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?*

La determinazione del numero di individui presenti è compito dell'Antropologo. Maggiore è il numero di inumati, maggiore è la difficoltà

Fosse del GUATEMALA (eccidio del 1982)

Chichupac, sito III

Ossario di Amerindiani (188 individui)

Analisi di tutti i reperti anche se frammentati

Es., resti scheletrici soldati americani morti durante la prigionia in Vietnam e inviati negli USA nel 1975.

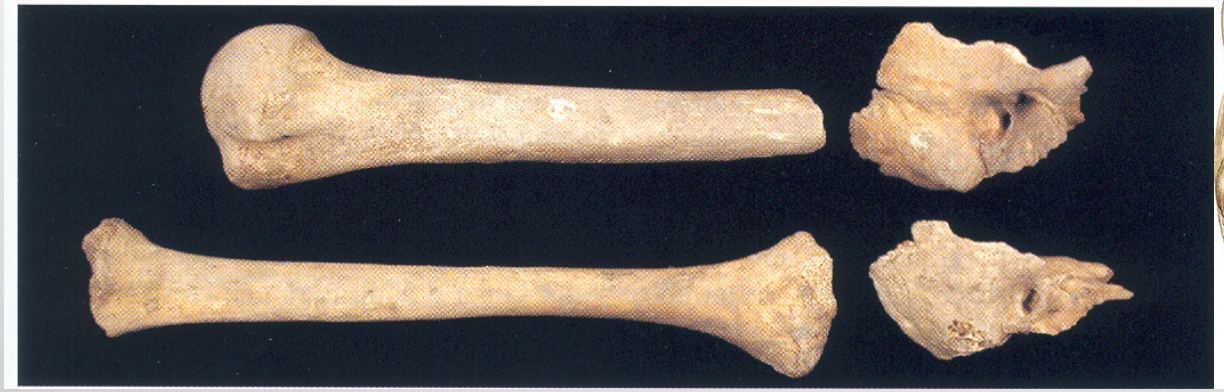
1 vertebra del collo in più → altro individuo

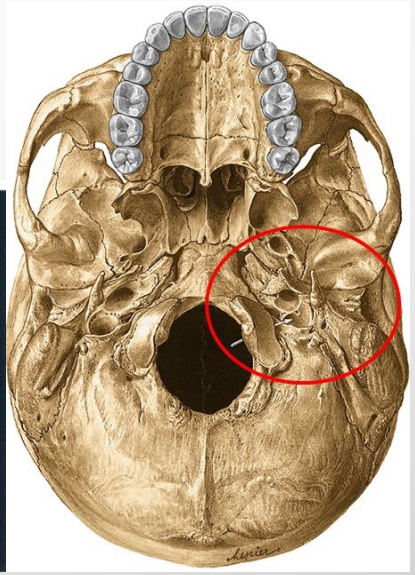
ESEMPI DI CALCOLO del NMI: si tiene conto dell'osso più rappresentato

1- abbiamo 2 tibie dx e 1 sn

N.min.individui= 2

2- 2 omeri dx, 1 con epifisi non saldata 2 Rocche p dx





N.min.individui= 3 (2 adulti + 1 soggetto in crescita)

OTTO DOMANDE PER L'ANTROPOLOGO:

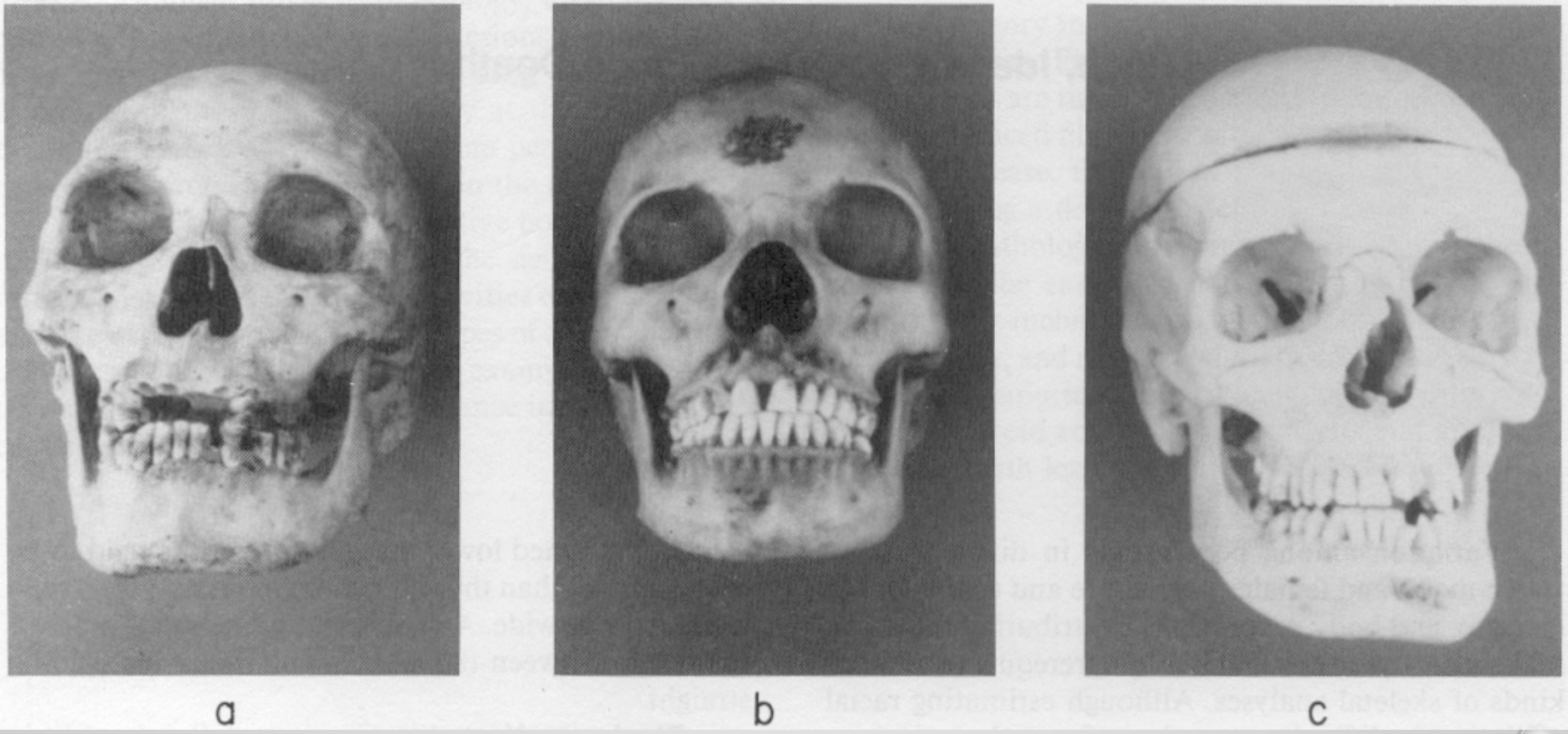
1. *è un osso umano?*
2. *è un reperto recente?*
3. *sono presenti più persone?*
4. *di quale origine etnica?*
5. *di che sesso?*
6. *di che età ?*
7. *di quale statura?*
8. *con quali caratteristiche?*

Biodiversità

Differenze analoghe anche per lo scheletro anche se più difficoltose da rilevare

Tre grandi gruppi etnici

Fig. 139. Racial differences in the facial bones. **a,** Mongoloid. **b,** Negroid/Black. **c,** Caucasoid/White.



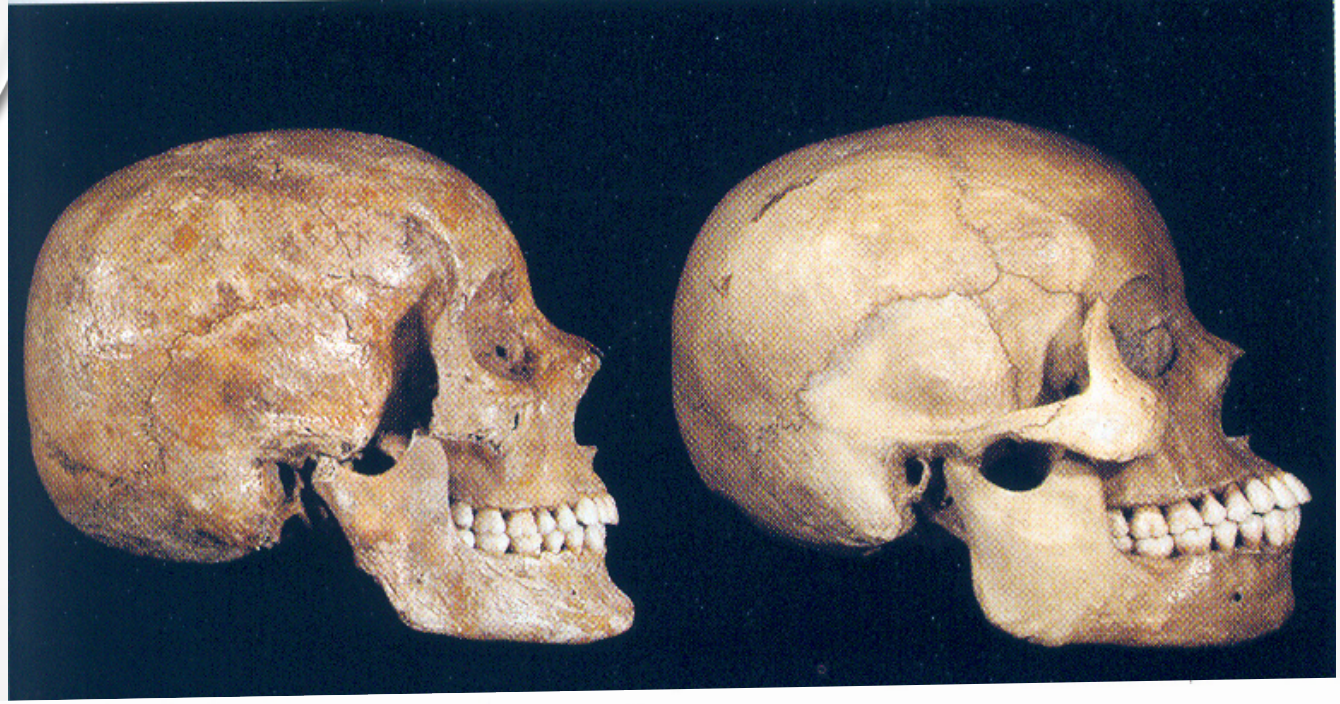


Tabella 7.3.22. Distribuzione geografica del prognatismo

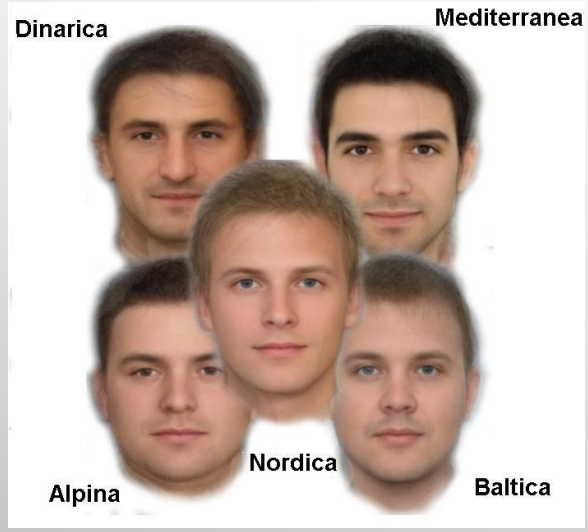
	valori d'angolo	**popolazioni**
prognati	< 70°	forme australoidi (prognatismo totale): Australiani, Papua, Neocaledoni,, Vedda; forme negroidi: forme dell'Africa occidentale, sudanesi, nilotici, cafri, melanesiani
mesognati	70°-73°	forme negroidi: Pigmei africani, andamanesi, aeta-semang, khoisanidi forme mongolidi: indonesiani, sud-Mongolici forme "derivate": paleoindiani, amerindiani (specie nelle forme pueblo-andine)
ortognati	> 73°	forme "negroidi": khoisanidi forme "mongolidi": siberiani, tibetani, tungusi, sinici, eschimesi forme particolari: Ainu, Uralici forme "europee": lapponi, nordici, mediterranei, alpini, dinarici, baltici, iraniani, anatolico-pamiriani, indiani di alta casta forme "derivate": etiopici, malgasci, polinesiani

CARATTERISTICHE DEL CRANIO NEI GRANDI GRUPPI UMANI

Carattere	Asiatici	Europei	Africani
Lun.Cranio	lungo	corto	lungo
Lar.Cranio	largo	largo	stretto
Alt.Cranio	medio	alto	basso
Lar.Faccia	molto larga	larga	stretta
Alt.Faccia	alta	alta	bassa
Forma Orbita	arrotondata	arrotondata	rettangolare
Apertura nas.	stretta	mod.larga	larga
Ossa nasali	larghe,piatte	strette,ad arco	strette
Profilo Faccia	diritto	diritto	prognato
Forma palato	U	V	U
Incisivi a pala	90%	<5%	<5%

CARATTERISTICHE DEL CRANIO IN POPOLAZIONI ITALIANE ATTUALI

Carattere	Mediterraneo	Alpino	Dinarico
Lun.Cranio	lungo	corto	corto
Lar.Cranio	stretto	largo	largo
Alt.Cranio	medio	medio	alto
Lar.Faccia	stretta	larga	stretta
Alt.Faccia	alta	medio	alta



- Mescolanza fra popolazioni
- Distribuzione nel passato

I popoli europei nei primi secoli dell' Età del Ferro

dal 1000 al 200 a.C.

I numeri accanto ai nomi rinviano ai capitoli del volume

Variabilità nel passato

Necropoli longobarda di Vicenne (Campobasso, VIII sec.)

$$\text{Indice cranico orizzontale} = \frac{\text{larghezza}}{\text{lunghezza}} \times 100$$

La presenza di crani brachimorfi in questa necropoli può essere messa in relazione con l'arrivo in Europa di popolazioni asiatiche (dall'oriente?) nel periodo delle "Migrazioni" tra il IV e il X secolo.

Bibliografia:

- Belcastro MG, Mariotti V. La BioArcheologia. CD-ROM Museo dell'Evoluzione, Università di Bologna.
- Bertoldi F. I resti umani in Archeologia. Studi Camuni. Edizioni del Centro.
- Borgognini Tarli S., Pacciani E. I resti umani nello scavo archeologico. Meto= diche di recupero e studio. Bulzoni ed.
- Gualdi E.2012. L'Antropologo sulla scena del crimine. In: (Gualdi, Russo, Eds) "La scena del crimine" libreriauniversitaria.it, Padova.

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CEES
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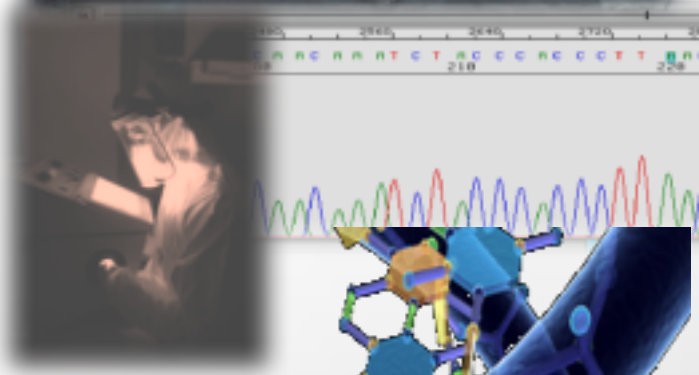

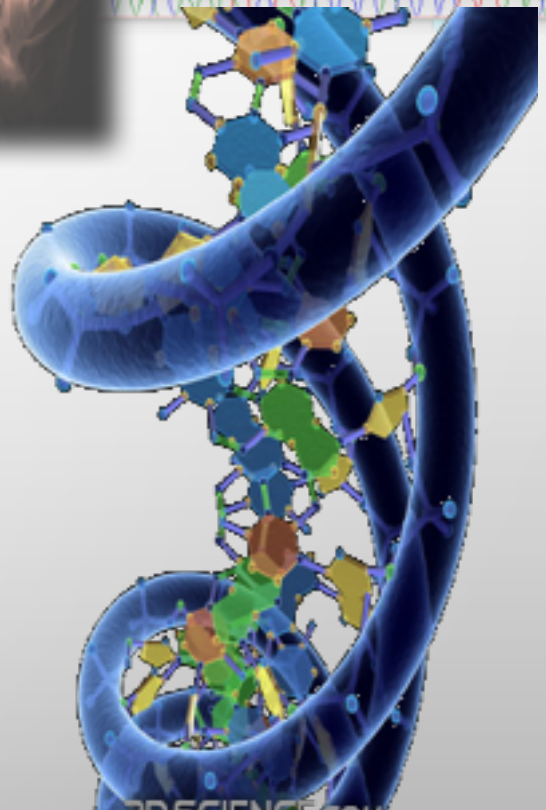

Ancient DNA (aDNA) Analyses of Human remains:

