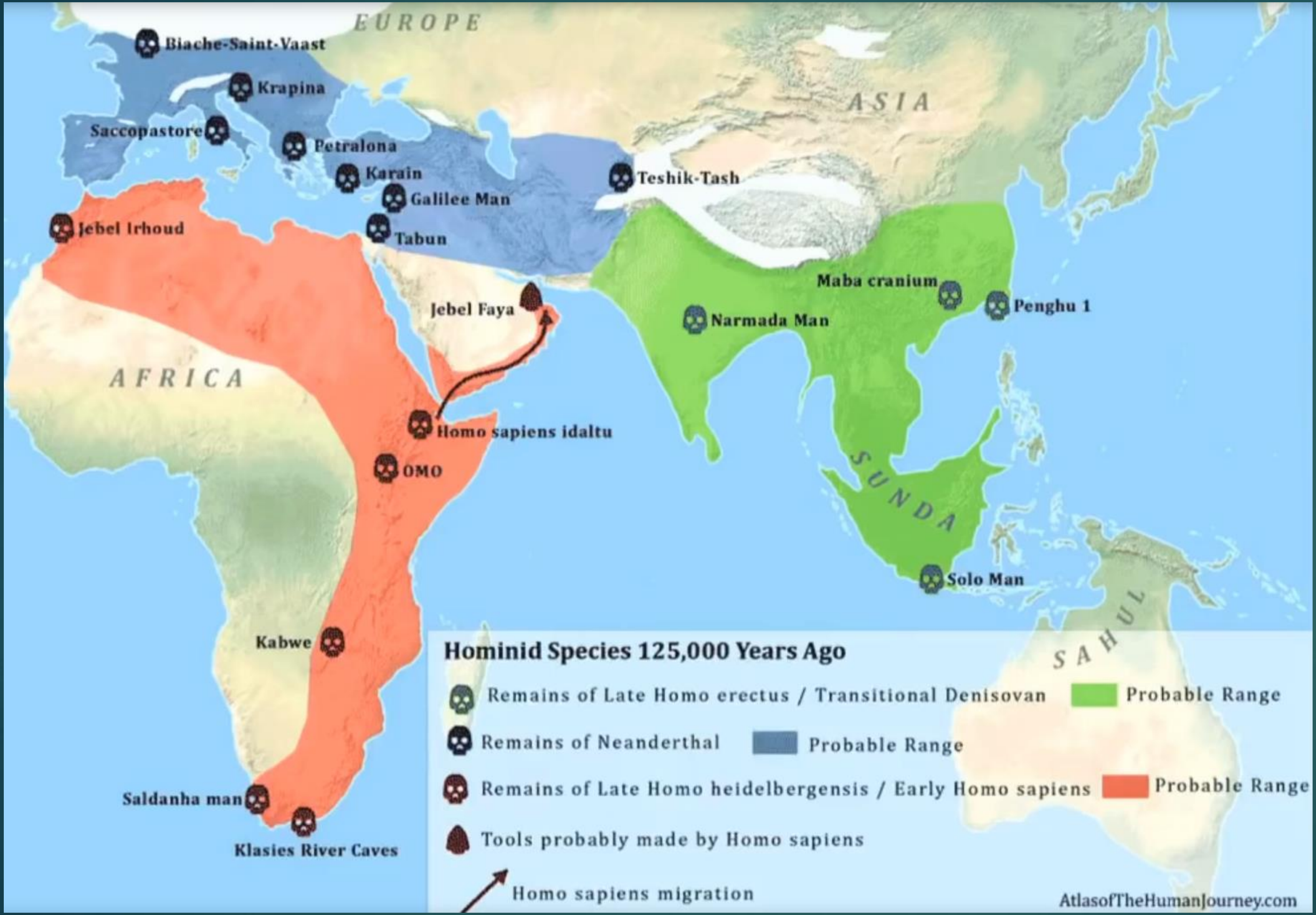
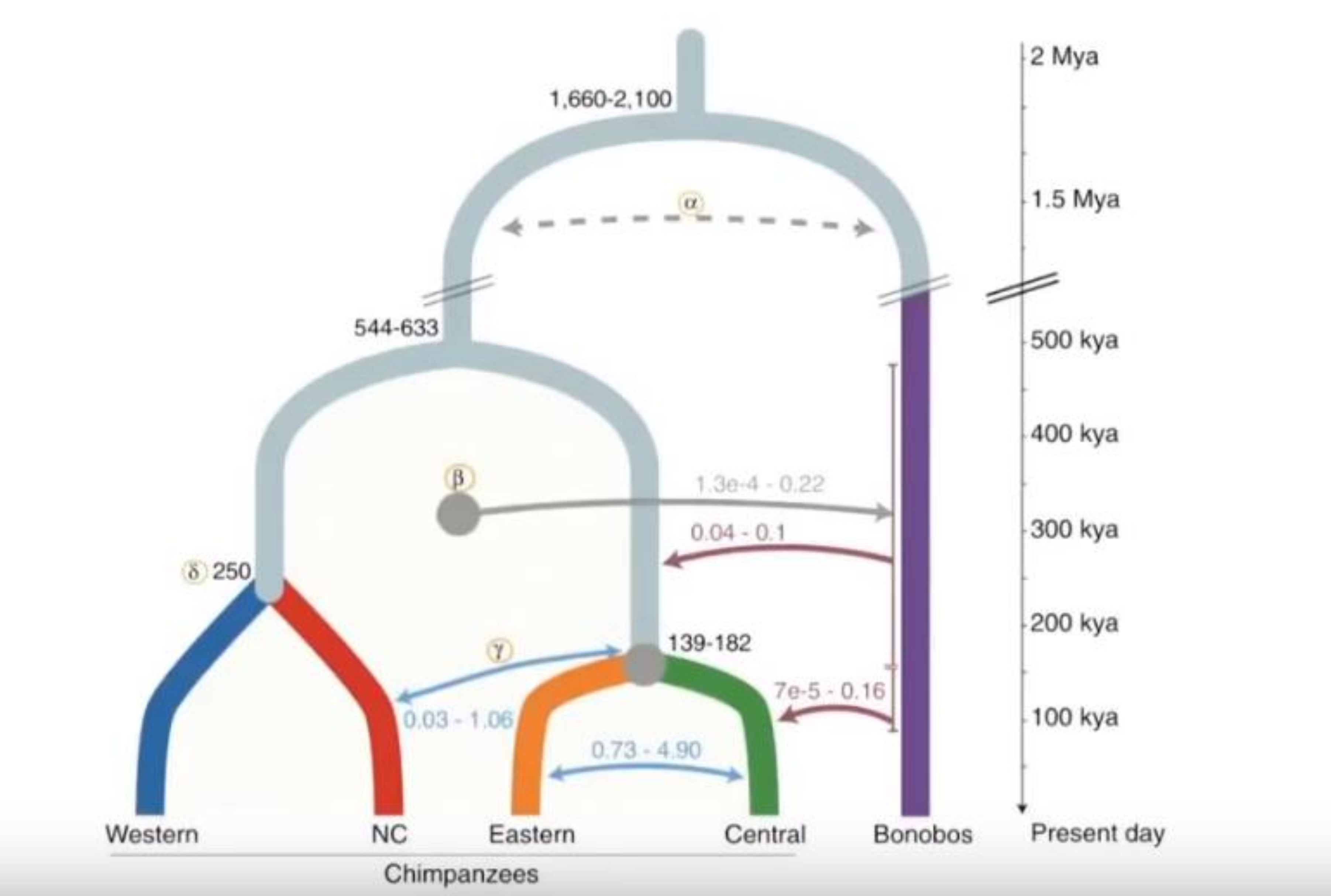
The Stranger from Siberia

The Denisovans --
(*Homo* sp. *Altai*, or
H. sapiens ssp. *Denisova, or*
H. denisova, or
H. altaiensis)

Charles J. Vella, PhD
September 2020



- Hominin World as of 2009:
- Archaic *Homo* in Africa
- Ns in Eurasia
- *H. erectus* in Far East



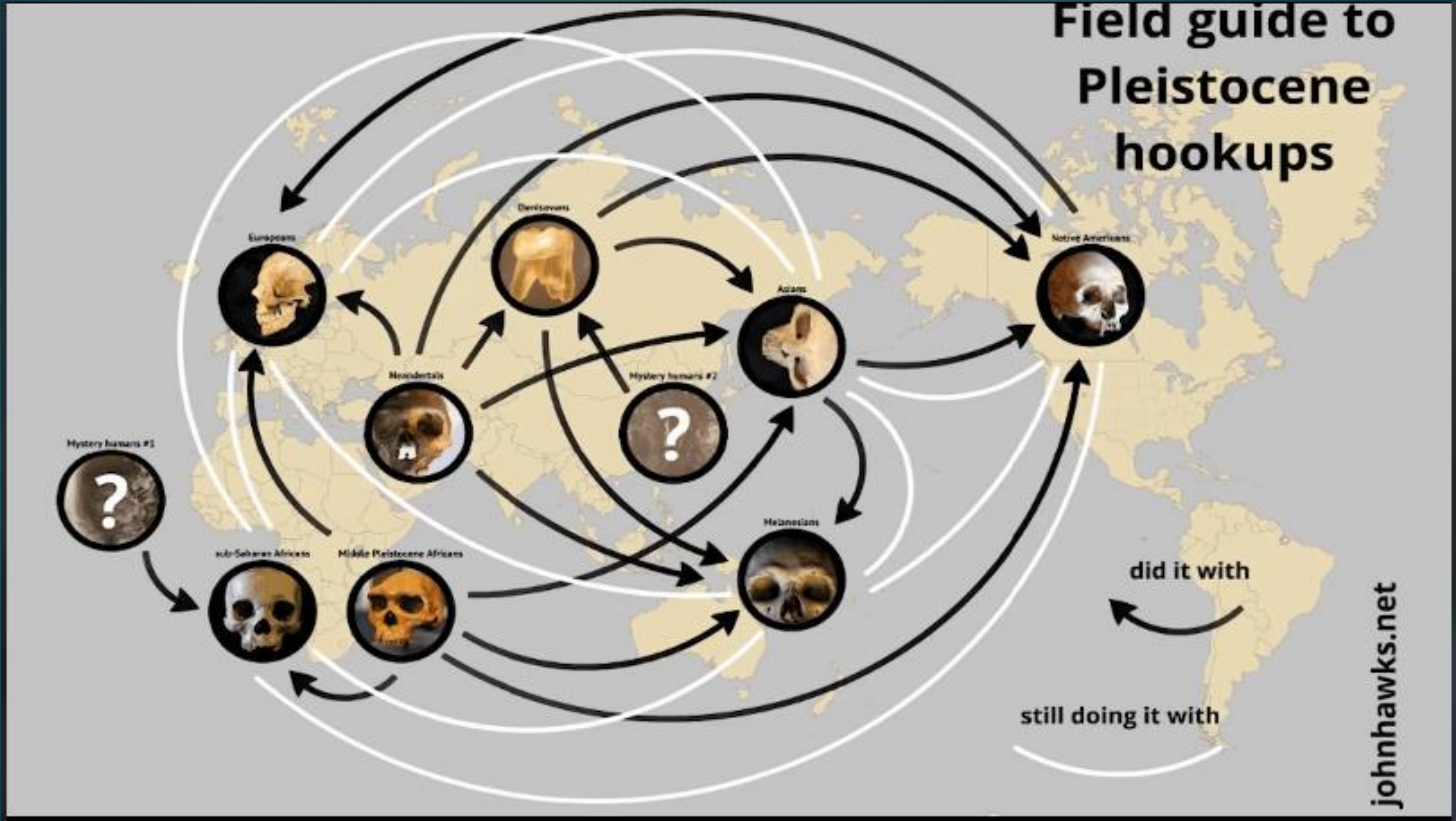
de Manuel *et al.* 2016 *Science* 10.1126/science.aag2602

Every genetic study of mammals (chimps, cave bears) gives evidence of hybridization

Bonobos and chimpanzees

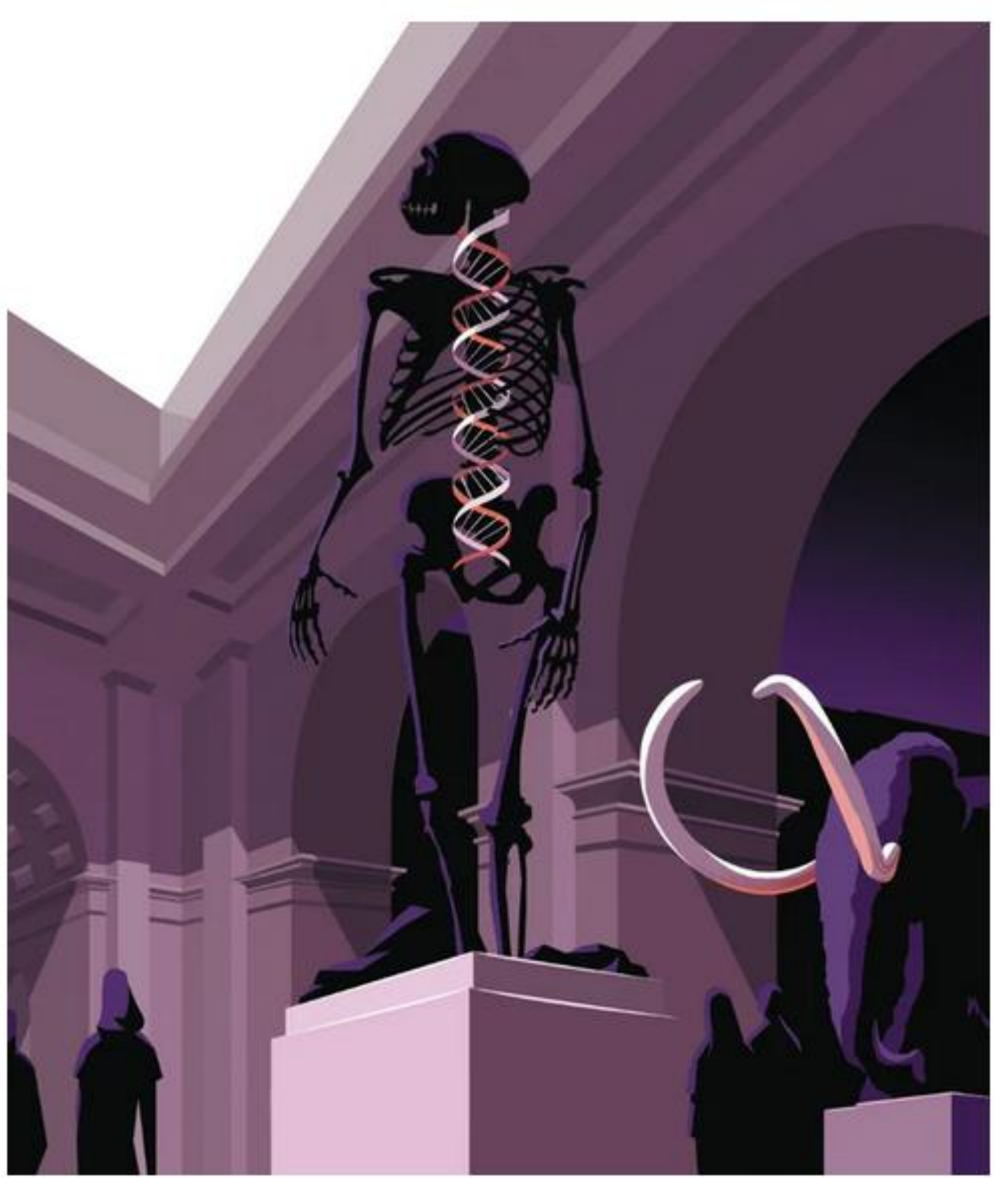
But rarely as high as 2-3%, as in Ns to MHs

Lots of historical genetic admixtures



Denisovan: A genome in search of an archaeology

The increasing significance of Molecular Genetics to Paleontology & Archeology



Human Fossil Record

- The hominin fossil record is spectacular (compare to feathered dinos)
- But it’s biased record: mostly skulls, teeth, mandibles
- Very few hominin skeletons for 1st 95% of our history
- But for Neandertals, many skeletons; i.e. Krapina, Sima de los Huesos
- Research bias: sites in Asia haven't been studied as systematically as Neanderthal sites in Europe,

1997, first molecular studies

- First DNA studies, circa 1997, were a bombshell in evolutionary studies: genetically excluded Ns from MH ancestry
- DNA of Ns was too different to be considered part of MHs
- Molecular studies indicated that date of divergence was far too early for them to be counted among our ancestors
- Early research conclusion: no interbreeding between N and MHs
- But all above based on early mitochondrial DNA not nuclear DNA; because mtDNA is far more common in fossils
- Recent: complete nuclear DNA from N, 1-2% N in modern humans

mtDNA located in mitochondria; and is inherited maternally

Mitochondrial genome

- Mitochondrial DNA or mtDNA is double stranded and circular.
- Mitochondrial DNA is not enveloped by a membrane.
- In Humans, the mitochondrial genome size is 16,569 DNA base pairs (A & T, G & C).
- Each mitochondrion contains dozens of copies of mt DNA. Several mitochondria in a cell accounts for 1000 of copies of mtDNA per cell.

mtDNA

- The mitochondrial genome contains 37 genes that encode 13 proteins, 22 tRNAs, and 2 rRNAs.
- Mitochondrion is a semi-autonomous organelle; majority of proteins required by cells are coded by nuclear DNA
- You can use mtDNA to establish a molecular clock and you can get an idea of when the last common ancestor of a species and when this population lived
- “Molecular clock”: use the mutation rate of biomolecules to deduce the time in prehistory when two or more life forms diverged

Nuclear DNA is in cell nucleus; from both parents

Nuclear genome

- Nuclear DNA is double stranded and linear
- Nuclear DNA or genome is enveloped by nuclear membrane.
- Nuclear genome is made of 3.3 billion DNA base pairs.
- Only two copies of nuclear genome per somatic cell.
- Nuclear genome has 20,000-25,000 protein encoding genes.
- Nuclear genes codes for all proteins required for cell function.
- Genes vary in size from a few hundred DNA bases to more than 2 million bases
- 2 copies of each gene: Alleles are forms of the same gene with small differences in their sequence of DNA bases

Gene deserts

- **Gene deserts** are regions of the genome that are devoid of protein-coding genes.

- Gene deserts constitute an estimated 25% of the entire genome.

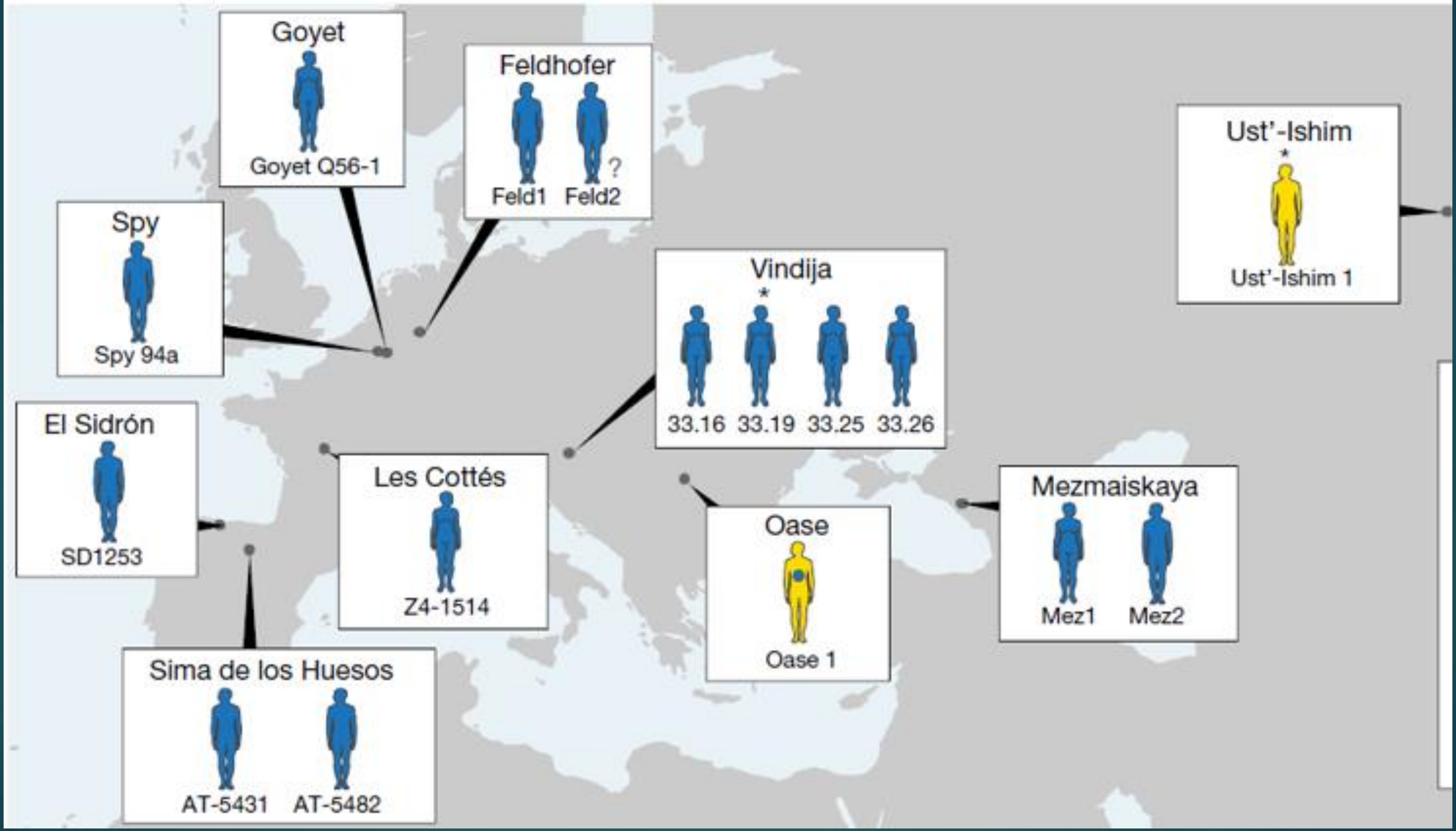
- Originally believed to contain inessential and “junk” DNA due to their inability to create proteins, gene deserts have since been linked to several vital regulatory functions

- Gene deserts contain DNA sequences that can switch genes on and off over considerable distances along the DNA molecule. Stable gene deserts represent ancient treasure boxes of multiple gene regulatory elements. Variable sections of gene deserts (65%) may be non-essential.

Entire scientific literature on the Denisovans

- 1997 to 2008: 3 Neandertal genome studies
- No books or textbook chapters on the Denisovans; closest is D. Reich, 2018
- 2010-2020: 53 journal articles about Denisovans + dozen science press descriptions (see bibliography); also large unavailable archeological literature on Denisova Cave in untranslated Russian journals
- The 53 articles are the basis of this lecture

2020: Ancient Hominin DNA: N DNA from 14 Ns and 2 MHs



Denisova Cave: early research years

- Soviet archaeologists began to excavate the cave in the 1970s and early 1980s, discovering tens of thousands of stone tools and bone fragments of animals, many gnawed and digested by hyenas or other carnivores that had lived in the cave.

- The average annual temperature of the cave remains at 0 °C (32 °F), which has contributed to the preservation of archaic DNA among the remains discovered

Denisovans

- Denisovans are members of a hominin group
    - who are currently only known directly from 8 very small fragmentary skeletal fossils,
    - genomes of which have been studied primarily
    - from a single site, Denisova Cave, in Altai mountains of Siberia.

- They are also known indirectly from their genetic legacy through gene flow into several modern populations

- Discovery of Denisovan DNA is the first case in the history of physical anthropology when a new taxon was described by the results of genetic analysis, rather than by a morphological study of fossil bones.

- The lack of morphologically informative Denisovan fossils hinders our ability to research their geographic and temporal dispersion and to understand their relation to recent Asian populations.

Neandertal DNA sequences and the origin of modern humans - M. Krings, et al., 1997

- 1997: DNA was extracted from the Neandertal-type specimen found in 1856 in western Germany.
- A hitherto unknown mitochondrial (mt) DNA sequence was determined.
- The Neandertal mtDNA falls outside the variation of modern humans.

A draft sequence of the Neandertal genome – R. Green, et al. 2010

- A draft nuclear sequence of the Neandertal genome composed of more than 4 billion nucleotides from three individuals.

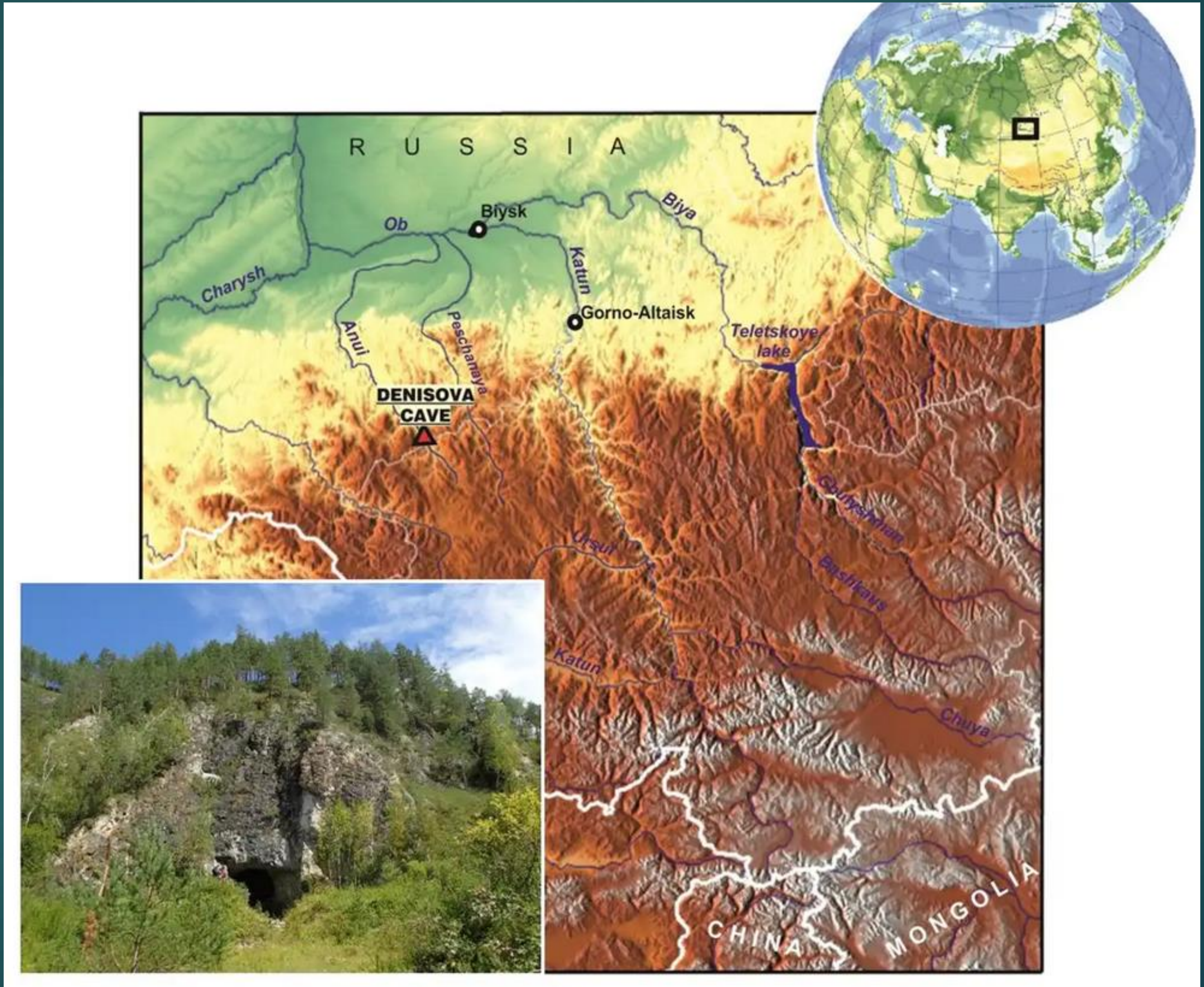
- Identified a number of genomic regions that may have been affected by positive selection in ancestral modern humans, including genes involved in metabolism and in cognitive and skeletal development.

- We show that Neandertals shared more genetic variants with present-day humans in Eurasia than with present-day humans in sub-Saharan Africa

- MHs have 1 to 4% N DNA

Denisova Cave, Siberia: (51°40’ N; 84° 68’ E) in the Altai Mountains

11-hour drive southeast from Novosibirsk, Russia



Locations of Denisova Cave in the Altai Mountains of Siberia and Baishiya Karst Cave on the Tibetan Plateau

View of the Altai Mountains from just above Denisova Cave

The Denisova Cave is located in the Altai Mountains of Central Siberia, not far from where borders of Russia, Mongolia, and China meet.

Altai Mountains foothills in area of Denisova Cave

The foothills of the Altai Mountains in the area of Denisova Cave.

Nearby Karmal cave: 800 Ka stone tools

Denisova Cave basecamp

D Cave

Denisova Cave

Located 2,198 feet (670 meters) above sea level

Denisova Cave

The cave, formed from Silurian sandstone, is ~28 meters above the right bank of the Anui River near its headwaters.

