The Stranger from Siberia

The Denisovans --
Part 2

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October 2020

** Basic Denisovan facts

▶ It is unknown whether Denisovans actually lived in Denisova Cave

▶ They didn't bury their dead there, so any bone fragments found inside were likely regurgitated by predators like cave hyenas.

▶ Site has evidence of Denisovan and Neanderthal bone fragments and teeth layered in both cold and warm periods from approximately 300 to 50 Ka.

▶ Both Neanderthals and Denisovans were less genetically diverse than modern humans

Alert from CJV:

- I am doing a historical review of the scientific discoveries related to Denisovans based on 53 journal articles over 10 years.

- As a consequence, Denisovan information I present earlier in my presentation may change later due to later discoveries.

- This especially applies to genetic data.

- Given that I was not trained as a geneticist, my understanding of genetic studies is clearly not perfect.

- You have been warned!

3 separate Denisovans individuals; lived in Siberia x 60 K years

▶ 2012: Genetic variation among D specimens: two Denisovan molars and finger bone = 3 different individuals of the same species.

▶ The mtDNA of one molar (Denisova 8) has accumulated fewer substitutions than the mtDNAs of the other two specimens,

▶ This suggested that D8 molar was on the order of 60,000 years older than the other two specimens, a difference that suggests "that the Denisovans were present in the Altai Mountains in southern Siberia over a very long time"

By 2017, 5 Known Denisovan Individuals

▶ 1) 2010 - phalanx of finger (Denisova 3) – female; D with some N DNA

▶ 2) 2012 - molar (Denisova 4) – adult male

▶ 3) 2010 - molar (Denisova 8) – adult male; 2nd oldest

▶ 4) 2014 – N toe bone (Denisova 5) = Altai Neandertal

▶ 5) 2017 – deciduous molar (Denisova 2)

+++ Denisova Cave by 2014

▶ Europeans have N but not D DNA, with 1 exception

▶ 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 (N & D) HLA haplotypes

Sawyer, 2015: Denisova 4 and 8 – molar teeth

▶ Sawyer et al. 2015: full mitochondrial and partial nuclear genomes for two other Denisovan individuals represented by Denisova 4 and 8 adult molar teeth

▶ Teeth:

  ▶ very large size; with large splayed roots

  ▶ lack N derived dental traits: absence of crown traits typical of either N or MH upper molar

▶ Deciduous molar Denisova 2: This molar derives from the even deeper layer 22, dated by luminescence to ~170 Ka

What to do?: Bags of some of 40 K bone fragments from Asia

13. Identification of a new hominin bone from Denisova Cave, Siberia using collagen fingerprinting and mitochondrial DNA analysis. - S. Brown, 2016

▶ Bones at Palaeolithic sites: majority of bones lack features necessary for traditional morphological identification.

▶ New identification method:

  ▶ Collagen is the most dominant and time-stable protein in bone

  ▶ collagen fingerprinting on more than 2000 fragmented bones, ranging from 3–5 cm, from the site of Denisova Cave

▶ Only found a single hominin bone (= Denisova 11--later identified as Denny); was later found to carry mitochondrial DNA of the Neandertal type. Subsequent radiocarbon dating revealed the bone to be >50 Ka old.

ZooMS: Proteins – how to identify a hominin bone

▶ Prior recovery of proteins from
  ▶ 1.8 Ma animal teeth and a 3.8 Ma eggshell.

▶ Mass spectrometry — a technique used to study modern proteins — could also be applied to ancient proteins.

▶ Mass spectrometry: break down proteins into their constituent peptides (amino acids) and analyzing their masses to deduce their chemical make-up.

▶ Comparison of the peptide signatures of a bone against a library of such signatures from known animals; assign the unidentified bones to the correct family, genus and rarely species, i.e. a hominin

ZooMS: Proteins

▶ <u>Sift through thousands of bone fragments</u> to identify the types of animal they came from.

▶ <u>ZooMS: "Zooarchaeology by mass spectrometry"</u>, researchers analyze collagen found in bone

▶ Cost = $5-10 per specimen.

▶ <u>Minimally destructive</u>—it requires only around <u>10 to 20 milligrams</u> of bone for analysis.

▶ Rapid; one person can screen <u>hundreds of bones a week</u>.

▶ First 1226 samples from Denisova cave were not Hominidae.

▶ First bone of next sample was DC1227= Denny

Current Leipzig analysis of 1000s of bone fragments

Denisova 11

New proteinomic analysis of collagen of bone fragments from Denisova cave: vast majority are animals; In testing 2200 fragments, they found 1 hominin bone

S. Brown, 2016: Proteins

Figure 2. Photograph of DC1227, detailing each visible surface of the bone.

Mass spectrometer at Oxford
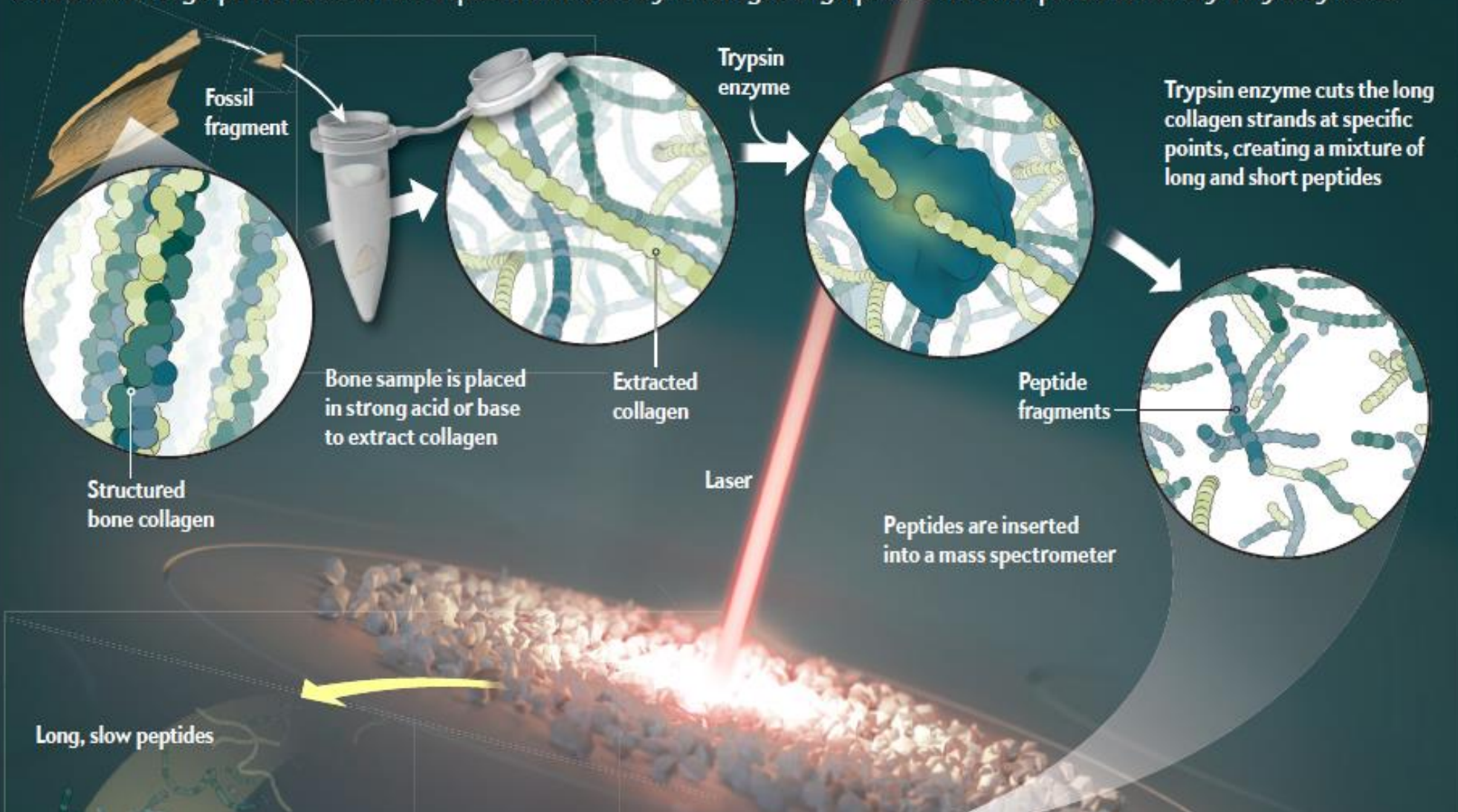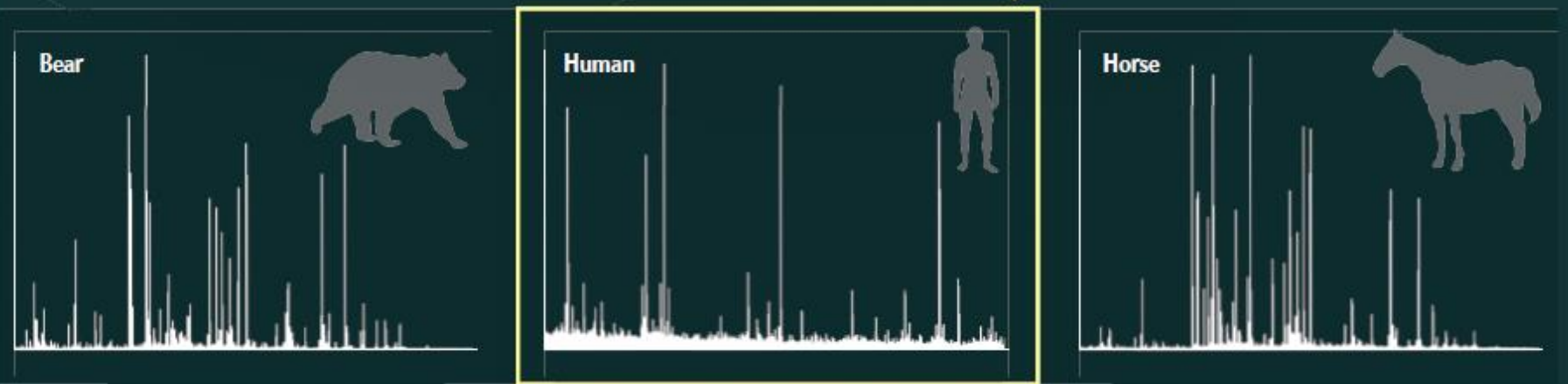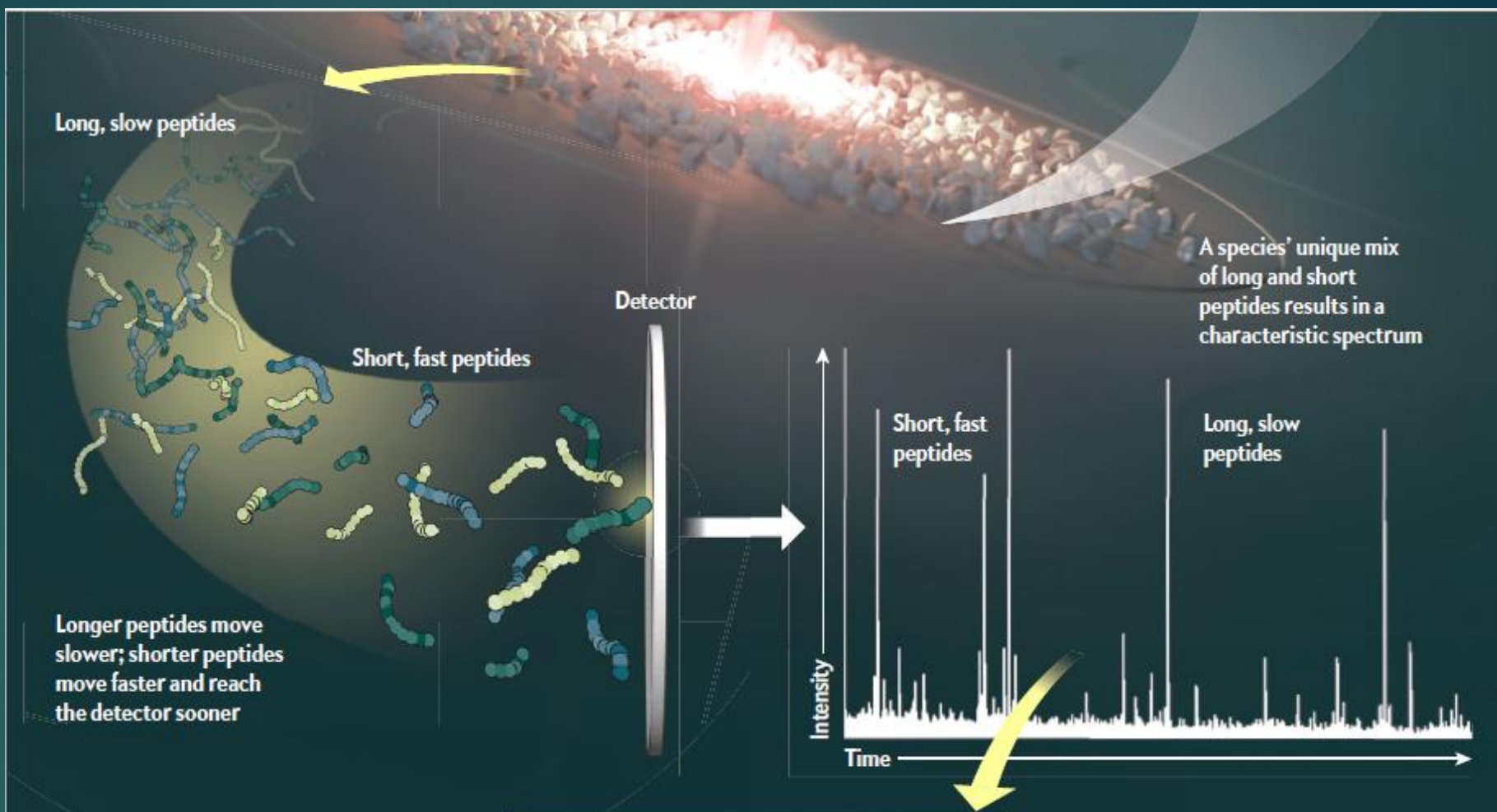
ZooMS

- Analyzing fossil fragments using ZooMS requires sawing off a 20-milligram sample from each tiny specimen

- Collagen extracted from bone

- Enzyme breaks up peptides from collagen

Whose Bone Is It, Anyway?

With ZooMS, researchers can assign bone fragments to their proper taxonomic group. ZooMS analyzes collagen protein preserved in bone. An enzyme cuts the collagen into its component peptide chains. A mass spectrometer uses a laser to impart an electric charge to the peptides, which then race toward a detector that measures when each peptide strikes it. The resulting spectrum of values is a distinctive "fingerprint" that can be compared with a library of collagen fingerprints of known species to identify a mystery bone.

Fossil fragment

Trypsin enzyme

Trypsin enzyme cuts the long collagen strands at specific points, creating a mixture of long and short peptides

Structured bone collagen

Bone sample is placed in strong acid or base to extract collagen

Extracted collagen

Laser

Peptide fragments

Peptides are inserted into a mass spectrometer

Long, slow peptides

A species' unique mix of long and short peptides results in a characteristic spectrum

Long, slow peptides

Short, fast peptides

Detector

Longer peptides move slower; shorter peptides move faster and reach the detector sooner

A species' unique mix of long and short peptides results in a characteristic spectrum

Short, fast peptides

Long, slow peptides

Intensity

Time

Bear

Human

Horse

Problem with proteins: there are very few of them in ancient bones

▶ Problem: Ancient teeth and bones contain a very, very small amount of protein.

▶ A Denisovan bone contained 8 kinds of collagen protein (2,000 amino acids).

▶ Only 1 of those 2000 amino acid differed from N and MH sequences,

▶ There simply might not be enough information in the amino-acid sequences to say anything definitive about its relationship to modern or archaic humans.

▶ In contrast, a single ancient DNA genome has three million variants compared with any other genome

Proteins: issues

▶ And because proteins often perform crucial functions — forming the structure of bone, say — they don't always change much as species evolve.

▶

▶ Example: Proteins that are specific to enamel are exactly the same in Ds, MHs and Ns, so can't be used to distinguish between these groups.

▶ Some geneticists are skeptical of the methodology

▶ But successes so far include: Gigantopitechus is related to orangutans at 1.9 Ma; H. antecessor is sister clade to ancestor of MHs, Ns & Ds; identified Denny bone as hominin

▶ What of *H. naledi, H. floresiensis, H. erectus?*

When MH left Africa: entered Neandertal & Denisovan Territories
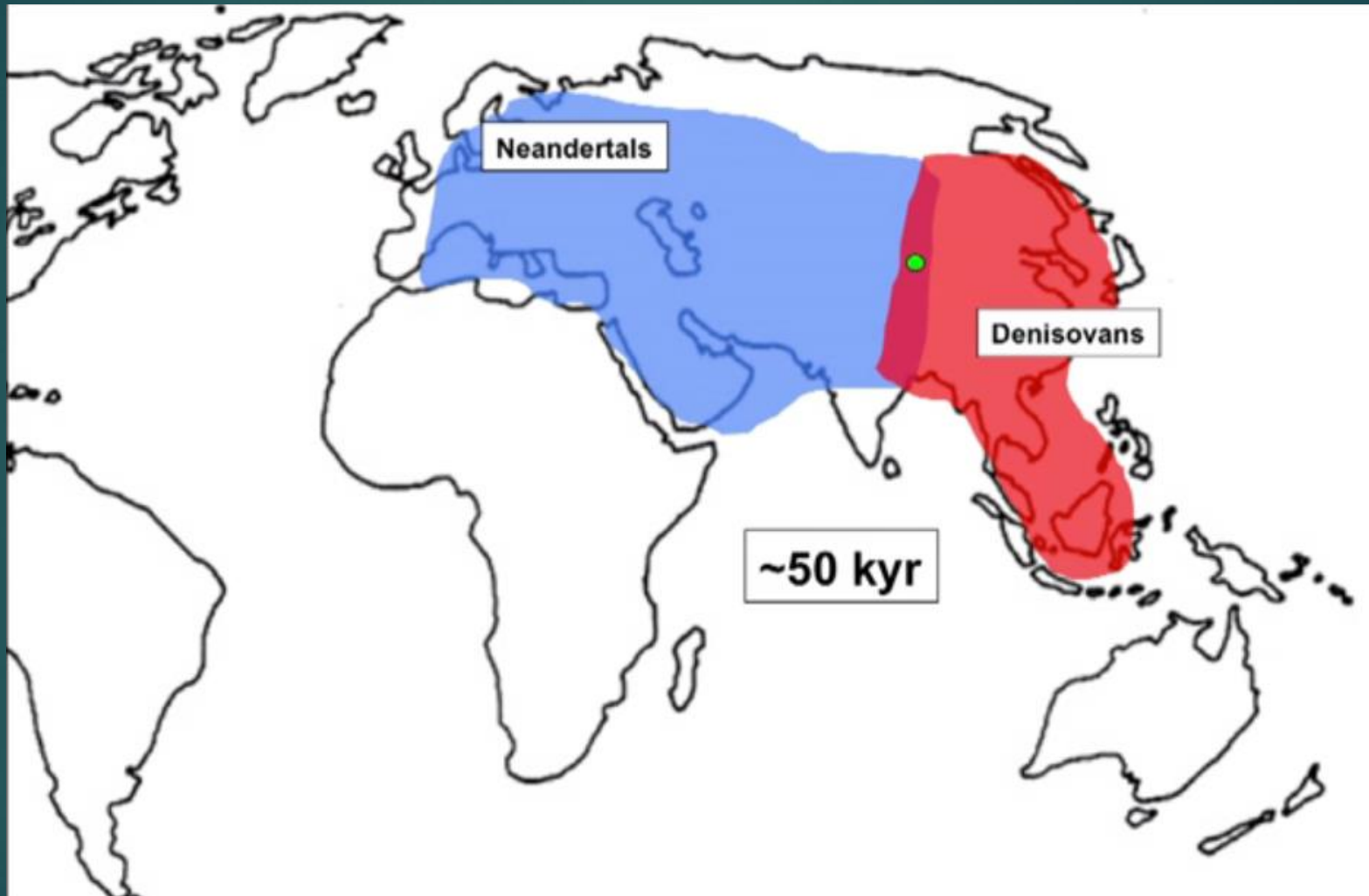
N in West, D in East; Both in southern Siberia
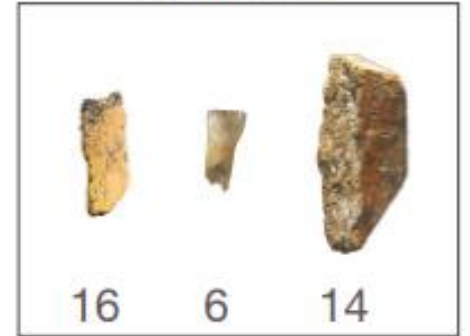
Current 11 Denisova specimens numbering

Pleistocene Occupations at Denisova Cave: 13 layers

▶ Excavations in the central chamber at Denisova have revealed

  ▶ 13 Pleistocene "occupations" (evidence of hominin presence)

  ▶ between ~125 to 30 Ka.

▶ The chronological dates are radio thermal luminescence dates (RTL) taken on sediments

▶ Most recent: Layer 9, Upper Paleolithic (UP), Mousterian and Levallois, ~46 Ka (OIS-2)

• Layer 11, Initial UP, Altai Mousterian, ~48,6 to 29 Ka (OIS-3)

• Layers 12-20, Later Middle Paleolithic (MP) Levallois, ~69-155 Ka

• Oldest Layers: 21 & 22, Initial MP Levallois, Mousterian, ~171-182 Ka (OIS-5)

Sequential occupation of Denisova Cave

▶ As of 2013, Russians propose sequential occupations:

  ▶ The Denisovans are present at the cave about 76-52 Ka

  ▶ Neanderthals appear briefly about 45 Ka ago

  ▶ modern humans followed.

  ▶ However, researchers are continuing to date Level 11 of the cave, where the remains and artifacts have been found, in order to get a clearer picture.

Sima de los Huesos (Pit of the Bones), Atapuerca, Spain

Sima de los Huesos, Atapuerca, Spain

The Sima Humans Illustration by Mauricio Antón

Sima de los Huesos
Homo heidelbergensis hominins, 400K

Human fossils, Sima de los Huesos
E436/0172 Rights Managed

28 people's body parts from 400 Ka

Matthias Meyer, 2013: 400k Denisovan Mitochondrial DNA

► SH hominins considered to be early Ns

► 2013: femur from Sima de los Huesos (Pit of Bones) in Atapuerca, Spain: Denisovan mitochondrial DNA;

► mtDNA closer to that of Denisovans than to Ns or MHs.

Figure 2 | Femur XIII reassembled from three parts after sampling. The natural fractures are visible in the proximal third of the femur.

