Paleogentics, Part II

Charles J Vella, PhD, 2021

Joshua Lederberg, Nobel Prize bacteriologist

- In this time of pandemic, a voice from the past:

- “The single biggest threat to man's continued dominance on the planet is the virus.”
- “It’s a never-ending battle between viruses’ spectacular genetic capability and our wits.”

Lethal coalitionary attacks of chimpanzees (*Pan troglodytes troglodytes*) on gorillas (*Gorilla gorilla gorilla*) in the wild

- First information of two lethal coalitionary attacks of chimpanzees (*Pan troglodytes troglodytes*) on another hominid species, western lowland gorillas (*Gorilla gorilla gorilla*), that occur sympatrically in the Loango National Park in Gabon. In both events, the chimpanzees significantly outnumbered the gorillas and victims were infant gorillas.
- The first encounter involved a party of 27 chimpanzees and a group of five gorillas. The second involved a party of 27 chimpanzees and a group of seven gorillas

Stepwise synaptic plasticity events drive the early phase of memory consolidation

- **Where and when of memory consolidation**
- Synaptic plasticity underlies learning and plays a critical role in memory consolidation.
- Goto *et al*.; By selectively manipulating sLTP: 3 step process to memory
- 1 - Local circuitry in hippocampal area CA1 is required for memory formation shortly after the encoding event.
- 2- hippocampus for offline memory consolidation within 24 hours.
- 3 - The anterior cingulate cortex, connected with area CA1, is crucial for memory consolidation during sleep on the second night.

Akihiro Goto, et al., Science • 11 Nov 2021 •

Memory erasure

- Memories are initially encoded in the hippocampus but subsequently consolidated to the cortex. Changes in synaptic plasticity is key to these processes, via LTP.
- Using optogenetics to selectively erase long-term potentiation (LTP) within a defined temporal window, found that distinct phases of synaptic plasticity play differential roles.
- The first wave acts locally in the hippocampus to confer context specificity.
- The second wave, during sleep on the same day, organizes these neurons into synchronously firing assemblies.
- Finally, LTP in the anterior cingulate cortex during sleep on the second day is required for further stabilization of the memory.
- This demonstrates the precise localization, timing, and characteristic contributions of the plasticity events that underlie the early phase of memory consolidation.

1 - Human brain has gotten smaller

- Jeremy DeSilva, 2021: a change-point analysis to a dataset of 985 fossil and modern human crania. They found that human brains increased in size at:
  - 2.1 million years ago and
  - 1.5 million years ago but
  - But decreased in size around 3,000 years ago (Holocene), which is more recent than previous estimates.
- As for the decrease in brain size, a new hypothesis, finding clues within ant societies.
- Studying computational models and patterns of worker ant brain size, structure, and energy use in some ant clades, such as the Oecophylla weaver ant, Atta leafcutter ants, or the common garden ant Formica, showed that group-level cognition and division of labor may select for adaptive brain size variation.
- This means that within a social group where knowledge is shared or individuals are specialists at certain tasks, brains may adapt to become more efficient, such as decreasing in size.

Jeremy DeSilva et al 2021

Human and ant brains

- Ants also share with humans important aspects of social life such as group decision-making and division of labor, as well as the production of their own food (agriculture).

- The externalization of knowledge in human societies, thus needing less energy to store a lot of information as individuals, may have favored a decrease in brain size.

- This decrease was due to increased reliance on collective intelligence, the idea that a group of people is smarter than the smartest person in the group, often called the 'wisdom of the crowds’

Social Brain

- Not due to: body size reduction, a result of a shift to an agricultural diet, or a consequence of self-domestication.
- Analysis supports the hypothesis that the recent decrease in brain size may instead result from the externalization of knowledge and advantages of group-level decision-making due in part to the advent of social systems of distributed cognition and the storage and sharing of information.
- Humans live in social groups in which multiple brains contribute to the emergence of collective intelligence.

2 - Sociality may have been important in the evolutionary success of sauropods, the largest animals that ever walked the Earth

Illustration of the breeding ground of a herd of *Mussaurus patagonicus*, showing differently-aged individuals, including newborns in nests, young dinosaurs and fully-grown adults in what is now Patagonia.

200 Ma dinosaurs moved in herds and socialized by age groups

- Paleontologists have found the earliest known evidence that dinosaurs lived in herds and socialized with each other by age groups.
- The scientists, working a rich deposit of fossils at a site in Argentina's province of Santa Cruz, at the southern tip of South America, found more than 100 eggs and the skeletons of 80 individuals ranging in age from embryos to adults.
- Named *Mussaurus patagonicus*, which, based on tiny fossilized hatchling specimens found in 1979, was inappropriately given a scientific name that means "mouse lizard."
- The bones and eggs are spread over about 250 acres — a small area for finding so many fossils of the same species. Most of the eggs were found in clutches of eight to 30 in nests close together, which suggests that the animals used a common breeding ground. Within the nests, the eggs are arranged in trenches that the animals apparently excavated for the purpose.

Social Dinosaurs

- Eggs, neonates, juveniles and adults were clustered close to each other, which indicates that the animals lived in socially cohesive groups, rather than gathering only temporarily to breed and lay eggs.
- Age groupings like this suggest that the animals maintained social connections with each other across their life spans. They were a group of herbivorous, long-necked dinosaurs — lived in herds.
- But these Mussaurus bones were found in deposits made from windblown dust, and the authors conclude that they probably died simultaneously in periodic droughts. There were at least three episodes of mass death at the site. These dinosaurs likely died because of a drought. Many of the animals died in a resting pose, meaning they died laying down and then were covered by windblown dust.

3 - Human head carvings and phallus-shaped pillars discovered at 11,000-year-old site in Turkey

Researchers excavate at the site of Karahantepe in Turkey on Sept. 30, 2021.

Turkey

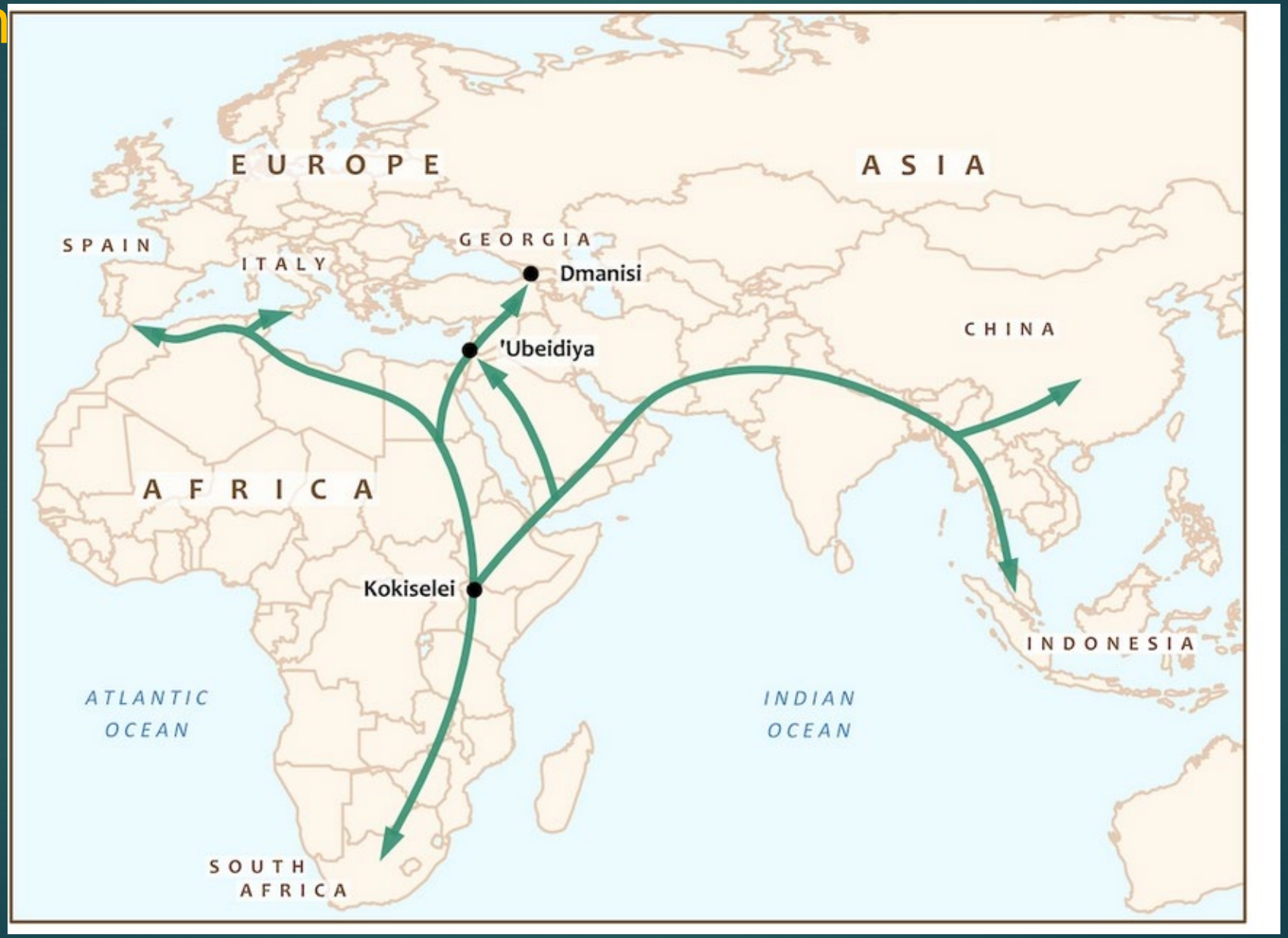
- Archaeologists in Turkey have found evidence that an 11,000-year-old prehistoric site was used for a ceremonial parade through a building containing phallus-shaped pillars and a carving of a human head.
- Called Karahantepe, the site is located in southern Turkey, archaeologists found carvings of human heads, snakes and a fox,
- Discovered 11 pillars near a carving of a human head. All pillars are erected and shaped like a phallus.
- Rather than being abandoned, the buildings were filled in with dirt, possibly during a decommissioning ceremony of sorts..
- Gobekli Tepe is also located near Şanlıurfa

3 - Archeologists find rare 2,700-year-old toilet in Israel in royal mansion

4 - Arabia as the exit from Africa

- The Arabian Peninsula has long served as a key crossroads between Africa, Europe and Asia.
- First large-scale analysis of the genetics of a Middle Eastern population, examining DNA from 6,218 adults randomly recruited from Qatari health databases and comparing it with the DNA of people living in other areas of the world today and DNA from ancient humans
- DNA from Middle Eastern groups made significant genetic contributions to European, South Asian and even South American communities, likely due to the rise and spread of Islam across the world over the past 1,400 years, with people of Middle Eastern descent interbreeding with those populations

5 - The Travels of *H. erectus*: did not live just in Savannah



Forested ‘Ubeidiya, Israel, and *H. erectus*

- ‘Ubeidiya, Israel, is one of the earliest-known sites settled by *H. erectus* (sometimes called *Homo ergaster*) en route out of Africa
- The ‘Ubeidiya site, which *H. erectus* reached between 1.2 and 1.6 million years ago, was a way station en route through the Levant
- For many years, scholars subscribed to the “Savannahstan” hypothesis to explain hominin journeys out of Africa.
- According to this idea, *H. erectus* dispersed out of East Africa about 2 million years ago as climate change triggered the expansion of East African savanna into the Southern Levant. They trailed new savannas out of Africa. Stuck close to water sources.
- Study: findings at ‘Ubeidiya have complicated the notion that *H. erectus* passively followed the spreading savanna. ‘Ubeidiya wasn’t a savanna at all: It was a woodland, covered in trees, Hundreds of thousands of fossilized animal bones excavated at ‘Ubeidiya: teeth and bones of rhinoceroses, hippopotami, crocodiles, bears, pigs, camels, and a saber-toothed tiger.

‘Ubeidiya: home to H. erectus was forested

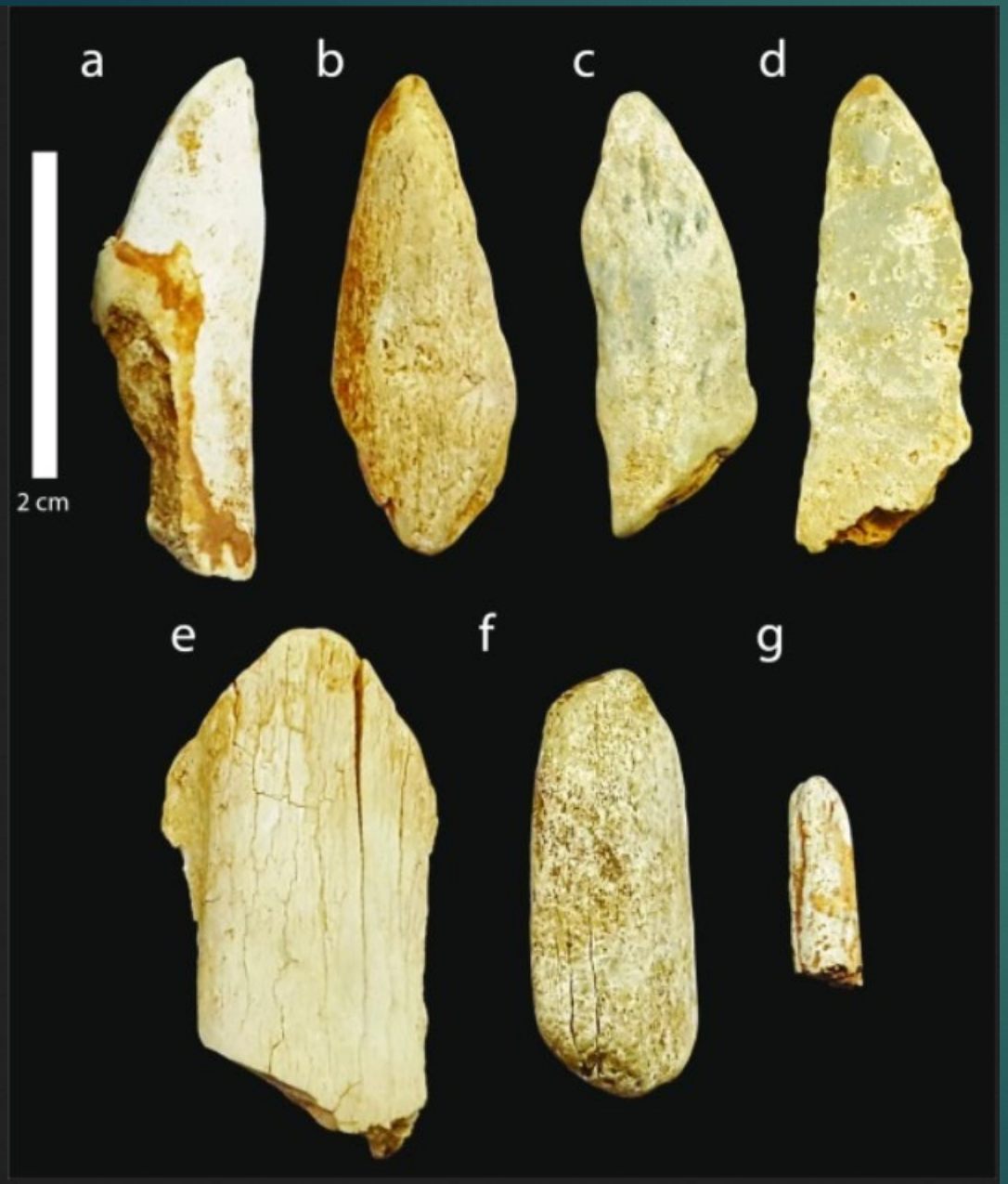
- Belmaker’s findings suggest *H. erectus* could thrive in more than one habitat type and was not limited to savannas. Building on this point, she has put forward a different theory of migration: *H. erectus* was innately adapted to a diversity of landscapes before groups even left Africa, both hunting antelope on open plains and scavenging in patches of forest.
- The majority of hoofed mammals found at ‘Ubeidiya were Eurasian, such as deer and elk, which indicates that the site was not an African savanna..
- *H. erectus* was not simply following spreading savanna as the climate changed but rather had the capacity to adjust to a variety of environments.
- By around 1.4 to 1.6 million years ago, *H. erectus* was occupying tropical Southeast Asia and Indonesia.

Rick Potts and climate change

- Several major hominin milestones, including the dispersals of *H. erectus* and *H. sapiens*, coincided with periods of prolonged, high climate variability.
- One important *H. erectus* innovation that first appears in the fossil record 1.76 million years ago—at Kokiselei near Lake Turkana in Kenya—are bifacial Acheulean axes

5 - Oldest shell beads recovered from Bizmoune Cave in western Morocco, determined to be between 142 and 150 Ka

7 - Paranthropus robustus: stone tools



- New bone tool associated with paranthropic remains has been presented at Cooper's D site (South Africa), dated at 1.0-1.4 million years (Ma), and containing 7 remains of *P. robustus* and 50 lithic tools.
- It joins the set of 102 bone tools in different South African sites (Sterkfontein, Swartkrans, Kromdraai and Drimolen)

8 - Body size and temperature

- The growth of the body and brain in the evolutionary process within the genus *Homo* can be explained in relation to climate, according to a work that combines data from more than 300 fossils with paleoclimatic models that consider temperature, precipitation and other conditions.
- Temperature is strongly linked to body size: larger bodies generate more heat but lose relatively less, because the surface does not grow in the same proportion.
- This correlation of heavy bodies with cold climates is already known in other mammals. As for the brain, this organ underwent selective pressures other than the size of the body. The results do not show an association of brain size with temperature, but with more stable climates and dietary needs to maintain a large brain.

9 - Middle Pleistocene fire use: The first signal of widespread cultural diffusion in human evolution

- Control of fire is one of the most important technological innovations within the evolution of humankind. The archaeological signal of fire use becomes very visible from around 400,000 y ago onward.
- Interestingly, this occurs at a geologically similar time over major parts of the Old World, in Africa, as well as in western Eurasia, and in different subpopulations of the wider hominin metapopulation.
- We interpret this spatiotemporal pattern as the result of cultural diffusion, and as representing the earliest clear-cut case of widespread cultural change resulting from diffusion in human evolution.
- This fire-use pattern is followed slightly later by a similar spatiotemporal distribution of Levallois technology, at the beginning of the African Middle Stone Age and the western Eurasian Middle Paleolithic

Katharine MacDonald, et al., 2021

Fire use by cultural diffusion

- These archaeological data, as well as studies of ancient genomes, lead us to hypothesize that at the latest by 400,000 y ago, hominin subpopulations encountered one another often enough and were sufficiently tolerant toward one another to transmit ideas and techniques over large regions within relatively short time periods.
- Furthermore, it is likely that the large-scale social networks necessary to transmit complicated skills were also in place.
- Most importantly, this suggests a form of cultural behavior significantly more similar to that of extant *Homo sapiens* than to our great ape relatives.

Pre-400 Ka lack of fire in Europe

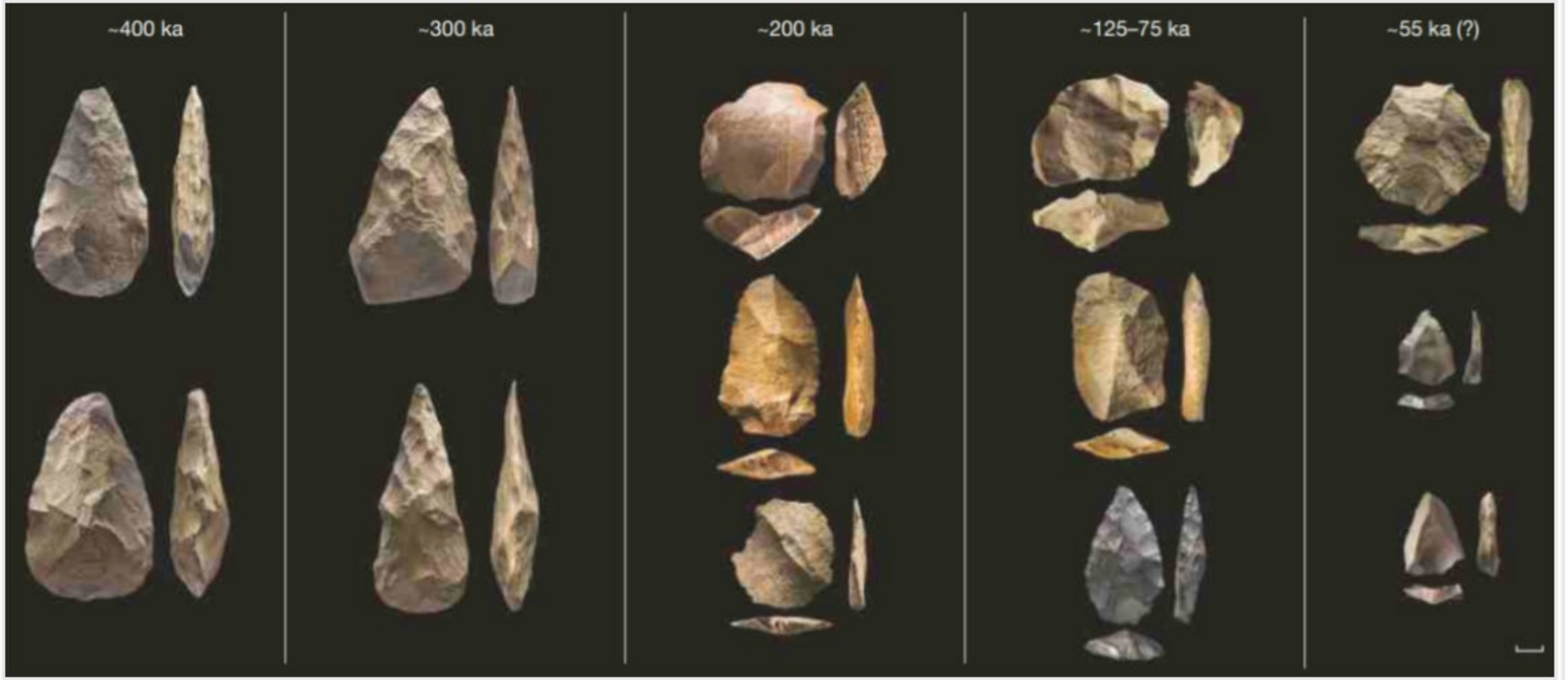
- A review by Roebroeks and Villa identified a clear pattern for Europe: there the record strongly suggests that anthropogenic fire use was very rare to nonexistent during the first half of the Middle Pleistocene pre-400 Ka, as exemplified by the absence—bar a few dispersed charcoal particles—of fire proxies in deeply stratified archaeological karstic sequences, such as the Atapuerca site complex in Spain or the Caune de l'Arago at Tautavel (France), as well as from such prolific open-air sites as Boxgrove in the United Kingdom.
- In contrast, the record from 400 ka onward is characterized by an increasing number of sites with multiple fire proxies (e.g., charcoal, heated lithics, charred bone, heat-altered sediments) within a primary archaeological context.
- Studies published following this review have strengthened this pattern and identified its existence beyond Europe.

11 - Multiple hominin dispersals into Southwest Asia over the past 400,000 years

- We are still at the interesting threshold of about 400 ka. At that time, different human migrations were taking place through the so-called Green Arabia to southwest Asia (and surely also from Asia "back" to Africa).
- Climatic changes led to wet periods in the present-day Nefud desert region, producing lakes and rivers that transformed the landscape into resource-rich grasslands.
- Six of these climatic phases have been identified at the Khall Amayshan 4 site (KAM 4), with human presence in five of them: 400 ka, 300 ka, 200 ka, 130-75 ka and 55 ka. The lithic tools illustrate well the technological evolution in each of these phases, from the Acheulean bifaces to the Middle and Upper Paleolithic industries.

