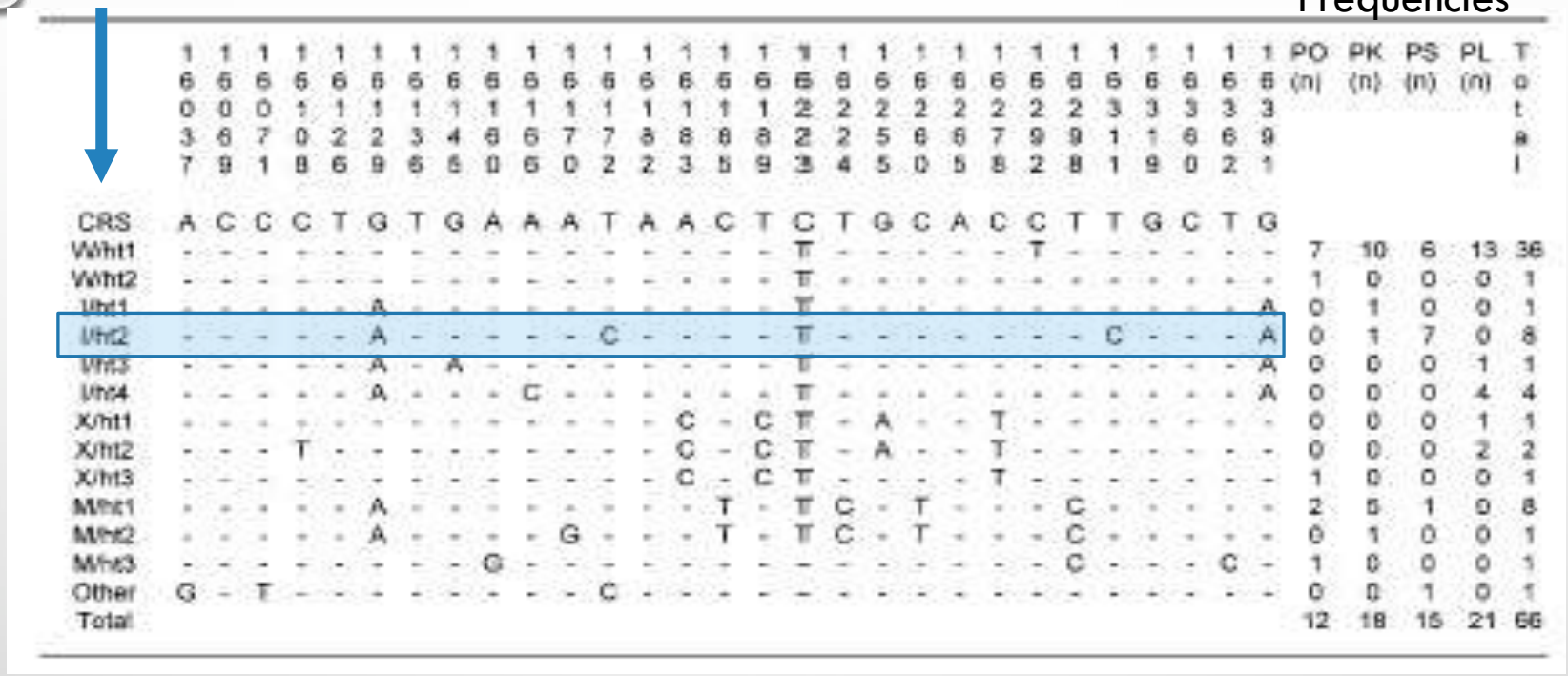
Some examples of aDNA analysis from human remains



Sources of aDNA in mammalian cells



Haplotyp (*haploid genotype*)

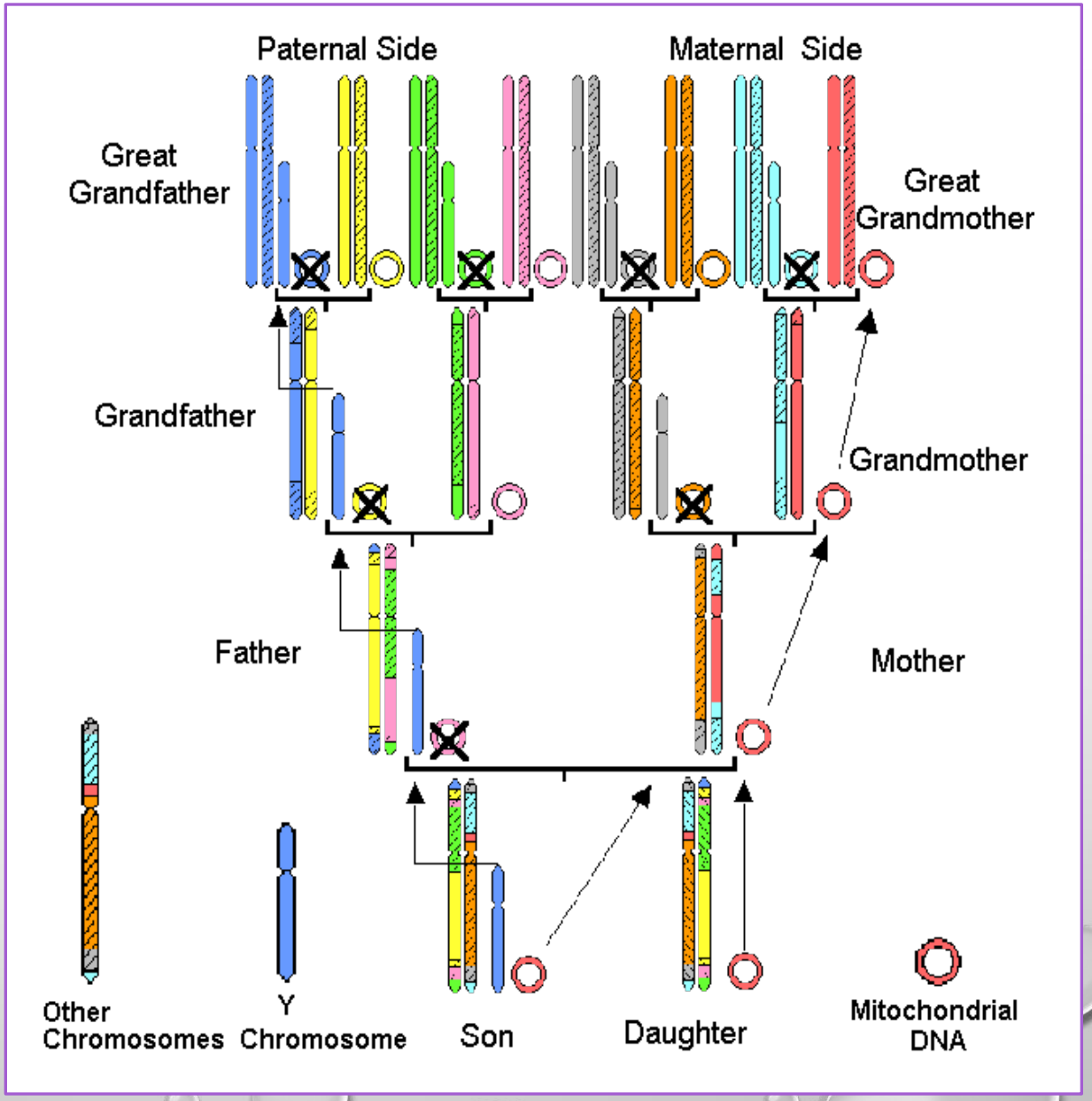


	16037	16069	16071	16108	16126	16129	16136	16145	16160	16166	16170	16172	16182	16183	16185	16189	16223	16224	16255	16260	16265	16278	16292	16298	16311	16319	16360	16362	16391	Frequencies PO (n)	PK (n)	PS (n)	PL (n)	Total
CRS	A	C	C	C	T	G	T	G	A	A	A	T	A	A	C	T	C	T	G	C	A	C	C	T	T	G	C	T	G					
Wht1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	T	-	-	-	-	-	-	7	10	6	13	36
Wht2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0	1
Uht1	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	A	0	1	0	0	1
Uht2	-	-	-	-	-	A	-	-	-	-	-	C	-	-	-	-	T	-	-	-	-	-	-	-	C	-	-	-	A	0	1	7	0	8
Uht3	-	-	-	-	-	A	-	A	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	A	0	0	0	1	1
Uht4	-	-	-	-	-	A	-	-	-	C	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	A	0	0	0	4	4
X/ht1	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	C	T	-	A	-	-	T	-	-	-	-	-	-	-	0	0	0	1	1
X/ht2	-	-	-	T	-	-	-	-	-	-	-	-	-	C	-	C	T	-	A	-	-	T	-	-	-	-	-	-	-	0	0	0	2	2
X/ht3	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	C	T	-	-	-	-	T	-	-	-	-	-	-	-	1	0	0	0	1
M/ht1	-	-	-	-	-	A	-	-	-	-	-	-	-	-	T	-	T	C	-	T	-	-	-	C	-	-	-	-	-	2	5	1	0	8
M/ht2	-	-	-	-	-	A	-	-	-	-	G	-	-	-	T	-	T	C	-	T	-	-	-	C	-	-	-	-	-	0	1	0	0	1
M/ht3	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	C	-	1	0	0	0	1
Other	G	-	T	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	1	0	1
Total																														12	18	15	21	66

(data from Meinilä et al. 2001)

Nuclear genomic DNA vs. mtDNA

No recombination!