D MtDNA: 16,561 ATGC bases

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10381 acaaaaggga ttagactgag ccgaattggt atatagttta aacaaaacga atgatttcga
10441 ctcattaaat tatgataatc atatttacca aatgcccctc atttacataa atattatact
10501 agcatttacc atctcacttc taggaatact agtatatcgc tcacacctta tatcctccct
10561 actatgccta gaaggaataa tactatcact gttcattata gctactctca taaccctcaa
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10681 agcagcggta ggcctagccc tactagtctc aatctccaac acatatggcc tagactacgt
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10801 ctgact...
10861 attag
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10981 cccct
11041 ctcta
11101 gaact
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11221 ctagt
11281 ctaaa
11341 ttaat
11401 ttatg
11461 gtact
11521 acaaa
11581 tccat
11641 atagc
11701 attct
11761 tacga
11821 ctaat
11881 aacct
11941 ctact
12001 acgca
12061 aacac
12121 attac
12181 aacag
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15. Nuclear DNA sequences from the Middle Pleistocene Sima de los Huesos hominins - M. Meyer, et al., 2016

- A unique assemblage of 28 hominin individuals, found in Sima de los Huesos in the Sierra de Atapuerca in Spain, has recently been dated to approximately 430 Ka.

- mtDNA retrieved from one individual from Sima de los Huesos is more closely related to the mtDNA of Denisovans than to that of Neanderthals.

- But nuclear DNA: the Sima de los Huesos hominins were related to Neanderthals rather than to Denisovans,

- Conclusion: Population divergence between Neanderthals and Denisovans predates 430 Ka.

Sima de los Huesos: Implications

▶ Meyer suggests that the ancestors of Ds and Ns carried mtDNA that is reflected in both — but which is not present in later Neanderthals.

▶ Mitochondrial lineages go extinct whenever a female does not give birth to a daughter, so the Neanderthals could have simply lost that sequence while it lived on in Denisovan women.

▶ This elimination could have

  ▶ happened by chance

  ▶ Meyer favors the hypothesis that an as yet unknown species from Africa migrated to Eurasia and bred with Neanderthals, replacing the mtDNA lineages

Why that D mtDNA in SH

▶ Stringer proposes that Ds interbred with an ancestral population to both Denisovans and Sima de los Huesos hominins, introducing the newly decoded mitochondrial lineage to both populations

▶ This scenario explains another oddity thrown up by the sequencing of ancient hominin DNA: Pääbo's team suggests that Denisovans interbred with a superarchaic hominin group

Archaic Human Contribution: *Homo erectus?*

- Estimate that <u>2.7–5.8% of the Denisova genome comes from superarchaic hominin</u> which diverged from the other hominins <u>900 Ka to 1.4 MA</u> ago

- <u>Second method</u> estimates that <u>0.5–8% of the Denisovan genome comes from an unknown hominin</u> which split from other hominins between <u>1 to 4 MA</u> ago.

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

12. Small Amounts of Archaic Admixture Provide Big Insights into Human History - Selina Vattathil and Joshua M. Akey, 2015

▶ Did our ancestors interbreed with archaic humans?

▶ All current non-Africans inherited approximately 2% of their genome from Neanderthal ancestors and individuals of Melanesian ancestry inherited approximately 4%–6% of their genome from Denisovan ancestors.

▶ Note, the number of hybridization events compatible with the observed levels of archaic ancestry is currently unknown and difficult to estimate precisely as it requires tenuous assumptions about a range of parameters such as historical population sizes and fitness of hybrid offspring

Vattathil & Akey, 2015

▶ <u>Widespread Purging of Neanderthal DNA segments in Modern Humans:</u>

▶ The <u>amount of surviving Neanderthal genes varies considerably across the current MH genome</u>.

▶ Such <u>marked heterogeneity</u> (2 alleles are different) suggests that there may have been

    ▶ fitness consequences to hybridization and

    ▶ <u>some N sequences were deleterious in MH genome and were eliminated</u>.

Vattathil & Akey, 2015: Genetic deserts

► **Maps of Neanderthal admixture:** number of specific genomic regions that exhibit especially strong depletion of Neanderthal haplotypes.

► These "deserts" of Neanderthal genes are particularly interesting because they may delineate regions that contribute to uniquely modern human phenotypes.

► For example, the largest Neanderthal desert on the autosomes spans a 15Mb region on chromosome 7 and contains the gene FOXP2, a developmentally important transcription factor that has previously been implicated in speech and language.

► Natural selection weeds out deleterious genes

Vattathil & Akey, 2015

▶ Consistent with above hypothesis, the

- ▶ frequency of Neanderthal alleles is negatively correlated with inferred functional importance = more important the MH gene area, the fewer N genes present

- ▶ More genetic divergence between MHs and Ns, less chance of seeing N genes in that region = odds of observing Neanderthal genes in a region is inversely proportional to the amount of gene divergence between the modern human and Neanderthal genome

▶ Both of these actualities are expected if introgressed Neanderthal sequence in MHs experienced widespread purifying selection = removal of deleterious genes

Vattathil & Akey, 2015: N male hybrid sterility

▶ X chromosome (in both sexes) = 153 million base pairs. It represents about 800 protein-coding genes compared to the Y chromosome containing about 70 genes, out of 20,000–25,000 total genes in the human

▶ X chromosome is significantly depleted of Neanderthal genes,

  ▶ with an approximate 5-fold reduction in Neanderthal genes compared to autosomes (non-sex chromosomes)

  ▶ reduced fitness in male hybrid offspring (and perhaps resulting in male hybrid sterility), a phenomenon observed in many other species (Orr, 1997).

▶ MH Testis-specific genes are significantly enriched in regions depleted of Neanderthal genes, further supporting the inference of reduced fitness in male hybrid offspring.

Vattathil & Akey, 2015

▶ Contact with N and D hominins resulted in episodes of adaptive introgression (genes that give you evolutionary advantage).

▶ Examples:

▶ Haplotype of EPAS1 that was previously shown to confer adaptation to high altitude in Tibetans (likely by lowering hemoglobin concentration) was found to be inherited from a Denisovan ancestor (Huerta-Sanchez et al., 2014).

▶ Similarly, HLA (Abi-Rached et al., 2011) and STAT2 (Mendez et al., 2012), both of which play important roles in the immune response.

Vattathil & Akey, 2015: N admixture

▶ 1 - Most parsimonious model to explain these data was that N-MH hybridization occurred once in the ancestral population to all present-day non-Africans, likely in the Levant shortly after the dispersal of modern humans out of Africa.

▶ East Asian individuals were found to have on average 20% more Neanderthal sequence than European individuals.

▶ 2 - New models suggest at least two distinct admixture events—

    ▶ an initial pulse of admixture into the common ancestor of all present day non-African populations

    ▶ an additional pulse of admixture into the ancestors of East Asians after their divergence from European populations.

Vattathil & Akey, 2015

▶ D DNA in Melanesians; but very low levels of Denisovan ancestry in East Asians

▶ Papua New Guineans carry the highest level of Denisovan ancestry (4%–6% of the genome) among all populations studied

▶ Other Southeast Asian island populations and Chinese may have gotten their lower amount of D DNA via recent gene flow from populations that had admixed with Papua New Guineans

16. Ancient gene flow from early MHs into Eastern Neanderthals - Martin Kuhlwilm, et al., 2016

▶ Comparisons of Altai N and D genomes with the sequences of chromosome 21 of two Ns from Spain and Croatia: evidence of multiple gene flow events

▶ Ancient African MHs

  ▶ contributed genetically to the ancestors of Altai N ~100 Ka ago

  ▶ but not to Ds or the two European Ns.

▶ Ancestors of Altai Ns and of MHs met and interbred, possibly in the Near East, many thousands of years earlier than previously reported.

Kuhlwilm, 2016: Lots of gene flow

▶ This divergence was followed by

  ▶ N gene flow into ancient MHs outside of Africa (3.3–5.8%)

  ▶ D gene flow into the ancestors of MHs in Oceania and mainland Asia

  ▶ N gene flow into the Ds

  ▶ Superarchaic gene flow into the Ds (0.0–0.5%)

  ▶ Early MHs gene flow into Ns ~100 Ka (1.0–7.1%).

Kuhlwilm, 2016: pre 60 Ka N gene flow into MHs

▶ The Altai N genome shares 5.4% more derived alleles with present-day Africans than does the D genome

▶ These gene flow events occurred in the African MH genomes 100–230 Ka ago, suggesting that they

  ▶ entered into the ancestors of the Altai N well before

  ▶ the gene flow from Neanderthals into MHs outside Africa 65-50 Ka ago

▶ MH introgression into Ns occurred mainly after the divergence of the Altai N from western El Sidrón and Vindija N lineages ~110 Ka years ago

▶ Consistently small Altai Neanderthal population perhaps reflecting a long period of isolation in the Altai Mountains.

17. Excavating Neandertal and Denisovan DNA from the genomes of Melanesian individuals – Benjamin Vernot, et al., 2016

▶ Comparison of ancient DNA and whole-genomes from 1523 geographically diverse living individuals, including 35 Island Melanesian genomes.

▶ Neandertal admixture:

  ▶ occurred multiple times in different MHs populations,

  ▶ characterize genomic regions that are significantly depleted of archaic sequences,

  ▶ identify signatures of adaptive introgression.

Vernot, 2016

▶ All current MHs derive ~2% of their ancestry from Neandertals,

▶ 2 to 4% Denisovan ancestry are only found in Oceanic populations, low levels of Denisovan ancestry is more widespread

▶ Melanesians have both N and D DNA, whereas Europeans only have N DNA.

▶ These Melanesian samples had 2 to 3% D DNA, genes involved in metabolism and immunity; none involved in brain development

Vernot, 2016

▶ Skoglund suspects that <u>SE Asia</u> is where the ancestors of Melanesians admixed with Denisovans.

▶ <u>Some African groups (Esan, Luhya and Gambians)</u> <u>show evidence of having</u> <u>some Neanderthal ancestry</u>, most likely inherited indirectly through recent admixture with non-Africans.

▶ <u>In East Asians, South Asians, and Europeans</u>: <u>virtually all of the archaic sequence is Neandertal in origin</u>

▶ <u>Only small fraction</u> <u>(<1%) of D DNA in East and South Asians</u>

Vernot, 2016: Three N encounters, but only 1 D

▶ Conclusion: the ancestors of MHs acquired Neanderthal DNA on three occasions.

▶ The first N encounter: common ancestor of all non-Africans interbred with Neanderthals (60-50 Ka)

▶ The second occurred among the ancestors of East Asians and Europeans, after the ancestors of Melanesians split off.

▶ The third happened when the ancestors of East Asians — but not Europeans — interbred with Neanderthals.

▶ The Melanesians took a different course.

▶ After a single interbreeding with Neanderthals, their ancestors went on to interbreed just once with Denisovans.

19. Archaic Adaptive Introgression in TBX15/WARS2 – F. Racimo, et al., 2016

▶ Study of signal of positive selection in 200 Greenlandic Inuit from Denisovan genome.

▶ The region contains two genes, WARS2 and TBX15, and has previously been associated with adipose tissue differentiation and body-fat distribution in humans.

▶ Adaptation to the cold by promoting heat-generating brown body fat

Australia: ~4% Denisovan DNA

► <u>Aboriginal Australian and Papuan genomes</u> show an excess of Denisovan introgressed sites.

► Evidence for Denisovan admixture estimate of <u>~ 4.0%;</u> <u>Denisovan/Australo-Papuan admixture took place</u> <u>~ 44 Ka</u>

► Our results imply that Aboriginal Australian groups are the descendants of the ancestral population that first colonized Australia.

20. The Morphology of Permanent Molars from the Paleolithic Layers of Denisova Cave - A.V. Zubova, et al., 2017

▶ 2017: morphology of two permanent molars:

  ▶ Denisova 4 is an upper left third or second molar, and

  ▶ Denisova 8 is an upper left third molar.

▶ Extremely large dimensions, and morphological affinities with *Homo erectus* of Sangiran and the Middle Pleistocene hominins of China, such as Xujiayao.

▶ Proposed that the unidentified part of the Denisovan genome may stem from *Homo erectus*. Dentally, Homo altaiensis is a very conservative taxon.

Morphology of D 4 and D 8
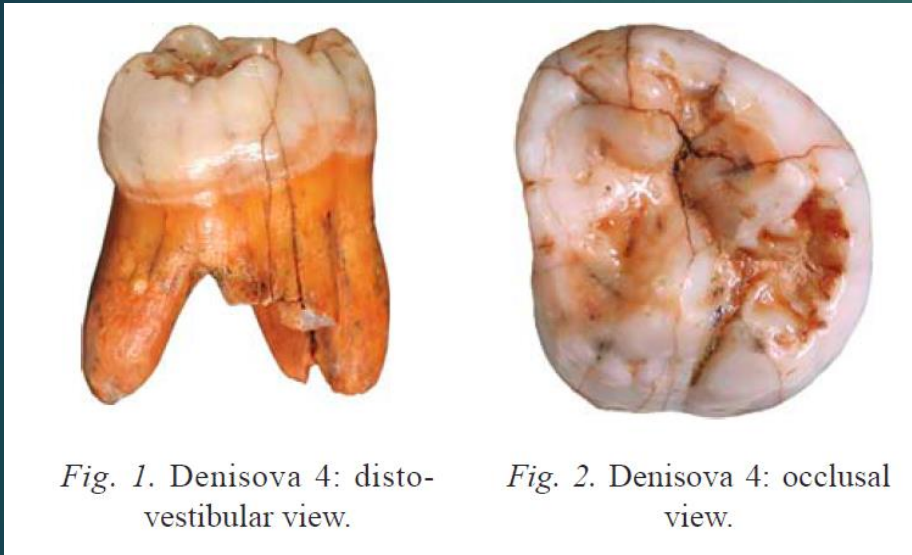
Fig. 1. Denisova 4: disto-
vestibular view.

Fig. 2. Denisova 4: occlusal
view.
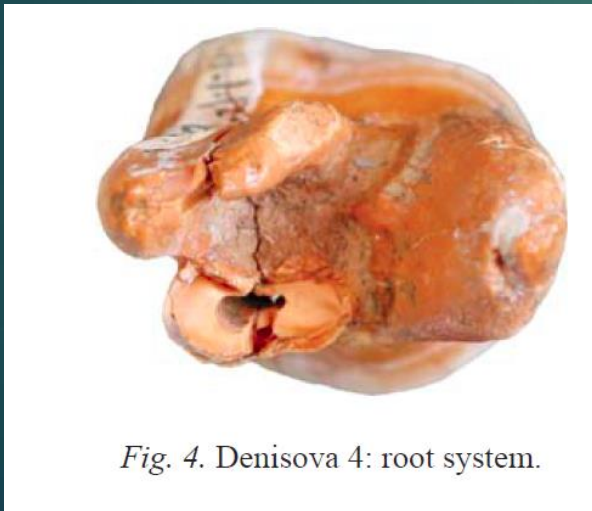
Fig. 4. Denisova 4: root system.
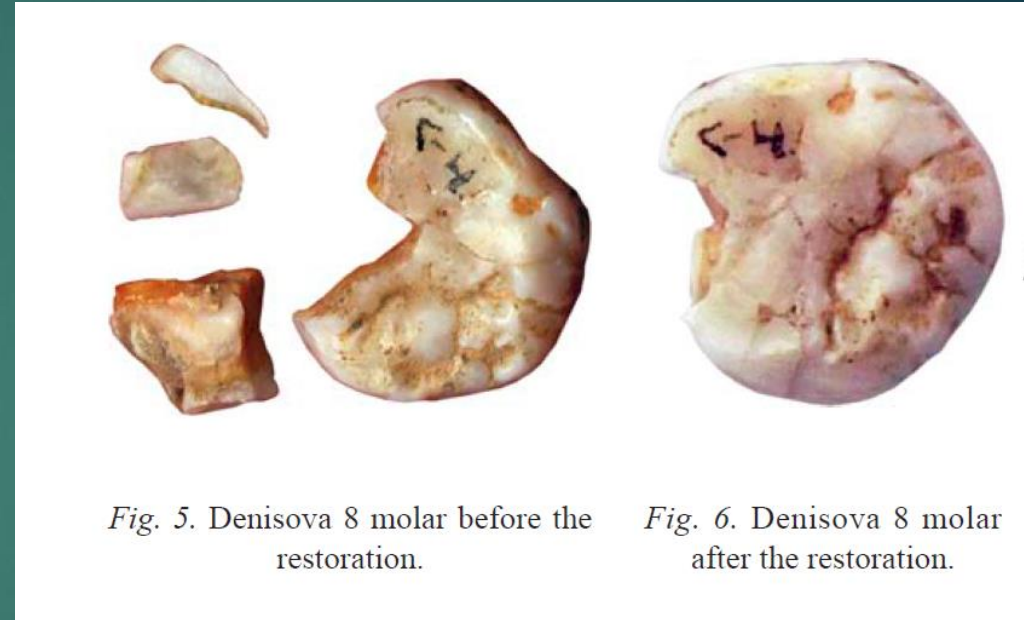
Fig. 5. Denisova 8 molar before the
restoration.

Fig. 6. Denisova 8 molar
after the restoration.

Zubova, 2017: Very large D molars are *H. erectus*-like

▶ Denisovan molars do not match the dental patterns of any European hominin taxa: highly archaic markers; lacks Neanderthal features

▶ They are mostly similar to the dental complexes found in *H. erectus* of Southeast Asia; in particular, in Sangiran specimens; as well as the Chinese Xujiayao molars

▶ The admixture between these two species most probably took place in Southeast Asia.

▶ The paleogenetic data also suggest that Denisovan genes were widespread in this region before the advent of modern humans.

Zubova, 2017: Multiregionalist interpretation of molars

▶ Conclusions: The most prominent feature: set of erectoid traits found in both molars studied.

▶ The peculiar morphology similar to the Denisovan molars is found only in Asian hominins, but not in any European specimens.

▶ Thus, the origin of *H. altaiensis* is most probably related to Asian *H. erectus*

▶ Denisovan genome includes an unknown hominin species (Krause et al., 2010), possibly *H. erectus*.

▶ Peculiar features of dental morphology of this population are megadontia; Thus, *H. altaiensis* exhibits a very conservative mode of morphological evolution.

21. A fourth Denisovan individual – V. Slon et al., 2017

▶ We report the retrieval of DNA from a deciduous lower second molar (*Denisova 2*), from a female Denisovan individual.

▶ *Denisova 2* is substantially older than two of the other Denisovans,

▶ The tooth came from a geological layer formed between 227 to 128 Ka, making it potentially the oldest of the four specimens.

▶ Estimate that the Denisova 2 individual lived approximately 50 to 100 Ka earlier than Denisova 3

Slon, 2017: 3rd tooth, Denisovan 2, 227-128 Ka

A B C

D E F

5 mm

Slon et al. Sci. Adv. 2017; 3: e1700186
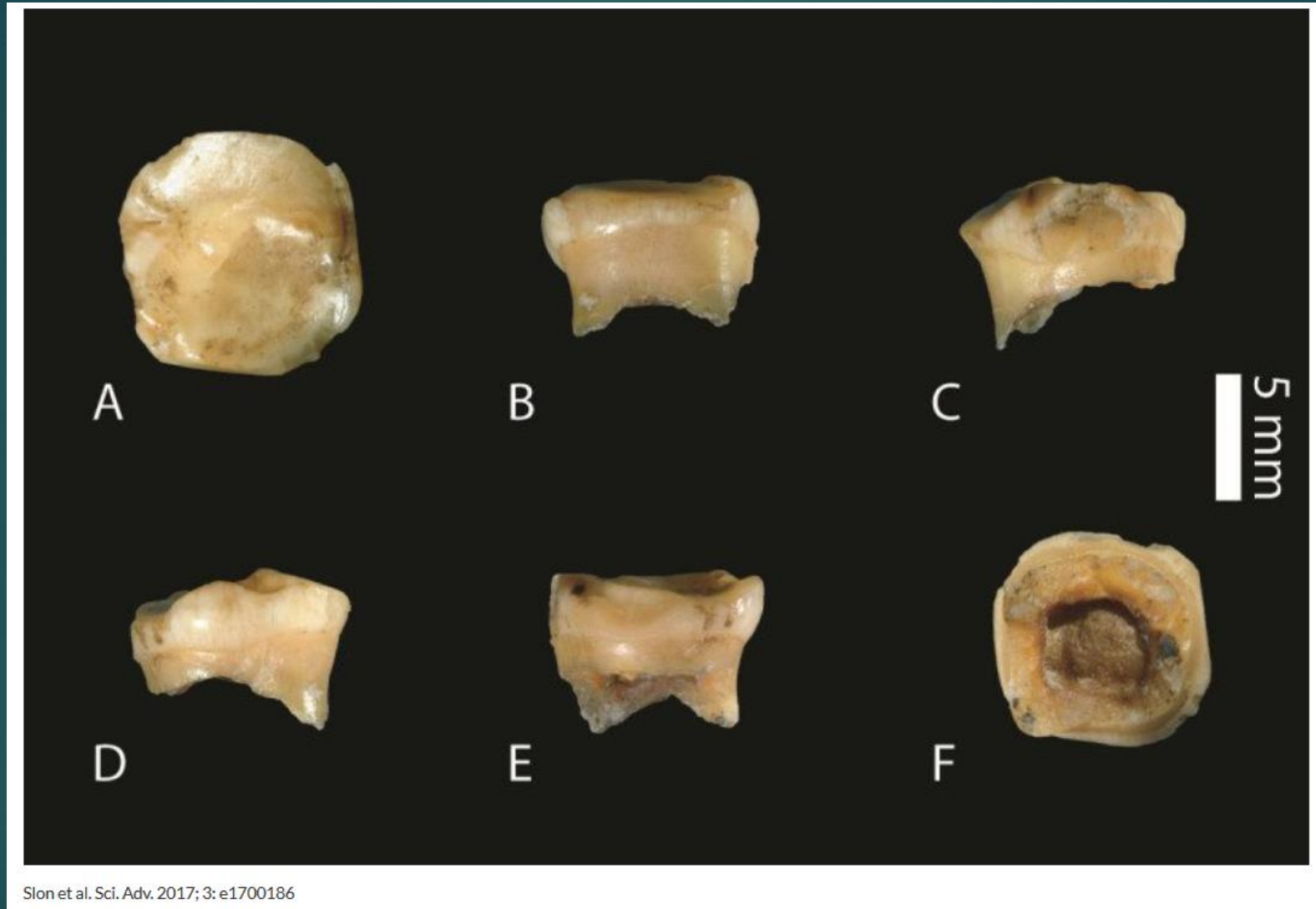
Slon et al.
Sci. Adv.
2017

DNA in the new specimen is genetically remarkably similar to that extracted from the three younger fossils. This fits with the idea that the Denisovan population was small and had a low genetic diversity

45. Neandertal and Denisovan DNA from Pleistocene sediments – V. Slon, et al., 2017

▶ Study of just dirt: looked in cave sediments for ancient DNA of hominids and other mammals, even those lacking skeletal remains.