35 years of evolution of a scientific discipline

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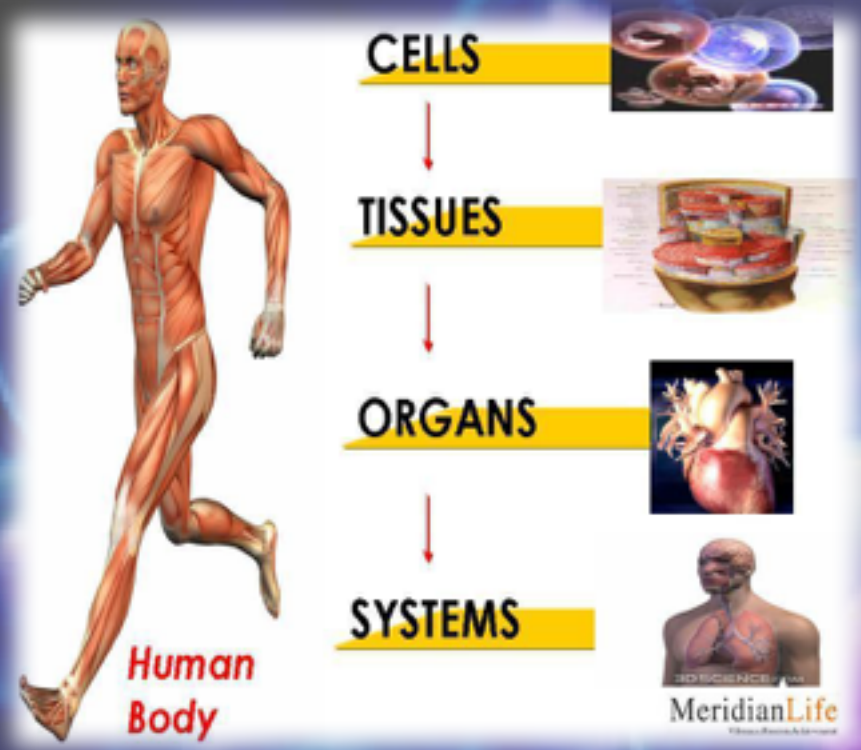
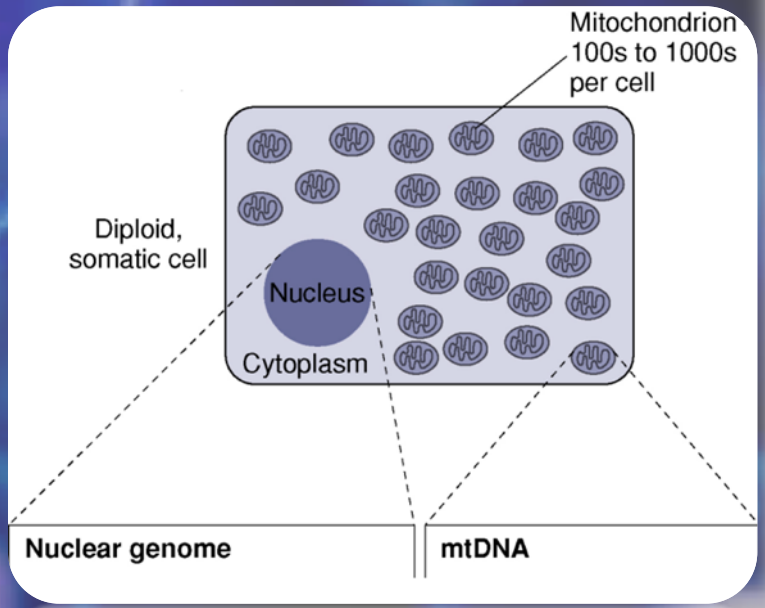
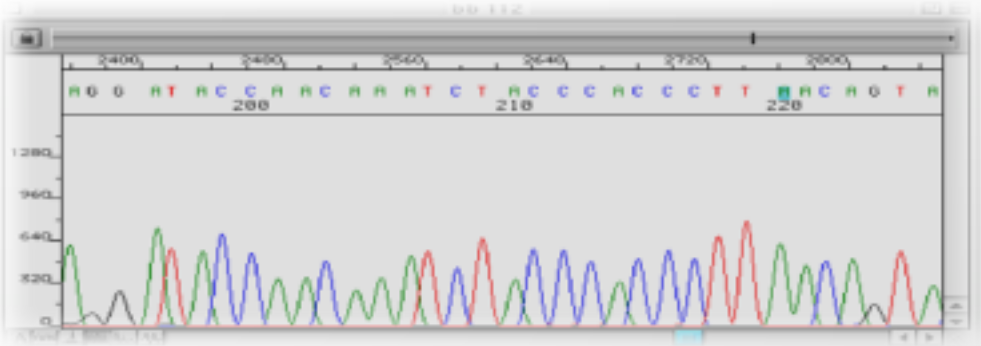
What is ancient DNA (aDNA)?


CELLS
TISSUES
ORGANS
SYSTEMS
Human Body
MeridianLife


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

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



Variability

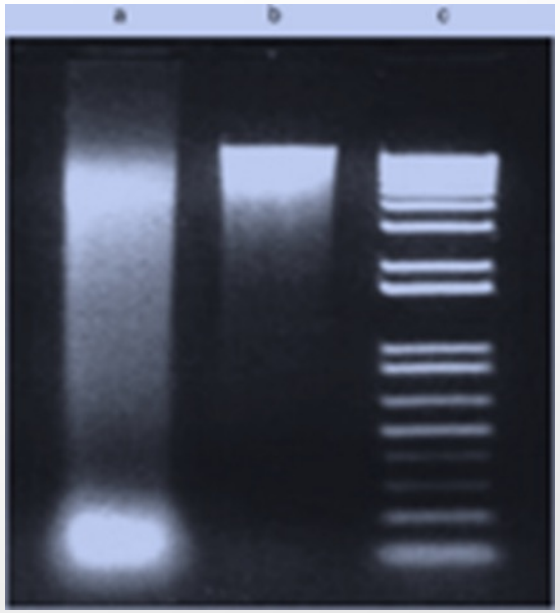
	16037	16069	16071	16081	16126	16129	16136	16145	16160	16166	16170	16172	16182	16183	16185	16189	16223	16224	16255	16260	16265	16278	16292	16298	16311	16319	16360	16362	16391	PO (n)	PK (n)	PS (n)	PL (n)	Total
CRS	A	C	C	C	T	G	T	G	A	A	A	T	A	A	C	T	C	T	G	C	A	C	C	T	T	G	C	T	G					
W/ht1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	T	-	-	-	-	-	-	7	10	6	13	36
W/ht2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0	1
I/ht1	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	A	0	1	0	0	1
I/ht2	-	-	-	-	-	A	-	-	-	-	-	C	-	-	-	-	T	-	-	-	-	-	-	-	C	-	-	-	A	0	1	7	0	8
I/ht3	-	-	-	-	-	A	-	A	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	A	0	0	0	1	1
I/ht4	-	-	-	-	-	A	-	-	-	C	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	A	0	0	0	4	4
X/ht1	-	-	-	-	-	-	-	-	-	-	-	-	C	-	C	T	T	-	A	-	-	T	-	-	-	-	-	-	-	0	0	0	1	1
X/ht2	-	-	-	T	-	-	-	-	-	-	-	-	C	-	C	T	T	-	A	-	-	T	-	-	-	-	-	-	-	0	0	0	2	2
X/ht3	-	-	-	-	-	-	-	-	-	-	-	-	C	-	C	T	T	-	-	-	-	T	-	-	-	-	-	-	-	1	0	0	0	1
M/ht1	-	-	-	-	-	A	-	-	-	-	-	-	-	T	-	T	T	C	-	T	-	-	C	-	-	-	-	-	-	2	5	1	0	8
M/ht2	-	-	-	-	-	A	-	-	-	-	G	-	-	T	-	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

The process of decomposition

0-10 days	4-10 days	20-50 days	50-365 days
			 
Autolysis and putrefaction (**bacteria)** processes: release of putricine and cadeverine. **Insects** (Sarcophagidae and Calliphoridae) spread digestive enzymes and bacteria.	**Bacteria** Anaerobic decomposition - *Clostridium sp.* (Fermentation) releases methane (CH4) Aerobic decomposition - *Bacillus sp.* (Respiration) releases CO2 Increase in T$_o$	Most of the soft tissues are gone	All soft tissues are gone

Ancient DNA (aDNA)

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

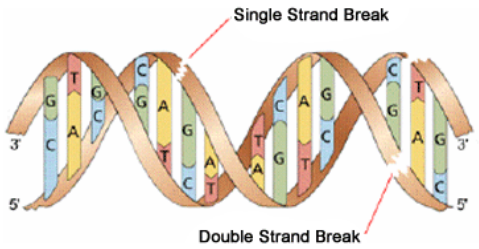


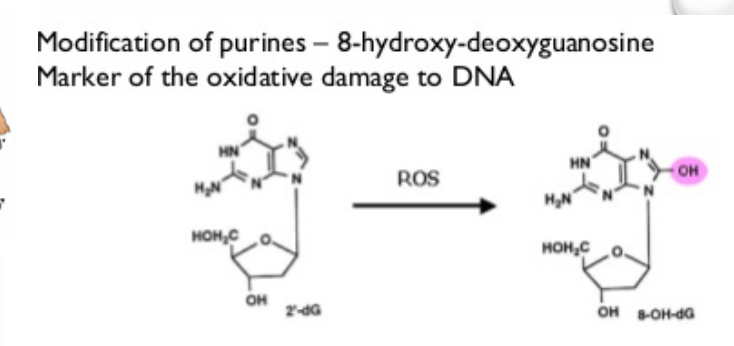
aDNA

Modern DNA

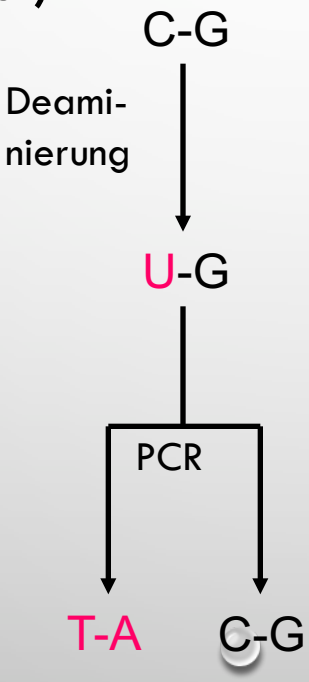
Typical aDNA damages

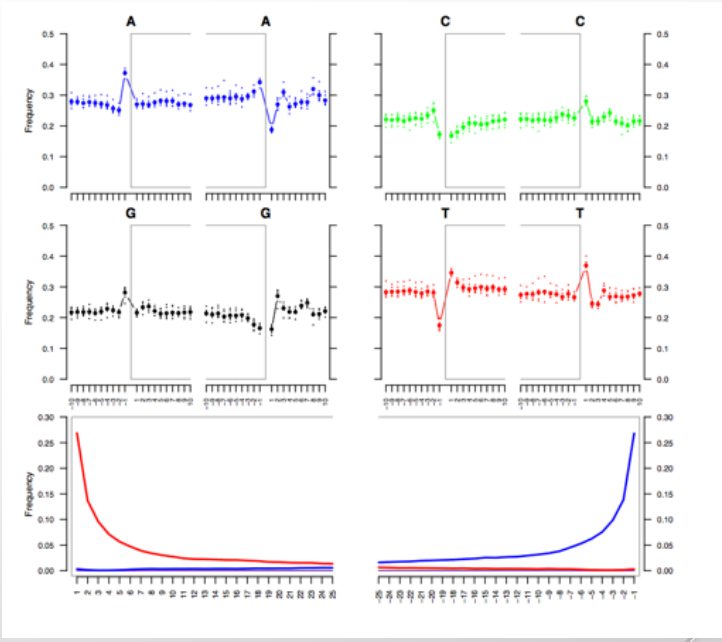
Oxidative lesions





Hydrolytic lesions (water)

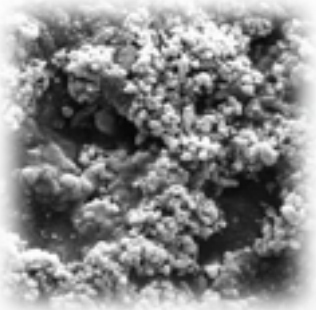


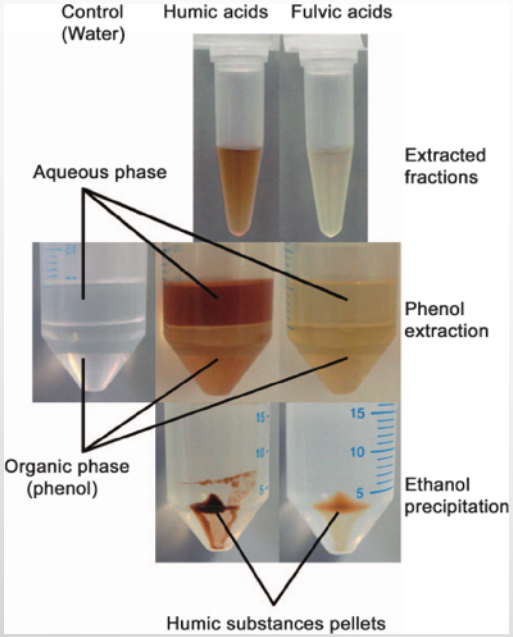


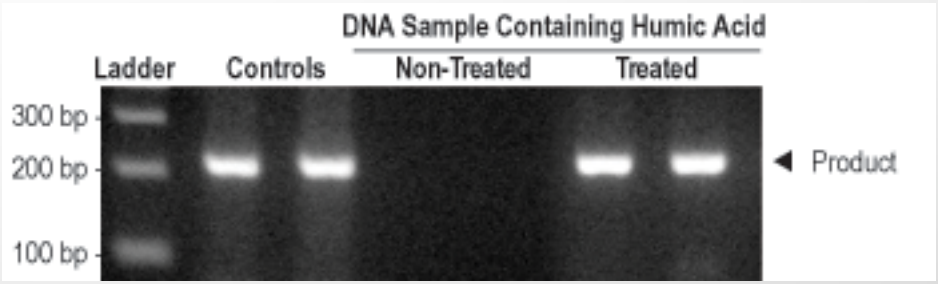
Typical aDNA issue

DNA binds to inhibitors

- Humin acids can **inhibit the PCR reaction** (Tsai 1991, Bourke 1999, Watson 2000, Tebbe 1993)







How long can aDNA survey?

Ideal environments!

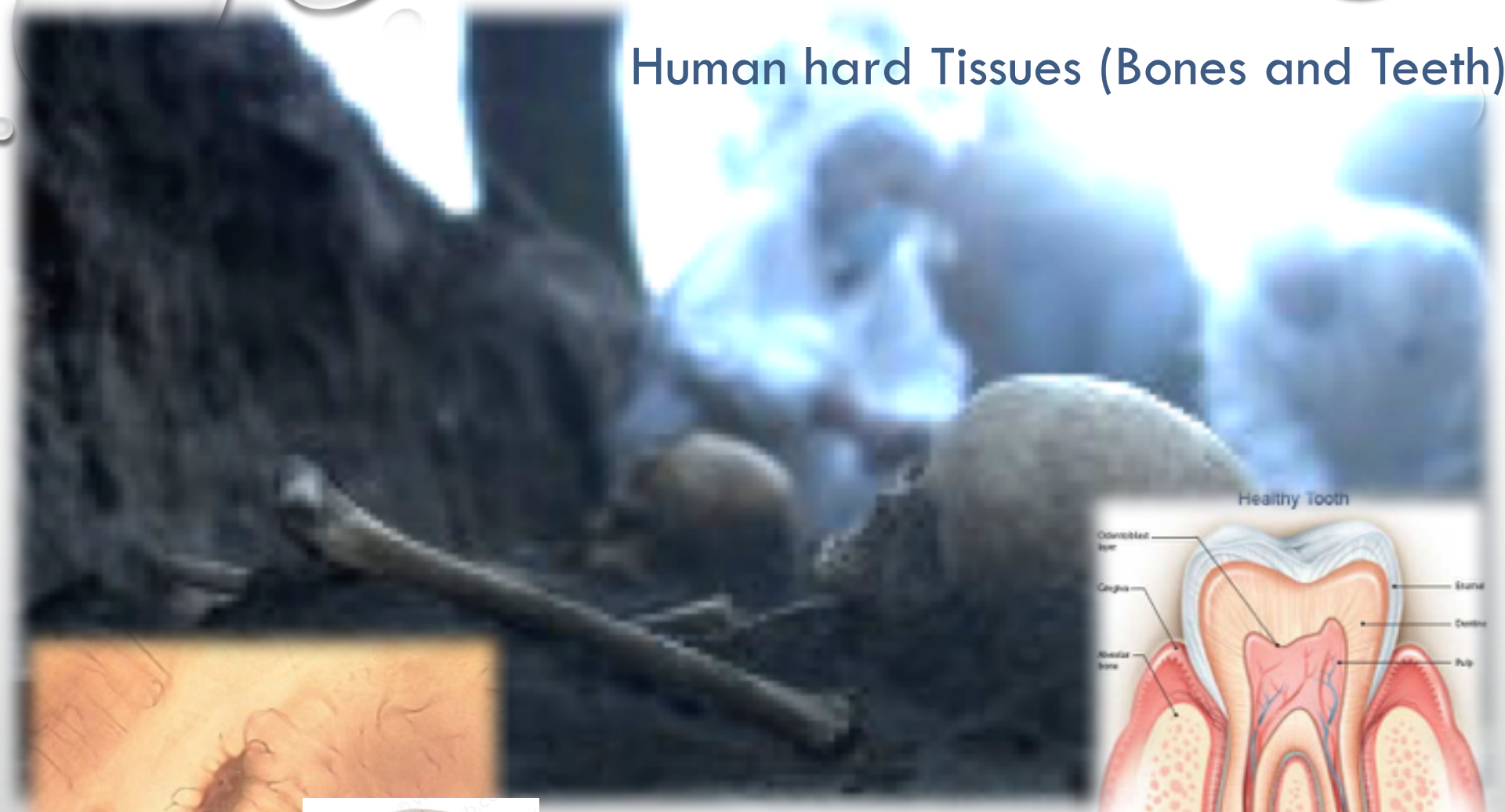
Types of decay inducing environments:

- Presence of moisture
- High temperatures
- Presence of Micro-Organisms, insect, fungi
- Acidity (-pH)