H. Grucutt, et al., 2021

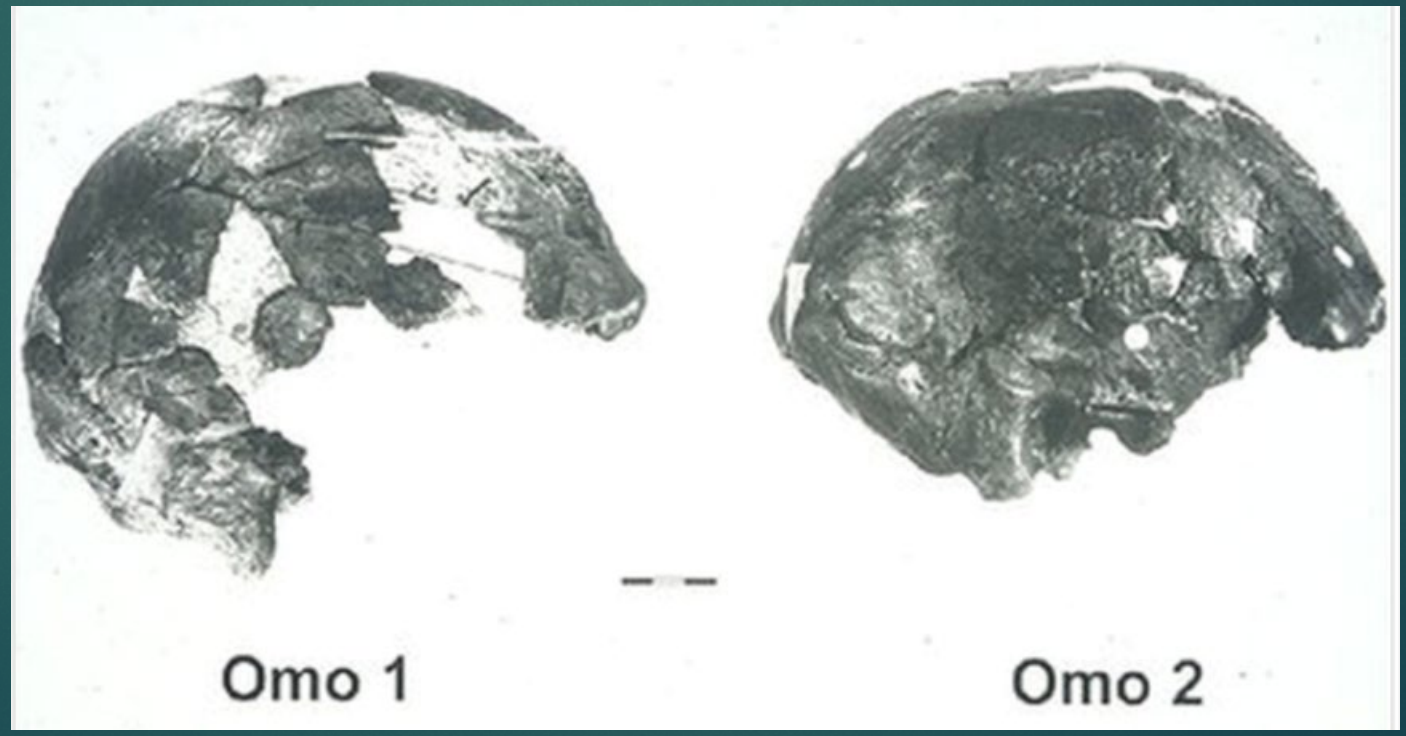
Arabia



Stone tools from KAM 4 and Jebel Umm Sanman 1 (JSM 1). Credit: Groucutt et al (2021)

12 - New Omo dating: 212 Ka, not 196 Ka

- The geochemical revision of the volcanic layer that is superimposed on the Omo-Kibish fossils establishes a new minimum dating of 212 ka for the two skulls Omo I and Omo II, older than the estimated until now of 196 ka



13 - Neandertal kids used teeth as third hand

- Neanderthal and sapiens children from the Cantabrian region used their mouths as a third hand and participated in group tasks, as indicated by the marks and tooth wear of 8 individuals from the Axlor , El Castillo , Las Caldas , Tito Bustillo and Santa caves. Catalina, in contexts ranging from the Mousterian to the Magdalenian.

- We also found evidence of habitual dental hygienic practices in the form of toothpicking on a deciduous premolar. Orientation of the cultural striations indicates similar handedness development as in modern children.

- Taken together, these dental wear patterns support the participation of young individuals in group activities, making them potential contributors to group welfare.

14 - Tibetan Plateau: new site Jiangjunfu 01

- Human ecological and genetic adaptation to life in high altitudes.
- Recent work on the Tibetan Plateau has documented hominin occupations by Denisovans at Baishiya Karst Cave (BKC) from at least ca. 160 Ka, and again around 100 and 60 thousand years ago (ka), followed by modern human occupation at Nwya Devu (ND) around 30–40 ka.
- Here we report a newly discovered well-stratified and well-dated Paleolithic site, Jiangjunfu 01 (JJF01), from the northeastern margin of the plateau. The site was occupied by hominin who employed simple core-and-flake technology, during warmer interglacial environments ~90–120 ka. T
- Confirming that hominins, potentially Denisovans, occupied and inhabited the highest region of our planet at least by the early Upper Pleistocene.

15 - Early Homo sapiens groups in Europe faced subarctic climates

- Evidence from Bacho Kiro Cave, humans have been enduring very cold climatic conditions, similar to the ones typical for present-day northern Scandinavia

- The first *sapiens* who entered Europe were not accompanied by a warm climate, but suffered for millennia a cold climate similar to the current one in the Scandinavian region, which once again demonstrates their resilience and ability to adapt to the environment.

16 - Neandertal blood types

- Study of high-quality sequences of three Neanderthals and one Denisovan individuals for 7 blood group systems that are used today in transfusion (ABO including H/Se, Rh (Rhesus), Kell, Duffy, Kidd, MNS, Diego).
- These hominins already possessed the full range of blood variability found in modern humans.
- In addition, it confirms that they had:
  - an African origin,
  - low genetic variability,
  - weak fertility and
  - susceptibility to viral infections that lead to a high infant mortality rate.
  - In a Neanderthal cross with *sapiens*, there would be an 18% chance that the child would develop a hemolytic disease and die.
- .

Condemi S, et al., 2021

N blood types

- We show that Neanderthal and Denisova were polymorphic for ABO and shared blood group alleles recurrent in modern Sub-Saharan populations.

- Furthermore, we found ABO-related associated with a high risk of hemolytic disease of the fetus and newborn

- Such a common blood group pattern across time and space is coherent with a Neanderthal population of low genetic diversity exposed to low reproductive success and with their inevitable demise.

17 - Oldest evidence of humans in Siberia at 26 Ka

- Ancient cut marks on mammoth bones unearthed on a remote island in the frozen extremes of Siberia are the northernmost evidence of Paleolithic humans ever found
- The bones from the woolly mammoth skeleton, dated to about 26,000 years ago, were excavated this summer by a Russian expedition to Kotelny Island, in the far northeast of Siberia — 615 miles (990 kilometers) north of the Arctic Circle.
- Found cut marks and notches, made by stone or bone tools, on almost every bone.
- Found two ivory tools made from the tusks

18 - Identical twins share epigenetic markers

- All identical twins share a common signature of twinhood, not in their DNA, but on it.
- This signature is part of the epigenome, chemical markers that dot many spots along DNA and influence the activity of genes without altering their sequence.
- Identical twins everywhere largely share a specific set of these epigenetic marks that persists from birth to adulthood,
- These shared epigenetic tags could be used to identify people who were conceived as identical twins but lost their sibling in the womb or were separated at birth.
- Looked for epigenetic differences at over 450,000 sites along the genomes of nearly 6,000 monozygotic twins and dizygotic, or fraternal, twins.
- Comparing identical twins with fraternal twins, as opposed to comparing twins to non-twins, allowed the researchers to rule out any epigenetic changes that stemmed from the unusual experience of sharing a womb.

Epigenetics in twins

- At 834 spots along the genome, identical twins were strikingly similar. These shared epigenetic marks were concentrated in certain parts of the genome, including centromere and telomere regions on chromosomes.
- The shared marks are so uniquely common to identical twins that the researchers were able to devise a test that can determine, with up to 80 percent accuracy, whether an individual is an identical twin. That includes individuals who don't know that they lost their twin during pregnancy, a phenomenon known as vanishing twin syndrome, and twins who were separated at birth.
- Whether this epigenetic signature is a cause, consequence or by-product of monozygotic twinning remains unclear. It's possible that some of these epigenetic changes tell a zygote to split. Alternatively, these chemical marks could reflect the epigenetic aftermath of the splitting event.

19 - Native Americans and Japanese Jomon

- Analysis of ancient teeth questions theory that Native Americans originated from Japan

- For years, archaeologists had predicted that the first people to live in North America descended directly from a group called the Jomon, who occupied ancient Japan about 15,000 years ago, the same time people arrived in North America around 15,000 years ago via the Bering Land Bridge

- This theory is based on archaeological similarities in stone tools, especially projectile weapons, found in Native American and Jomon settlements.

A collection of stone arrowheads found in ancient Jomon settlements

Jomon

Dental morphology

- Scott and his team compared 25 dental morphology traits in around 1,500 sets of ancient teeth from Native American and Jomon people dating back over 10,000 years, as well as other ancient groups from East Asia, Southeast Asia and the Pacific.
- This analysis of tooth traits and DNA within the teeth revealed that the Native Americans were not closely related enough to the Jomon people to consider them ancestors but that they may have descended from another unknown group from East Asia, Scott said.
- Convincing argument for eliminating Japan's Jomon people as direct ancestors of the first Native Americans

20 - Domestication of cassowaries at 18 Ka in New Guinea: imprint on first person they see

The World's Deadliest Bird Was Raised by People 18,000 Years Ago

- Researchers studying ancient cassowary eggshells in New Guinea found signs that the sharp-taloned bird was being domesticated at 18 Ka; thousands of years before domestication of the chicken at 8 Ka

- Usually shy and secretive, In 1926, a cassowary attacked by an Australian teenager kicked him in the neck with its four-inch, velociraptor-like talons, slitting his throat.

- First people arrived on New Guinea at least 42,000 years ago.

Cassowary eggs and talons

A clutch of Southern cassowary eggs. Gerry Pearce/Alamy

The ferocious talon of a Southern cassowary. Trevor Collens/Alamy

Cassowaries

- Some eggs — early in development — showed burn patterns, suggesting they'd been cooked. But a large number of fragments — particularly those from around 11,000 to 9,000 years ago — came from almost fully developed eggs. And while people might have been eating the embryos, there's a great possibility that people were hatching those eggs and rearing cassowary chicks.
- Some Indigenous groups on the island prize cassowary meat and feathers as ritual and trade goods. They still raise cassowary chicks from eggs taken out of wild nests. Hatchlings imprint on humans easily and are relatively manageable. (It's only once they reach adulthood that the danger begins.)

21 - Camel sculptures in Arabia

Arabian sculptures

- A series of camel sculptures carved into rock faces in Saudi Arabia are likely to be the oldest large-scale animal reliefs in the world.
- But a fresh study puts the camels at between 7,000-8,000 old. Older than Stonehenge (5,000 yo) or the Pyramids at Giza (4,500 yo). They even predate the domestication of camels.
- Created during green phase in Arabia. Many of the reliefs are high above the ground, meaning their carvers would have had to build scaffolding to create them.

22 Wonderwerk Cave, South Africa

23 - Oldest footprints in Americas in New Mexico

Used carbon-dating methods on seeds found in sediments within the prints.
Mostly adolescents and children; flat footed

Into the Americas

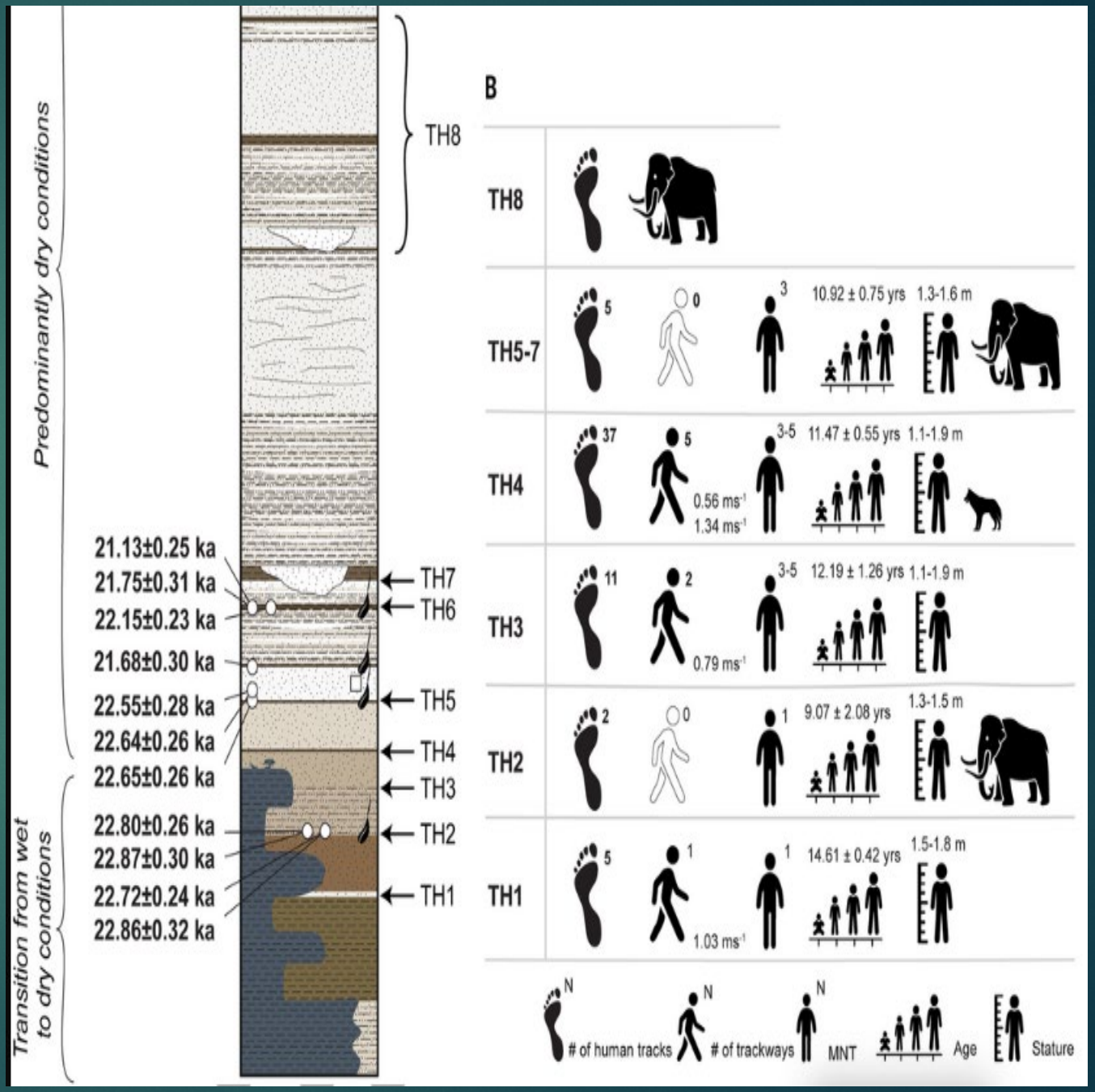
- Hypotheses range from those that favor:
  - Clovis as the earliest occupation [~13 Ka] to those that favor
  - older (“pre-Clovis”) sites dating to ~16.5 ka [i.e., post–Last Glacial Maximum (LGM);
  - 26.5 to 19 or 20 ka] or even older.
- Viable migration routes from Asia were dependent on timing and associated environmental conditions and could have occurred via an inland route through the Ice-Free Corridor, the Pacific Coastal Route, or both; however, these routes would have been closed or at least unlikely during the LGM.
- The New Mexico footprints Indicate the presence of humans in North America for approximately 2000 years during the Last Glacial Maximum south of the migratory barrier created by the ice sheets to the north

Footprints

- Humans were present on the landscape in New Mexico by at least ~23 ka, with evidence of occupation spanning approximately two millennia.

- These data provide definitive evidence of human occupation of North America south of the Laurentide Ice Sheet during the LGM.

Trench levels



21-23 Ka footprints

- Fossilized footprints in New Mexico are earliest 'unequivocal evidence' of people in the Americas; during the height of the Last Glacial Maximum,
- Stone artifacts discovered in Chiquihuite Cave in central Mexico were at least 26,500 years old;
- 60 footprints embedded in an ancient lakebed in what is now White Sands National Park in south central New Mexico are strong evidence that humans occupied the New World between about 21,000 and 23,000 years ago.
- These new findings suggest that people made their way to the Americas millennia before the Last Glacial Maximum prevented migration via Beringia

Michael Bennett, et al., 2021

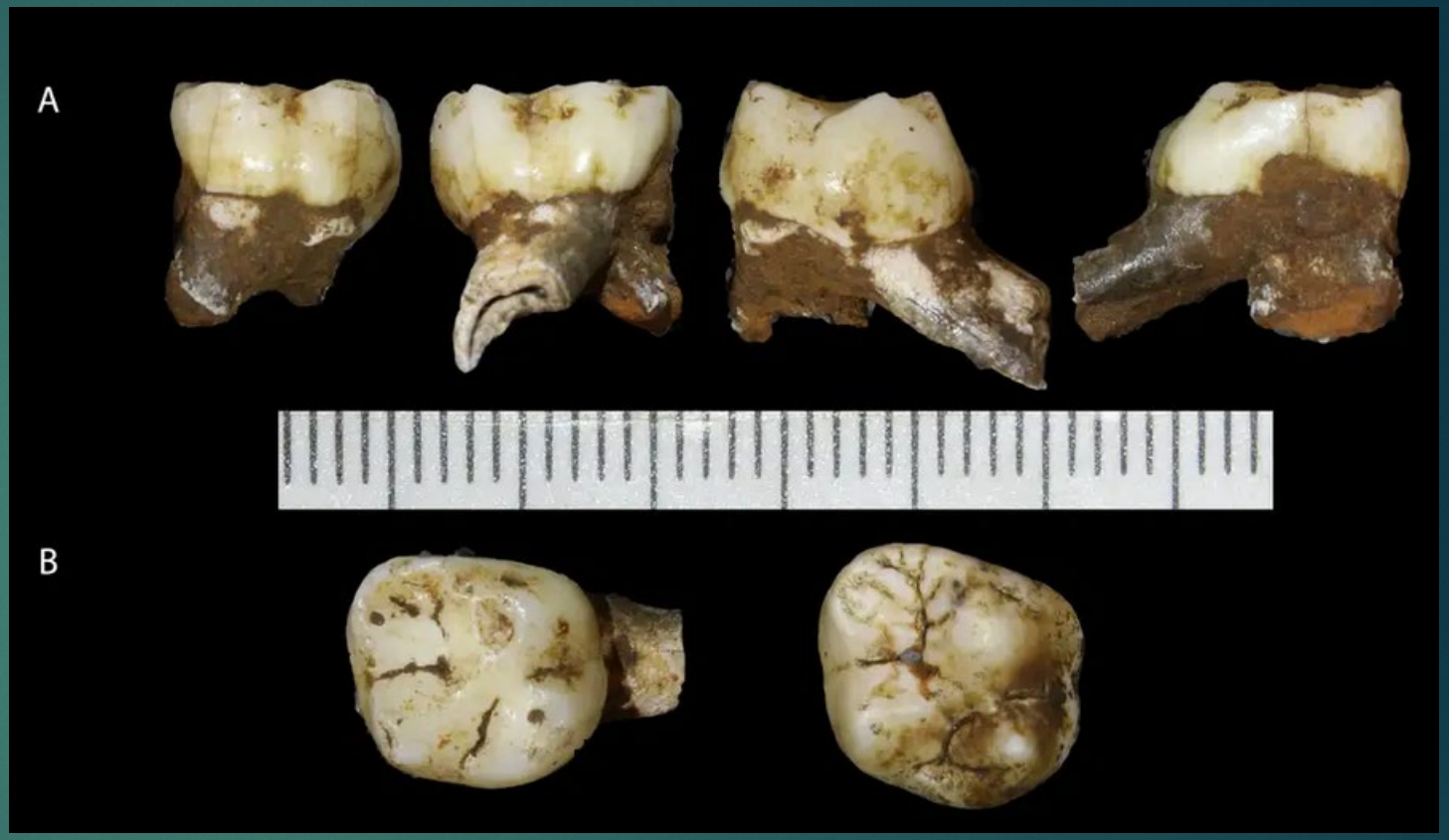
Other footprints found in the wetland area include those of mammoths, ground sloths, canines, felines, bovines and camels

24 - Secret Vanguard cave chamber may be one of the last Neanderthal hideouts

Chamber in Vanguard Cave, Gibraltar

- A cave chamber sealed off by sand for some 40,000 years has been discovered in Vanguard Cave in Gibraltar
- Given that the sand sealing the chamber was 40,000 years old, and that the chamber was therefore older, it must have been Neanderthals
- 43 feet (13 meters) in length, with stalactites hanging like eerie icicles from the chamber ceiling. Along the surface of the cave chamber, the researchers found the remains of lynx, hyenas and griffon vultures, as well as a large whelk, a type of sea snail
- A milk tooth of a 4-year-old Neanderthal was found close to the chamber four years ago. The tooth "was associated with hyenas, and it is suspected the hyenas brought the child [who was likely dead] into the cave.

25 -Leti, the “Lost One”



Fragmented skull discovered by itself in a tight crevice.
(20 cm by 80 cm tall

New child skull of *Homo naledi*

- On a limestone shelf: teeth and fragments of skull belonging to a *Homo naledi* child. The only child ever discovered at Rising Star cave.
- Lee Berger argues that the remote location of the finding implies it was a burial.
- The researchers named the child "Leti," after the word "letimela" in the local Setswana language, meaning "the lost one."
- All told, they found six teeth and 28 skull fragments in 2017, which were used to reconstruct Leti's skull over the last four years.
- Estimated Leti's age to be between four and six years old using its teeth,

Leti

Lee Berger holds a reconstruction of Leti's skull. Wits University

26 - Bodo, Ethiopia, 1976, *Homo heidelbergensis, 600 Ka, 1250 cc*

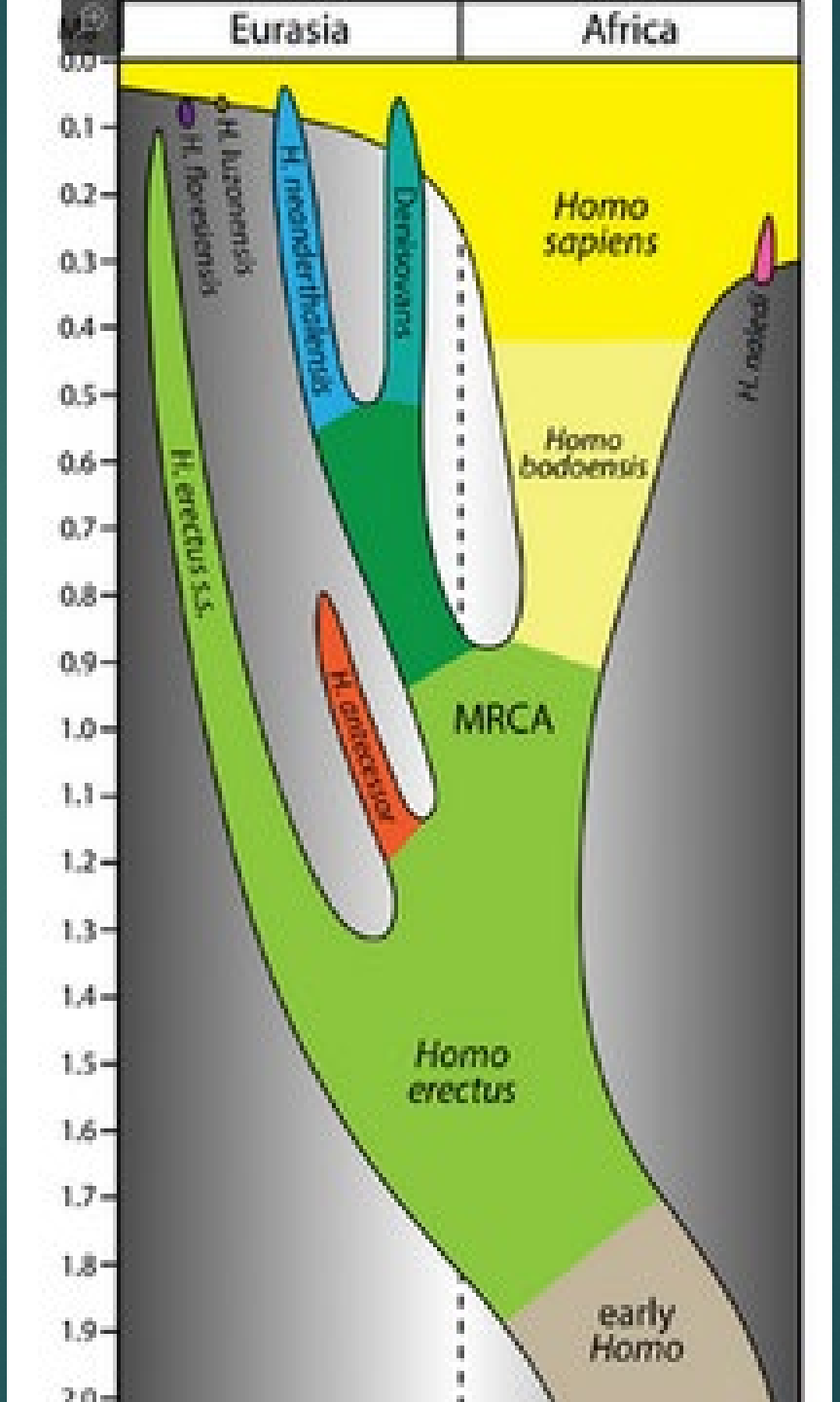
Photograph by Donald Johanson.