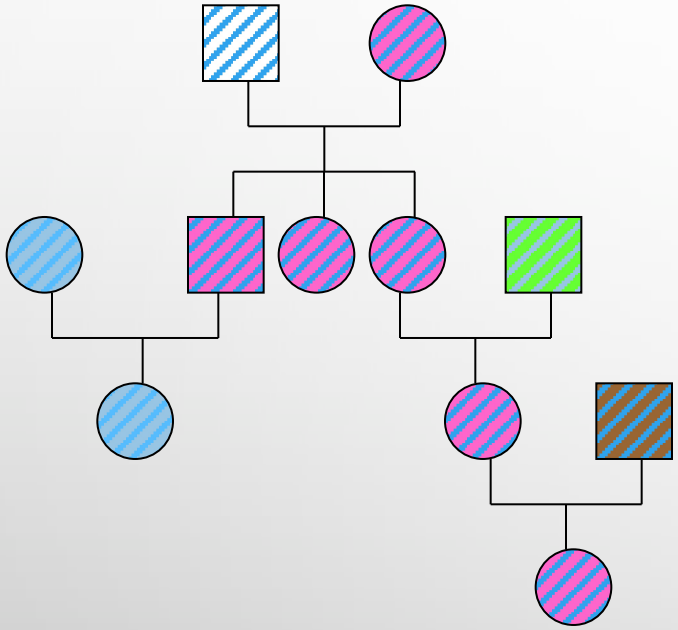
Attribution of skeletal elements

Westerhausen, Iron Age (ca. 270 CE).

- Nine individuals, nine mtDNA haplotypes
- No maternal relationship
- Reconstruction of the individual skeletons
- nDNA confirmed the gender (8 male, 1 female ind.)

Identification and Family reconstruction

Maternal lineage

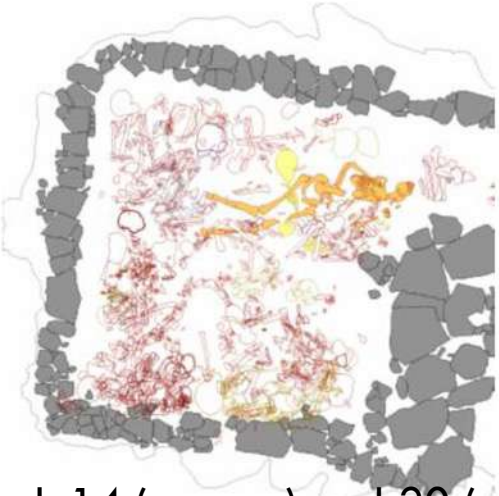


The Romanov

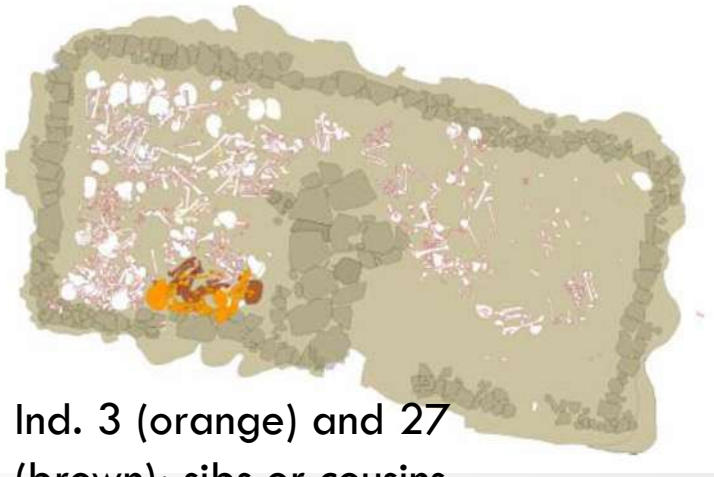
(Gill *et al.* 1996)

The relatives of Benzingerode

Bernburg culture (BEC), 3100 cal BC; mtDNA from 17 out of 21 individuals

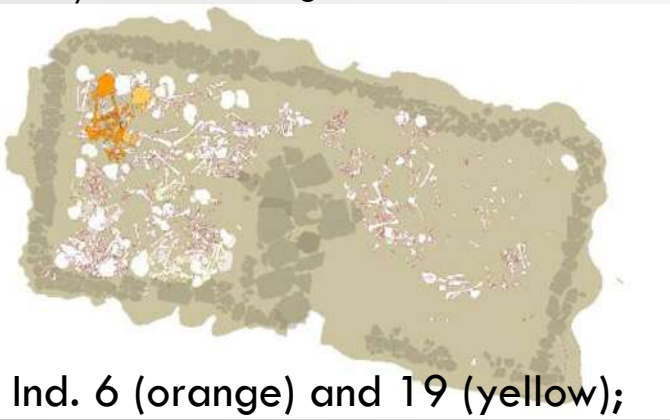


Haplotyp	Ind.	Haplogruppe
1.	1	U
2.	14, 20	
3.	35	
4.	18	
5.	3, 27	K
6.	33	
7.	6, 19	T
8.	17, 36	H
9.	29	
10.	40	
11.	39	V ?
12.	15	W
13.	37	X