Denisova Cave, Siberia: Temperature ideal for DNA preservation

- 0-5 degrees Celsius (32 °F - 41 ° F) year round

Entrance: 8 meters wide, 4 meters high

3 entrances

Denisova Cave

Central chamber; chimney at top

Floor area of 270 meters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КАНУР

Local village young people: summer money job

Michael Shunkov, Katerina Douka, Tom Higham, Maxim Kozlinkin

Holocene layer at top: highly laminated, with goat droppings layer

Lower layers: brown, cave loam deposits; 200 to 20 Ka

Sediment profiles (stratigraphy) in Denisova Cave: a, Main Chamber; b, East Chamber. The string lines in each photo ar
50 cm apart. Institute of Archaeology and Ethnography of the Siberian Branch of the Russian Academy of Sciences, Aut
provided

East gallery: 2.5 x 6 m long; 50 to 15 Ka

Denisovan excavation

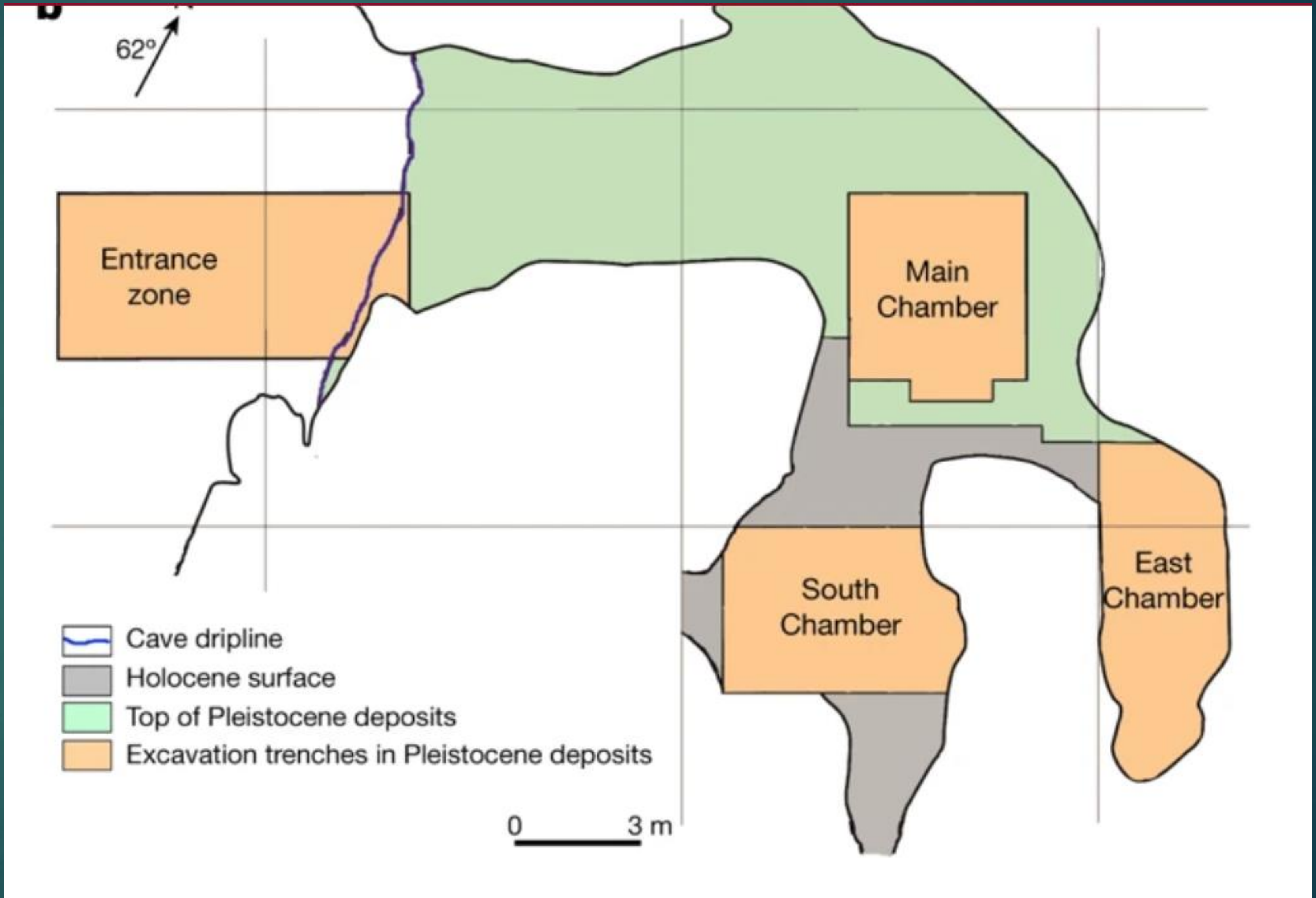
Fossils are mere scraps,
Many of them leftovers from hyena meals

Denisovan bone fragments

Most of caves in Altai region were not habituated by hominins, but by hyenas

Denisova Cave Chambers

- It consists of several short galleries extending out from a central chamber, with a total cave area of some 270 sq. m.
- The central chamber measures 9 x 11 meters, with a high arched ceiling & ceiling hole.



Denisova Cave

- Denisovan remains are known only from the type locality of Denisova Cave, which is located in the foothills of the Altai Mountains in southern Siberia (51° 23′ 51.3″ N, 84° 40′ 34.3″ E).
- Neanderthal remains have also been recovered from the deposits of this cave.
- The cave consists chiefly of three large chambers—named Main Chamber, East Chamber and South Chamber —each containing meters of deposit. South chamber has been barely touched.
- The stratified sequences are numbered by layer; layers with the same number in the different chambers are not necessarily equivalent in age.

Denisova Cave

- Denisova Cave is formed in Silurian limestone and covers a total area of about 270 m^2.
- Main Chamber is about 10 m high; 6 m of deposits, first excavated 40 years ago.
- East Chamber and South Chamber are less than 3 m wide, off of the Main Chamber; deposit depths of 7 and 4.5 m, respectively.
- The upper 1.5–2 m of deposit in each chamber (layers 0–8) are Holocene (last 11,700 y) in age.
- Holocene: Goat and sheep herders kept their animals in the cave for centuries and burned out the poop annually. Below those layers of goat and sheep poop are older Pleistocene layers made of cave loam. Those layers contain fossils like stone tools, beads and other ornaments, bones with cut marks and other evidence of life in the cave.

Denisova Cave: Two teeth in 1988 – N or bison?

- Excavations have yielded Middle Palaeolithic stone artefacts and a variety of Upper Palaeolithic artefacts, as well as the remains of fauna and flora.

- Two teeth were first described in 1988 by a dental anthropologist named Christy Turner. He proposed that these two teeth are Neanderthals. This was interesting because at the time nobody had a clue that Neanderthals lived this far east.

- When Bence Viola was working on his Ph.D. dissertation, he examined these teeth from Denisova Cave and came to a different conclusion than Christy Turner did. Viola found that the tooth that didn’t seem human or Neanderthal actually turned out to be a worn-down bison tooth - and that’s what it said in his thesis.

First came the discovery of Tools and Pendants at Denisova Cave: Châtelperronian debate all over again?

Denisovan tools unearthed

- Excavations at the Denisova cave in Siberia have uncovered almost 80,000 stone artefacts that extinct humans left over a 300,000-year period.
  - 4300 specimens have been found in the cave entrance,
  - 14,000 in the cave’s main chamber, and
  - 60,000 in the east chamber.

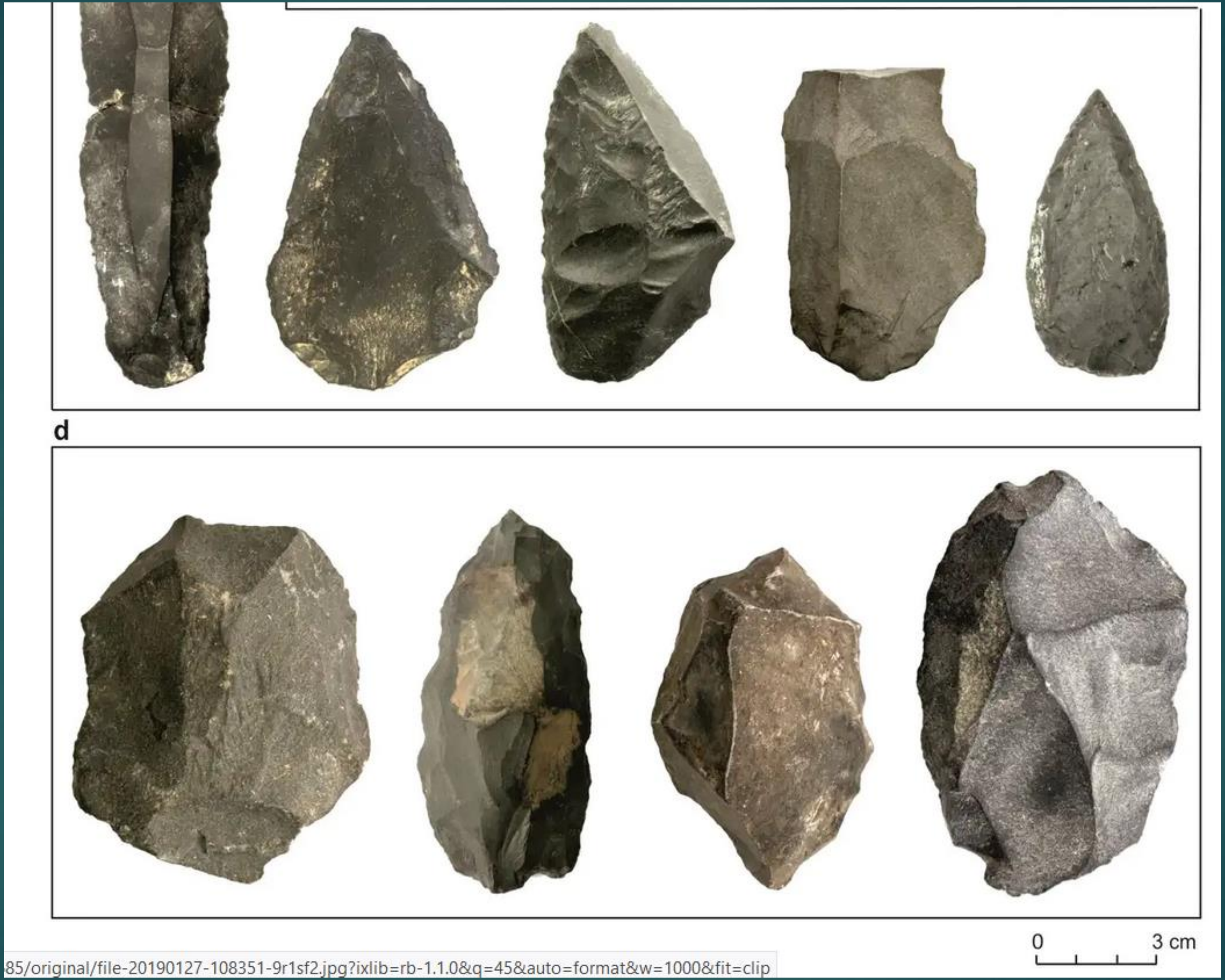
- Culminating with the production of spectacular bracelets, beads and tiaras about 50 Ka ago.

Denisovan tools unearthed

- The oldest specimens are in dirt layers more than 200 Ka old. The artefacts show that the cave’s inhabitants used the so-called “Levallois technique” to make tools

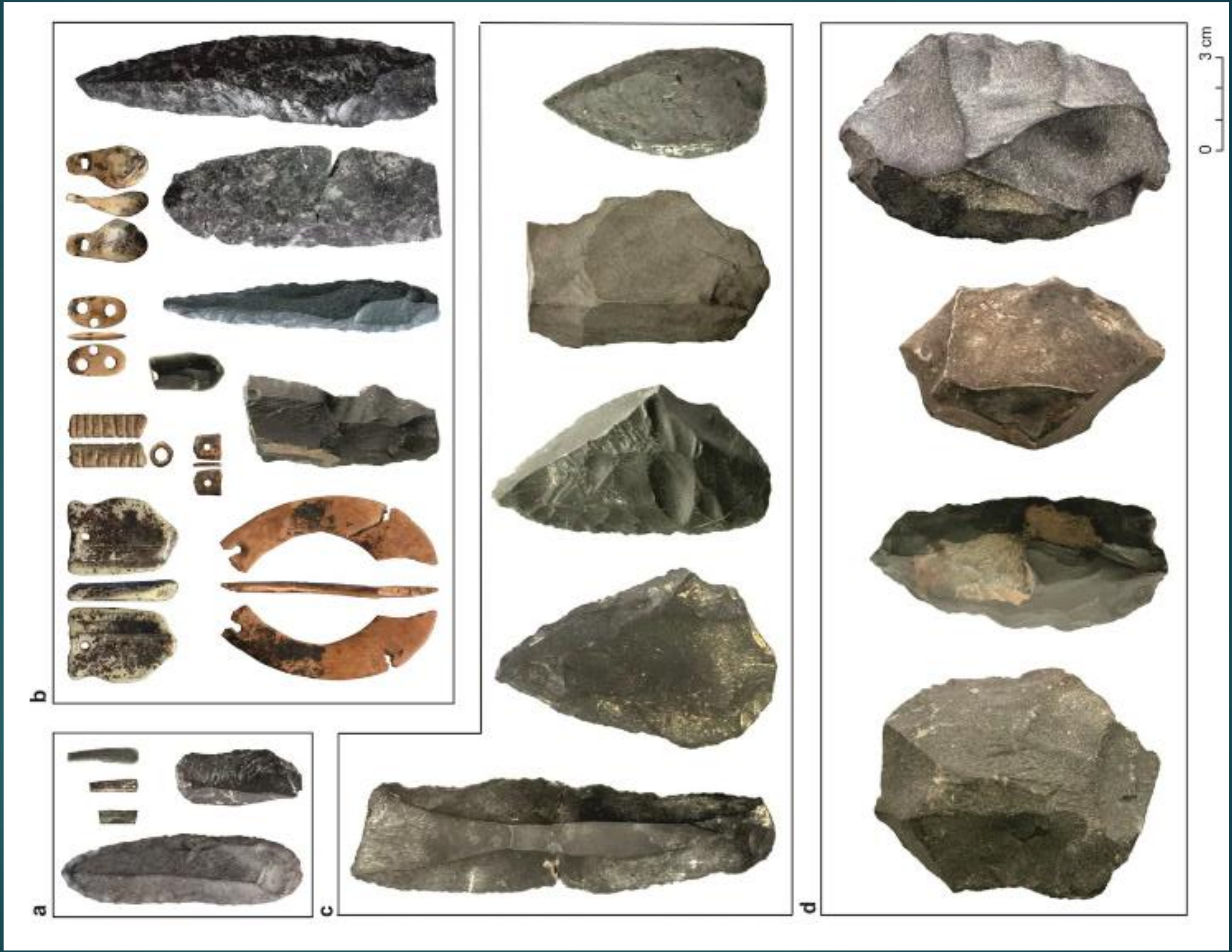
- By about 150 Ka ago, the people using Denisova cave were shifting away from producing older flakes.

- They began using the Levallois technique to produce narrow, parallel-sided stone blades, some about 3 centimeters wide and 10 centimeters long.



Mostly silex stone tools; silex is high silica stone that fractures well

Selection of artefacts from Denisova Cave (Siberian Branch of the Russian Academy of Sciences)



Artifacts

- C. Early Upper Paleolithic artifacts date to
  - 44 Ka in the Main Chamber,
  - 63 Ka in the East Chamber, and
  - 47 Ka in the South Chamber, though some layers of the East Chamber seem to have been disturbed.
- There was blade production and Levallois production, but scrapers were again predominant.

- Around 36 Ka in Main chamber: A well-developed, Upper Paleolithic stone bladelet technology

UP Bone Tools at Denisova

- In the Upper Paleolithic layers, there were also several bone tools and ornaments:
  - a marble ring,
  - an ivory ring and ivory pendant,
  - a red deer tooth pendant,
  - an elk tooth pendant,
  - a chlorite bracelet,
  - a bone needle.

Who produced the UP ornaments of Denisova Cave?

- However, Denisovans are only confirmed to have inhabited the cave until 55 Ka.

- Dating of Upper Paleolithic artifacts overlaps with modern human migration into Siberia

- But there are no MH occurrences in the Altai region

- The DNA of the only specimen in the cave dating to the time interval (Denisova 14: c. 46 Ka) is too degraded to confirm a species identity, so the attribution of these artifacts is unclear.

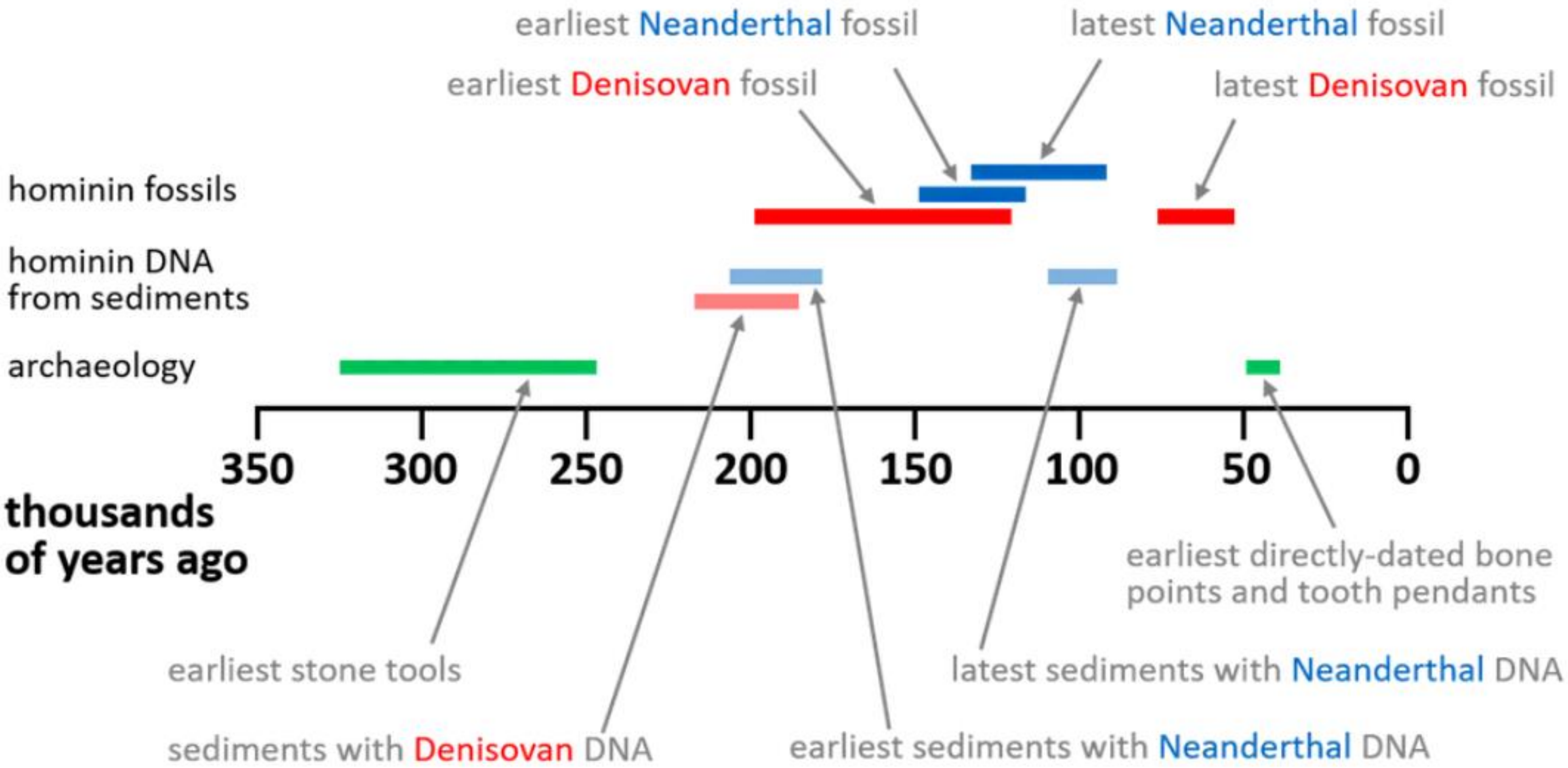
UP artifacts at Denisova Cave

Pendants

- Bence Viola of Univ. of Vienna: Neanderthals were present at Denisova sporadically between about 190 and 100 Ka ago.

- He doubts that the Denisovans really made the jewellery 50 Ka ago, particularly since a 45 Ka old bone (Ust'-Ishim) from a site 1500 kilometers to the north-west shows that MHs may have been present in the area.

- But 1500 kilometers is a large distance: there is no evidence – yet – that MHs were any nearer to the Denisova cave so early in prehistory. There is no evidence of MHs at Denisova during the time period when the jewellery was made,



Summary timeline for the archaeology, hominin fossils and hominin DNA retrieved from the sediments at Denisova Cave.

(Bert Roberts)

None of D bone fragments has been directly dated. Dating comes from dating of sediment layers

Layers of Denisova Cave: 0 (latest) to 22 (oldest

- Rich paleontological findings (at the least 66 species of mammals and 50 of birds found there) and its archaeological record, which is composed of the following layers:

- Oldest Levels 21 and 22 (194-123 Ka) contain Levallois artifacts, highlighting scrapers and denticulate, reflecting the initial stages of the middle Paleolithic.

- Levels 12 to 20 (118-79 Ka) very different characteristic types of lithic industry of middle Paleolithic

Layers of D Cave: 0 (latest) to 22 (oldest

- Level 11: Initial Upper Paleolithic. Contains useful artifacts and ornaments made of ivory and bone. Age usually estimated as between 40-45 Ka.
- Its date is difficult and is bounded between 50 Ka (edge of the lower level 12) and 30 Ka (edge close to the upper level 10).
- Dating problem: Major discontinuity separates the two UP levels 11 and 9

- Level 9: Upper Paleolithic knives and tools composed with microlites.

- Finally, Levels 0 to 8 (after 12 Ka) cover different stages of the Holocene, from the age of metals to the Middle Ages.

Art objects in UP layers of D Cave

- Several remarkable art objects at Level 11 have been
  - recovered within the Altai Mousterian layers of the cave,
  - including decorative objects of bone, mammoth tusk, animal teeth, fossilized ostrich eggshell and mollusk shell.
- Two fragments of a stone bracelet made of drilled, worked and polished dark green chlorite was discovered in these UP levels at Denisova.
- A set of bone tools including small needles with drilled eyes, awls and pendants, and a collection of cylindrical bone beads has also been found in the Upper Paleolithic deposits.
- Denisova contains the earliest evidence of eyed needle manufacture in Siberia.

UP objects at D Cave

- Impressive collection of objects at level 11
- **1. Lion figurine:**
  - 42x8x11 mm
  - Possibly made of ivory brought from a long distance.
  - For its similarity to other figures of the Aurignacian in Germany (35-40 Ka), it has been interpreted as a fragment of cave lion (*Panthera spelaea*) retaining its hind legs, but not the front, not the head. On both sides of the body it has numerous lines recorded in groups of four, which could be the coat, and the entire piece is polished. The legs are bent to a position that suggests that the animal is running, jumping or preparing to jump. The belly has traces of ocher.
- **2. "Pencil" hematite stone and marble with traces of ocher**
  - A slice of hematite that would be used for recording marks reddish brown as a "pencil" and a marble pebble presence of traces of ocher

(Top left) from the Denisova Cave lion
&
(Down) Colored 'pencil' and marble pebble with traces of ocher.

(Top right) hybrid Cave lion "lion-man" from the Swabian Aurignacian representations.
1, 2, 4, 5: Vogelherd, 3: Hohle Fels, 6: Geissenklösterle, 7: Hohlenstein-Stadel.
Unscaled.

D Cave ornaments

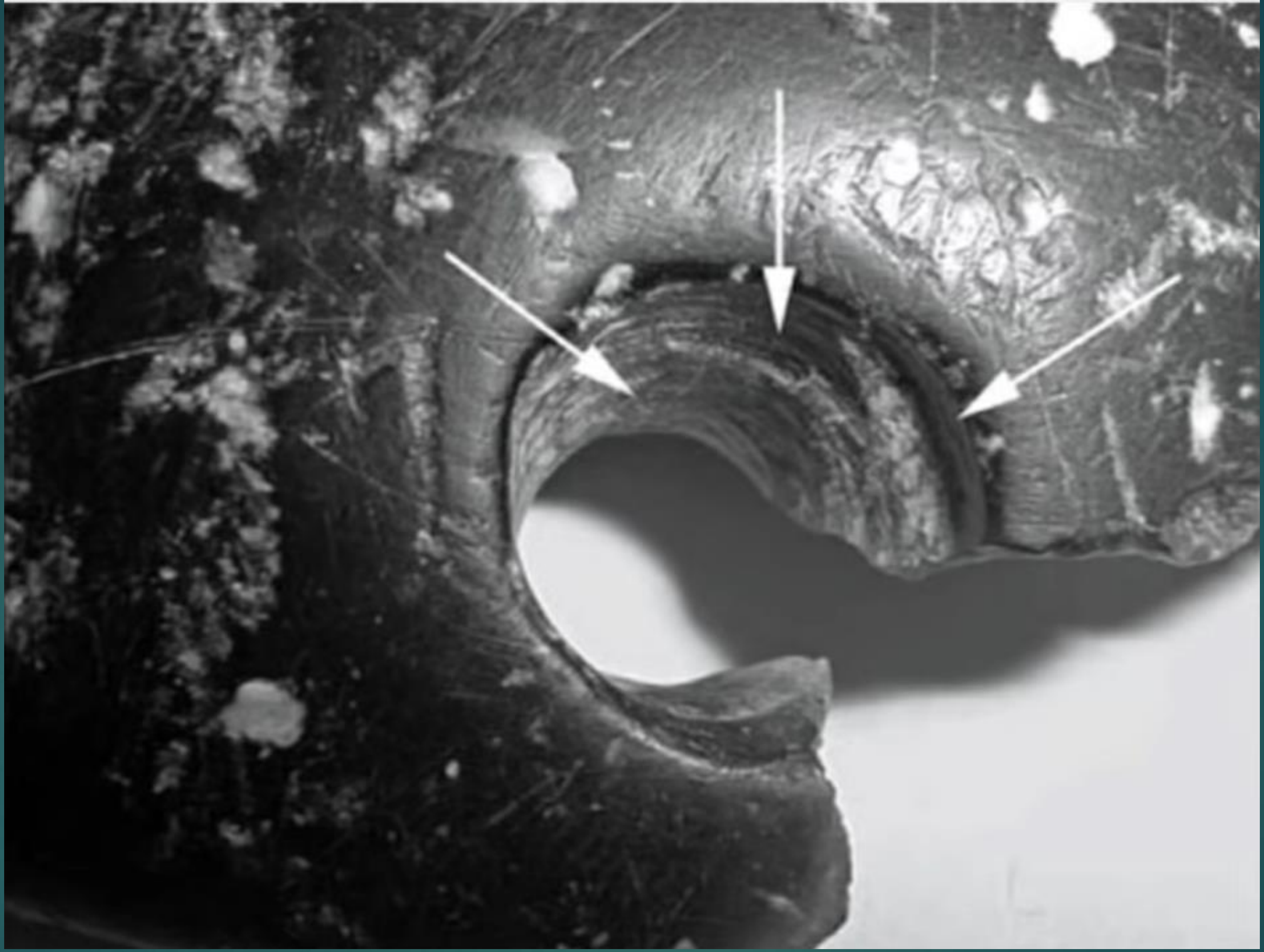
- **3. Diadem/Tiara:**
  - This ivory object is interpreted as a diadem fragment would have a practical use (hold the hair) or identifying a special individual.
  - At one end, the headband has a hole where a rope could be used to tie behind the head. Wear indicates that it was used frequently.
  - Diadem fragments have been found in other Siberian fields, but with a much younger age (20-28 Ka).
- **4. Bracelet:**
  - A perforated green bracelet made od Chlorite (from a distance of 200 km).
  - Measures 27 × 9 mm, and the diameter of the entire object would be 70 mm.
  - It has numerous signs of having been worked and polished with abrasive materials using different techniques, some of which are more typical of the Neolithic period, such as drilling. Oldest jewelry piece ever discovered & oldest made of stone.

Paleolithic tiara from Denisova Cave.

World’s oldest stone bracelet found in 11th layer of Denisova cave. Made from green-hued chlorite

The bracelet was carefully polished and grinded, with a heavy pendant added in the center, probably hanging from a short leather strap.