Photograph by Donald Johanson.

- Homo heidelbergensis
- 1976
- Discoverers: Alemayhew Asfaw, Paul Whitehead, and Craig Wood
- Date: c 600 Ka

Resolving the “muddle in the middle”: The case for *Homo bodoensis* sp. nov.

- Middle Pleistocene, also known as the Chibanian (774,000 to 129,000 years ago), represents a key transitional stage for the human genus; poor fossil record during this period.
- Declaration of a new taxon, or species, of ancestral human: *Homo bodoensis*. This species is not based on any new fossil discovery but is instead a reworking of pre-existing fossils found in Africa and Eurasia, all of which date back some 700,000 to 500,000 years ago.
- Previously: All fossils assigned to *H. bodoensis* are traditionally assigned to one of two ancestral human species: *Homo heidelbergensis* or *Homo rhodesiensis*.
- The newly described species, *H. bodoensis,* is based on a skull found in 1976 in Bodo D’ar Ethiopia that’s thought to be a direct ancestor of *H. sapiens*. The Bodo cranium is currently assigned to *H. heidelbergensis*.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H. floresiensis
H. neanderthalensis
Denisovans
Homo sapiens
H. naledi
Homo bodoensis
H. erectus s.s.
H. antecessor
MRCA
Homo erectus
early Homo

Homo bodoensis

- They want to reassign Mauer jaw, now H. heidelbergensis, to early Neanderthal.
- Chris Stringer disagrees.
- *H. heidelbergensis* fossils from Western Europe should be assigned as early Neanderthals

26 - Depression genetic risk factors in non Europeans

- Genetic Risks for Depression Differ Between Ancestral Groups
- A large genome-wide association study in East Asians uncovers novel genetic links to depression, calling attention to the consequences of underrepresentation of non-European groups in genetic research data.
- Study assessed 102 known depression risk factors from previous studies, and found that only 11 percent of them were associated with depression in the East Asian cohort.
- found five novel genes associated with depression in their dataset.
- Not only were a majority of genetic variants associated with depression in European populations not applicable in East Asian ancestral cohorts, but novel indicators emerged in East Asians that had not been discovered in studies on Europeans. The study's authors caution that the existing knowledge on genetic risk factors for depression is not generalizable to a global population.

Depression

- In European studies, clinical depression is correlated with heart problems and a high body mass index (BMI), while among people of East Asian descent, clinical depression correlated with a lower average BMI than controls without the condition

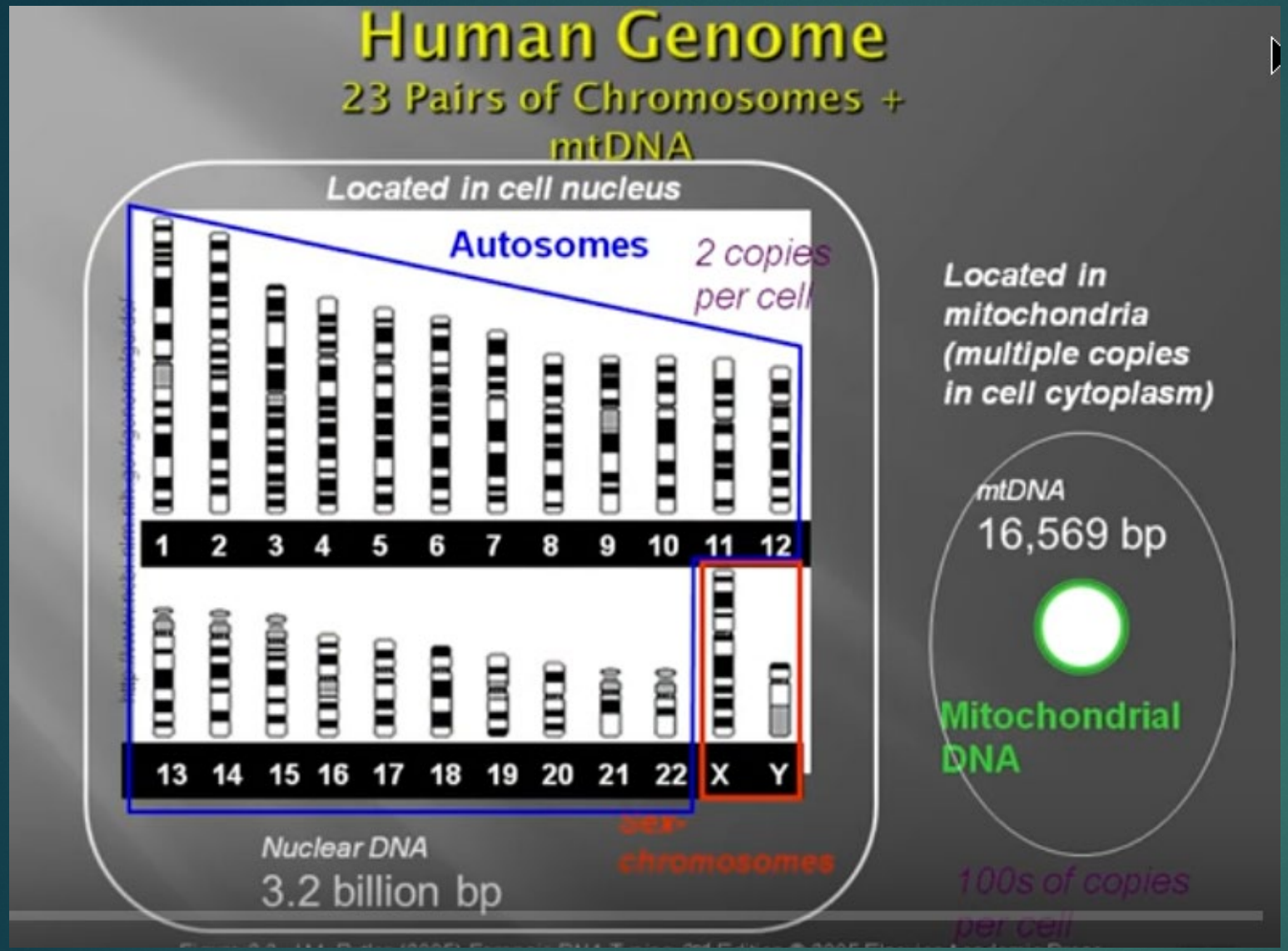
Women are the targets

- Being pregnant or recently having had a baby nearly doubles a woman's risk of being killed.
- Study of pregnancy deaths in all 50 US states from 2018 and 2019: Pregnant women in the United States die by homicide more often than they die of pregnancy-related causes — and they're frequently killed by a partner,
- US women who are pregnant or were pregnant in the past 42 days (the post-partum period) die by homicide at more than twice the rate that they die of bleeding or placental disorders — the leading causes of what are usually classified as pregnancy-related deaths. Also, becoming pregnant increases the risk of death by homicide: between the ages of 10 and 44 years, women who are pregnant or had their pregnancy end in the past year are killed at a rate 16% higher than are women who are not pregnant. 66% of the homicides recorded in their data occurred in the person's home, suggesting that the woman was killed by her partner.
- Male partners are the killers in nearly half of homicides involving women in the United States.
- 57% of mass shootings involve incidents of domestic violence.

Paleogenetics, Part II

DNA Review, Ancient DNA, and Sequencing Methodologies

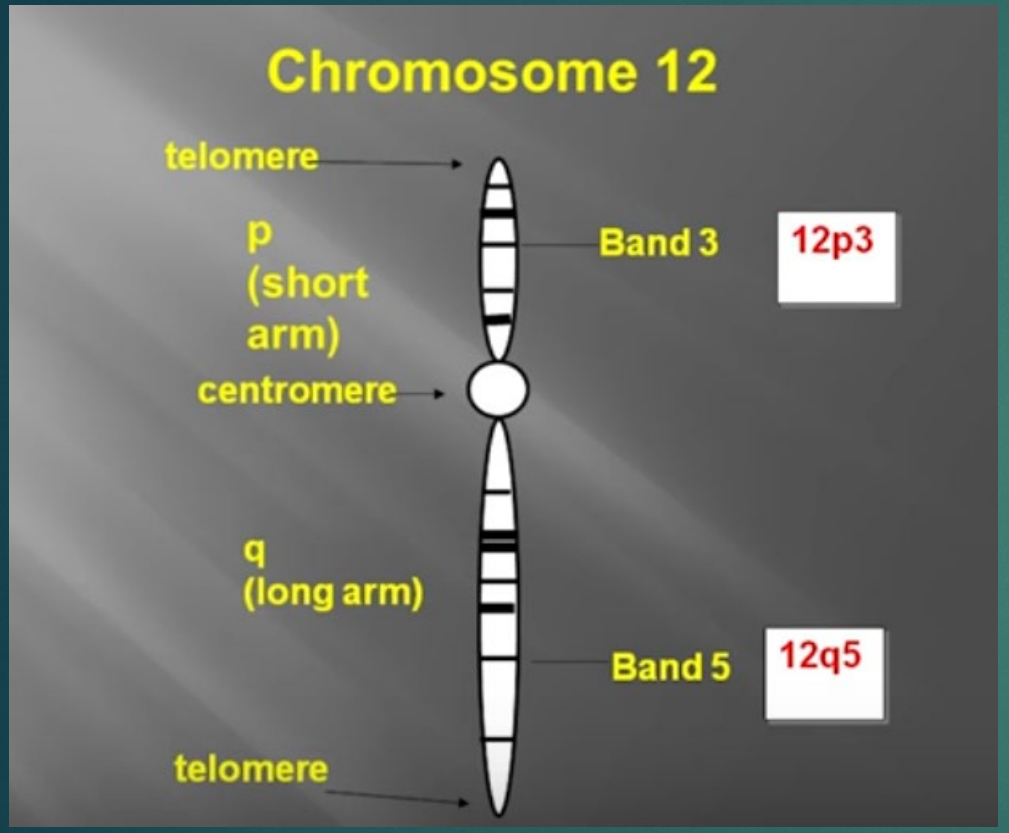
Nov. 17, 2021



- Human Genome: located in cell nucleus
  - 23 pairs of chromosomes
  - 2 copies per cell
  - 3.2 Billion basepairs
- Mitochondrial DNA:
  - Located in mitochondria in cell
  - 16,569 bps

Chimps have 24 chromosomes; 2 fused in humans = 23 chrom
Humans: females have 2 Xs, males have X and Y

Telomeres in Chromosome



- Telomeres (caps at end of chromosome) indicate biological longevity:
-
- Longer your telomeres, longer you live
- They are repeat regions that shorten with age

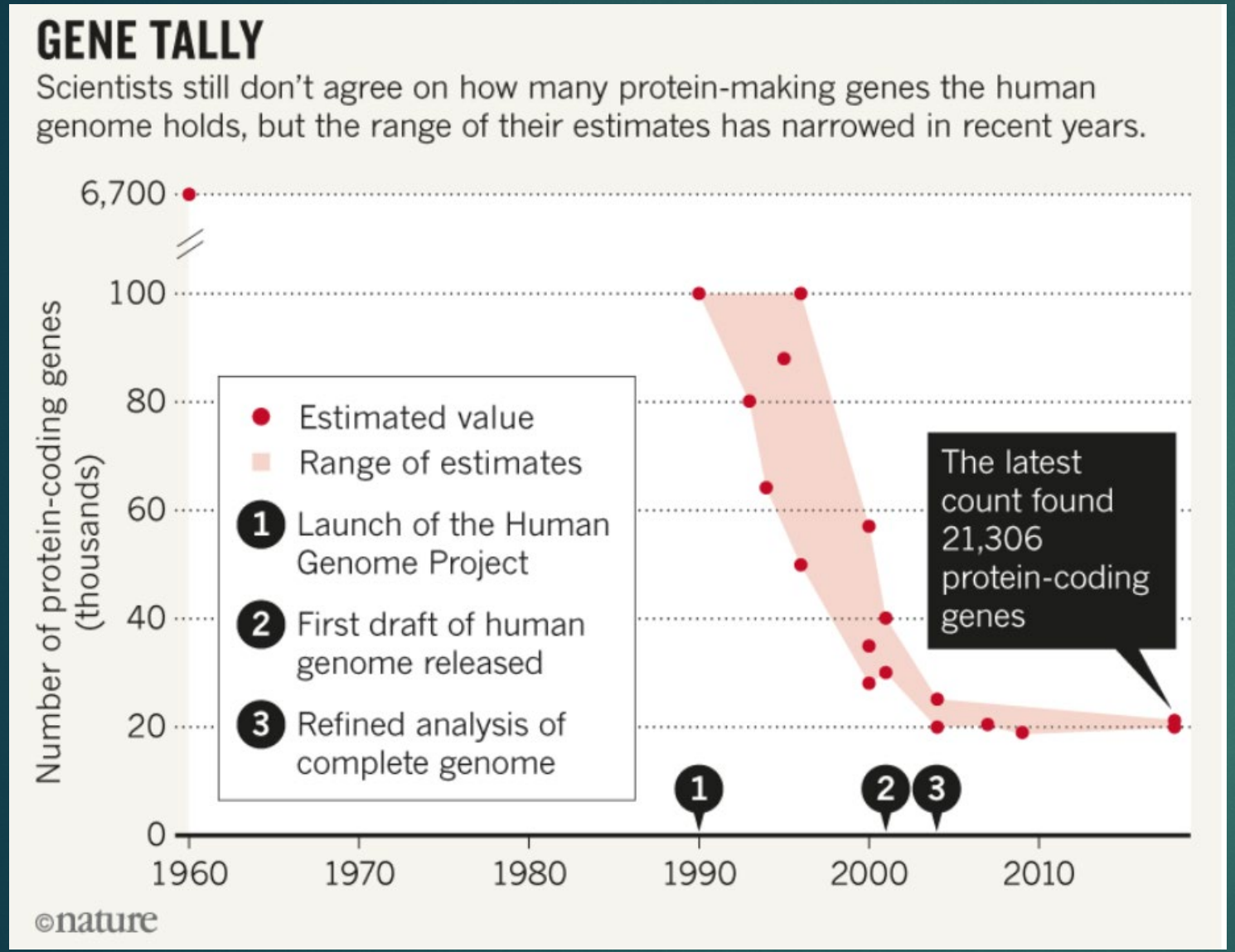
The Gene gambling bet in 2000

- In 1990s, Human Genome Project; Nature, 2001; predicted that in a matter of months, we would discover genetics of all diseases

- Ewan Birney at the Cold Spring Harbor bar in 2000 with most of world's greatest geneticists: $1 bet on number of genes: n = 1000 geneticists, range was 312 K to 26 K, average 40 K

- Now: Only 19 K human genes

- Most of genome is not protein coding gene



1960s: 2 M gene estimate

2018:
21,306 protein-coding genes and
21,856 non-coding genes

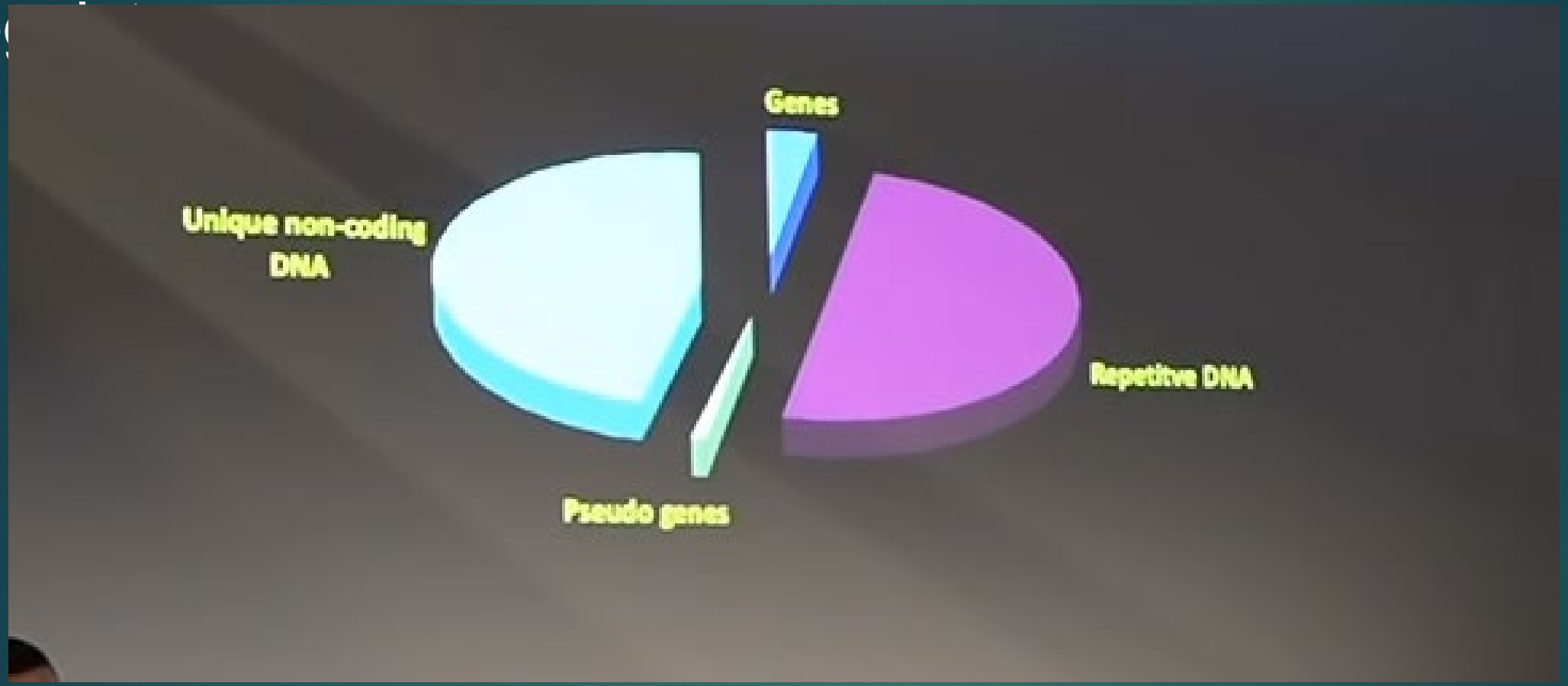
Fewer genes than nematode, banana, rice

From several hundred to 2 M SNPS per gene

13 K nonfunctional pseudogenes (via duplication)

50% of DNA are repetitive sequences

Genome: 97% not coding genes;
some noncoding regions are composed of
re



Pseudogenes are nonfunctional segments of DNA that resemble functional genes;
Originally discovered by Barbara McClintock, dispersed repeats have been increasingly recognized as a potential source of genetic variation and gene regulation.

DNA Contains

- Coding Sequences (genes) (2-3%) 19,000 genes
- Non-Coding Sequences (97-98%)

Before Human Genome Project, thought we had 100 to 250 K genes based on number of proteins we make; 1 gene – 1 protein idea

In fact, we have only 19 K genes that make unknown number of proteins (19 K confirmed)

DNA contains

- Non-coding areas:
- Originally thought it was "junk DNA"; now seen as more functional, regulatory:
- Absorbs errors; some genes more susceptible to errors; can get by with only 1 allele of 2 (25-30% of enzyme activity)

Not all DNA is functional; not necessary for survival

- Humans, onions, and other organisms lose functional DNA when mistakes are made during mutations or reproduction
- Uncomfortable idea for creationists who struggle to explain why an intelligently designed genome would consist mostly of rubbish

- Challenge for those who still think most non-coding DNA is vital is to explain why an onion has 15.9 billion bps; or amoebas with 290 billion bps; vs our 3.2 billion bps
- Junk DNA is not actively affected by natural selection
- Crickets possess 11 times more genetic material than fruit flies, who have 14,000 genes. Crickets lose junk DNA 40 times slower than fruit flies.

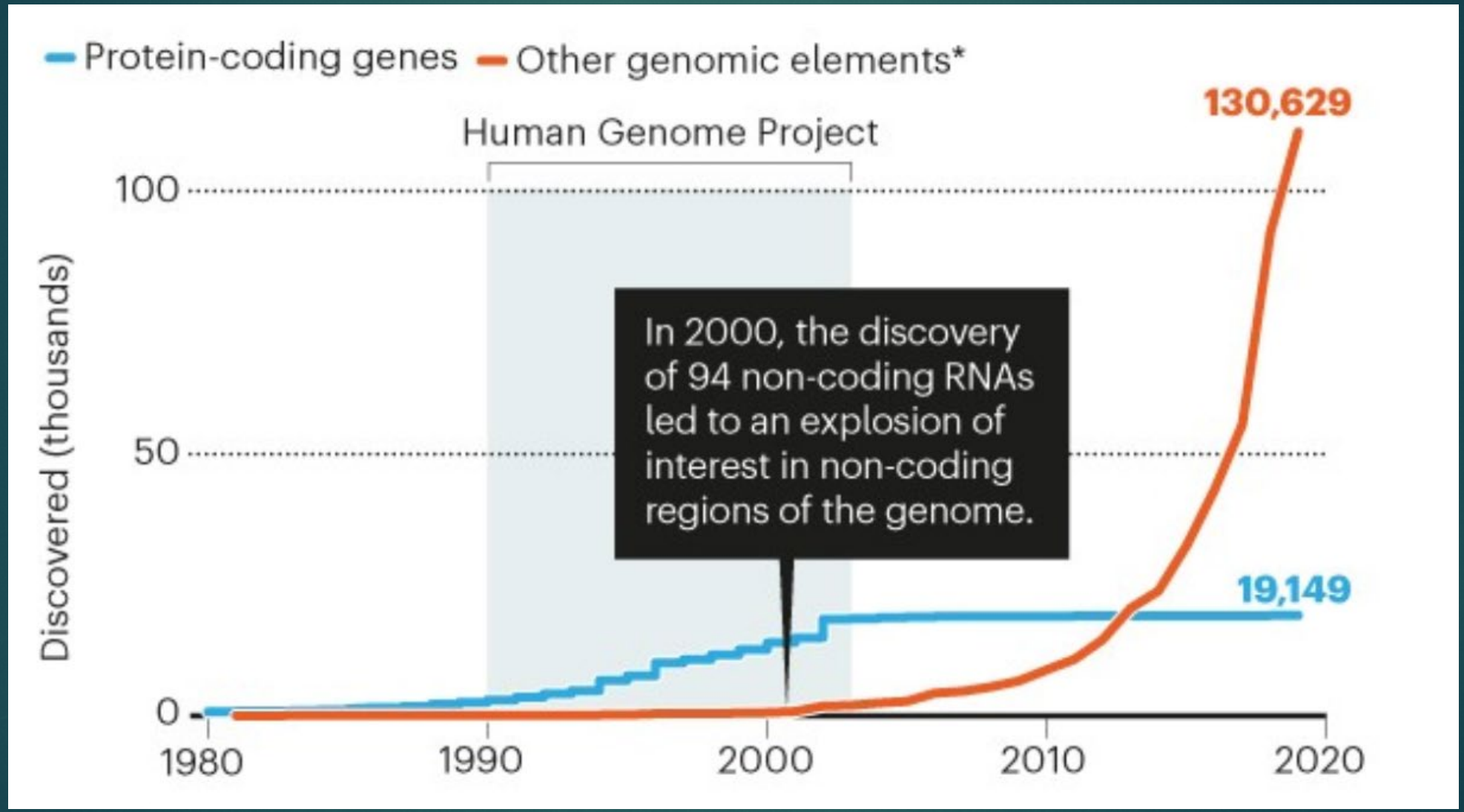
New genomics: Genes vs Junk DNA

- Junk DNA: now appreciated that the majority of functional sequences in the human genome do not encode proteins. Rather, elements such as long non-coding RNAs, promoters, enhancers and countless gene-regulatory motifs work together to control gene and protein expression.