2014: mitochondrial genome of a hominin that lived more than 400,000 years ago; Exomes from two Neanderthal individuals (more than 40,000 YBP); nearly complete nuclear genome from a 45,000-year-old modern human fossil.
2016: 430,000-year-old DNA of a pre-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.[4]

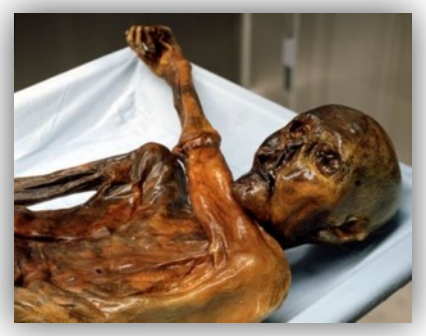
Human hard Tissues (Bones and Teeth)



Petrous part of temporal bone (Pars petrosa; Pinhasi et al. 2015)

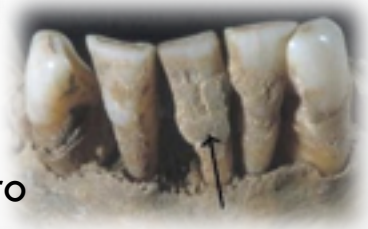
Other sources of aDNA

Corpi imbalsamati

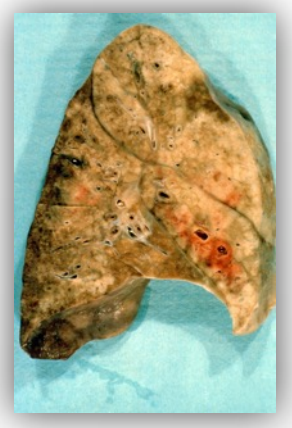


Mummie naturali

Capelli



Tartaro



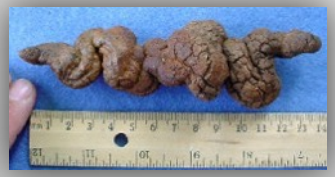
Preparati anatomici

Insetti

Artefatti

Sedimenti

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



Coproliti

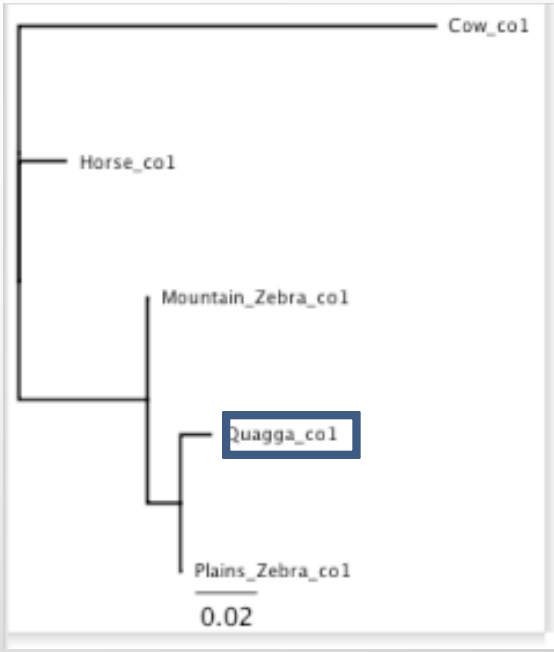
Piante, frutti

Chewing-gum
(di 5000 anni fa)

A bit of History…

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

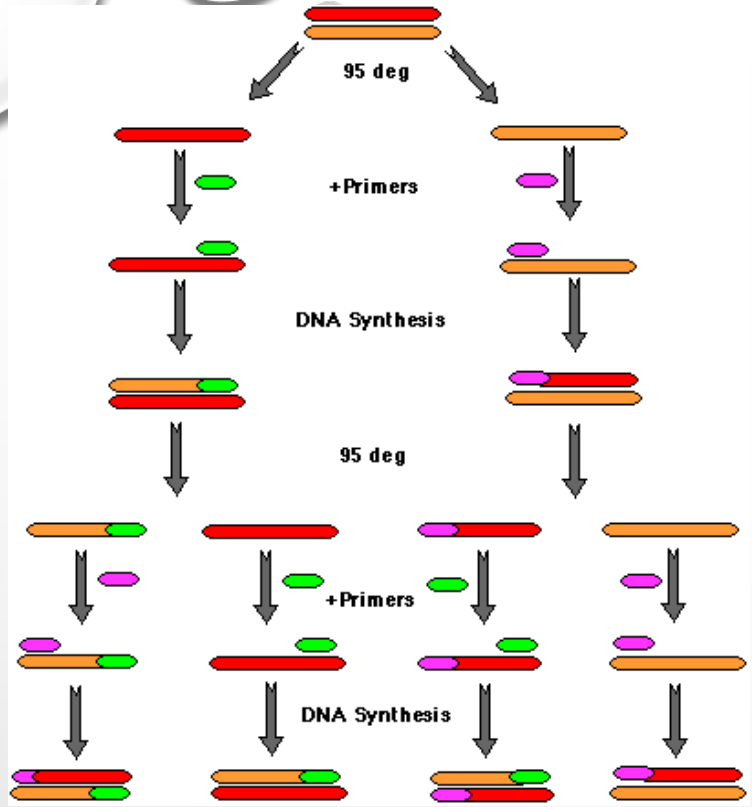
Higuchi R, Bowman B, Freiberger M, Ryder OA, Wilson AC, *DNA sequences from the quagga, an extinct member of the horse family*, in *Nature*, vol. 312, n° 5991, 1984, pp. 282–4



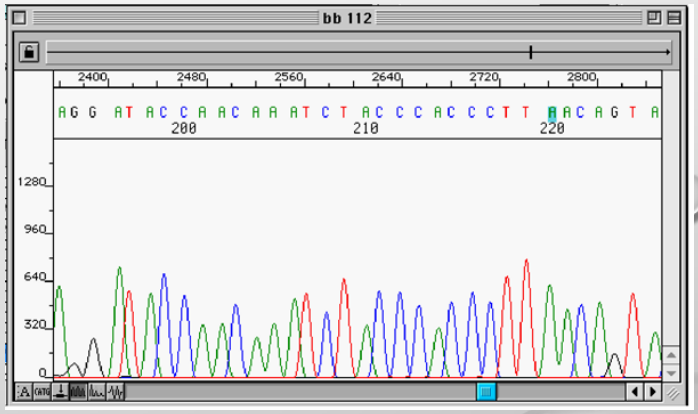
Family of quaggas (*Equus quagga quagga*), 150 years old, at the Naturhistorische Museum in Mainz

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

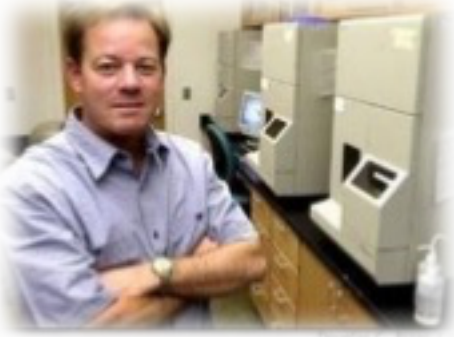
The first ancient human sequence (ca. 2,400 YBP) contained only two sequencing errors (1989).



1984 K. Mullis invented the PCR

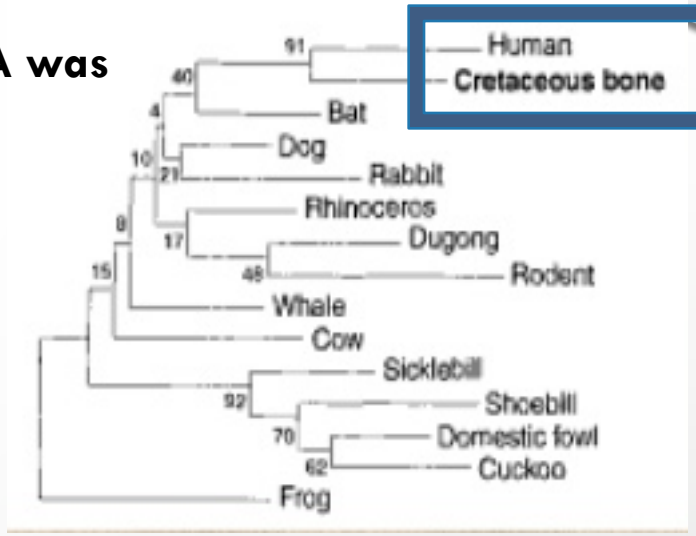


1994. Scott Woodward claimed to have sequenced aDNA from an 80 million years old Dinosaur bone



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.

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*For important discoveries, additional criteria are also essential.

References

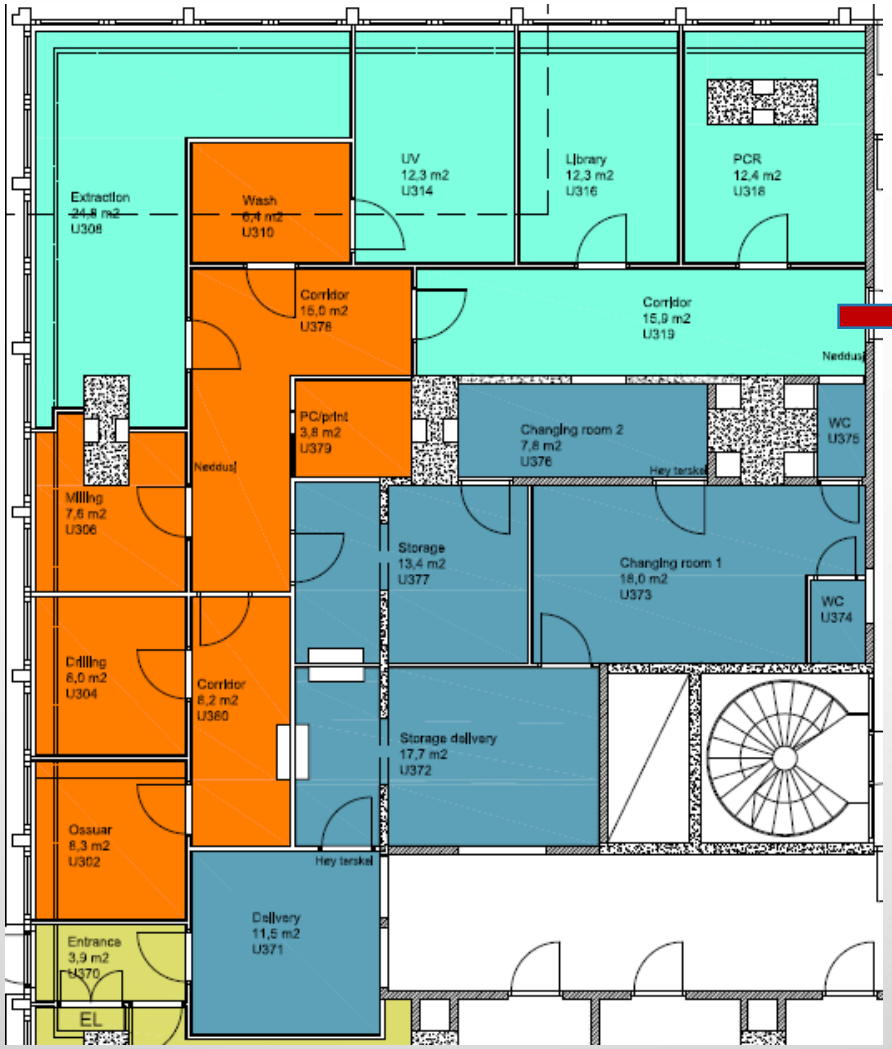
1. J. J. Austin, A. J. Ross, A. B. Smith, R. A. Fortey, R. H. Thomas, *Proc. R. Soc. London B* **264**, 467 (1997).
2. S. R. Woodward, N. J. Weyand, M. Bunnell, *Science* **266**, 1229 (1994).
3. H. Zischler *et al.*, *Science* **268**, 1192 (1995).
4. O. Handt, M. Krings, R. H. Ward, S. Pääbo, *Am. 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).
9. A. Cooper, in *Ancient DNA*, B. Herrmann and S. Hummel, Eds. (Springer-Verlag, New York, 1993), pp. 149-165.
10. A. D. Greenwood, C. Capelli, G. Possnert, S. Pääbo, *Mol. Biol. Evol.* **16**, 1466 (1999).
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

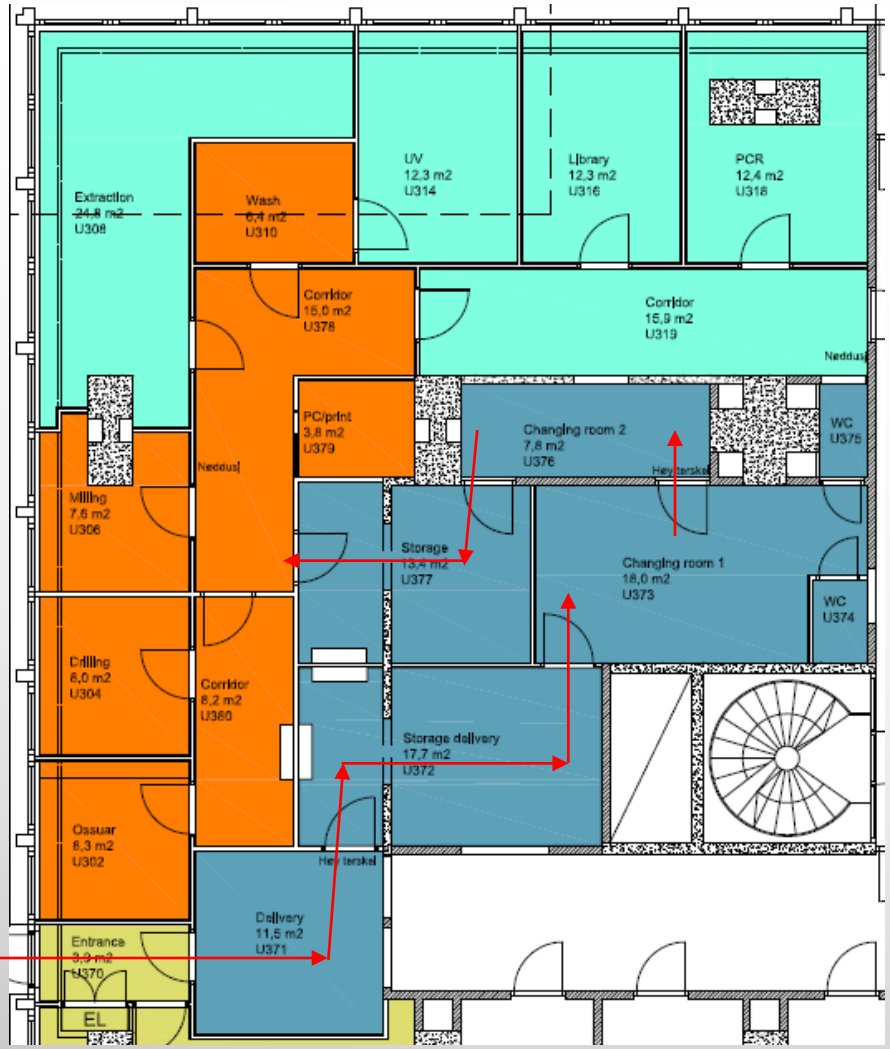


Entrance with Special Kay

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



Wear protective clothes.