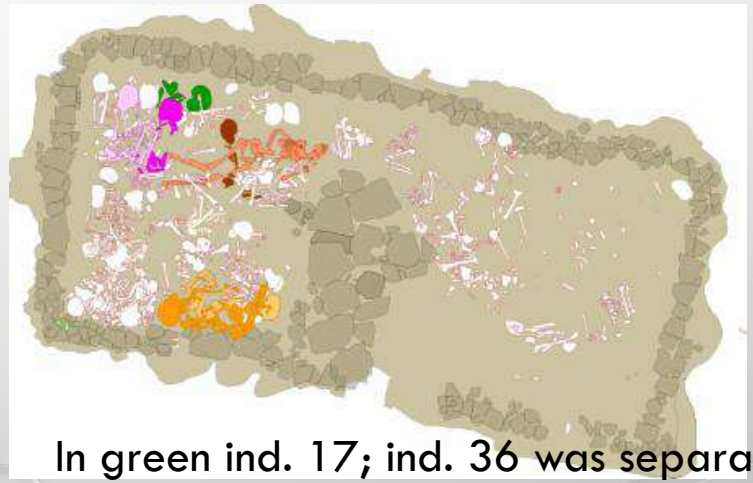


Ind. 3 (orange) and 27 (brown); sibs or cousins

Ind. 14 (orange) and 20 (yellow); child/mother or gramma

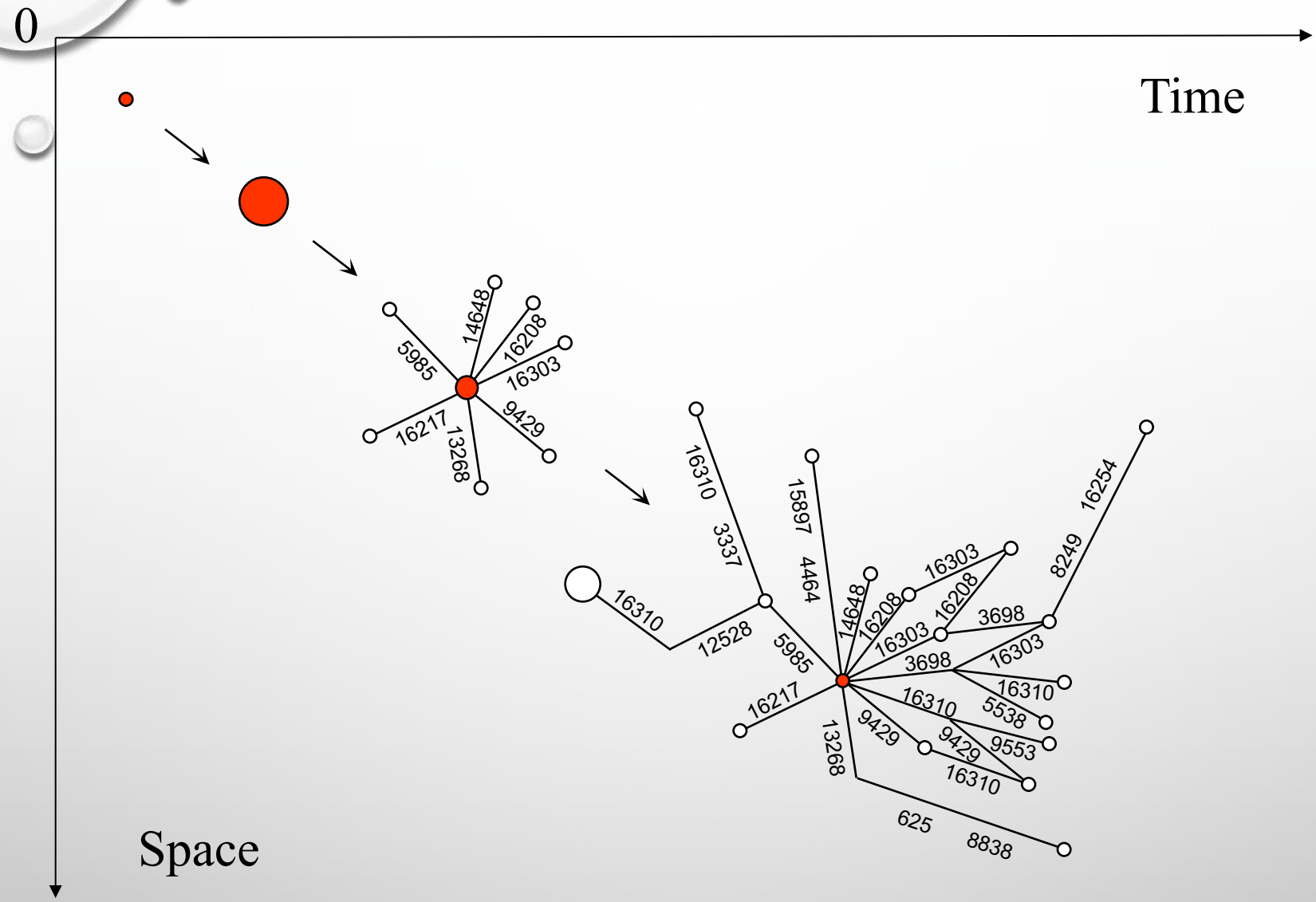


Ind. 6 (orange) and 19 (yellow); daugther/mother or gramma; sibs or cousins



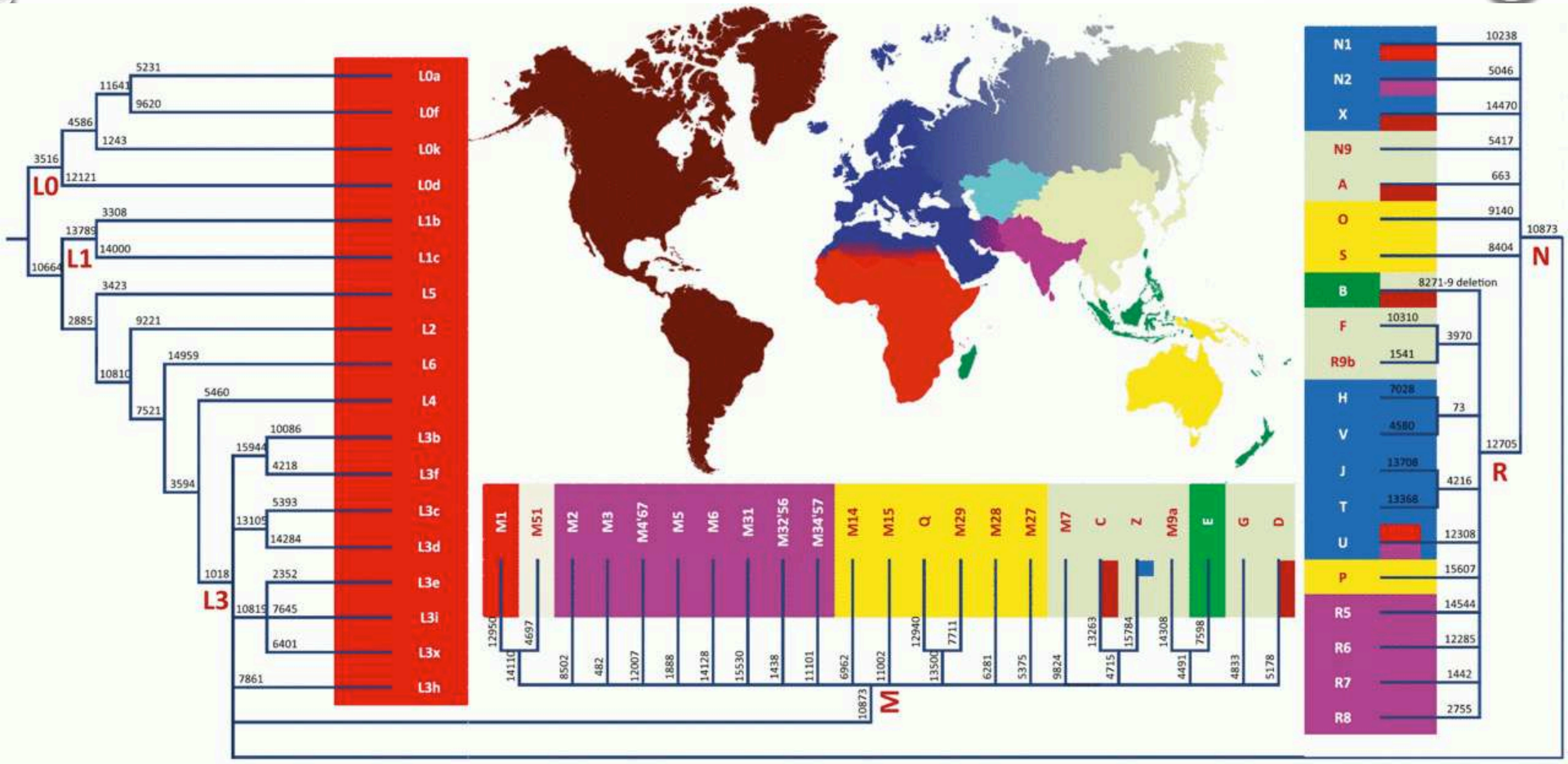
In green ind. 17; ind. 36 was separated.

Haplogroups



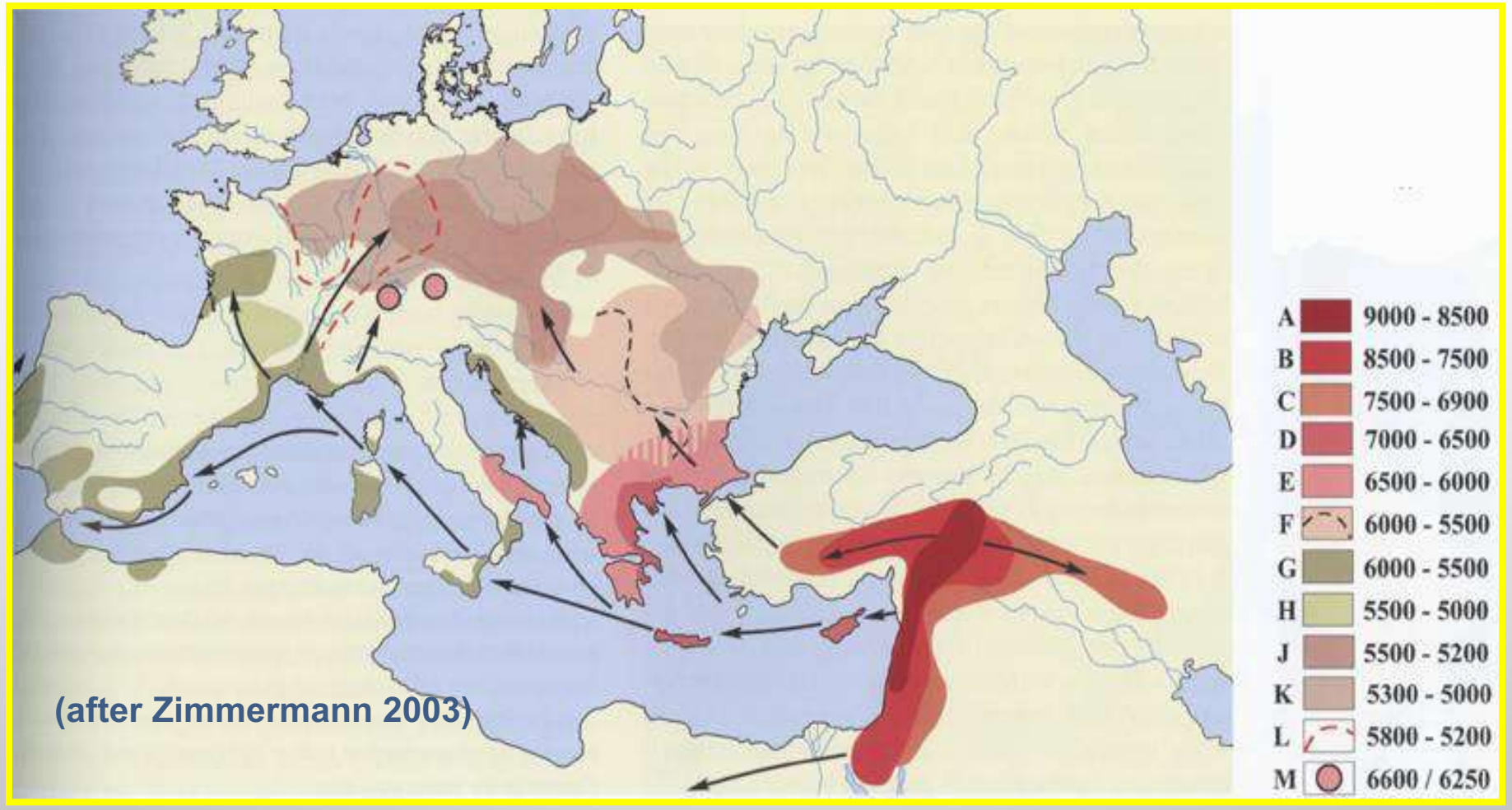
Courtesy of Peter Forster

PHYLOGENETIC TREES and PEOPLING OF THE WORLD at different TIMES



Population Genetics

The Neolithic Transition was due to migrations?

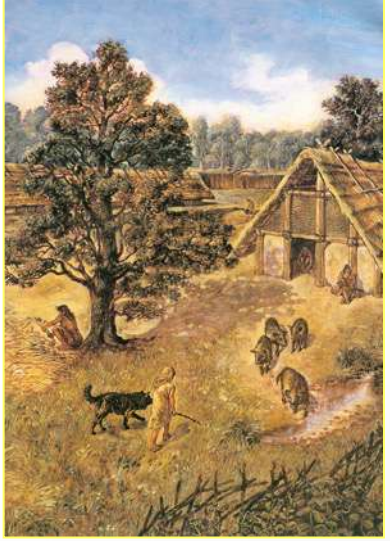


Hunter-gatherers (Palaeo-Mesolithic periods) 45,000-4,000 YBP

- Hunting
- Fishing
- Gathering
- ➢ Nomadism (tents or portable shelters)

Acculturation or immigration

?