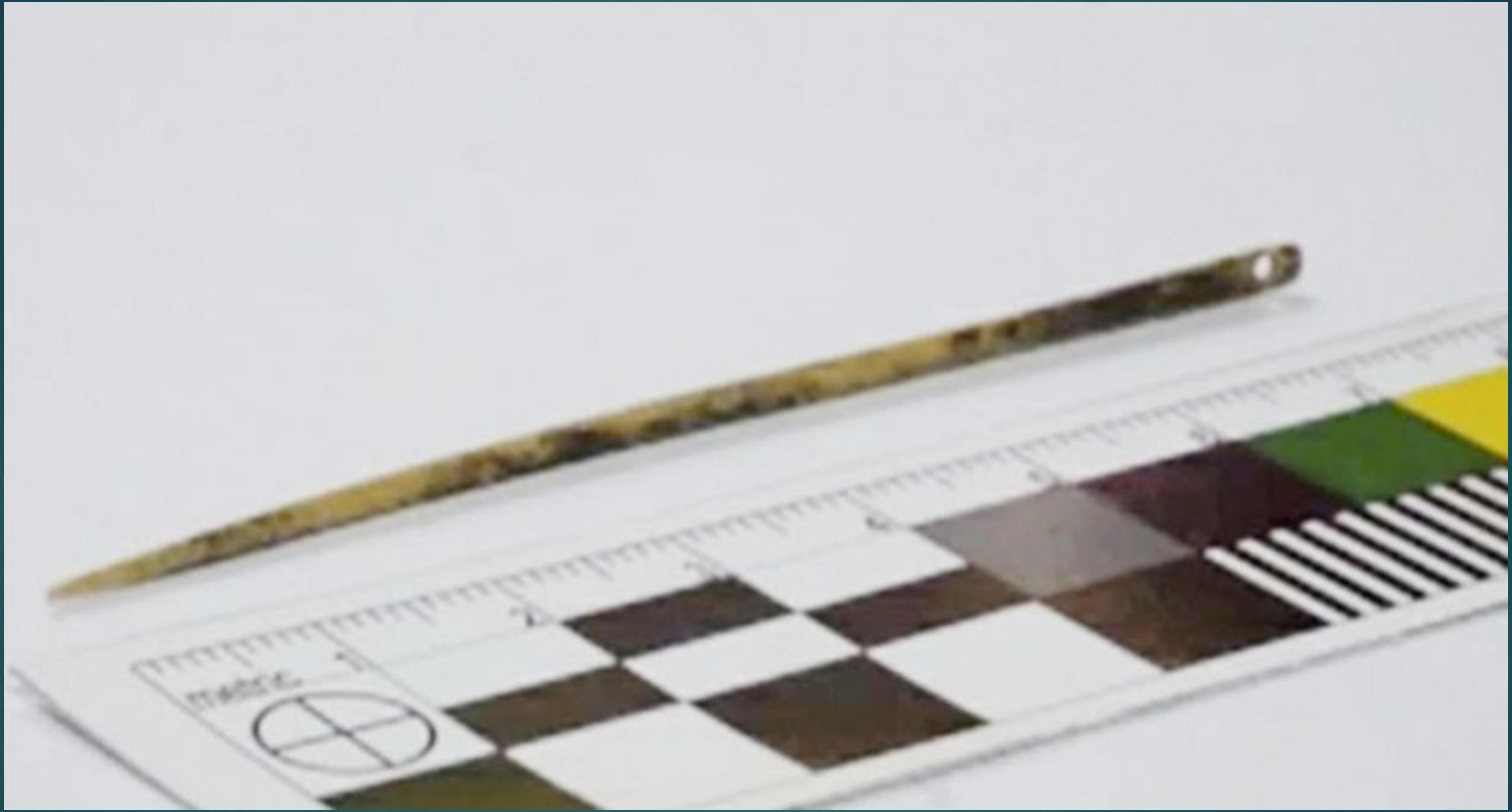
Color depends on the light



How did they drill?

Bone points and pierced animal teeth dating to around 45Ka ago

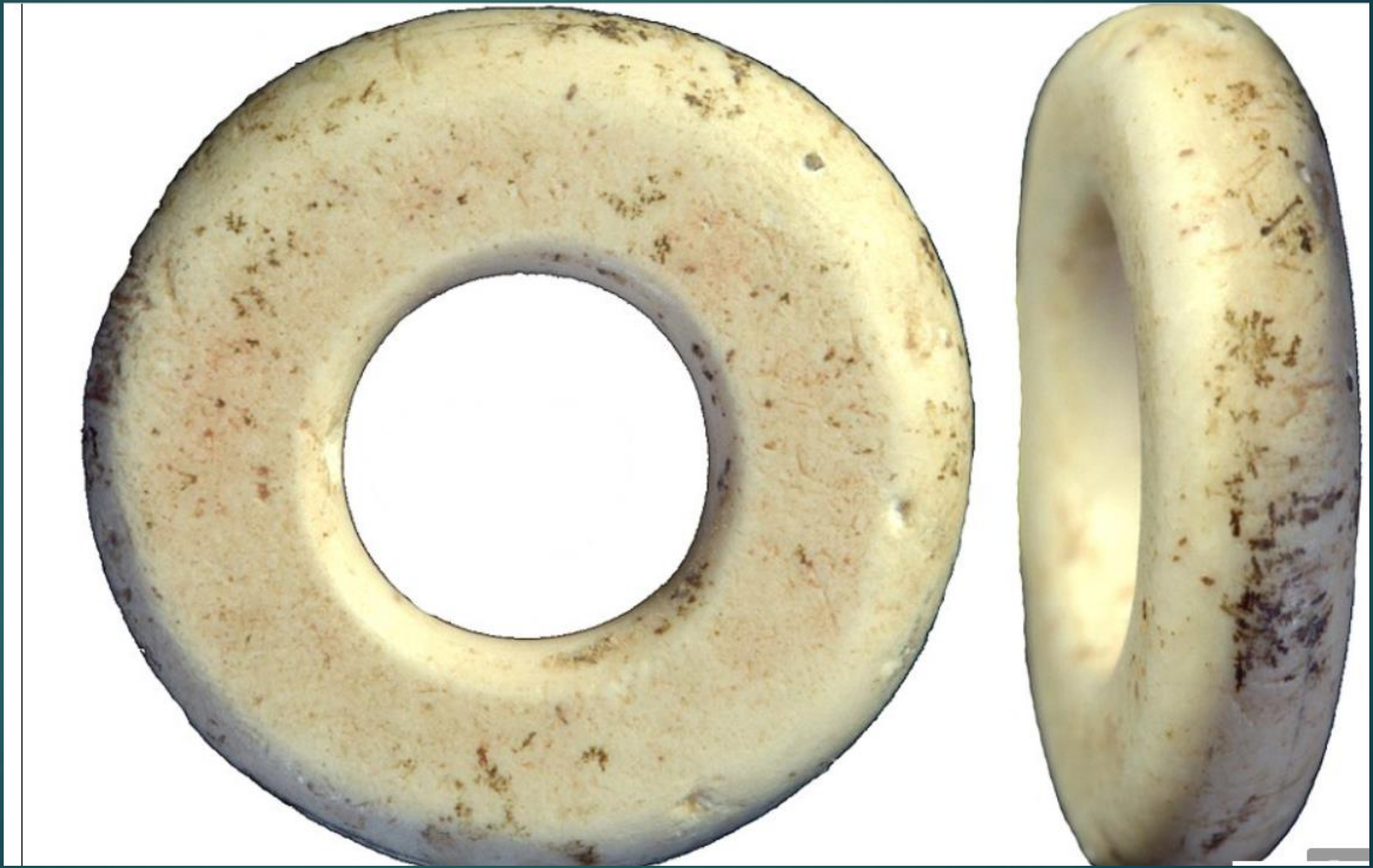
Denisovan bird bone needle: 50 Ka



The 7 centimeter (2 3/4 inch) needle was made and used by our long extinct Denisovan; 10 K older than pendant; found in the same layer where Denisovan remains were previously found.

Denisova Cave

Bead jewellery made from ostrich eggs



Ancient hominin-made items from the Denisova Cave

Rings (**a**), pendants (**b**) and a needle (**c**).

(Left) Bracelet of green-hued chlorite, bead jewelery ostrich eggs Comprising, and a needle - still useable today. (Right) Ivory and talc (soapstone) beads. Credit: SB RAS Institute of Archeology and Ethnography.

Denisova Cave – Ds or MHs

- Bead jewellery made from ostrich eggs and a bone needle – which is still usable today – testify to the talents of Denisovans.

- Two bone artefacts dated to 48-43 Ka and 46-42 Ka old.

- On the basis of human DNA identified at a site called Ust'-Ishim, it is estimated that *H. sapiens* reached Siberia at least 47 to 43 years ago.

- This raises the possibility that MHs contributed to the deposits in the Initial Upper Palaeolithic at Denisova.

Pendants

- Estimates that animal-tooth pendants and bone points previously unearthed at Denisova Cave date from 49 to 43 Ka.
- Russian archaeologists regard Denisovans as the likely makers of these finds.
- Denisova Cave investigations, which began 40 years ago, have yielded no *H. sapiens* fossils or MH DNA.
- *H. sapiens* lived elsewhere in Siberia around 45 Ka ago.
- Since Denisovans date to no later than 52Ka ago in the new studies, C. Stringer: “my money would be on early modern humans” as the makers of Denisova Cave’s pendants and points”

Pendants

- But it wouldn’t be surprising if offspring of Denisovans and *H. sapiens* fashioned those artifacts, writes archaeologist Robin Dennell of the University of Exeter in England
- All Neanderthal fossils at Denisova date to between 140 to 80 Ka.
- In any case, we need to avoid further speculation for now, and wait for the publication of materials and dating.

Note on dating

- Radiocarbon dating reaches its limit at 50 Ka ago or thereabouts; anything older than this cannot be dated.

- Optical ages date the deposition of the sediment rather than the human remains directly, and sometimes tiny human bones might be subject to imperceptible post-depositional movement.

- Genetic dates (via Bayesian statistical analysis of genomes) are relative age estimates, with uncertainties relate to mutation rate variations.

Statistical methods in genetic studies of ancient DNA

- Genetic algorithms are statistical search algorithms which are classified as an evolutionary computing algorithms, which use techniques inspired by natural evolution

- For study, must decide on estimates of variables to include in your statistical model:
- Recombination rate: **Recombination** occurs when two molecules of DNA exchange pieces of their genetic material with each other, i.e. during meiosis when homologous chromosomes line up in pairs and swap segments of DNA.

- This process is also known as **crossing over;** creates gametes that contain new combinations of genes, which helps maximize the genetic diversity of any offspring; creates genetic diversity; creating new allelic combinations
- Transmission-**genetic** process by which the combinations of alleles observed at different loci in two parental individuals become shuffled in offspring individuals

Statistical methods

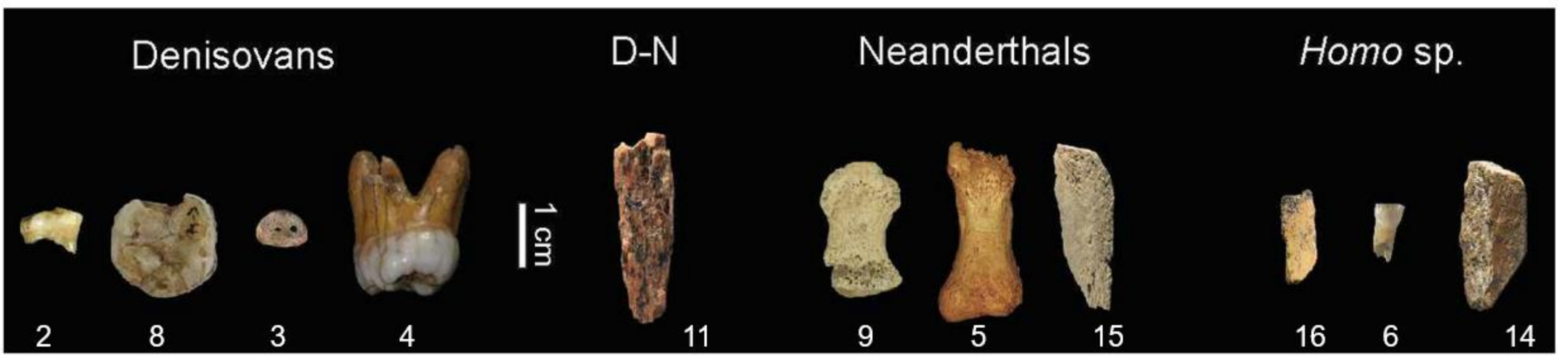
- Mutation rate: Mutation rates quantify the number of errors per nucleotide copied, irrespective of the fate (increase or decrease in frequency) of the error copy produced. The source of **genetic** variation in a population is **mutation**.
- **Mutation rates** in humans have been estimated to be on the order of 10^{-4} to 10^{-6} per **gene** per generation.
- Demographic model: estimates of birth, deaths, life span, etc.
- Generation time: the average **time** between two consecutive **generations** in the lineages of a population. In human populations, **Generation time** typically ranges from 22 to 33 years

- Researcher must choose specific numbers for each of these variables; ultimately effect results and cause criticisms

Concept of mutation rate for genetic dating

- Genetic dates (via Bayesian statistical analysis of genomes) are <u>relative age estimates</u>, with uncertainties in mutation rates
- Ust'-Ishim MH in western Siberia dated to 45 Ka (46,880–43,210)
- Example of a mutation rate estimate: "We estimate an autosomal mutation rate of 0.4×10^{-9} to 0.6×10^{-9} per allele site per year, a Y chromosomal mutation rate of 0.7×10^{-9} to 0.9×10^{-9} per site per year based on the additional substitutions that have occurred in present-day non-Africans compared to this genome, and a mitochondrial mutation rate of 1.8×10^{-8} to 3.2×10^{-8} per site per year based on the age of the bone."
- Researchers must decide on a mutation rate for changes in an allele site. Not all scientists will agree.

Current 11 Denisovan Specimens: Fit on the palm of your hand



Almost all less than 2 cms.(less than 1 inch); mostly acid etched (digested and regurgitated or pooped out by hyenas); some have hyena DNA;
All from different individuals; mostly children.

Denisovans: No morphological information

- No complete D crania or whole postcranial bones:
- Almost no phenotypic morphological information exists about Denisovans
- Except for small fragmentary bones and 1 larger bone, we have no fossil evidence for this group.
- But we do have several Denisovan mtDNA and nuclear genomes.

Denisova Cave

- Neolithic and later Turkic pastoralists took shelter, gathering their herds around them to ride out the Siberian winters
- Denis the Hermit was a Russian Orthodox religious contemplative who lived in the cave named for him during the 18 th Century.
- More recently, sheep herders sporadically occupied it.

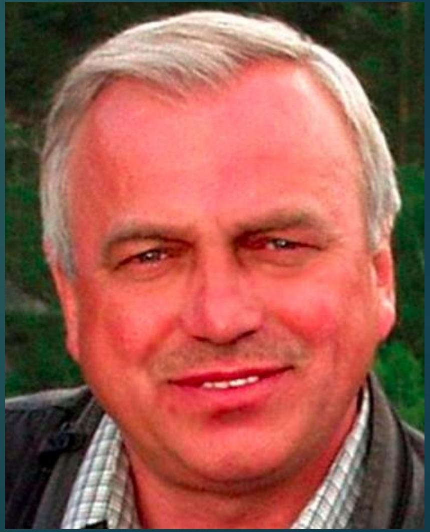
First discovery

- The cave was originally explored in the 1970s by Russian paleontologist Nikolai Ovodov, who was looking for remains of canids.
- In 2008, Michael Shunkov from the Russian Academy of Sciences and other Russian archaeologists from the Institute of Archaeology and Ethnology of Novosibirsk investigated the cave
- Russian archaeologist named Alexander Tsybankov was digging in side chamber in July 2008, in deposits believed to be 76-52 Ka, when he came upon a tiny piece of bone.
- .

Anatoly Derevianko and Michail Shunkov:
Homo sp. Altai*, or *Homo sapiens ssp. Denisova

- Anatoly Derevianko and Michail Shunkov of the Institute of Archaeology and Ethnology of Novosibirsk of the Russian Academy of Sciences ,

- 2000 & 2008: working at the site of Denisova Cave in the Altai Mountains of Siberia, discovered in 2000, a huge adult molar and in 2008 uncovered a small bone fragment from the fifth finger of a juvenile hominin, dubbed the "X woman"



- They are multiregionalists

Denisova Cave: Was bone MH or N?

- Anatoly Derevianko, leader of the Altai excavations and director of the Institute of Archaeology and Ethnography in Novosibirsk, thought it was a MH bone.

- But DNA from a fossil found earlier in a nearby cave had proved to be Neanderthal, so it was possible this bone was Neanderthal as well.

Denisova Cave: a juvenile female pinkie bone in 2008

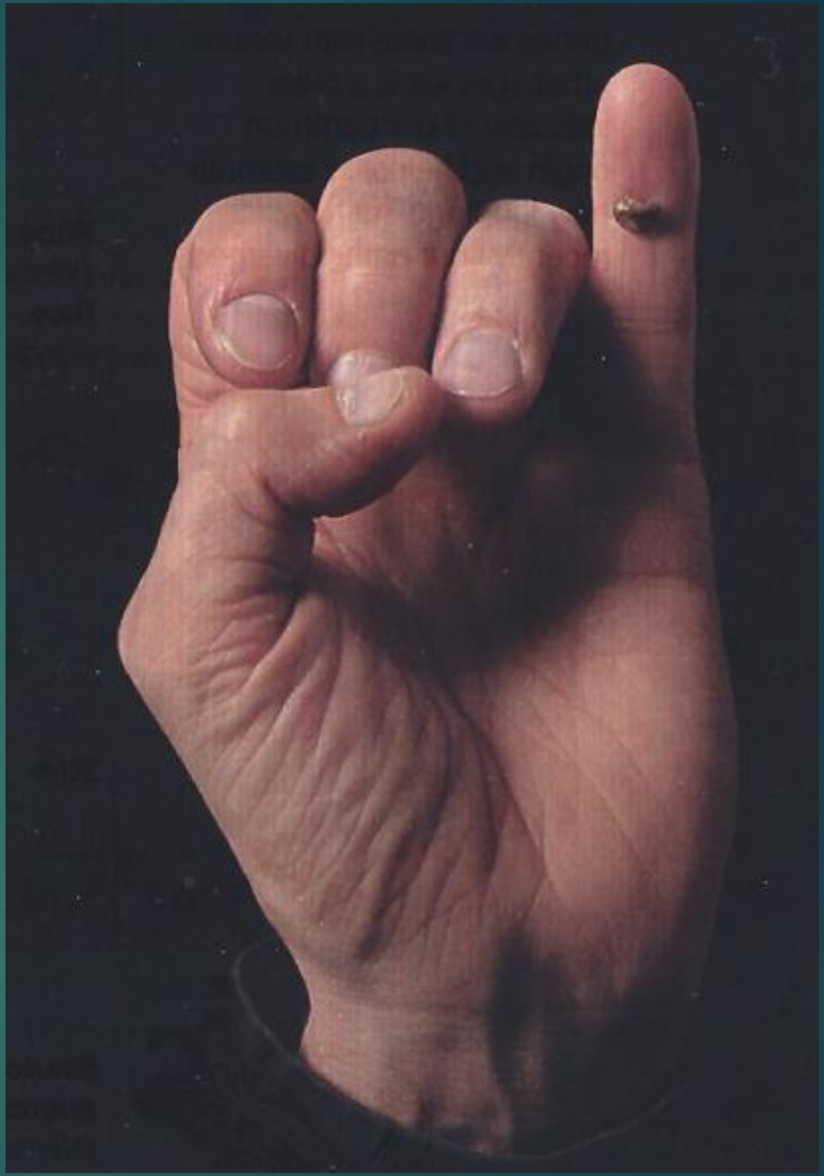
- It was hardly promising: a rough nubbin about the size and shape of a pebble or the size of a lentil seed (1/4 inch)...a chip from a primate fingertip—specifically the part that faces the last joint in the pinkie.
- Judging by the incompletely fused joint surface, the human in question had died young, perhaps as young as eight years old.
- Since its publication in 2010: hominin group for which the only significant source of information has been the genome sequence

2008 Discovery: Denisova 3: finger bone, before its split

1 Finger bone changed our conception of the hominin universe

2008 Discovery:
"X Woman" (girl), 83-63 Ka

The X woman finger bone was about the size of a lentil and weighed less than 40 milligrams



Pinkie bone, Denisova cave

Paabo's hand & bone
Laid around in lab for 6 months

Denisova 3: Pinky bone, size of 2 grains of rice

The Meandering trail of Denisova 3, the finger bone: Two competing pieces of bone

- In 2009, Derevianko decided to split the fingertip bone into two pieces.

- The pictures of the phalanx tip taken prior to its cutting have been lost.

- The smaller proximal part of the bone went into an envelope and was hand-delivered to Svante Pääbo, at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany.

- The larger distal part was sent to Edward Rubin, a leading paleogeneticist at UC Berkeley’s genetics laboratory in California.

Meeting of the minds. Archaeologist Anatoly Derevianko (left) and paleogeneticist Svante Pääbo worked together to discover the Denisovans

The meandering trail of Denisova 3

- Svante Pääbo, a transplanted Swede, is arguably the world's leading expert in ancient DNA, especially human DNA.
- When Pääbo received the package from Derevianko, his team was hard at work producing the first sequence of the entire Neanderthal genome.
- Pääbo’s expectations were low because the bone was so small and therefore unlikely to contain much DNA.
- It was actually laying around for 6 months in a lab drawer before his team analyzed it.

The finger bone at Leipzig

- It wasn't until late 2009 that the finger bone drew the attention of Johannes Krause, at the time a senior member of Pääbo's team. (He's now at the University of Tübingen.)
- Like everyone else, Krause assumed the bone was hominin.
- Using 30 mg (2 Ts) of the finger bone powder, Krause and his student Qiaomei Fu extracted its mitochondrial DNA (mtDNA), which is easier to find in ancient bone than is nuclear DNA.
- None of the Neanderthal fossils Pääbo and his colleagues had ever tested contained even 5 percent endogenous DNA (DNA belonging to the fossil), and most had less than one percent.

Extracting DNA is difficult

- DNA degrades over time, so usually very little remains in a bone tens of thousands of years old.

- Moreover, the DNA from the bone itself—called endogenous DNA—is typically just a tiny fraction of the total DNA in a specimen; 90-95% is made up of contaminants, usually soil bacteria.

- To their amazement, the DNA in the finger bone, when removed, was some 70 percent endogenous DNA.

DNA Extraction: It’s a girl

- The cold cave had preserved it well.
- Given so much DNA, the scientists easily ascertained that there was no sign of a male Y chromosome in the specimen.
- Denisova 3: The fingertip had belonged to a little girl who had died in or near Denisova cave tens of thousands of years before.

Denisova 3 at Leipzig

- They compared the DNA sequence with those of living humans and Neanderthals.

- Then they repeated the analysis, because they couldn't believe the results they'd gotten the first time around.

- Krause himself recalls that Friday as "scientifically the most exciting day of my life."

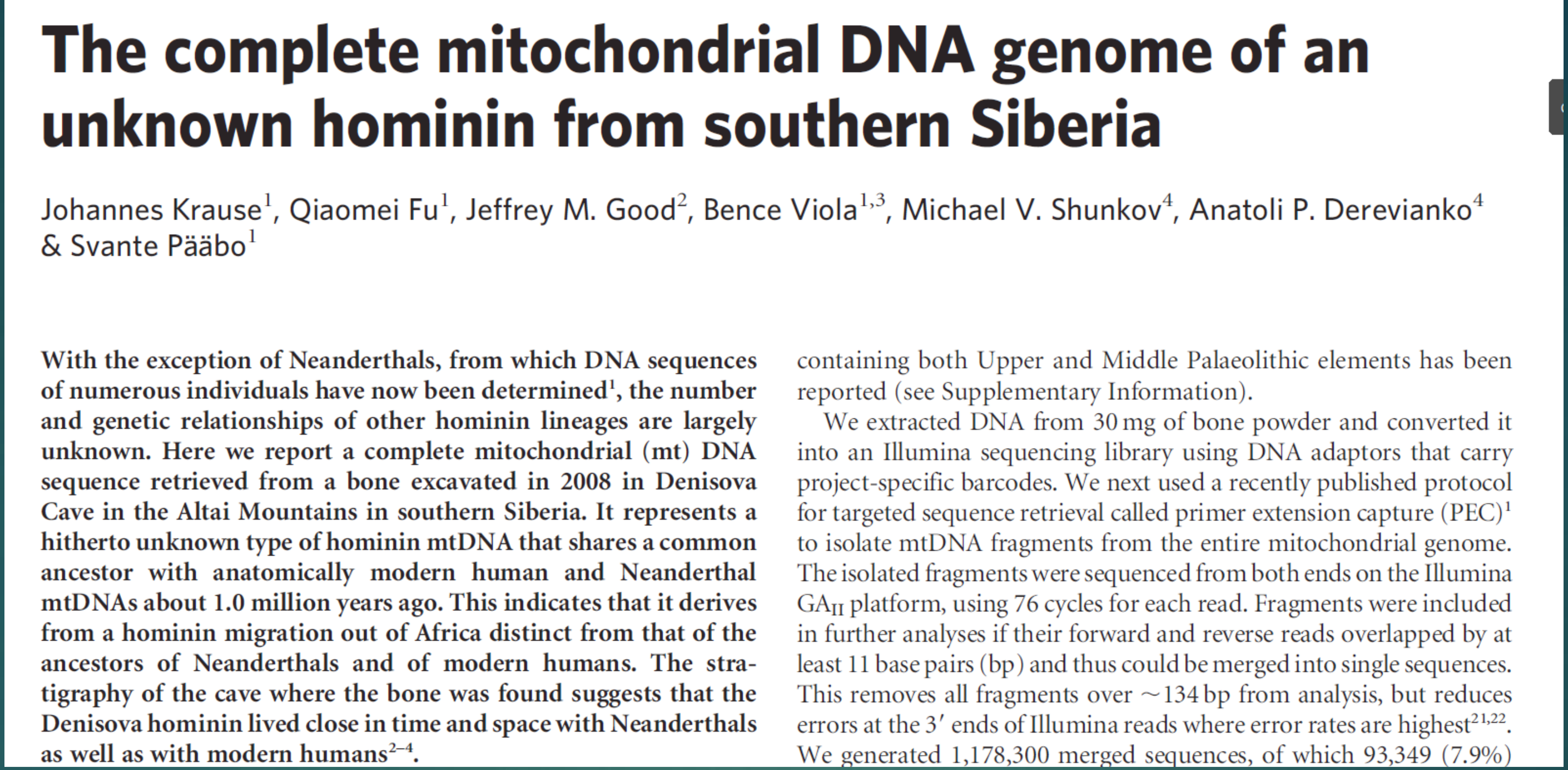
- The tiny chip of a finger bone was neither MH nor N. It belonged to a new kind of human being, never before seen.

Denisova 3: “Are you sitting down?”

- When the analysis of the first bits was completed, Pääbo happened to be in the United States.
- When he called his office, Krause said to him, “Are you sitting down?”
- The DNA showed that the digit could not have belonged to a Neanderthal nor to a modern human.
- Its owner must have been part of some entirely different and previously unsuspected type of hominin.

Elizabeth Kolbert, The New Yorker, August 15, 2011

2010, Nature: A new unknown archaic hominin: “Denisovan”



The complete mitochondrial DNA genome of an unknown hominin from southern Siberia

Johannes Krause[1], Qiaomei Fu[1], Jeffrey M. Good[2], Bence Viola[1,3], Michael V. Shunkov[4], Anatoli P. Derevianko[4] & Svante Pääbo[1]

With the exception of Neanderthals, from which DNA sequences of numerous individuals have now been determined[1], the number and genetic relationships of other hominin lineages are largely unknown. Here we report a complete mitochondrial (mt) DNA sequence retrieved from a bone excavated in 2008 in Denisova Cave in the Altai Mountains in southern Siberia. It represents a hitherto unknown type of hominin mtDNA that shares a common ancestor with anatomically modern human and Neanderthal mtDNAs about 1.0 million years ago. This indicates that it derives from a hominin migration out of Africa distinct from that of the ancestors of Neanderthals and of modern humans. The stratigraphy of the cave where the bone was found suggests that the Denisova hominin lived close in time and space with Neanderthals as well as with modern humans[2–4].

containing both Upper and Middle Palaeolithic elements has been reported (see Supplementary Information).

We extracted DNA from 30 mg of bone powder and converted it into an Illumina sequencing library using DNA adaptors that carry project-specific barcodes. We next used a recently published protocol for targeted sequence retrieval called primer extension capture (PEC)[1] to isolate mtDNA fragments from the entire mitochondrial genome. The isolated fragments were sequenced from both ends on the Illumina GA_{II} platform, using 76 cycles for each read. Fragments were included in further analyses if their forward and reverse reads overlapped by at least 11 base pairs (bp) and thus could be merged into single sequences. This removes all fragments over ~134 bp from analysis, but reduces errors at the 3′ ends of Illumina reads where error rates are highest[21,22]. We generated 1,178,300 merged sequences, of which 93,349 (7.9%)

First species to be discovered by DNA alone: mitochondrial DNA sequence of an unknown hominin from Siberia

1. The complete mitochondrial DNA genome of an unknown hominin from southern Siberia – J. Krause, et al., 2010

- First genetic discovery of Denisovan DNA from finger bone at Denisova Cave, Siberia, Russia:

- 2010 - J. Krause: report a complete mitochondrial (mt) DNA sequence retrieved from a juvenile female finger bone excavated in 2008 in Denisova Cave in the Altai Mountains in southern Siberia.

- It represents a hitherto unknown type of hominin mtDNA that shares a common ancestor with anatomically modern human and Neanderthal mtDNAs about 1.0 million years ago.

Newspaper title: “Giving accepted prehistoric history the finger”

- ran the headline on the story in the Sydney Morning Herald about this study.

- The stratigraphy of the cave indicates the Denisova hominin lived close in time and space with Neanderthals as well as with MHs

- Episodic hominin occupation since 125 Ka
- The finger bone was found in a stratum (layer 11) dated to 48–30 kyr ago

Debate over *"Homo altaiensis"*

- The Russians in Pääbo's group: name of "*Homo altaiensis*" for the new Denisovan hominin

- But Pääbo was uneasy with idea of creating a new Latin "species" name.

- Note long contention over whether Ns were a species or subspecies, based on idea that species do not interbreed; but Ns interbreed with MHs and Ds; raises question as to whether Ds are a species, esp. since species decisions are usually done on basis of morphology

- He prefers talking about metapopulations rather than species. His group does not use Latin species names in their publications.

Debate over *“Homo altaiensis”*

- They rewrote the paper and removed any reference to a new species, awaiting nuclear DNA description [did not happen then either].

- Later agreed to simply call them “Denisovans”

- The 2010 finding transformed the cave into one of the world’s most important archaeological sites.