Shower and fresh washed 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

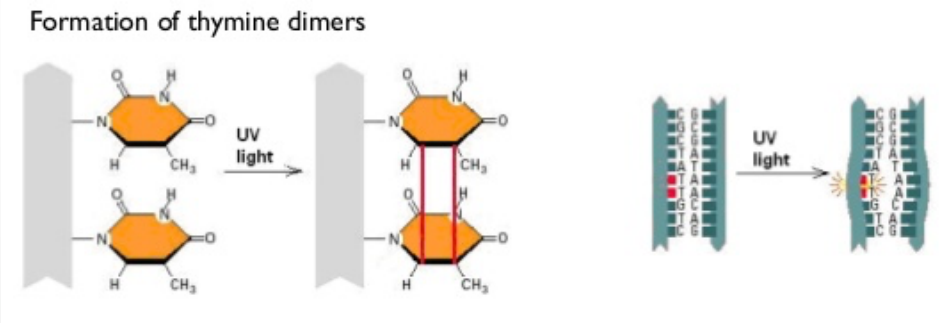
2. cover skin, prevent loss of eyelashes and hair in the lab to protect aDNA-lab environment from worker's DNA:

- 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

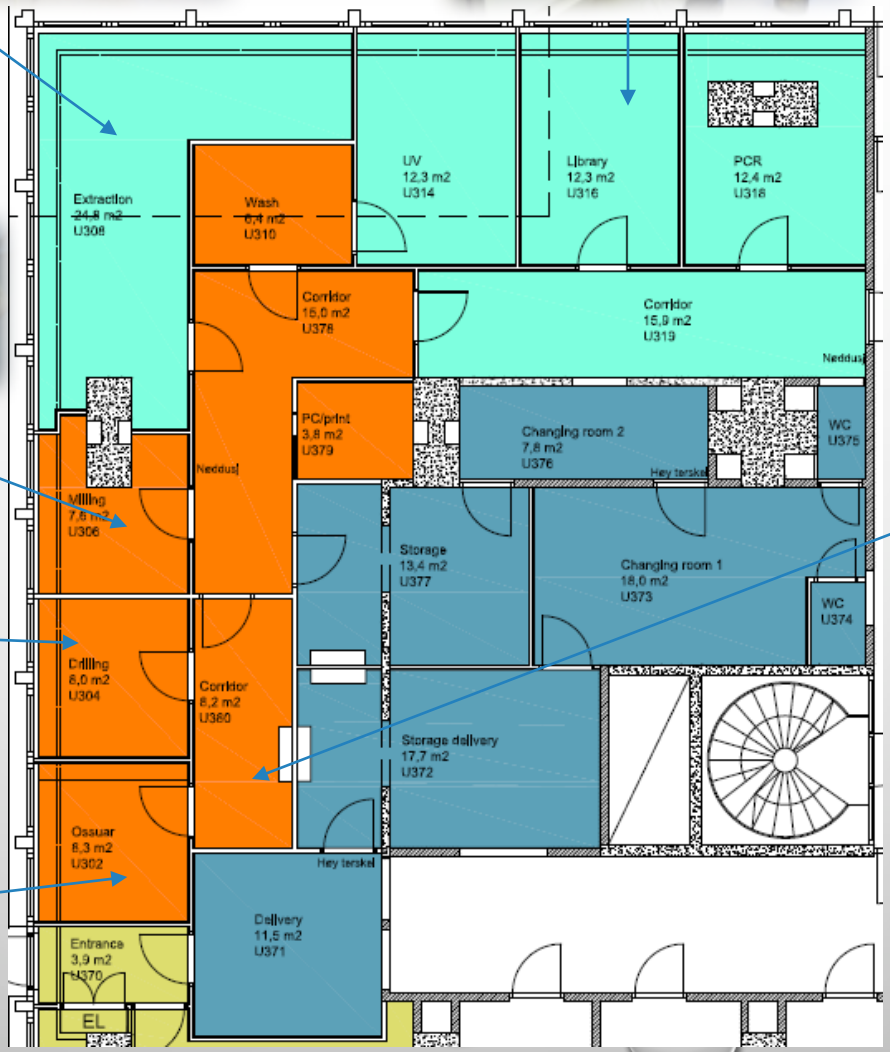
- Wear protective clothes by handling even in the repository (at least gloves and face mask)
- Don't wash the samples for aDNA analyses!!!
- Don't use glue or other chemicals!!!
- Don't write on the specimens!!! Use bags.
- **If possible, isolate two samples of each individual for aDNA analyses during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

Extraction

Preparation of PCRs and libraries

Milling

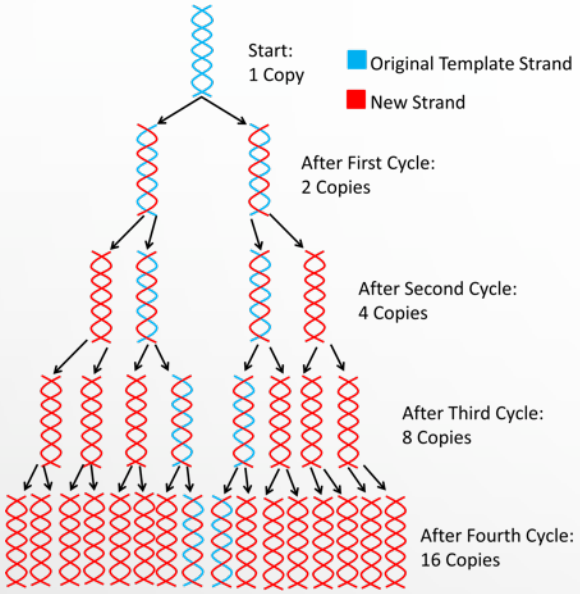
Sandblasting



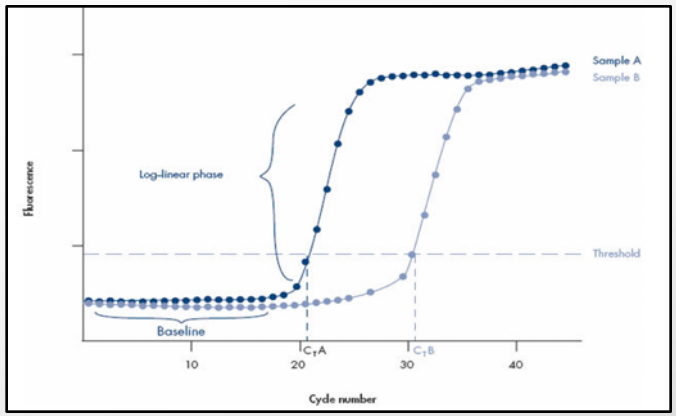
Introduction

Cataloging & UV

(q)PCR (outside the aDNA lab)



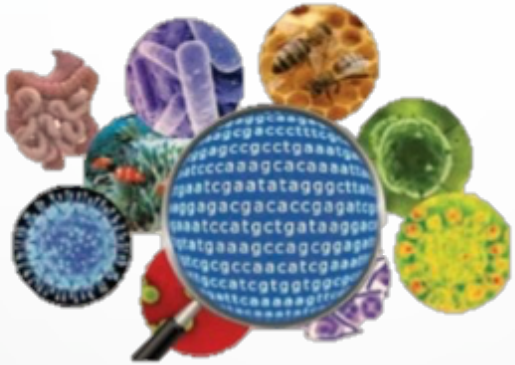
- During RealTime PCR (or qPCR wird) the number of copies of the target is determined thanks to a fluorescence marker (SYBRR® Green) , which is intercaled in the DNA double strains.



Quantification

Shotgun (Metagenomic analysis)

(outside the aDNA)

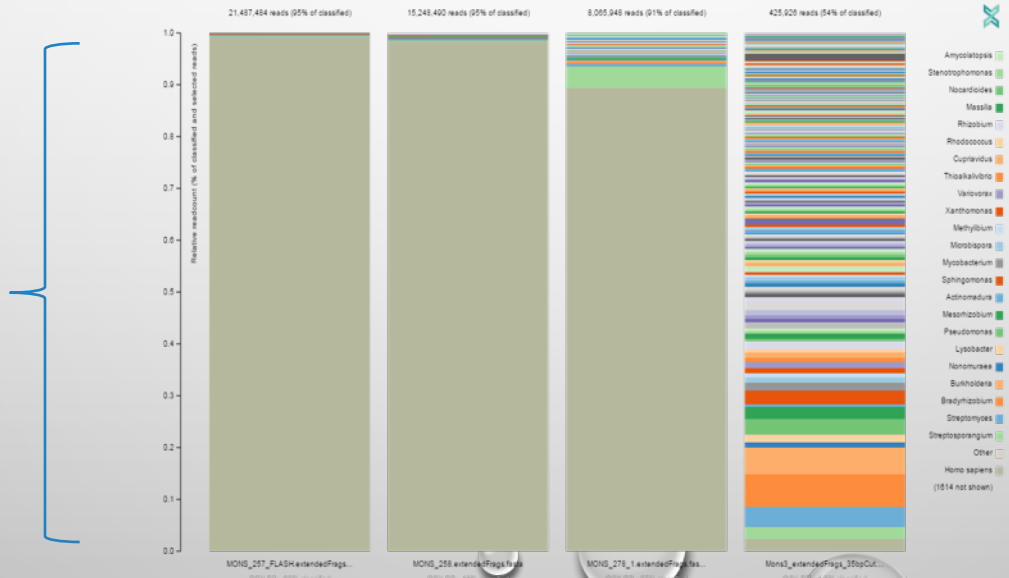


Whole collection of genomes isolated from a sample.

Pars petrosa

Tooth

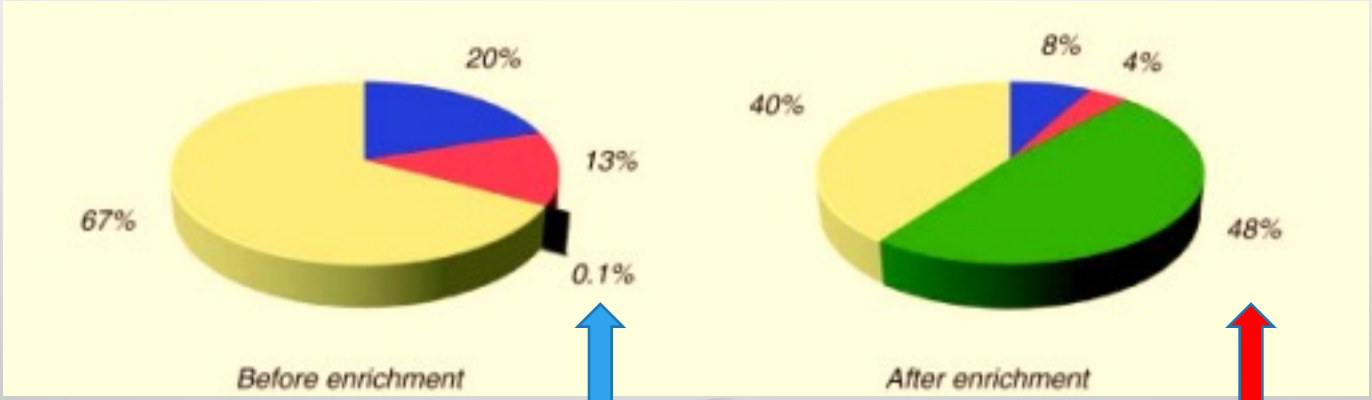
Human DNA



Credit: M. Guellil

Target enrichment / Capture

(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).

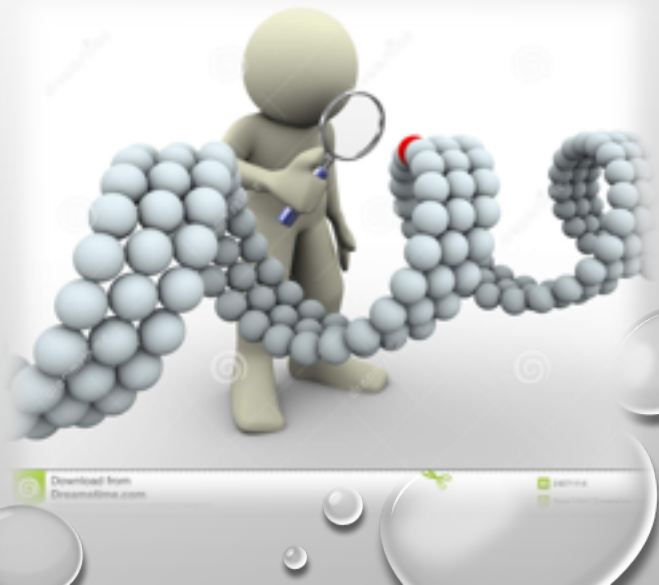
- Calculating global statistics on the project.

- Aligning the reads against the reference sequence(s).

- SNPs (or SNVs) calling.

- BLASTing

- …



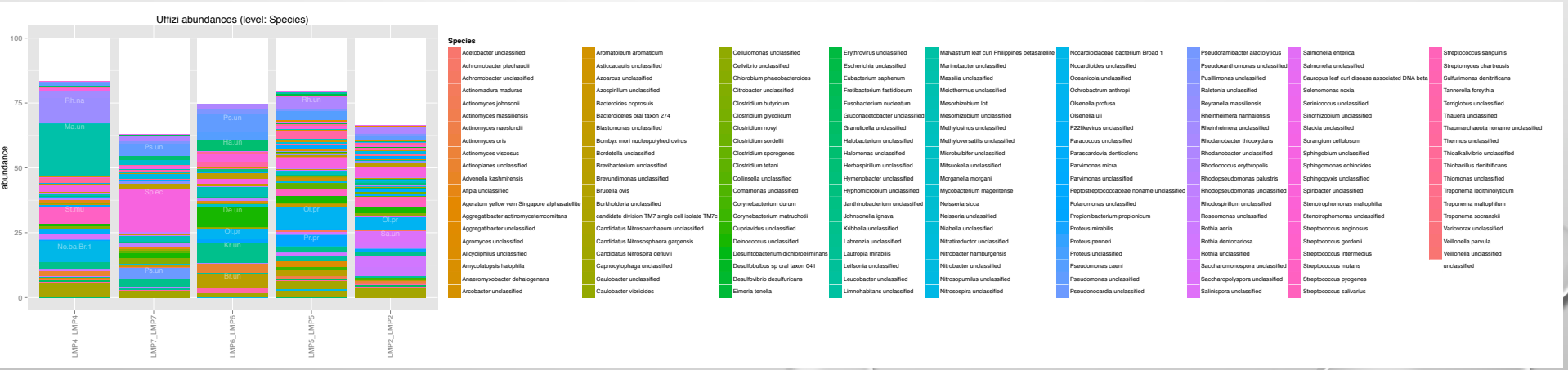
Assembling of aDNA

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

Metagenomics (bioinformatics for shot-gun)

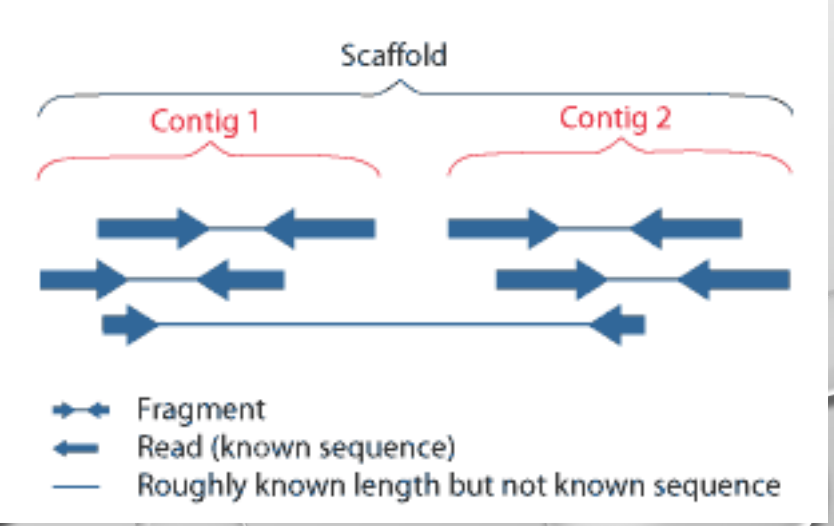
Different packages:

- Metaphlan (Metabit pipelines)
- Cracken
- Kaiju
- …

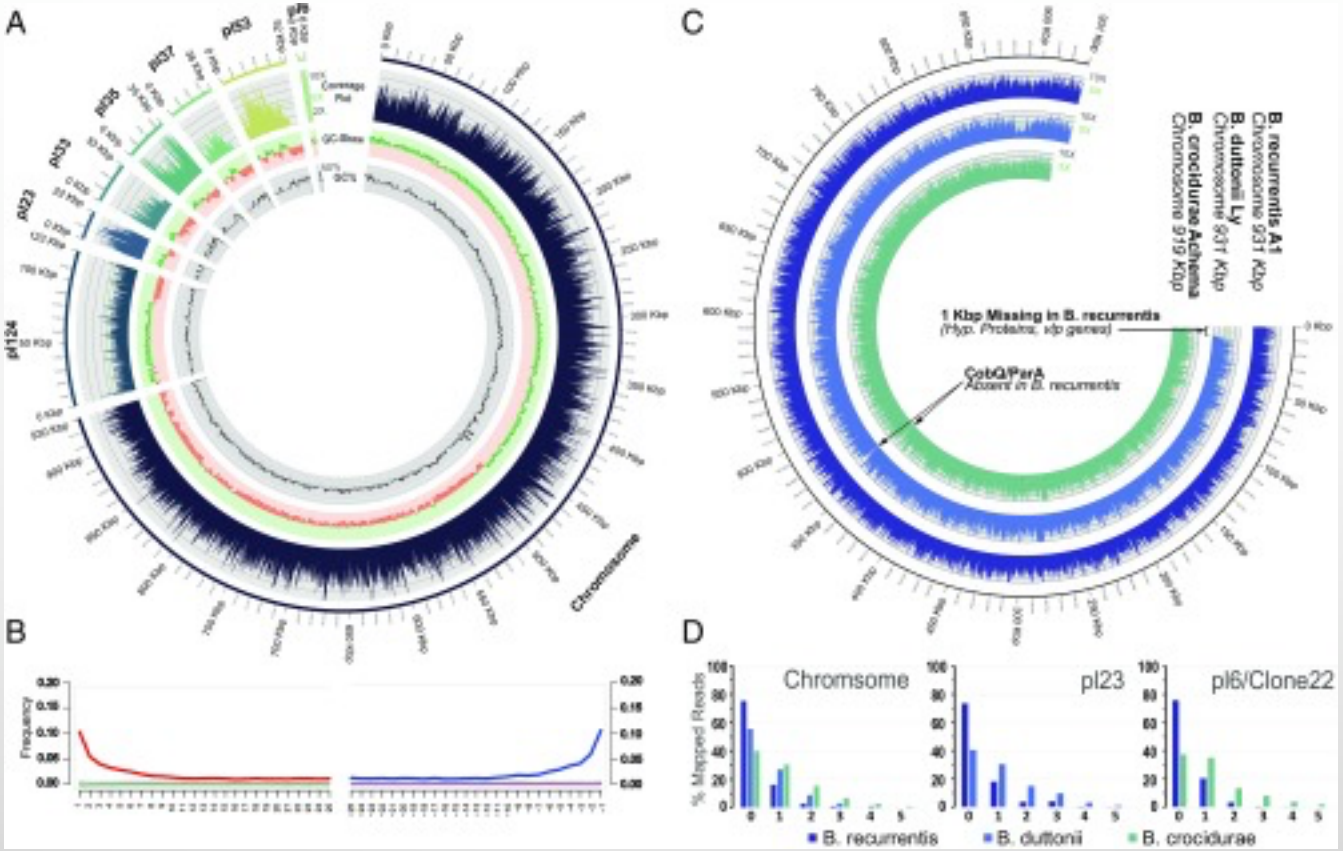


Major issues with bioinformatics

- Low coverage
- Short reads are difficult to attribute
- Incomplete data (scaffolds)
- Databanks are not (yet) complete (no reference for any organism)
- Misattribution of reads to another species
- Individual variability can be lost