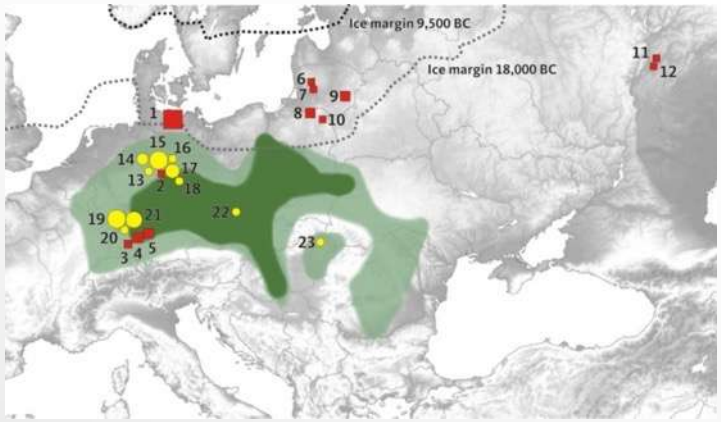


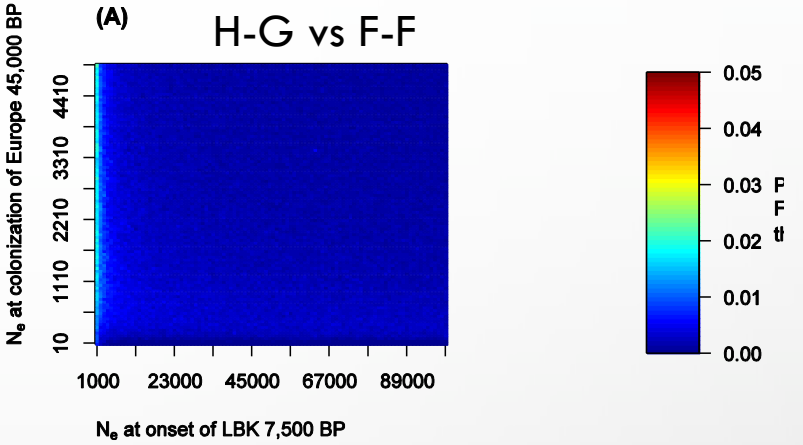
Farmers (Neolithic period) 10,000-4,000 YBP

- Use of pottery
- Agriculture
- Animal husbandry
- ➢ "Urbanisation"
- ➢ Social structures
- ➢ Technology

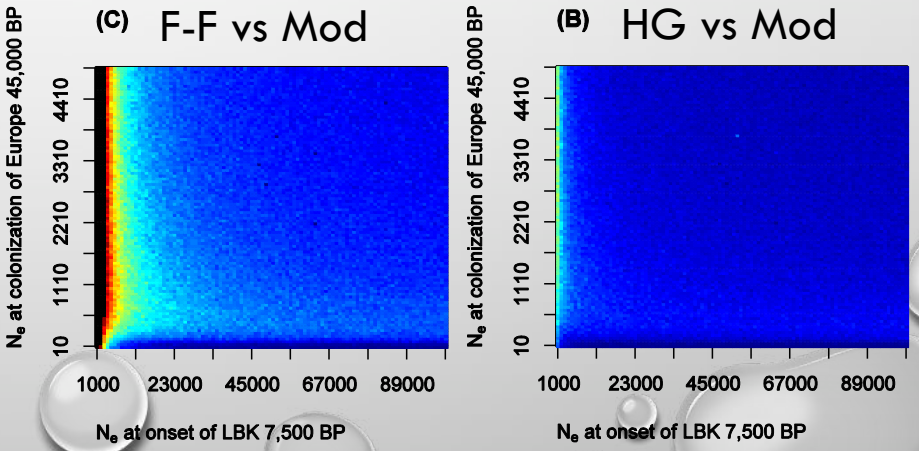
Bramanti et al. *Science* (2009)

1) No genetic continuity between Hunter-Gatherers (**U4 & U5**) & First Farmers (**other haplogroups**)

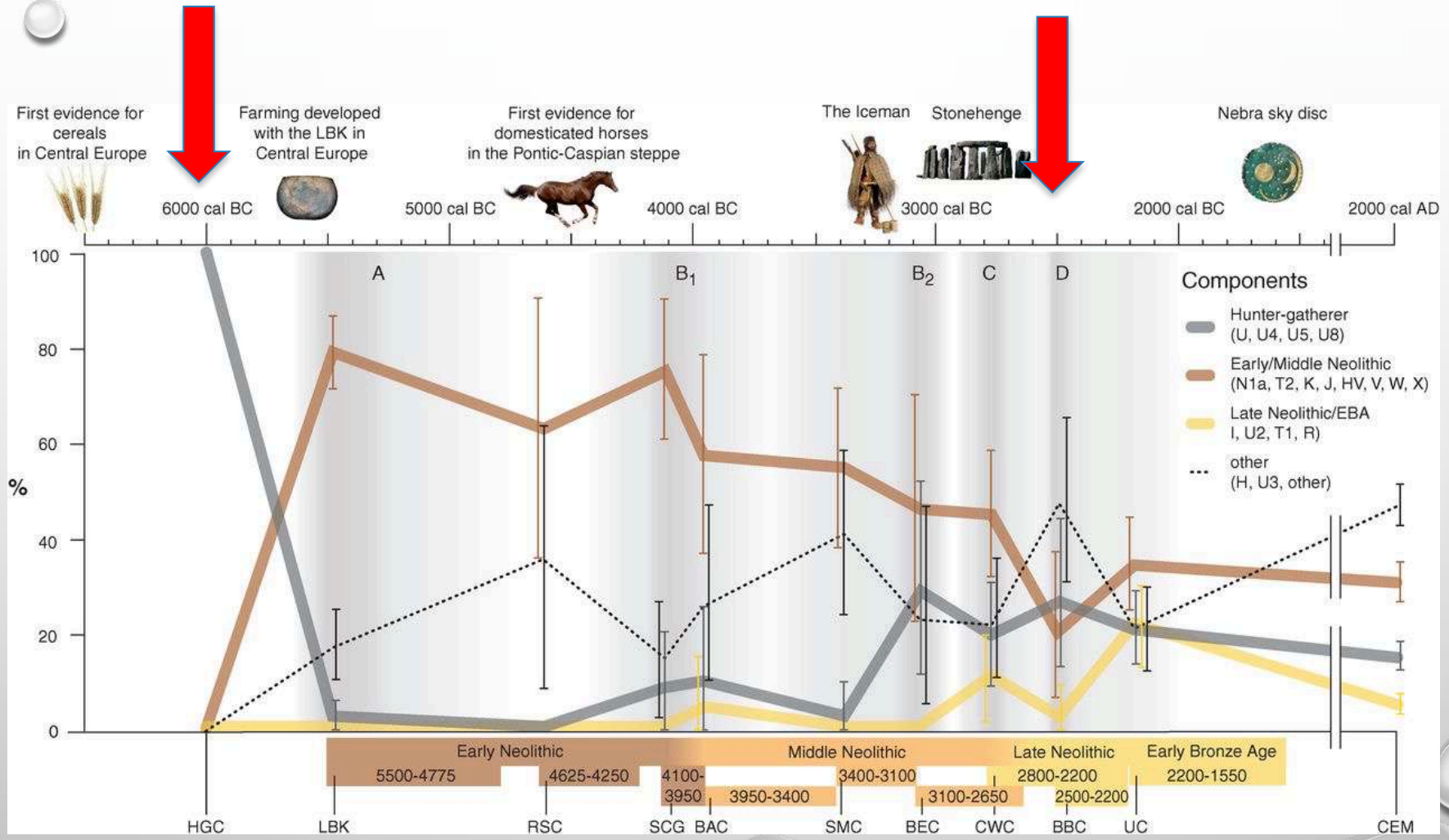




2) No direct genetic continuity between Hunter-Gatherers, First Farmers and modern Europeans