Lost second half of the finger

- But Pääbo did not know about the 2nd half of the finger bone.
- The whereabouts of UC Berkeley finger bone was unknown for 9 years.
- Eva-Maria Geigl is a paleogeneticist at the Institute Jacques Monod in Paris.
- According to Geigl, Rubin (who left the UCB’s LBNL in 2016 for industry) sent his half of the finger bone to her lab in 2010.

Lost second half of the finger

- But after Pääbo's team published the Denisovan nuclear genome, Rubin asked Geigl to return the fossil.
- After she extracted its DNA and took detailed photographs first, she returned the fragment in 2011 to UC Berkeley.
- Geigl sat on her data for years, but in 2016, she decided, at the suggestion of Pääbo, to publish them (Bennett et al., 2019)

Whereabouts unknown

- Her team sequenced the mitochondrial genome and discovered — unsurprisingly — that it exactly matched the sequence Pääbo’s team had published in 2010.
- According to Derevianko, Rubin sent the finger bone sample to the ancient-DNA lab of Eske Willerslev, at the University of Copenhagen and the Natural History Museum of Denmark, around 2011.
- Pääbo and his team had to grind up an inner portion of their piece of the bone to produce a high-quality genome sequence; he returned the rest to Derevianko
- Geigl is unsure if the second half she analyzed is gone.

Denisova 3 is no more

- Group debated a name for Denisova 3 who was a 3 to 5 yo girl. The self-dubbed "X-Man" team first decided on "X-Girl", but thought it was too Japanese manga-like. Pääbo decided on "X-Woman".

- But what of the little girl herself? The tiny bit of bone that is all we ever had of her—or at least the half that went to Leipzig—is gone now.

- In pulling DNA from it, Johannes Krause and Qiaomei Fu eventually used it all up.

- The little girl has been reduced to a computerized "library" of DNA genetic data that can be exactly copied again and again forever.

- The genetic library tells us that she probably had dark hair, dark eyes, and dark skin.

MH & Ns share 99.7% of 3 billion SNPs

- Neanderthal DNA is 99.7 percent identical to present-day human DNA
- 98.8 percent identical to chimpanzee DNA.
- 9 million SNPs difference between N & MHs
- MHs have 1-2% N nuclear DNA.
- N and D mtDNA variants have not contributed to MH mtDNA pool.
- Based on mtDNA, split of the N and MH lineages is dated to between 760 to 550 Ka

mtDNA differences

- MH differences in mtDNA from:
  - Neanderthals: an average of 202 nucleotide positions, out of approximately 16,500
  - Denisovans: 385 positions,
  - Chimpanzees: 1,462 positions

mtDNA differences

- The mtDNA differences suggested that
  - Denisovan mtDNA diverged from that of modern humans and Neanderthals about 1,3 Ma–779 Ka ago
  - Modern human and Neanderthal mtDNA diverged 618–321 Ka ago.
  - Krause and colleagues then concluded that Denisovans were the descendants of an earlier migration of *H. erectus* out of Africa, completely distinct from modern humans and Neanderthals.

Two approaches to understanding hominin relationships

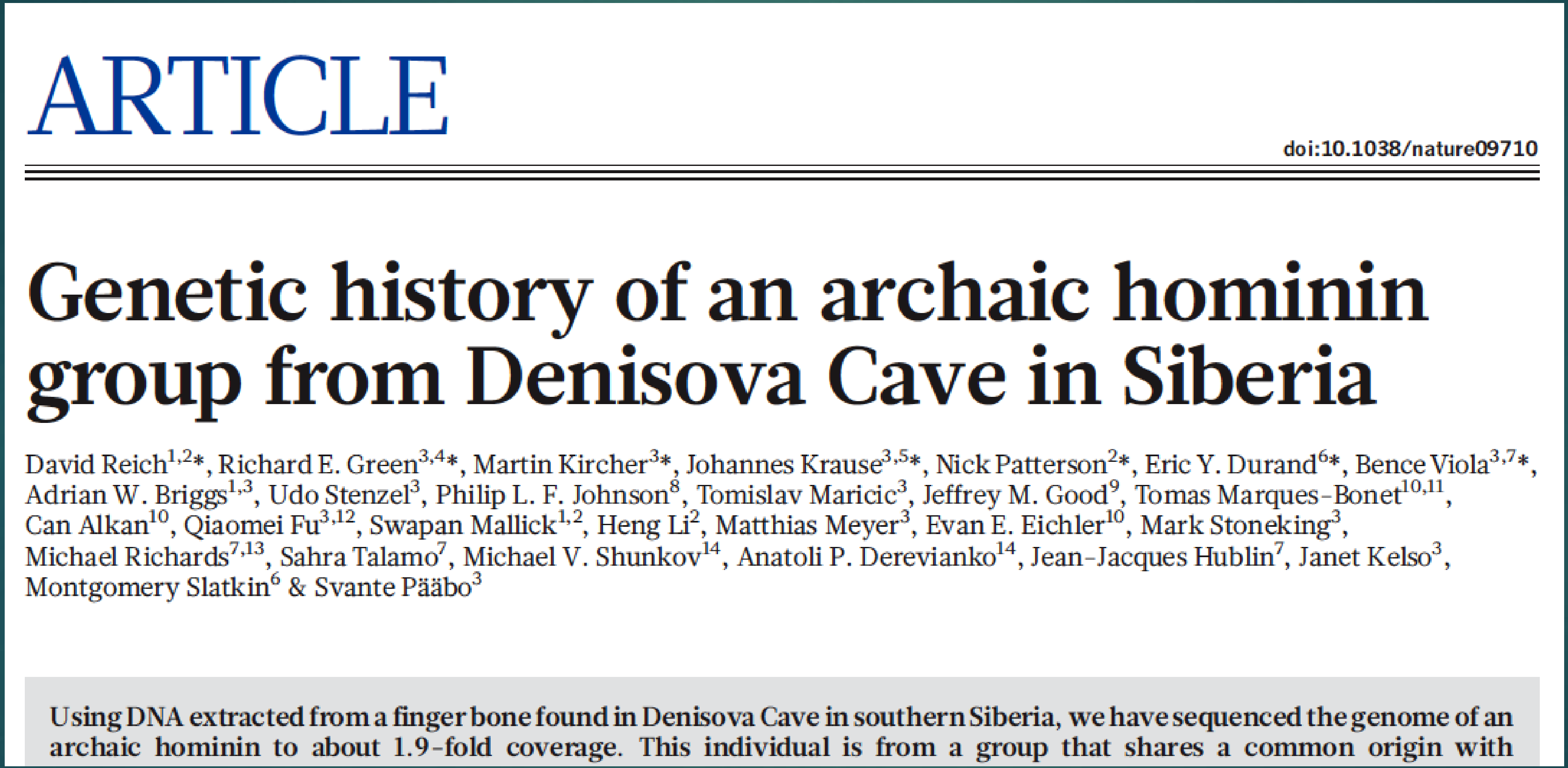
- Two approaches to understanding hominin relationships:
  - DNA sequences retrieved from hominin remains and
  - fossil morphology.

- Neandertal Nuclear genome:
  - Ns and MHs share common ancestors ~ 800 Ka
  - population split of N and MH ancestors occurred 440-270 Ka.

- Neanderthals shared more genetic variants with present-day humans in Eurasia than with present-day humans in sub-Saharan Africa

D. Reich et al., 2010

2010: Later work allowed for complete genome sequencing of this hominin (now named the "Denisovan hominin"):



ARTICLE

doi:10.1038/nature09710

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[3,7]*, 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]

Using DNA extracted from a finger bone found in Denisova Cave in southern Siberia, we have sequenced the genome of an archaic hominin to about 1.9-fold coverage. This individual is from a group that shares a common origin with

2. Genetic history of an archaic hominin group from Denisova Cave in Siberia. D. Reich, et al., 2010

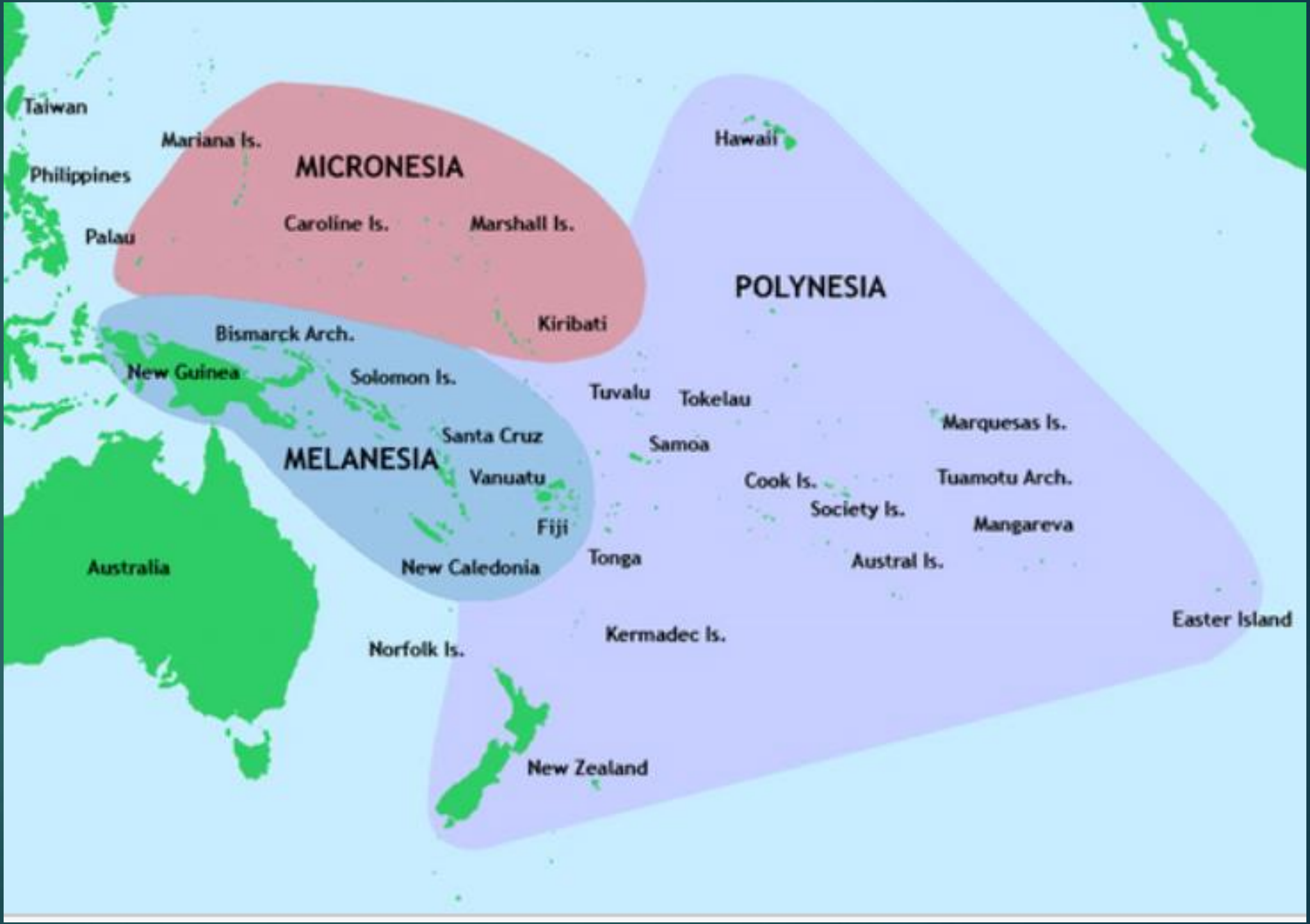
- Using DNA from Denisova 3 finger bone found in Denisova Cave, we have sequenced the nuclear genome of an archaic hominin to about 1.9-fold coverage.

- Denisovans share a common origin with Neanderthals.

- Ds contributed 4–6% of its genetic material to the genomes of present-day Melanesians.

Denisovans:

Not just in cold Siberia, but also in Melanesian Islands



Denisovans

- Ns diverged from ancestor of MHs ~770-550 Ka.
- Ns were in Europe & western Eurasia
- Ds were in eastern Eurasia
- 17% of the Denisovan genome is Neandertal
- They lived among and interbred with the ancestors of some modern humans in SE Asia.

D Nuclear DNA

- D Nuclear DNA paints different picture than the mtDNA
- First: N and D were more closely related to each other than either was to MHs
- N and D populations separated 470-380 Ka
- Ds were cousins of Ns, but were different, having separated from Ns before many N traits appeared in classical period fossil record [~200 Ka].

Reich, 2010: a tooth

- Designated this hominin population as 'Denisovans' and suggest that it may have been widespread in Asia during the Late Pleistocene epoch.
- A tooth found in Denisova Cave carries mtDNA highly similar to that of the Denisova 3 finger bone.
- This tooth shares no derived morphological features with Neanderthals or modern humans, further indicating that Denisovans have an evolutionary history distinct from Neanderthals and modern humans.

Denisovans

- In addition, a minimum of 0.5% of the genome of the Denisova 3 individual was derived from a
    - Neandertal population more closely related to the Altai Neandertal from Denisova Cave
    - than to Neandertals from more western locations.

- Ds were in the Altai Mountains,

- They also lived in other parts of Asia

Reich, 2010

- Vindija Neanderthals share more derived alleles with non-Africans than with Africans, consistent with Neanderthals contributing 1-2% of the genomes of present-day humans across Eurasia
- ~4.8% of the genomes of Melanesians derive from Denisovans.
- Altogether, as much as ~7.4% of the genomes of Melanesians may derive from recent admixture with Ns and Ds.

Denisovans are closer to Neandertals, but different

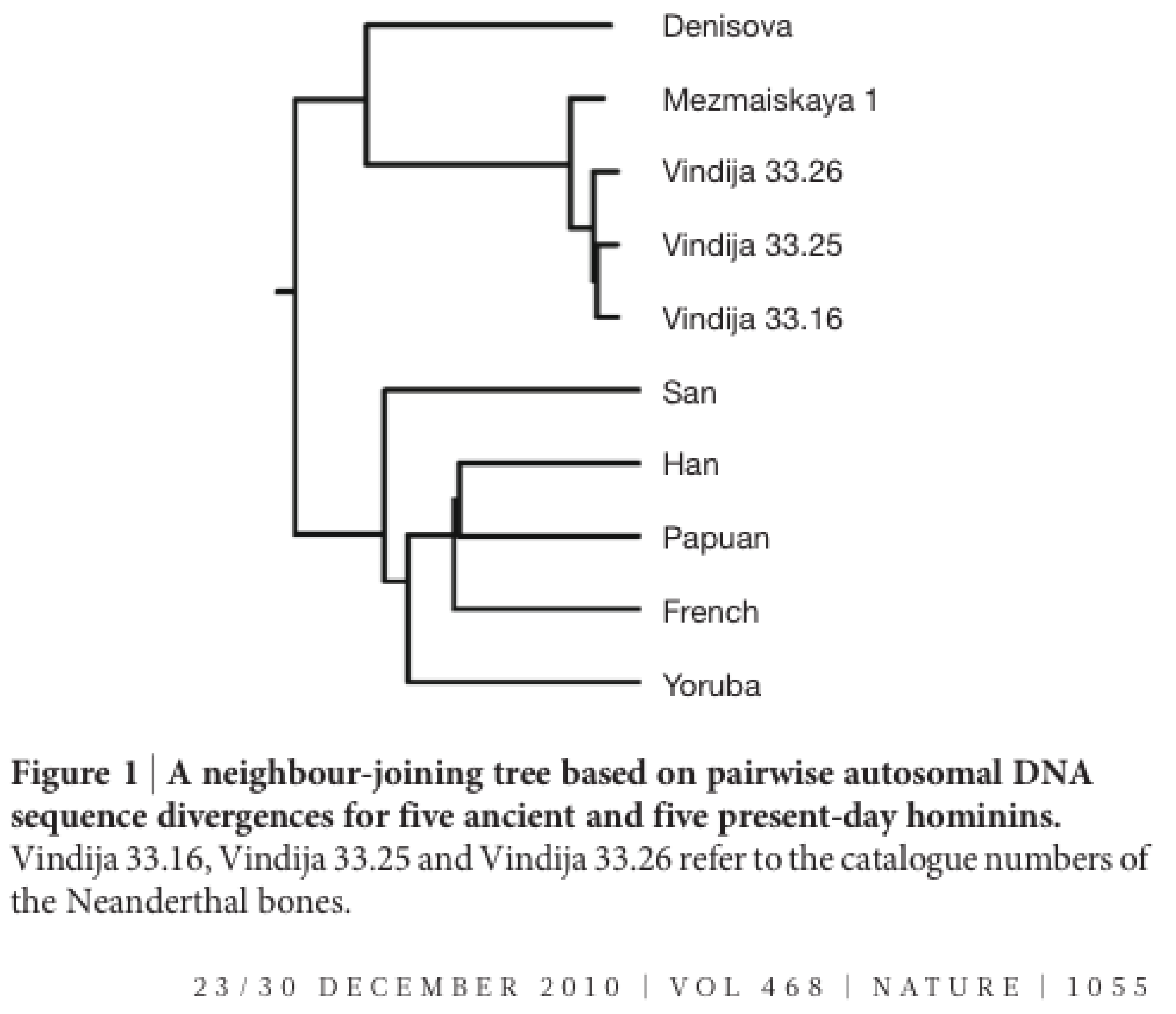


Figure 1 | A neighbour-joining tree based on pairwise autosomal DNA sequence divergences for five ancient and five present-day hominins. Vindija 33.16, Vindija 33.25 and Vindija 33.26 refer to the catalogue numbers of the Neanderthal bones.

23/30 DECEMBER 2010 | VOL 468 | NATURE | 1055

Neandertals

Modern Humans

Reich, 2010

- Denisovans are a sister group of Neanderthals with a population divergence time of one-half to two-thirds of the time to the common ancestor of Neanderthals and humans.
- After the divergence of the Denisovans from Neanderthals, there was gene flow from Neanderthals into MHs.
- Later there was admixture between the Denisovans and the MH ancestors of Melanesians
- The population history indicated by the D nuclear genome is different from that indicated by the mtDNA phylogeny. There are two possible explanations for this.

Reich, 2010

- One possible explanation:
  - both Ns & Ds descend from a common ancestral population that separated earlier from ancestors of present-day humans.
  - Such a scenario would predict a closer relationship between the Denisova individual and Neanderthals than between either of them and present-day humans.

Reich, 2010: A common ancestral population

- Another theory is that the mtDNA lineage was introduced into Denisovan ancestors by admixture from another archaic hominin lineage for which we have no data.

- Another idea is that the discordance is the result of ‘incomplete lineage sorting’, that is, the random assortment of genetic lineages due to genetic drift which may have allowed a divergent mtDNA lineage to survive in Denisovans by chance while becoming lost in Neanderthals and modern humans. A large ancestral population size makes incomplete lineage sorting more likely to occur

Reich, 2010: Denisova 4 tooth

- In 2000, a hominin tooth (Denisova 4) was discovered in layer 11.1 of the south gallery of Denisova.

- Its mtDNA genome differs at only two positions from the mtDNA of the finger bone, making it D, whereas it differs at about 380 positions from both Neanderthal and present-day humans.

- The tooth and the finger bone are both Denisovan but derive from two different individuals

Denisova 4 molar: Primitive morphology unlike Ns

- The tooth is very large; As a third molar, it is outside the range of normal size variation of all fossil taxa of the genus Homo, with the exception of *H. habilis*, and comparable to the Australopithecines. Large molars are usually associated with eating tough uncooked plants.
- Compared to second molars, it is larger than Ns or early MHs, but similar to *H. erectus* and *H. habilis*.
- Has strongly diverging roots, unlike the closely spaced and frequently fused roots of Neanderthals
- Denisova 4: less than 0.2% of the DNA in the tooth derives from a hominin source, but it is an unprecedented 70% endogenous DNA. By contrast, in all Neanderthal remains studied so far, the relative abundance of endogenous DNA is below 5%, and typically below 1%.

Reich, 2010: Conclusions

- The Denisova individual and the population to which it belonged have:
  - some exceptionally archaic mtDNA
  - Different morphological dental features
  - nDNA indicates the Denisova population is a sister group to Neanderthals.
  - D and Ns split before Ns developed their final morphology

Reich, 2010: Separate D and N histories

- After their split, Denisovans and Neanderthals had largely separate population histories:

- First, Ds did not contribute genes to most present-day Eurasians, whereas Neanderthals did. Ns at some point interacted with ancestors of present-day Eurasians independently of Denisovans.

- Second, the genetic diversity of Neanderthals across their geographical range in their last 30-40 K years of their history was extremely low, indicating that they experienced one or more strong genetic bottlenecks independently of the Denisovans.

Reich, 2010

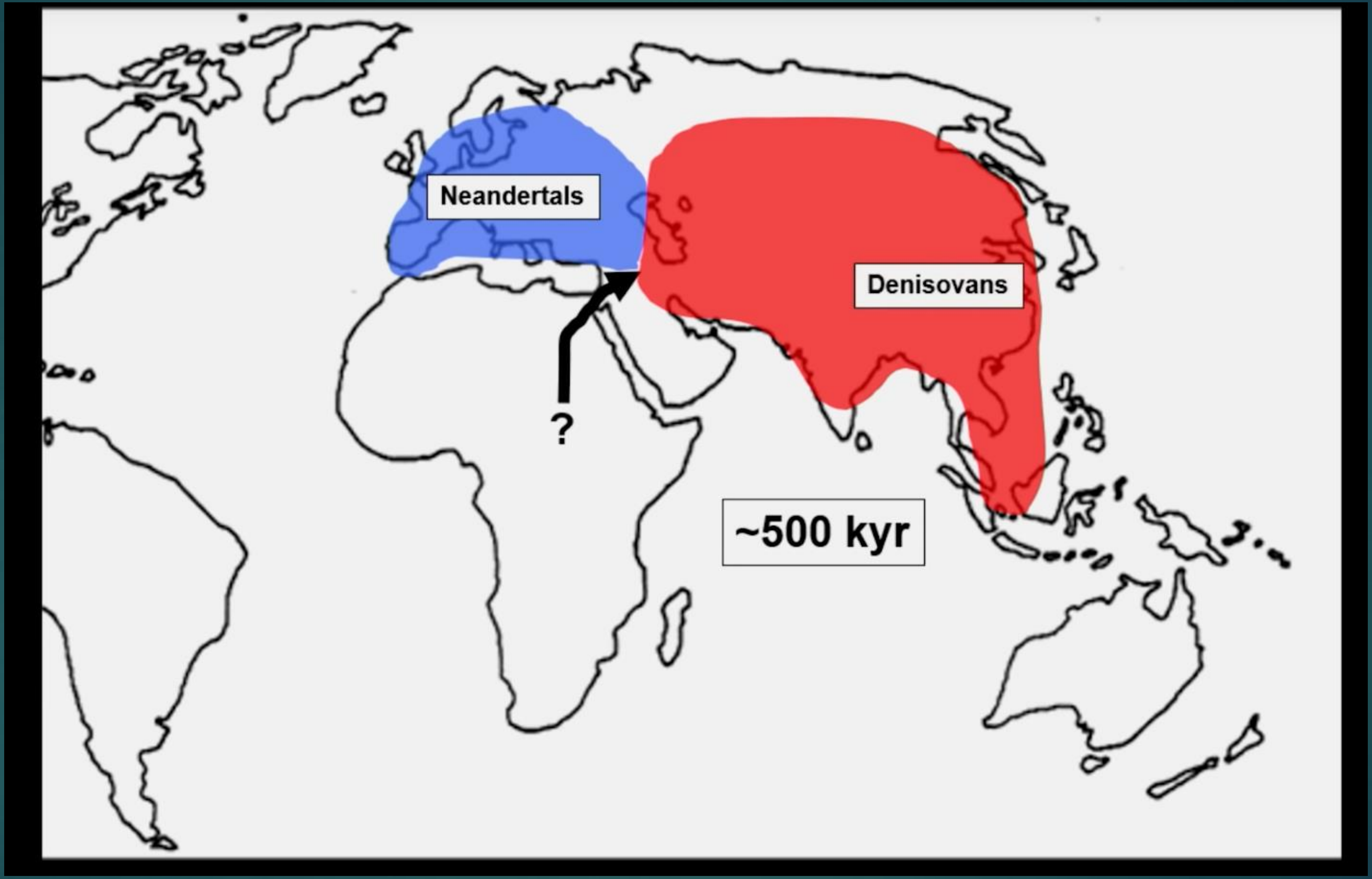
- Ds also had remarkably low divergence—which is 30% of that in present-day humans
- Indicates that the Vindija (29 Ka) and Mezmaiskaya (40 Ka) Neanderthals descend from a common ancestral population that experienced a drastic bottleneck since separating from the ancestors of the Denisova individual
- These late Neanderthals share a strong population bottleneck not experienced by the ancestors of the Denisovans

Reich, 2010: Western and Eastern hominins

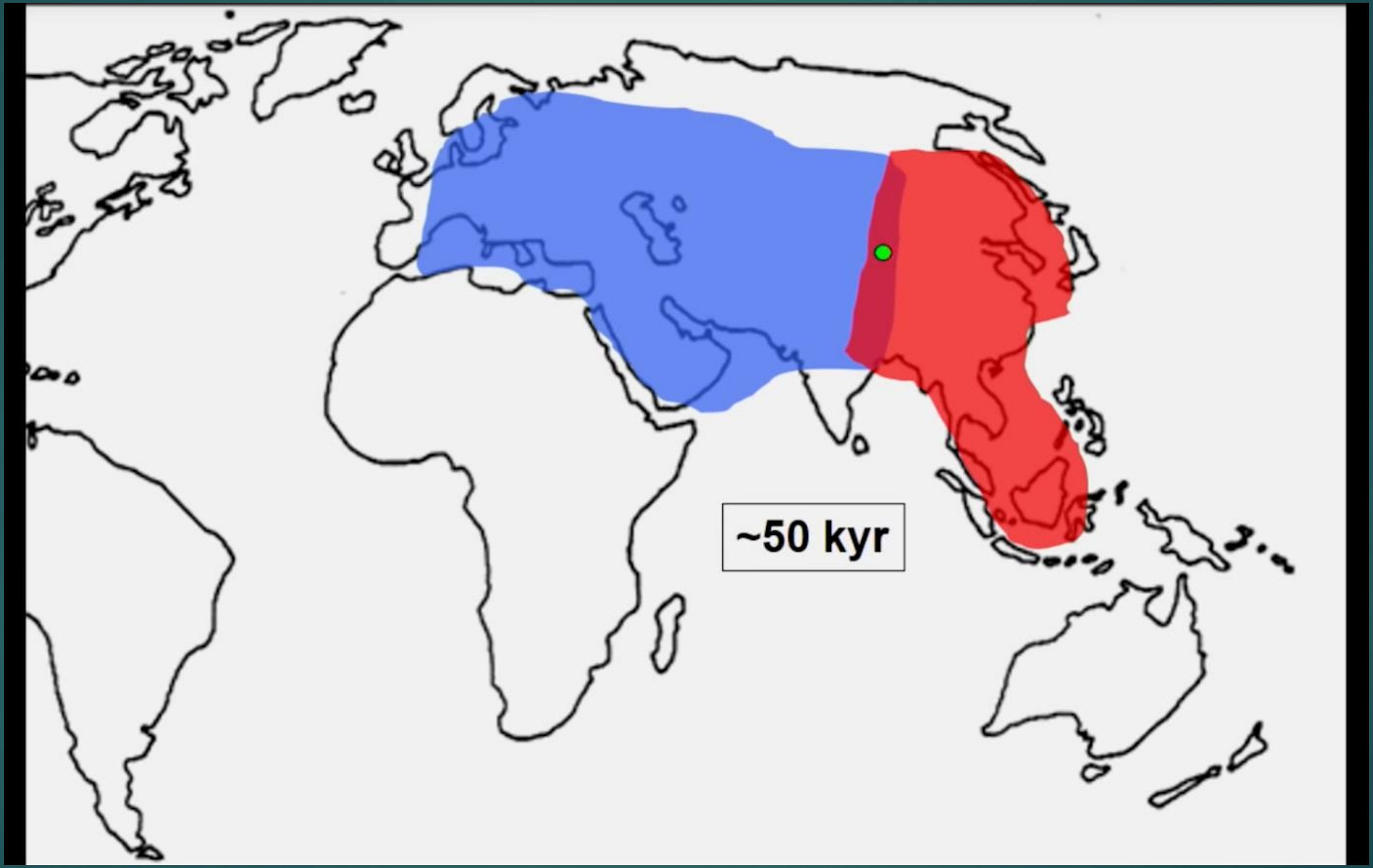
- Third, Denisovans but not Neanderthals, contributed genes to ancestors of present-day Melanesians.

- Fourth, the dental morphology of Ds shows no evidence of any derived features seen in Neanderthals. In fact, dental remains from the Sima de los Huesos of Atapuerca, dated to 430 Ka, already carry Neanderthal-like morphological features that are not seen in the Denisova molar.

- Conclusion: on the Eurasian mainland there existed at least two forms of archaic hominins in the Upper Pleistocene: a western Eurasian form with N morphological features, and an eastern form to which the Denisova individuals belong.


Neandertals
Denisovans
?
~500 kyr

Ns and Ds



nuclear DNA of Denisova 3

- nDNA of Denisova 3—Denisovans and Neanderthals were more closely related to each other than they were to modern humans.

- Denisovans/Neanderthals split from modern humans about 760 to 550 Ka ago

- Using a mutation rate of $1\text{x}10^{-9}$ or $0.5\text{x}10^{-9}$ per base pair (bp) per year, the Neanderthal/Denisovan split occurred around either 236–190 Ka or 473–381 Ka ago, respectively.

nuclear DNA of Denisova 3

- Using 1.1x10^{-8} per generation with a new generation every 29 years, the time is 744 Ka ago. Using 5x10^{-10} nucleotide site per year, it is 616 Ka ago.