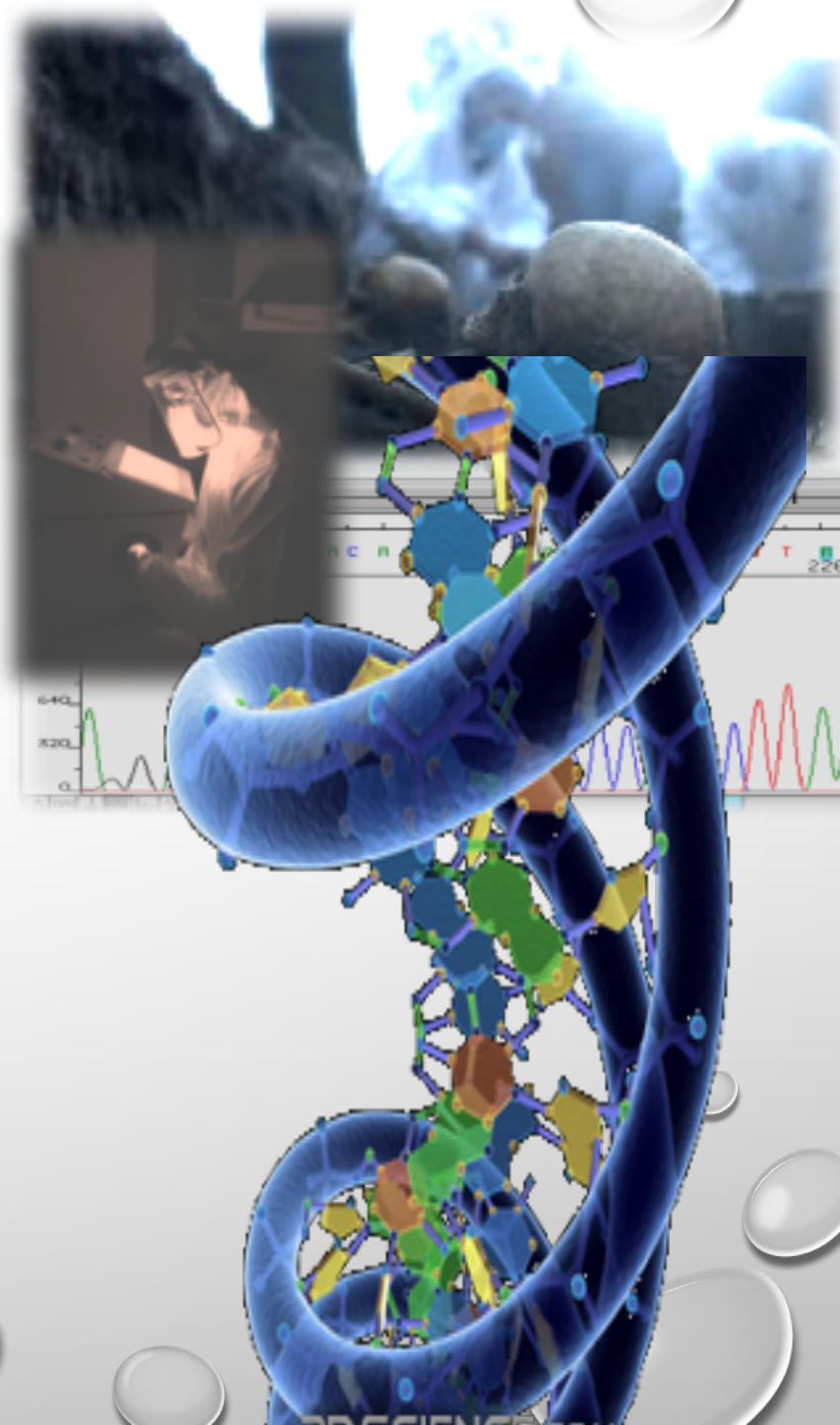


(Almost) complete genomes

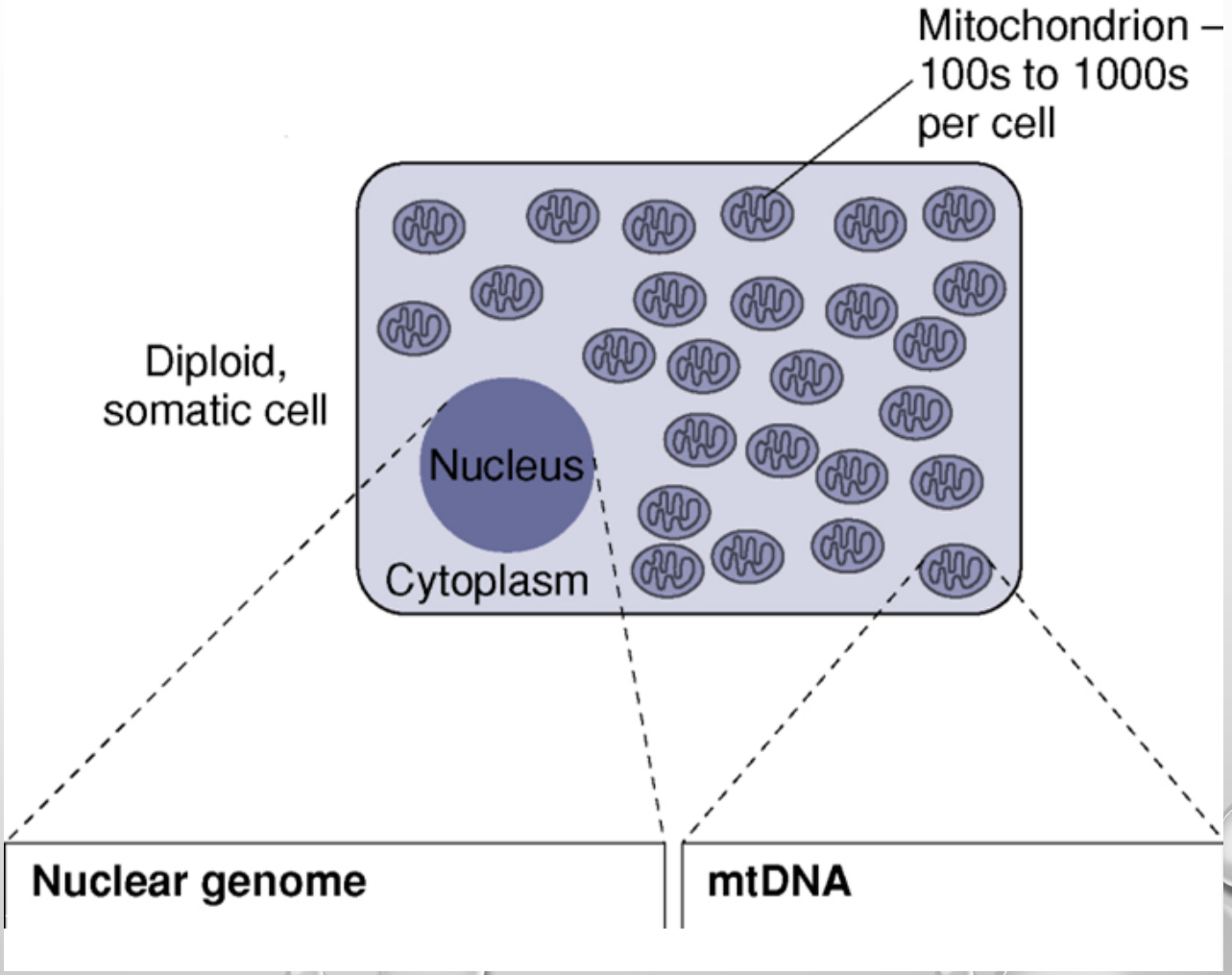


Guellil et al. 2018

Some examples of aDNA analysis from human remains

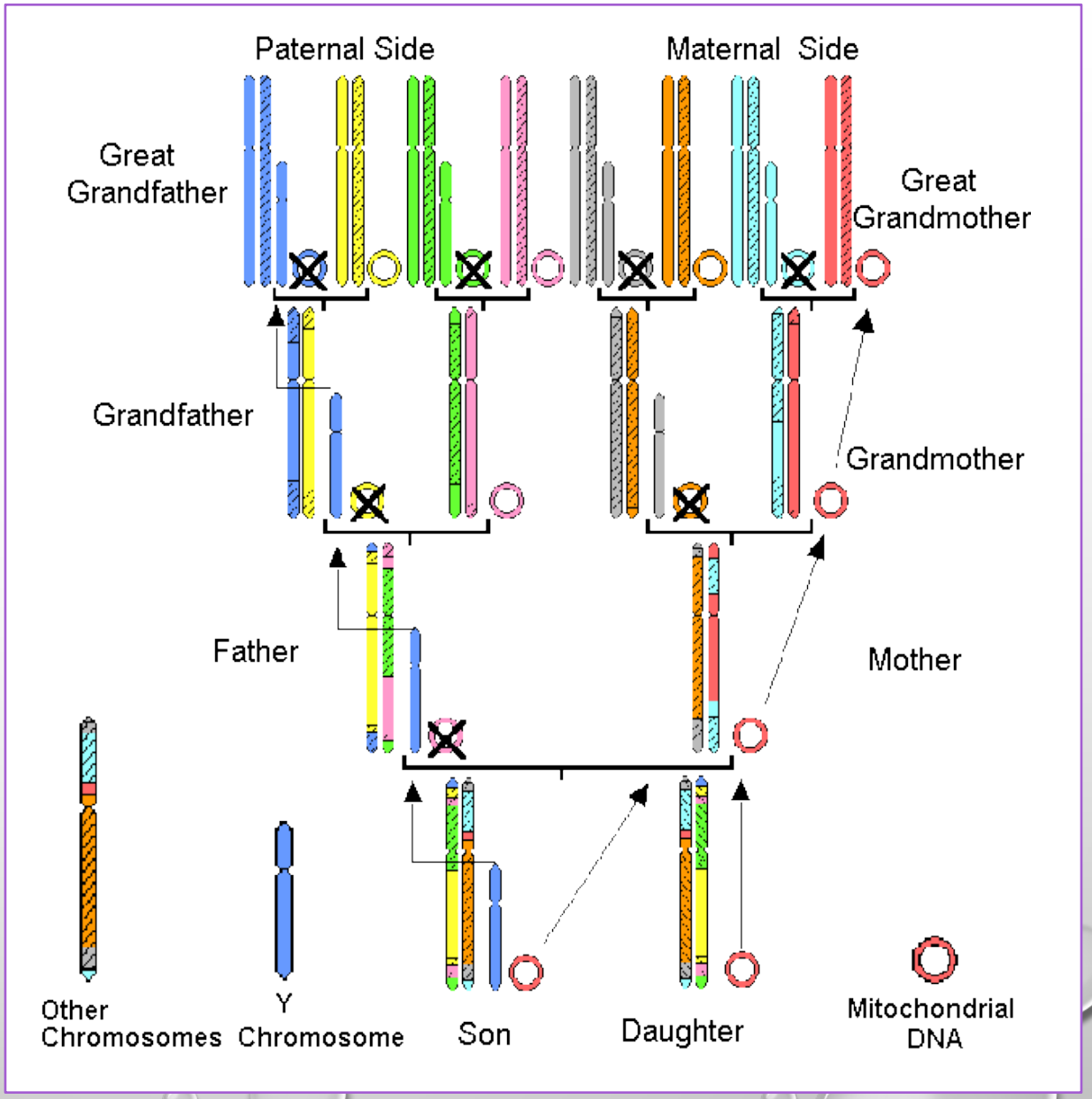


Sources of aDNA in mammalian cells



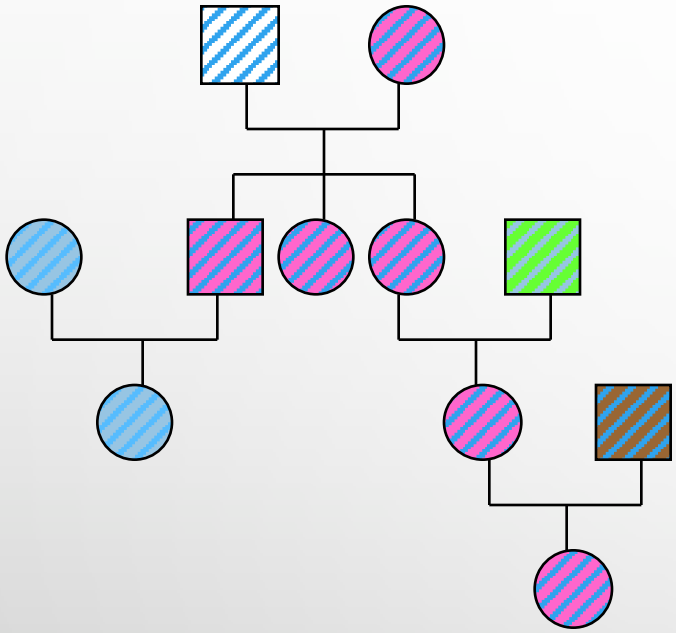
Nuclear genomic DNA vs. mtDNA

No recombination!



The Romanov

Maternal lineage

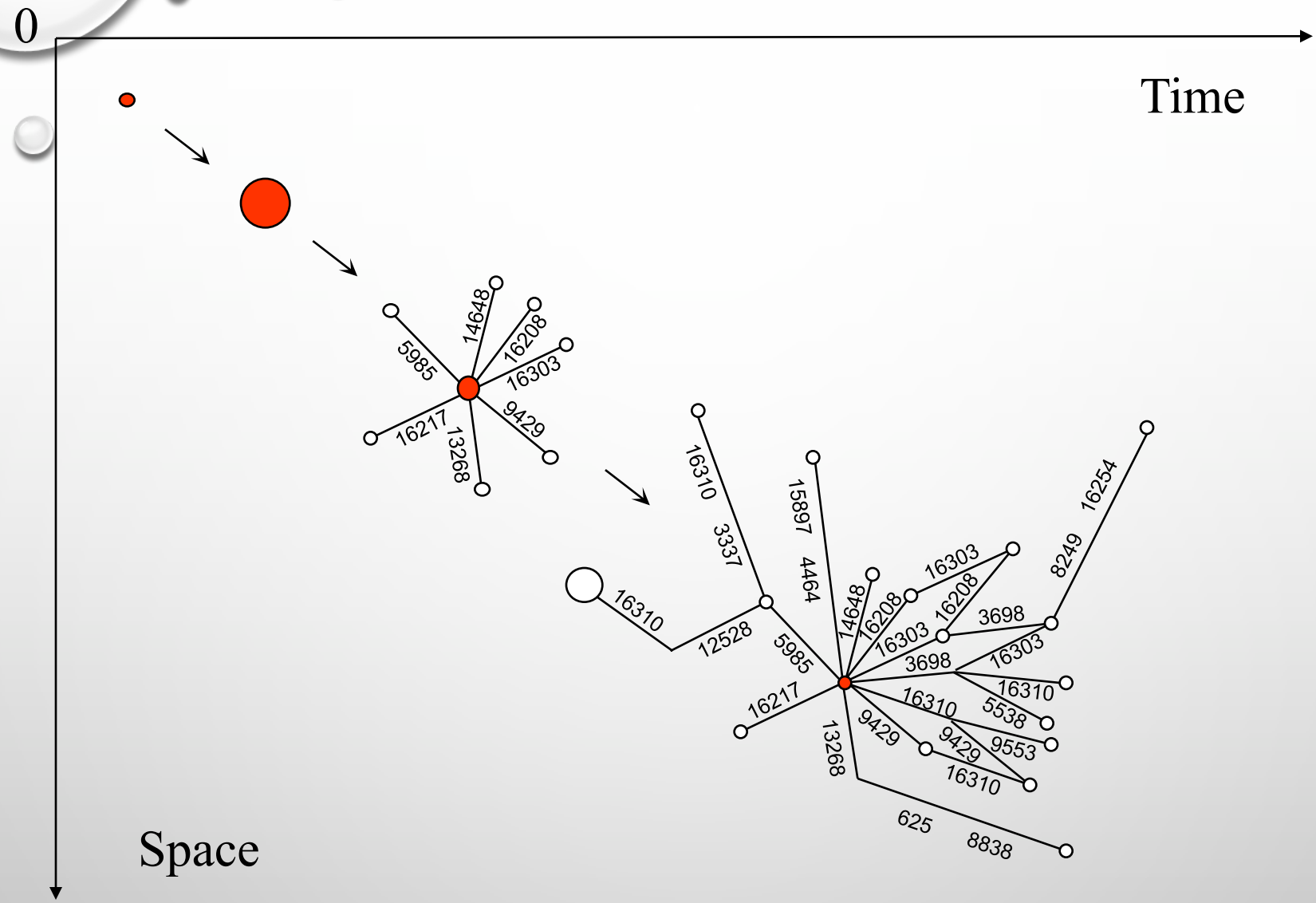


Haplotyp (*haploid genotype*)

	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	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					
W/ht1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	T	-	-	-	-	-	-	7	10	6	13	36
W/ht2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0	1
I/ht1	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	A	0	1	0	0	1
I/ht2	-	-	-	-	-	A	-	-	-	-	-	C	-	-	-	-	T	-	-	-	-	-	-	-	C	-	-	-	A	0	1	7	0	8
I/ht3	-	-	-	-	-	A	-	A	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	A	0	0	0	1	1
I/ht4	-	-	-	-	-	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)

Haplogroups



Courtesy of Peter Forster

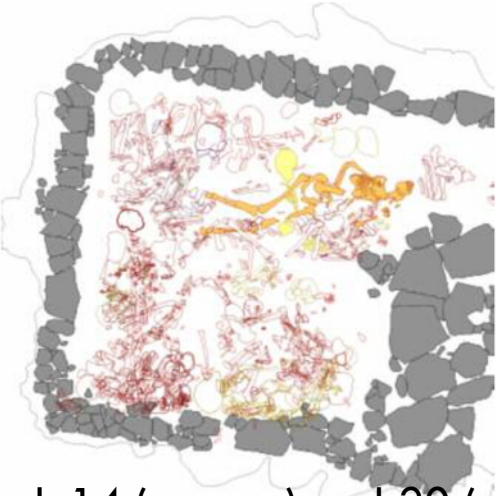
Attribution of skeletal elements

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.)