▶ Found N and D mtDNA in cave sediments at multiple sites.

▶ Investigated whether hominin DNA may survive in sediments at archaeological sites in the absence of visible skeletal remains. Mineral and organic components in sediments can bind DNA

You only need dirt…to find mtDNA
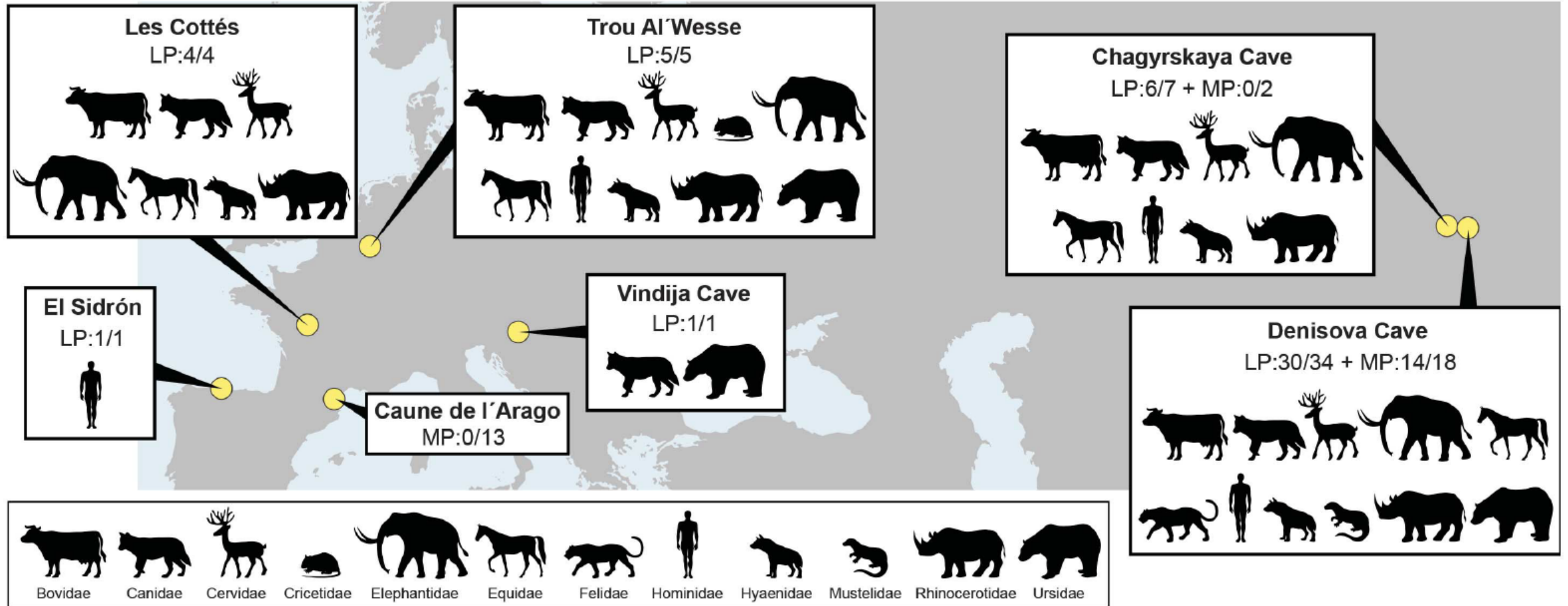
Fig. 1. Ancient taxa detected in Late Pleistocene (LP) and Middle Pleistocene (MP) sediment samples from seven sites. For each time period, the fraction of samples containing DNA fragments that could be assigned to a mammalian family and authenticated to be of ancient origin is indicated. The shaded symbols representing each family are not to scale.

Slon, 2017: sediments

▶ Collected 85 samples from seven archaeological sites with known hominin occupation, varying in age between ~14 Ka and ≥550 Ka; 49 samples from Denisova Cave and 9 from Chagyrskaya Cave

▶ Found mtDNA fragments from 12 mammalian families (mostly hyaenids, bovids, equids, cervids, and canids)

▶ For eight sediment samples (from El Sidrón, Trou Al'Wesse, Chagyrskaya Cave, and Denisova Cave), found N mtDNA in 87 to 98% of sequences; 4-11% for MHs, 0 to 2% for Ds, 0 to 2% for SH Ns

▶ Compatible with the DNA originating from excreta or the decay of soft tissue

Rogers, 2017: N fitness and population size

▶ They estimate that

  ▶ the effective population size (Ne) of Neandertals was ~15,000 individuals and

  ▶ that Neandertals and Denisovans separated from each other shortly after their ancestor separated from modern humans (~300 generations).

▶ These estimates are at odds with previous results that suggested a smaller Ne for Neandertals [ranging from 1,000–5,000 individuals

Multiple Early MH-N Admixtures: before 60 KA

▶ MH group 1: Early MHs emerged in Africa between 300 to 200 Ka ago, and mixed with Neandertals in the Middle East or the Arabian Peninsula, where fossils and stone tools of both groups date back to about 120 to 125Ka.

▶ This group of MHs went extinct, but their DNA persisted in the Neandertals that headed east to eventually settle in Siberia.

▶ MH group 2: A later group of modern humans left Africa much later and interbred 50-60 Ka ago with Neandertals that had headed south from Europe to the Middle East.

Multiple Early MH Mixtures

▶ In the 60 Ka migration, Neandertals interbred with the ancestors of living Europeans and Asians, who then spread throughout Eurasia.

▶ Some of this group of MHs also encountered Ds, picking up the DNA that persists today in Melanesians and some Asians.

▶ Thus MHs mixed with Ns and Ds at least three times after they migrated out of Africa. But that's just a fraction of the intermingling that must have taken place.

Multiple Admixtures

▶ Neandertals interbred with Denisovans.

▶ Denisovans interbred with a "superarchaic" hominin, possibly *H. erectus,* whom they encountered as early as 400 Ka ago.

▶ Denisovans interbred with MHs in Asia more than once, based on different patterns in the distribution of Denisovan DNA in some Chinese and Melanesians.

Example of DNA segmentation based on recombination during meiosis

DNA segments
can be longer,
if more recent,
or shorter,
if more ancient
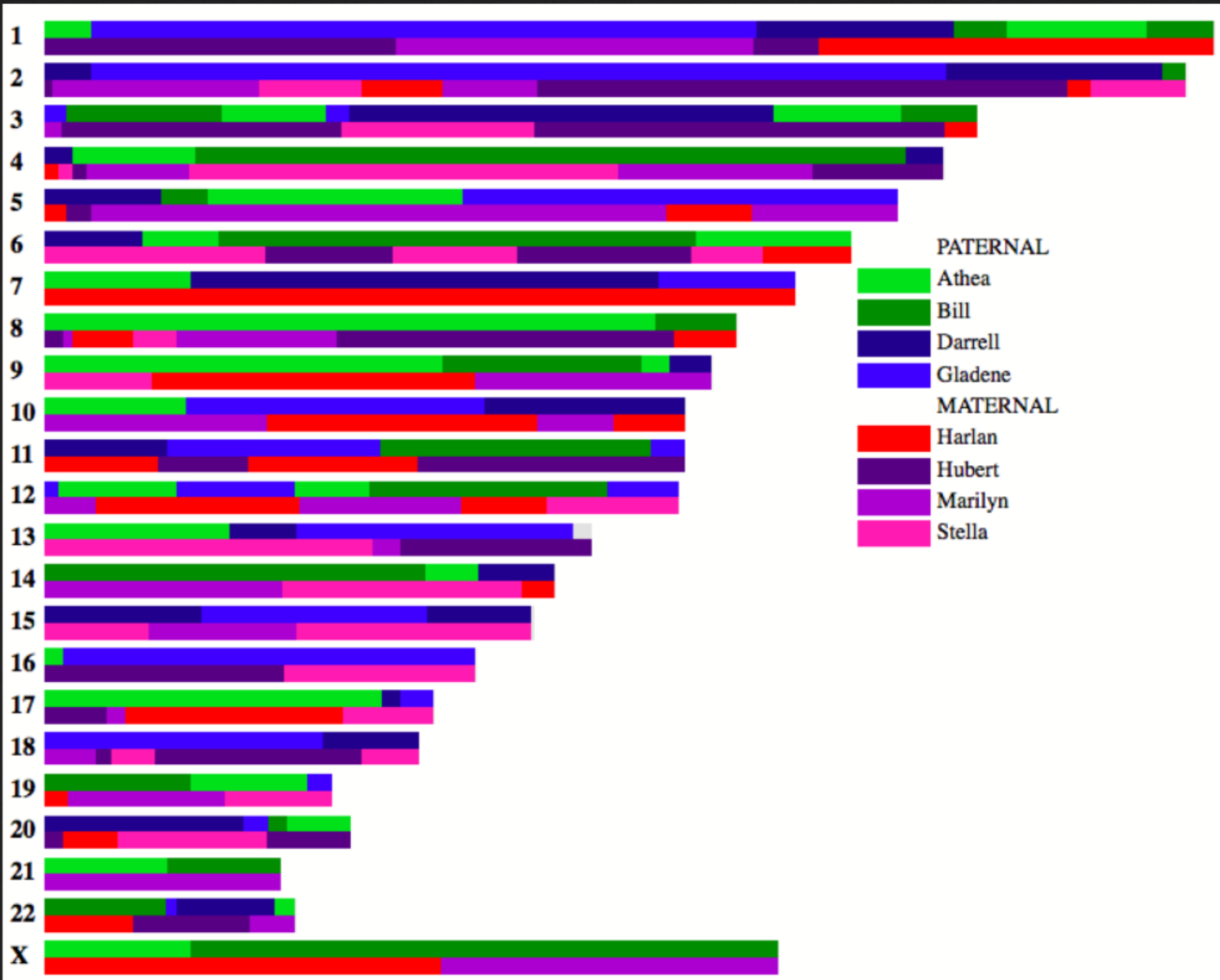
Use of ancient DNA segment length: D and New Guineans

▶ <u>DNA as a clock</u>: As soon as archaic populations mix with modern ones,

  ▶ <u>DNA segments</u> <u>contributed by archaic humans are chopped up by the process of recombination at conception,</u>

  ▶ <u>spliced together with MH segments at the rate of one or two splices per chromosome per generation</u>.

▶ Measure the size of intact archaic ancestry segments: D segments in New Guineans were 12% longer than N segments which implies <u>D</u> segments had been introduced that <u>much more recently</u>

Ancient DNA segment length: D and New Guineans

▶ <u>Based on</u> <u>how much longer the D segments were than the N segments in New Guineans</u>, interbreeding between Ds and NGs occurred <u>59 to 44 Ka.</u>

▶ 5-8% of New Guinean ancestry is from archaic humans:

  ▶ N = 2%

  ▶ D = 3 to 6%

▶ New Guineans have <u>largest known contribution of archaic DNA of any present- day human population</u>

D. Reich, 2018: <u>Superarchaics</u>: a Ghost population in D DNA

▶ "Ghost population": a genetic lineage we do not have data from in unmixed form, but whose past existence can be detected from its genetic contribution to later people's genome.

▶ Ns are slightly more closely related to ancient Africans than to Ds.

▶ Implies Ds interbreed with a superarchaic lineage (from which Ns and current Africans have no DNA)

  ▶ evidence: at locations in genome where all Africans share a mutation, the mutation is more often see in Ns than in Ds;

Ghost Superarchaics

▶ given that all Africans carry the mutation, they occurred long time ago (takes 1 million years for new mutation to fixate in 100% of population)

▶ This superarchaic group split from MH lineage 1.4 M to 900 Ka ago; represents 3 to 8% of D DNA

H. erectus?: Archaic Human genetic Contribution To Denisova Genome

► D DNA harbors a component that derives from a population that lived before the separation of Ns, Ds, & MHs.

► One method estimates that 2.7–5.8% of D DNA comes from superarchaic hominin which diverged from the other hominins 0.9–1.4 million years ago

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

► The estimated population split time is when *Homo erectus existed*.

Peștera cu Oase, Romania: MH mandible

Teeth were much larger than our current teeth

N interbreed with MH

▶ Peștera cu Oase, Romania: Male MH fossil jaw

▶ 5–11% of the bone's genome is Neanderthal, including large chunks of several chromosomes

▶ Had a close Neanderthal ancestor 4–6 generations back – i.e. great, great, grandfather

▶ Current MHs have 1-2% N DNA in our genomes;
  ▶ each of us have different N segments;
  ▶ equals 20-30 % of total N genome currently present

Two Pulses of Archaic Denisovan Admixture

Modern humans · Denisovans · Neanderthals

Admixed genomes

Two waves of Denisovan admixture detected in East Asians

Ancestry proportions not drawn to scale
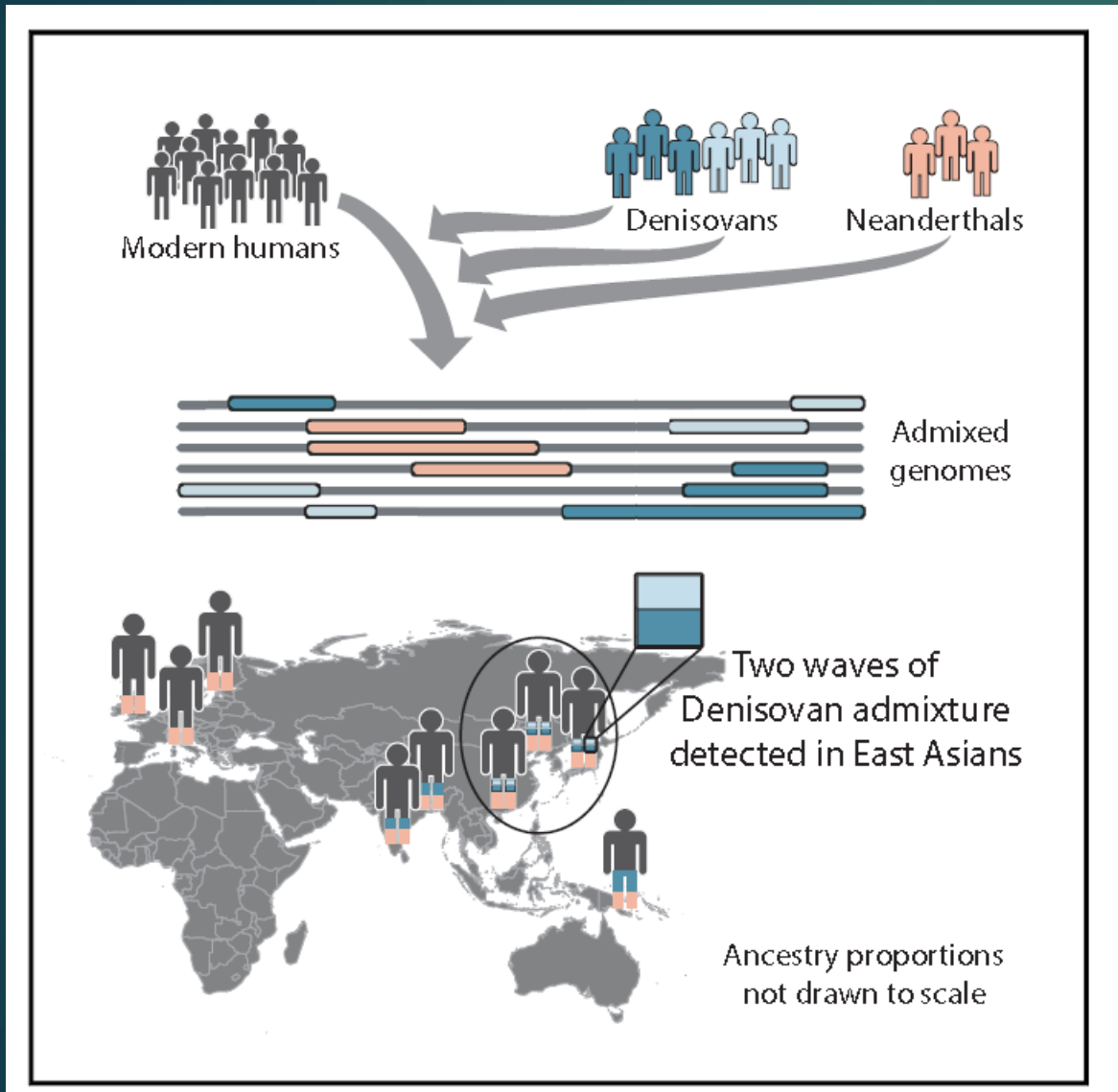
- S. Browning, 2018:

- Asian genomes carry N and D DNA

- East Asians show evidence of introgression from two distinct Denisovan populations

- South Asians and Oceanians carry introgression from only one Denisovan population

Sharon R. Browning,, et al., Cell, 2018

Browning, 2018: 2 Denisovan Lineages

▶ 2018: Genetic evidence of a second introgression event from Denisovans in various modern East Asian populations: the Han Chinese, Japanese people and the Dai people.

▶ The Denisovan component in these East Asian populations is closer to the sequenced Siberian Denisovan genome than that found in Papuans.

▶ Thus, the Papuans must have derived their elevated Denisovan DNA component from a separate D introgression event.

Browning, 2018: 2 Denisovan lineages

▸ New evidence for two waves of Denisovan admixture,

 ▸ one from a population more distantly related to the Altai Denisovan; primarily present in Papuans and South Asians

 ▸ one from a population closely related to the Altai Denisovan individual; primarily present in East Asians,

  ▸ The East Asian populations are the only populations with relatively equal and non-negligible contributions from both components.

Browning, 2018

▶ Overall, <u>East Asians and South Asians</u> carry similar amounts of detected Denisovan ancestry, while <u>Papuans</u> carry much more detected Denisovan ancestry

▶ Lack of evidence for multiple waves of Neanderthal ancestry: The frequency of Neanderthal introgression is substantially higher (30%) in East Asians than in Europeans.

▶ Generally, <u>Asian populations carry about 0.1% Denisovan ancestry</u>. So, only one-thousandth of their genome comes from Denisovans

Viviane Slon, Max Planck Institute, chosen one of *Nature's 10* in 2018

Viviane Slon takes sediment samples in Denisova cave to look for ancient DNA. Slon did the DNA analysis of the bone fragment that showed it was a Neanderthal-Denisovan hybrid. (Courtesy Bence Viola)

'Could I have accidentally mixed two samples together?'
Took 6 more samples.

Denisova 11: Bone of 13 yo girl

▶ A <u>fragment of a long bone, "Denisova 11",</u> was identified <u>among over 2,000 undiagnostic bone fragments</u> excavated in Denisova Cave <u>as being of hominin origin</u> using collagen peptide mass fingerprinting.

▶ Its <u>mtDNA</u> was found to be <u>Neandertal</u> and direct radiocarbon dating showed it to be > 50 Ka old.

▶ From its cortical (outer surface of bone) thickness, Denisova 11 was an <u>adult woman, but possibly an adolescent girl as young as 13 years old</u>.

▶ First discovery of an <u>ancient-hominin hybrid</u>

Denisova 11: 2 cm bone: "Denny"

DC1227 was a bone fragment weighing 1.68 g (0.059 oz), measuring in with a maximum length of 24.7 mm (0.97 in) and maximum width of 8.39 mm (0.330 in).

Nature: Denny - Article does not use the word "hybrid"

The first-generation offspring of a Neanderthal mother and a Denisovan father
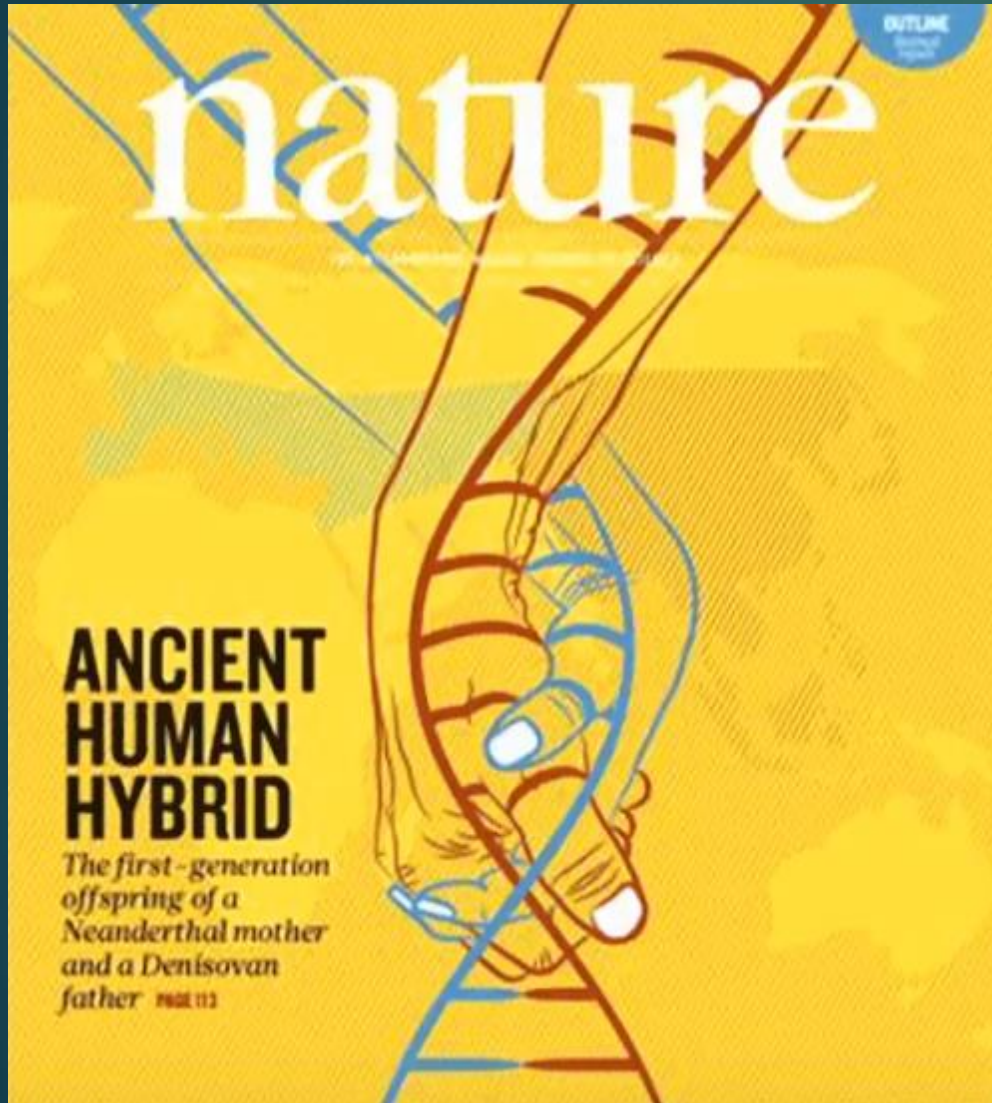
- Denny:
  - Mom's a Neanderthal Dad's a Denisovan

- Denisova Cave

- Why does journal Nature use word "hybrid", but the article does not? Hybrid is a species concept

26. The genome of the offspring of a Neandertal mother and a Denisovan father – V. Slon, et al., 2018

▶ Genome of *"Denisova 11"*, a bone fragment from Denisova Cave,

▶ from an individual who had a Neandertal mother and a Denisovan father.