- Using the latter dates, the split had likely already occurred by the time hominins spread out across Europe.

- The more divergent Denisovan mtDNA has been interpreted as evidence of admixture between Denisovans and an unknown archaic human population, possibly a relict *H. erectus* or *H. erectus*-like population

3. A proximal pedal phalanx of a Paleolithic hominin from Denisova cave, Altai - M. B. Mednikova, 2011

- Denisova 5: A toe bone (pedal phalanx) of Homo from layer 11 at Denisova Cave

- Found in the same layer as the original finger bone (manual phalanx).
- The new specimen has been identified as a left proximal phalanx of the fourth or fifth pedal digit, apparently that of an adult.

- The bone is rather elongated and has a very robust and broad shaft; certain traits that are intermediate between the Neanderthal and the early modern morphology.

4. Denisova Admixture and the First Modern Human Dispersals into Southeast Asia and Oceania - David Reich, et al., 2011

- Denisova admixture in 33 additional MH populations from Asia and Oceania.

- D DNA in Aboriginal Australians, Near Oceanians, Polynesians, Fijians, east Indonesians, and Mamanwa (Philippine “Negrito” group); but not mainland East Asians, or western Indonesians.

- Suggests that relatives of present-day East Asians were not in Southeast Asia when the Denisova gene flow occurred.

D. Reich, 2011: Ds had large territory

- Fact that descendants of the earliest inhabitants of Southeast Asia do not all harbor Denisova admixture is inconsistent with a history in which the Denisova interbreeding occurred in mainland Asia and then spread over Southeast Asia, leading to all its earliest modern human inhabitants.
- Instead, the data can be most parsimoniously explained
  - if the Denisova gene flow occurred in Southeast Asia itself.
  - Thus, archaic Denisovans must have lived over an extraordinarily broad geographic and ecological range, from Siberia to tropical Asia.

D. Reich, 2018

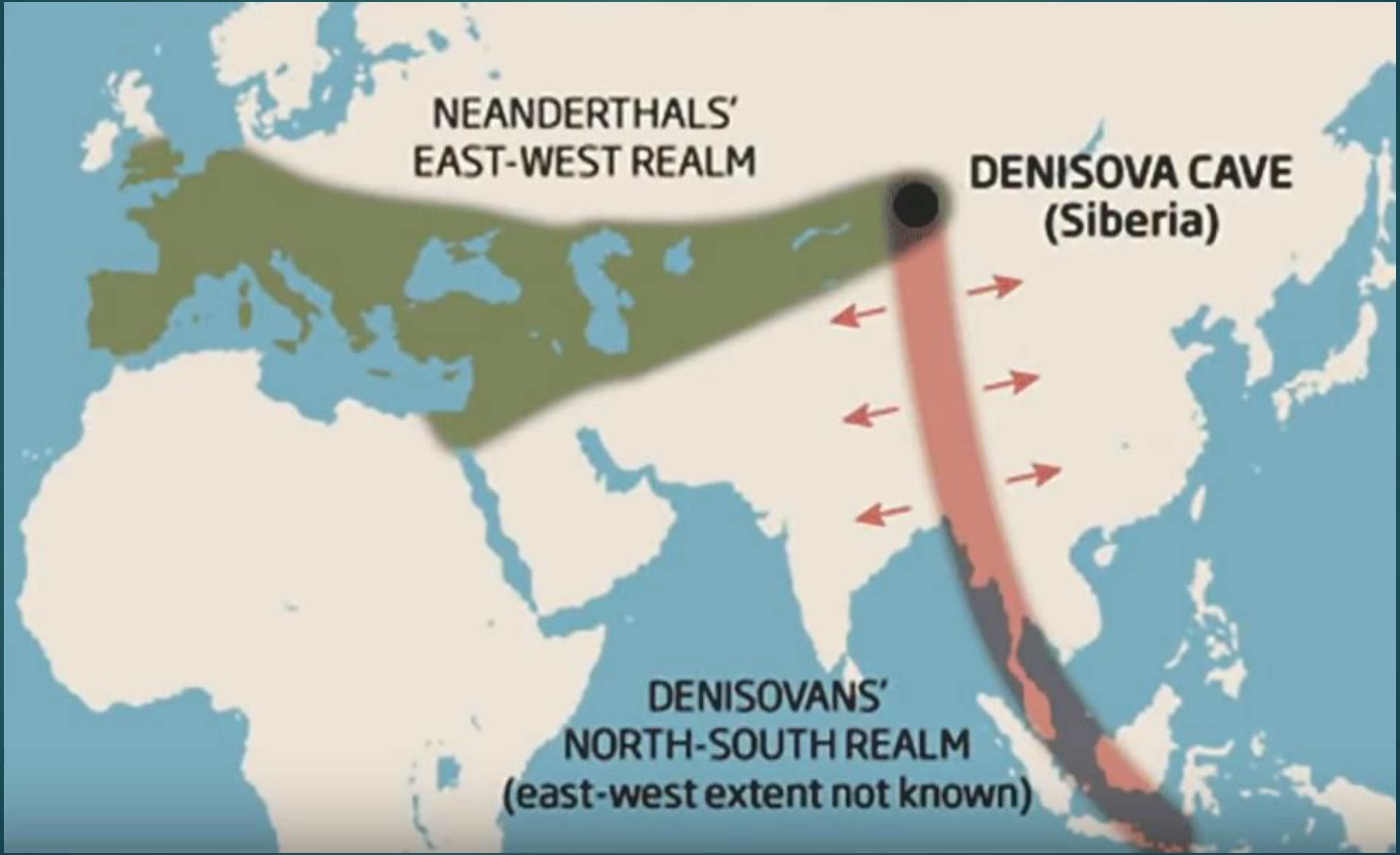
- Ds were genetically closer to New Guineans than to any population from mainland Eurasia: NG ancestors had interbreed with Ds.
- Where did Ds and NGs intermix? First guess: mainland Asia = Wrong
- Lack of much D DNA in mainland E or S Asia;
  - largest amounts of D DNA in islands of SE Asia, esp. in Philippines and New Guinea and Australia
  - east of Huxley's line, deep ocean trench, then sea levels 100 meters lower
- Better theory: interbreeding near the islands (on islands themselves or in mainland SE Asia) but in either case in tropical region; but no artifacts in SE Asia; more likely admixture in S China or mainland SE Asia.
- Dali, Jinniushan, Maba fossils are possible D like at 200 Ka; but no DNA

Reich, 2011

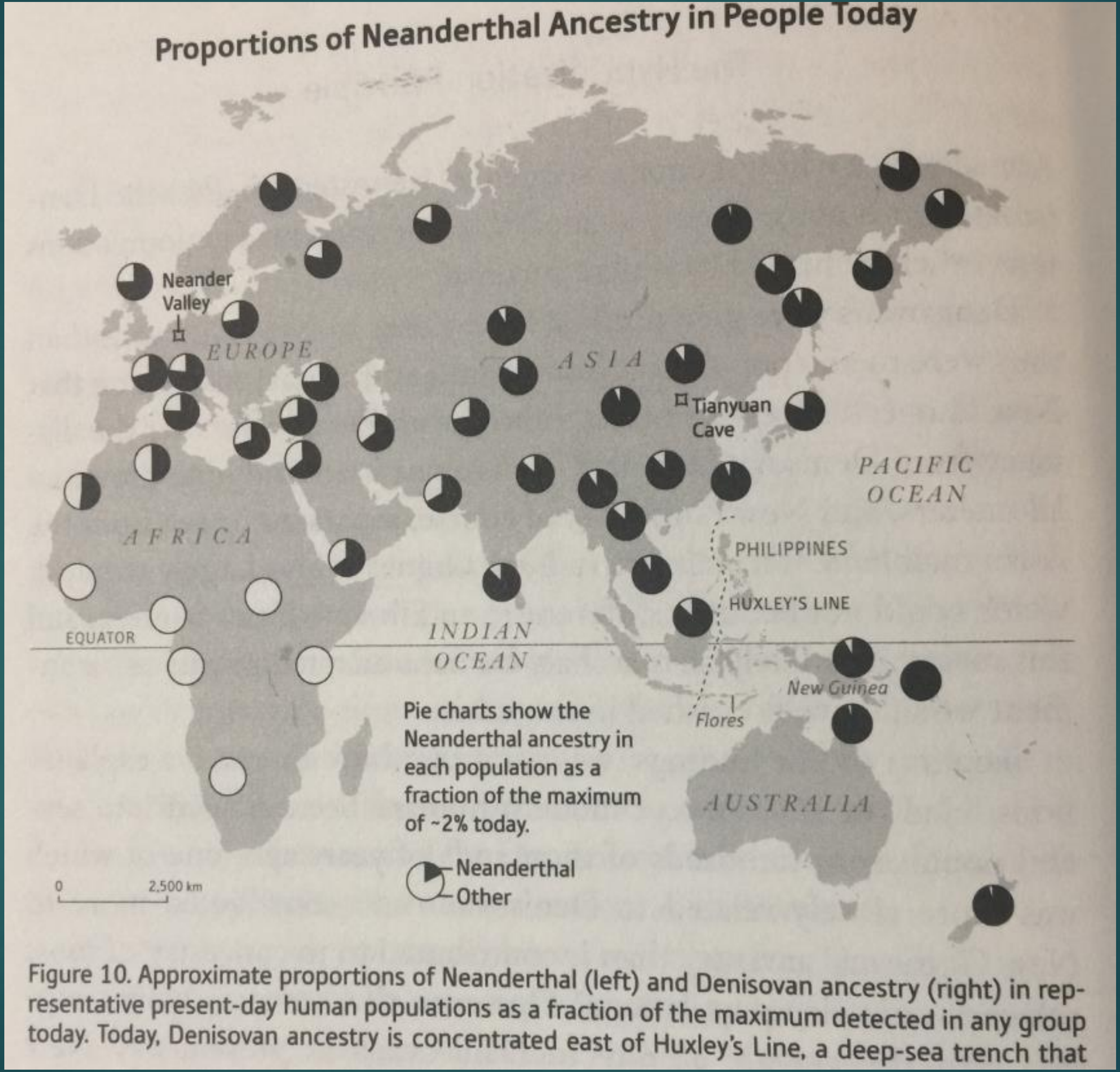
- The recent finding that Near Oceanians (New Guineans and Bougainville Islanders) have received 4%–6% D DNA

- New Guineans and Australians: Denisova gene flow into the common ancestors of Australians and New Guineans prior to their entry into Sahul (Pleistocene New Guinea and Australia), ~ 44 Ka (now 65 Ka)

Range of N and Ds



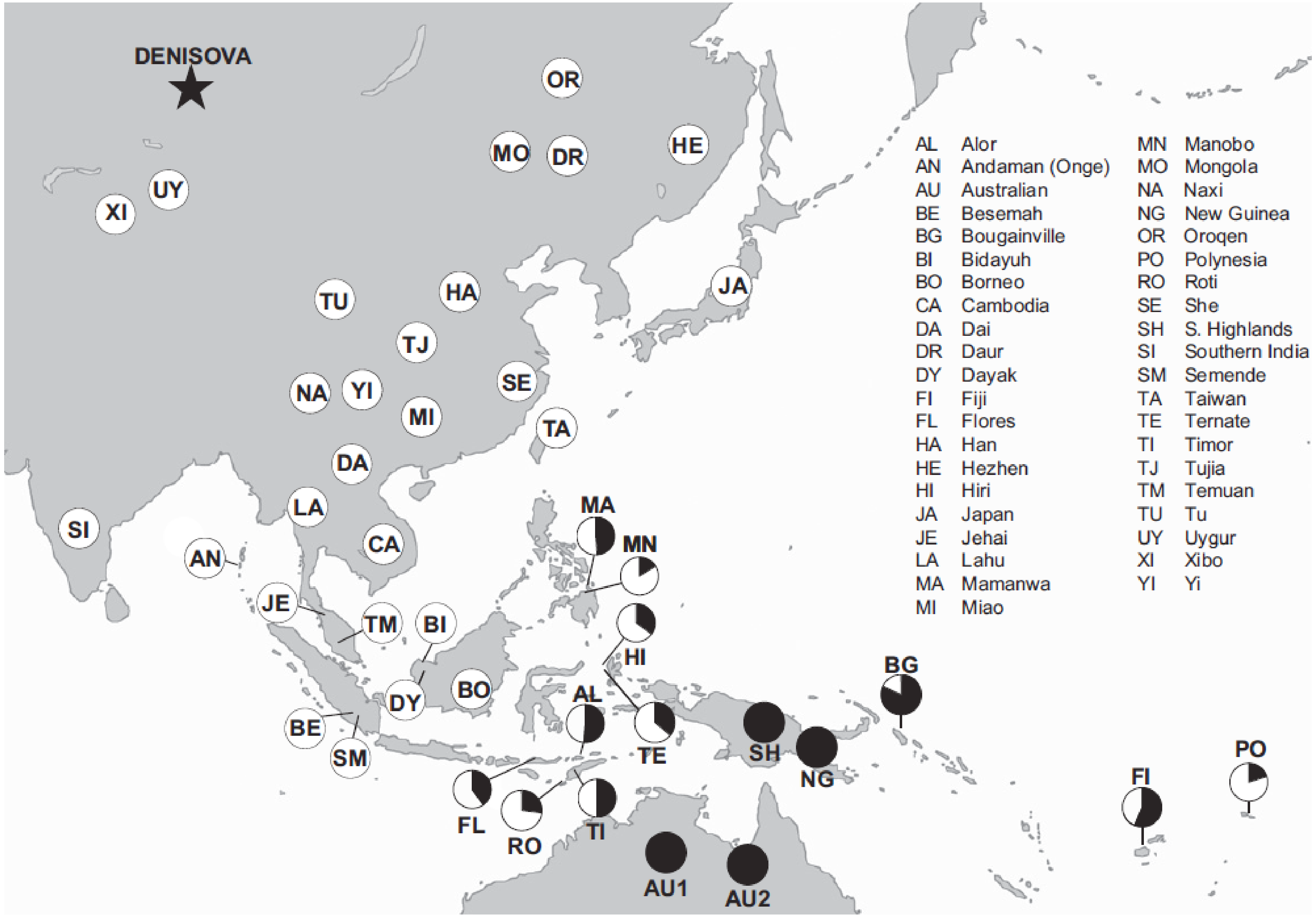
Neandertal
Ancestry
In MHs



Proportions of Neanderthal Ancestry in People Today

Pie charts show the Neanderthal ancestry in each population as a fraction of the maximum of ~2% today.

Figure 10. Approximate proportions of Neanderthal (left) and Denisovan ancestry (right) in representative present-day human populations as a fraction of the maximum detected in any group today. Today, Denisovan ancestry is concentrated east of Huxley's Line, a deep-sea trench that



AL	Alor	MN	Manobo
AN	Andaman (Onge)	MO	Mongola
AU	Australian	NA	Naxi
BE	Besemah	NG	New Guinea
BG	Bougainville	OR	Oroqen
BI	Bidayuh	PO	Polynesia
BO	Borneo	RO	Roti
CA	Cambodia	SE	She
DA	Dai	SH	S. Highlands
DR	Daur	SI	Southern India
DY	Dayak	SM	Semende
FI	Fiji	TA	Taiwan
FL	Flores	TE	Ternate
HA	Han	TI	Timor
HE	Hezhen	TJ	Tujia
HI	Hiri	TM	Temuan
JA	Japan	TU	Tu
JE	Jehai	UY	Uygur
LA	Lahu	XI	Xibo
MA	Mamanwa	YI	Yi
MI	Miao		

Figure 1. Denisovan Genetic Material as a Fraction of that in New Guineans

Black = proportion of Denisovan DNA

Reich et al., 2011

Reich, 2011

- Archaeologic evidence of an early southern route migration leading to the colonization of Sahul and East Asia

- Interbreeding might have occurred in Southeast Asia itself.

- Our evidence of a Southeast Asian location for the Denisovan admixture thus suggests that Denisovans were spread across a wider ecological and geographic region—from the deciduous forests of Siberia to the tropics—than any other hominin with the exception of modern humans

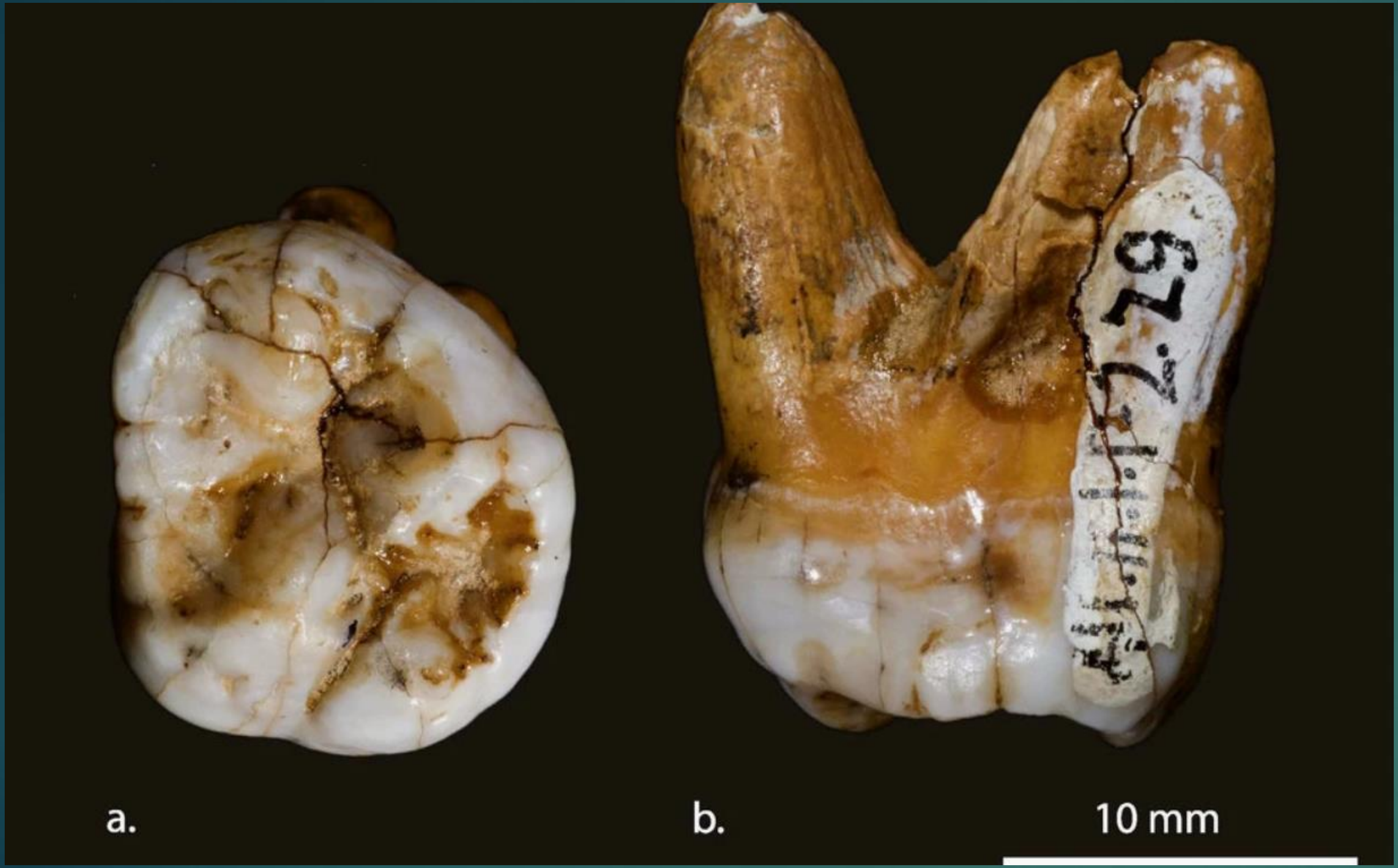
Derevianko’s Institute in Novosibirsk, 40 years of excavations:
Now where did I put that tooth?

Two new teeth: Denisova 4 & 8

- In July 2011, three years after Tsybankov unearthed the finger bone, Anatoly Derevianko organized a scientific symposium at the archaeological camp a few hundred yards from Denisova cave...
- The year before, two molars had been found to contain DNA similar to that of the finger bone.
- The first tooth had discovered among the boxed specimens from Denisova housed at Derevianko's institute in Novosibirsk.

Two new teeth

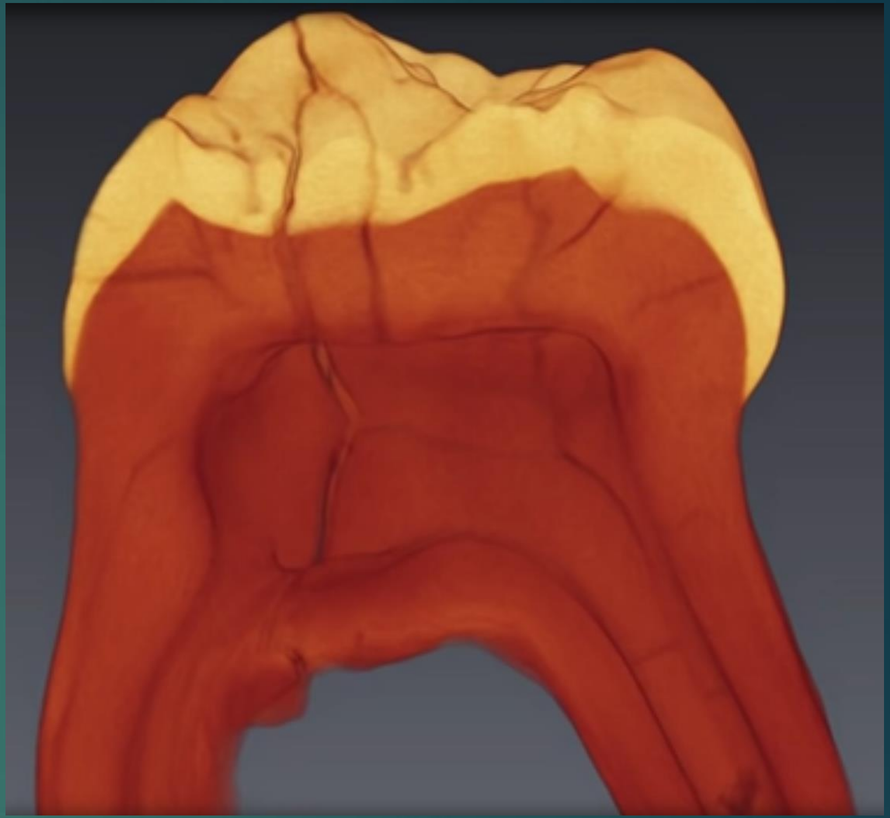
- It was bigger than either a modern human or a Neanderthal tooth, in size and shape resembling the teeth of much more primitive members of the genus Homo who lived in Africa millions of years ago.
- Tooth was 50% larger than N or MH molars. Crown traits and roots were also different; roots were strongly divergent (unlike the closely spaced or fused N roots).
- The second molar had been found in 2010 in the same cave chamber that had yielded the finger bone—indeed, near the bottom of the same 30 to 50 Ka deposits, called Layer 11.



Denisova 4 tooth

Denisova 8

Massive teeth



Yellow = enamel
Orange = dentine
Huge pulp cavity for blood and nerve

Ns had thin enamel

Denisova 4

After DNA extraction

Big teeth

- Remarkably, the second tooth (Denisova 8) was even bigger than the first, with a chewing surface twice that of a typical human molar. It was so large that Max Planck paleoanthropologist Bence Viola mistook it for a cave bear tooth.
- Only when its DNA was tested was it confirmed to be Denisovan, as the scientists had taken to calling the new ancestors.
- “It shows you how weird these guys are," Viola said at the symposium. “At least their teeth are just very strange.”
- The two teeth were not published until 2017.

Denisovan teeth are big!

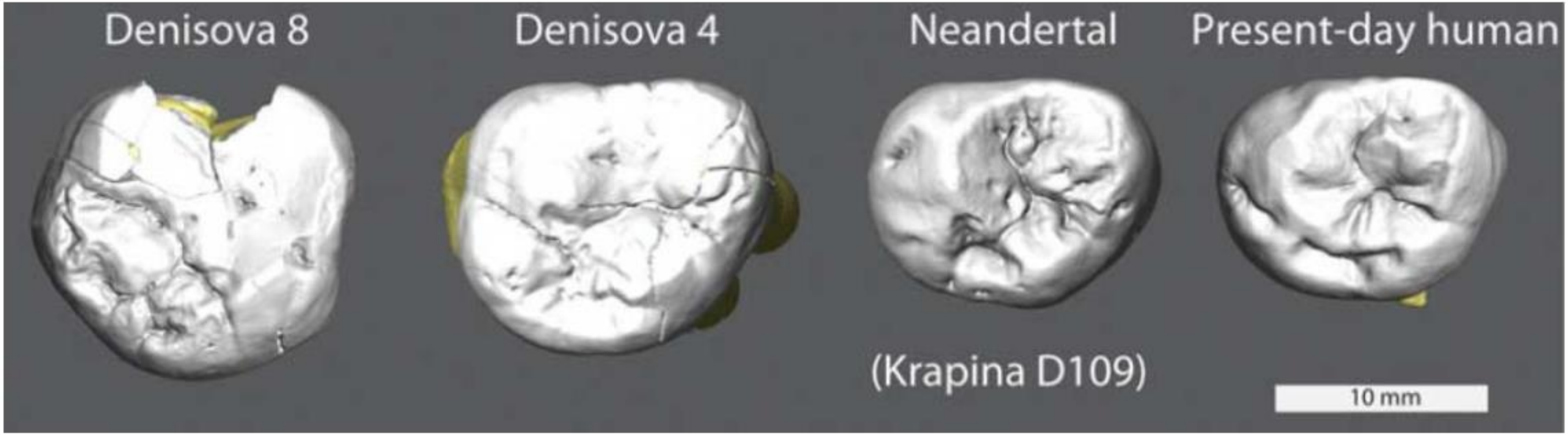


Figure 1 from Sawyer and colleagues 2015. Original caption: "Fig. 1. Occlusal surfaces of the Denisova 4 and Denisova 8 molars and third molars of a Neandertal and a present-day European."

Bigger than most living humans, bigger than any Neandertals, as big as some third molars of *Australopithecus*.

Denisova 8

Denisova Cave: Paleolithic telenovela characters

- Denisova 3: a young female
- Denisova 4 an adult male
- In 2010, Denisova 8: third Denisovan known
- 2012: Denisova 4: third molar
- 2010/2014: Denisova 5, toe bone; the Altai Neandertal genome;

D DNA has low genetic diversity

- Denisovan DNA: The two molars (Denisova 4 and 8) clearly group with the original pinky bone (Denisova 3) genome in their nuclear and mitochondrial DNA.
- Denisova 8 is an outlier to the other two, more different from them in mtDNA sequence than any Neandertals are from each other; lived some 60 Ka earlier in time than first two.
- Denisovans and Neandertals have low genetic diversity relative to today's people
- Ds: a bit less inbred than Neandertals

Teamwork. Anthropologist Maria Mednikova (*left*) analyzed fossils, and geneticists Susanna Sawyer and David Reich studied the DNA of the ancient Denisovans

Denisova 5: N toe bone – *Altai Neanderthal*

- In 2010, a toe bone was discovered in the cave, in layer 11.4 of the East Gallery, and therefore contemporary with the Denisovan 3 finger bone.

- mtDNA suggested it belonged to a Neanderthal, not a Denisovan.[Later analysis confirmed the toe bone as coming from a Neanderthal.

- Denisova 5: Produced the first high-coverage genome of Neanderthals was taken from this toe bone.

- This Neanderthal is referred to as the *Altai Neanderthal*. Dated to 120 Ka.

Maria Mednikova: The Altai Neandertal
Neandertal toe bone

- Russian Academy of Science, Moscow
- Denisova 5: Originally thought to be a Denisovan toe bone, 120K, but DNA showed it was Neandertal
- Found in same layer 11 as Denisova 3 finger, but distinct from it

Differences between *Vindija* and *Altai* Neandertals

- First high coverage genomes:
- Eastern, Siberian: *Altai* Neanderthal from Denisova Cave.

- Western: *Vindija 33.19:* first high coverage N genome from Vindija Cave, Croatia (bear cave). At around 30-fold coverage; 47 Ka; female

- 4 N genomes from *Vindija:* genetically closest to each other than any other Neanderthal individuals on record

- *Mezmaiskaya* Neandertal from Mezmaiskaya cave in the Caucasus; closer to Vindija.

Altai Neanderthal = Eastern Siberian N

- Western Ns: other Neanderthals for which nuclear DNA has been recovered are all genetically closer to each other than to the *Altai Neanderthal*.

- Modern humans and Ust'-Ishim man share more alleles with all other Neanderthals than with the *Altai Neanderthal*

- The admixture event of Ns and MHs likely took place after the split of the lineage of the *Altai Neanderthal* from that of other Neanderthals.

New DNA extraction method leads to full D genome

- In 2012, Pääbo's group published a new complete version of the Denisova 3 finger bone's genome—the only high-coverage Denisovan genome that is currently available -- equivalent to recent current MH genome that has been sequenced.