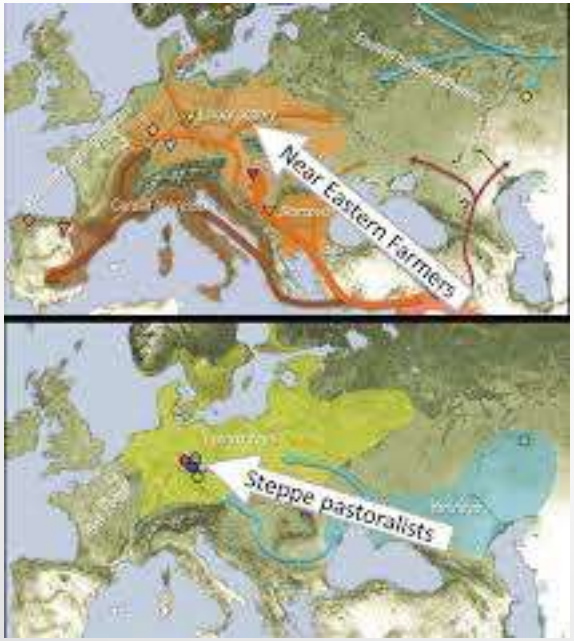


H-G and Farmers in Central Europe

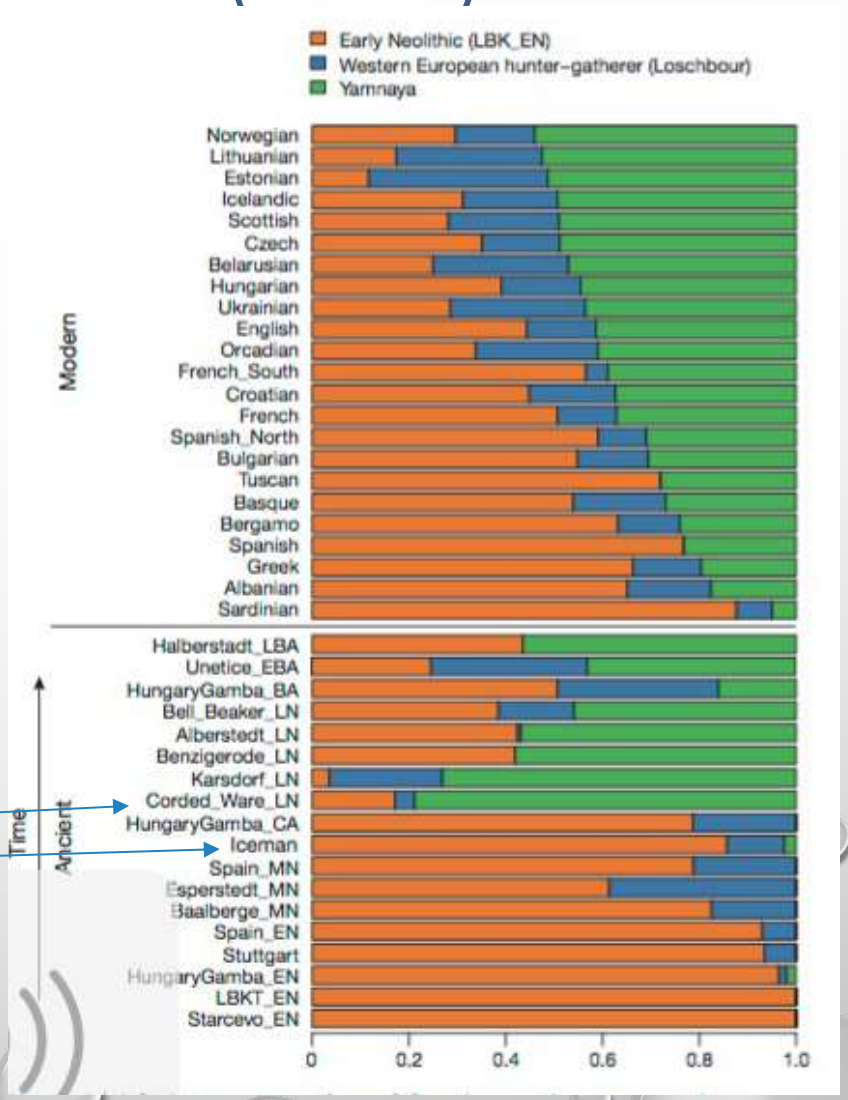


Brandt et al. 2013

Today Europeans are a mixture of at least three different ancestral populations (mtDNA).



2400 BCE
3200 BCE

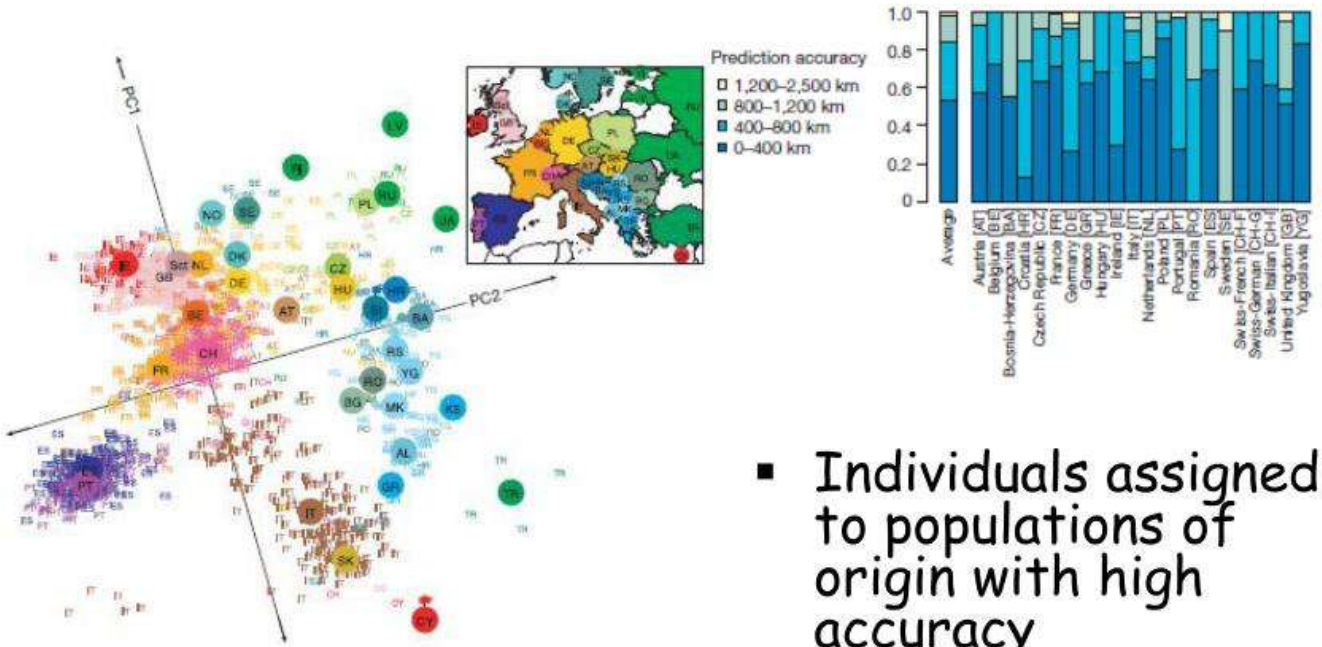


Admixture proportion inferred in ancient and modern samples (Haak et al. 2015).

Determination of individual origins (nDNA)

Human Population Assignment with SNP

- Assayed 500,000 SNP genotypes for 3,192 Europeans
- Used Principal Components Analysis to ordinate samples in space
- High correspondence betweeen sample ordination and geographic origin of samples

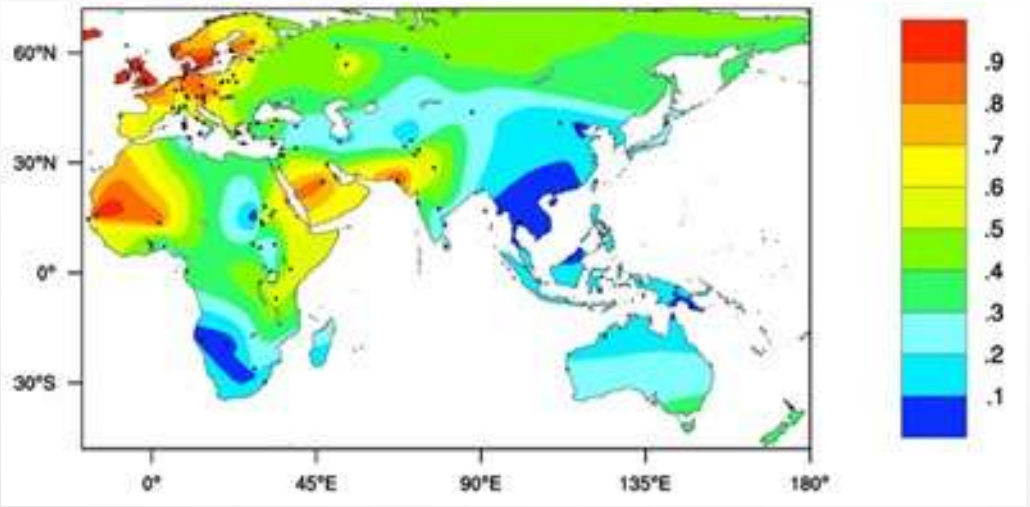


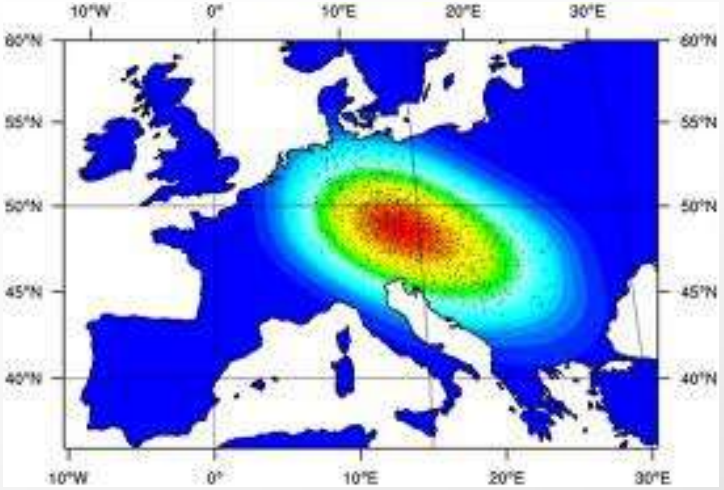
- Novembre et al. 2008 Nature 456:98

- Individuals assigned to populations of origin with high accuracy

Genetic disorders & particularities (nDNA)

Lactase-persistance





PNAS

Absence of the lactase-persistence-associated allele in early Neolithic Europeans

J. Burger[†‡], M. Kirchner[†], B. Bramanti[†], W. Haak[†], and M. G. Thomas[§]

[†]Johannes Gutenberg University, Institute of Anthropology, Saarstrasse 21, D-55099 Mainz, Germany; and [§]Department of Biology, University College London, Wolfson House, 4 Stephenson Way, London NW1 2HE, United Kingdom

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Lactase persistence (LP), the dominant Mendelian trait conferring the ability to digest the milk sugar lactose in adults, has risen to high frequency in central and northern Europeans in the last 20,000 years. This trait is likely to have conferred a selective advantage in individuals who consume appreciable amounts of unfermented

would have provided a selective advantage in the absence of a supply of fresh milk, and because of observed correlations between the frequency of LP and the extent of traditional reliance on animal milk, the culture-historical hypothesis has been proposed (8–12). Under this model, LP was driven from