- Research of non-protein-coding elements is now 5x greater than on genes.

- There are now more than 30,000 papers per year linking SNPs and traits. A large fraction of these associations are in the once-dismissed non-coding regions

Published papers on non-coding elements of DNA



Still mostly junk DNA

- Current assessment of DNA:
  - 8 % to 14% is protein coding;
  - rest is junk;
  - only 1 in 10 bases actually matters; 90% is still junk

- DNA sequence is functional only if it evolved to do something useful and if a mutation disrupting it would have harmful effect.

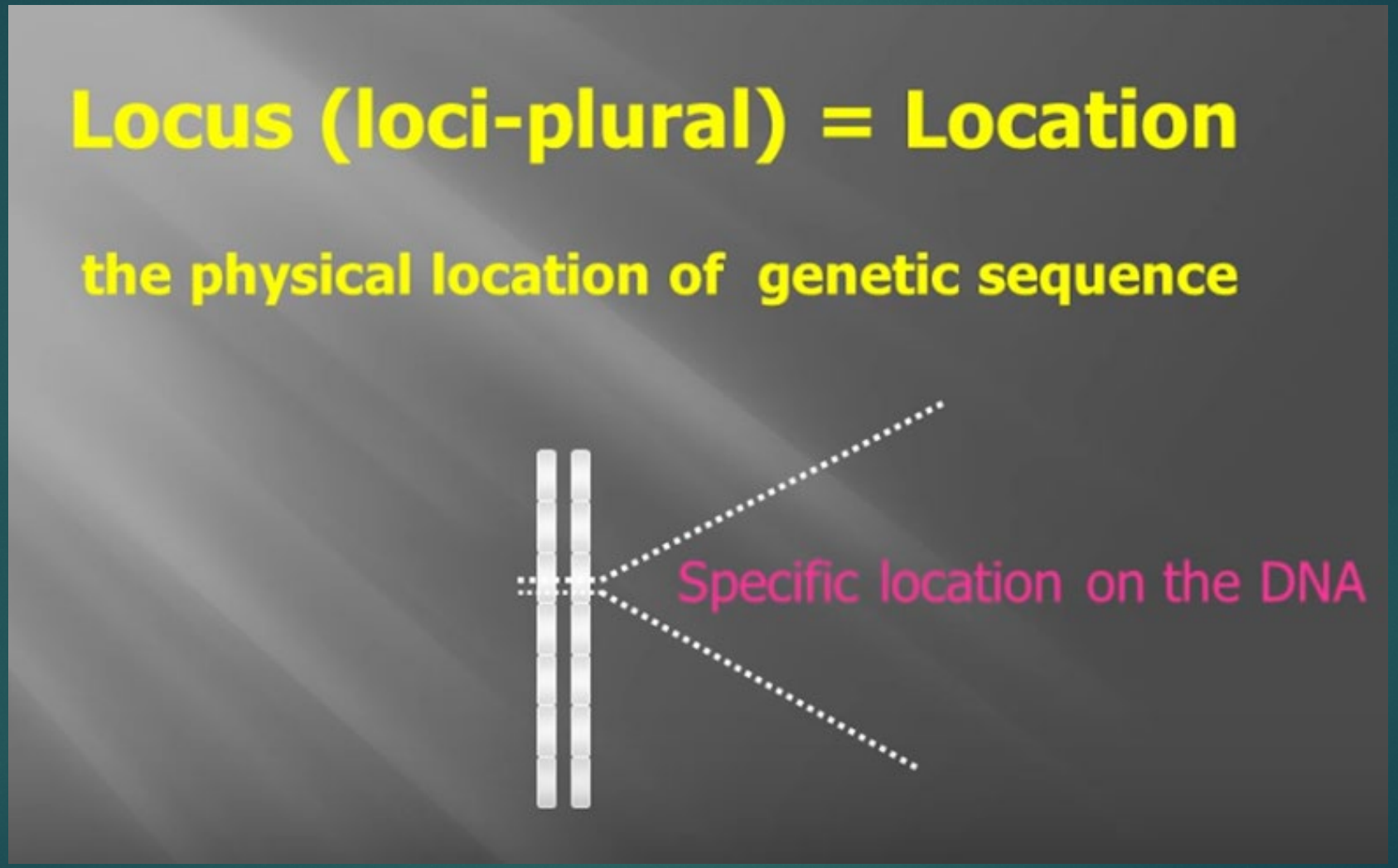
- DNA mutates at random due to UV radiation, or errors during cell division;

- Having too many bad mutations will kill you;
  - if most DNA was functional, most mutations would fall in good sequences & be bad for us;
  - if most DNA is junk, most mutations would not affect us; which is the actual reality

Genetics issues

- Eye color dominantly inherited (brown over blue), as was ability to roll one's tongue
- In reality, complete spectrum of eye color
- Genes for polydactyly (6 fingers), wet or dry earwax
- GWAS for particular trait: most significant disorders (i.e. IQ, psychiatric) are very polygenetic (dozens or hundreds of genes)
- Media nonsense: gene for….addiction, schizophrenia, transsexuality, politically liberal, adulterous, warrior, mass murder, evil
- Human behavior and its genetic base is incredibly complex

A genetic locus is specific location of a basepair or allele



Polymorphic = genetic variation

- **Polymorphism** is a term for a condition where there are *at least 2 genetic variants* (alleles) of some sign in the population (i.e. hair color) and its frequency of occurrence in the population exceed 1%.

- If the percentage of occurrence is smaller, we're talking about a random occurrence – mutation.

- Polymorphisms could be just a single-letter change (C instead of T).

- They could also be something more elaborate, like a whole stretch of DNA, that is either present or absent. You might call that a copy number variant (sections of the genome are repeated via duplication or deletion); those are all polymorphisms

SNPs: Single nucleotide polymorphisms – single letter difference

- Single nucleotide polymorphisms, frequently called SNPs (pronounced “snips”), are the most common type of genetic variation among people.

- Each SNP represents a difference in a single DNA building block, called a nucleotide (letters A, C, G, &T).

- The differences between individual genomes are largely due to **single nucleotide polymorphisms (SNPs)**, positions in the genome where some individuals have one nucleotide (e. g. an A) and others have a different nucleotide (e. g. a G).
- Over 1.4 million SNPs have been identified for function, an average of one for every 2.0 kb of sequence

SNPs: basis of human variability

- SNPs occur normally throughout a person's DNA.
- They occur almost once in every 2,000 nucleotides on average, which means there are roughly 4 to 5 million SNPs in a person's genome.
- These variations may be unique or occur in many individuals.
- More than **335 million** SNPs have been found across humans from multiple populations.

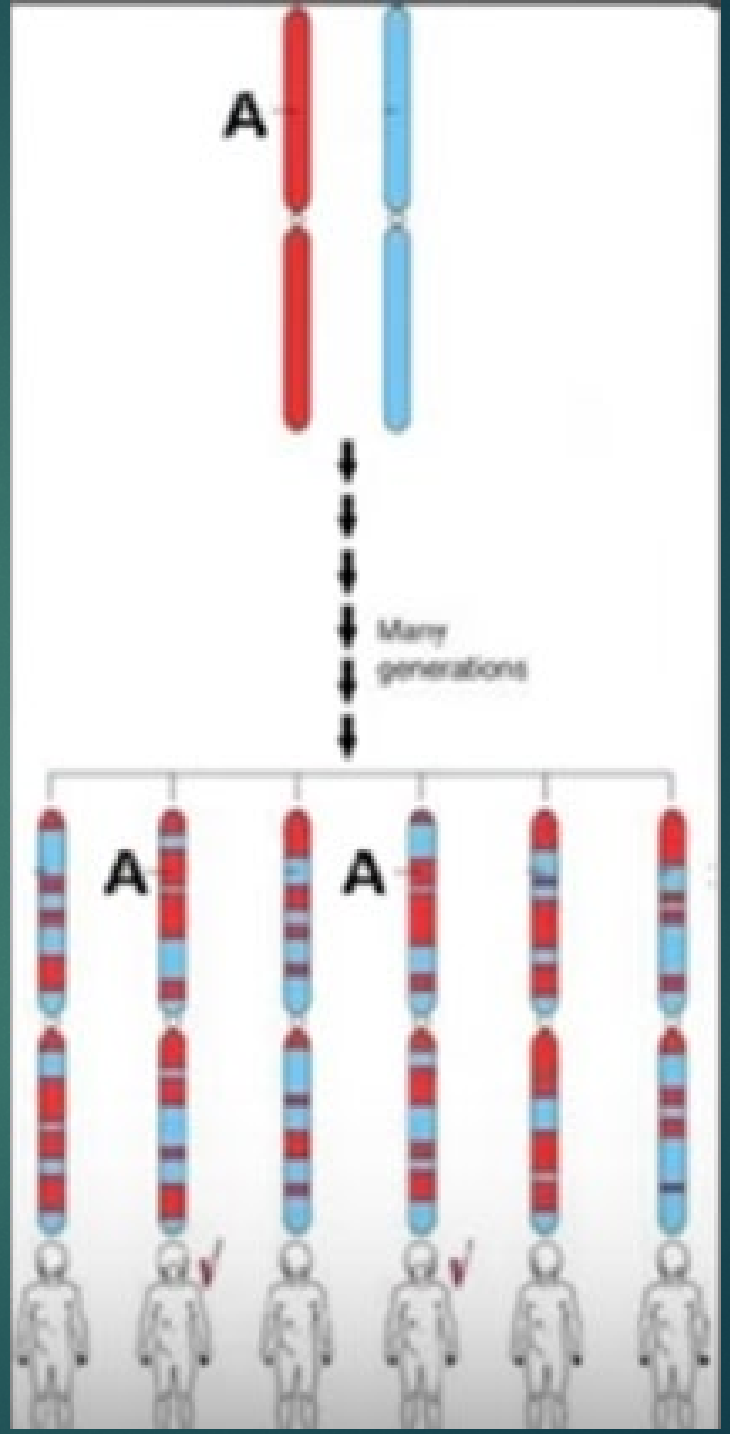
SNPs: mostly in noncoding areas

- If more than 1% of a population does not carry the same nucleotide at a specific position in the DNA sequence, then this variation can be classified as a SNP.
- If a SNP occurs within a gene, then the gene is described as having more than one allele. In these cases, SNPs may lead to variations in the amino acid sequence.
- SNPs, however, are not just associated with genes; they can also occur in noncoding regions of DNA.
- Most commonly, these variations are found in the DNA between genes, but 60 000 SNPs (of 4-5 M) lie within a gene

Haplotypes

A Haplotype is a combination of alleles at different chromosome regions that are closely linked and that tend to be inherited together

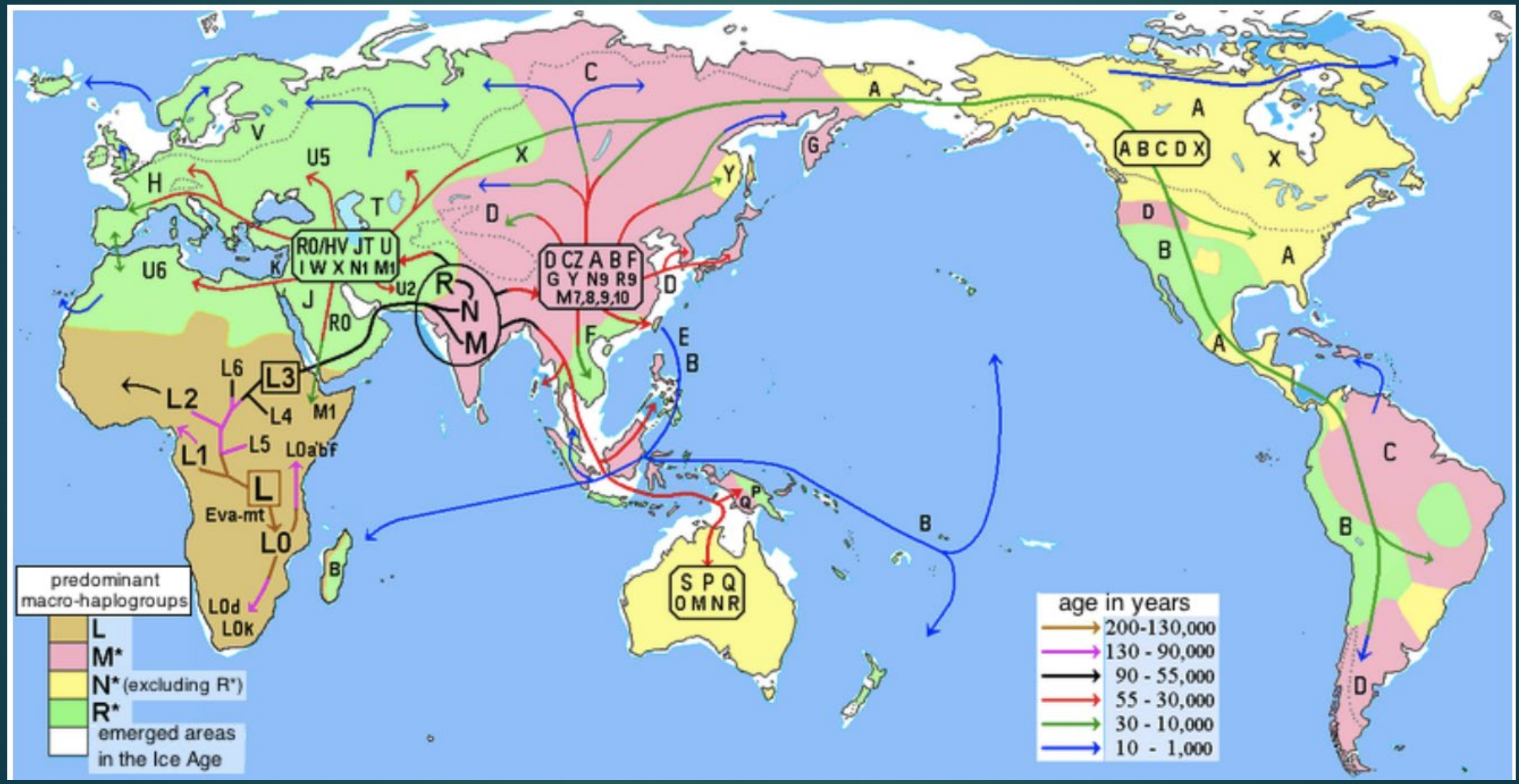
In both mt and nuclear DNA



Humans show haplotypes

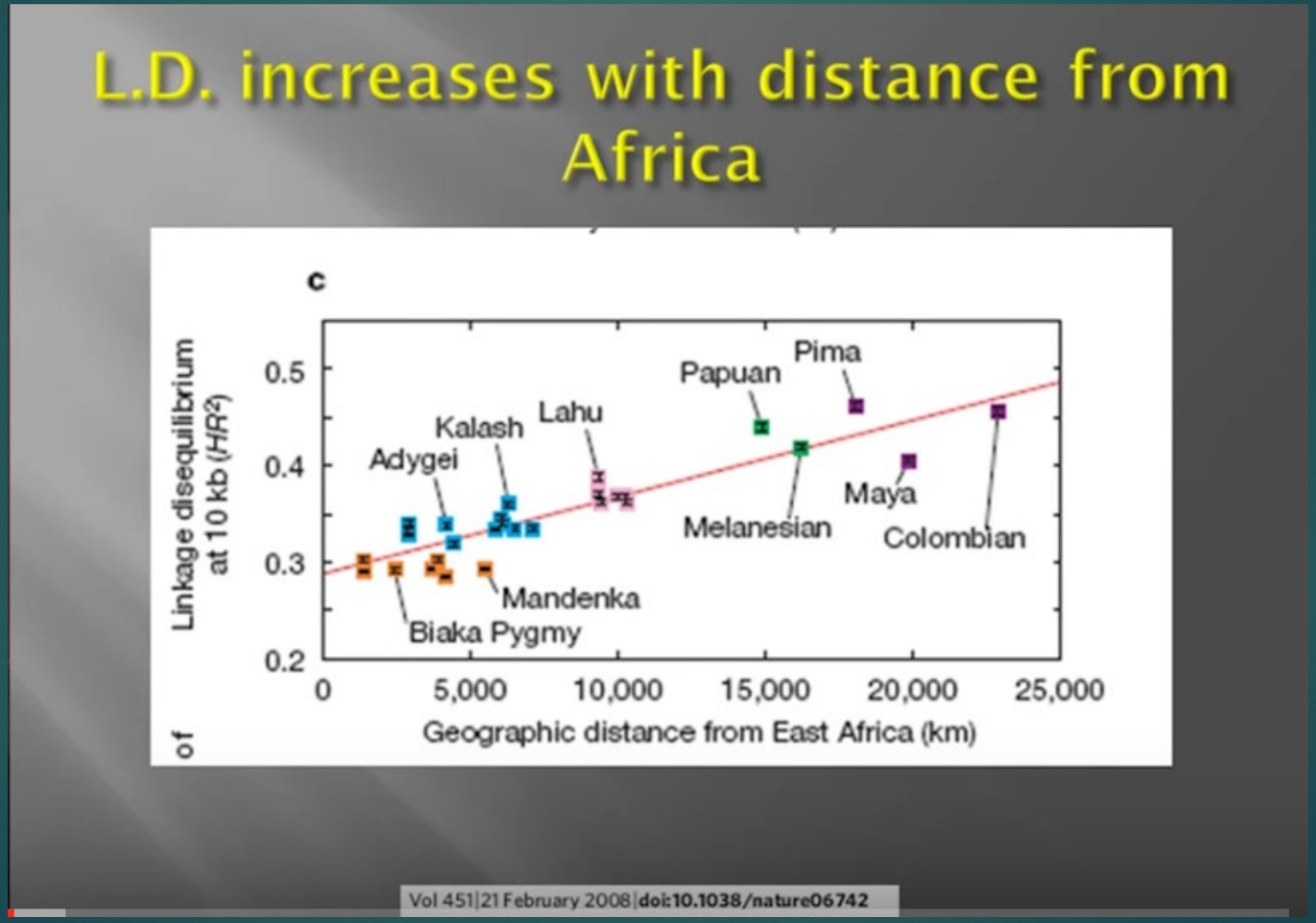
- We are believed to have descended from a human who originated ~200,000 years ago
- These humans originated in Africa
- As humans spread throughout the world in small groups, only some of the ancestral variation was present in each group
- The most haplotype variation is in Africa.

Haplogroup migrations: L is original



Lineage disequilibrium:

the non-random association of alleles at different loci in a given population



Linkage Disequilibrium

- Linkage disequilibrium (LD) is the correlation between nearby variants such that the alleles at neighboring polymorphisms (on the same chromosome) are associated within a population more often than if they were unlinked; form of haplotype

- LD is important to GWAS as it allows identifying genetic markers that tag the actual causal variants to complex human diseases.

*** History of aDNA Research

Ancient DNA

- Ancient DNA is a field of molecular evolutionary biology that uses DNA sequence data recovered from poorly preserved organisms, usually deceased for hundreds to hundreds of thousands of years.

- Involves extracting and manipulating sequence data from samples that are old and decayed in some way.

- Current estimate of perseveration of DNA: 100,000 to 1.6 M years

Insights into human history from the first decade of ancient human genomics

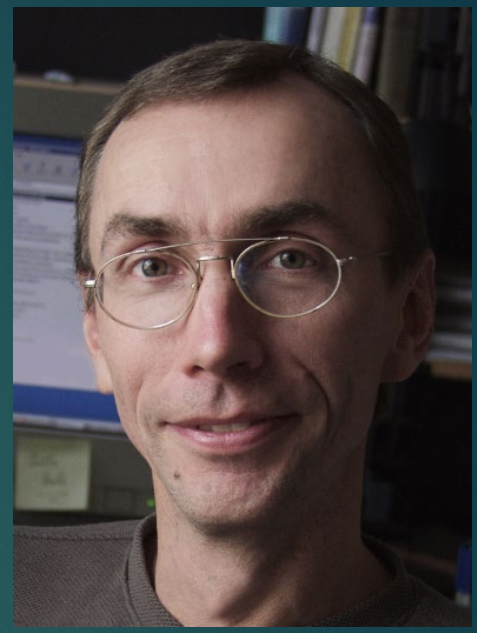
- Recent advancements in DNA sequencing technologies and laboratory preparation protocols have rapidly expanded the scope of ancient DNA

- Discoveries include:
  - interactions between archaic and modern humans
  - modern human population dynamics,
  - including the settlement history of most world regions.

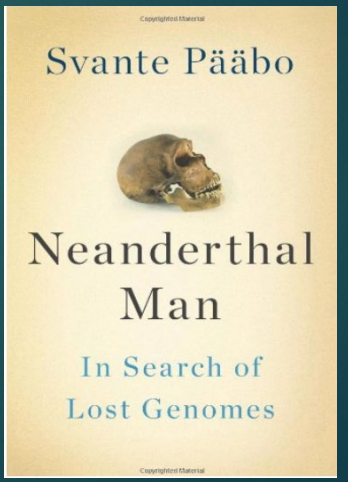
- In 2001, a draft sequence of the human genome was published.

Yi. Liu, X. Mao, J. Krause, Qiaomei Fu, 2021

Svante Pääbo (1955-): Grandfather of Evolutionary Genetics



- Swedish biologist specializing in evolutionary genetics
- Student of Allan Wilson
- Director of genetics at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany)
- A leader in the field of molecular evolution & one of the founders of paleogenetics
- 1997: retrieve DNA from Feldhofer Cave Neanderthal; different species



Svante Pääbo: a short biography

- Born of an extramarital affair in Stockholm, Sweden in 1955. During his childhood in Sweden, Svante Pääbo developed a fascination with archeology, which only deepened after his mother, Karin Pääbo, a chemist, took him to Egypt when he was 13.

- Pääbo switched from archeology to medicine at Uppsala University, influenced, he says, by his father, the 1992 Nobel Prize-winning biochemist Sune Bergström.

- Svante Pääbo studied Egyptology, Russian and the history of science at the University of Uppsala from 1975 and medicine from 1977, before earning his doctorate in cell biology in 1986.

Svante Pääbo: a short biography

- He switched from his medical studies in 1981 to do doctoral research on adenoviruses and their interaction with the immune system.

- Yet he still had Egyptology on his mind. He wondered if it was possible to obtain DNA from archeological remains.

- At the time, scientists did not know if DNA could survive intact for a hundred let alone several thousand years.

- Without telling his PhD advisor (who Pääbo worried might not approve) and with the help of one of his former Egyptology professors, Pääbo obtained mummy tissue samples from a German museum and went to work trying to isolate some DNA from them

Svante Pääbo: a short biography

- His finding— a demonstration that DNA survived in the cell nuclei of some Egyptian mummies—was published in 1984 in a small East German journal, and then a year later as the cover story in Nature.
  - Das Altertum, 1984: DNA from Egyptian child mummy
  - Journal of Archeological Science, 1985: DNA from Egyptian adult mummy
  - Nature, 1985: Molecular cloning of Ancient Egyptian mummy DNA

- One of the scientists who read—and was impressed—with Pääbo's work was the most famous evolutionary molecular biologist of his time, Allan Wilson at UC Berkeley.

- Wilson had just announced that his lab had isolated a small section of DNA from a zebra-like animal known as a quagga, which had become extinct in the 19th century.