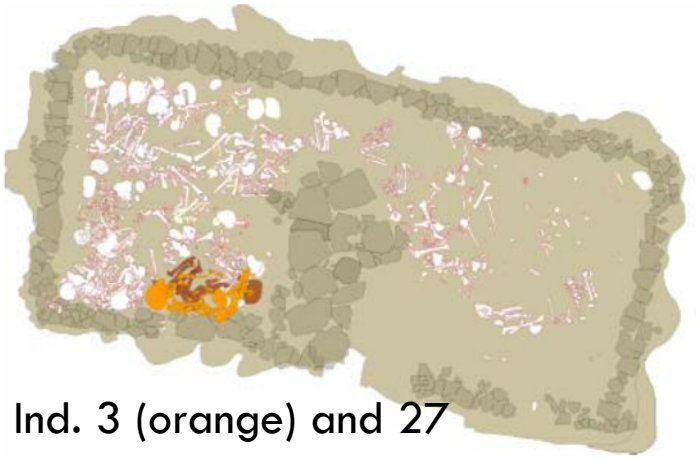
The relatives of Benzingerode

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

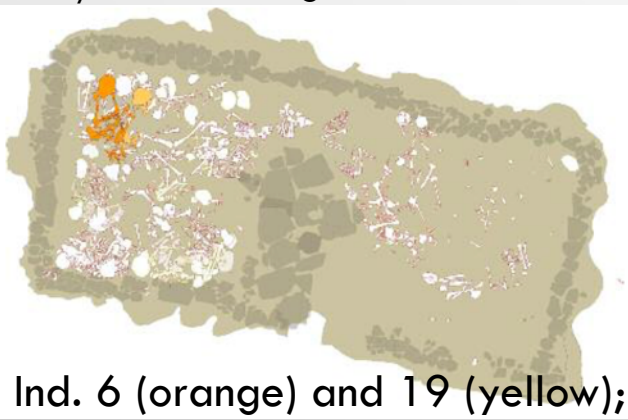


Ind. 14 (orange) and 20 (yellow); child/mother or gramma

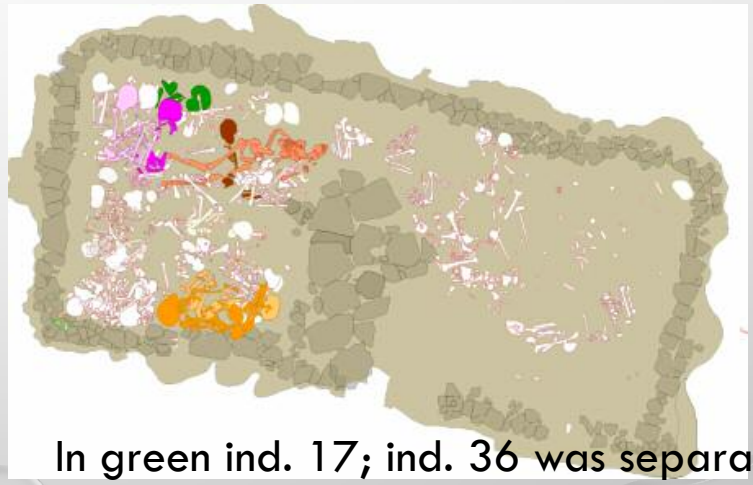
Haplotyp	Ind.	Haplogruppe
1.	1	U
2.	14, 20	
3.	35	
4.	18	
5.	3, 27	K
6.	33	
7.	6, 19	T
8.	17, 36	H
9.	29	
10.	40	
11.	39	V ?
12.	15	W
13.	37	X



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

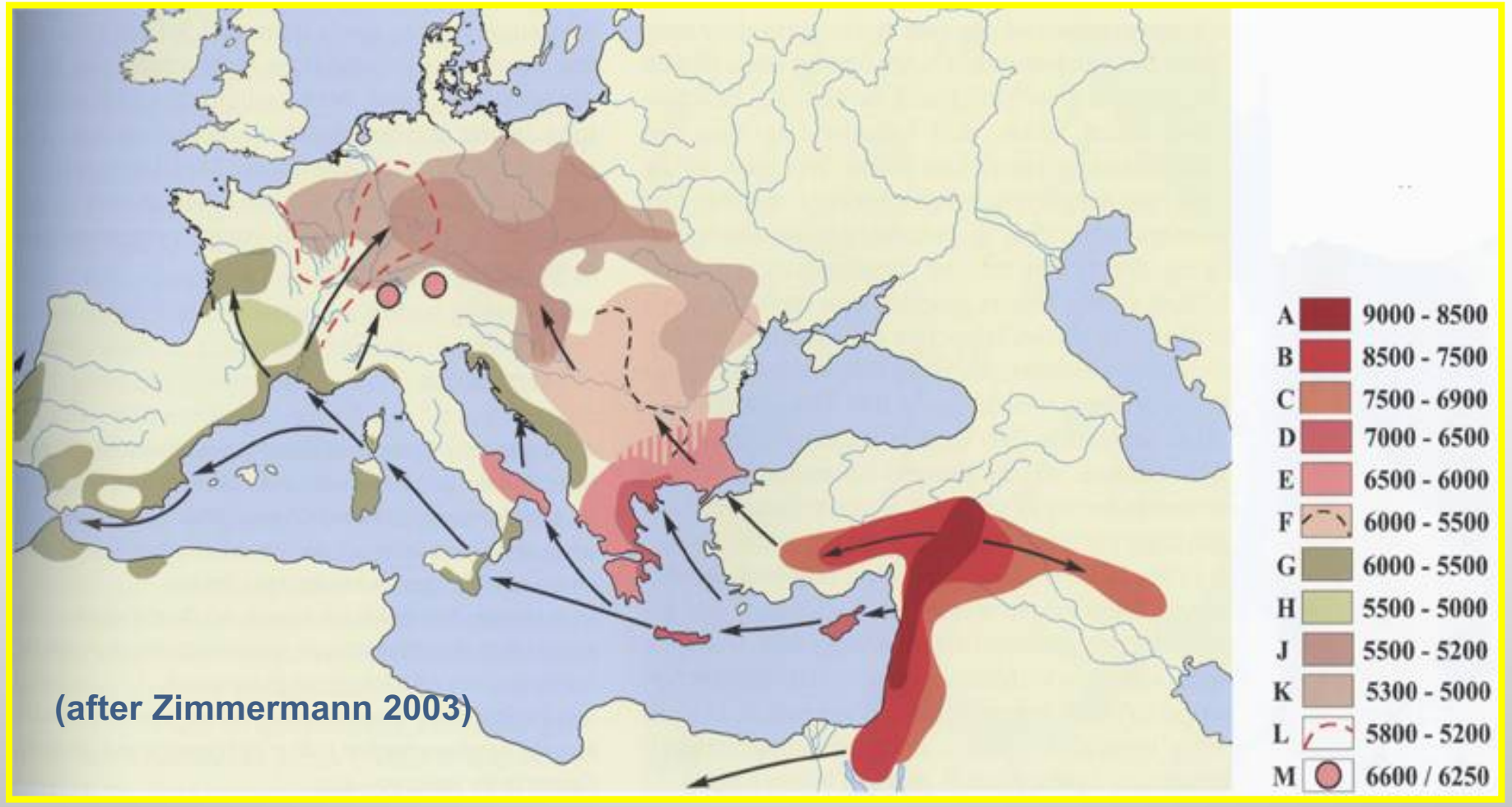


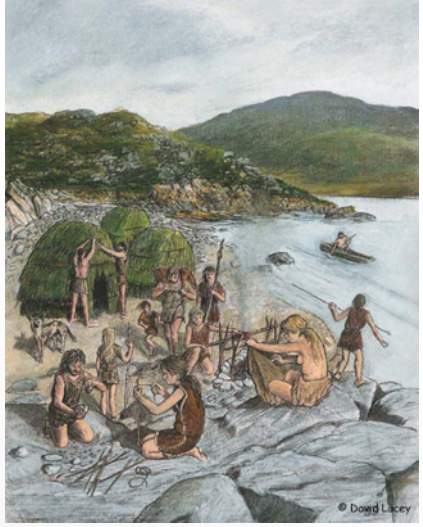
Ind. 6 (orange) and 19 (yellow); daugther/mother or gramma; sibs or cousins



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

mtDNA in Population Genetics: The Neolithic Transition



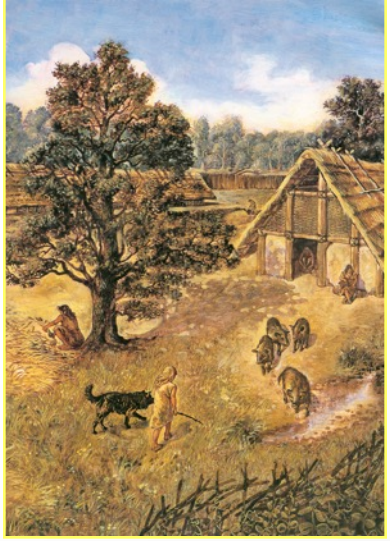


Hunter-gatherers (Palaeo-Mesolithic periods) 45,000-4,000 YBP

- 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

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 Tänzer,[1] Richard Villems,[3] Colin Renfrew,[2] Detlef Gronenborn,[4] Kurt Werner Alt,[1] Joachim Burger[1]

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.

Agriculture originated in the Fertile Crescent of the Near East about 12,000 years ago, from where it spread via Anatolia all over Europe (*1*). It has been widely suggested that the global expansion of farming included not only the dispersal of cultures but also of genes and languages (*2*). Archaeological cultures such as the Linear pottery culture (*Linearbandkeramik* or LBK) and Alföldi Vonaldiszes Kerámia (AVK) mark the onset of farming in temperate regions of Europe 7500 years ago (*3*). These early farming cultures originated in Hungary and Slovakia, and the LBK then spread rapidly as far as the Paris Basin and the Ukraine (*4*, *5*). The remarkable speed of the LBK expansion within a period of about 500 years, and the general uniformity of this archaeological unit across a territory of nearly a million square kilometers (Fig. 1), might indicate that the spread was fueled to a considerable degree by a migration of people (*6*–*8*). On the other hand, a number of archaeological studies suggest that local European hunter-gatherers had shifted to farming without a large-scale uptake of genes from the first farmers (*9*–*11*). Genetic studies carried out on modern Europeans have led to conflicting results, with estimates of Neolithic input into the present population ranging from 20 to 100% (*12*–*20*). A theoretical simulation study by Currat and Excoffier (*21*) has recently suggested a minor contribution, clearly less than 50%, and possibly much less. Conclusive ancient DNA studies on skeletons of the first European farmers have so far not been published to our knowledge.

To resolve the question regarding the extent of the Neolithic female contribution to the present European population, we collected 57 Neolithic skeletons from 16 sites of the LBK/AVK culture from Germany, Austria, and Hungary. These include well-known archaeological sites such as Flomborn, Schwetzingen, Eilsleben, Asparn-Schletz, and several new excavations; for example, from Halberstadt and Derenburg Meerenstieg II. All human remains were dated to the LBK or AVK period (7500 to 7000 years ago) on the basis of asse finds. We extracted DNA from b from the morphologically well-pres uals, and we amplified nucleotide 15997–16409 [see supporting o (*22*)] of the mitochondrial geno overlapping primer pairs. In additi number of coding-region mtD phisms, which are diagnostic for n in the mtDNA tree (*22*).

From a total of 57 LBK/AV analyzed, 24 individuals (42%) re ducibly successful amplification primer pairs from at least two extractions usually sampled from of the skeleton. Eighteen of t belonged to typical western Eur branches; there were seven H or five T sequences, four K sequen quence, and one U3 sequence (tal 18 sequences are common and modern Europeans, Near Eastern

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Response to Comment on "Ancient DNA from the First European Farmers in 7500-Year-Old Neolithic Sites"

Joachim Burger,[1]* Detlef Gronenborn,[2] Peter Forster,[3] Shuichi Matsumura,[3] Barbara Bramanti,[1] Wolfgang Haak[1]

The discovery of mitochondrial type N1a in Central European Neolithic skeletons at a high frequency enabled us to answer the question of whether the modern population is maternally descended from the early farmers instead of addressing the traditional question of the origin of early European farmers.

Our study (*1*) described the discovery of the mitochondrial type N1a in 6 out of 24 Central European Neolithic skeletons, which was unexpected because today this type is found at 150-times lower frequency in Europe. We offered two possible explanations for our observations. First, female Early Neolithic farmers could have been replaced by immigrant women after the early Neolithic (post–early-Neolithic replacement theory). Second, the female early Neolithic farmers could have been genetically diluted by resident native hunter-gatherers (Paleolithic survival theory). Both interpretations are compatible with our genetic data. Because there is so far no archaeological evidence for a major post–early-Neolithic population replacement, we suggested that the Paleolithic survival theory is more likely.

In their comment, Ammerman et al. (*2*) raise concerns about our study and call for further ancient DNA studies. First, the authors may have misread the central question asked in our study. We tackled the question of the fate of the early European farmers [as represented by the Neolithic skeletons of the Linear pottery culture (LBK)], that is, whether modern central Europeans are descended from them or not. In contrast, Ammerman *et al.* imply that our study deals with questions on the origin of the early European farmers, such as whether the female lineages in the farmer skeletons were immigrants from southeastern Europe or whether they were local Mesolithic women who intermarried with incoming males. Irrespective of this misunderstanding, the origin of the farmers remains an important question, and the plight of the early farmers' descendants outlined in our study, along with the intriguing ancient DNA data, may one day contribute to a better understanding of farming origins.

We believe it is worthwhile to clarify the points that Ammerman and colleagues usefully raise. Regarding the point that we should have analyzed far more than 24 samples, we point out that our main conclusions (*1*) were based on statistically significant results. Furthermore, we carefully examined the sample locations and mitochondrial DNA types to exclude the possibility of biased sampling. Ammerman *et al.* (*2*) are correct that one of our 24 skeletons, namely the one from Ecsegfalva, is not a "first" farmer but only an "early" farmer, as far as eastern Hungary is concerned. We included this skeleton in our analysis because it is culturally and chronologically closely related to our actual focus, the first farmers in the LBK area of neighboring Central Europe (Fig. 1). The other 23 skeletons represent the first full farming populations in their local LBK regions; this is particularly the case for the Flomborn site, which is among the first LBK colonies west of the Rhine and is also the type-site for the "Flomborn"

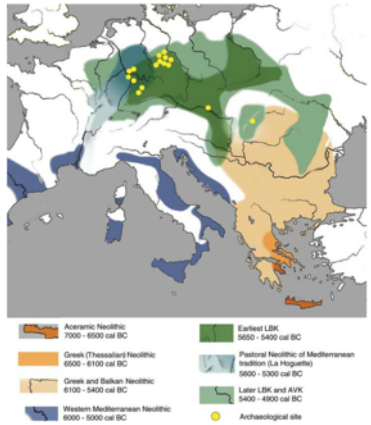

Fig. 1. The spread of farming across Europe. The colors indicate time scales for the spread of the early Neolithic in Europe. All 24 samples of our ancient DNA study belong to the same LBK/AVK (Linear pottery and Alföld linear pottery culture) chronostratum, representing the first farmers in much of central Europe.

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Genetic Discontinuity Between Local Hunter-Gatherers and Central Europe's First Farmers

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After the domestication of animals and crops in the Near East some 11,000 years ago, farming had reached much of central Europe by 7500 years before the present. The extent to which these early European farmers were immigrants or descendants of resident hunter-gatherers who had adopted farming has been widely debated. We compared new mitochondrial DNA (mtDNA) sequences from late European hunter-gatherer skeletons with those from early farmers and from modern Europeans. We find large genetic differences between all three groups that cannot be explained by population continuity alone. Most (82%) of the ancient hunter-gatherers share mtDNA types that are relatively rare in central Europeans today. Together, these analyses provide persuasive evidence that the first farmers were not the descendants of local hunter-gatherers but immigrated into central Europe at the onset of the Neolithic.

Europe has witnessed several changes in archaeological cultures since anatomically modern humans displaced the Neanderthal population 30,000 to 40,000 years ago (*1*, *2*). Palaeolithic hunter-gatherers survived the Last Glacial Maximum (LGM) about 25,000 years ago in southern and eastern refugia (*3*) and resettled central Europe after the retreat of the ice sheets. With the end of the Ice Age at ~9600 B.C.E., their Mesolithic descendants or successors had recolonized large parts of the deglaciated northern latitudes (*4*, *5*). From around 6400 B.C.E., the hunter-gatherer way of life gave way to farming cultures in a transition known as the Neolithic Revolution (*6*). The extent to which this important cultural transition was mediated by the arrival of new peoples, and the degree of Mesolithic and early Neolithic ancestry in Europeans today, have been debated for more than a century (*7*–*10*). To address these questions directly, we obtained mitochondrial DNA (mtDNA) types from 22 central and northern European post-LGM hunter-gatherer skeletal remains (Fig. 1) and compared 20 of these (those for which full sequence information was available) to homologous mtDNA sequences from 25 early farmers (*11*, *12*) and 484 modern Europeans from the same geographic region (*13*). Our ancient sample spans a period from circa (ca.) 13,400 to 2300 B.C.E. and includes bones from Hohler Fels in the Ach valley (Late Upper Paleolithic) and Hohlenstein-Stadel in the Lone valley (Mesolithic). Extensive precautions were taken to ensure sequence authenticity (*14*), including extracting independent samples from different skeletal locations of the same individuals and examining remains only from high latitudes or cave sites with good biomolecular preservation.