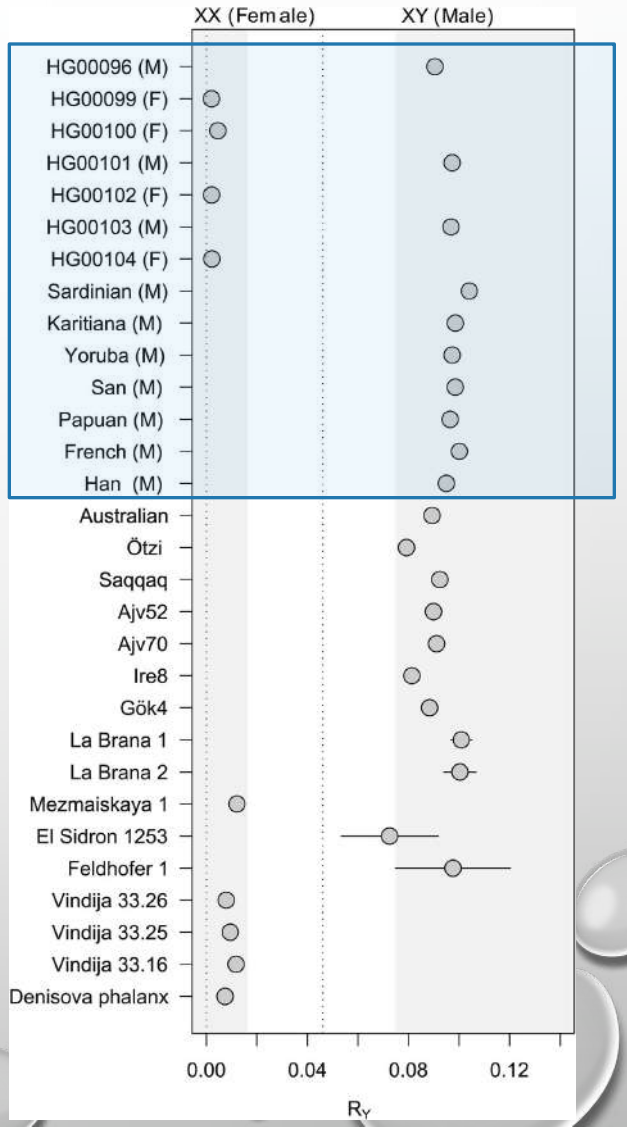
Itan et al. 2009
(Burger et al. 2007,
Malmström et al. 2010
Sverrisdottir et al. 2014)
...

Determination of sex (nDNA)

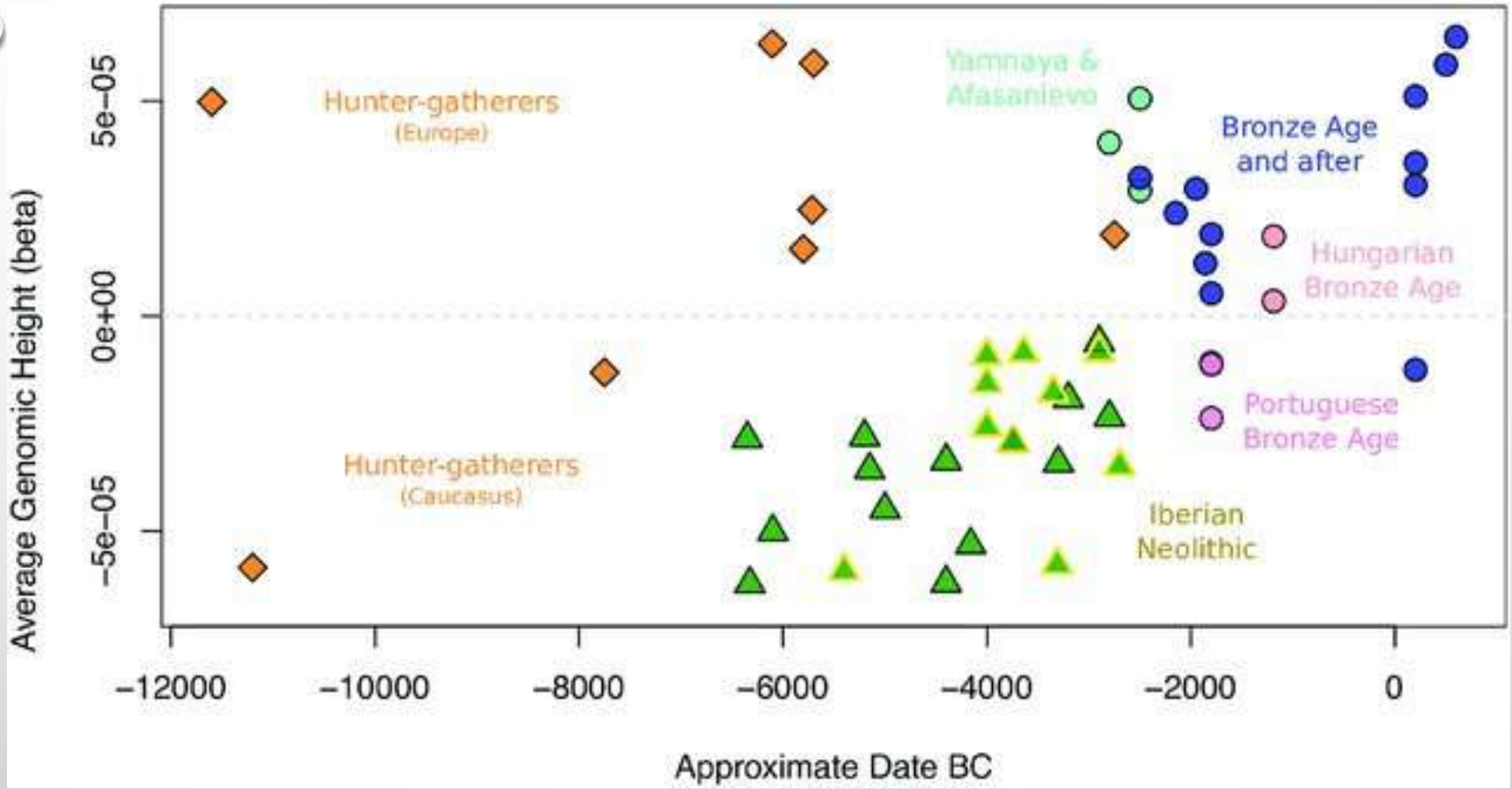
Metodo di Skoglund (et al. 2013):

„even relatively **sparse shotgun sequencing (about 100,000 human sequences)** can be used to reliably identify chromosomal sex simply by considering the **ratio of sequences aligning to the X and Y chromosomes**“.

- Also in subadults
- Also on fragmented bones (no skull, no pelvis)
- Most accurate



Genomic Height (nDNA)



Martiniano et al. 2017: „Average genomic height for each of the Western Eurasian samples in the imputed dataset, plotted against its approximate date, highlighting temporal trends in genetic height."

Somatic traits (nDNA)

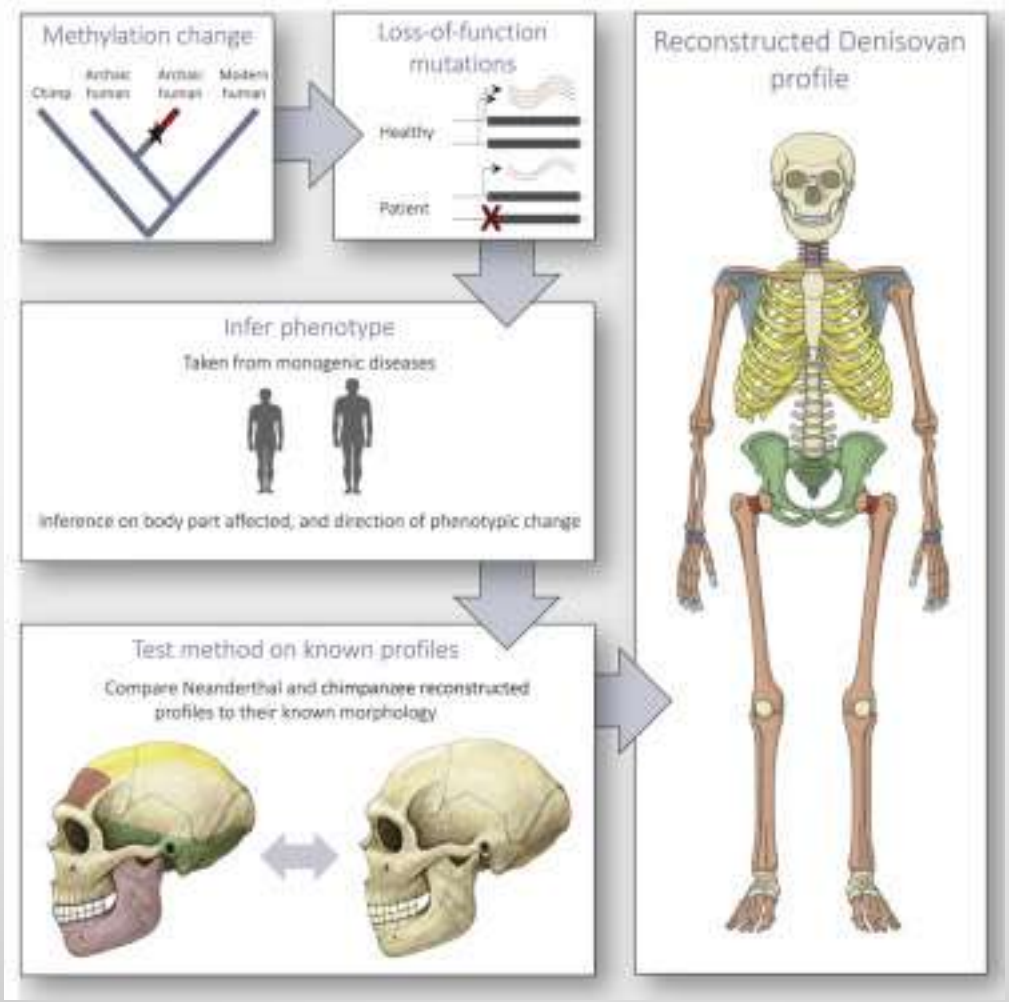
La Braña 1, a 7,000-year-old individual from the Mesolithic Period, had blue eyes and dark skin. Credit: Spanish National Research Council

La Braña 1 has a common ancestor with the settlers of the Upper Paleolithic site of Mal'ta, located in Lake Baikal (Siberia)

Olalde et al. 2014
(Wilde et al. 2014)