Svante Pääbo: a short biography

- Wilson wrote to Pääbo to ask if he could do a sabbatical in Pääbo's laboratory. "He had no way of knowing that I didn't have a lab, that I was just a graduate student." Pääbo wrote back to explain the situation—and to ask for a postdoctoral position in Wilson's lab.

- In 1987, Pääbo began working with Wilson in UC Berkeley, CA. With the aid of the new PCR (polymerase chain reaction) technology, they analyzed mitochondrial DNA (mtDNA) from a 7,000-year-old human brain.

- But they soon came to realize that contamination was a major difficulty.

- Pääbo switched to extracting DNA from non-human ancient creatures, such as mammoths, giant sloths, and the marsupial wolf.

- It led to some interesting discoveries, such as the finding that moas, the giant flightless birds that were hunted into extinction on New Zealand about 500 years ago, are more closely related to Australian emus than to kiwis, the flightless birds that populate New Zealand today.

Svante Pääbo: a short biography

- In 1990 he returned to Europe and became professor of general biology at the University of Munich, where he focused on development of techniques to study ancient DNA and started applying them to Neandertals.

- Using specimens from a German museum, he successfully sequenced mtDNA from the Feldhofer Neandertal's upper arm bone.

- That achievement, which was published in the journal Cell in 1997, is considered a watershed in evolutionary genetics: The first DNA analysis of another hominin species

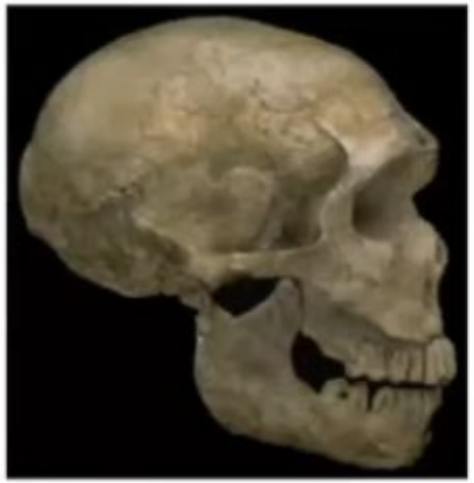
- Pääbo's results had shown not only that DNA could be successfully extracted and sequenced from Neandertal remains, but also that Neandertals and humans were distinctly different groups that split off from each other about 500,000 years ago.

2010:

First Neandertal Nuclear DNA Draft Sequence

From left to right: Johannes Krause, Adrian Briggs, Richard E. Green, Svante Pääbo.
Credit: Copyright Max Planck Institute for Evolutionary Anthropology

Two publications that created the field of aDNA



Svante Pääbo

A Draft Sequence of the Neandertal Genome

Richard E. Green,[1]*†‡ Johannes Krause,[1]†§ Adrian W. Briggs,[1]†§ Tomislav Maricic,[1]†§ Udo Stenzel,[1]†§ Martin Kircher,[1]†§ Nick Patterson,[2]†§ Heng Li,[2]† Weiwei Zhai,[3]†‖ Markus Hsi-Yang Fritz,[4]† Nancy F. Hansen,[5]† Eric Y. Durand,[3]† Anna-Sapfo Malaspinas,[3]† Jeffrey D. Jensen,[6]† Tomas Marques-Bonet,[7,13]† Can Alkan,[7]† Kay Prüfer,[1]† Matthias Meyer,[1]† Hernán A. Burbano,[1]† Jeffrey M. Good,[1,8]† Rigo Schultz,[1] Ayinuer Aximu-Petri,[1] Anne Butthof,[1] Barbara Höber,[1] Barbara Höffner,[1] Madlen Siegemund,[1] Antje Weihmann,[1] Chad Nusbaum,[2] Eric S. Lander,[2] Carsten Russ,[2] Nathaniel Novod,[2] Jason Affourtit,[9] Michael Egholm,[9] Christine Verna,[21] Pavao Rudan,[10] Dejana Brajkovic,[11] Željko Kucan,[10] Ivan Gušic,[10] Vladimir B. Doronichev,[12] Liubov V. Golovanova,[12] Carles Lalueza-Fox,[13] Marco de la Rasilla,[14] Javier Fortea,[14]¶ Antonio Rosas,[15] Ralf W. Schmitz,[16,17] Philip L. F. Johnson,[18]† Evan E. Eichler,[7]† Daniel Falush,[19]† Ewan Birney,[4]† James C. Mullikin,[5]† Montgomery Slatkin,[3]† Rasmus Nielsen,[3]† Janet Kelso,[1]† Michael Lachmann,[1]† David Reich,[2,20]*† Svante Pääbo[1]*†

2010: Discovery of interbreeding between Neandertals and modern humans

Genetic history of an archaic hominin group from Denisova Cave in Siberia

David Reich[1,2]*, Richard E. Green[3,4]*, Martin Kircher[3]*, Johannes Krause[3,5]*, Nick Patterson[2]*, Eric Y. Durand[6]*, Bence Viola[3], Adrian W. Briggs[1,3], Udo Stenzel[3], Philip L. F. Johnson[8], Tomislav Maricic[3], Jeffrey M. Good[9], Tomas Marques-Bonet[10,11], Can Alkan[10], Qiaomei Fu[3,12], Swapan Mallick[1,2], Heng Li[2], Matthias Meyer[3], Evan E. Eichler[10], Mark Stoneking[3], Michael Richards[7,13], Sahra Talamo[7], Michael V. Shunkov[14], Anatoli P. Derevianko[14], Jean-Jacques Hublin[7], Janet Kelso[3], Montgomery Slatkin[6] & Svante Pääbo[3]

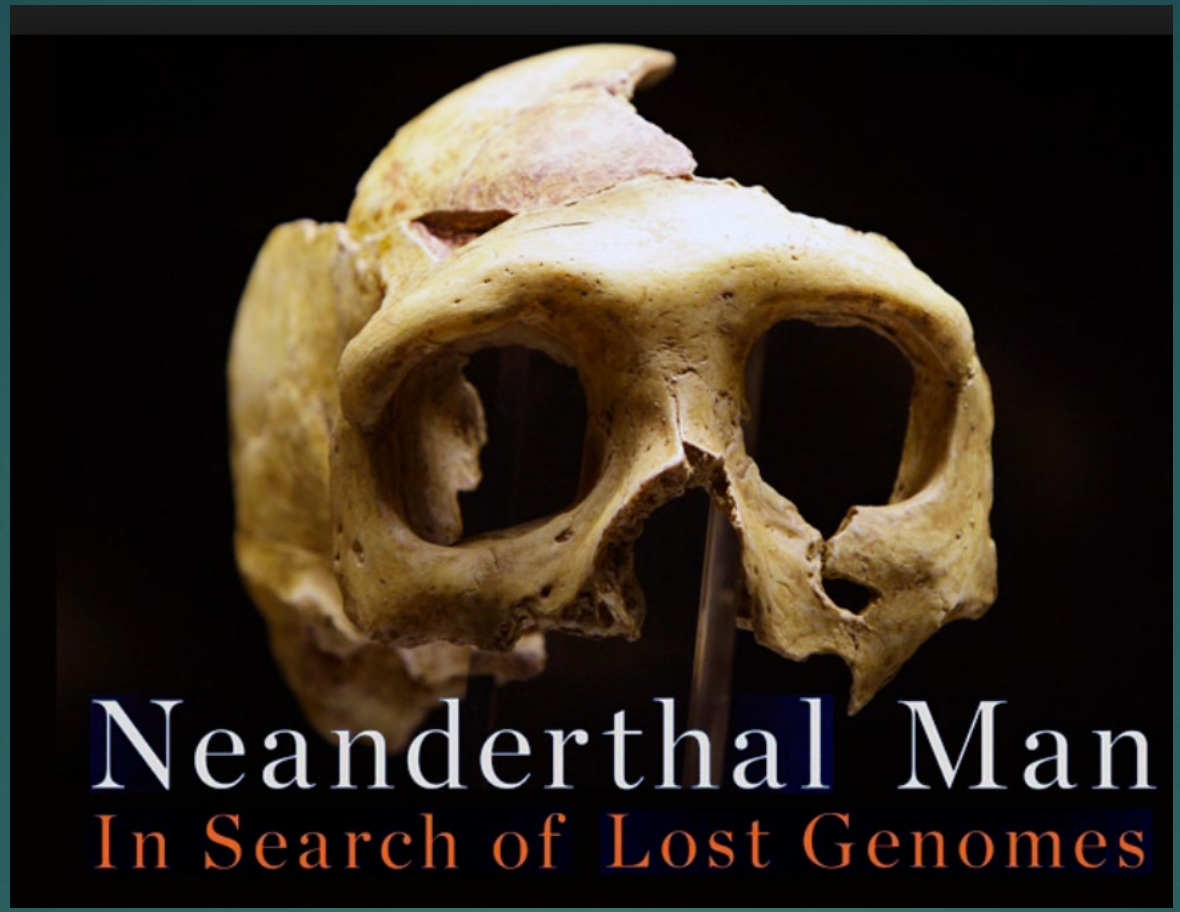
2010: Discovery of the "Denisovans," a previous unknown archaic population who also interbed with modern humans

Svante Pääbo: a short biography

- In 1997, Pääbo became director of the Department of Genetics at the new Max Planck Institute for Evolutionary Anthropology in Leipzig, a position he continues to hold today.
- Over the past decades, he has led the efforts to sequence
  - Draft version of the nuclear genome of the Neandertals. In 2010, he and his colleagues at the Institute published the draft version of that genome, with the discovery of 2% N DNA in MHs. The finding shows that a small number of Neandertals and early humans have interbred.
  - That same year: A DNA analysis of a finger bone found in 2008 in a Siberian cave showed that the bone belonged to a previously unknown hominin group, which the researchers named Denisovans. It was the first time a new hominin had been identified by genetic analysis alone. They also showed that Denisovans have contributed DNA to people living in Melanesia today.

Svante Pääbo: a short biography

- Pääbo has also investigated the genetic relationship between humans and great ape populations, particularly how differences in gene expression evolve.
- What makes humans special: genes critically important in human evolution, for example FOXP2, which is associated with language development.
- In 2002, Pääbo reported that the protein encoded by the FOXP2 gene in Neandertals was identical (T in chimps, A in N and MH) to that of present-day humans, which raised the tantalizing possibility that they may have had language capabilities similar to present-day humans.
- Published more than 562 papers between 1974 and 2021 (= 1 paper a month for 47 years.

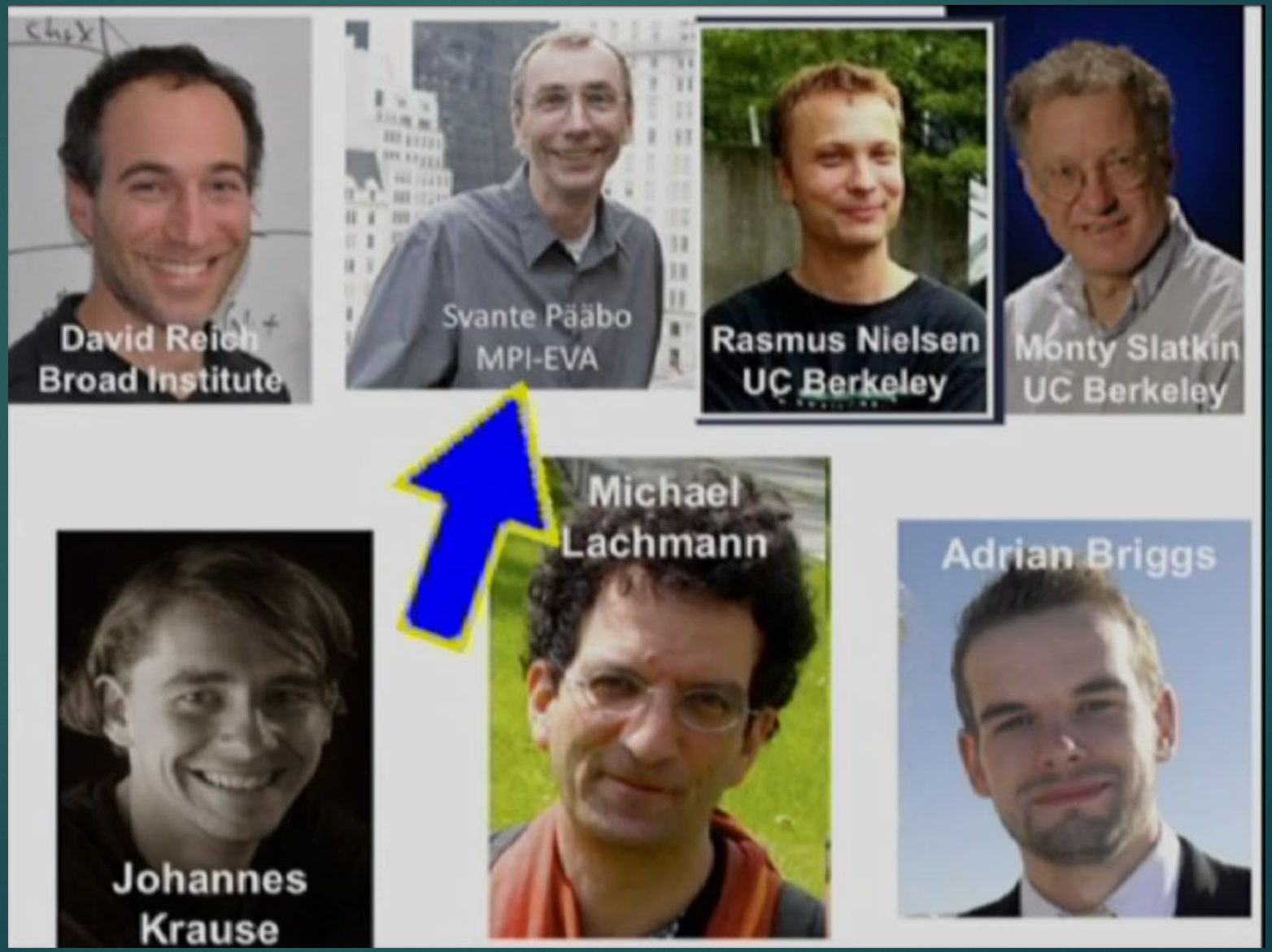


By Svante Pääbo, 2014

Awards

- Pääbo has won numerous awards, including
  - Gottfried Wilhelm Leibniz Prize of the German Research Foundation (1992)
  - the Louis Jeantet Prize for Medicine (Switzerland)
  - the Virchow Medal (2005)
  - the order Pour le mérite (2008)
  - the H.M. The King's Medal by the King of Sweden (2012)
  - the Gruber Prize for Genetics (2013)
  - the Kistler Prize (USA)
  - the Leibniz Prize (Germany),
  - the Breakthrough Prize in Life Science, 2015
  - the Keio Medical Science Prize, 2016
  - the Körber Prize for European Science in 2018
  - the Japan Prize in 2020
- He is a member of Sweden's Royal Academy of Sciences, and in 2004 he became a foreign member of the National Academy of Sciences. One of Time's 100 most influential humans in 2007.
- Pääbo, who is openly bisexual, lives in Leipzig with his wife, Linda Vigilant, an American primatologist. They have a son and a daughter.

Paleogenetics gang



Leipzig Paleogenetics Gang

Front row, from left: Hernán Burbano, Anja Buchholz, Svante Pääbo, Janet Kelso, Qiaomei Fu & Martin Kircher; back row from left: Adrian Briggs, Jesse Dabney, Matthias Meyer, Tomislav Maricic, Johannes Krause, Udo Stenzel and Kay Prüfer

*** Ancient DNA - aDNA

Ancient DNA: Ancient Biomolecules and Evolutionary Inference

- Over the last few decades, studies of ancient biomolecules have transformed our understanding of the evolutionary history of life on Earth.

- Evolutionary inferences are drawn almost exclusively from
  - observation of phenotypic traits in fossils.
  - molecular analyses of living organisms

- Ancient biomolecules include: DNA, proteins, lipids

- The sequencing of ancient DNA has enabled the reconstruction of speciation, migration and admixture events for extinct species.

Enrico Cappellini…Eske Willerslev, et al., 2018

Ancient Biomolecules

- Since then, the focus of aDNA studies has progressed from:
  - small mitochondrial DNA fragments retrieved from a single species to
  - multiple species to
  - full genomic sequencing of one or a few specimens, to
  - single-nucleotide polymorphism capture-based population genomics and
  - whole-genome shotgun sequencing, often including over a hundred individuals.

- The age of specimens from which DNA can be successfully recovered has also increased significantly from a relatively modest couple of hundreds of years to more than 1 Ma years.

aDNA and aProteins

- The irreversible post-mortem degradation of ancient DNA has so far limited its recovery—outside permafrost areas—to specimens that are not older than approximately 500 K years.

- By contrast, tandem mass spectrometry has enabled the sequencing of approximately 1.5 M-old collagen proteins, and suggested the presence of protein residues in fossils of the Cretaceous period (145 to 65 K)—although with limited phylogenetic use

Applications in Evolutionary Biology

- Analyses of ancient biomolecules have led to some of the biggest breakthroughs in the field of evolutionary biology.

Archaic Hominins

- Ancient genomics has been central to furthering our understanding of
  - human evolution after our divergence from archaic hominins, as well as
  - the evolutionary consequences of human encounters with archaic hominin groups in the Late Pleistocene.

- The comparison of the Neanderthal genome with non-African individuals showed that the ancestors of all present-day non-African people contain around 2% Neanderthal DNA, indicating that they admixed with Neanderthals shortly after the dispersal of humans from Africa approximately 65–55 kyr ago.

Ancient biomolecules: nucleic acids, proteins, and lipids

- The categories of ancient molecules that have arguably made the biggest contribution to elucidating evolutionary history to date are:
  - nucleic acids,
  - proteins,
  - lipids.

- Deoxyribonucleic acids (DNA) can show evolutionary processes with the highest resolution, but proteins and lipids are important on longer temporal scales and in geographic areas that are less favorable to DNA preservation

Sources of Ancient biomolecules

- Bones and teeth remain the most widely used mineralized specimens for extracting aDNA

- Wealth of other suitable calcified and mineralized substrates, such as eggshells, invertebrate shells, coprolites, and dental calculus, the latter two being particularly valuable for investigating ancient microbiomes.

- Keratinous material, e.g., hair, claws, and feathers; but are scarce

- Archaeobotanical remains, such as fossilized seeds, fruit, and wood, have been the dominant source of ancient plant DNA,

Sources of aDNA

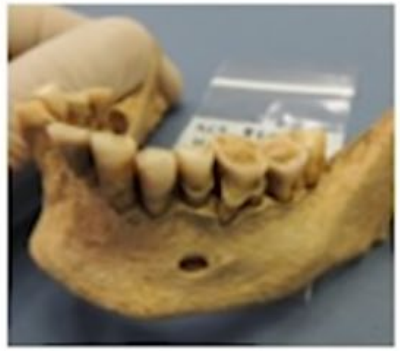
Hair
(Bonnichsen et al., 2001; Rassmussen et al., 2010)

Plants
(Goloubinoff et al., 1993; Medović et al., 2011)

Coprolites
(Sutton, 1996; Poinar et al., 1998a,b; Hofreiter et al., 2000)

Quids
(LeBlanc et al., 2007)

Tobacco lump



Dental calculus
(Ozga et al., 2016)

Ice/Soil cores
(Willerslev et al., 2003; Haile et al., 2009)

Parchments
(Parry et al., 1996; Teasdale et al., 2014)

Clothing
(LeBlanc et al., 2007; Schröder et al., 2016)

Sources of Ancient biomolecules

- Archeological artefacts, e.g., lithics and ceramics; source of aDNA originating primarily from food sources.

- Environmental DNA or eDNA is DNA that is collected from a variety of environmental samples such as soil, seawater, snow or even air rather than directly sampled from an individual organism

aDNA: Lipids

- Like aDNA and ancient proteins, ancient lipids (fatty acids, waxes etc.) persist in a wide range of substrates, including ocean and lake sediments, sedimentary rocks, soils, fossil and subfossil remains, archaeological artefacts (e.g., pottery, stone tools), animal and human remains (soft tissue and bone lipids), coprolites, botanical remains, and a wide range of other deposits (tars, pitches, and bitumens) and bog butters (i.e., fatty substances, made either of animal carcass or dairy products found in peat bogs).
- A notable difference between lipids and other classes of biomolecules is that, under favorable preservation conditions, they are frequently recovered in high concentrations.

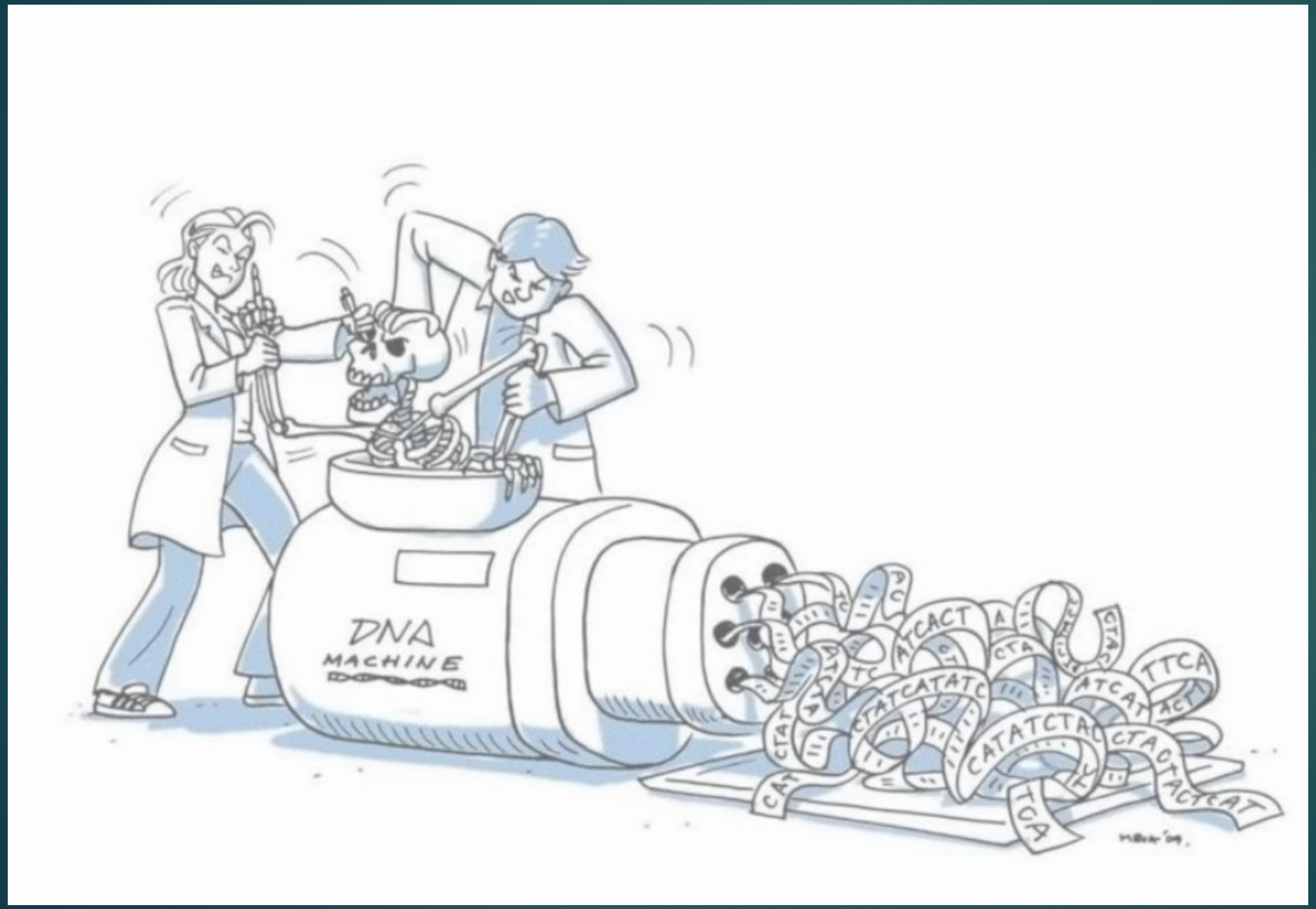
Degradation of Ancient Biomolecules

- The chemical structure of ancient biomolecules is heavily altered by a series of complex diagenetic reactions that begin upon the death of the organism and continue until their recovery.