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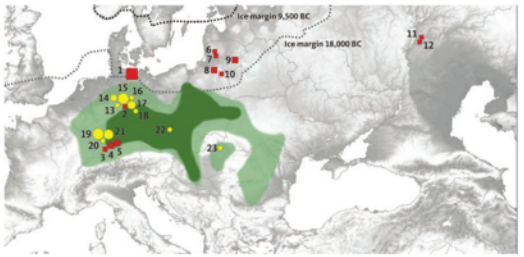
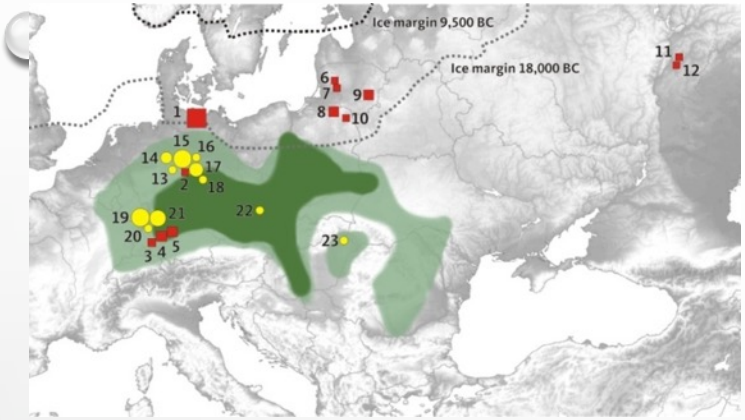


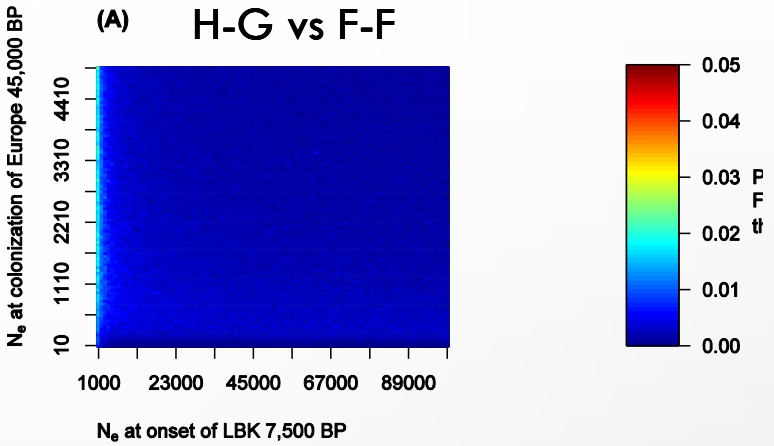
Fig. 1. mtDNA types from prehistoric samples of hunter-gatherers and farmers. The green shading represents the first farming areas [dark green: early LBK, 5650 to 5400 calibrated years B.C.E. (calBC); light green: LBK, 5400 to 4900 calBC] in central Europe, based on archaeological finds, whereas squares represent successfully analyzed Late Palaeolithic, Mesolithic, and Ceramist hunter-gatherers dating from 13,400 to 2300 B.C.E. The term "Neolithic" is sometimes applied to the Eastern European Ceramist culture because of their use of pottery, but this does not imply a farming economy (*13*). Previously analyzed (*11*, *12*) LBK farming sites are marked with circles for comparison. The area of each square or circle is proportional to the number of individuals successfully investigated. In red are labeled archaeological sites with one or more U4/U5 individuals; in yellow, sites with other mtDNA types, highlighting the specificity of U types in the prehistoric hunter-gatherers. The sites are as follows: 1, Ostorf; 2, Bad Dürrenberg; 3, Falkensteiner Höhle; 4, Hohler Fels; 5, Hohlenstein-Stadel; 6, Donkalnis; 7, Spiginas; 8, Dudka; 9, Kretuonas; 10, Drestwo; 11, Chekalino; 12, Lebyazhinka; 13, Unseburg; 14, Unterwiederstedt; 15, Derenburg/Meerenstieg; 16, Eilsleben; 17, Halberstadt; 18, Seehausen; 19, Flomborn; 20, Vaihingen an der Enz; 21, Schwetzingen; 22, Asparn/Schletz; 23, Ecsegfalva.

Downloaded from www.sciencemag.org on October 6, 2009

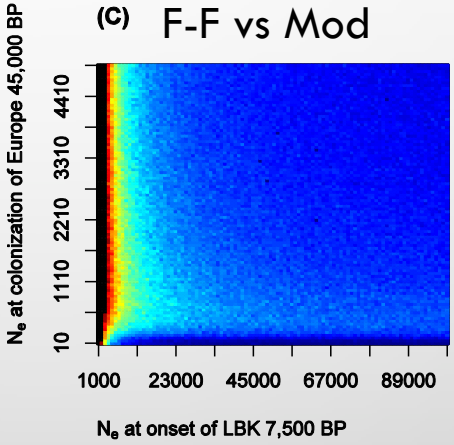
Bramanti et al. (2009)

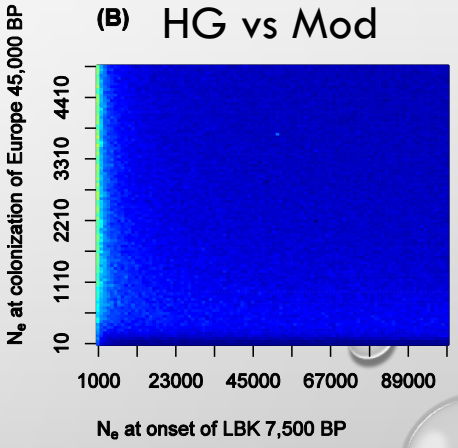
1) No genetic continuity between Hunter-Gatherers & First Farmers



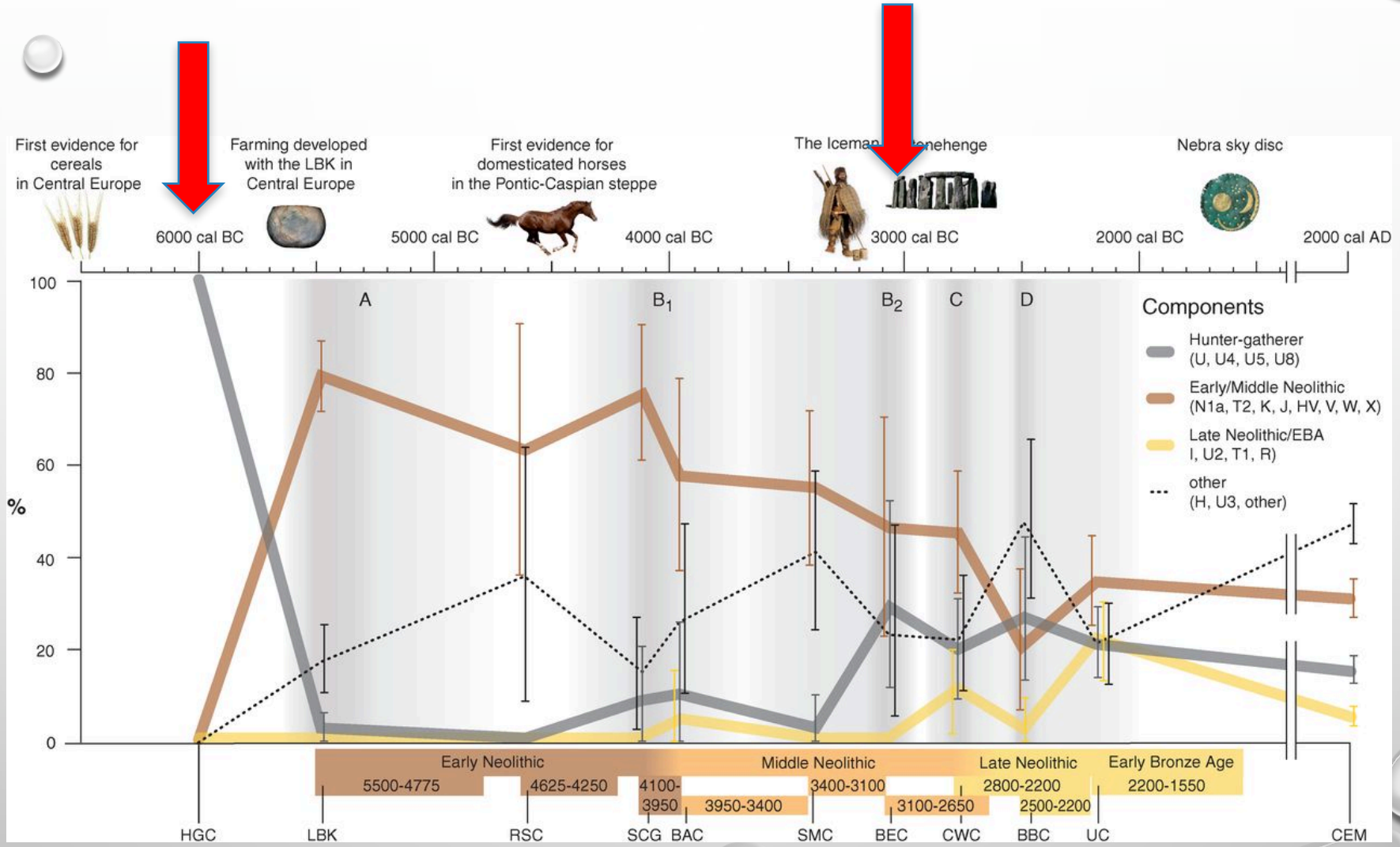


2) No direct genetic continuity between Hunter-Gatherers, First Farmers and modern Europeans



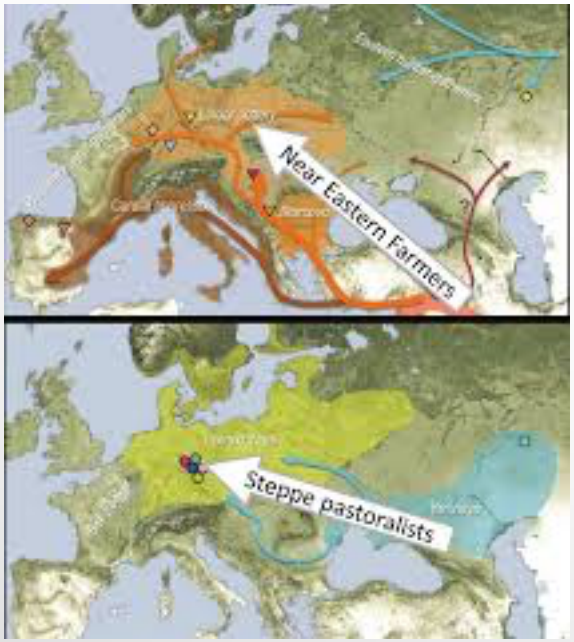


H-G and Farmers in Central Europe

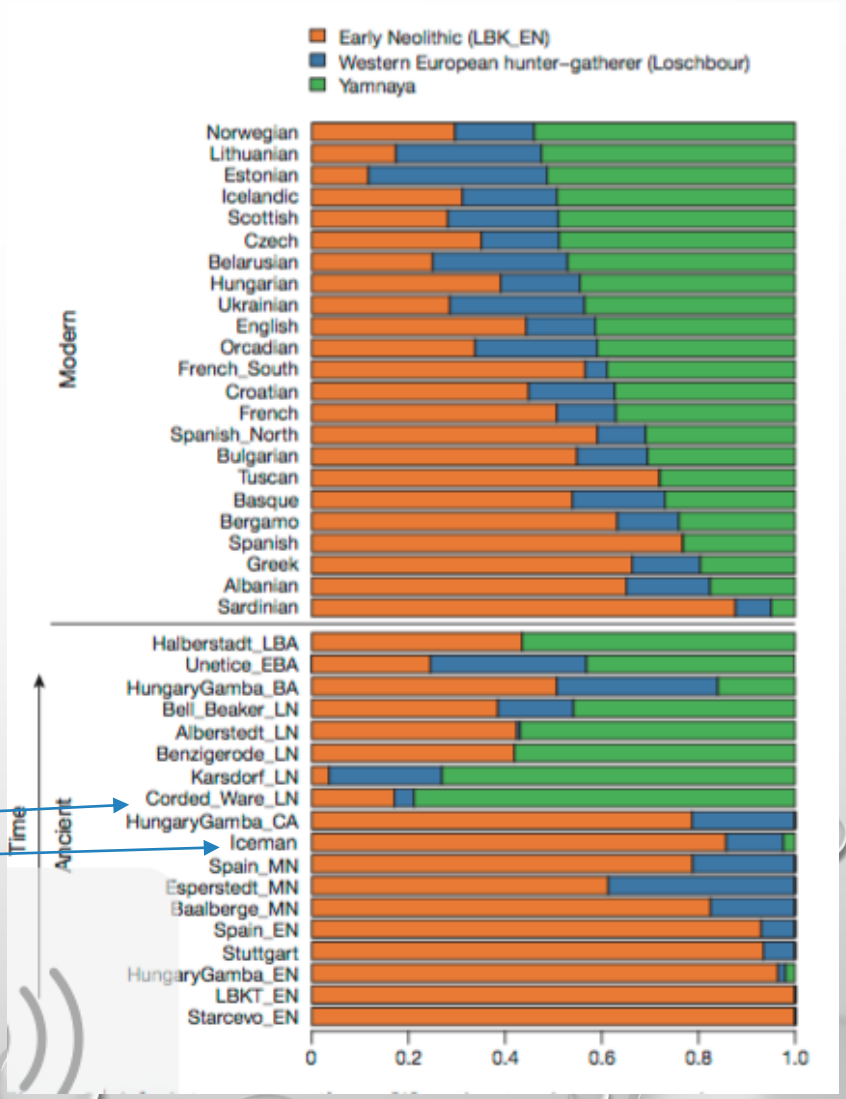


Brandt et al. 2013

Today Europeans are a mixture of not two but three different ancestral populations (mtDNA).

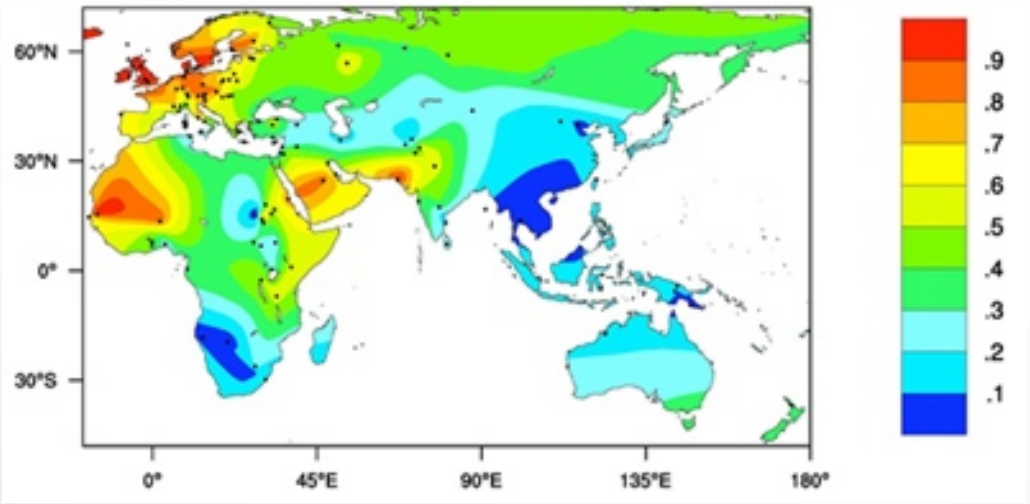


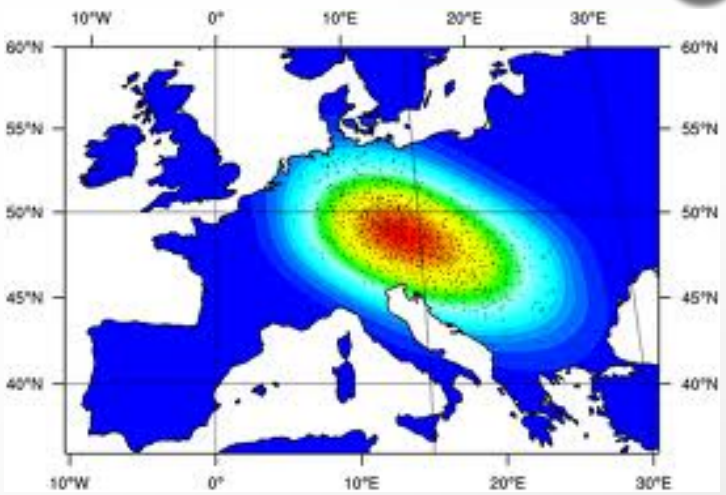
2400 BCE
3200 BCE



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

Nuclear DNA: Lactase-persistance





PNAS

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

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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)
...

nDNA: Somatic traits

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)

Kirsanow et al. Submitted

(85 prehistoric and 138 historic individuals analysed)

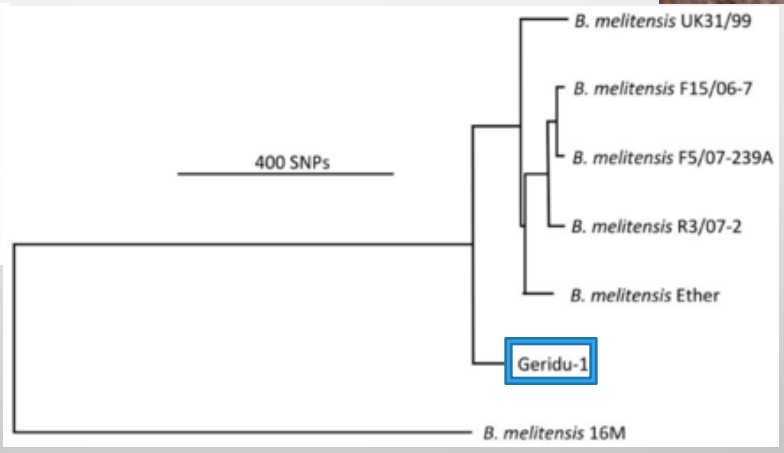
Identification and phylogeny of pathogens