Somatic traits (nDNA)

GoKhman et al. 2019



Microbioma and diet

Jensen et al. 2019

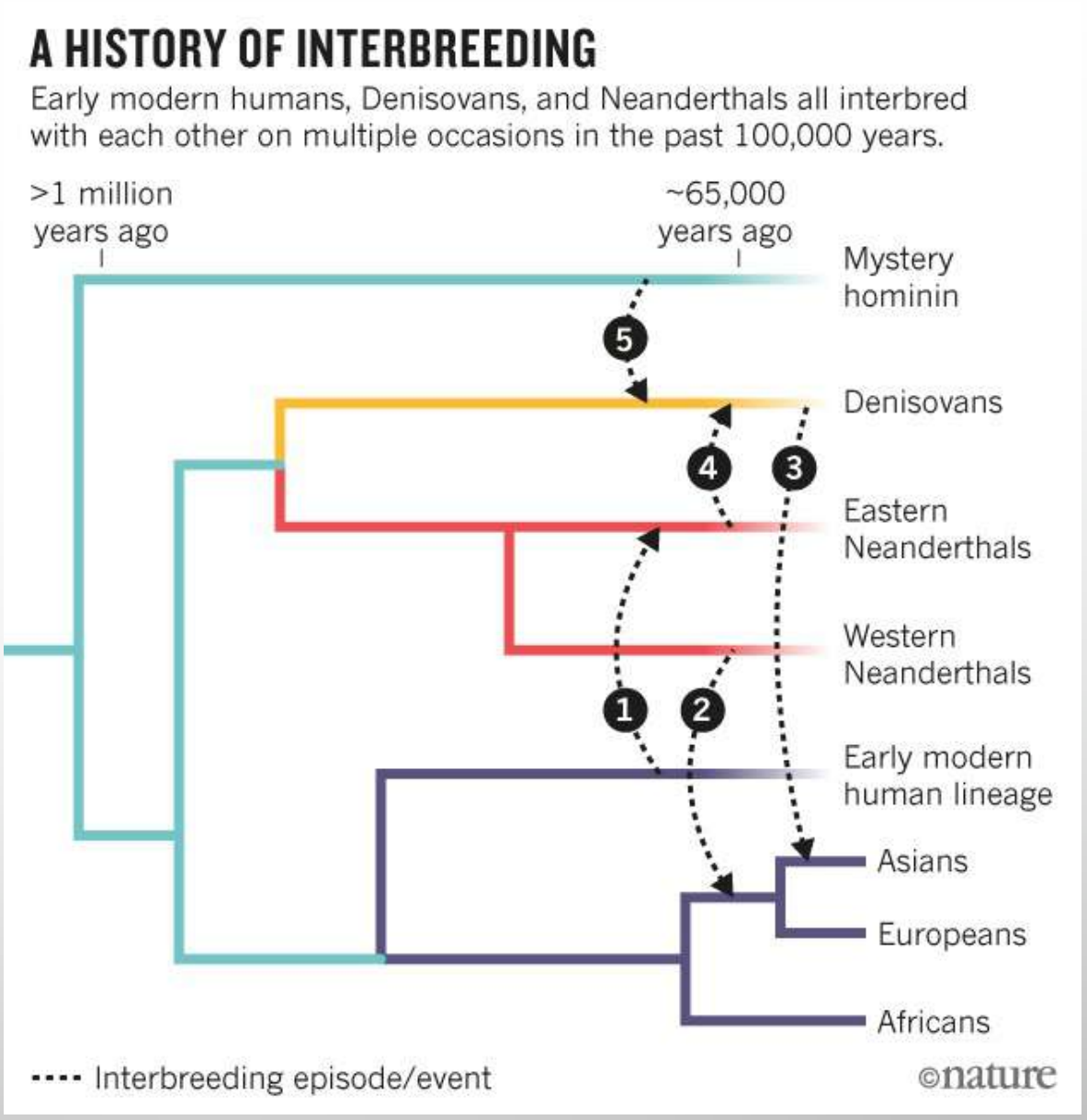
(Illustration by Tom Björklund)

Lola's portrait, "reconstructed" by a 5,700 years old chewing-gum (**chewed birch pitch**) – no human bones!

- Entire genome
- Oral microbiome
- Meal (hazelnuts and mallard duck but no milk)

Attribution to different species

Introgression:
Permanent incorporation of genes from a genetically distinct group/species



IL DNA di Neanderthal

1-4 % nelle popolazioni euro-asiatiche (Green et al. 2010)
3.4 - 7.9 % nelle popolazioni Europee (Lohse et al. 2013)
1.8 – 2.4 % nelle popolazioni Europee (Prüfer et al. 2017)
2.3 – 2.6 % nelle popolazioni dell'Est asiatico (Prüfer et al. 2017)

Introgressioni in associazione con:

- ✓ Celiachia (Taskent et al. 2017)
- ✓ Severità della malaria (Taskent et al. 2017)
- ✓ Sindrome di Costello (Taskent et al. 2017)
- ✓ Capelli rossi e pelle chiara (Ding et al. 2014), ma la variante è rara (Dannemann, M.; Kelso, J. 2017)
- ✓ Molti geni legati al sistema immunitario (e.g. Nédélec et al. 2016) => maggior difesa.

Da McCoy et al./Cell 2017:

- ✓ Statura
- ✓ Schizofrenia
- ✓ Lupus
- ✓ Fibrosi cistica
- ✓ Dipendenza dal fumo

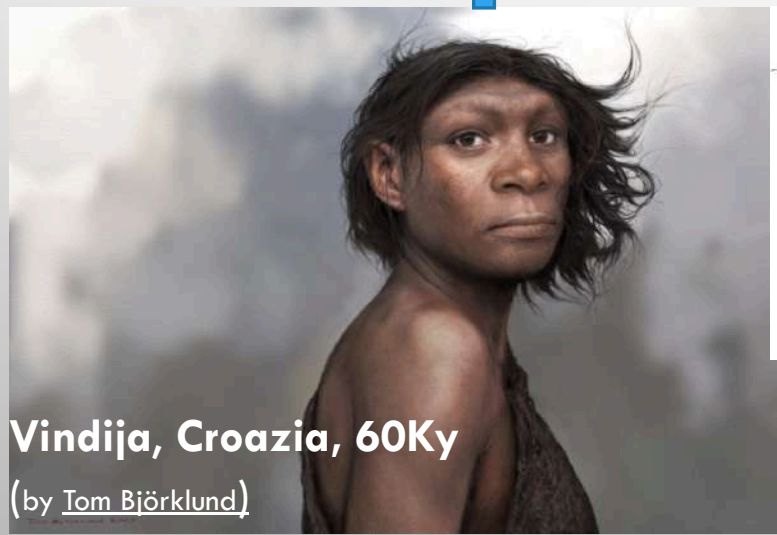
NEANDERTHAL E COVID-19

ZEBERG, H., PÄÄBO, S. THE MAJOR GENETIC RISK FACTOR FOR SEVERE COVID-19 IS INHERIT
FROM NEANDERTHALS. *NATURE* **587**, 610–612 (2020). HTTPS://DOI.ORG/10.1038/S4158
020-2818-3

Aplotipo che induce un percorso clinico più critico, maggior problemi respiratorii

3p21.31

Introgressione da Neanderthal



Vindija, Croazia, 60Ky
(by Tom Björklund)

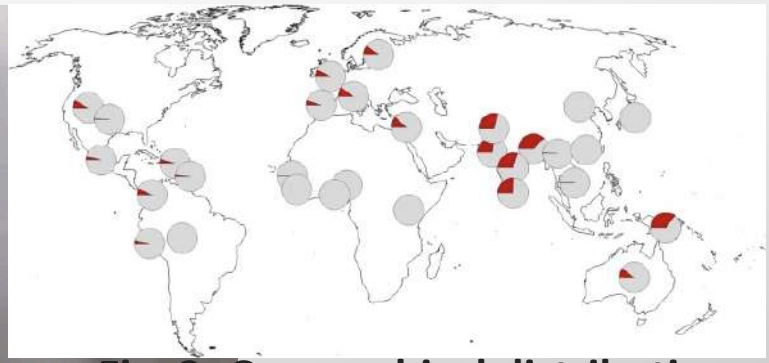
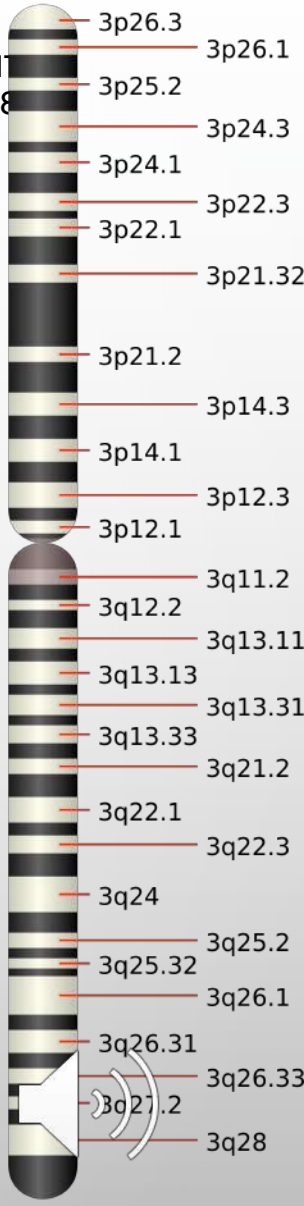


Fig. 3: Geographical distribution of the Neanderthal core haplotype that confers risk for severe COVID-19.