- Ancient DNA is normally:
  - heavily fragmented and
  - chemically modified.

- After the death of an organism, DNA is initially degraded via
  - internal enzymes.
  - followed by external degradation processes, such as oxidation, hydrolysis (water breaks chemical bond), and background radiation,
  - which alter the bases and cleave the sugar-phosphate backbone of the DNA molecules, leading to their destabilization and fragmentation.

aDNA Genome Sequencing: actual DNA converted into computer digital code



Only in 2003, was sequencing ability capable of doing nuclear DNA (3 Billion letters)

Molecular biology definitions

- DNA sequencing is the process of determining the nucleic acid sequence – the order of nucleotides in DNA. It includes any method or technology that is used to determine the order of the four bases: adenine, guanine, cytosine, and thymine.

- Polymerase chain reaction (PCR) is a method to rapidly make millions to billions of copies of a specific DNA sample

- Amplification refers to the production of one or more (usually millions) copies of a genetic fragment or target sequence; i.e. via PCR

Reference genome: a digital summarized copy

- A reference genome is a digital nucleic acid sequence database (digital genotype), assembled by scientists as a representative example of the set of genes in one idealized individual organism of a species; usually based on multiple real genomes

- As they are assembled from the sequencing of DNA from a number of individual donors, reference genomes do not accurately represent the set of genes of any single individual organism. A reference provides a mosaic of different DNA sequences from each donor.

- There are now reference genomes for multiple species.

Reference genome: a digital summarized copy

- All reference genomes are updateable.

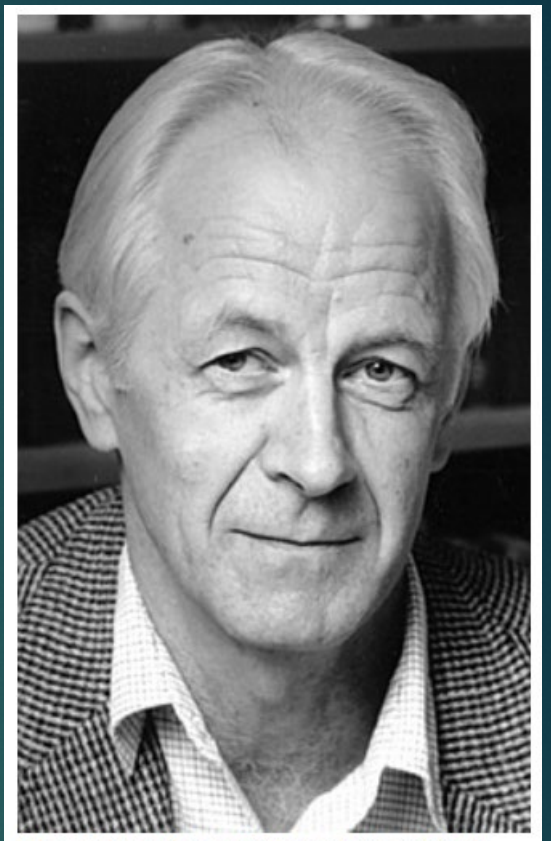
- Reference genomes are typically used as a guide on which new genomes are built. A basic comparison step in DNA sequencing

- The most recent is the Human Reference Genome, *GRCh38*, from the Genome Reference Consortium is derived from thirteen anonymous volunteers. First version (1990-2003) had roughly 150,000 gaps.

Allan Charles Wilson (1934-1991):

Molecular phylogenetics – Mitochondrial Eve

- New Zealand molecular evolutionist at UC Berkeley
- Invented the field of molecular phylogenetics, the modern application of genomics to the study of evolution, via 1984 Quagga study
- When he read Pääbo's 1985 mummy study, he asked to do a sabbatical in Pääbo's lab, not knowing that the latter was still a predoctoral student.
- Mitochondrial Eve study, 1987



Allan Wilson *(Jane Scherr photo, 1990)*

1984: Birth of Paleogenetics: Quagga

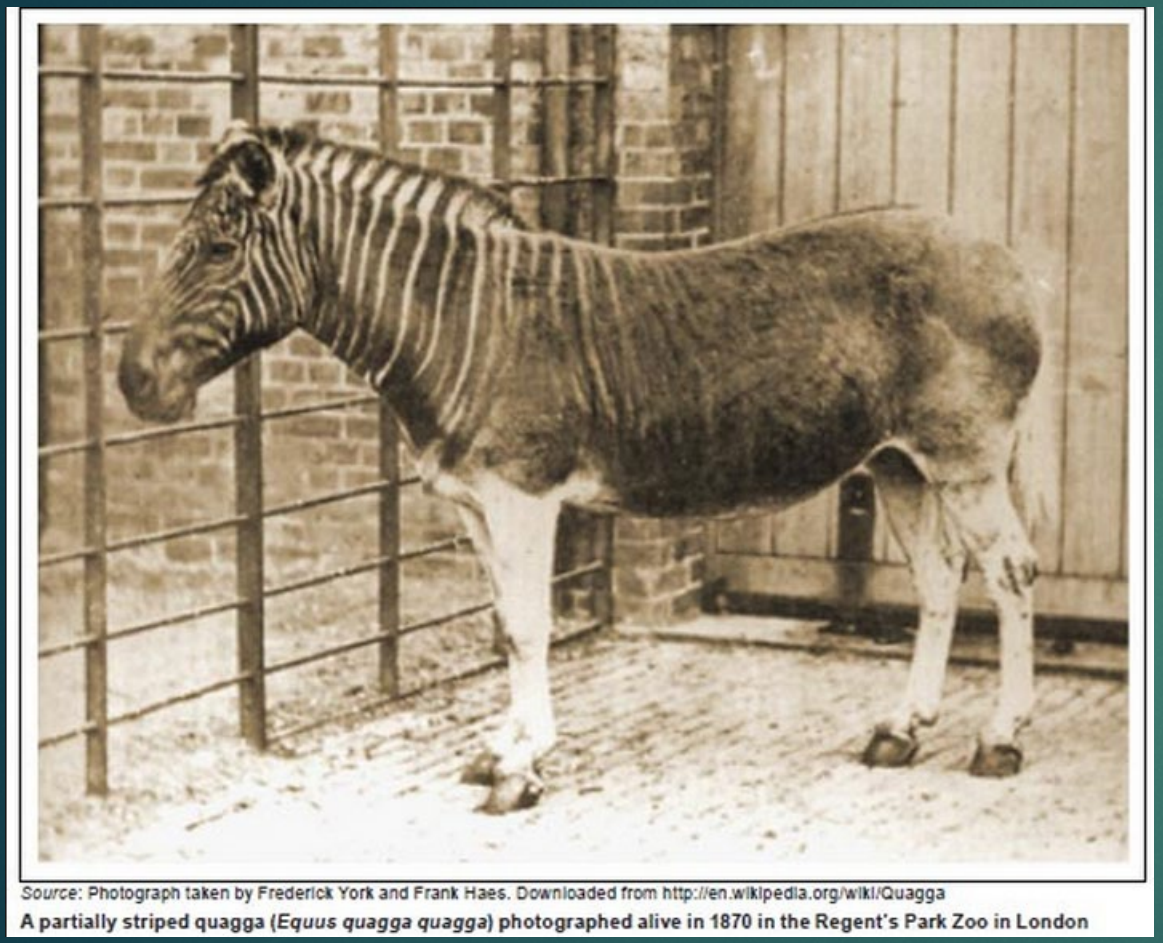
- In 1984, a team led by Allan Wilson of the University of California, Berkeley, published the first partial sequences of ancient mitochondrial DNA (mtDNA), from a museum specimen of a 140 year old quagga, a zebralike animal that had gone extinct almost exactly 100 years before.

- They cloned two short fragments of mitochondrial DNA (mtDNA).

- The first study to describe DNA preserved in nonliving tissues in a mainstream scientific journal.

(Higuchi et al. 1984)

Quagga: 1st mtDNA from extinct species, 1984



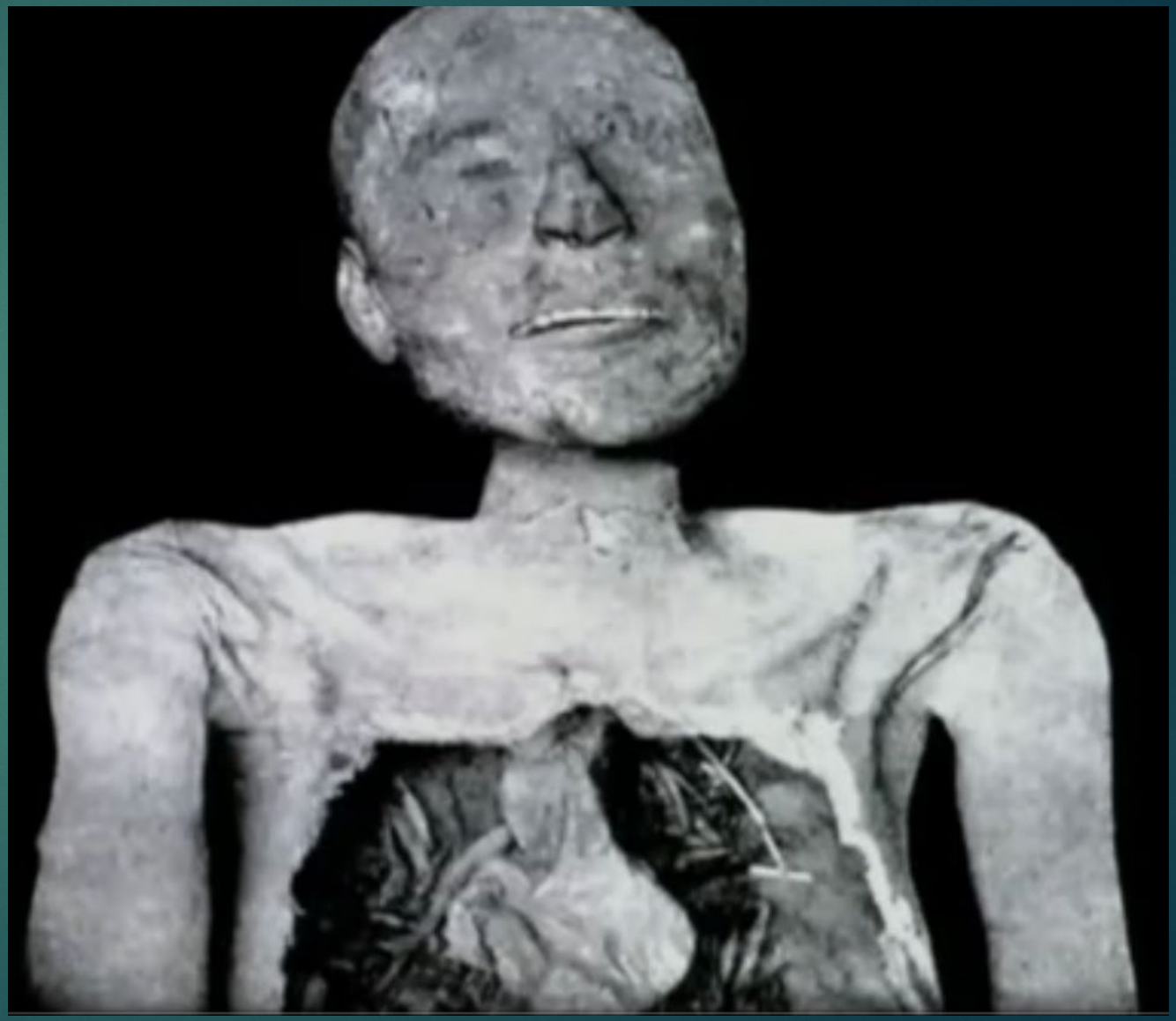
Source: Photograph taken by Frederick York and Frank Haes. Downloaded from http://en.wikipedia.org/wiki/Quagga

A partially striped quagga (*Equus quagga quagga*) photographed alive in 1870 in the Regent's Park Zoo in London

- Last South African zebra subspecies died at the Amsterdam Zoo in 1883.
- In 1984, Allan Wilson at UC Berkeley recovered 229 base pairs of genetic mt DNA code from a quagga.
- Achievement proved DNA could survive in dead things and spurred a new field of science: paleogenetics.
- Recent Project has succeeded in producing animals that closely resemble the original quagga.

Egyptian Mummies, 1985

Very first Human aDNA, 2400 ya

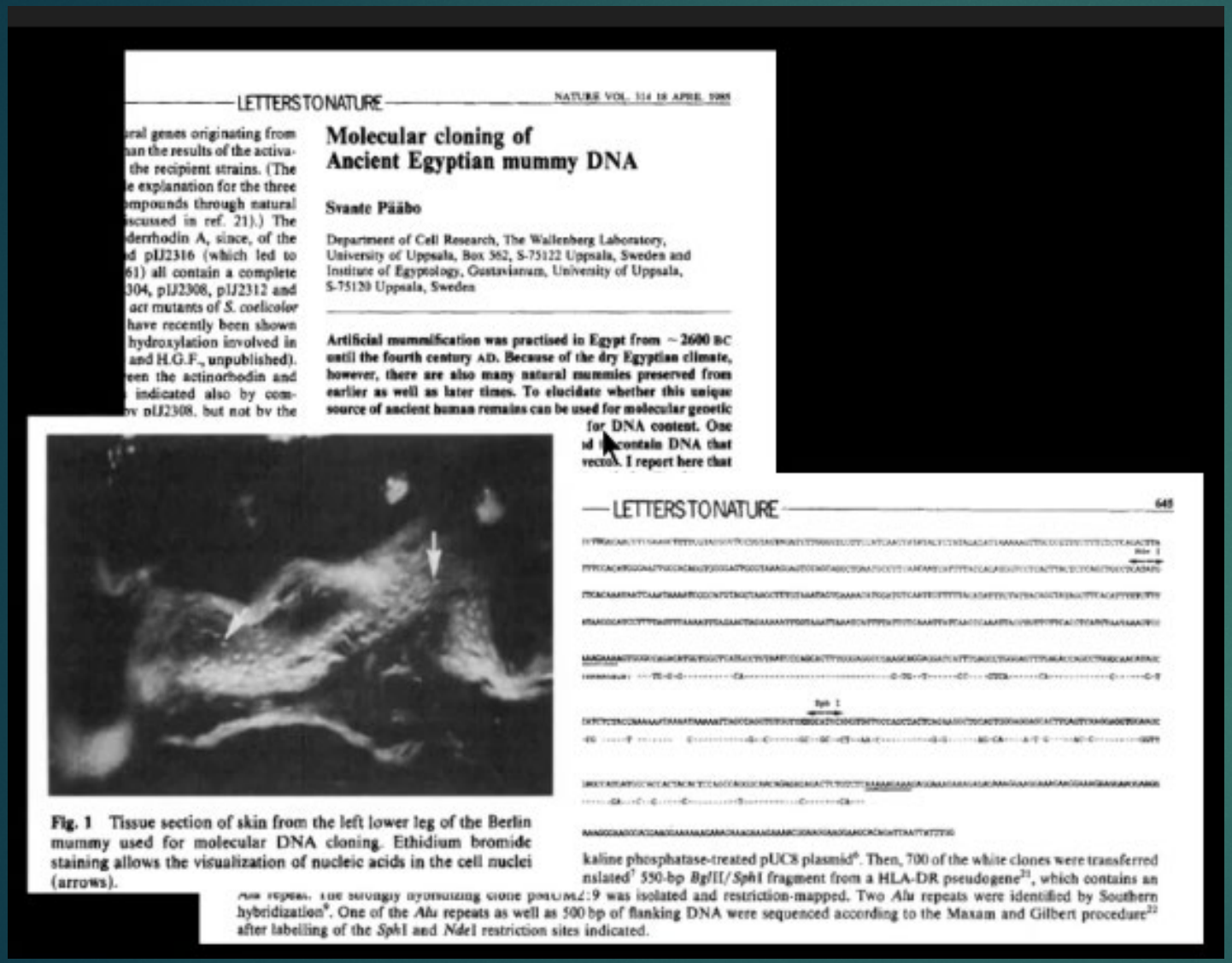


1985: Egyptian Mummies

- "The father of ancient DNA," Svante Pääbo began his work with the aim of genetically characterizing the evolutionary history of Egyptian mummies.
- In 1985, he recovered two members of the Alu family of human repetitive DNA sequences from a 2400-year-old Egyptian mummy. Used bacterial cloning technique.
- DNA could be recovered from only one of the 23 mummies he tested.
- Only 3.4 kilobases if mtDNA

Pääbo, S. Das Altertum 30,(1984); S **Pääbo** , *Nature*, 1985

Pääbo, 1985



LETTERS TO NATURE

NATURE VOL. 314 18 APRIL 1985

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304, pIJ2308, pIJ2312 and
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have recently been shown
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and H.G.F., unpublished).
een the actinorhodin and
indicated also by com-
by pIJ2308, but not by the

Molecular cloning of Ancient Egyptian mummy DNA

Svante Pääbo

Department of Cell Research, The Wallenberg Laboratory, University of Uppsala, Box 562, S-75122 Uppsala, Sweden and Institute of Egyptology, Gustavianum, University of Uppsala, S-75120 Uppsala, Sweden

Artificial mummification was practised in Egypt from ~2600 BC until the fourth century AD. Because of the dry Egyptian climate, however, there are also many natural mummies preserved from earlier as well as later times. To elucidate whether this unique source of ancient human remains can be used for molecular genetic for DNA content. One contain DNA that vectors. I report here that

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Fig. 1 Tissue section of skin from the left lower leg of the Berlin mummy used for molecular DNA cloning. Ethidium bromide staining allows the visualization of nucleic acids in the cell nuclei (arrows).

kaline phosphatase-treated pUC8 plasmid[6]. Then, 700 of the white clones were transferred nslated[7] 550-bp *Bgl*II/*Sph*I fragment from a HLA-DR pseudogene[21], which contains an hybridization[8]. One of the *Alu* repeats as well as 500 bp of flanking DNA were sequenced according to the Maxam and Gilbert procedure[22] after labelling of the *Sph*I and *Nde*I restriction sites indicated.

Later discovered to be contaminated DNA

For the next 15 years, Pääbo & his lab became obsessed with defeating contamination in aDNA research

Lots of aDNA resources: museum collections

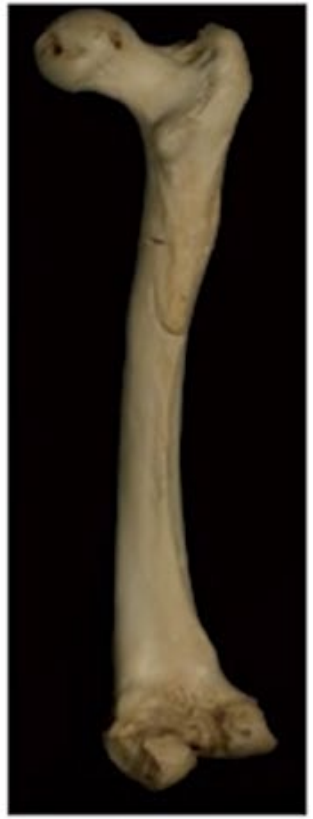
Ancient DNA sources

- Ancient DNA is analyzed from:
  - Mummies
  - Organisms preserved in amber
  - Plant materials found in ancient tombs
  - Bacteria
  - Bones
  - Pages in books
  - Dirt
  - Etc.

Sources of ancient DNA

First Neandertal DNA
(Krings et al., 1997)

First Denisovan DNA
(Krause et al., 2010)



Oldest DNA outside permafrost
First Middle Pleistocene hominin DNA
(Meyer et al., 2014)

Oldest DNA sequences
(Orlando et al., 2013)

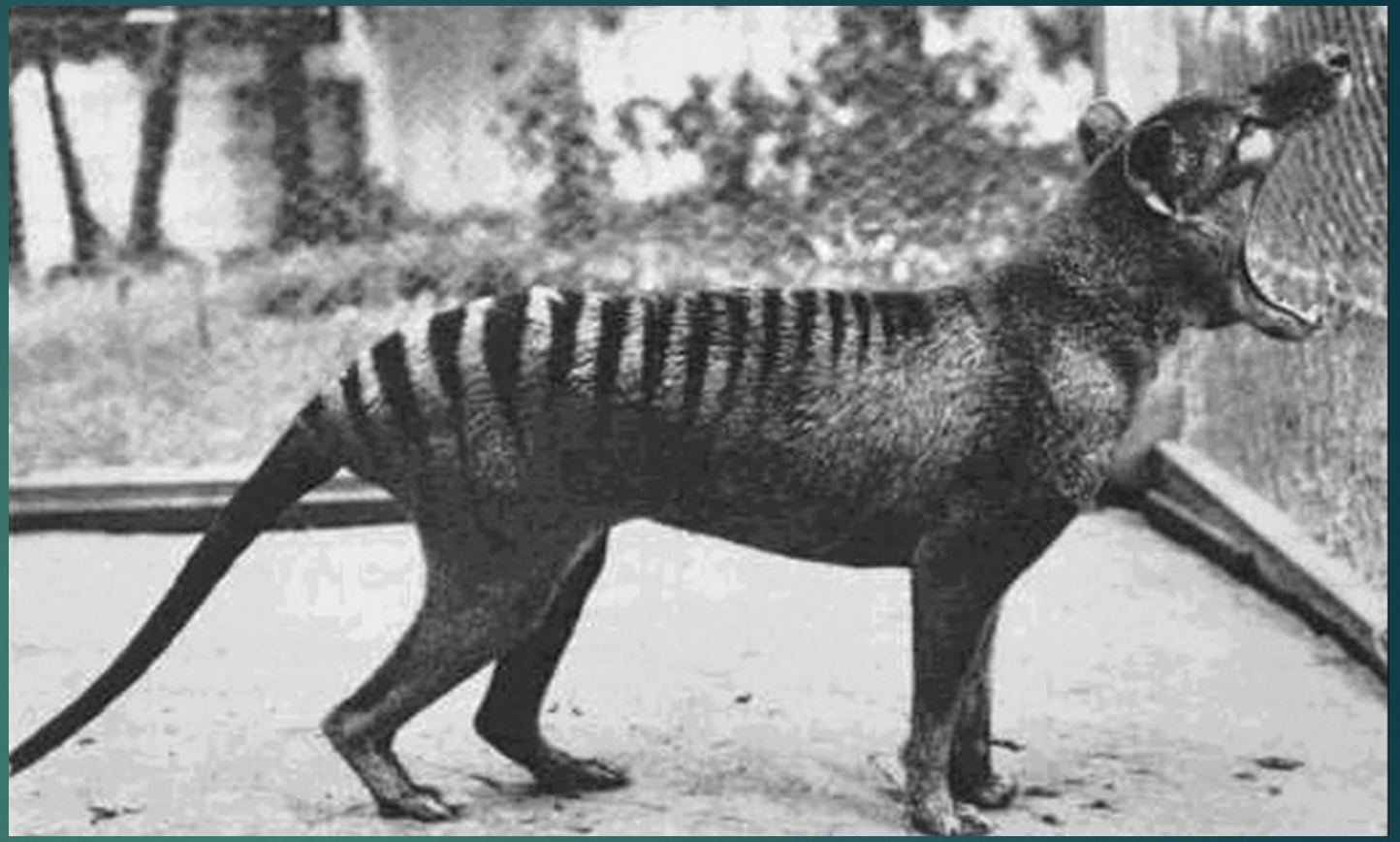
Oldest modern human DNA
(Fu et al., 2014)

2nd oldest DNA: 560–780 Ka Horse

Sima de los Huesos Neandertal, 400 Ka

Ust'-Ishim Siberian MH, 45 Ka

1989: Marsupial “Wolf”

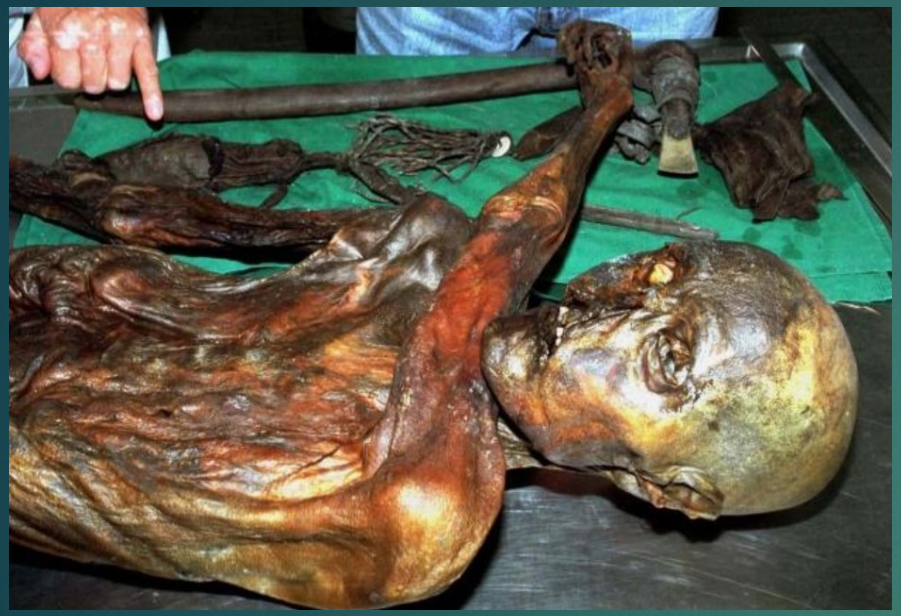


- Marsupial Wolf, Thylacine
- Largest carnivorous marsupial in the world
- Species extinct in 1936
- 219 bases of mitochondrial mtDNA from museum specimen

Thomas, RH, et al.,. Nature,1989

See YouTube: Tasmanian Tiger: colorized film from 1933

1994: Otzi, the 5000 yo Iceman



The frozen mummy of a person known as Ötzi, the Tyrolean Iceman (reconstructed here), provided some of the first evidence that ancient Europeans were genetically distinct from their modern counterparts.