▶ dated to 79-118 Ka ago.

▶ Neandertal MtDNA from the bone fragment, which is inherited only from the mother; western N

▶ Nuclear DNA, which is inherited from both parents, was half Neanderthal, and half Denisovan. The child was a hybrid of a N mother and a D father.

▶ The D father, has N ancestry; from a N population related to a later Siberian Denisovan found in the cave, from a different N population than her mother

Denny: Ancient Girl's Parents Were Two Different Human Species

► 2014: A fragment of a long bone, 'Denisova 11', was identified among 2000 these undiagnostic bone fragments.

► It was one of these thousands of bone chips, most from ibexes, deer and horses, recovered from the cave using river water and a sieve.

► Sample DC1227 (*Denisova 11*) was then screened by researchers at the University of Oxford and identified as being of hominin origin using collagen peptide mass fingerprinting. Then it was sent to Leipzig.

Denny

▶ This was the <u>first time that this protein technique was used to successfully identify the presence of an extinct human.</u>

▶ Half the bone was ground up for radiocarbon dating; > 50 Ka old.

▶ The other half was ground up for DNA analysis. Nuclear DNA analysis redone 6 times.

▶ <u>Denny bone no longer exists</u>.

▶ Bone was <u>acid etched</u>. It was probably digested by a hyena.
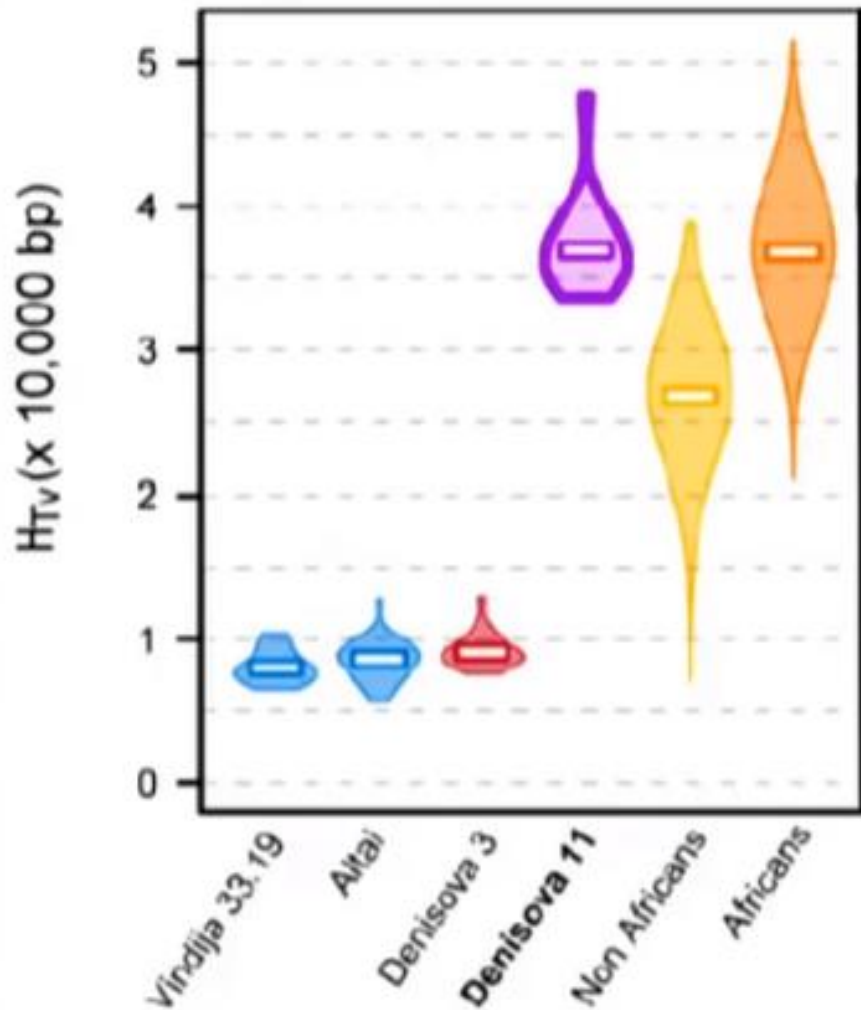
Slon et al., Nature 2018.

Genetic variation:

Africans have most diverse (most (40 K) basepairs due to longer mutation rate period)

More than Non-Africans

But Ds and Ns had significantly less genetic diversity (10 K bps); small groups, 3-4x less variation

Denisova 11 =
Hybrid had more genetic variation being combination
Mom N, father D (with N ancestry, circa 10-20 K earlier); first generation

Slon, 2018: Mixing between Late Pleistocene hominin groups was common

▶ The Neandertal <u>mother</u> came from
  ▶ a population more closely related to Neandertals who lived later in Europe
  ▶ than to an older Neandertal found in Denisova Cave,
  ▶ suggesting that migrations of Neandertals across Eurasia occurred sometime after ~120 Ka ago.

▶ Denisova 11 bone: <u>38.6% N alleles; 42.3% D alleles</u>

▶ Denny was a female who died around 90 Ka ago
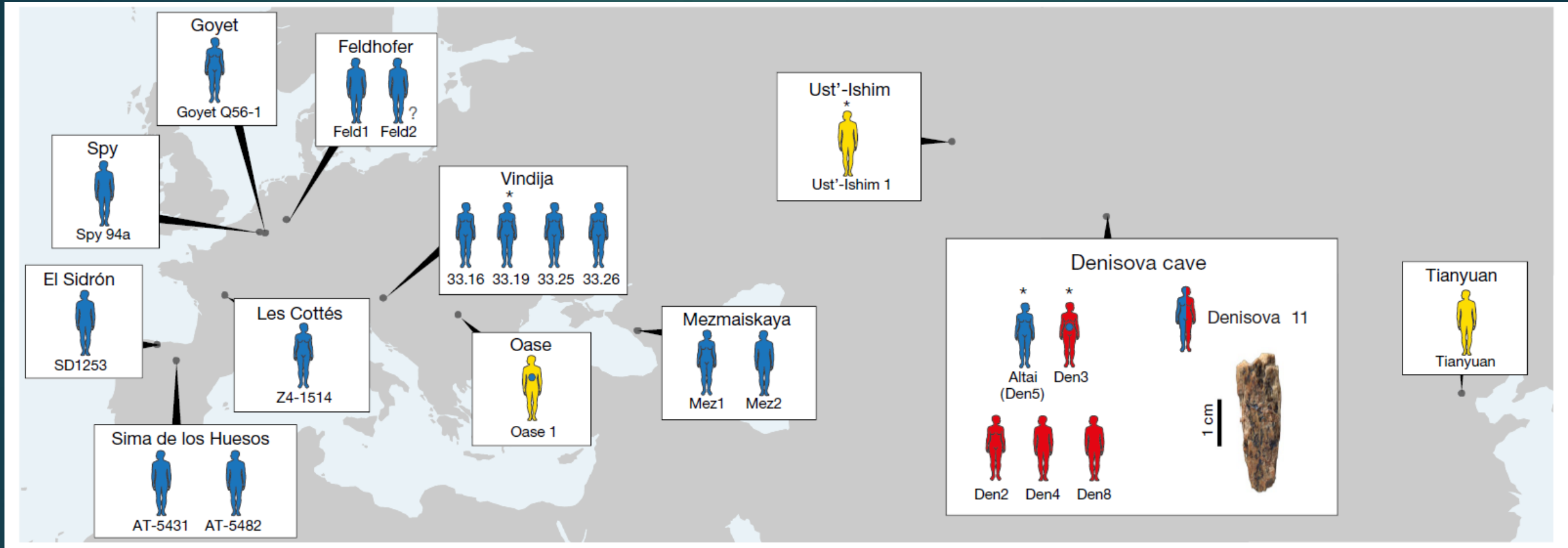
Fig. 1 | Location of Ns, Ds and ancient MHs dated to approximately 40 Ka or earlier.
Blue, Neanderthals; red, Denisovans; yellow, ancient modern humans.

Asterisks indicate that the genome was sequenced to high coverage; individuals with an unknown sex are marked with a question mark.
Note that Oase 1 has recent N ancestry (blue dot) that is higher than the amount seen in non-Africans.
Denisova 3 has also been found to carry a small percentage of Neanderthal ancestry

Tangled Tree

An unknown hominin species interbred with Denisovans or their ancestors.

The Altai Neanderthal's genome revealed mixture with an early *Homo sapiens* lineage.

>1 million years ago

~90,000 years ago

Mystery hominin species

Denny's father

Denisovans

Neanderthals

Altai Neanderthal — Denny's mother — Vindija Neanderthal

Early modern-human lineage

Asians

Europeans

Africans

Interbreeding episode or event

The ancestry of Denny's mother was a surprise. She was more closely related to a 55,000-year-old Neanderthal from Vindija cave in Croatia than to an 'Altai Neanderthal', who lived in Denisova Cave roughly 30,000 years before Denny.

All non-African humans carry traces of Neanderthal DNA, owing to pairings some 47,000–65,000 years ago.

©nature

Denny: N and D gene flow

▶ In conclusion, the genome of Denisova 11 provides direct evidence for genetic mixture between Neandertals and Denisovans on at least two occasions:

  ▶ once between her Neandertal mother and her Denisovan father, and

  ▶ at least once in the N ancestry of her Denisovan father.

  ▶ Therefore, of the six individuals from Denisova Cave from whom nuclear DNA is available, two (Denisova 3 and Denisova 11) show evidence of gene flow between Neandertals and Denisovans.

University of Toronto researcher Bence Viola holds a 3D printed replica of the bone fragment from Denisova

Bence Viola, an assistant professor of anthropology at the University of Toronto, who is in charge of the catalogue of fossils found at Denisova Cave.

Denisova 11: related to Vindija N

➤ Like *Denisova 3*, this father carries some Neanderthal DNA from an admixture event far in the past, estimated at more than 300 generations earlier, and similar in sequence to the Altai Neanderthal genome.

➤ The N mother of *Denisova 11* was genetically closer to Neanderthal specimen *Vindija 33.19* from Croatia than to the *Altai Neanderthal, f*ound less than 1 meter away in the same cave.

➤ The Croatian N died much more recently than Denny, ~55 Ka ago — while the Neanderthal from Denisova Cave are dated to 120 Ka old.

V. Slon, 2018: Ns moved great distances

▶ That a ~90 Ka Neandertal in Siberia shared more alleles with Neandertals that lived at least ~20 ky later in Europe than with an earlier Neandertal from the same cave suggests that

- ▶ eastern Neandertals spread into Western Europe sometime after ~90 Ka or that

- ▶ western Neandertals spread to Siberia before that time and partially replaced the local population.

- ▶ Clearly, Neanderthals, at least at one point in their history, moved thousands of kilometers between eastern and western Eurasia.

Ns and Ds

▶ Despite interbreeding, <u>Neanderthals and Denisovans never merged </u>into a single genetic population.

▶ For hundreds of thousands of years, they <u>remained distinct</u>.

▶ It's possible they simply didn't have much opportunity to mate because they lived in small groups spread out across a vast landscape.

5 Ancient Admixtures
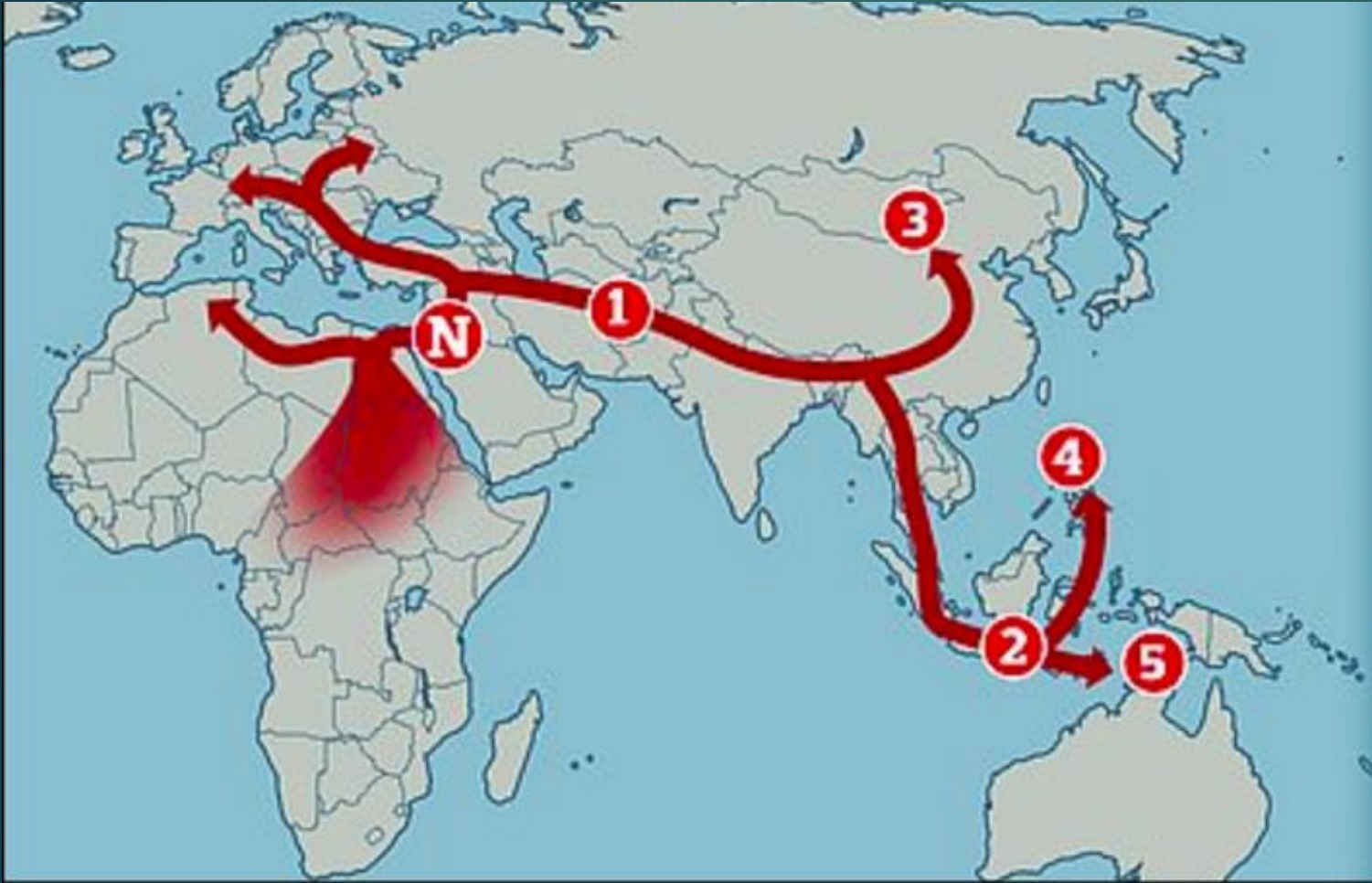
(N) Ancient MHs mated with Ns in Eurasia

1 = MHs interbred with superarchaic (EH1)

2 = MH ancestors of Australo-Papuans mate with Ds.

3 = Ds mated again with MHs

4 = Ancestors of MHs in Philippines mate with Ds.

5 = Ancestors of Flores pygmies mate with Ds and EH2

2 Ghost species: EH1 and EH2

▶ The <u>first unknown extinct hominid - named EH1</u> - was roughly <u>genetically equidistant from Ds and Ns</u>.

  ▶ Using a statistical technique called Bayesian inference, the researchers found evidence of what they call a "third introgression" – a 'ghost' archaic population (EH1) that MHs interbred with during the African exodus. This population is either related to the Neanderthal-Denisova clade or diverged early from the Denisova lineage

  ▶ The <u>ancestor of all Asian and Australo-Papuan populations bred with EH1</u>, resulting in <u>2.6 to 3.4 percent shared EH1 ancestry</u>.

▶ EH1 <u>genetic signal can still be detected in the DNA of Aboriginal Australians, East Asians and Andaman Islanders</u>. <u>EH1 likely occupied a region in northern India (1 on map).</u>

▶ Modern humans also seemed to have interbred with Denisovans in a number of locations, such as East Asia, the Sunda Shelf, and the Philippines (2, 3, and 4 on the map).

Flores pygmies and EH2

▶ Evidence for EH2 - an extinct hominin that interbred with MHs on Flores.

   ▶ It only appears in current human pygmy people that live near Liang Bua Cave, on Flores. (5 on the map).

   ▶ Flores pygmies have D, N and EH2 DNA

▶ The Island Southeast Asia region was clearly occupied by several archaic human groups, living in relative isolation from each other for hundreds of thousands of years before the ancestors of MHs arrived.

▶ Sadly, it also looks like the arrival of MHs was followed by the extinction of all the archaic hominids in each area. Talk about being ghosted.

27. Neanderthal and Denisovan ancestry in Papuans: A functional study - Evgeny E Akkuratov, et al., 2018

▶ Papuan genome: both N and D alleles, with different gene functional groups inherited from each.

   ▶ N alleles in genes: responsible for the regulation of transcription (copying DNA to RNA) and neurogenesis.

   ▶ D alleles: functionally responsible for bone and tissue remodeling.

Akkuratov, 2018: Papuan New Guinea

▶ Found D DNA in Melanesia, Papua New Guinea, Bougainville, Australia, Polynesia, Philippines

  ▶ Pacific Islanders and Aboriginal Australians: 3% to 6% D DNA

  ▶ Papuans: ~7% D & N DNA: 2.5 % N; later ~4.8 % D DNA

▶ Still unclear why so little D Dna (>.2%) is present in Mongolia, China, Cambodia, or mainland Asia: Did MHs admix with Ds and take southern coastal Asian route before MHs populated rest of Asia and then met Ds in Indonesia

A Multiplicity of Archaic Human DNA Lineages and lineage splits

▶ **1.4 Ma-900 Ka**: Main ancestral population of MHs, Ns, & Ds separates from the superarchaic African lineage.

▶ **1 Ma to 800 Ka**: The Denisovan and Sima de los Huesos mtDNA lineages separate from those of Neanderthals and modern humans.

▶ **770-550 Ka**: Ns and MH population split

▶ **700-60 Ka**: The "Hobbits" persist on the island of Flores in Indonesia.

▶ **470-380 Ka**: Neanderthal-Denisovan population split

470 Ka to Present

▶ 470-360 Ka: MH mtDNA split from Neanderthals

▶ 430 Ka: Sima de los Huesos skeletons and DNA show that the Neanderthal lineage was already evolving in Europe.

▶ 400-270 Ka: Siberian Denisovan (D0) and Australo-Denisovan (D1) lineages separate.

▶ <200 Ka and 54-49 Ka: Neanderthals and MHs interbreed.

▶ 49-44 Ka: Denisovans and MHs interbreed.
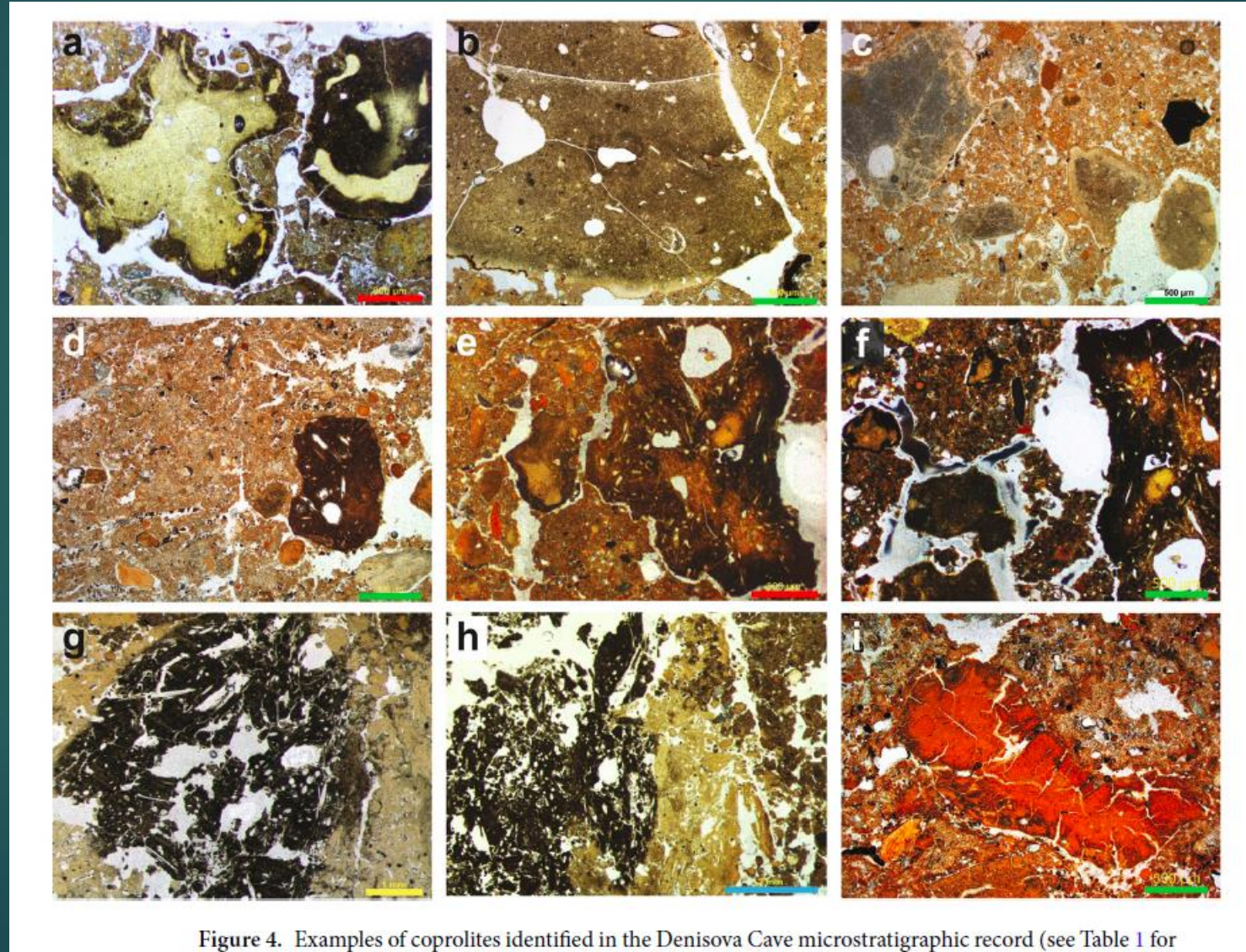
Figure 4. Examples of coprolites identified in the Denisova Cave microstratigraphic record (see Table 1 for

30. Hominin and animal activities in the microstratigraphic record from Denisova Cave - MW Morley, et al., 2019

▶ Dishing the dirt: ~4.5 meters of Pleistocene sedimentary deposit in D cave

▶ Denisovan sediments contain abundant fossil droppings, but surprisingly scant evidence of human activity such as fires.