- Matthias Meyer: a German postdoc in Pääbo’s lab invented method

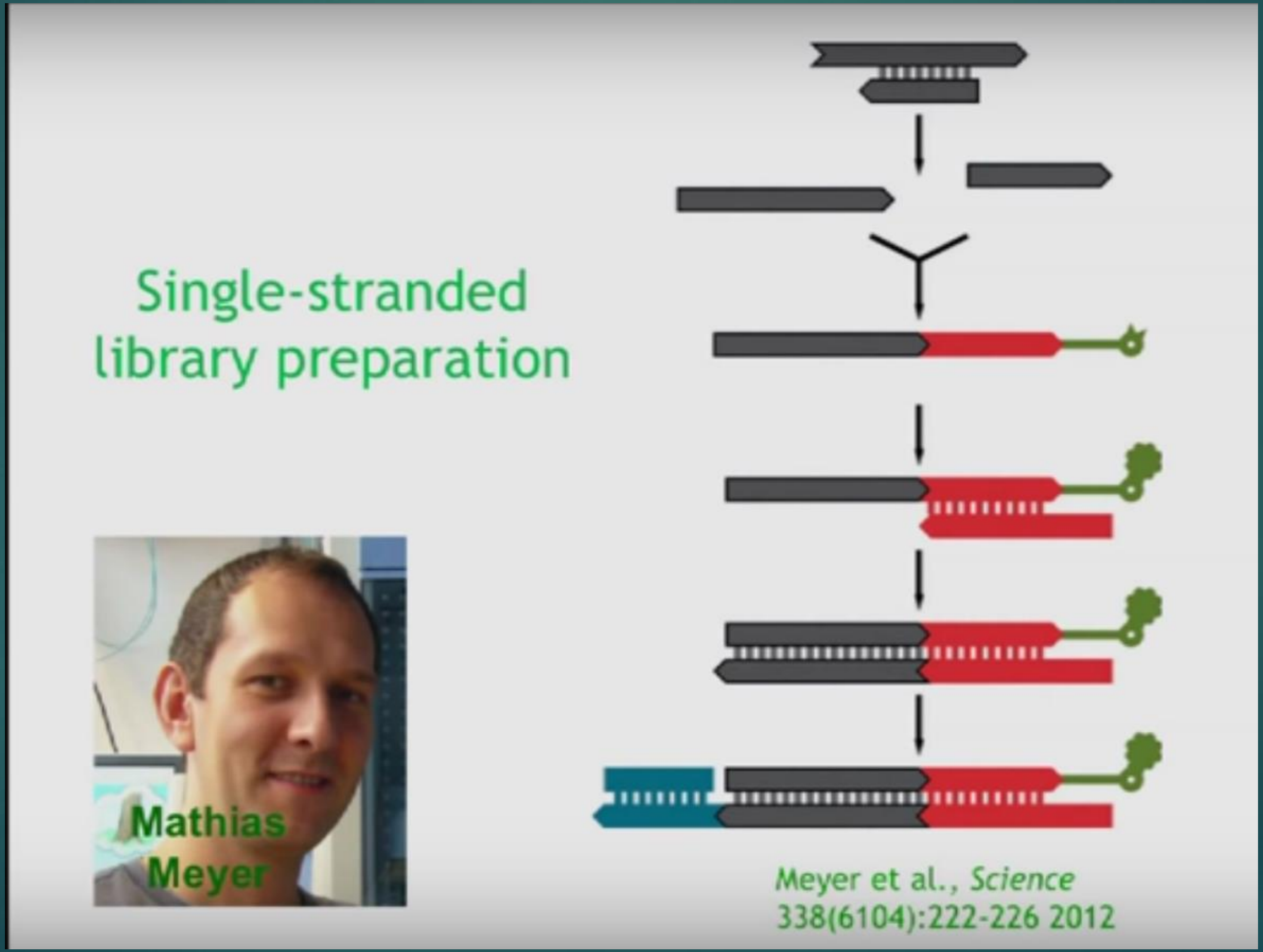
- DNA consists of two interlocking strands—the familiar double helix. Previous methods for retrieving DNA from fossil bone could read out sequences only when both strands were preserved.

6. A high-coverage genome sequence from an archaic Denisovan individual - Matthias Meyer, 2012

- 2012: a new DNA library preparation method; reconstructed a high-coverage (30×) genome sequence of a Denisovan.

- Allows a direct estimation of Denisovan heterozygosity indicating that genetic diversity in these archaic hominins was extremely low.
- It also allows
  - tentative dating of the specimen on the basis of "missing evolution" in its genome
  - detailed measurements of D and N admixture into present-day MHs
  - produced a catalog of genetic changes that swept to high frequency in modern humans since their divergence from Denisovans.

Better DNA replication technology: use of only single strand of DNA: increased Denisovan DNA data – 30-fold coverage (can see any base 30 times)

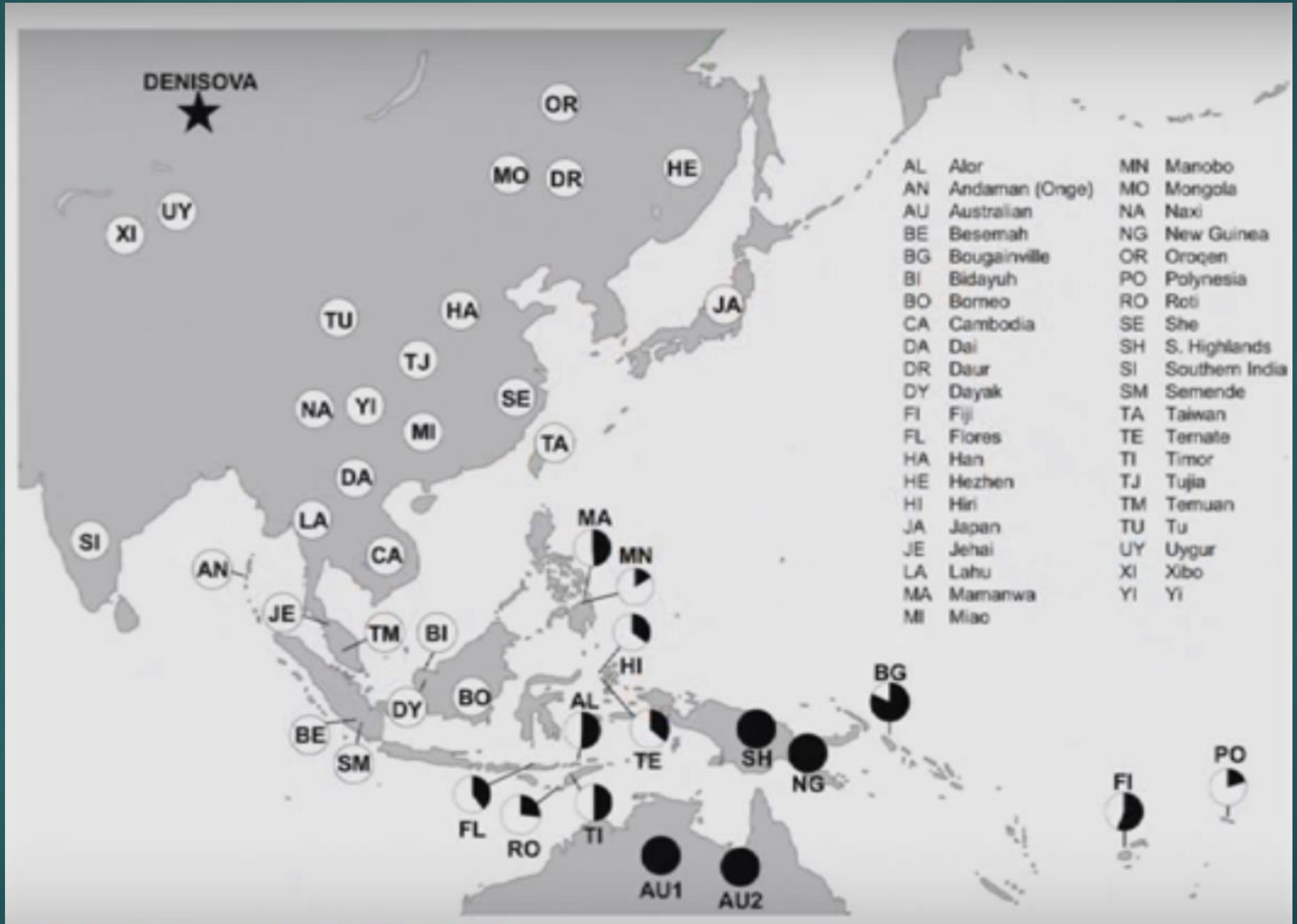


Single-stranded library preparation

Mathias Meyer

Meyer et al., *Science* 338(6104):222-226 2012

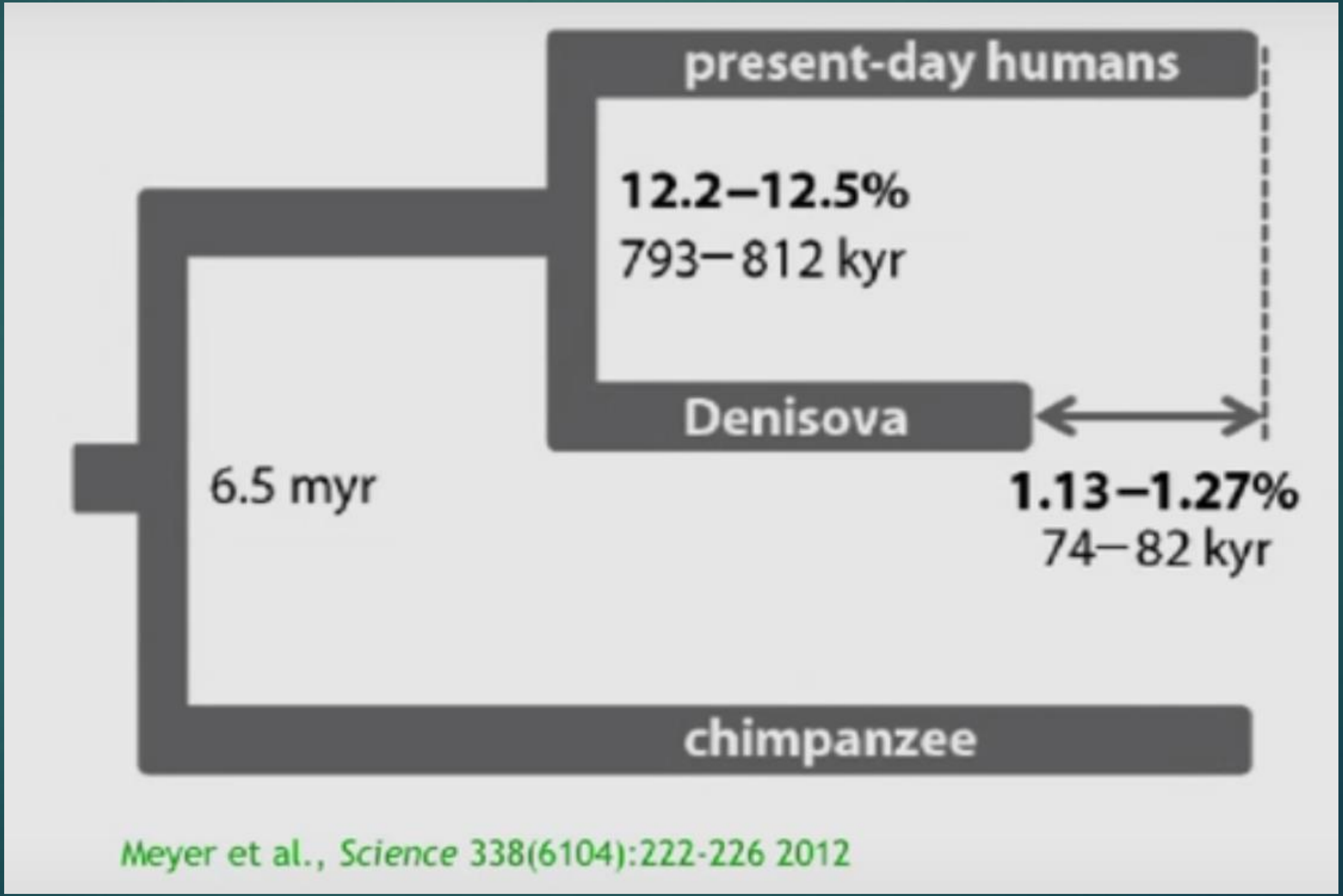
D signal operates mostly east of Wallace line (Australia & Polynesians) but much less in mainland Asia



AL	Alor	MN	Manobo
AN	Andaman (Onge)	MO	Mongola
AU	Australian	NA	Naxi
BE	Besemah	NG	New Guinea
BG	Bougainville	OR	Oroqen
BI	Bidayuh	PO	Polynesia
BO	Borneo	RO	Roti
CA	Cambodia	SE	She
DA	Dai	SH	S. Highlands
DR	Daur	SI	Southern India
DY	Dayak	SM	Semende
FI	Fiji	TA	Taiwan
FL	Flores	TE	Ternate
HA	Han	TI	Timor
HE	Hezhen	TJ	Tujia
HI	Hiri	TM	Temuan
JA	Japan	TU	Tu
JE	Jehai	UY	Uygur
LA	Lahu	XI	Xibo
MA	Mamanwa	YI	Yi
MI	Miao		

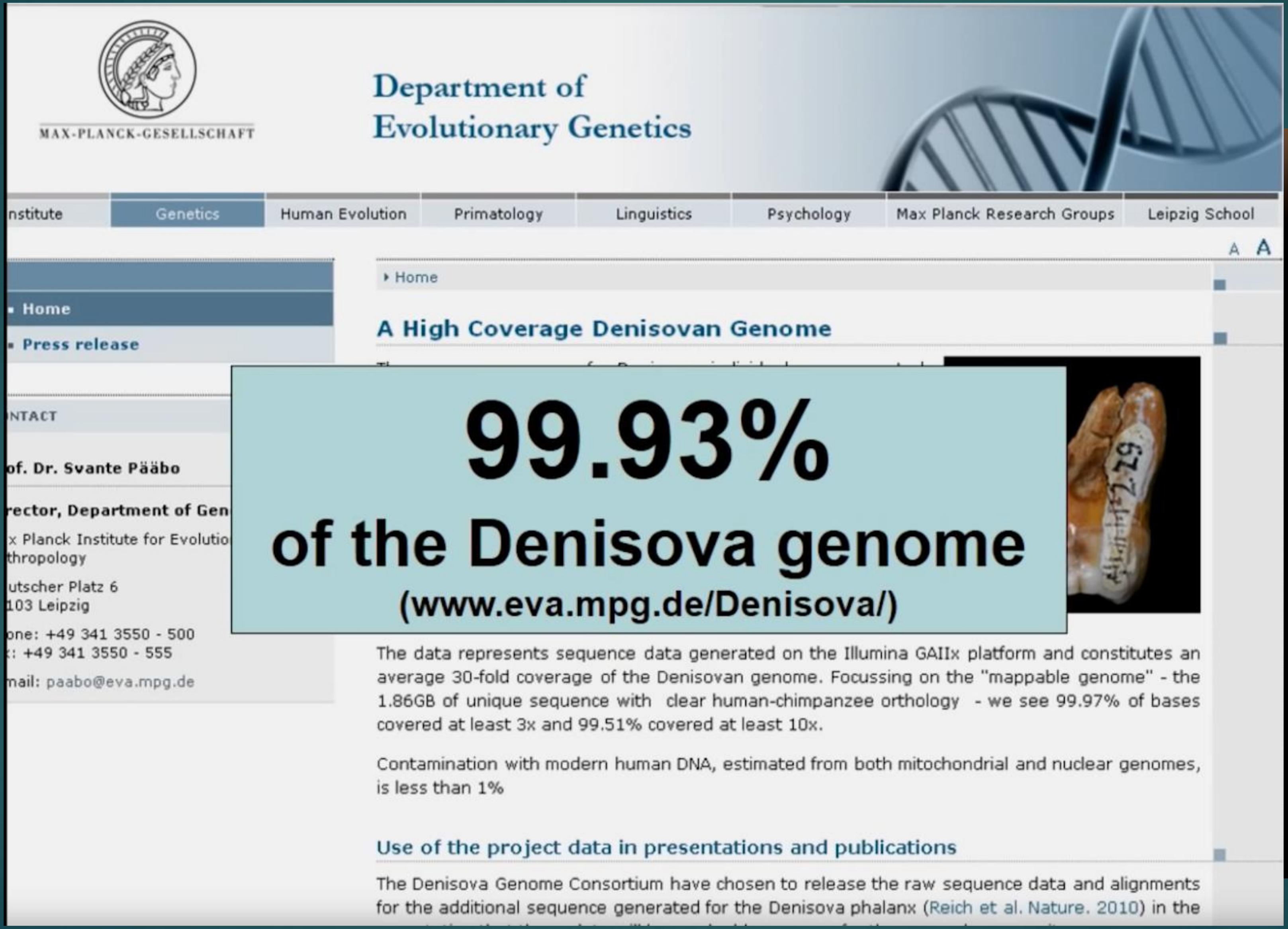
Reich et al., *AMHJ* 89(4):516 2011

“Missing evolution”: Rather than having excess branch leading to archaics, Ds have shorter branch: estimation of mutations that is new way to date bones based on missing mutations



Meyer et al., *Science* 338(6104):222-226 2012

Denisova Cave pinky bone: Denisovan genome



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A High Coverage Denisovan Genome

99.93%

of the Denisova genome

(www.eva.mpg.de/Denisova/)

The data represents sequence data generated on the Illumina GAIIx platform and constitutes an average 30-fold coverage of the Denisovan genome. Focussing on the "mappable genome" - the 1.86GB of unique sequence with clear human-chimpanzee orthology - we see 99.97% of bases covered at least 3x and 99.51% covered at least 10x.

Contamination with modern human DNA, estimated from both mitochondrial and nuclear genomes, is less than 1%

Use of the project data in presentations and publications

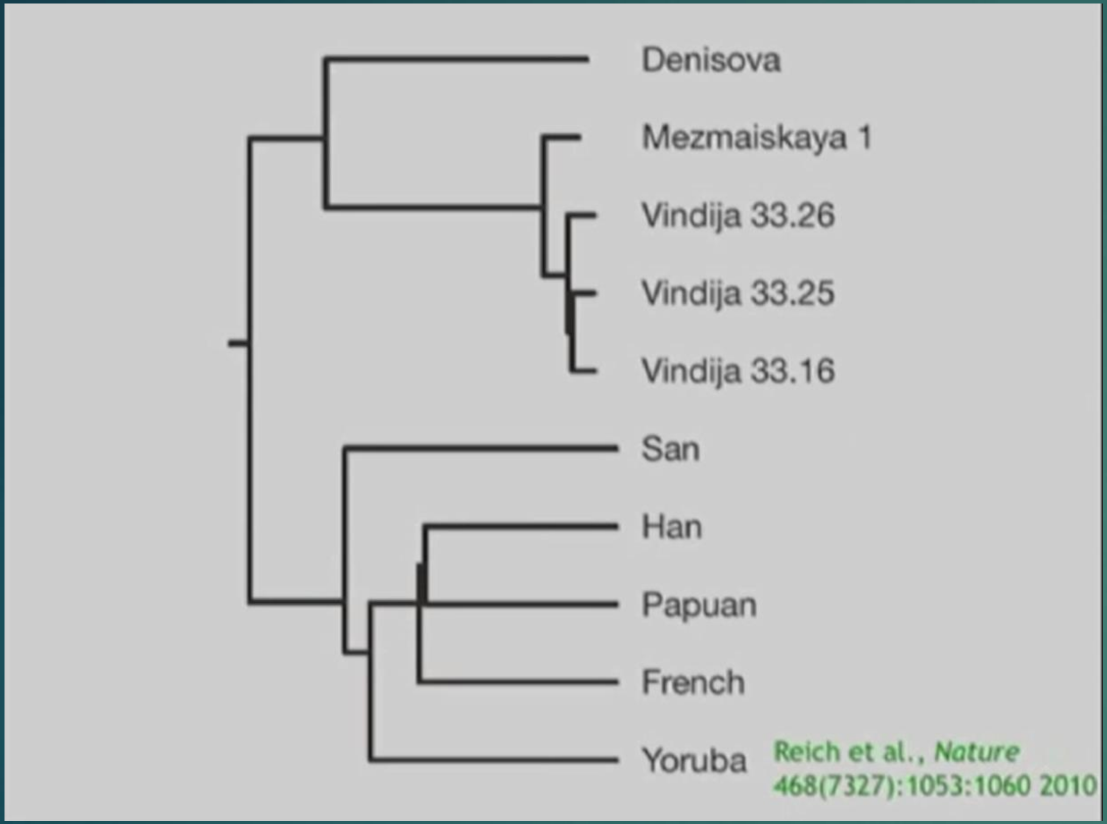
The Denisova Genome Consortium have chosen to release the raw sequence data and alignments for the additional sequence generated for the Denisova phalanx (Reich et al. Nature. 2010) in the

2012: A High-Coverage Genome Sequence from an Archaic Denisovan Individual

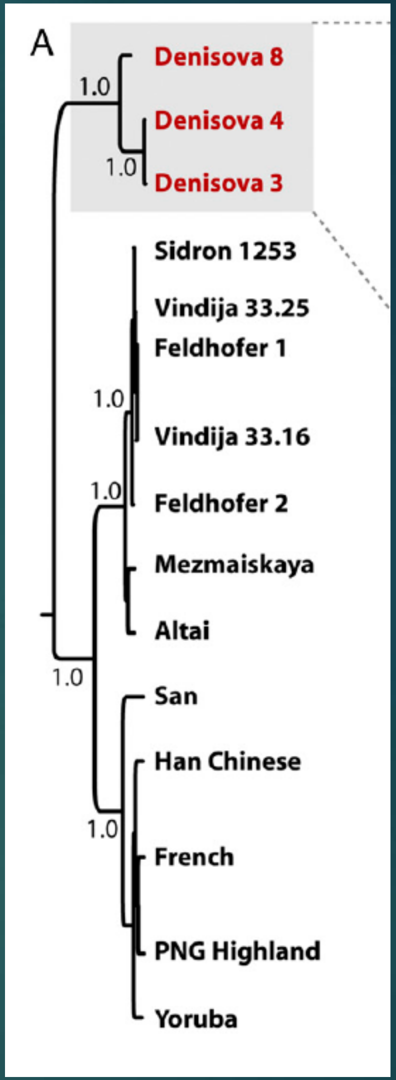
- Used new single strand method
- 30-fold coverage of the genome was generated using the Illumina GAIIx sequencing platform.
- This data is freely available without passwords.
- New technique obtained 99.93% of D genome
- Genetic diversity in these archaic hominins was extremely low
- > than 200 Ka separate D from N

Matthias Meyer et al. Science 338, 222 (2012)

N & D divergence



Reich et al., *Nature* 468(7327):1053:1060 2010



2015 study

- D are sister group to N in nuclear DNA, but deeply divergent mDNA; more distinct from N than any 2 MH populations today, but more closely related to N than to MH

New single strand method

- His method could discriminate heterozygosity: nucleotide sites that are different between a person’s maternal and paternal genomes

- One immediate revelation was how little variation there was between the parents’ genomes—about 30% as much as there is between any two living humans. Parents were highly related.

Meyer, 2012: high coverage D genome

- It is <u>possible to determine dates of fossils based on genome sequences</u>:
- Phalanx dated to between 82-74 Ka
- 6.0% of the genomes of present-day Papuans derive from Denisovans; 3% from Neandertal admixture
- Denisovans share more alleles with Papuans than with mainland Eurasians
- Denisovan individual carried alleles that in present-day humans are associated with dark skin, brown hair, and brown eyes

Meyer, 2012: high coverage D genome helps reveal MH recipe

- 111,812 single-nucleotide changes and 9499 insertions and deletions where MHs are fixed for the derived state
- Among conserved sites:
  - 8 affect genes that are associated with brain function or nervous system development.
  - 4 of these are involved in axonal and dendritic growth and synaptic transmission,
  - 2 have been implicated in autism.
  - CNTNAP2 is also associated with susceptibility to language disorders and is regulated by FOXP2, a transcription factor involved in language and speech development as well as synaptic plasticity.
  - Tempting to speculate that crucial aspects of synaptic transmission may have changed in modern humans

Denisovans: A small population with low genetic diversity

- Differences sprinkled across the genomes ruled out inbreeding as cause of low D genetic diversity: If the girl's parents had simply been closely related by interbreeding, they would have had huge chunks of exactly matched DNA, which Ds did not have.

- Denisovan population represented by this fossil had never been large enough to have developed much genetic diversity.

- It suffered a drastic decline sometime before 125 Ka ago—the little girl in the cave may have been among the last of her kind.

Denisovan nuclear DNA

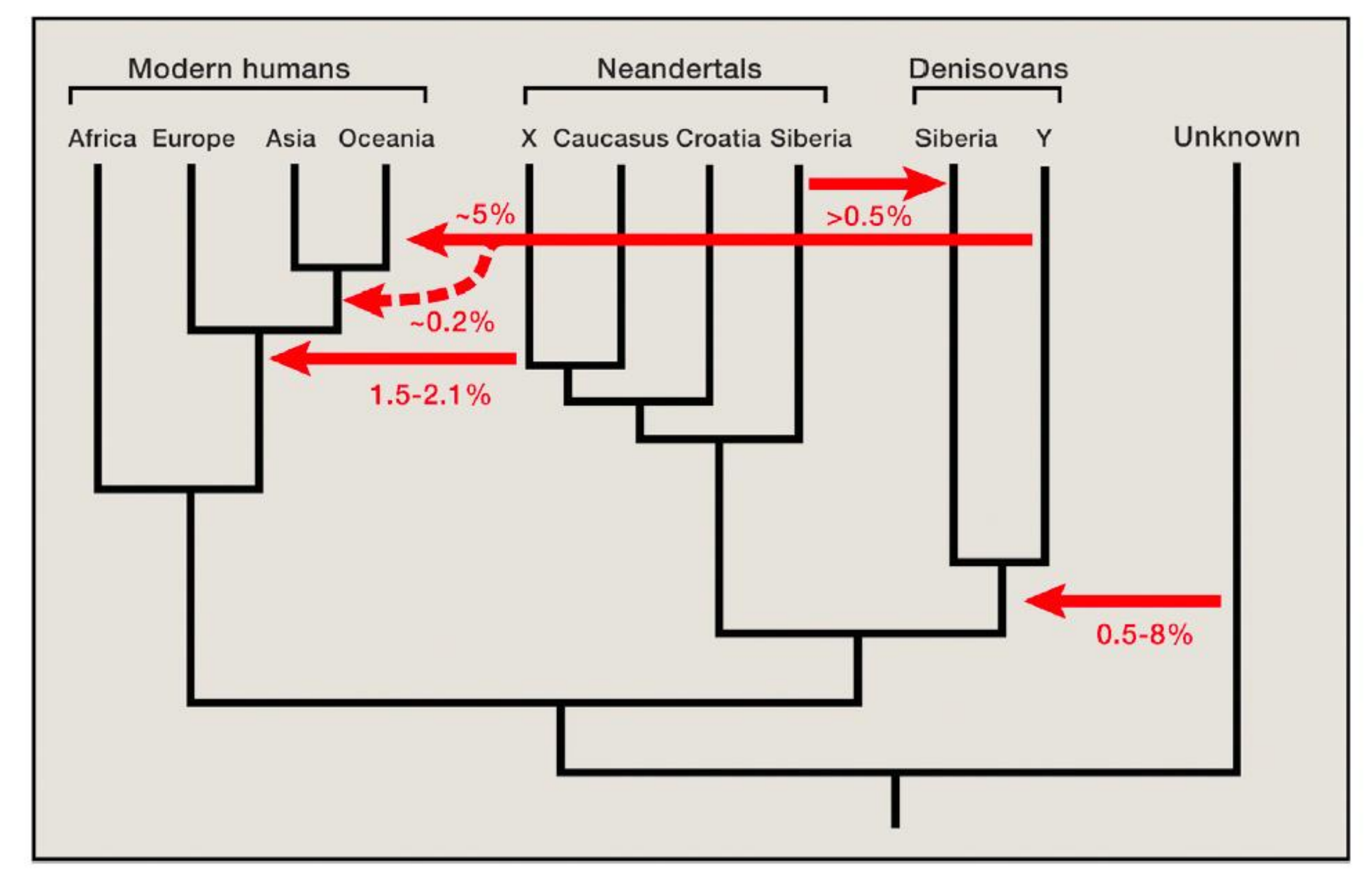
- Comparison with 5 MHs in N analysis: the D share more derived SNP alleles with the Papuan individual than with Chinese, European or 2 African groups.
- Found very few Y chromosomal fragments; X –Woman was a girl
- Ancestor of Denisovans and Ns first diverged from MHs and then Ds and Ns diverged (D and N more closely related to each other than to MHs)

7. The complete genome sequence of a Neanderthal from the Altai Mountains – Kay Prüfer, et al., 2013

- A high-quality genome sequence of a Neanderthal woman from Denisova, Siberia. Her parents were related at the level of half-siblings and that mating among close relatives was common among her recent ancestors. Also sequenced the genome of a N from the Caucasus with low coverage.
- Several gene flow events occurred among Neanderthals, Denisovans and early modern humans, possibly including gene flow into Denisovans from an unknown archaic group.
- Interbreeding, albeit of low magnitude, was a feature of the Late Pleistocene.

Neandertal and Denisovan Genomes

- Ns and Ds are more closely related to one another than they are to modern humans.
- At least two events of gene flow from these groups into modern humans have been found:
  - Neanderthal-derived DNA in people outside Africa is 1.5–2.1%
  - Denisovan gene flow into the common ancestors of Australians and New Guineans.
- Genomes allows for analyses of gene flow into the archaic lineages.
  - evidence for two gene flow events into Denisovans:
    - Gene flow from Neandertals and
    - gene flow from an unknown group of archaic humans.