1994: Handt et al., 1994
2008: oldest complete *Homo sapiens* mtDNA genome generated to date
2012: Full genome: reported by Andreas Keller (Keller et al.,2012).
2012: Hawks: Otzi had more N DNA: baseline that Europeans have an average of 3.5 percent Neandertal, Ötzi had around 5.5 percent
2014: Otzi's paternal side now live in Sardinia and Corsica (ancient farmers)

2000: Shasta Giant Ground Sloth

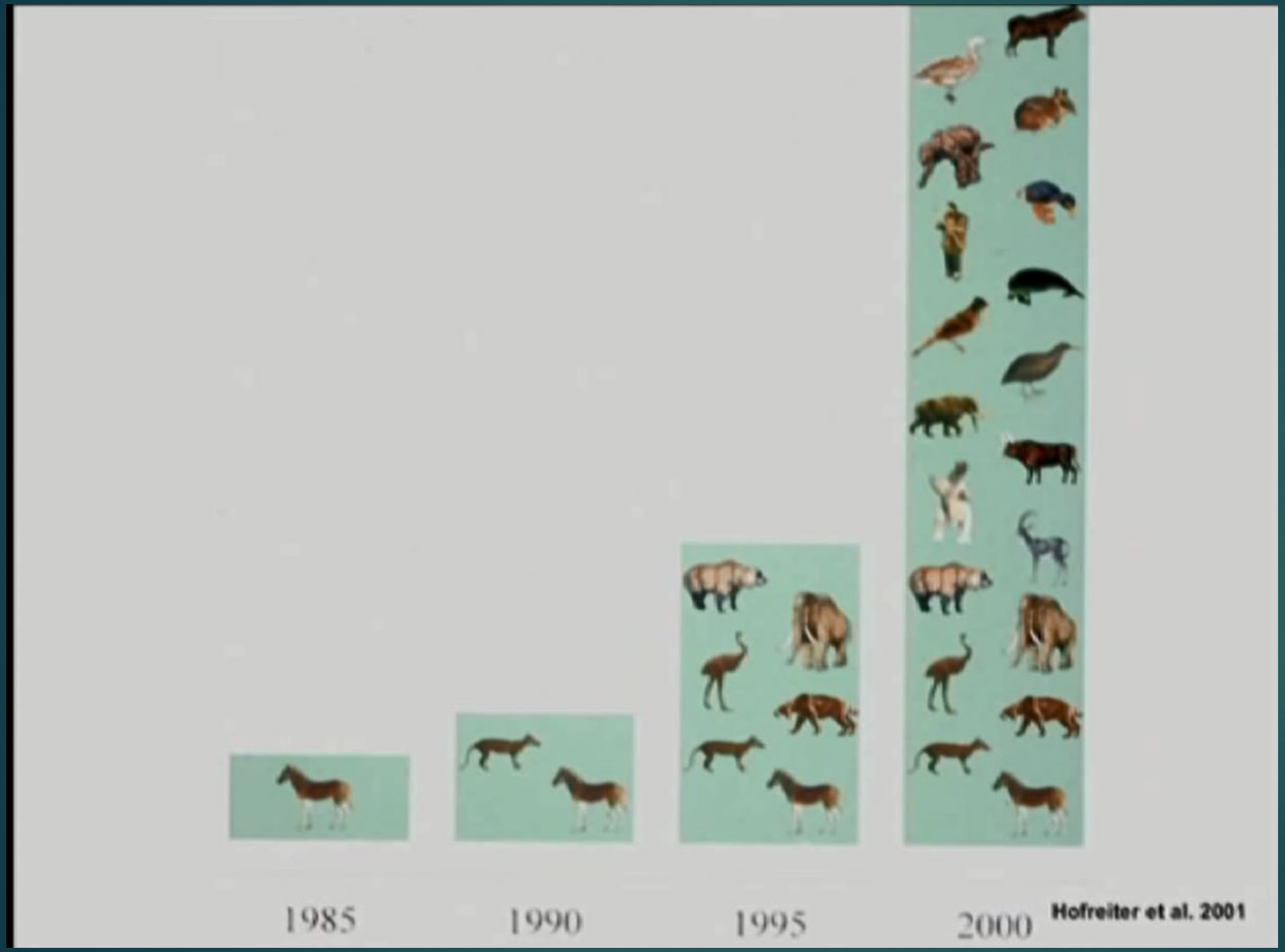
- DNA from 20,000 yo giant ground sloth coprolite
- 2008: Siberian mammoth population had been completely replaced by mammoths of North American origin

Hofreiter M, Poinar HN, Spaulding WG, Bauer K, Martin PS, et al. 2000

Mammoths, Mastodons

How to clone a mammoth by Beth Shapiro

George Church at Harvard: Colossal Project focused on de-extinction

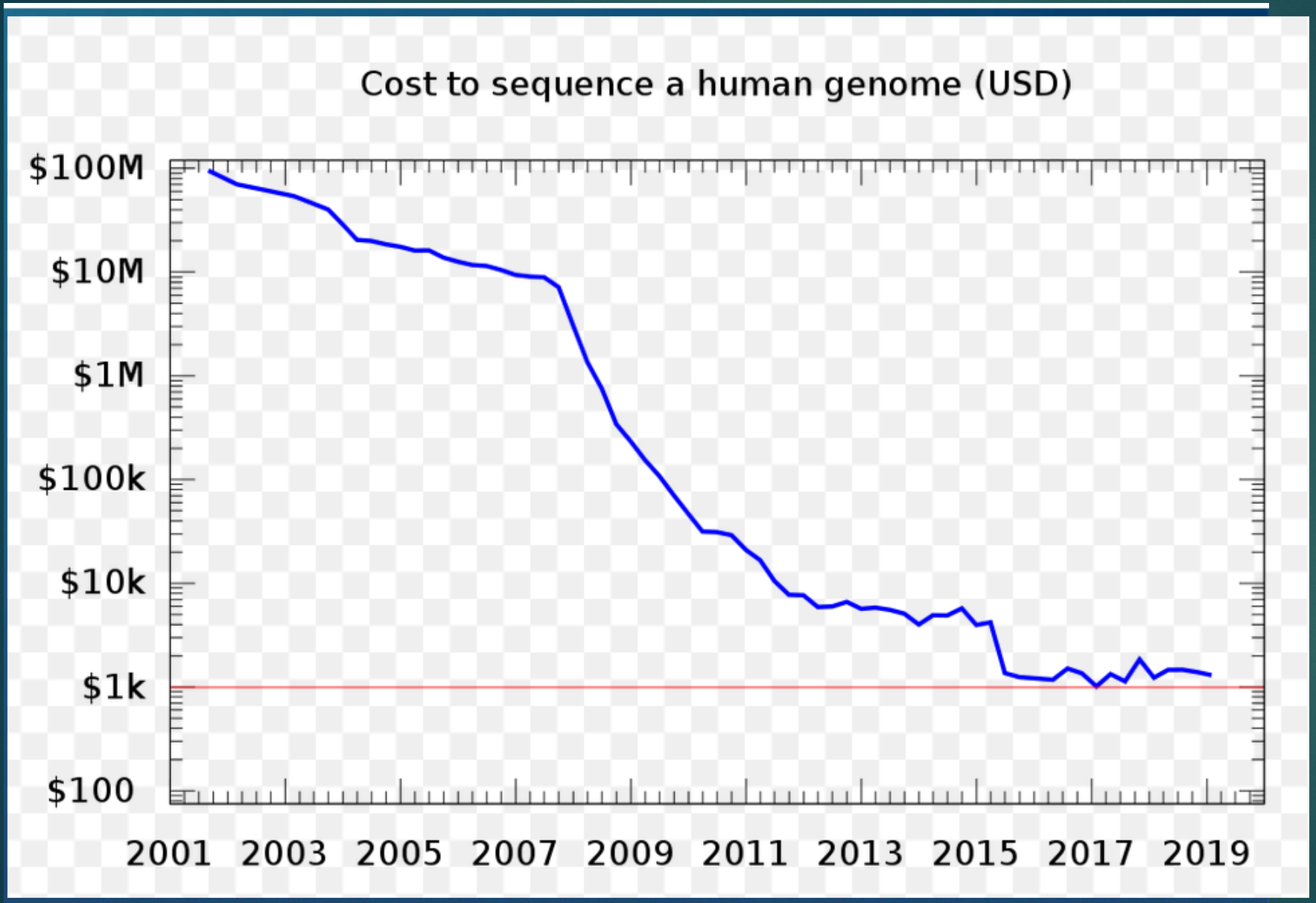


2018: 3,500 species of complex life; but only about 100 have been sequenced at “reference quality”

2018: 181 horticultural plants

2019: 1,100 plant species

2021: project to genotype all vertebrate species



1 genome = 400 GB of raw data

Amazon & Google will store your genome for $25 a year

85,000 full human genomes currently

Australian = 100,000 Genome Project

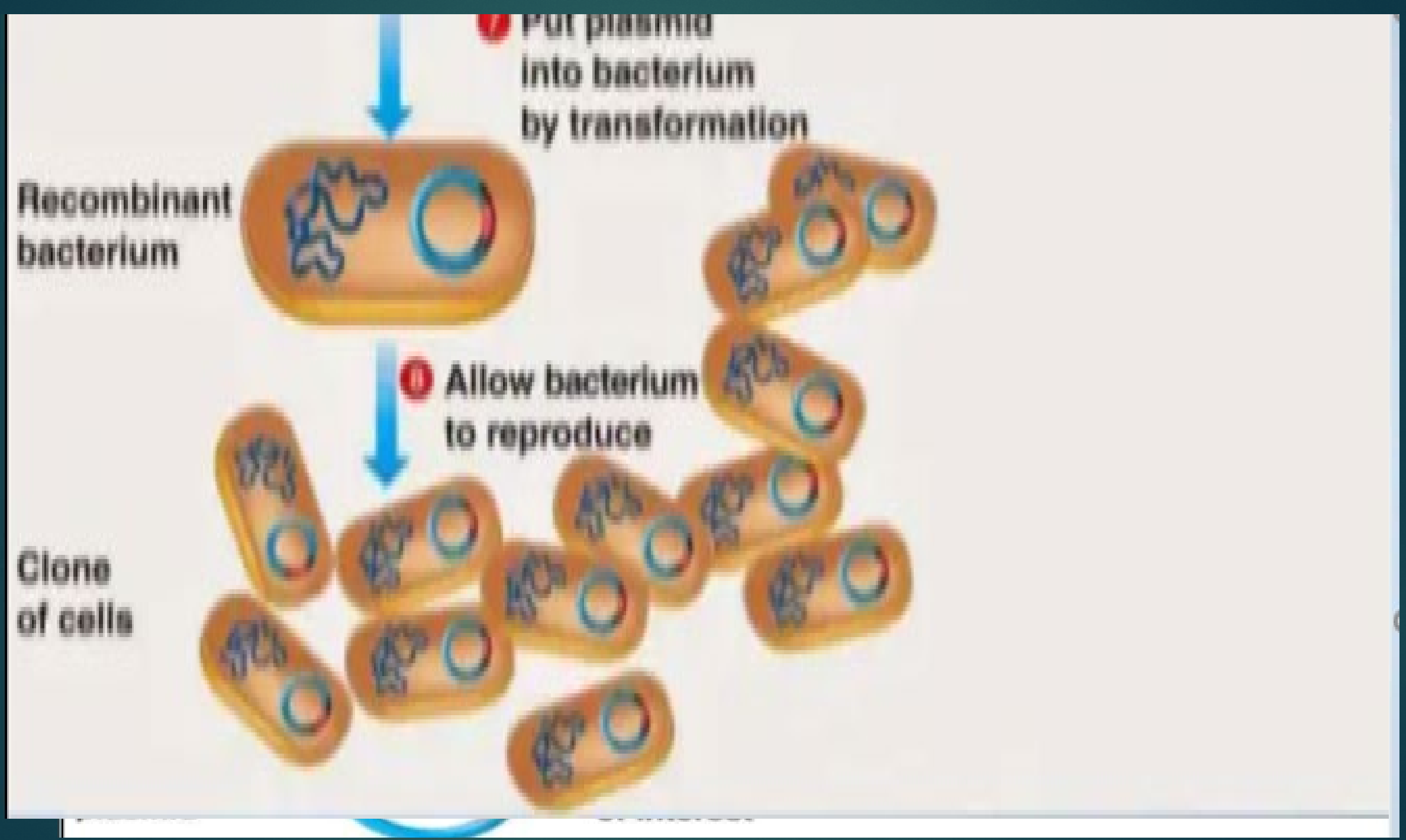
France = plan 235,000 WGS a year

GenomeAsia/100K

China = aiming for 1 Million

How is DNA cloning done

- Take DNA from any organism
- Join it to a plasmid
  - Bacterial Plasmid is an autonomously replicating circular double stranded extra-chromosomal DNA which is physically separated from chromosomal DNA and replicates independently.
- Introduce plasmid into the bacteria, where it would replica along with its host, making thousands/millions of copies of foreign DNA


Put plasmid into bacterium by transformation
Recombinant bacterium
Allow bacterium to reproduce
Clone of cells

Bacterial cloning method of DNA replication

- Then determine sequence of captured foreign DNA
- Find differences in sequences between the DNA of 2 species
- The more similar the two sequences were, more closely related they were; method for determining how closely related 2 species are
- From number of shared mutations, can infer how they evolved from common ancestor and how long ago they diverged
- PCR technique mostly replaced bacterial cloning in aDNA research. Added ability of repeat replication, which cloning did not guarantee.

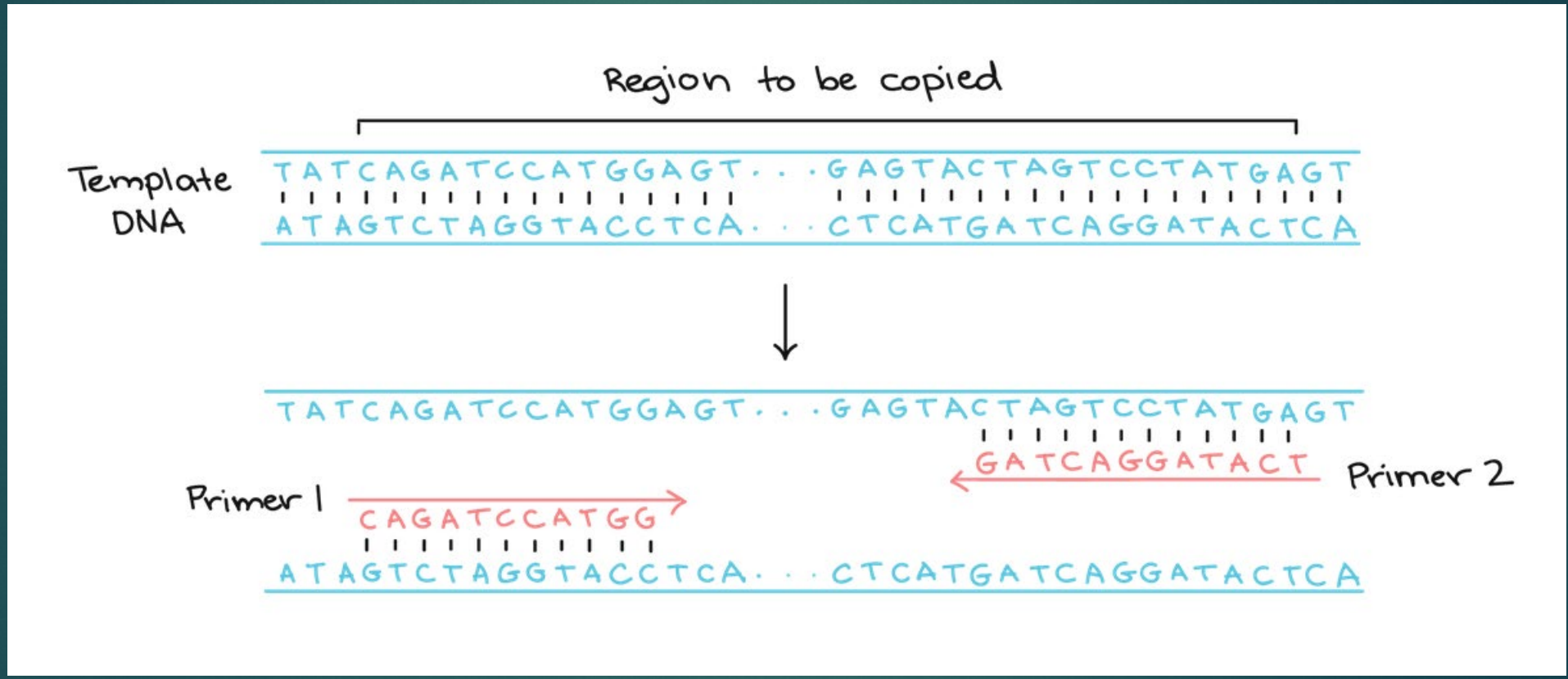
Polymerase chain reaction (PCR)

- Polymerase chain reaction is a widely used DNA manipulation technique, one with applications in almost every area of modern biology.

- PCR reactions produce many copies of a target DNA sequence starting from a piece of template DNA, present in trace amounts (e.g., in aDNA or a droplet of blood at a crime scene).

- PCR relies on a thermostable DNA polymerase, ***Taq* polymerase**, and requires DNA **primers** (a short sequence of nucleotides) designed specifically for the DNA region of interest.
- The primers bind to the template by complementary base pairing.

PCR copying


Region to be copied
Template DNA
TATCAGATCCATGGAGT. . .GAGTACTAGTCCTATGAGT
ATAGTCTAGGTACCTCA. . .CTCATGATCAGGATACTCA
TATCAGATCCATGGAGT. . .GAGTACTAGTCCTATGAGT
GATCAGGATACT
Primer 2
Primer 1
CAGATCCATGG
ATAGTCTAGGTACCTCA. . .CTCATGATCAGGATACTCA

PCR

- In PCR, the reaction is repeatedly cycled through a series of temperature changes, which allow many copies of the target region to be produced.

- Using PCR, a DNA sequence can be amplified millions or billions of times. Under perfect conditions could amplify one DNA molecule to become 1.07 billion molecules in less than two hours.

- Typically, the goal of PCR is to make enough of the target DNA region that it can be analyzed or used in some other way.

- For instance, DNA amplified by PCR may be sent for sequencing, visualized by gel electrophoresis, or cloned into a plasmid for further experiments.

- The results of a PCR reaction are usually visualized (made visible) using gel electrophoresis

Jurassic Park Hypothesis: dinosaur aDNA

Ancient DNA were awarded a special place in the public imagination by the 1993 release of Steven Spielberg's "Jurassic Park."

Would it be possible to resurrect the dinosaurs?

Michael Crichton & Jurassic Park

- Michael Crichton's 1990 book and Stephen Spielberg's 1993 movie Jurassic Park coincided with the development of PCR, and its instantaneous worldwide popularity situated aDNA research and its scientists in the spotlight.

- In the early 1990s, the hype around the search for DNA from fossils took form as two different but not unrelated expectations:
  - (1) that scientists could recover multi-million-year-old DNA and
  - (2) that they could one day use that DNA to resurrect extinct creatures such as dinosaurs.

Ancient human DNA: A history of hype (then and now), Elizabeth D Jones & Elsbeth Bosl, 2021

Million-year-old aDNA?

- Following Jurassic Park's 1990 publication, two research teams in the US set out in a race to extract and sequence multi-million-year-old DNA.

- In 1993, George Poinar et al. claimed to have recovered DNA from a 125–135-million-year-old amber-encased weevil, announcing it as the oldest DNA ever recovered (Cano et al., 1993).

- Nature published the paper on June 10, 1993 – one day after the Jurassic Park movie premiere and one day before its release in theaters across the United States

“Antediluvian” DNA

- Nicknamed “Antediluvian” DNA
- Reports of several sediment-preserved plant remains dating to the Miocene were published.[
  - 1992 – 30 Ma termite encased in Dominican amber
  - 1993 – 120 and 135 Ma weevils in Lebanese amber; 35 Ma leaf (Paul Cano, Cal Tech)
  - 1994, Woodward *et al.* reported what at the time was called the most exciting results to date[— mitochondrial cytochrome b sequences that had apparently been extracted from dinosaur bones dating to more than 80 million years ago.

“Antediluvian” DNA

- When in 1995 two further studies reported dinosaur DNA sequences extracted from a Cretaceous egg, it seemed that the field would revolutionize knowledge of the Earth's evolutionary past.

- Even these extraordinary ages were topped by the claimed retrieval of 250-million-year-old halobacterial sequences from halite.

Genetic analysis of Ancient DNA - Svante Pääbo, et al., 2004

- This article = Best analysis of very technical difficulties (and solutions) to aDNA analysis
- Ancient DNA research = retrieval of DNA sequences museum specimens, archaeological finds, fossil remains, and other unusual sources of DNA.
- aDNA analysis only really became feasible with the advent of techniques for the enzymatic amplification of specific DNA sequences.
- Today, reports of analyses of specimens hundreds, thousands, and even millions of years old are almost commonplace. But can all these results be believed?