▶ Large carnivores (hyenas, wolves, & bears) were common in the cave.

▶ Overall, the microstratigraphic record for Denisova Cave indicates that human activity was intermittent (>300 Ka to ~20 Ka).

Morley, 2019: Denisova Cave stratigraphy: few fires, lots of animals
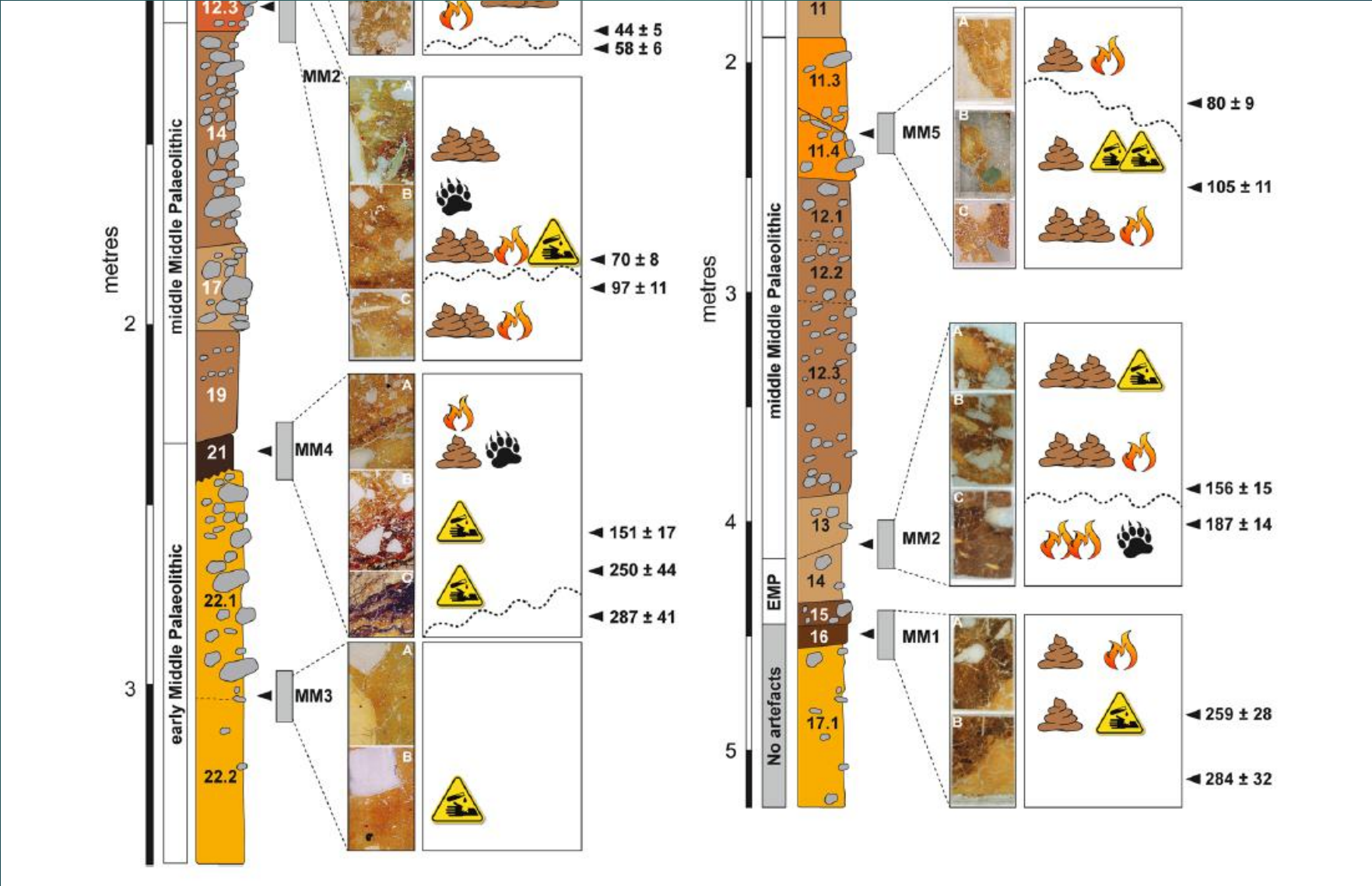
Morley, 2019: Denisova Cave as primarily a hyena den?

▶ <u>Very few hominin activities</u> in the microstratigraphic record.

▶ On the other hand, <u>coprolitic evidence for cave-dwelling carnivores is ubiquitous</u> and suggests that the site often <u>served as a den for hyenas</u> and, to a lesser extent, for wolves.

▶ The <u>cave was visited sporadically by hominins, who appeared not to have been prolific users of fire</u>, at least in the Middle Palaeolithic deposits that constitute the majority of the Pleistocene sequence.

Karst Baishiya cave in Xiahe, China

The Baishiya Karst Cave above the Jiangla riverbed in the Gansu province of China.
It is both a locally famous Buddhist cave and a tourist site.

Chen, et al., 2019

Finally, the mandible of a Denisovan!

DENISOVAN HANG-OUTS

The ancient-human group known as the Denisovans was first discovered from a bone fragment in Denisova Cave in Siberia. Researchers have now uncovered the remains of a Denisovan from outside that cave, at a site high on the Tibetan Plateau.

RUSSIA

Denisova Cave, 700 metres.

Baishiya Karst Cave, 3,280 metres. The altitude of the latest remains suggests that, as suspected, Denisovans evolved a genetic adaptation that helped them to cope in low-oxygen environments. This was eventually passed down to some modern Tibetans.

CHINA

Indian

36. A late Middle Pleistocene Denisovan mandible from the Tibetan Plateau - Fahu Chen, et al., 2019

▶ Discovery of a Denisovan mandible, identified by protein analysis, found on the Tibetan Plateau, at 3200 meters, in Baishiya Karst Cave, Xiahe (sh-a-he), Gansu, China.

▶ Dated to 160 Ka (MIS 6) through U-series dating of an adhering carbonate matrix.

▶ The Xiahe specimen provides first direct evidence of the Denisovans outside the Altai Mountains

▶ Denisovans occupied the Tibetan Plateau in the Middle Pleistocene epoch and successfully adapted to high-altitude hypoxic environments long before the regional arrival of modern *Homo sapiens*

The lateral photograph of Xiahe mandible showing two attached molars. The mandible is the first confirmed discovery of a Denisovan fossil outside of Denisova Cave.

Denisovan jaw, 160 Ka, Tibet, 10,000 feet

Xiahe half mandible

The Xiahe mandible (a, b, d, e, views after digital removal of the adhering carbonate matrix).

No chin
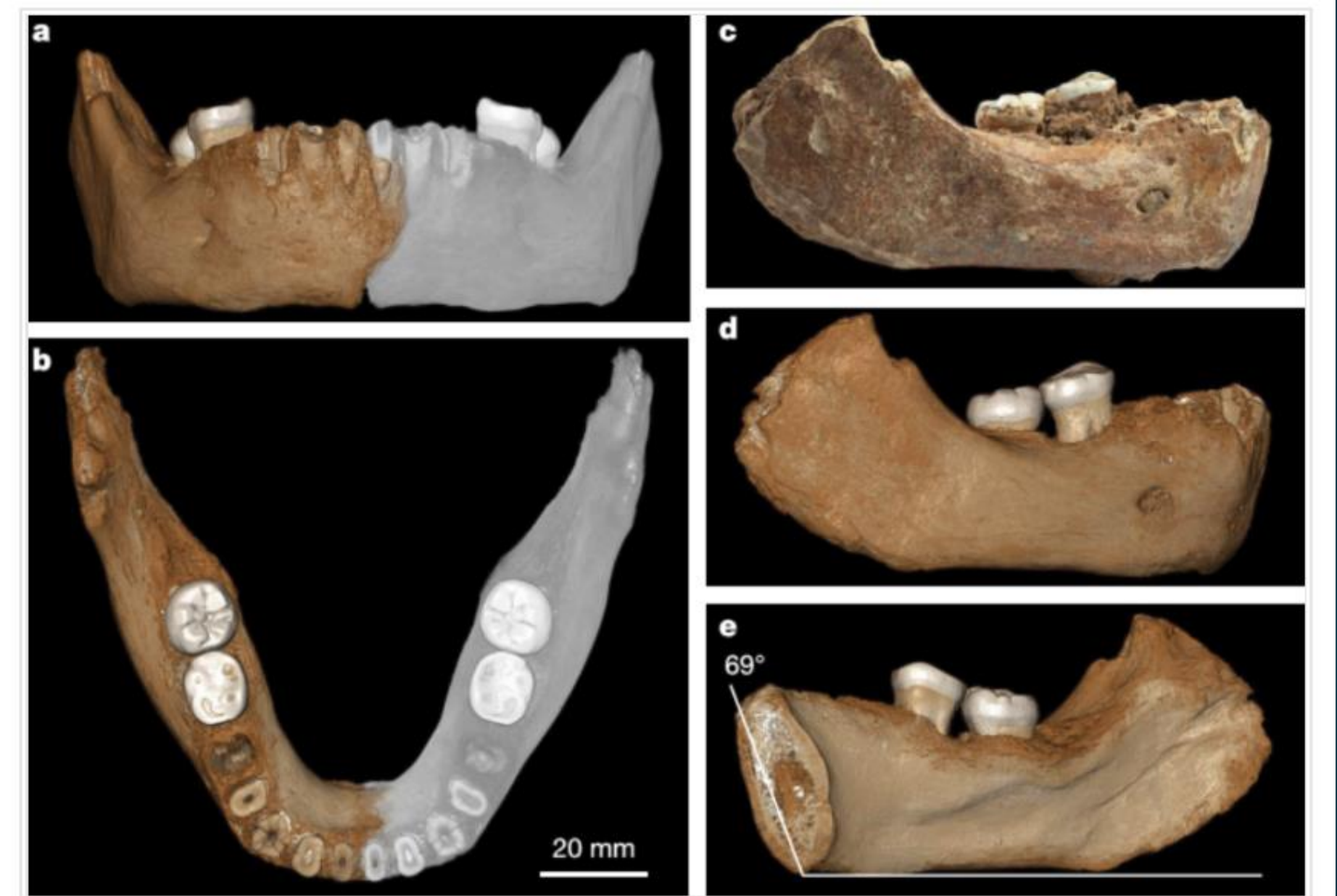
Denisovan jaw, 160 Ka, Tibet

▶ In 2019, a 160 Ka old right half of a mandible with 2 teeth of an adolescent belonging to Ds has been published.

▶ It was found in the Karst Baishiya cave in Xiahe (China) at 3280 meters of altitude.

▶ The new fossil is the first evidence of this species found outside the Denisova Cave in Siberia, buttressing the theory that Ds lived across much of central and eastern Asia.

▶ Earliest prior MH presence in Nwya Devu, Tibet dated 40 Ka.

Chen F. et al , *Nature(2019)*.

Protein analysis: Denisovan jaw, 160 Ka, Tibet

▶ The research marks the <u>second time an ancient human has been identified solely through the analysis of proteins</u>.

▶ Although no traces of DNA were preserved in the fossil, it was possible to <u>extract proteins from one of the molars</u>.

▶ A phylogenetic analysis concludes that **the** individual belonged to "a population closely linked to the Denisovans of Siberia."

Phylogenetic Tree: closest to Denisovan
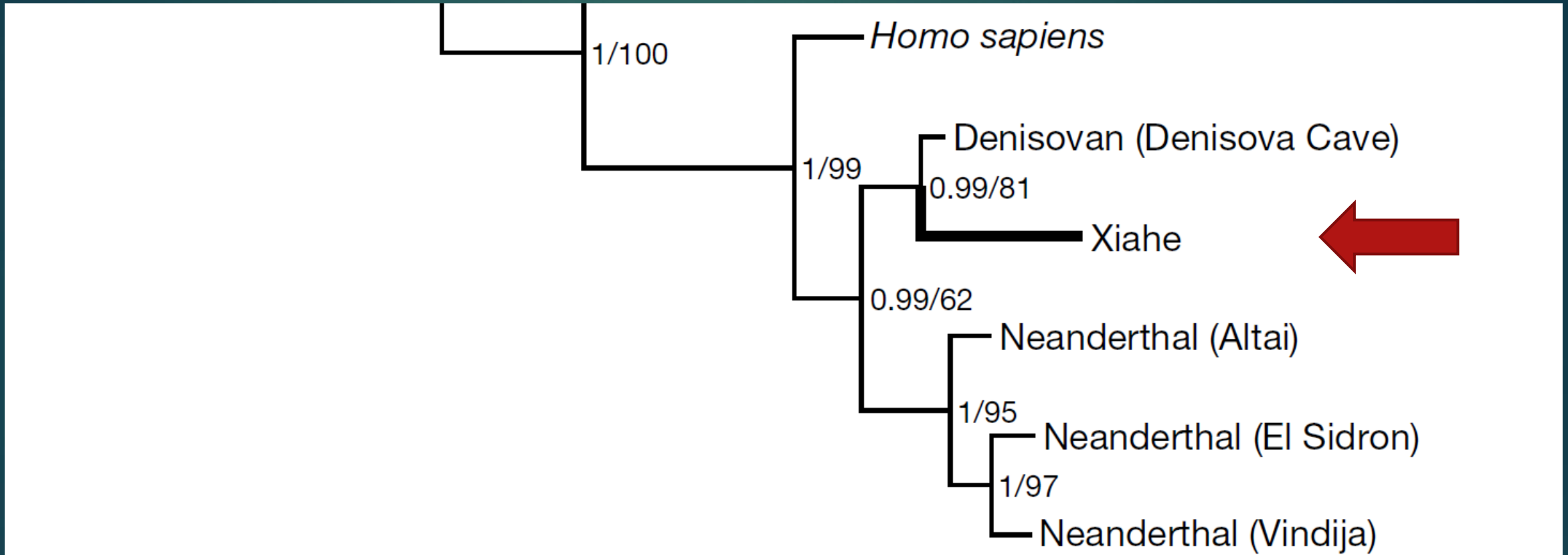
| Phylogenetic position of the Xiahe proteome within Hominidae.

In dentine from the teeth, they found collagen proteins suitable for analysis. Using phylogenetic analysis, the team compared these with equivalent proteins in groups including Denisovans and Neandertals, and found that they lined up closest with sequences from Denisovans.

The history of the mandible

▶ Cave is at an altitude of 3200 meters on the margins of the Tibetan Plateau

▶ 39 years ago, a Buddhist monk meditating in a cave on the edge of the Tibetan Plateau found a human jawbone with giant molars

▶ Recognizing the jaw's unusual nature, the monk gave it to the sixth Gung-Thang living Buddha, one of China's officially designated "living Buddhas," who consulted scholars and then gave the jaw to Lanzhou University.

▶ The jawbone was so "weird" that researchers there didn't know how to classify it, and it sat on shelves for years.

Karst Baishiya cave in Xiahe, China

▶ The fossil became part of the collection of Lanzhou University, where it remained unstudied until 2010.

▶ Chinese researchers showed the jaw to Jean-Jacques Hublin of the Max Planck Institute.

▶ After seeing its large molars—as big as ones found in Denisova Cave— Hublin immediately suspected it was Denisovan.

The history of the mandible

▶ In 2019 a research group led by F. Chen, et al. described a partial mandible discovered in 1980 by a Buddhist monk in the Baishiya Karst Cave on the Tibetan Plateau in China.

▶ Max Planck paleogeneticists couldn't get DNA from the jaw, but Hublin's graduate student Frido Welker had found in his doctoral work that Ns, MHs, & Ds differ in the amino acid sequence of key proteins

▶ It was determined by ancient protein analysis to contain D collagen, while uranium decay dating of the carbonate crust enshrouding the specimen indicated it was more than 160 Ka old.

Karst Baishiya cave in Xiahe, China

► Welker, now a postdoc at the University of Copenhagen, was able to extract collagen, a common structural protein, from a molar of the Xiahe jawbone.

► He found its amino acid sequence most closely matched that of Denisovans.

► The jaw's features could be a template for spotting other Denisovans. "Its distinct large molars and premolar roots differ from those of Neanderthals," and the jawbone "is very primitive and robust."

Denisovan jaw, 160 Ka, Tibet

▶ The results indicate that Ds

  ▶ occupied the Tibetan Plateau in the Middle Pleistocene epoch

  ▶ successfully adapted to high-altitude hypoxic environments

  ▶ long before the regional arrival of modern *Homo sapiens*.

▶ The mandibular morphology in general is primitive,

  ▶ short and very robust, and

  ▶ close to *Homo erectus* specimens.

▶ But some features such as the less elongated shape of the dental arcade and the morphology of the dentition (marked retromolar space) are closer to Neanderthals and *Homo sapiens*.

Xiahe Mandible

▶ The feature of the Xiahe specimen that best links it to the fragmentary fossils from Denisova Cave is its large dentition

▶ Marked retromolar space that is commonly observed in Neanderthals

▶ This feature is of particular interest as three-rooted mandibular molars are very rare in non-Asian *H. sapiens* but appear much more frequently in recent Asian populations

a

Third premolar

Mesial — Distal

Mesial

Lingual

Distal
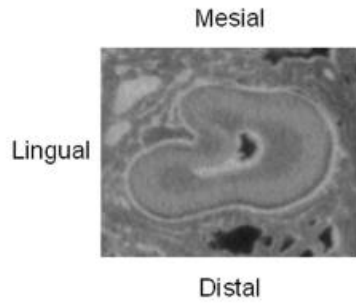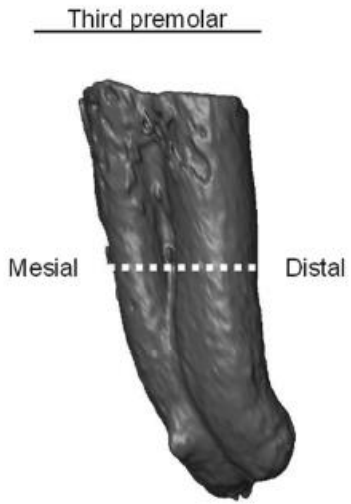
cross-section through root (at level of white dotted line above)

First molar

Mesial

Lingual

Distal

Lingual

Second molar

Mesial

Lingual

Distal

Lingual
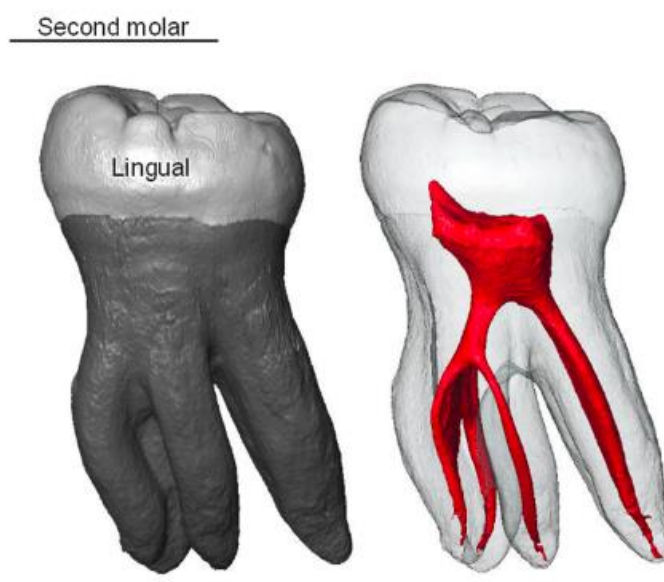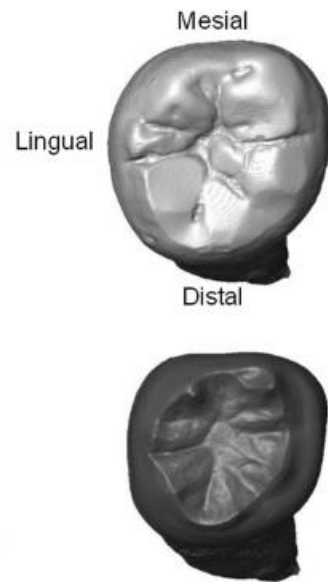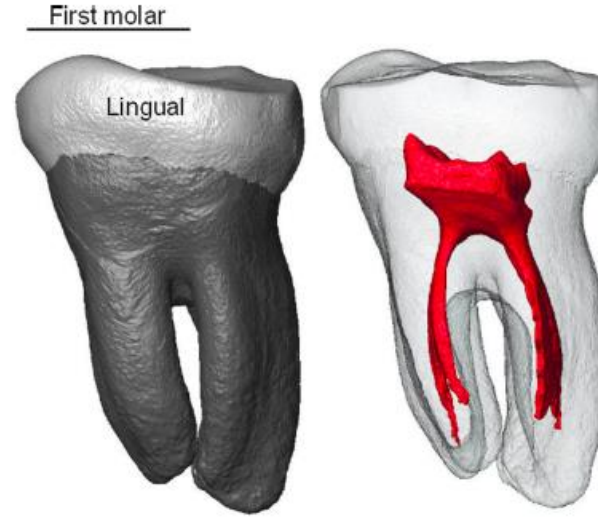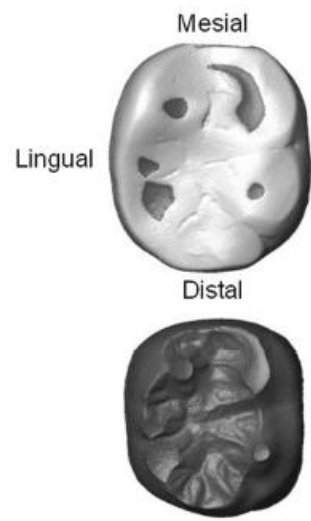
Getting fossils to speak:

Attempts at extracting DNA & Proteins

Although ancient DNA can provide unrivalled information about the identity of fossils and their evolutionary relationships to modern species, researchers might be unable to recover DNA from some specimens that are too old or resided in climates that do not favour DNA preservation. By contrast, some proteins are hardier than DNA and have been sequenced in some non-human fossils dating back millions of years. Sequences from ancient proteins could reveal insights about some iconic human ancestors.