PATHOGENS‘ DNA

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Distinct Clones of *Yersinia pestis* Caused the Black Death

Stephanie Haensch[1], Raffaella Bianucci[2,3], Michel Signoli[3,4], Minoarisoa Rajerison[5], Michael Schultz[6], Sacha Kacki[7,8], Marco Vermunt[9], Darlene A. Weston[10,11,12], Derek Hurst[13], Mark Achtman[14], Elisabeth Carniel[15], Barbara Bramanti[1]*

1 Institute for Anthropology, Johannes Gutenberg University, Mainz, Germany, 2 Laboratory of Criminalistic Sciences Department of Anatomy, Pharmacology and Legal Medicine, University of Turin, Turin, Italy, 3 Unité d'Anthropologie Bioculturelle, Faculté de Medecine, University of Mediterranean-CNRS-EFS, Marseille, France, 4 Centre d'études Préhistoire, Antiquité, Moyen-âge, UMR 6130 CNRS–250 University of Nice, Valbonne, France, 5 Center for Plague, Institute Pasteur de Madagascar, World Health Organization Collaborating, Antananarivo, Madagascar, 6 Department of Anatomy and Embryology Medical Faculty, Georg-August University, Göttingen, Germany, 7 Inrap, Villeneuve-d'Ascq Archaeological Center, Villeneuve-d'Ascq, France, 8 Laboratoire d'Anthropologie des Populations du Passé, Université Bordeaux 1, Talence, France, 9 Department of Monuments and Archaeology, Municipality of Bergen op Zoom, Bergen op Zoom, The Netherlands, 10 Bargo's Anthropologica, Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, The Netherlands, 11 Division of Archaeological Sciences, University of Bradford, Bradford, West Yorkshire, United Kingdom, 12 Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany, 13 Worcestershire Historic Environment and Archaeology Service, Worcestershire County Council, Worcester, United Kingdom, 14 Environmental Research Institute, University College Cork, Cork, Ireland, 15 Yersinia Research Unit, Institut Pasteur, Paris, France

Abstract

From AD 1347 to AD 1353, the Black Death killed tens of millions of people in Europe, leaving misery and devastation in its wake, with successive epidemics ravaging the continent until the 18th century. The etiology of this disease has remained highly controversial, ranging from claims based on genetics and the historical descriptions of symptoms that it was caused by *Yersinia pestis* to conclusions that it must have been caused by other pathogens. It has also been disputed whether plague had the same etiology in northern and southern Europe. Here we identified DNA and protein signatures specific for *Y. pestis* in human skeletons from mass graves in northern, central and southern Europe that were associated archaeologically with the Black Death and subsequent resurgences. We confirm that *Y. pestis* caused the Black Death and later epidemics on the entire European continent over the course of four centuries. Furthermore, on the basis of 17 single nucleotide polymorphisms plus the absence of a deletion in *glpD* gene, our aDNA results identified two previously unknown but related clades of *Y. pestis* associated with distinct medieval mass graves. These findings suggest that plague was imported to Europe on two or more occasions, each following a distinct route. These two clades are ancestral to modern isolates of *Y. pestis* biovars Orientalis and Medievalis. Our results clarify the etiology of the Black Death and provide a paradigm for a detailed historical reconstruction of the infection routes followed by this disease.

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* E-mail: bramanti@uni-mainz.de

Introduction

Of the numerous epidemics in human history, three pandemics are generally accepted as having been caused by plague. Justinian's plague (AD 541–542) spread from Egypt to areas surrounding the Mediterranean [1]. In 1347, an epidemic known as the Black Death spread from the Caspian Sea to almost all European countries, causing the death of one third of the European population over the next few years [2]. This second pandemic persisted in Europe until 1750, causing successive and progressively declining epidemic waves. A third plague pandemic began in the Yunnan region of China in the mid-19th century, and spread globally via shipping from Hong Kong in 1894. During this last pandemic, the etiological cause of plague was identified as *Yersinia pestis*, a Gram-negative bacterium [3,4]. Most microbiologists and epidemiologists believe that *Y. pestis* was also the etiological agent of the first two pandemics. This belief is supported by ancient DNA (aDNA) analyses which identified sequences specific for *Y. pestis* in the teeth of central European plague victims from the first and second pandemics [5–7]. Moreover, the *Y. pestis* F1 protein capsule antigen has been detected in ancient plague skeletons from Germany and France by immunochromatography [8,9].

Based on studies on modern strains, microbiologists have subdivided *Y. pestis* into three biovars: Antiqua, Medievalis, and Orientalis. These biovars can be distinguished depending on their abilities to ferment glycerol and reduce nitrate [10]. The Medievalis biovar is unable to reduce nitrates due to a G to T mutation that results in a stop codon in the *napA* gene [11], while the Orientalis biovar cannot ferment glycerol because of a 93 bp deletion in the *glpD* gene [11,12]. Conversely, the Antiqua biovar is capable of performing both reactions [10]. An apparent historical association of the routes of the three pandemics with the modern geographical sources of the three biovars led Devignat to propose that each plague pandemic was caused by a different biovar [10]. There is no doubt that the ongoing third pandemic

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Yersinia pestis DNA from Skeletal Remains from the 6th Century AD Reveals Insights into Justinianic Plague

Michaela Harbeck[1]*, Lisa Seifert[2], Stephanie Hänsch[3,4], David M. Wagner[5], Dawn Birdsell[5], Katy L. Parise[5], Ingrid Wiechmann[6], Gisela Grupe[1,2], Astrid Thomas[7], Paul Keim[4], Lothar Zöller[7], Barbara Bramanti[3,4]*, Julia M. Riehm[7], Holger C. Scholz[7]*

1 State Collection for Anthropology and Palaeoanatomy, Munich, Germany, 2 Department Biology I, Anthropology and Human Genetics, Ludwig Maximilian University of Munich, Martinsried, Germany, 3 Institute for Anthropology, Johannes Gutenberg University, Mainz, Germany, 4 Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, Oslo, Norway, 5 Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, Arizona, United States of America, 6 Institute of Palaeoanatomy, Domestication Research and the History of Veterinary Medicine, Department of Veterinary Sciences, Ludwig Maximilian University of Munich, Munich, Germany, 7 Bundeswehr Institute of Microbiology, Munich, Germany

Abstract

Yersinia pestis, the etiologic agent of the disease plague, has been implicated in three historical pandemics. These include the third pandemic of the 19th and 20th centuries, during which plague was spread around the world, and the second pandemic of the 14th–17th centuries, which included the infamous epidemic known as the Black Death. Previous studies have confirmed that *Y. pestis* caused these two more recent pandemics. However, a highly spirited debate still continues as to whether *Y. pestis* caused the so-called Justinianic Plague of the 6th–8th centuries AD. By analyzing ancient DNA in two independent ancient DNA laboratories, we confirmed unambiguously the presence of *Y. pestis* DNA in human skeletal remains from an Early Medieval cemetery. In addition, we narrowed the phylogenetic position of the responsible strain down to major branch 0 on the *Y. pestis* phylogeny, specifically between nodes N03 and N05. Our findings confirm that *Y. pestis* was responsible for the Justinianic Plague, which should end the controversy regarding the etiology of this pandemic. The first genotype of a *Y. pestis* strain that caused the Late Antique plague provides important information about the history of the plague bacillus and suggests that the first pandemic also originated in Asia, similar to the other two plague pandemics.

Citation: Harbeck M, Seifert L, Hänsch S, Wagner DM, Birdsell D, et al. (2013) *Yersinia pestis* DNA from Skeletal Remains from the 6th Century AD Reveals Insights into Justinianic Plague. PLoS Pathog 9(5): e1003349. doi:10.1371/journal.ppat.1003349

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Competing Interests: The authors have declared that no competing interests exist.

* E-mail: M.Harbeck@lrz.uni-muenchen.de (MH); holgerlscholz@bundeswehr.org (HCS); bramanti@uni-mainz.de (BB)

Introduction

In 541 AD, eight centuries before the Black Death, a deadly infectious disease hit the Byzantine Empire, reaching Constantinople in 542 and North Africa, Italy, Spain, and the French-German border by winter 543 [1]. The so called "Plague of Justinian", named after the contemporaneous emperor, led to mass mortality in Europe similar to that of the Black Death. It persisted in the territory of the Roman Empire until the middle of the 8th century and likely contributed to its decline, shaping the end of antiquity [1]. Based on historical records, this disease has been diagnosed as bubonic plague although discrepancies between historical sources and the progression of *Y. pestis* infections have led some authors to suppose that the Plague of Justinian was caused by a different pathogen (as discussed in [2]). This vivacious discussion was recently reinforced by an ancient DNA study of the second pandemic that also questioned whether *Y. pestis* was truly the causative agent of the first pandemic [3,4].

Western scientists have traditionally subdivided *Y. pestis* strains into three biovars: Antiqua, Medievalis, and Orientalis, depending on their abilities to ferment glycerol and reduce nitrate [5]. However, this system ignores many other *Y. pestis* biovars that have been designated and described by other scientists [see 6,7,8]. Biovars, which are based upon phenotypic properties, do not always correspond directly to specific molecular groups because the same phenotype can result from different mutations [9]. As a result, it has been suggested that groupings within *Y. pestis*, or assignment of unknown strains to specific populations should be based upon molecular signatures and not phenotypes [9]. Fortunately, the recent construction of highly-accurate rooted global phylogenetic trees for *Y. pestis* [10,11] (reproduced in Figure 1) have facilitated the assignment of isolates to distinct populations. The most recent global phylogeny is based upon single nucleotide polymorphisms (SNPs) identified from the genomes of 133 global strains [11]. All clones that caused the third pandemic belong to populations assigned to the molecular group 1.ORI [10,11]; the basal node for this group is N14 (Figure 1).

Two recent studies [3,12] have queried key SNPs in DNA samples obtained from victims of the second pandemic (14th century AD), facilitating the phylogenetic placement of these samples in the most recent global phylogeny [11]. These samples are along the branch between nodes N07 and N10 (Figure 1) close

DFG Deutsche Forschungsgemeinschaft