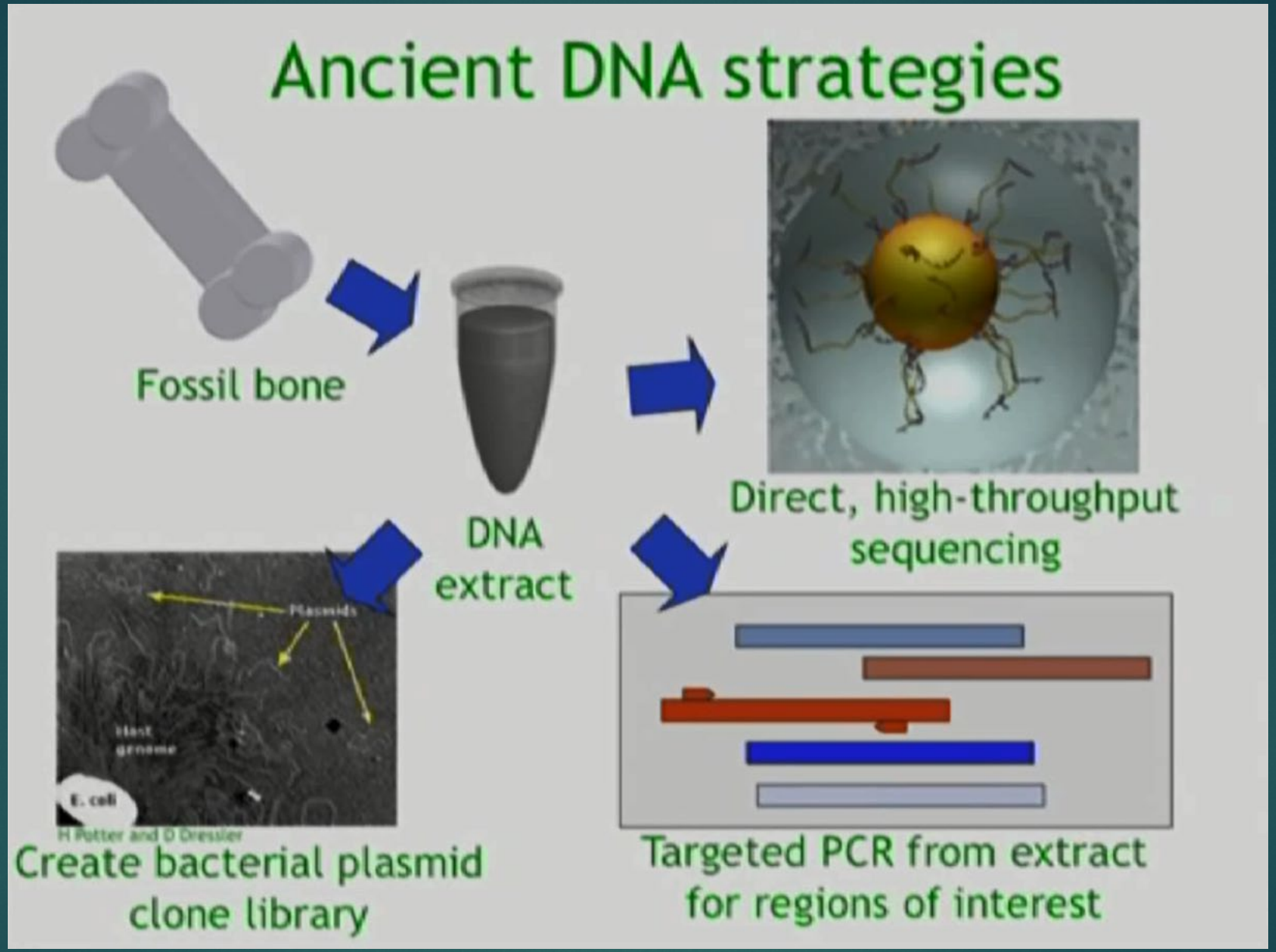
Genetic analysis of Ancient DNA

- The molecular cloning of DNA from a quagga and an Egyptian mummy were the first successes in the retrieval of ancient DNA sequences.

- But the amounts of DNA present in the old tissues were so small that the isolation of bacterial clones carrying the same DNA sequence was essentially impossible.

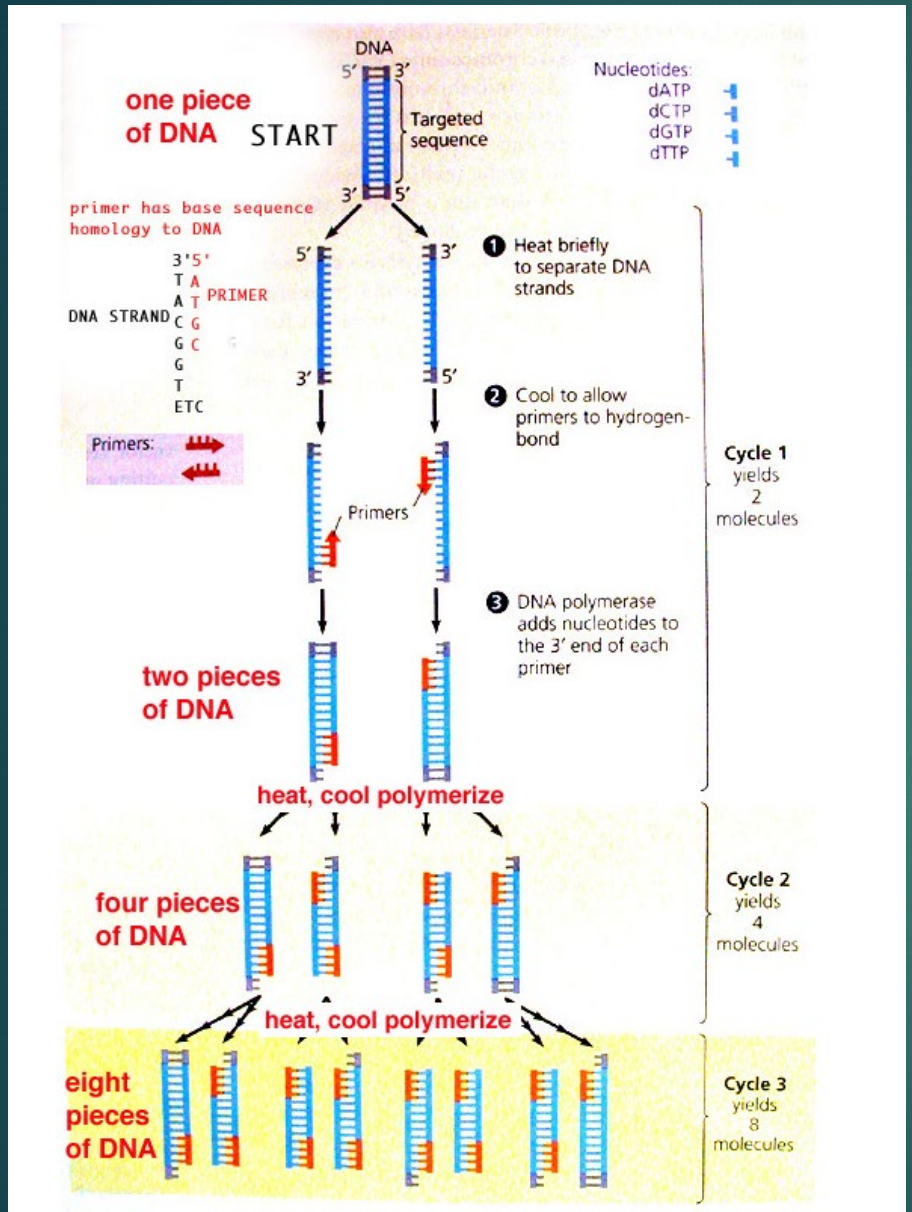
- The results could not be repeated in order to verify their authenticity.

- Thus, the litmus test of experimental science— reproducibility—was not achieved.

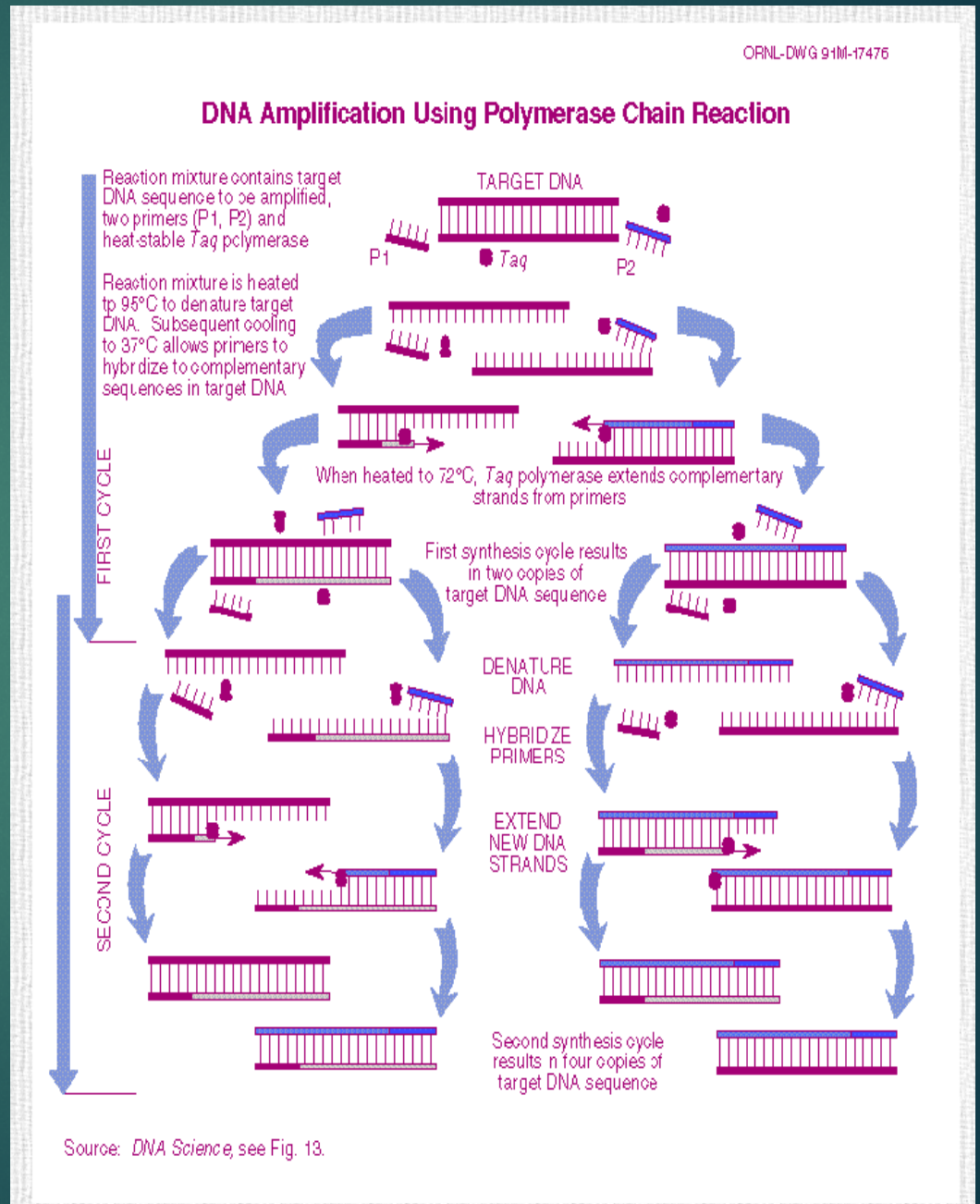


PCR method: for ancient DNA & Covid testing

The development of this PCR methodology won the Nobel Prize in 1993 for Kary Mulllis.



Every cycle, the amount of DNA doubles



Genetic analysis of Ancient DNA

- This changed with the development of the polymerase chain reaction (PCR).

- The PCR made it possible to produce essentially unlimited numbers of copies from very few or even single original DNA copies. Therefore, the same DNA sequence could be amplified multiple times from the same specimen and ancient DNA studied in a scientifically rigorous way.

- This study highlights that aDNA is very degraded:
  - Fragments are very short in length (less than 100 bps)
  - Contained many chemical modifications
  - Was hugely contaminated

Genetic analysis of Ancient DNA

- Two technical complications that remain the main challenges to the study of ancient DNA.

- The first complication, molecular damage, was evident from the fact that when PCR was used to reexamine the same quagga from which DNA had been cloned, two positions were shown to be incorrect in the original sequences.

- The second complication, DNA contamination, was evident from work showing that contemporary DNA contaminates almost all ancient remains and many laboratory environments.

- Molecular damage and DNA contamination give rise to erroneous DNA sequences used for final analysis.

Genetic analysis of Ancient DNA

- Within living cells, the integrity of DNA molecules is continually maintained by enzymatic repair processes.

- After the death of an organism, cellular processes that normally stop breakdown enzymes break down. As a consequence, the DNA is rapidly degraded.

- In addition, the DNA molecule faces an onslaught of bacteria, fungi, and insects that feed on and degrade macromolecules.

- Under rare circumstances, such as when a tissue becomes rapidly desiccated after death or the DNA becomes adsorbed to a mineral matrix, it may escape enzymatic and microbial degradation.

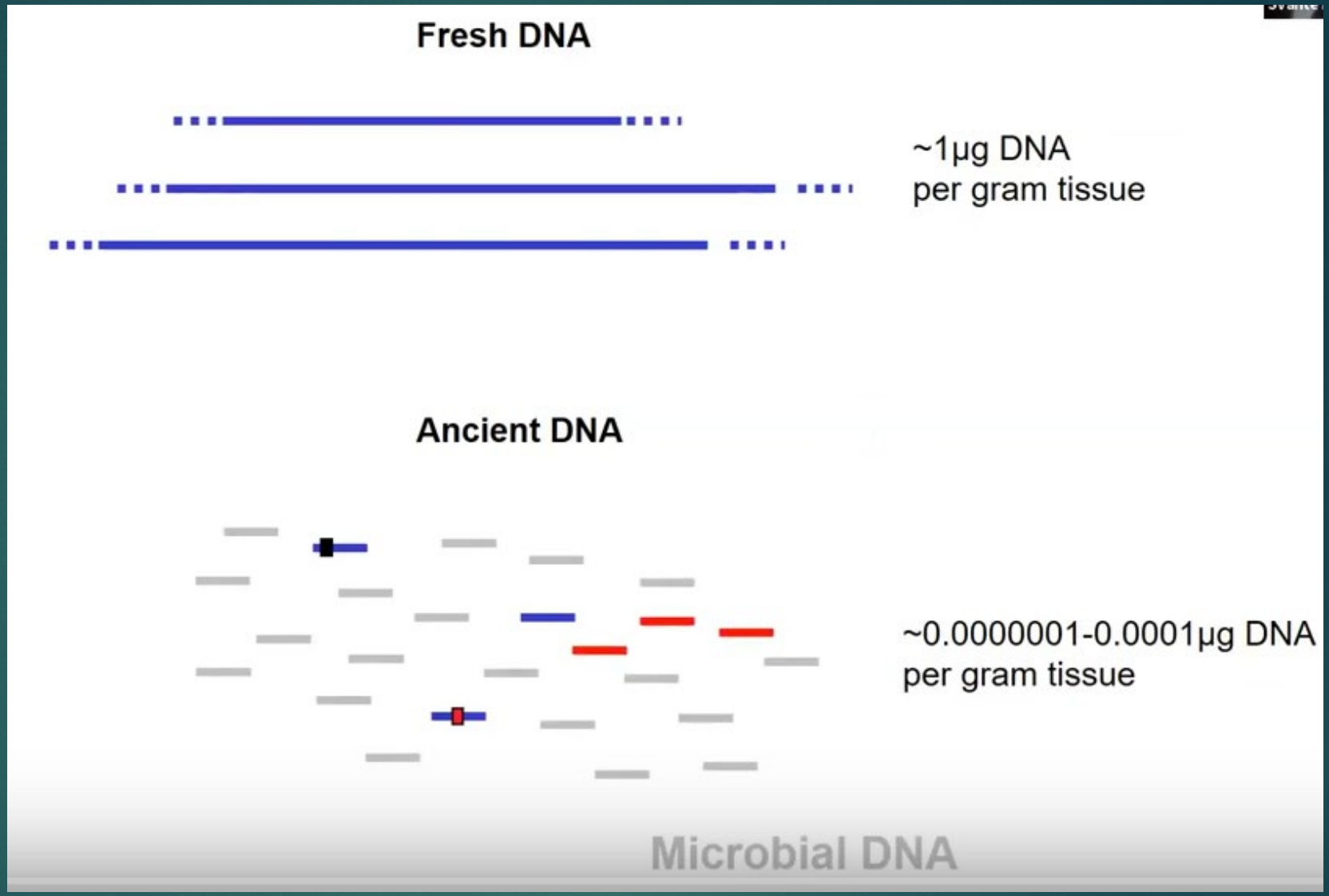
Genetic analysis of Ancient DNA

▶ On such occasions, slower but still relentless chemical processes start affecting the DNA. Many of these processes are similar or identical to those that affect the DNA in the living cell.

▶ However, after death they are not counterbalanced by cellular repair processes and thus damage accumulates progressively until the DNA loses its integrity and decomposes, with an irreversible loss of nucleotide sequence information.

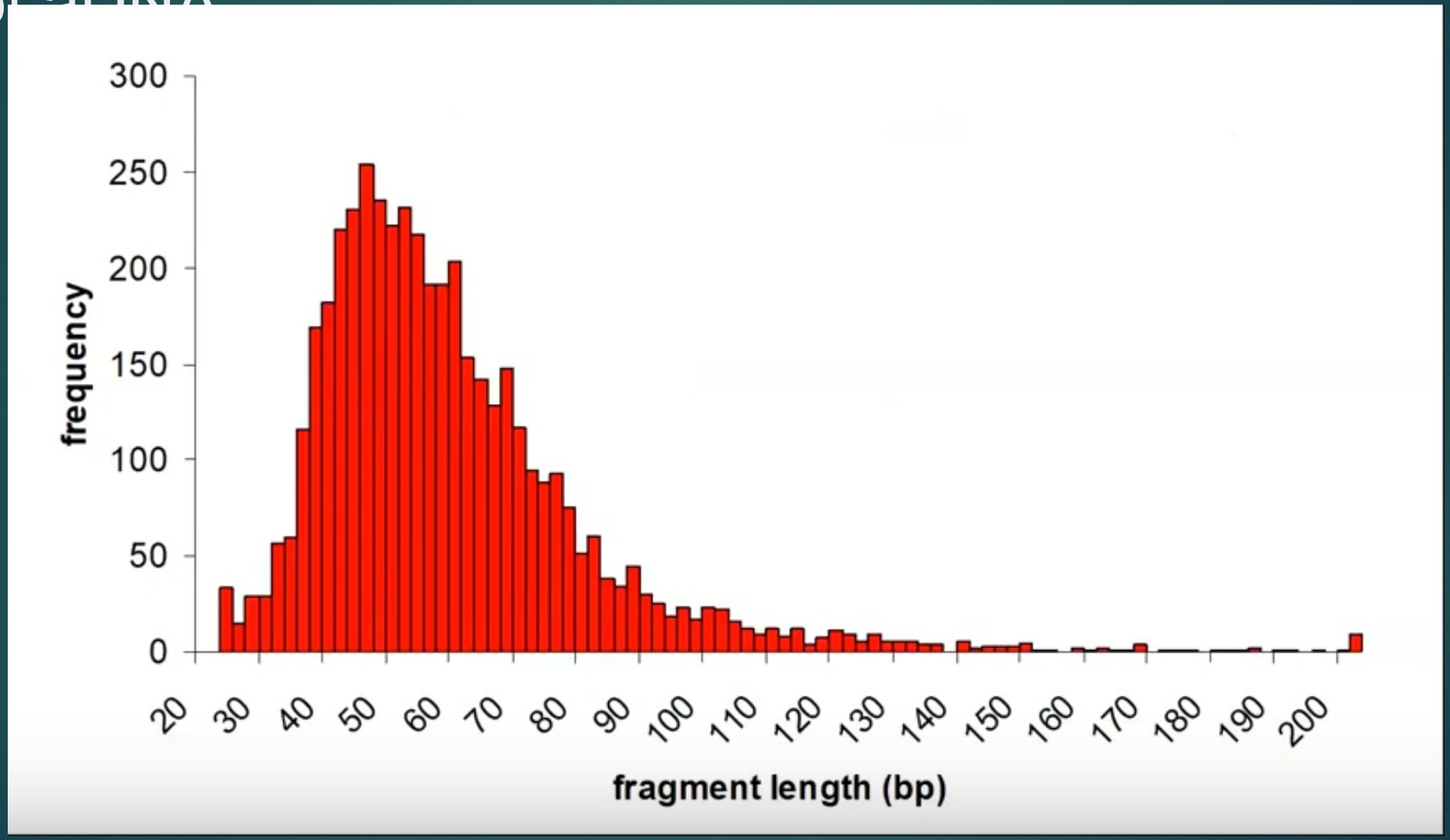
▶ What the PCR has made possible is the occasional salvage of information from some rare samples in which the disintegration of DNA is not yet complete.

aDNA



- Current DNA is in long segments; aDNA is very short, fragmented segments; 99% of aDNA is bacterial DNA and MH contamination

Very short fragments even from best aDNA: 60-70 bases in length; fragments that are much longer are not aDNA



1997: Serious test of Jurassic Park hypothesis

- 1997, Austin study: A comprehensive study to try to recover DNA from fifteen samples of insects in amber, each from different resin types and time periods.
- After performing DNA extractions and PCR amplifications, they carried out negative controls and conducted phylogenetic analyses to check for aDNA authenticity.
- Despite extensive experiments, however, they were unsuccessful and totally "failed to recover any authentic ancient insect DNA" (Austin et al., 1997: 470).

- The Jurassic Park hypothesis appeared debunked.
- Extraordinary claims should be accompanied by extraordinary evidence.

Universal Contamination

- Svante Paabo, very famously at a 1990 meeting, stood up and said, ‘Of course you can’t get DNA from bone!’ – just before Erika Hagelberg stood up and said, ‘Here’s my results on DNA from bone’”.
- Another researcher recalled a similar situation: “Svante had some very public fights with her in conferences saying it was all shit”.
- A colleague of hers: described her as being able to “get DNA out of a stone, just about“. Later famous for bone DNA analysis for forensic identification
- This 1990s disagreement came down to contamination and the difficulty of proving aDNA authenticity.

** Start here: Dec 29, 2021

Ancient DNA degradation

- Pääbo's lab: no replicable DNA from ancient amber
- His conclusion -- No dinosaur DNA: can't extract DNA from specimens that no longer have any.
- When organisms die, their DNA decomposes into minute fragments; the older the specimen, the smaller the DNA fragments.
- How long this takes depends on factors like temperature, burial conditions and the number of microbes making a meal of it.
- Eventual calculations then predict that in the optimal conditions — very cold ones — DNA could survive for around 1 million years.

Contamination

- By the end of the 1990s, aDNA researchers were concerned about contamination in a literal sense.
- This referred to unintentional and problematic exposure to modern DNA.
- Ancient DNA sequences, for example, were easily contaminated by environmental, bacterial, or recent human DNA introduced to a specimen over time or through handling in a museum collection or lab.
- This issue was heightened because of the degraded and damaged composition of aDNA, which resulted in fragmented genetic sequences. Therefore, it was difficult for researchers to determine what DNA sequences belonged to the actual specimen under study.
- This question of aDNA authenticity was a major problem for scientists.

Ancient DNA: fragmentation

- Direct quantitative comparisons of aDNA fragmentation in a large number of bone samples from different geographic regions, time periods, and environments have revealed that:
  - the number of aDNA fragments exponentially increases with the decrease of their length
  - as the random breakage of long molecules results in an accumulation of shorter ones.
- While the rate of the fragmentation depends on different environmental factors, e.g., temperature, pH, and water availability, it appears to be initially rapid, most likely due to high enzymatic activity, and followed by reduced rates over the long term.

Very Little DNA Survives Death

- Crucially, the quagga study noted what remains the most pervasive problem in the field of ancient DNA: that very little DNA survives postmortem.
- 10 K to 1 M less DNA than when fresh: .0000001 mg of DNA per gram
- Worse enemy of DNA: Release of water and oxygen which are destructive and break apart the DNA
- Dust particle (in labs, usually skin particles, full of DNA) contamination

DNA degradation

- Symbiotic and external bacteria degrade DNA postmortem
- End up with small fragments from 10 to several 100 basepairs long
- Ancient DNA is always damaged, which becomes an indicator if you are looking at right type of DNA (a way to exclude contamination which is better preserved)

Types of DNA damage

- Some damage results in changes to the DNA sequence.
  - Cytosine can change to uracil, which is read by copying enzymes as thymine, resulting in a C to T transition. This is the most common type of damage
  - Normally, in spontaneous chemical damage in functioning cells in the body, 10,000 C's per cell morph into U's each day, and are removed and correctly replaced with a T. Enzymes replace incorrect nucleotides before a bond rupture can occur.
  - Changes from G to A also occur.
  - Some of these DNA modifications are problematic because although they allow the amplification of the template molecules, they cause incorrect bases to be incorporated during the PCR.

- DNA errors are very common at the ends of molecules.

Setbacks: Contamination

- Setbacks and occasional disasters: paleogeneticists have discovered to their chagrin how easily ancient DNA samples can become degraded and contaminated with modern DNA, giving rise to erroneous and misleading conclusions.

- Contaminating DNA. DNA introduced into an experiment from the
  - preservation environment,
  - from excavation,
  - sample handling,
  - sample processing,
  - during the experiment itself;
  - DNA on dust particles