DNA Protein
- Extracted, sequenced
- Attempted, not extracted
- None reported

HOMO FLORESIENSIS
60,000–100,000 years old
Liang Bua Cave, Indonesia

DENISOVAN TOOTH AND FINGER PHALANX
30,000–50,000 years old
Denisova Cave, Russia

DENISOVAN–NEANDERTHAL HYBRID (DENNY)
90,000 years old
Denisova Cave, Russia

DENISOVAN JAWBONE
160,000+ years old
Baishiya Karst Cave, China

HOMO NALEDI
300,000 years old
Rising Star cave system, South Africa

STEPHANORHINUS (EXTINCT RHINOCEROS)
1.8 million years old
Dmanisi site, Georgia

AUSTRALOPITHECUS AFARENSIS (LUCY)
3.2 million years old
Hadar, Ethiopia

NEANDERTHAL
430,000 years old
Sima de los Huesos, Spain

HOMO ERECTUS
1.6 million years old
West Turkana, Kenya

OSTRICH EGGSHELL
3.8 million years old
Laetoli, Tanzania

44. Needle in the Haystack. *Scientific American* 319(6), Higham, T. and Douka, K. 2018.

ZooMS technique

39. In search of the Denisovans: biomolecular ways for the identification of new human fossils in the Eurasian archaeological record – K. Douka, et al., 2019 symposium

▶ K. Douka: Introduce an interdisciplinary project ("FINDER") that applies a ZooMS to identify, characterize and analyze new hominin remains from several sites in Eurasia with the aim of finding new Denisovan fossils.

▶ Using collagen peptide mass fingerprinting, or ZooMS.

▶ Can identify hominin bone remains from highly fragmentary bone assemblages.

▶ Find 1 hominin bone for about every 1,000 animal ones in this cache. At this rate, they could find as many as 400 more hominin specimens.

Michael Shunkov, Katerina Douka, Tom Higham

Epigenetics: DNA methylation
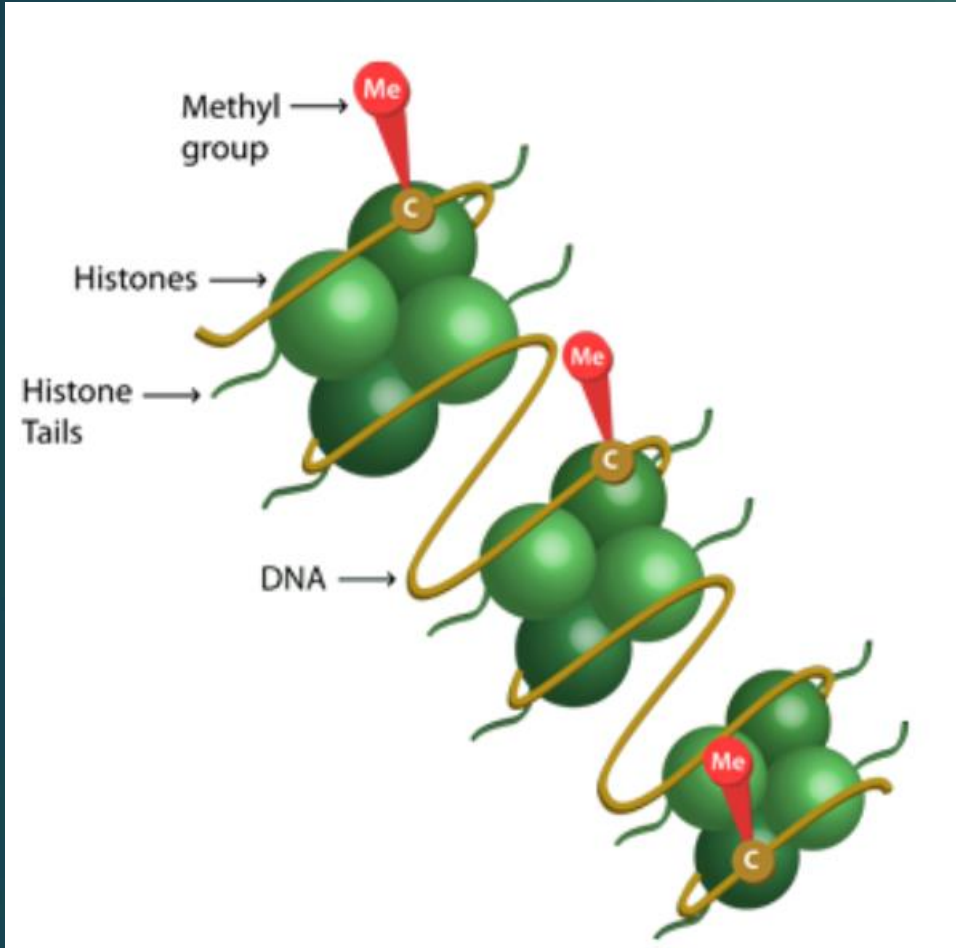
▶ **DNA methylation** is a biological process by which <u>experience induced methyl groups are added to the DNA molecule</u>.

▶ Methylation can change the activity of a DNA gene without changing the DNA sequence.

▶ DNA methylation typically acts to repress gene transcription.

What is DNA methylation

▶ When located in a gene promoter, DNA methylation typically acts to repress gene transcription/expression. One way evolution tamps down on gene activity is methylation.

▶ In intergenic regions it is linked to the activity of enhancers and insulators. Turns gene activity on or off.

▶ Two of DNA's four bases, cytosine and adenine, can be methylated. Cytosine methylation is widespread.

31. Reconstructing the DNA Methylation Maps of the Neandertal and the Denisovan - David Gokhman, et al., 2019

▶ Reconstruction of full DNA methylation maps of the Neandertal and the Denisovan by harnessing the natural degradation processes of methylated and unmethylated cytosines.

▶ Comparing these ancient methylation maps to those of present-day humans, identified ~2000 differentially methylated regions (DMRs).

▶ Found substantial methylation changes in the HOXD (body plan) cluster that may explain anatomical differences between archaic and present-day humans.

Gokhman, 2019: Conclusions

▶ 1 - DNA hypermethylation was used to infer gene downregulation

▶ 2 - Phenotype of downregulation was inferred from known loss of function phenotypes database

▶ They reconstructed Neanderthal and chimp profiles and tested against their actual known anatomy, with 85% accuracy

▶ Goal: a reconstructed Denisovan anatomical profile

Methylation change
Chimp / Archaic human / Archaic human / Modern human

Loss-of-function mutations
Healthy
Patient

Infer phenotype
Taken from monogenic diseases
Inference on body part affected, and direction of phenotypic change

Test method on known profiles
Compare Neanderthal and chimpanzee reconstructed profiles to their known morphology

Reconstructed Denisovan profile

DNA methylation maps can be used to predict anatomical features in hominins and chimpanzees, & allow for reconstruction of a putative anatomical profile of the Denisovan, currently absent from the fossil record.

Reconstructing Denisovan Anatomy Using DNA Methylation Maps

► <u>Liran Carmel and David Gokhman</u>, geneticists at the Hebrew University of Jerusalem, have <u>mapped out a proposed Denisovan skeleton</u> using information for <u>32 skeletal features</u> encoded in DNA that was extracted from a Denisovan pinky bone.

► <u>Using methylation data, they predict the Denisovans' physical features</u>.

► Over 99% of both Ns and Ds archaic genomes show no significant methylation differences compared to the present-day human.

► However, <u>in each archaic human we found ~1100 differentially methylated regions (DMRs)</u>

►
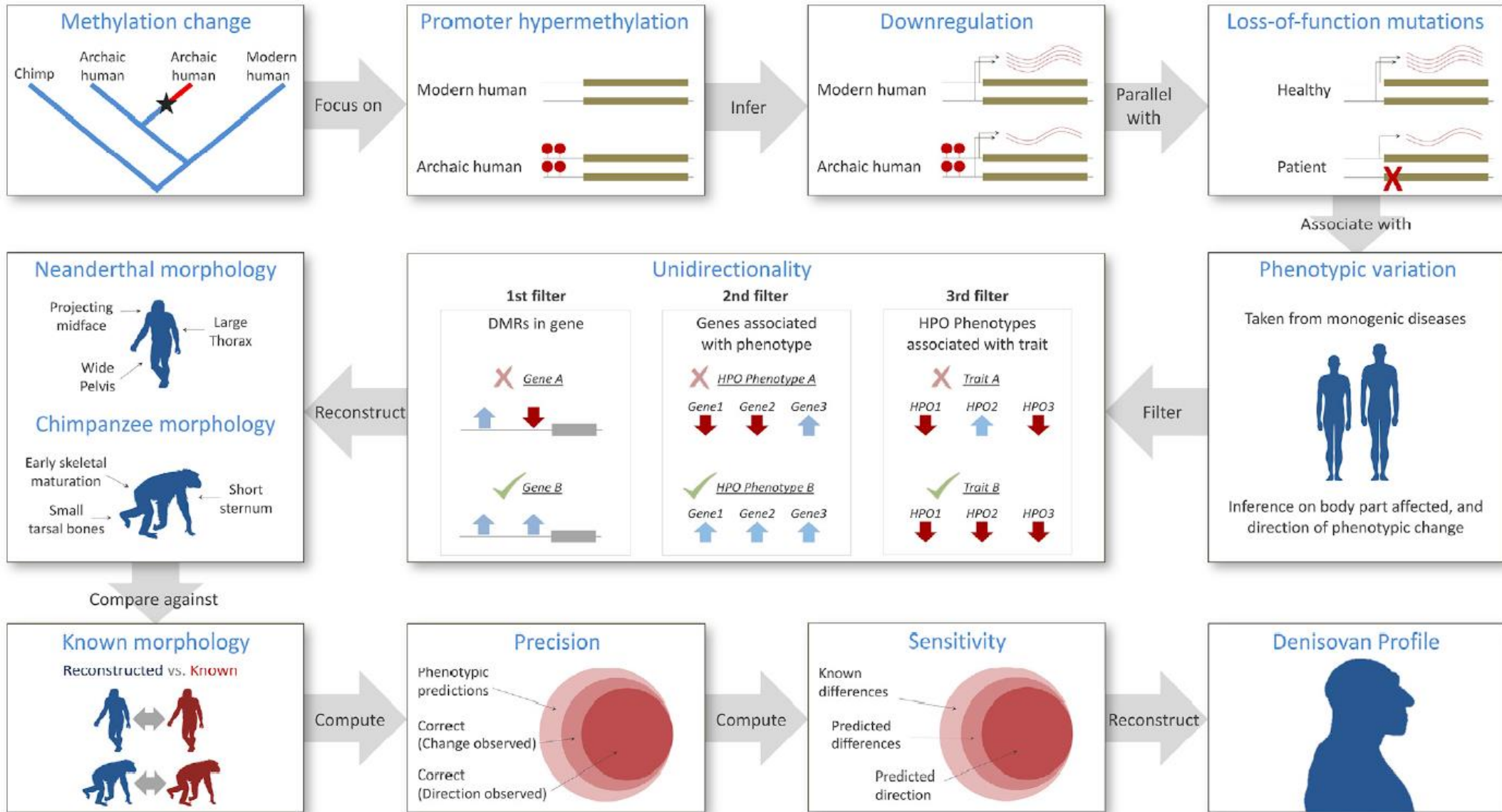
New method predicts N anatomy = 85% accuracy

Low forehead
HP:0000348 ✓

Flat cranial vault
HP:0000263 ✓

Narrow sutures and fontanelles
HP:0004492 ✗

Narrow temples compared to
anterior mandible width
HP:0000325 ✓

Projecting face
HP:0012368 ✓

Long face
HP:0000274 ✓

Enlarged dental pulp of molars
HP:0000679 ✗

Broad face
HP:0000275 ✓

Hyperplasia of the dental enamel
HP:0006297 ✓

Broad palate
HP:0000189 ✓

Delayed loss of permanent teeth
HP:0006357 ✓

Low palate
HP:0000218 ✓

Earlier eruption of primary teeth
HP:0000680 ✓

Macrognathia
HP:0000201 ✓

Increased cranial base growth
HP:0005451 ✓

High anterior mandible
HP:0000331 ✓

Wide mandible
HP:0000307 ✓

Figure 2. Unidirectional Promoter Methylation Changes Are Predictive of Neanderthal Anatomy

The 17 divergent skull phenotypes for which a direction of change could be assigned

Unidirectional Promoter Methylation Changes Are Predictive of Neanderthal Skeletal Anatomy

Hyperplastic cervical vertebrae
HP:0008434

Rounded epiphysis
HP:0003071

Outward bulging of the metaphyseal profile
HP:0003021

Posterior vertebral hyperplasia
HP:0008451

Wide greater sacrosciatic notches
HP:0003375

Long femoral neck
HP:0100864

Wide femoral neck
HP:0008819

Deeper acetabular fossae
HP:0003182

Hyperplastic scapulae
HP:0000882

Broad ribs
HP:0000883

Less curved long bones
HP:0006487

Accelerated skeletal maturation
HP:0002750

Hyperplastic pelvis
HP:0008839

Carpal bone hyperplasia
HP:0001498

Wide fingertips
HP:0001182

Increased bone mineralization
HP:0002749

Proposed Denisovan skeleton based on DNA methylation maps
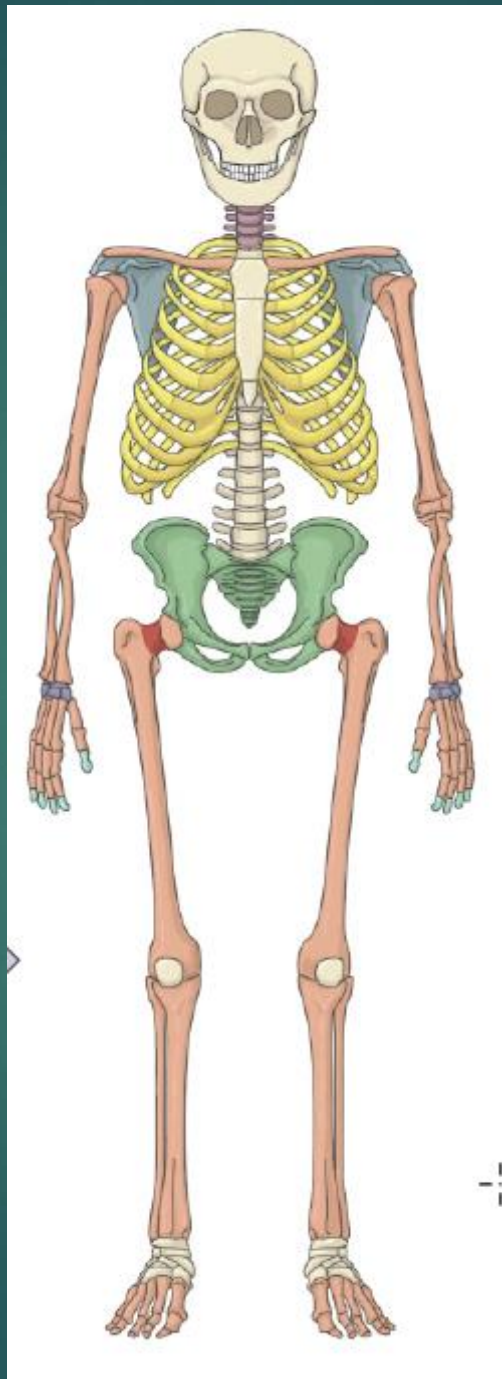
Gokhman, 2019: New D Jaw as successful test case

▶ <u>When this manuscript was in the process of peer review</u>, the first confirmed Denisovan <u>jawbone was reported </u>(Chen et al., 2019). This jawbone presents a <u>unique opportunity to test the accuracy of their</u> <u>predictions.</u>

▶ Discovery of Tibetan D jaw: They provided <u>four predictions</u> for the mandibular region: (1) high anterior mandible, (2) wide anterior mandible, (3) mandibular protrusion, and (4) long dental arch.

▶ <u>Seven out of eight predictions match the morphological description of the Denisovan jawbone (88% accurate)</u>—the authors report high anterior versus posterior mandible, a very wide anterior mandible, mandibular protrusion, and a long dental arch (Chen et al., 2019).

Gokhman, 2019: Critiques

▶ Chris Stringer: work "relies on a complex chain of extrapolations"

▶ Others caution against making species wide generalizations based on a single individual.

▶ Researchers have yet to evaluate whether methylation differences predict bone shapes in living people, Hawks says.

▶ Their conclusion: We conclude that unidirectional promoter methylation changes can be used to identify phenotypic divergence between closely related organisms. Even though we validated this approach on Neanderthals and chimpanzees, as well as on the Denisovan jawbone, its ultimate test would be to match the reconstructed profile to a more complete collection of Denisovan samples, once they are discovered

▶ Only more Denisovan fossils can confirm whether this portrait is accurate

Portrait of a juvenile female Denisovan based on a skeletal profile reconstructed from ancient DNA

This image is based on reconstructed skeletal features of a Denisovan girl, who lived in what's now Siberia around 50,000 years ago.

MAAYAN HAREL
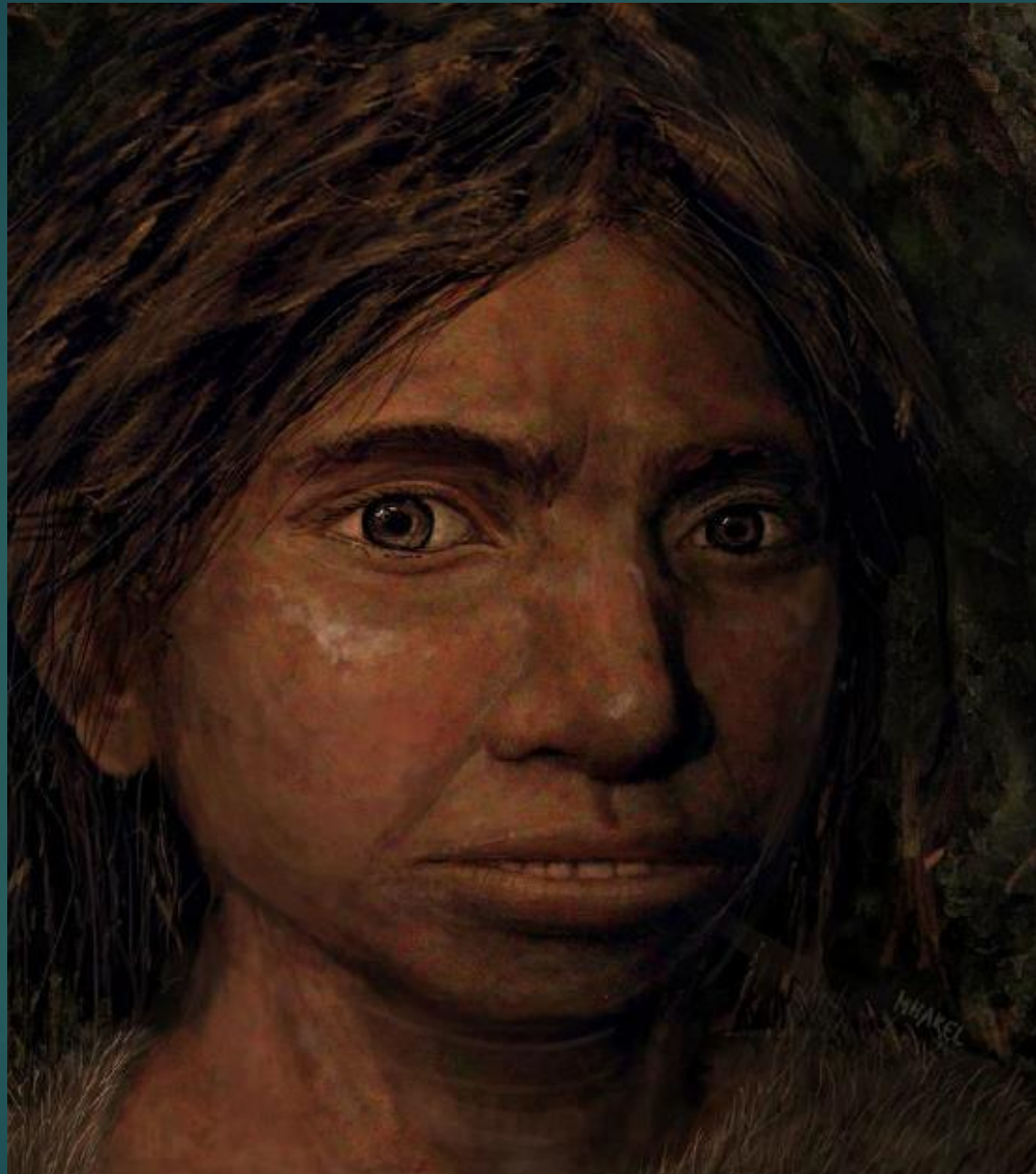
This artist's reconstruction, based on anatomical estimates from a new method, shows the face of a Denisovan girl from Siberia in Russia.

37. A parietal fragment from Denisova cave – Bence T. Viola, et al., 2019

▶ A newly discovered Parietal skull bone fragment, *Denisova 13,* attributed to the Denisovans based on its mtDNA,

▶ First glimpse at the Denisovan cranial morphology.

▶ From the South Gallery of Denisova cave, attributed it to Layer 22.

▶ 2 adjoining fragments of the posterior half of the left parietal, extending about 78 mm laterally of *lambda*, and about 51 mm anteriorly, preserving portions of both the sagittal and lambdoid sutures.