Four, Possibly Five, Gene-Flow Events that Have Been Documented by Analyses of the Genomes of Archaic and Present-Day Humans
X = Neandertal group that mixed with Eurasian modern humans,
Y = Denisovan group that contributed to ancestors of present-day people in Oceania.
The dotted arrow = mainland Asia contains small amounts of DNA from Denisovans that may have been contributed by a separate mixing event(s) or by the same event that affected Oceanian ancestors (Prüfer et al., 2014)

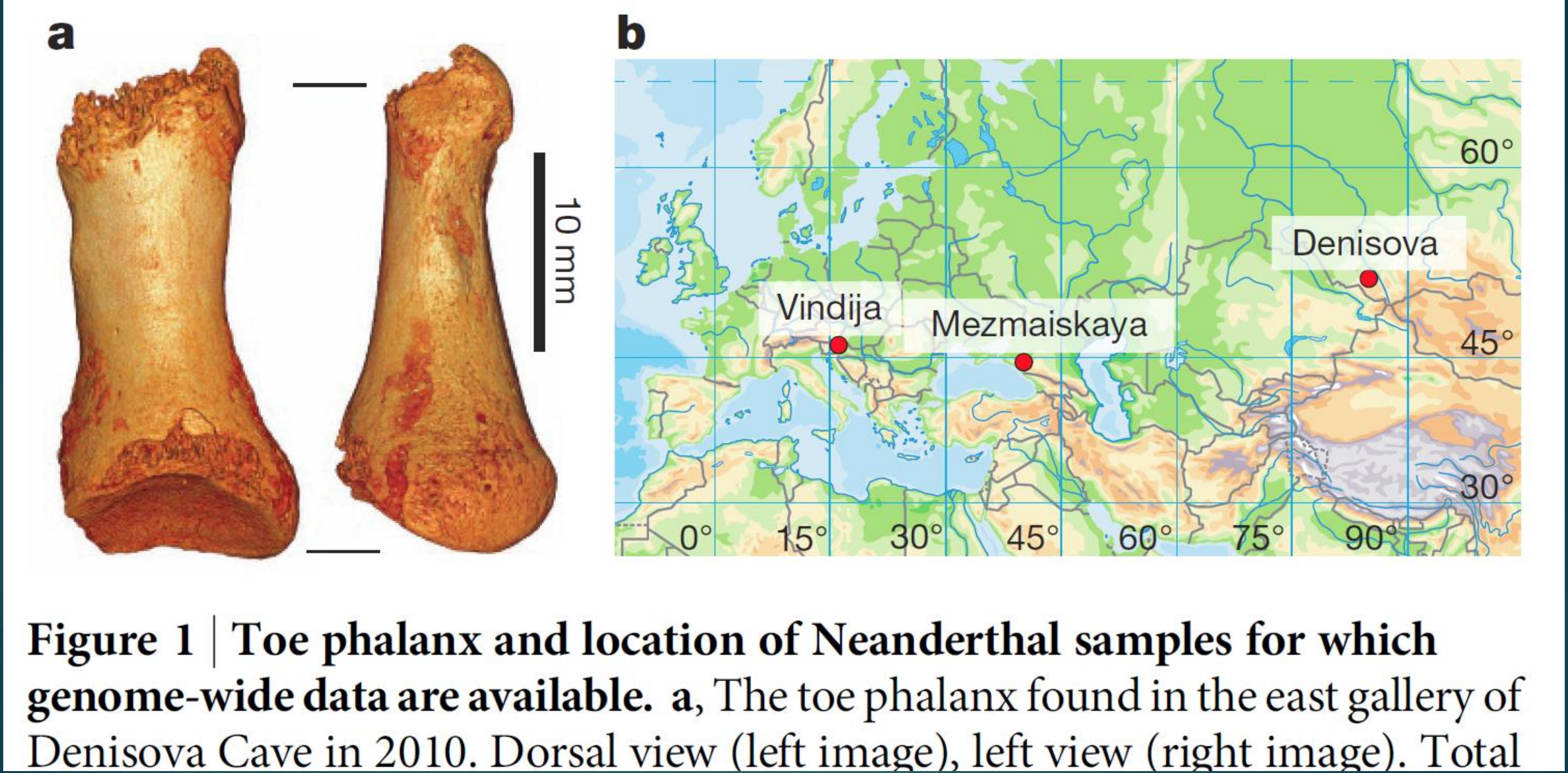


Figure 1 | Toe phalanx and location of Neanderthal samples for which genome-wide data are available. a, The toe phalanx found in the east gallery of Denisova Cave in 2010. Dorsal view (left image), left view (right image). Total

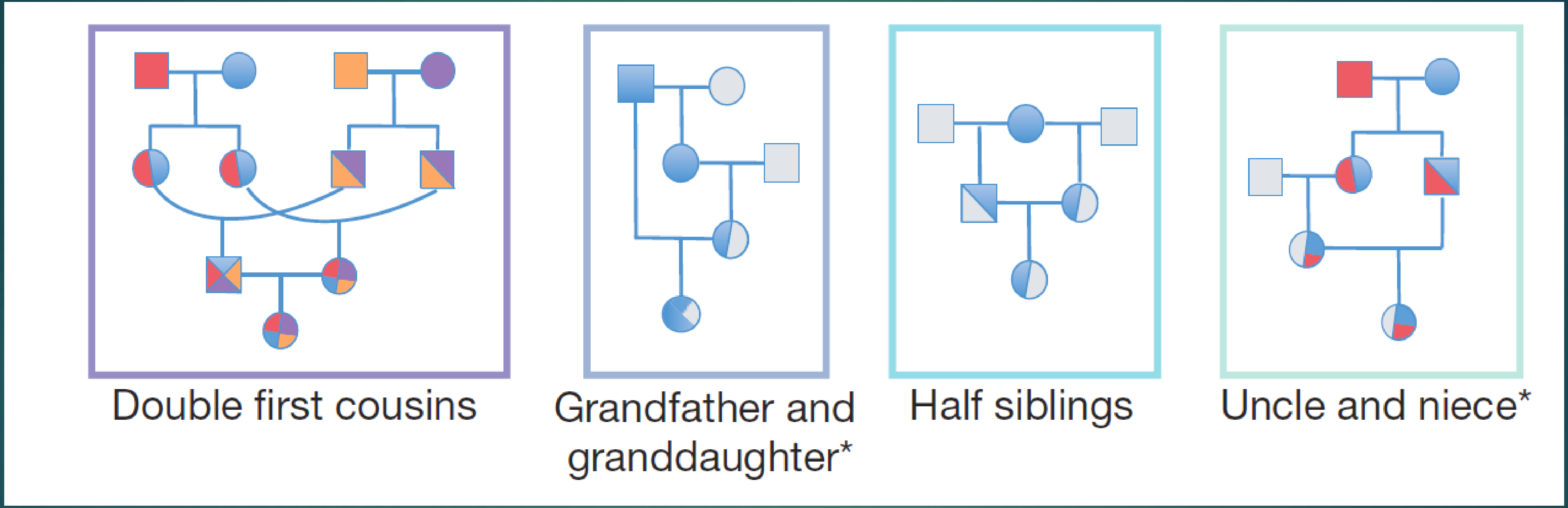
K. Prüfer, 2013: N genome from Denisova 5, toe bone

- A phylogenetic tree shows that the Neandertal toe phalanx mtDNA shares a common ancestor with six previously published Neanderthal mtDNAs

- Toe mtDNA is most closely related to the mtDNA from infant 1 from Mezmaiskaya Cave in the Caucasus

- Population split of
  - Neanderthals and Denisovans from modern humans to 765-550 Ka ago,
  - Neanderthals from Denisovans to 473-445 Ka ago

N Interbreeding: Relationship of parents of Altai Neanderthal genome



Altai Neanderthal genome contains several long runs of homozygosity, indicating that her parents were closely related: parents of this N individual were either half-siblings who had a mother in common, double first cousins, an uncle and a niece, an aunt and a nephew, a grandfather and a granddaughter, or a grandmother and a grandson

K. Prüfer, 2013: low genetic diversity in Ns and Ds

- Thus, heterozygosity in Neanderthals as well as Denisovans:
    - lower than in present-day humans
    - among the lowest measured for any organism

- Evidence of a reduction in population size that occurred sometime before 1 MA ago.

- Subsequently, the population ancestral to present-day humans increased in size, whereas the Altai and Denisovan ancestral populations decreased further in size.

K. Prüfer, 2013: D DNA in Native Americans

- Western Europeans have N DNA but not D DNA
- In contrast, in the Han Chinese, the Dai in southern China, and the Karitiana and Mixe in the Americas have both N and Denisovan DNA.
- Denisovan DNA in mainland Asian and Native American populations is 0.2% and thus about 25% less than in Papua New Guinea and Australia.
- D DNA in mainland Asia may be due to gene flow from ancestors of present-day people in Oceania after they mixed with Denisovans

Archaic Human Contribution: *Homo erectus?*

- Estimate that 2.7–5.8% of the Denisova genome comes from archaic hominin which diverged from the other hominins 0.9–1.4 million years ago

- Second method estimates that 0.5–8% of the Denisovan genome comes from an unknown hominin which split from other hominins between 1.1 and 4 million years ago.

- The estimated population split time is also compatible with the possibility that this unknown hominin was *Homo erectus*.

K. Prüfer, 2013: What makes us MH: Our genetic recipe

- N and D genomes with the 1000 Genome Project data: near complete catalog of allele sites in genome where all people today are different from Ns and Ds, and from apes
- MH DNA sequence changes that distinguish MHs from our nearest extinct relatives is small. In 3 billion base pairs, only:
  - 31,389 such single nucleotide substitutions
  - 4,113 short insertions and deletions (indels)
  - 105,757 substitutions and 3,900 indels shared by 90% of present-day humans.
  - only 96 fixed amino acid substitutions in a total of 87 proteins
  - 3000 fixed changes that potentially influence gene expression in present-day humans;
  - 5 genes effect neural stem cells in the adult subventricular zone.

K. Prüfer, 2013

- We also note that the introgressed Neanderthal DNA sequences suggest a population split from the Altai Neanderthal between 114-77 Ka ago, well after 200 Ka ago when Neanderthal features appear in the fossil record

- Allele sharing between Neanderthals and non-African populations is owing to recent admixture rather than ancient population subdivision, an alternative which we and others previously considered possible.

K. Prüfer, 2013: three to five cases of interbreeding among four distinct hominin populations

N to MH = 1.5-2.1%

N to D = 0.5%

D to MH = 3-6%

Unknown hominin
to D = 0.5-8.0%

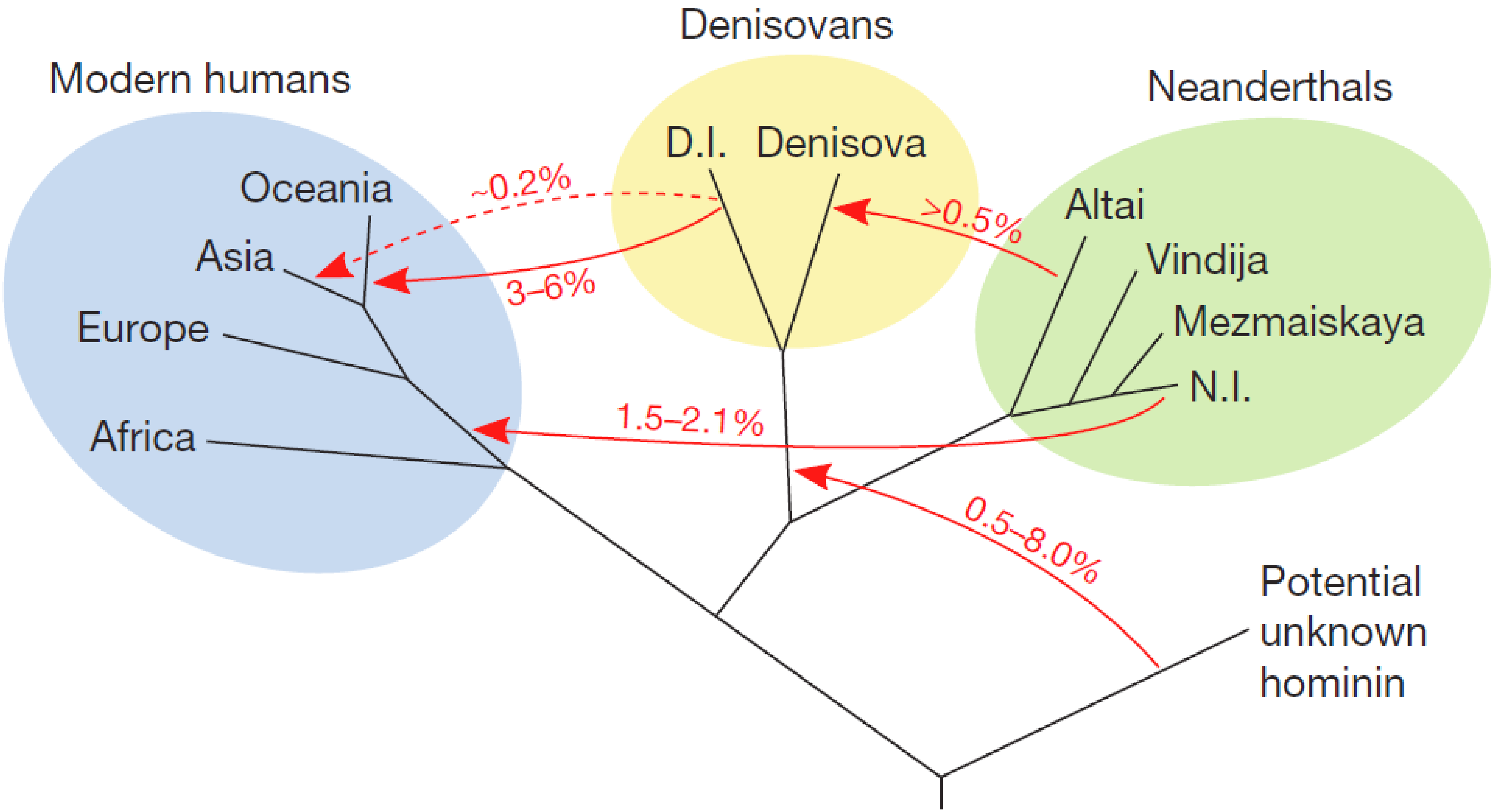
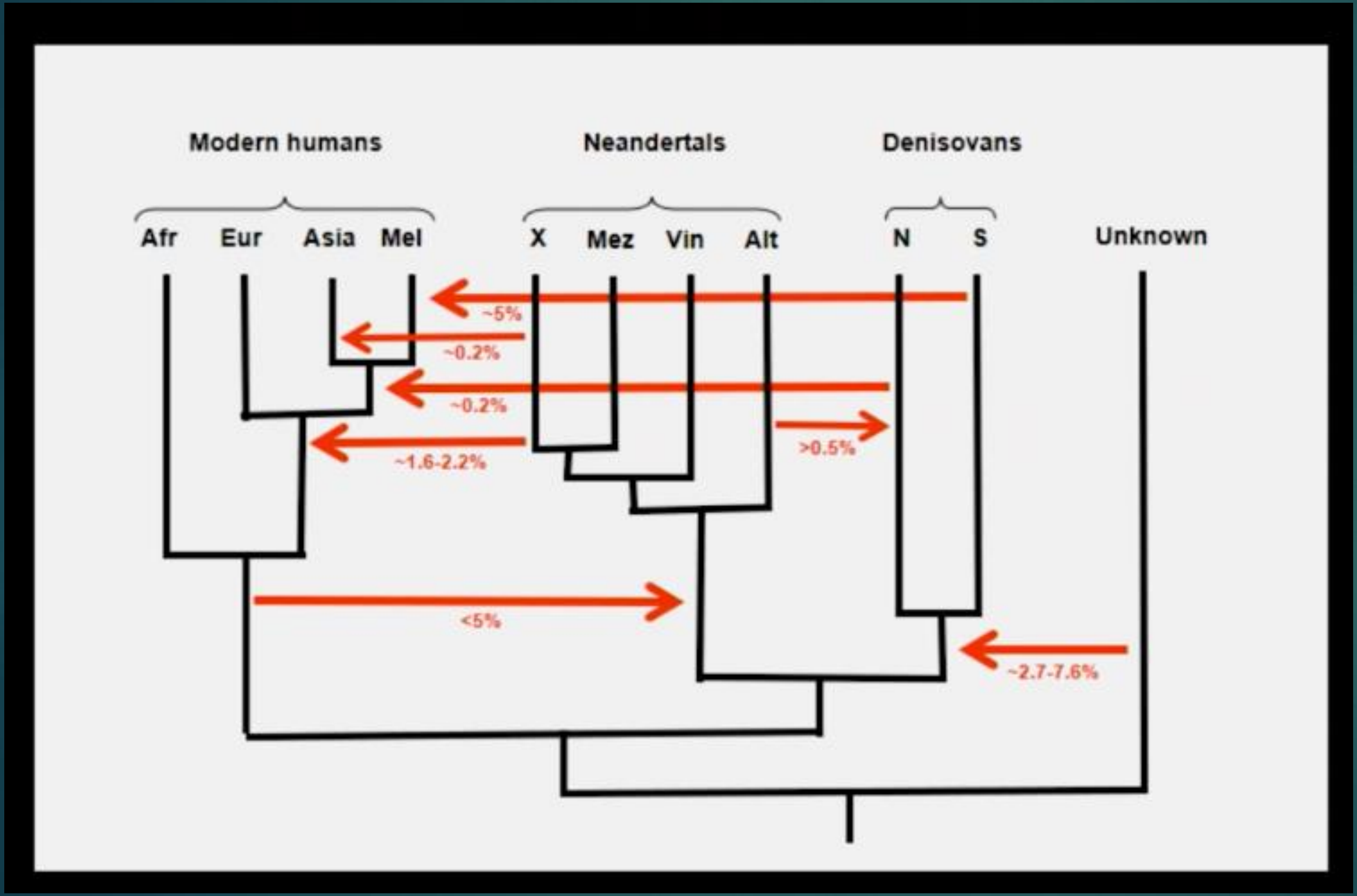


Figure 8 | A possible model of gene flow events in the Late Pleistocene. The

We have always mixed! (when we have met…)



7 gene flow events/ genetic introgressions:

2 D into MH

2 N into MH

1 N into D

1 MH into N

1 superarchaic (*H. erectus*?) into D

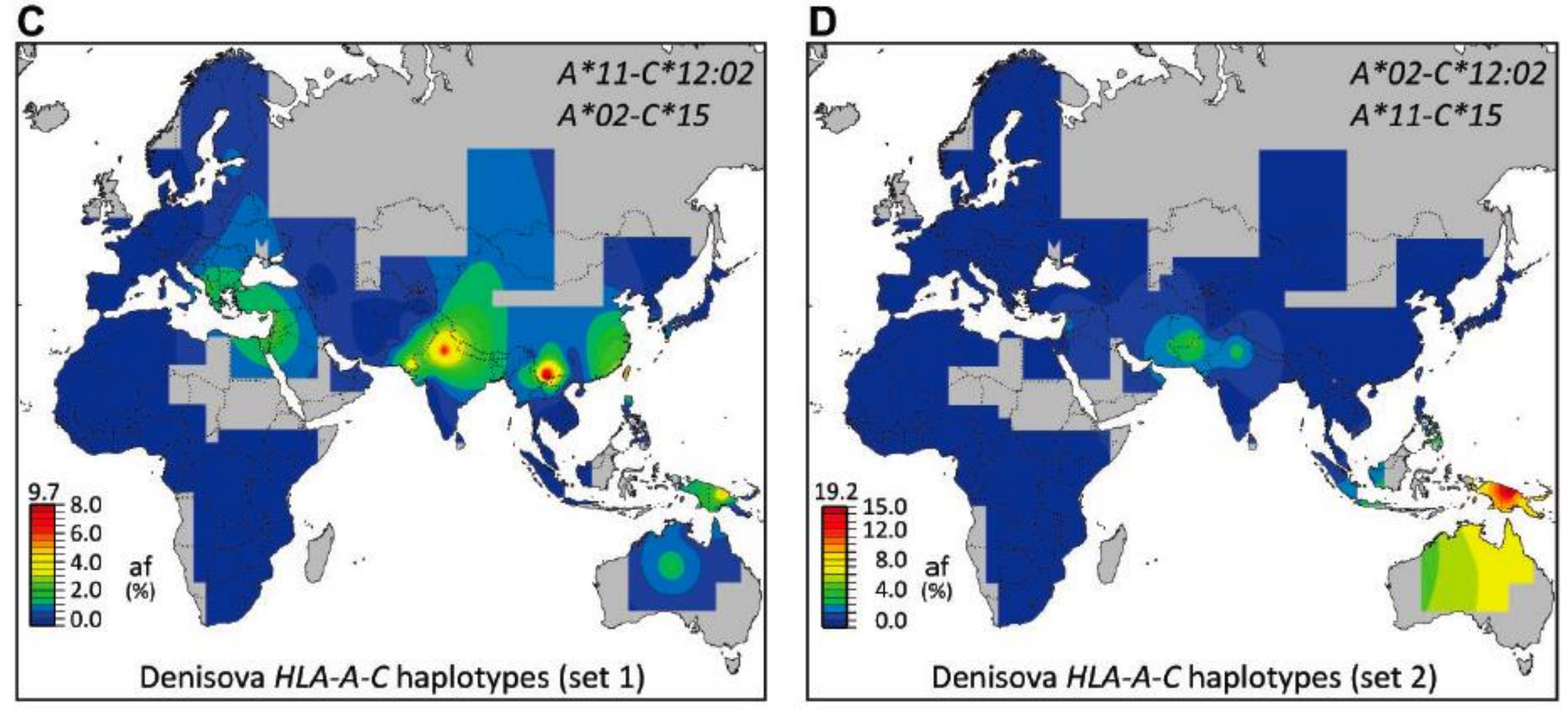
8. The Shaping of Modern Human Immune Systems by Multiregional Admixture with Archaic Humans - Laurent Abi-Rached, et al., 2013

- Analysis of how modern humans acquired the HLA-B*73 allele in west Asia through admixture with archaic humans called Denisovans

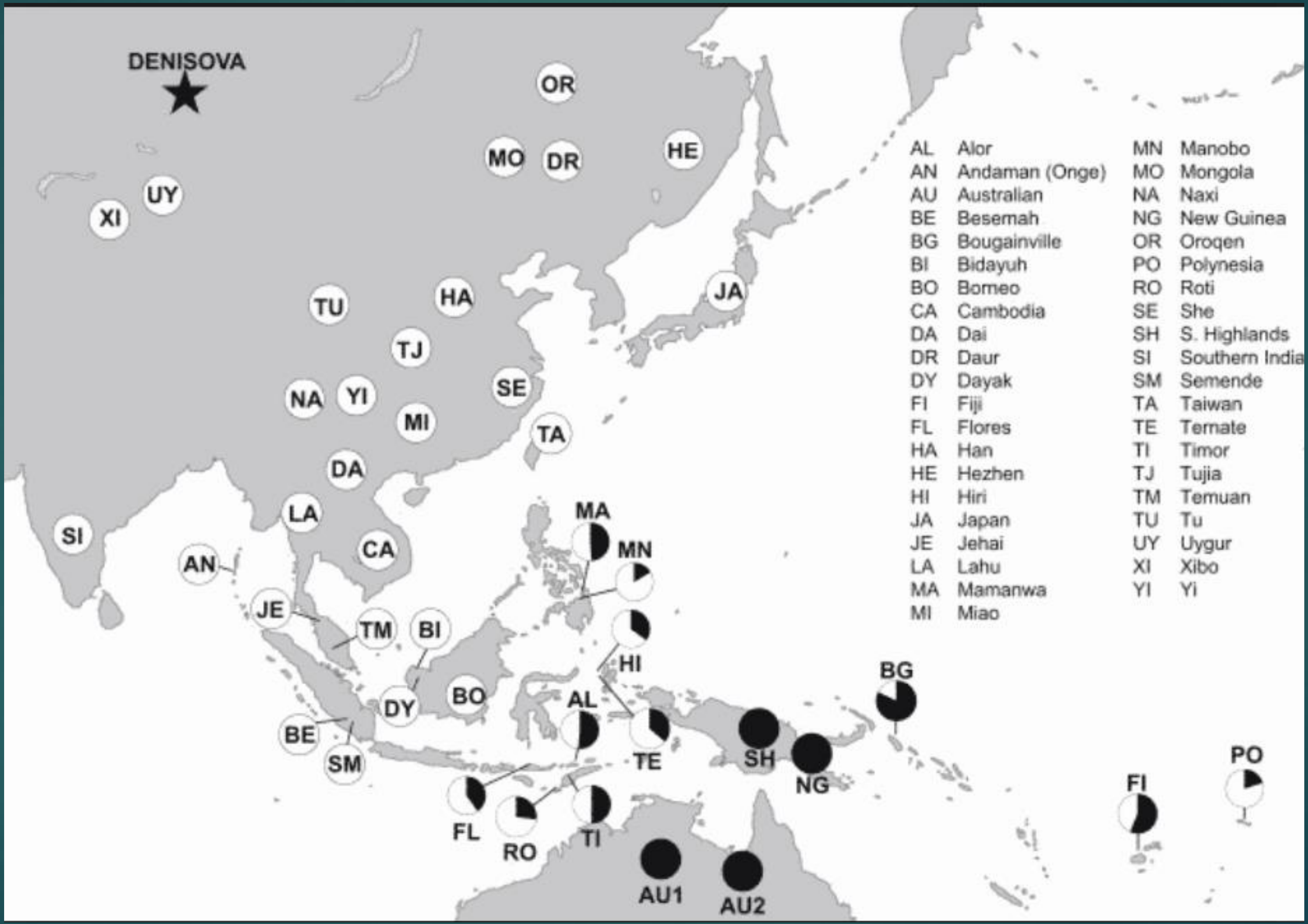
- These alleles represent more than half the HLA alleles of modern Eurasians

- Adaptive introgression of archaic alleles has significantly shaped modern human immune systems.