MACROSCOPICAL LESIONS

Tuberculosis

Lepra

Syphilis

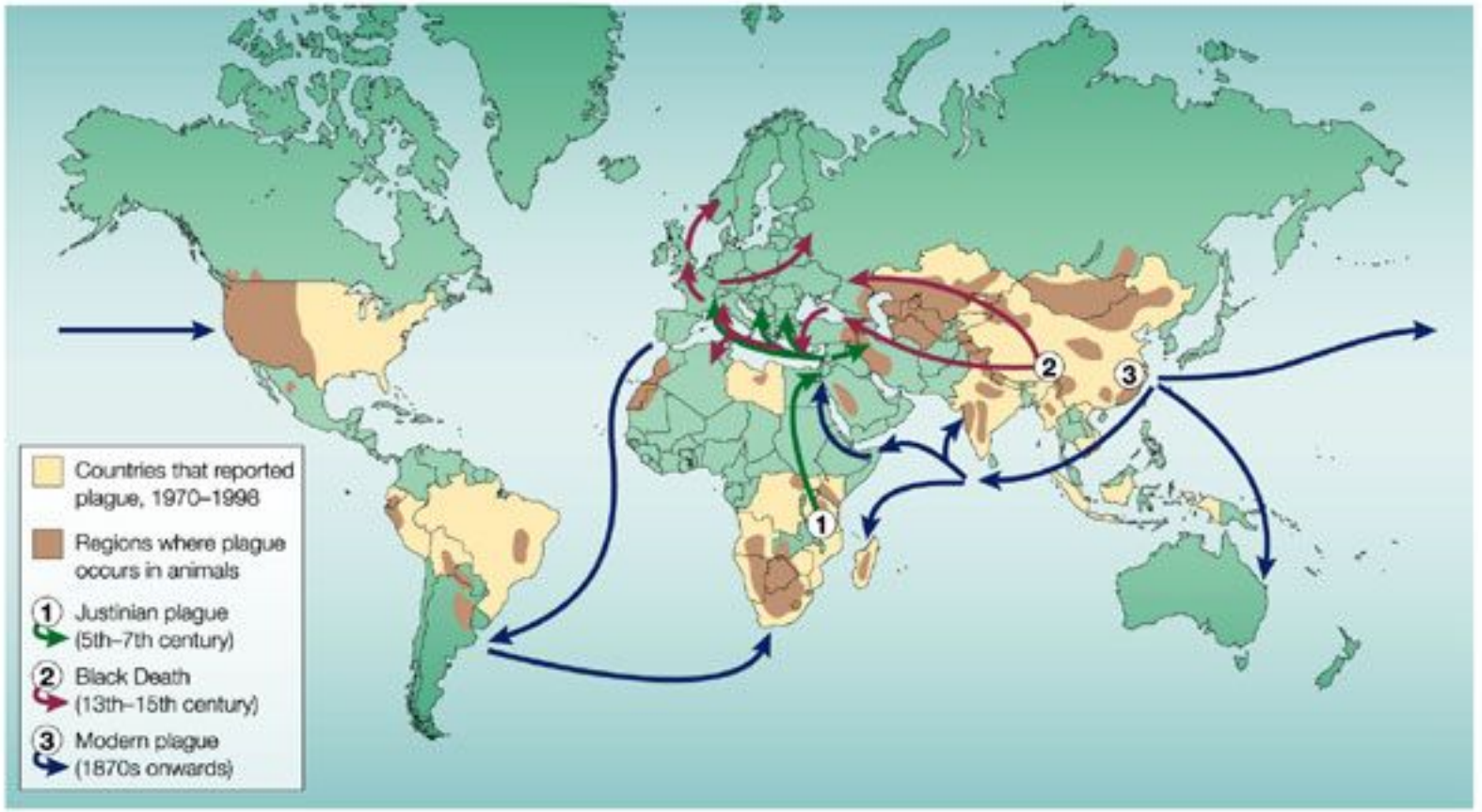


Brucellosis

Kay et al. 2014

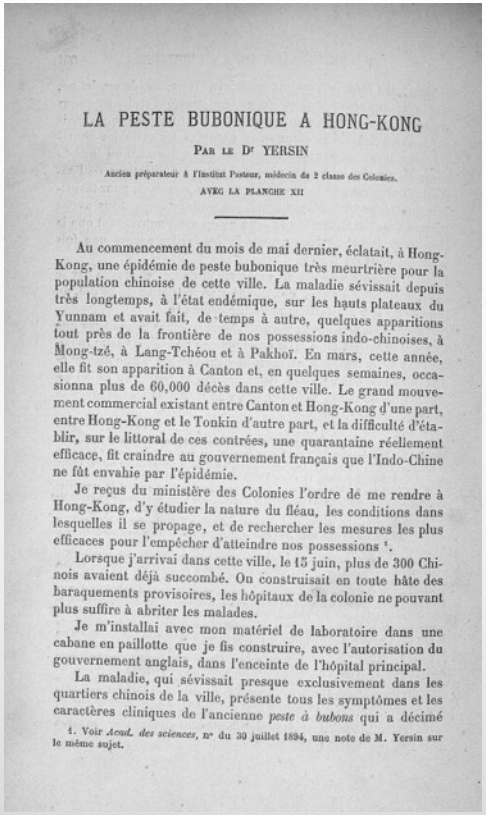
THE THREE PLAGUE PANDEMICS

WREN 2003



1894

YERSIN, Alexandre. - La peste bubonique à Hong-Kong. In : Annales de l'Institut Pasteur, 1894,Vol. 8, pp. 662-7



LA PESTE BUBONIQUE A HONG-KONG

PAR LE Dr YERSIN

Ancien préparateur à l'Institut Pasteur, médecin de 2e classe des Colonies.

AVEC LA PLANCHE XII

Au commencement du mois de mai dernier, éclatait, à Hong-Kong, une épidémie de peste bubonique très meurtrière pour la population chinoise de cette ville. La maladie sévissait depuis très longtemps, à l'état endémique, sur les hauts plateaux du Yunnam et avait fait, de temps à autre, quelques apparitions tout près de la frontière de nos possessions indo-chinoises, à Mong-tzé, à Lang-Tchéou et à Pakhoï. En mars, cette année, elle fit son apparition à Canton et, en quelques semaines, occasionna plus de 60,000 décès dans cette ville. Le grand mouvement commercial existant entre Canton et Hong-Kong d'une part, entre Hong-Kong et le Tonkin d'autre part, et la difficulté d'établir, sur le littoral de ces contrées, une quarantaine réellement efficace, fit craindre au gouvernement français que l'Indo-Chine ne fût envahie par l'épidémie.

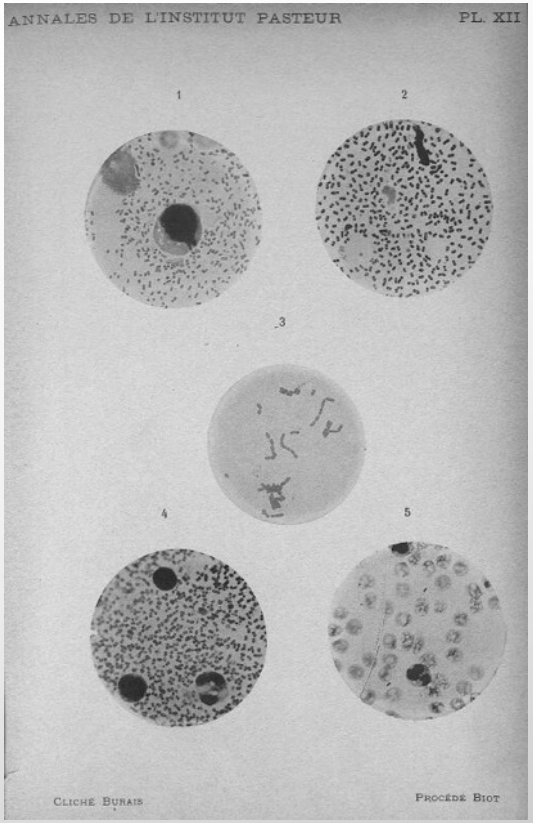
Je reçus du ministère des Colonies l'ordre de me rendre à Hong-Kong, d'y étudier la nature du fléau, les conditions dans lesquelles il se propage, et de rechercher les mesures les plus efficaces pour l'empêcher d'atteindre nos possessions [1].

Lorsque j'arrivai dans cette ville, le 15 juin, plus de 300 Chinois avaient déjà succombé. On construisait en toute hâte des baraquements provisoires, les hôpitaux de la colonie ne pouvant plus suffire à abriter les malades.

Je m'installai avec mon matériel de laboratoire dans une cabane en paillotte que je fis construire, avec l'autorisation du gouvernement anglais, dans l'enceinte de l'hôpital principal.

La maladie, qui sévissait presque exclusivement dans les quartiers chinois de la ville, présente tous les symptômes et les caractères cliniques de l'ancienne *peste à bubons* qui a décimé

1. Voir *Acad. des sciences*, n° du 30 juillet 1894, une note de M. Yersin sur le même sujet.



Pasturella pestis

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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]*

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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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Editor: Nora J. Besansky, University of Notre Dame, United States of America

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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

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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.

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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

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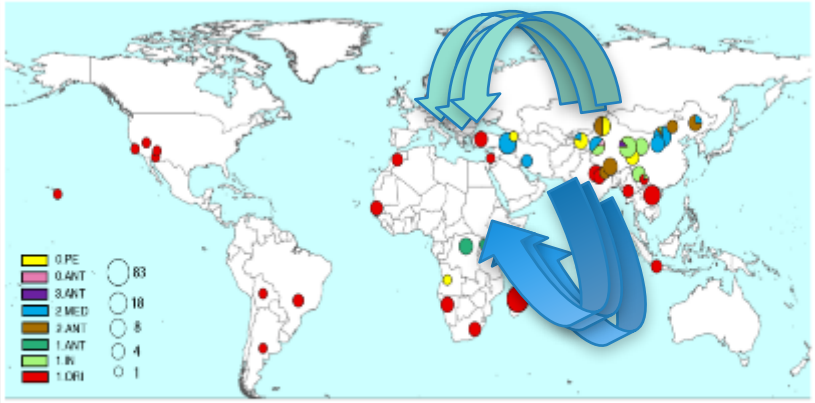
RESERVOIRS OF PLAGUE

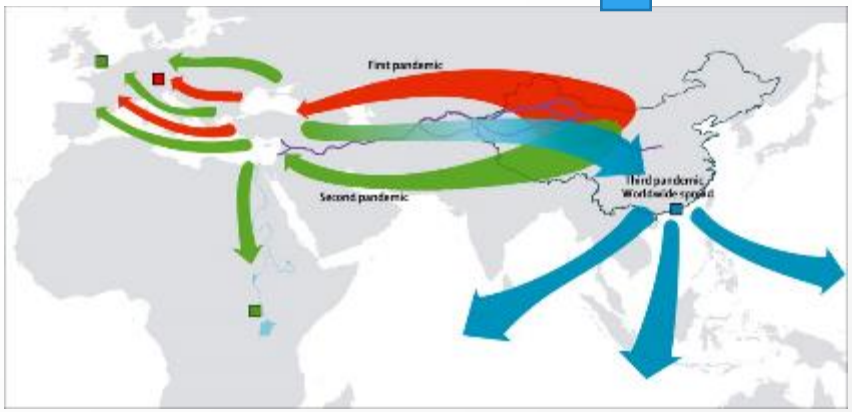
Rats

THREE THEORIES? YES!

One introduction?
Reservoir in (East)Eu



Supplementary Fig. 2. Global map showing the sources of all isolates. Filled circles or pie charts represent numbers of isolates whose groupings are indicated by colors (see legend at the left).



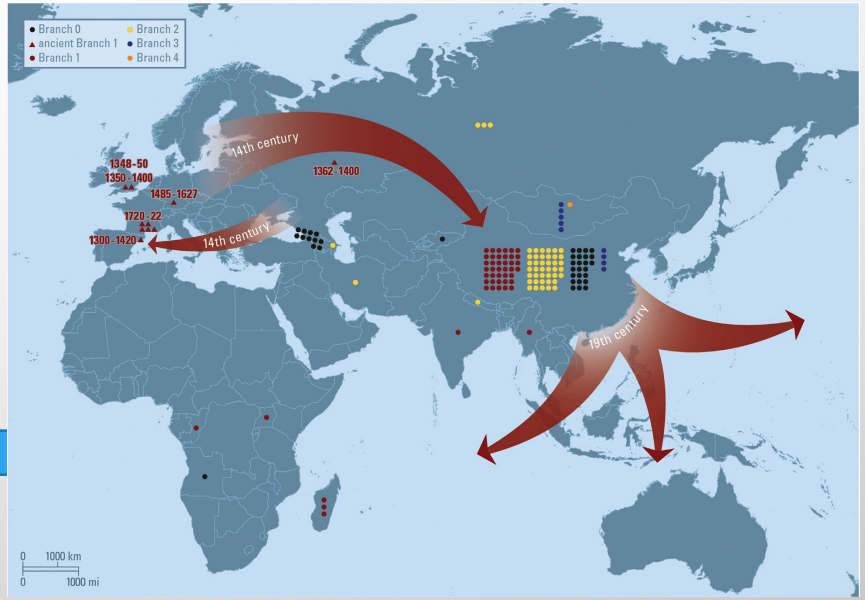
Wagner et al. 2014

Morelli et al. 2010/Schmid et al. 2015/Bramanti et al. 2016

Multiple introductions
No reservoir in West-Eu

One introduction
Reservoir in West-Eu

Spyrou et al. 2016



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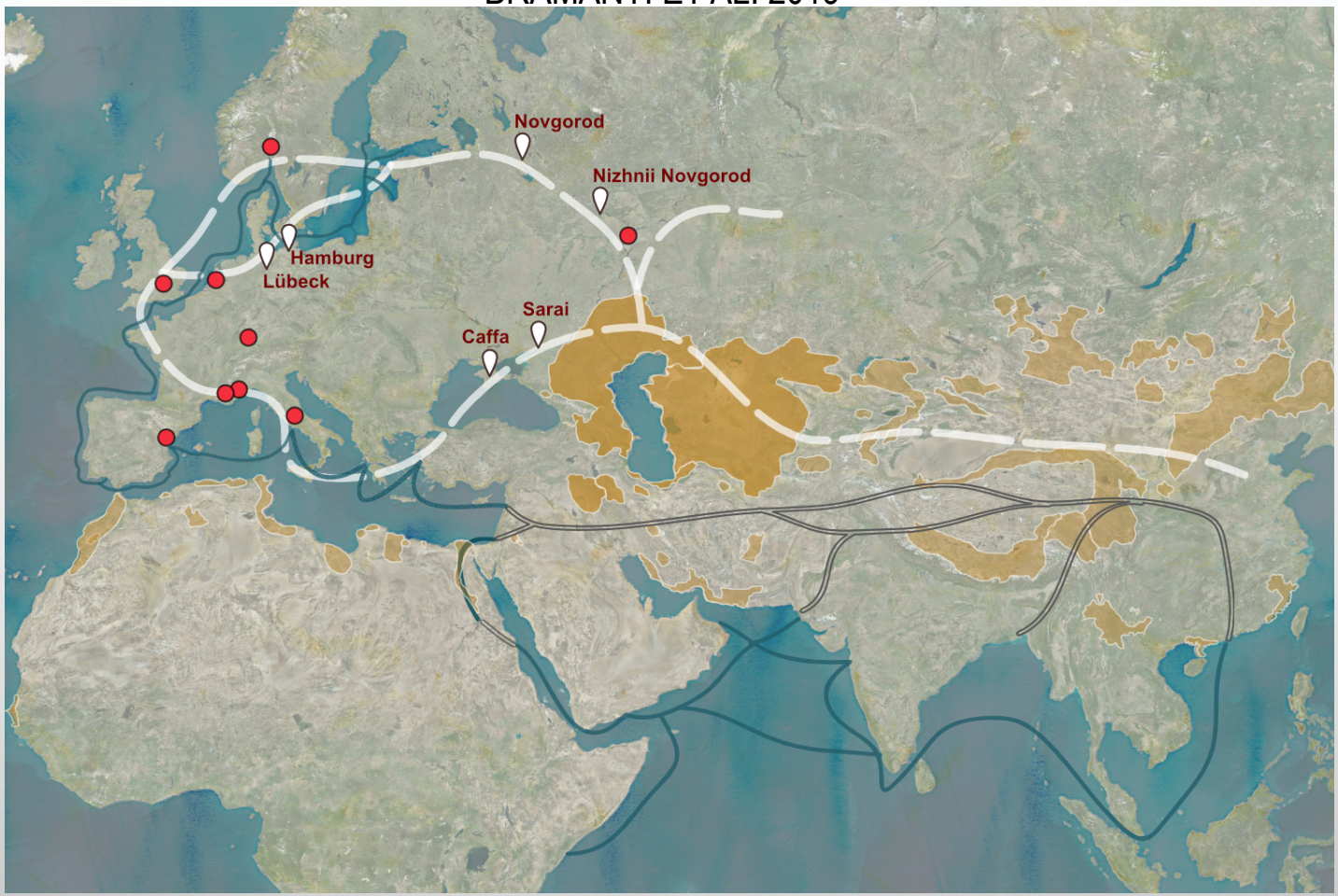
NAMOUCHI ET AL. 2018

BRAMANTI ET AL. 2019



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