2016 discovery: Denisova 13: 2 Parietal bone fragments
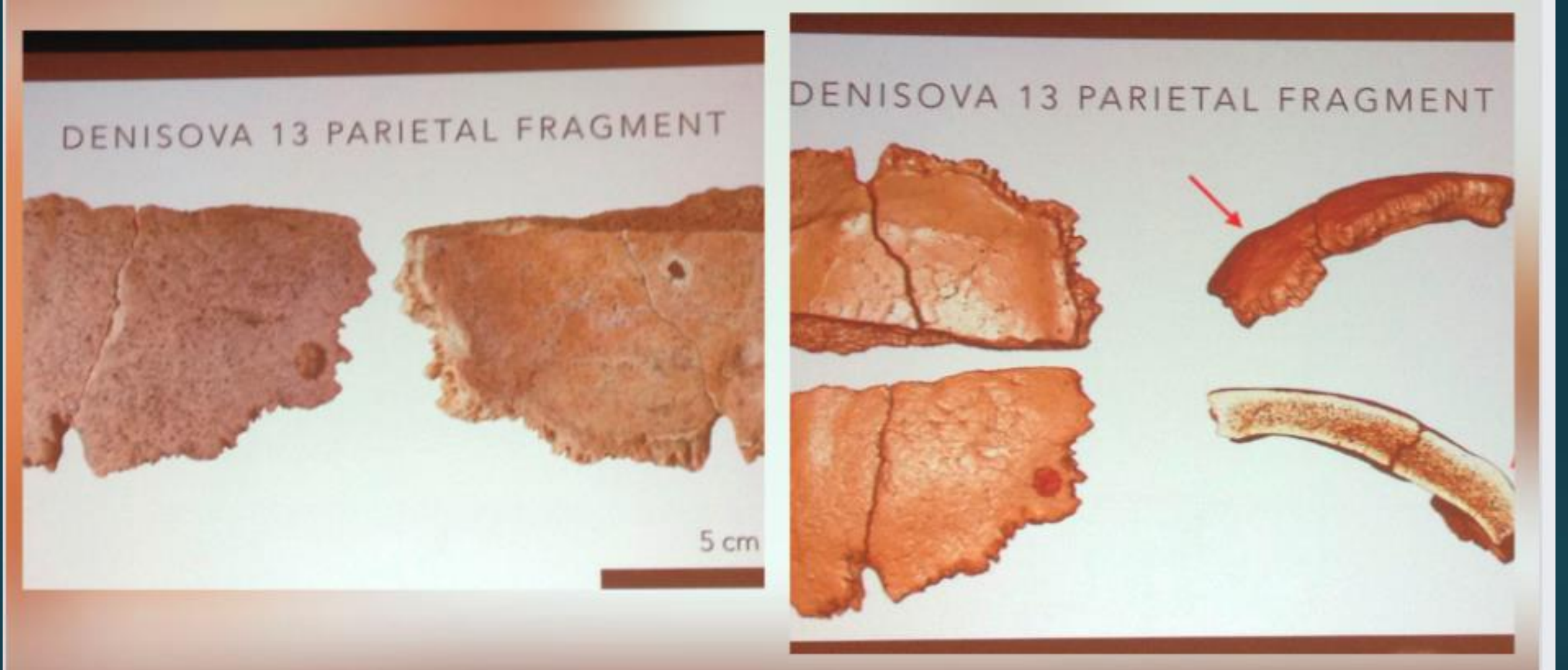
A palm-sized section of a braincase is the first Denisovan skull fossil ever found. Discovered in two pieces in Siberia's Denisova Cave in August 2016; Mitochondrial DNA extracted from the skull pegged it as Denisovan.

Much more angular, but it looked <u>very low and very robust</u>, which again is quite different from modern human shape nor like oval shape of N cranium; 10% larger than Kabwe skull

DENISOVA 13 PARIETAL FRAGMENT

a   b

Denisova 13 (left, photo of @VioLab_UofT) vs Ngawi (right, photo by Kaifu, Y. et al 2015), considered Homo erectus, and with which Denisova 13 can have morphological similarities.

And finally (for now) in 2019, a fifth Denisovan fossil, a fragment of parietal bone (Denisova 13), 10 cm x 7 ½ cm: more like *H. erectus*

32. Age estimates for hominin fossils and the onset of the Upper Palaeolithic at Denisova Cave – K. Douka, *et al. Nature,* 2019

- 50 radiocarbon determinations from the late Middle and Upper Palaeolithic layers of the site.

- A Bayesian age modelling approach used that combines chronometric (radiocarbon, uranium series and optical ages), stratigraphic and genetic data to calculate probabilistically the age of the human fossils at the site.

- Oldest Denisovan fossil suggests that Denisovans were present at the site as early as 195 Ka ago.

Denisova Cave Dating: 300 Ka to 55 Ka

▶ Judging by the oldest stone tools found there, the cave's first residents were present around 300 Ka ago and were, presumably, Denisovans or Neanderthals.

▶ Denisovans were present at the cave from at least 200 Ka ago to about 55 Ka ago — when the sediments in which Denisova 3 was found were laid down.

▶ Neanderthal remains = 190 to 100 Ka ago.

▶ K. Douka: Neanderthals are not present in the cave after ~80 Ka, although they persist in other places in the region,

▶ Denisovans remained at the cave, possibly as late as ~50 Ka ago.

Douka, 2019

- Dated 103 layers of sediment on the cave floor, scanning MIS 9 to MIS 2, as well as 50 items found in them, including bones, pieces of charcoal and tools.

- Neanderthal fossils (incl. hybrid Denisova 11) = 140-80 Ka ago.

- The youngest Denisovan dates to 76-52 Ka ago.

- UP tooth pendants and bone points = 49-43 Ka; earliest evidence for the production of these artefacts in northern Eurasia.

- On the basis of current archaeological evidence, it may be assumed that these artefacts are associated with the Denisovan population.

Douka, 2019: history of the Denisova Cave.

▶ 199 species: Remains of

  ▶ 27 species of large vertebrate,

  ▶ 100 species of small vertebrate (such as mammals and fishes)

  ▶ 72 species of plant

▶ Cave surroundings varied from being a broad-leafed forest in the warmest episodes to a tundra–steppe habitat during the coldest times.

▶ 4,527 bone fragments from Denisova have been analyzed in this way, with 2,212 of these being reported by Douka and colleagues.

Denisova 14 (?), 15 (N), 16 (?)

▶ Collagen peptide mass spectrometry fingerprinting (ZooMS) applied to

   ▶ 2,212 non-diagnostic bone fragments and identified

   ▶ three specimens that contained peptides consistent with the Hominidae.

▶ The bones come from layers:

   ▶ 9.3 (Denisova 14, ZooMS specimen code DC 3758): no DNA

   ▶ 11.4 (Denisova 15, DC 3573) in East Chamber; Neandertal mtDNA

   ▶ 9.1 (Denisova 16, DC 4114) in Main Chamber; too small for radiocarbon dating, and analyses of ancient DNA are ongoing.

Douka, et al. 2019

Douka, 2019:

▶ <u>Four pendants</u> made from red deer (*Cervus elaphus*) and elk (*Alces alces*) teeth—which are often associated with Upper Palaeolithic technocomplexes—provided results of <u>~32, ~40, & ~45 Ka</u>

▶ The <u>radiocarbon ages</u> for the <u>oldest pendants and the bone points at Denisova Cave overlap with the directly dated anatomically MH femur from Ust'-Ishim in western Siberia (47-43 Ka)</u>.

Douka, 2019:

Denisova 2 (a, b), Denisova 3 (c), Denisova 4 (d, e), Denisova 8 (f), Denisova 9 (g), Denisova 5 (h, i), Denisova 15 (j), Denisova 11 (k), Denisova 14 (l), Denisova 16 (m), Denisova 6 (n, o).

Legend: Denisovan (red), Neanderthal (blue), *Homo* sp. (grey).
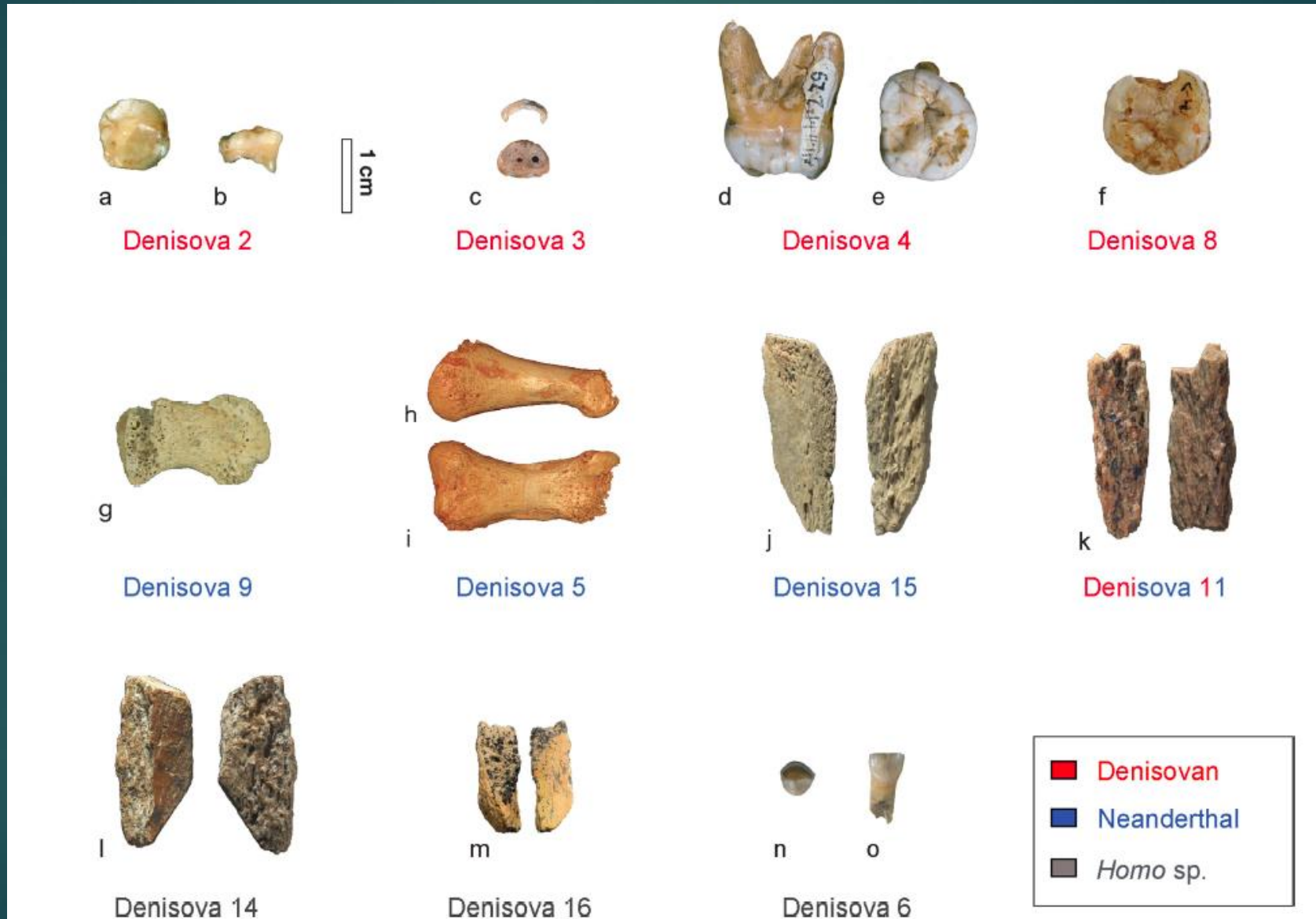
10 Denisova Cave Fossil Dates

▶ Denisova 14 (45,900-50,000 yr) – H. sp. – Most recent

▶ Denisova 3 (51,600-76,200 yr) - D

▶ Denisova 4 (55,200-84,100 yr) - D

▶ Denisova 11 (79,300-118,100 yr) – D & N

▶ Denisova 6 (91,200-130,300 yr) – H. sp.

▶ Denisova 15 (91.400-130,300 yr) - N

▶ Denisova 5 (90,900-130,000 yr) - N

▶ Denisova 9 (119,100-147,300 yr) - N

▶ Denisova 8 (105,600-136,400 yr) - D

▶ Denisova 2 (122,700-194,400 yr) – D -- Oldest

Douka, 2019:

▶ The earliest sediments with Neanderthal DNA (layer 14 in East Chamber) date to about 190 Ka ago.

▶ N and D groups lived in the region, met and—on occasion—interbred over the course of approximately 150 Ka.

Selection of artefacts from Denisova Cave
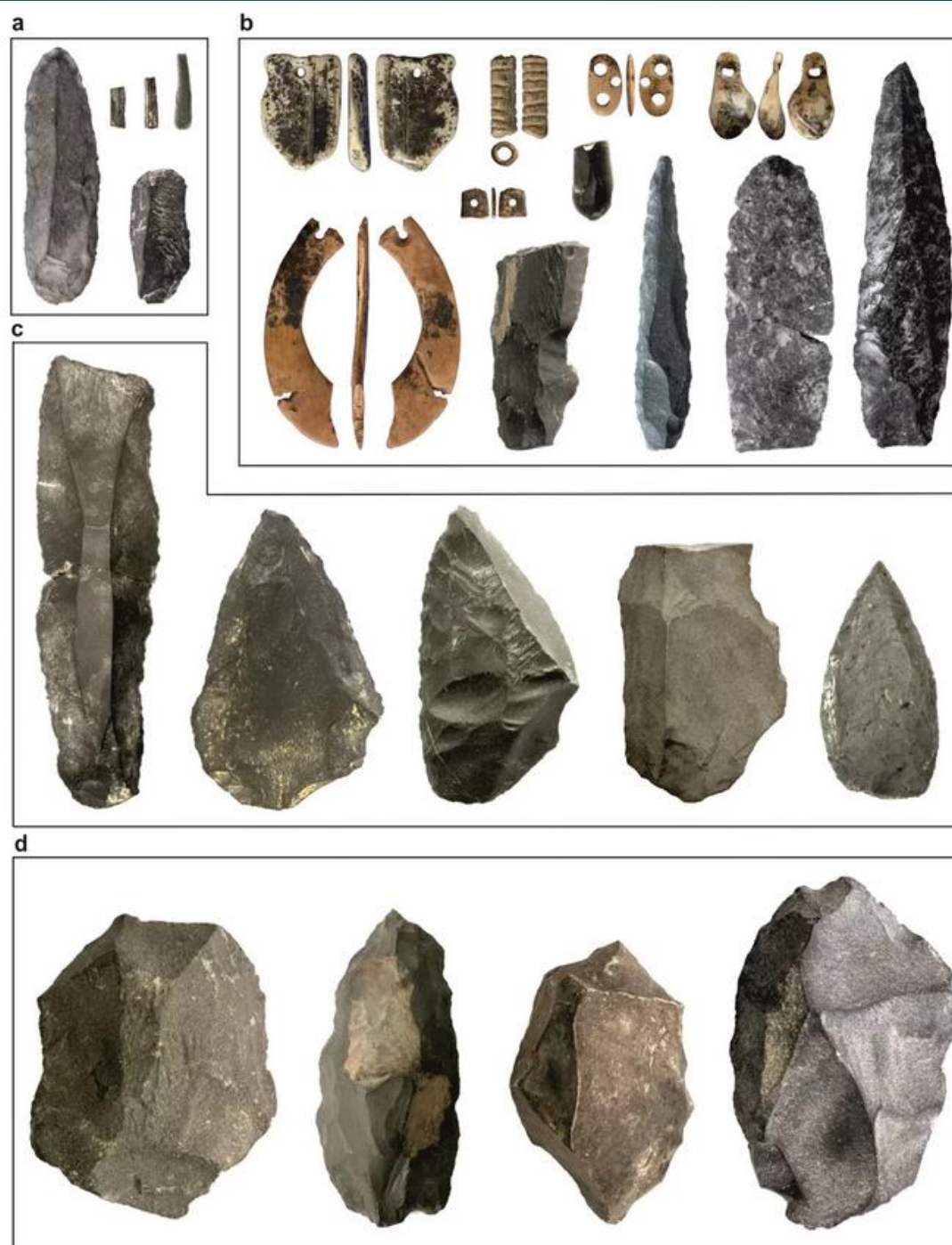
Douka, 2019:

▶ <u>Denisovans at the site appear to have survived later than Neanderthals</u>.

▶ <u>All known N and D fossils pre-date the appearance of the Initial UP (48-45 Ka ago) and the directly dated personal ornaments and bone points</u>.

▶ The <u>presence of AMHs to the northwest of Denisova Cave as early as 45 Ka at Ust'-Ishim</u>, synchronous with the dated pendants and bone points, raises the possibility that MHs may be the manufacture of these artefacts.

▶ <u>Parsimony gives Ds as the makers of these artefacts.</u>

▶ <u>No remains of modern humans have yet been recovered</u> that would confirm their presence at the site <u>during the Late Pleistocene.</u>

** Dating of Denisova Occupations

103 layers of sediment: continuous occupation since 300 Ka; No Denisovan remains have been dated directly

▶ Beginning at 300 Ka: Ns or Ds tools

  ▶ 287 Ka: oldest stone tools (non-Mousterian) by ??, MIS 9

  ▶ 217 to 185 Ka: oldest Denisovan DNA, MIS 7

▶ 287 (200 conservatively) to 55 Ka: Denisovans – fossils and DNA

  ▶ 49 to 43 Ka: Pendants

  ▶ 55 Ka: D skeletal

▶ 190 to 100 Ka: Neandertals – fossils and DNA

  ▶ 205 to 172 Ka: Neanderthal DNA

  ▶ ~118 to 79 Ka: Denny

▶ 140-110 Ka: Ns & Ds overlap

▶ No evidence of MHs in Late Pleistocene; only in Holocene.

K. Douka et al., 2019

Douka, 2019:
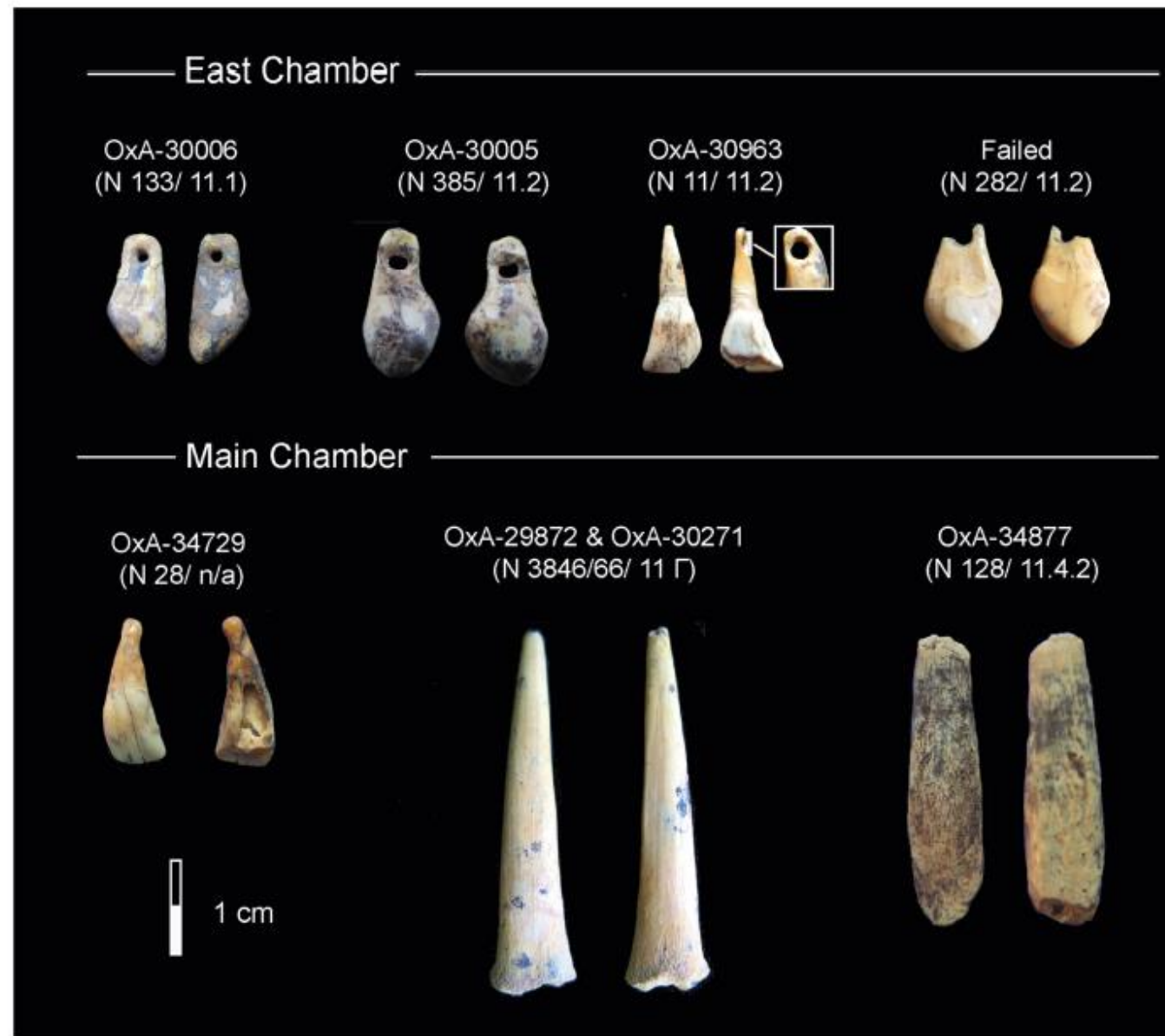
Extended Data Fig. 2 | Personal ornaments and bone points from Denisova Cave that were sampled for radiocarbon dating. N28 was discovered during section cleaning and is not assigned to a specific layer. N282 did not produce enough collagen, and was not dated. N3856/66 was dated twice. Direct dates are listed in Extended Data Table 1.
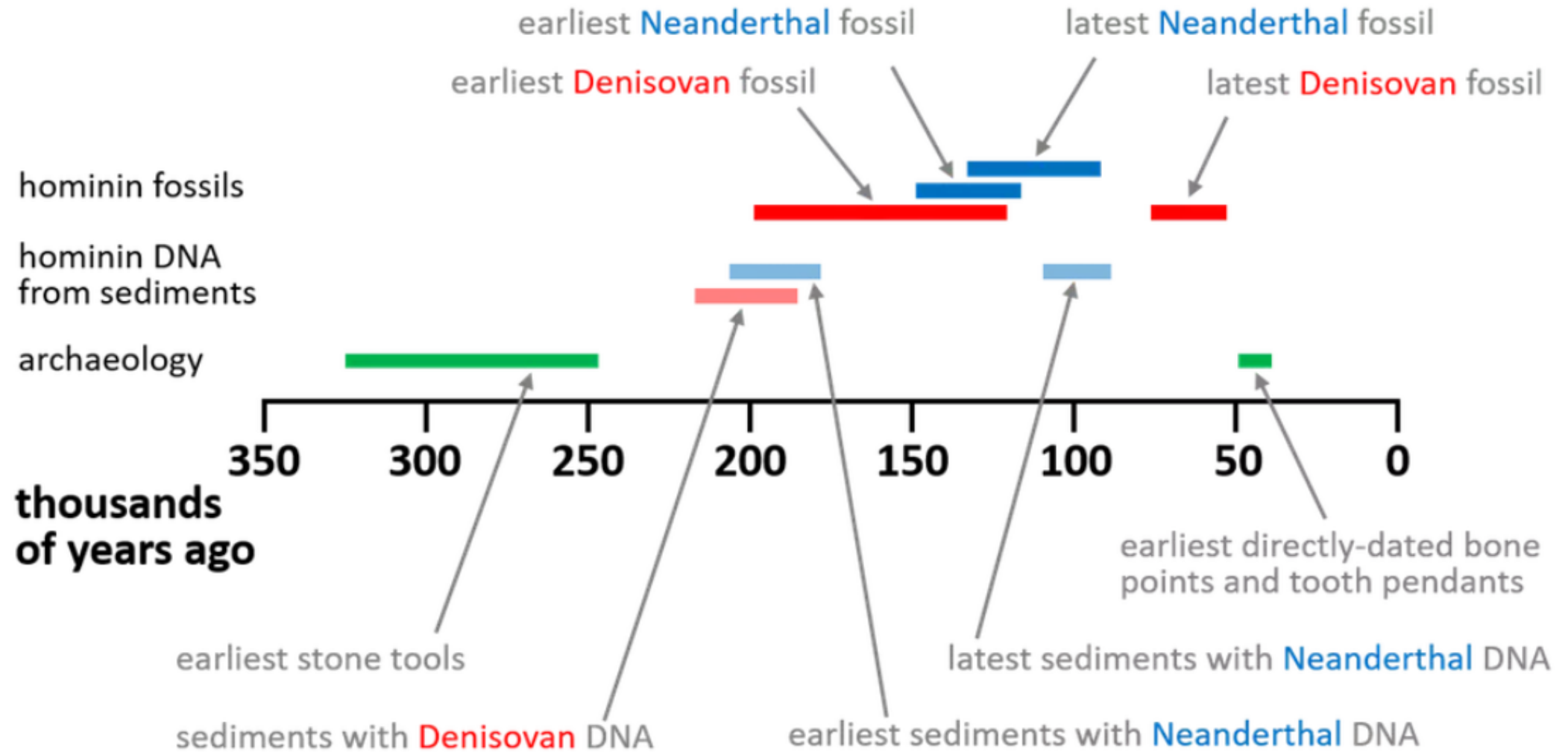
Summary timeline for the archaeology, hominin fossils and hominin DNA retrieved from the sediments at Denisova Cave.