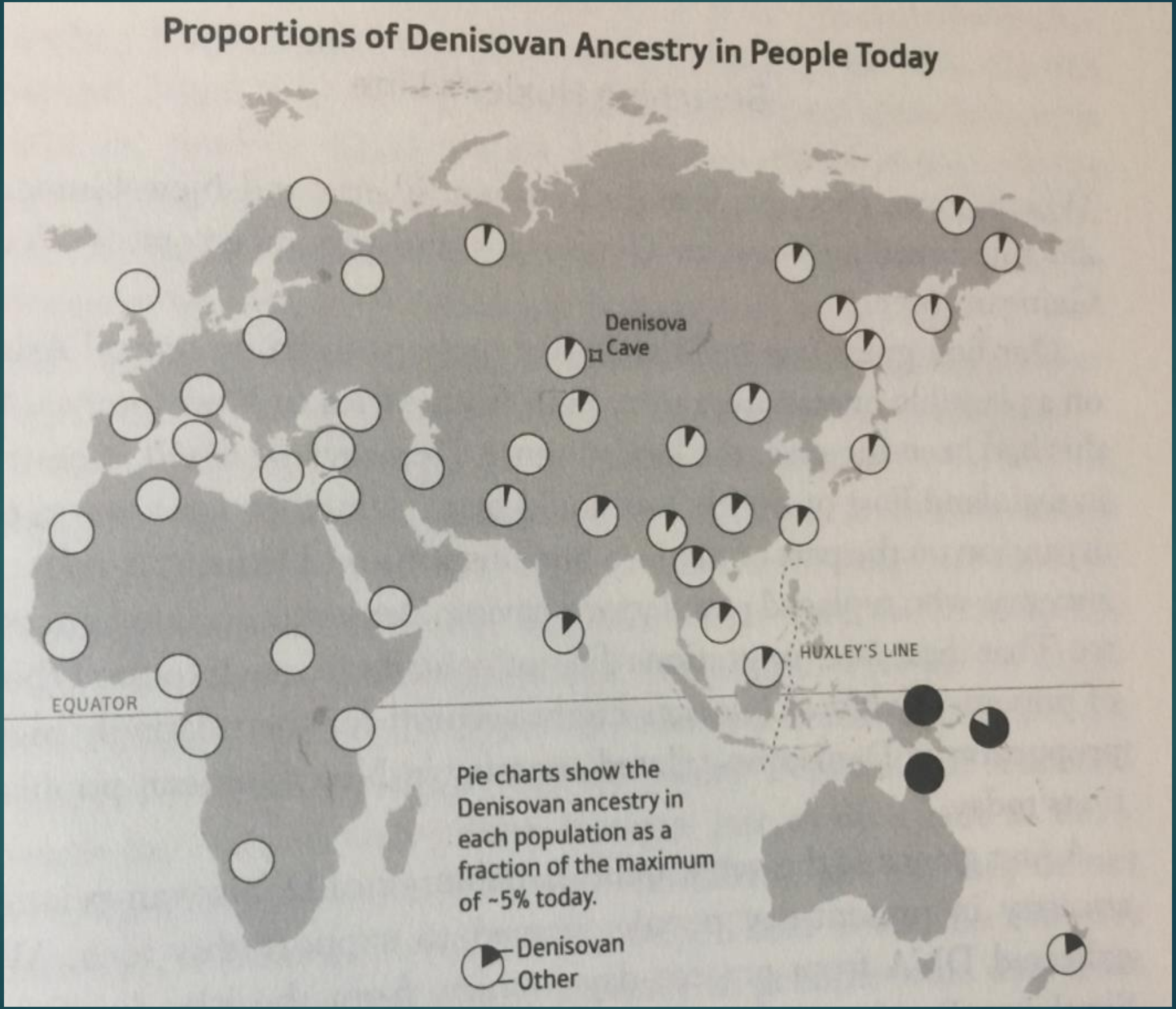
MHs leaving Africa acquired MHC I alleles of adaptive immune system from these hominin groups



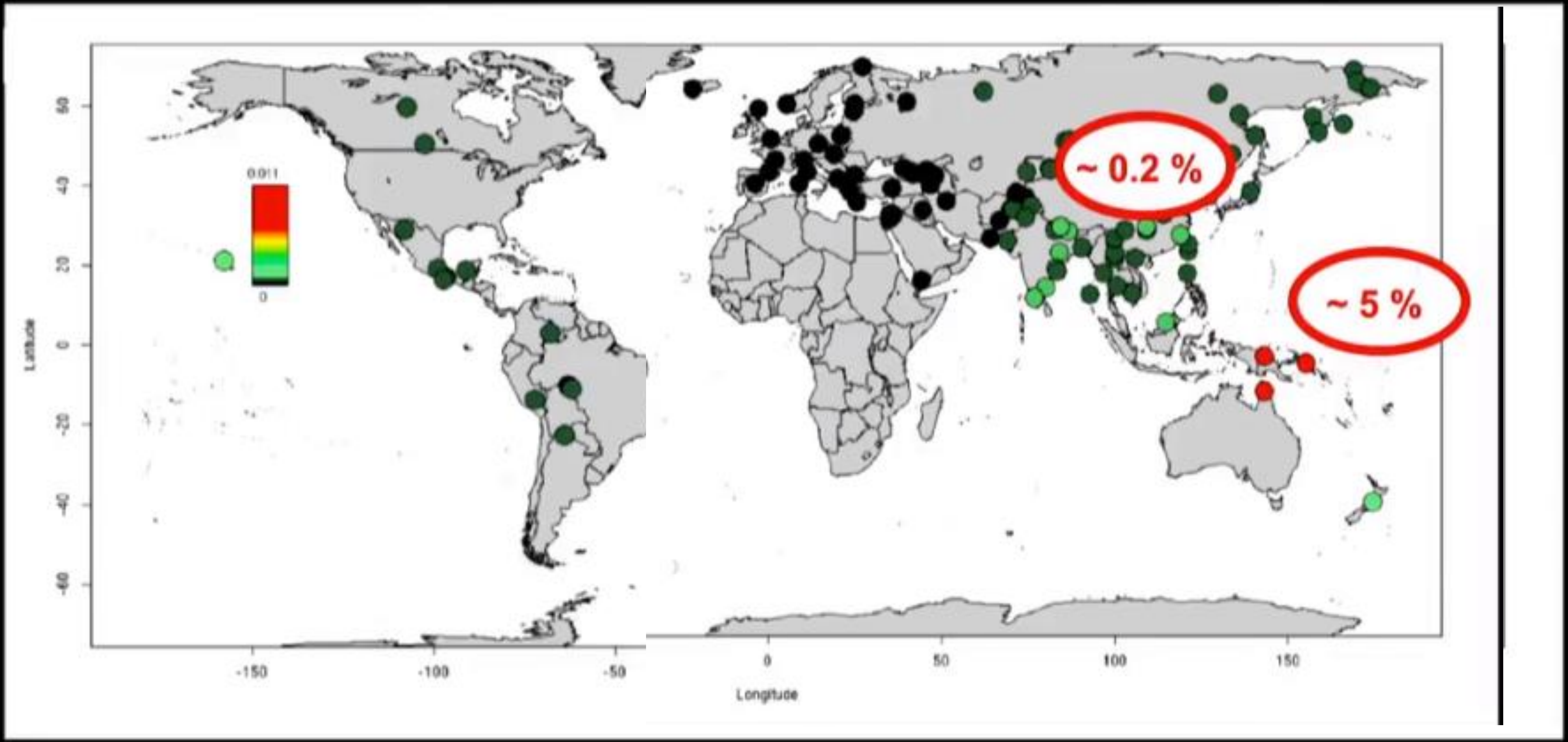
Spread of Denisovans: SE Asia to New Guinea & Australia



Denisovan Ancestry in MHs



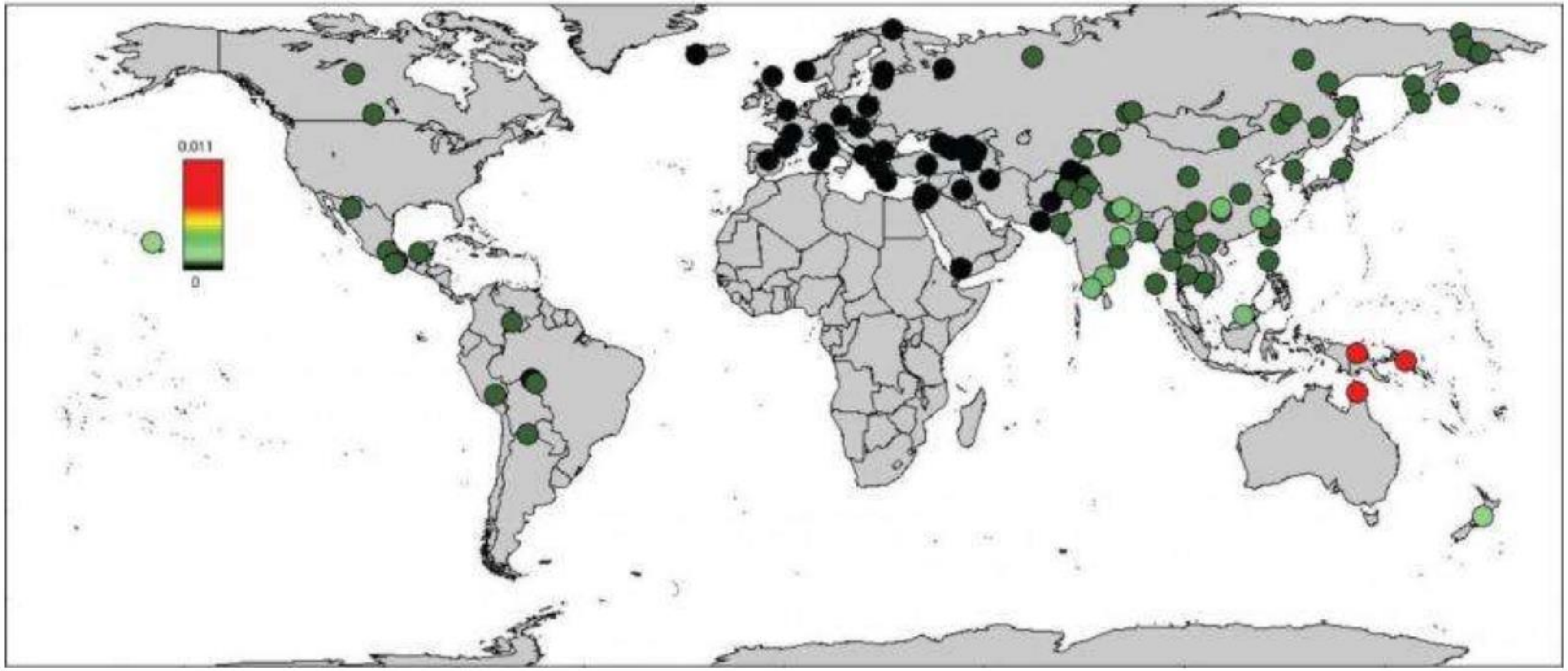
Denisovans: widespread in Asia



Sankaramanan *et al.*, Curr. Biol. 2016.

Not in Europe or Western Asia, but Eastern Asia;
0.2%; and 5% in Papua, New Guinea

Denisovan DNA: to the Americas



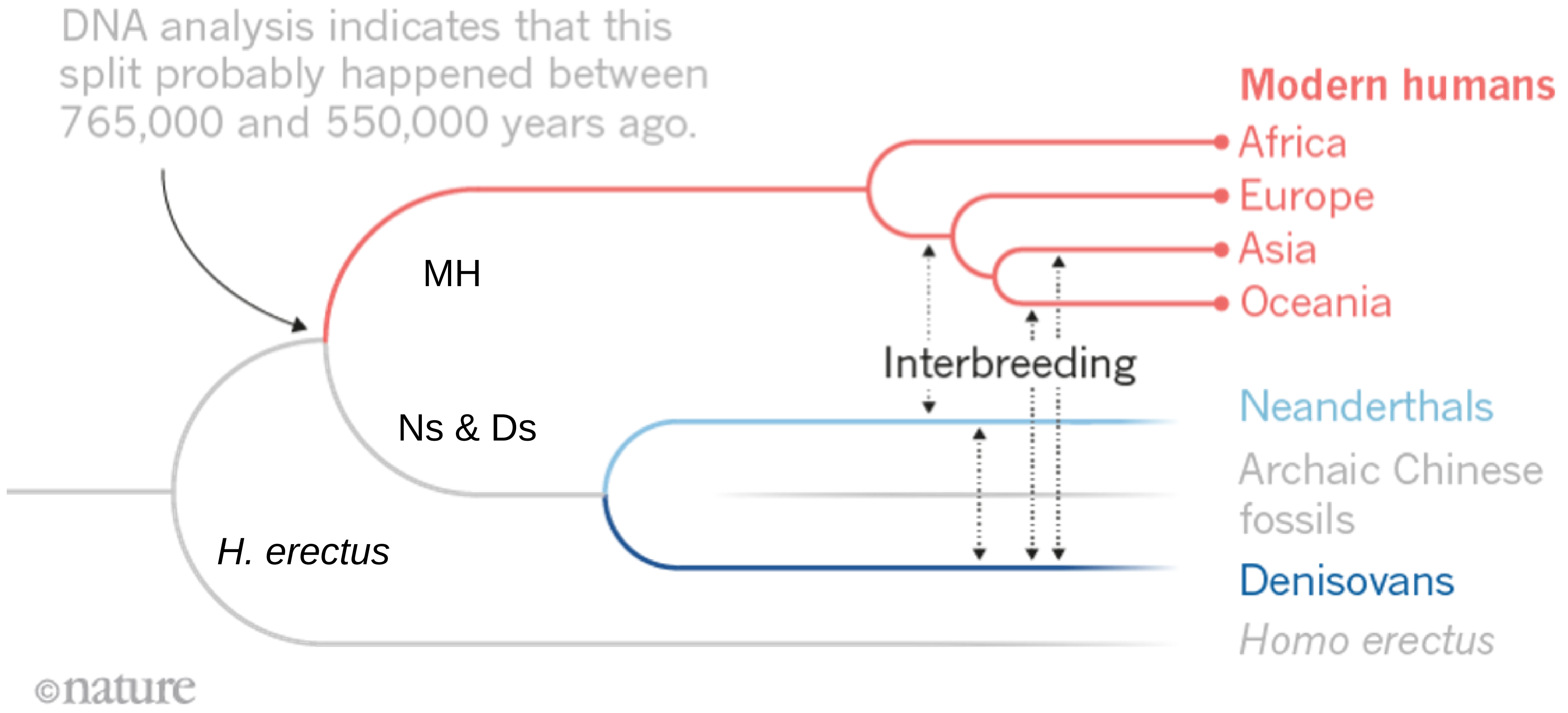
This map shows the proportion of the genome inferred to be Denisovan in ancestry in diverse non-Africans. The color scale is not linear to allow saturation of the high Denisova proportions in Oceania (bright red) and better visualization of the peak of Denisova proportion in South Asia. Credit: Sankararaman et al./Current Biology 2016

Native Americans carry both Denisovan and Neanderthal DNA, and they carry more than Europeans.

Denisova Cave by 2013

- Europeans have N but not D DNA
- Melanesians, N Guineans, Aboriginals carry 4–6% D DNA, 2-3% N DNA
- Mainland Asians and Native Americans have 0.2% D DNA
- Half of the HLA alleles of modern Eurasians were shown to represent archaic HLA haplotypes (N & D)
- Ds = dark skin, brown hair, and brown eyes

Tangled Tree



Sources: Ref. 3; C. B. Stringer & I. Barnes *Proc. Natl Acad. Sci. USA* **112**, 15542–15543 (2015).

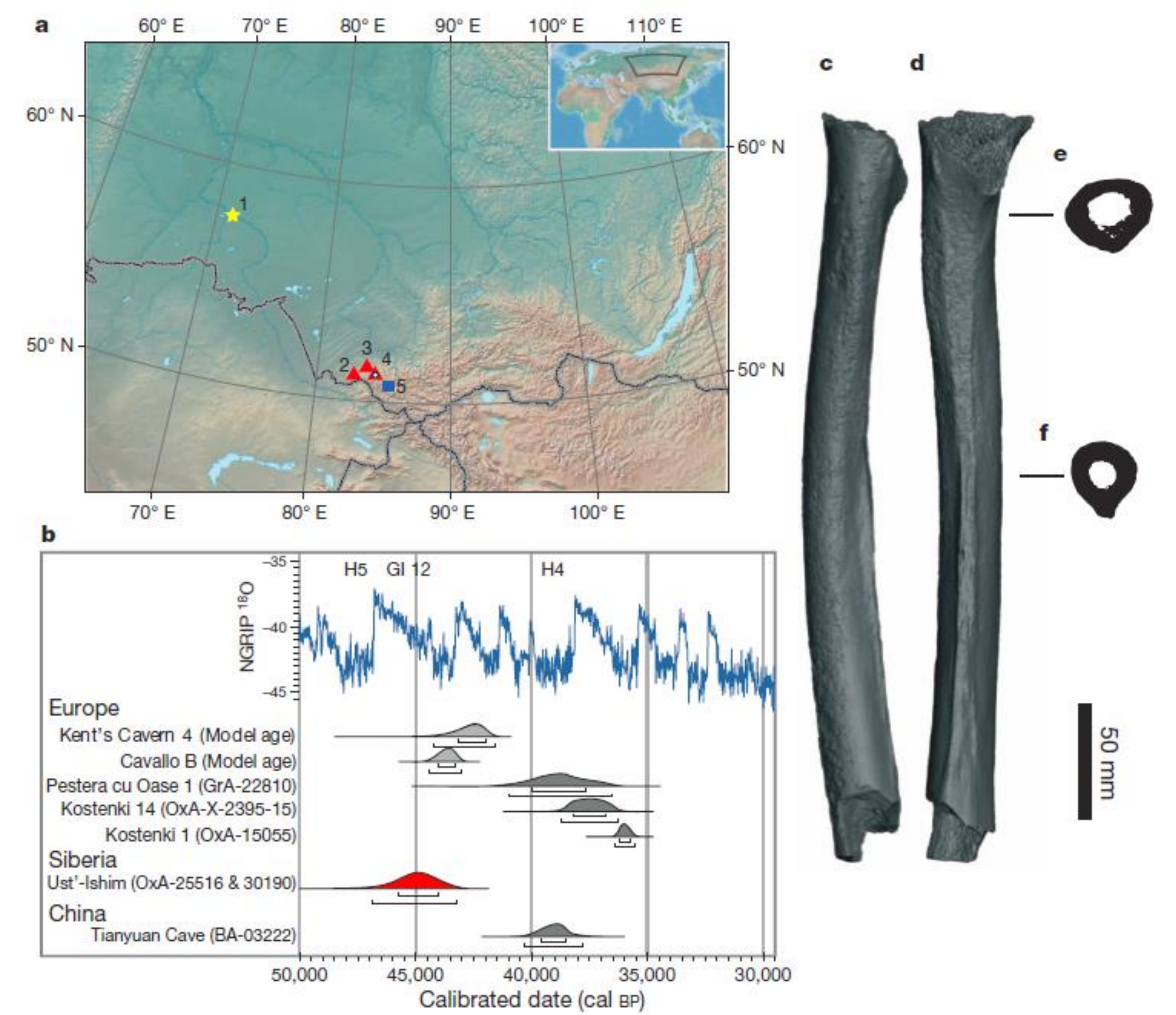
47. Genome sequence of a 45,000-year-old modern human from western Siberia - Qiaomei Fu, et al. 2014

- In 2008, a relatively complete left human femoral diaphysis (shaft) was discovered on the banks of the river Irtysh, near the settlement of Ust'-Ishim in western Siberia; about 45 Ka, MIS 3; modern human male

- Oldest directly radiocarbon-dated MH human fossil outside Africa

- Genomic segments of N ancestry are substantially longer than those observed in current MHs, indicating that Neanderthal gene flow into the ancestors of this MH occurred 7–13 Ka before he lived.

Ust’-Ishim, Siberian MH, ~45 Ka, femur



Ust’-Ishim

- 2.3% N DNA in the Ust’-Ishim individual (current east Asians (1.7–2.1%) & current Europeans (1.6–1.8%)). Admixture with Neanderthals had already occurred by 45 Ka ago.
- No Denisovan DNA in Ust’-Ishim individual
- Mean fragment sizes in the Ust’-Ishim genome are in the order of 1.8–4.2 times longer than in current MH genomes and that the Neanderthal gene flow occurred 232–430 generations before the Ust’-Ishim individual lived, at 45 Ka
- A model for the modern human colonization of Asia assumes that
  - an early southern coastal migration gave rise to the present-day people of Oceania,
  - a later more northern migration gave rise to Europeans and mainland Asians
- Modern Tibetans: most alleles in common with Ust'-Ishim man.
- Siberian and East Asian populations shared 38% of their ancestry with Ust’-Ishim man.

What’s special about Tibetans? Live at 13,000 feet above sea level

Only humans to possess the EPAS1 gene. A Denisovan gene.
80% of Tibetans have it..

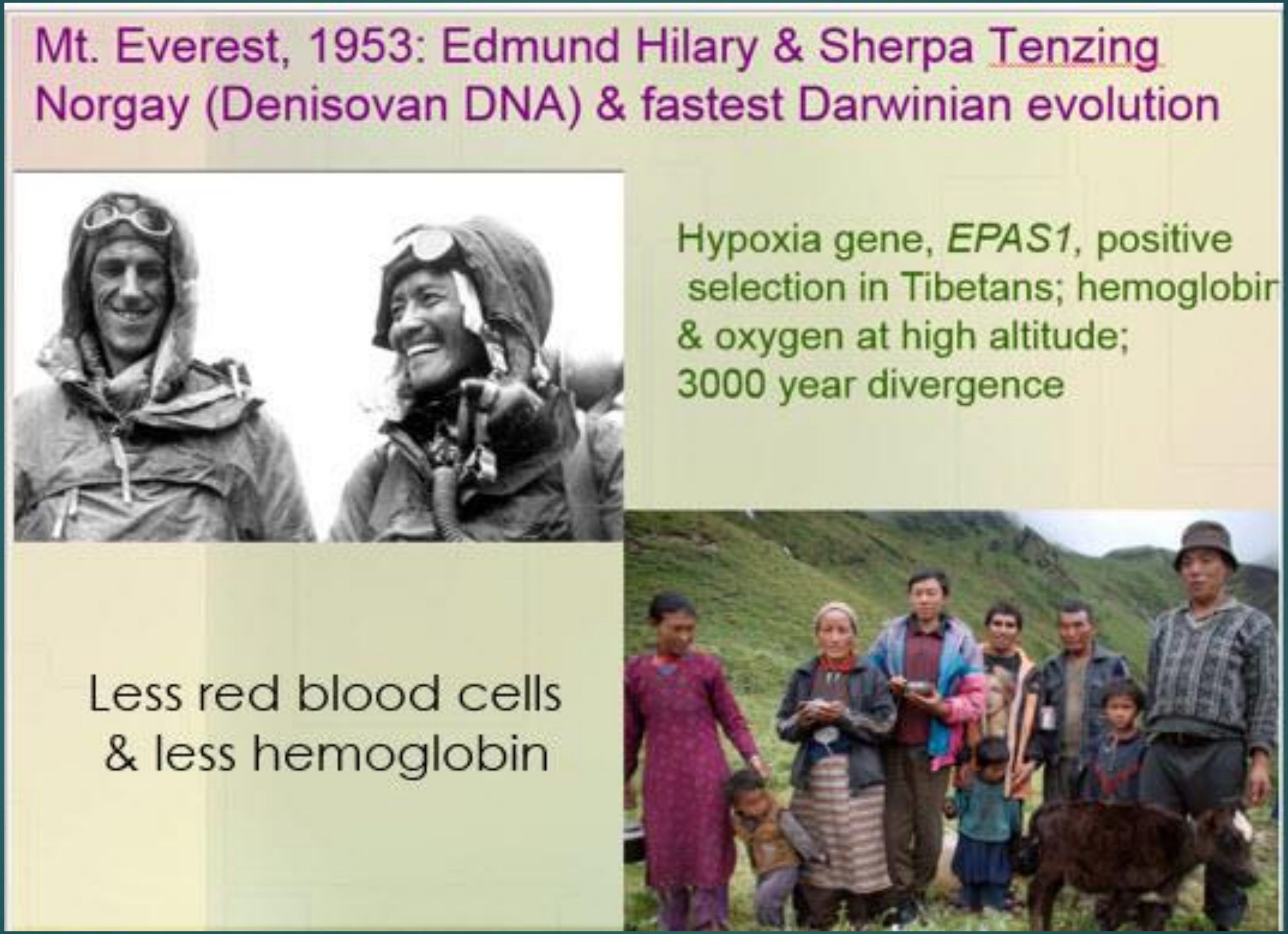
10. Altitude adaptation in Tibetans caused by introgression of Denisovan-like DNA - Emilia Huerta-Sanchez, et al., 2014

- Natural selection produced adaptation of Tibetans to the hypoxic environment of the high-altitude Tibetan plateau.
- EPAS1 gene: endothelial PAS domain-containing protein 1
- A hypoxia pathway gene, EPAS1, was previously identified as having the
  - most extreme signature of positive selection in Tibetans; and was shown to be
  - associated with differences in hemoglobin concentration at high altitude.
  - Tibetans have less hemoglobin and less red blood cells.

- EPAS1: has a Denisovan haplotype; it is not observed in modern-day Melanesians or in the high-coverage Altai Neanderthal

- 2019: Peruvians harbor a larger number of high frequency Denisovan variants than other admixed populations in the Americas.

Denisovan DNA: EPAS1 gene – Oxygen capacity of Sherpas



Mt. Everest, 1953: Edmund Hilary & Sherpa Tenzing Norgay (Denisovan DNA) & fastest Darwinian evolution

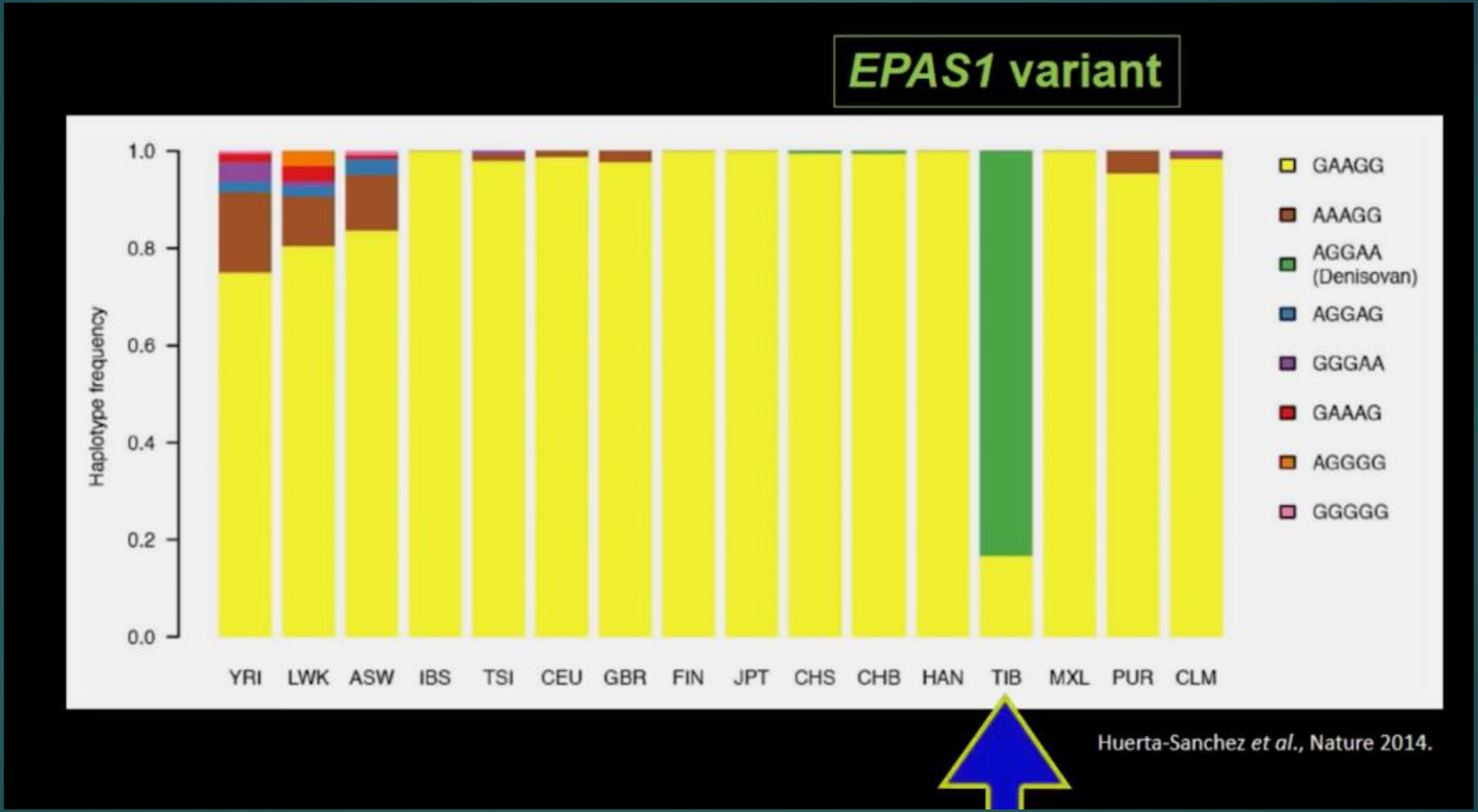
Hypoxia gene, *EPAS1*, positive
selection in Tibetans; hemoglobi
& oxygen at high altitude;
3000 year divergence

Less red blood cells
& less hemoglobin

EPAS1: increase in hemoglobin levels is limited, less blood viscosity

Denisovan functional contribution to modern humans?

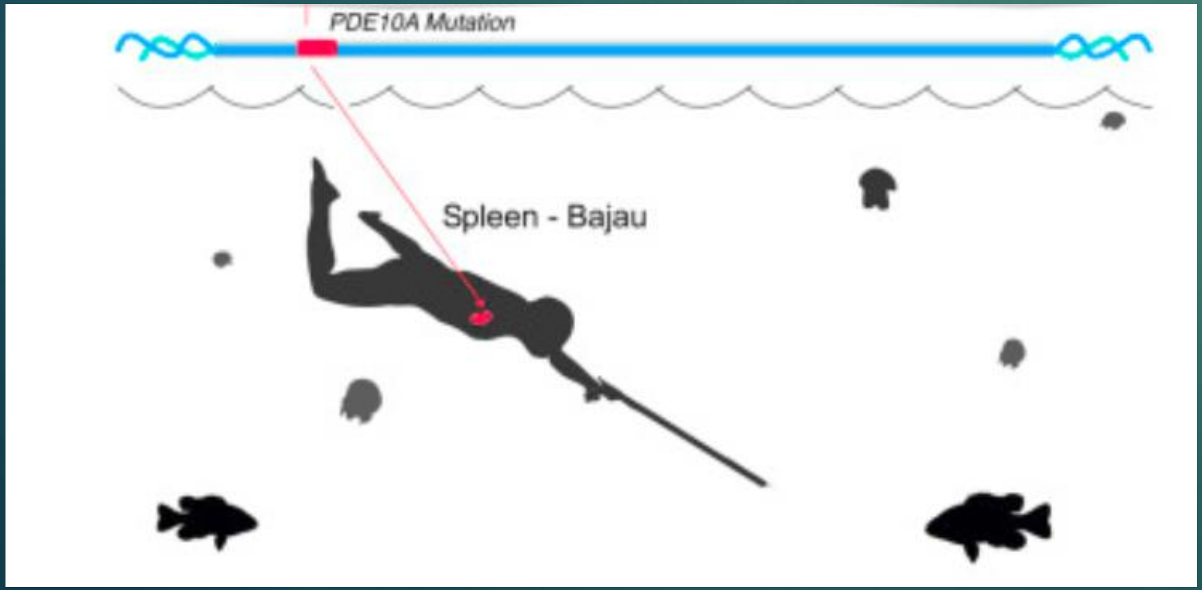
- High altitude adaptation in Tibet: 80% have EPAS1 Denisovan variant



Huerta-Sanchez *et al.*, Nature 2014.

- Only 9% of Han Chinese have it

World’s Best Freedivers -- Bajau people of Southeast Asia



- Extraordinary breath-holding abilities; Freediving several hundred feet down to fish
- Some spend as much as 60 percent of the day diving for food.
- To deal with hypoxia, 50% larger spleen, which stores oxygenated red blood cells
- FAM178B gene which prevent the build-up of carbon dioxide is Denisovan in origin

Denisova Literature

- Neandertal DNA sequences and the origin of modern humans - M. Krings, et al., 1997
- A complete Neandertal mitochondrial genome sequence determined by high-throughput sequencing – R. Green, *et al.* 2008
- A draft sequence of the Neandertal genome – R. Green, et al. 2010

- 1. The complete mitochondrial DNA genome of an unknown hominin from southern Siberia – J. Krause, et al., 2010
- 2. Genetic history of an archaic hominin group from Denisova Cave in Siberia. D. Reich, et al., 2010
- 3. A proximal pedal phalanx of a Paleolithic hominin from Denisova cave, Altai - M. B. Mednikova, 2011
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