(Bert Roberts)

33. Timing of archaic hominin occupation of Denisova Cave in southern Siberia - Z. Jacobs, *et al. Nature,* 2019

► The previous site chronology is based largely on radiocarbon ages for fragments of bone and charcoal that are up to 50 Ka old;

► Older ages of equivocal reliability have been estimated from

  ► thermoluminescence (optical dating)

  ► palaeomagnetic analyses of sediments, and

  ► genetic analyses of hominin DNA.

► Study of the stratigraphic sequences in Denisova Cave, establish a chronology for the Pleistocene deposits and associated remains from optical dating of the cave sediments, and reconstruct the environmental context of hominin occupation of the site from around 300 to 20 Ka ago.

Z. Jacobs, 2019: Dating

▶ Geochronologists Zenobia Jacobs and Richard Roberts led a team that dated sediment from Denisova Cave by analyzing hundreds of thousands of grains of quartz and feldspar.

▶ We used optical dating to determine when the sediments were last exposed to sunlight and deposited in the cave. Optical dating has been applied to archaeological sites around the world, with the minerals quartz and potassium feldspar most often used.

▶ Measured more than 280,000 individual grains of these minerals from more than 100 samples using a combination of well-established and new procedures.

Z. Jacobs, 2019: D Dating

▶ Dates for 103 samples from Denisova Cave sediment

▶ Zenobia Jacobs:
  ▶ Denisovans occupied the Siberian cave from 300 Ka to 55Ka ago,
  ▶ Neandertals until 97 Ka ago.

▶ But it's still uncertain whether hominin fossils from the cave derive from
  ▶ individuals who died during periodic occupations or
  ▶ whose remains were transported to the site by carnivores.

D. Reich, 2018: Australo-Denisovans

▶ Ds who mixed with New Guineans were not close relatives to Siberian Ds.

▶ 2 D groups: Australo-Ds split from Siberian Ds between 400-280 Ka

▶ 2 D groups probably had different adaptations, which may explain their geographic differences

▶ Reich calls Southern Ds "Australo-Ds"; Stringer prefers "Sunda Ds"

34. Multiple Deeply Divergent Denisovan Ancestries in Papuans - Guy S. Jacobs, et al., 2019

▶ Guy Jacobs Study of archaic haplotypes in 161 new genomes spanning 14 island groups in Island Southeast Asia and New Guinea

▶ Large stretches of DNA are inconsistent with a single introgressing Denisovan origin.

▶ Modern Papuans carry hundreds of gene variants from three deeply divergent Denisovan lineages that separated over 350 Ka ago.

▶ Papuans carry haplotypes from two Denisovan groups, with one unique to Oceania.

Jacobs, 2019:

Individuals in Island Southeast Asia carry ancestry from both Neanderthals and Denisovans
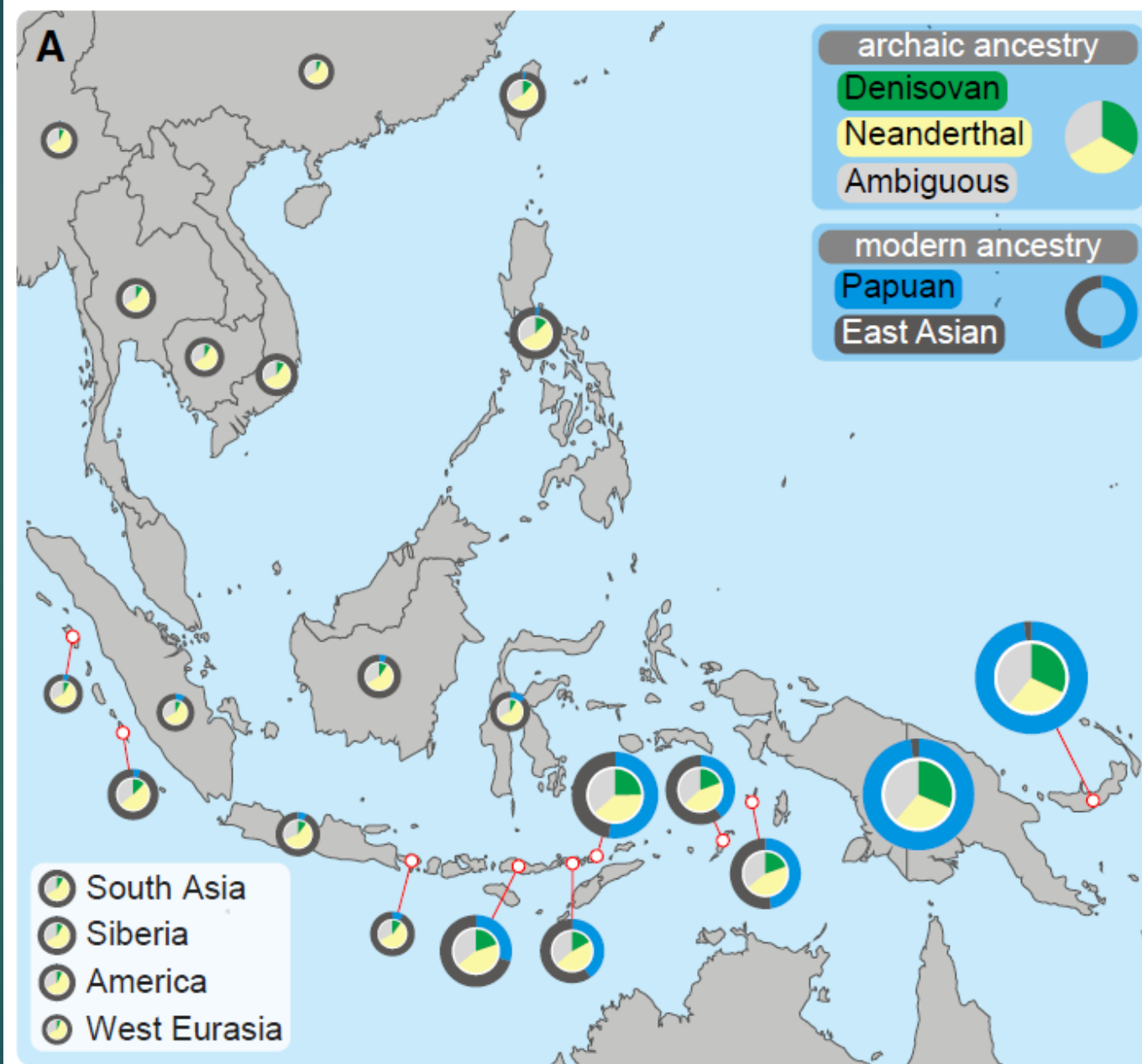
Figure 1. Modern and Archaic Ancestry
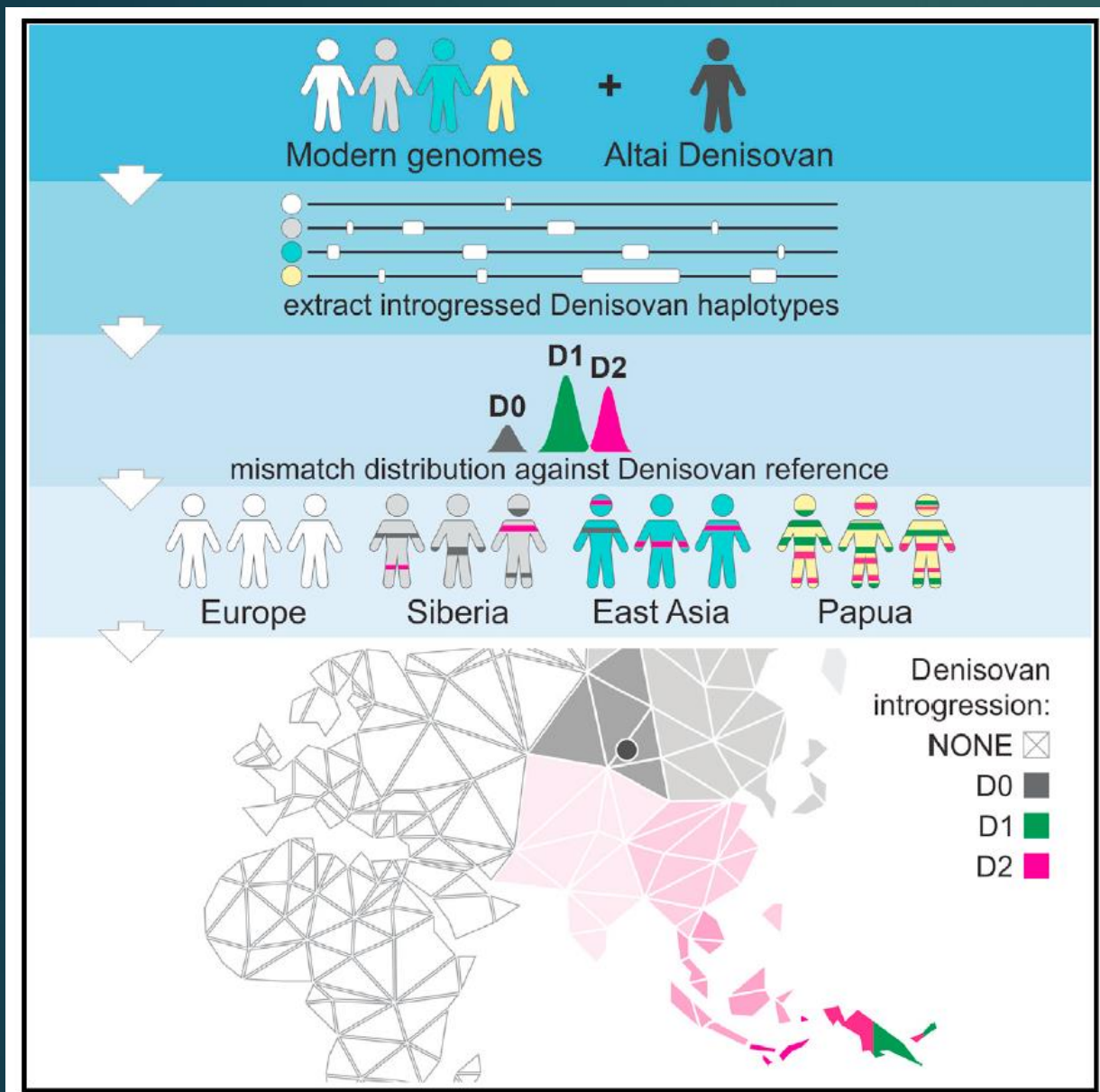
A new dataset of 161 genomes covering the understudied Indonesia-New Guinea region

Introgressing Denisovans comprise at least three genetically divergent groups

Papuans carry haplotypes from two Denisovan groups (D1 & D2), with one (D1) unique to Oceania

Some Denisovan introgression was recent (30-15 Ka) and likely occurred in New Guinea or Wallacea

Jacobs, et al. 2019

Jacobs, 2019: Three Denisovan Ancestries in Papuans

▶ The Denisovans comprised at least three diverse populations—including one lineage so distinct that it may even constitute an entirely separate species

▶ Indicates marked reproductive separation between Denisovan populations.

▶ These hominins likely interbred with *Homo sapiens* in two waves, creating varied genetic legacies still seen in contemporary residents of Oceania and East Asia.

Guy S. Jacobs, et al., 2019

Jacobs, 2019: Denisovan Locales

▶ The three groups were bounded by two large geographical impediments:

  ▶ the Himalayas in the east, and

  ▶ the oceanic gaps between the islands that make up Indonesia today.

▶ One group of Denisovans would have lived in or around present day Siberia, Kazakhstan and in China just north of the Himalayas.

▶ But a different group of Denisovans lived in Southeast Asia, around present day Thailand and Vietnam.

▶ The third group called the islands of Indonesia home.

▶ Those two southern groups diverged from the Siberian Denisovans over 250 Ka ago. That's before anatomically-modern humans even appeared.

Jacobs, 2019: 3 D lineages

▶ First Denisovan lineage (D0): closely related to the Denisovans of Denisova Cave, carrying DNA found in modern East Asians; also in indigenous Americans and at very low frequency elsewhere across Asia.

▶ Second lineage (D1): more limited genetic ties with the first Denisovan specimens and carried DNA evident in Papua New Guineans and South Asians.

▶ Third lineage (D2): is "about as different from the individual found in the Denisova cave (D0) as it is from Neanderthals." Needs a new name. D2 population is most distant from the Siberian Denisovans.

▶ Denisovan and modern human interbreeding occurring as recently as 15 Ka ago.

▶ If supported, this the last hominin cousin to disappear from the planet.

Jacobs, 2019: 3 lineages

▶ D2 was so distant that it likely split off approximately 363 Ka ago.

▶ Such distant divergence makes D1 and D2 as different from D0 as they were from Neanderthals.

▶ D2 might even have to be reclassified to give the group its own name. The researchers cannot name a new species without actual fossil evidence.

▶ Genetically, the group living in south-east Asia looked as different from the Denisovans in Siberia as it did from Neanderthals.

D1

▶ The D1 DNA is only seen in New Guinea; longer chunks of DNA that haven't been mixed over time, suggesting it entered the modern human genome recently—about 30 Ka ago, and perhaps just 15Ka ago.

▶ A group of Denisovans survived in the remote mountains or islands of New Guinea and mated with modern humans.

▶ East Asians had two sources of Denisovan DNA, suggesting at least two mixing events. New Guineans got Denisovan DNA from only 1 source.

▶ The multiple encounters with Denisovans gave living people in Indonesia and Papua New Guinea 400 new gene variants, including an immune gene variant (*TNFAIP3*) and a gene involved in diet (*WDFY2*) (metabolism of fat).

3 D Lineages	D0 Altai/Denisova	D1	D2	N	MH
Current Location	Denisova, Siberia; East Asia; across Asia; Papua; America	Only in Papua (New Guinea) (nearly complete absence in Asia)	Papua (New Guinea)	Everywhere	Everywhere
Introgression Site	East Asia	31 (15 ?) Ka introgression	46 Ka Introgression	Ns met MHs	
Restricted to:	Siberia, East Asia, SE Asia, America	Papua, S Asia?	Papua, Oceania, Asia	Western Eurasia	
Separation from D0		283 Ka	363 Ka; 3rd sister group?	N-D split: 473–381 Ka; sister groups	MH-N split: 765-550 Ka
Recent Splits	38 Ka split of Europeans & East Asia		51 Ka: Papuans split from Eurasians		Out of Africa 60 Ka
Divergence from D0: D1 & D2 as distantly related	to D0 as they were to Ns	Less divergent	More divergent; Different species?		
Unique genes		412 genes 3-5% of genome; last Ds	412 genes 4% of genome	1-2%	1-2% N

Jacobs, 2019:

► This, together with the nearly complete absence of D1 in continental Asia, is most consistent with the scenario that D1 Denisovans were present only in New Guinea or East ISEA (e.g., Wallacea).

► Implies that at least some Denisovan populations had the ability to cross large bodies of water, such as the one represented by the Wallace Line.

► This watercraft idea does not seem implausible given archaeological evidence of archaic hominin dispersals

   ► discovery of stone tools in the Philippines dating to 700 Ka

   ► the related finding *of H. floresiensis* on the island of Flores

   ► both across substantial water boundaries that persisted throughout the Pleistocene.

Jacobs, 2019: Ds as 2nd hominin left standing

▶ The late date for the D1 introgression suggests that Denisovans survived until 30 Ka, and perhaps as recently as 14.5 Ka.

▶ Not all agree with these late dates..

▶ This is longer than Neanderthals, who died out around 40 Ka, or H. floresiensis, who survived on Flores only to 60 Ka. The implication is that Denisovans living in ISEA may have been among the last of all the archaic hominins to survive

35. Morphology of the Denisovan phalanx closer to modern humans than to Neanderthals - Andrew Bennett, et al., 2019

► Morphometric analysis of a phalanx fragment = larger distal part of the original Denisova 3 phalanx, the genome of which had been published in 2010 and 2012.

3mm

► The Denisova 3 phalanx was identified in 2008 in layer 11.2 of the East Gallery, square D2, of the Denisova Cave, the date of which is assumed to be more than 50 Ka ago.

Denisovan finger
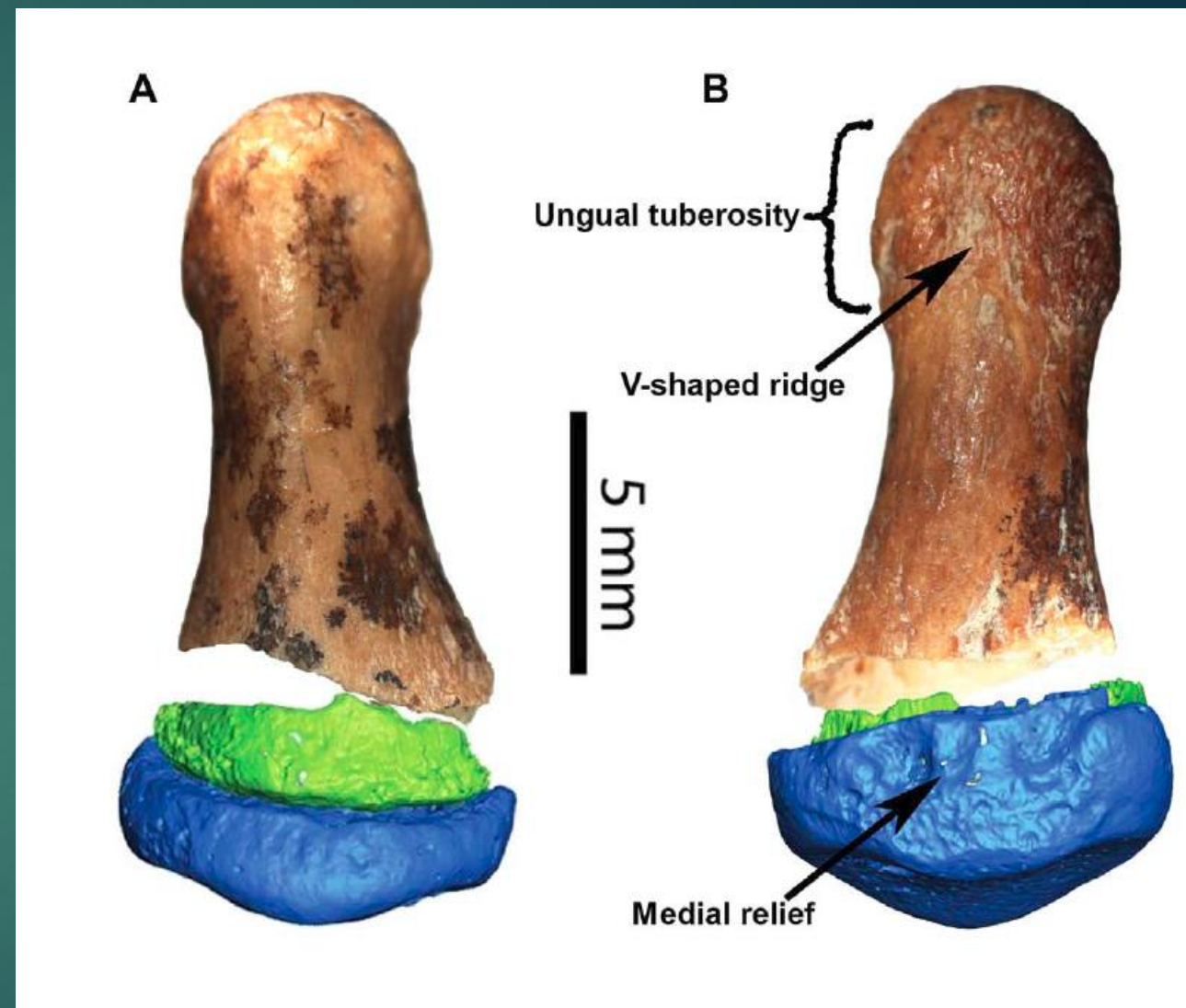
Krapina 206.12 — Recent human — Denisova 3

A   B

Ungual tuberosity
V-shaped ridge
Medial relief

5 mm

Andrew Bennett, et al., 2019

41. Neanderthal-Denisovan ancestors interbred with a distantly related hominin – Alan Rogers, et al., 2020

▶ Ancestors of Neanderthals and Denisovans interbred with their own Eurasian predecessors—members of a "superarchaic" population that separated from other humans about 2 million years ago.

▶ The superarchaic population was large, with an effective size between 20 and 50 thousand individuals.

▶ We confirm previous findings that

  ▶ (i) Denisovans interbred with superarchaics,

  ▶ (ii) Neanderthals and Denisovans separated early in the middle Pleistocene,

  ▶ (iii) their ancestors endured a bottleneck of population size

  ▶ (iv) Neanderthal population was large at first but then declined in size

  ▶ (v) Neanderthals interbred with the ancestors of modern humans

Rogers, 2020:

▶ <u>Superarchaics</u> may descend from the earliest human dispersal into Eurasia, as represented by the Dmanisi *H. erectus* fossils.

▶ Evidence for

   ▶ admixture of a <u>superarchaic population into Denisovans</u>

   ▶ admixture from <u>early moderns into Neanderthals</u>.

▶ Neandersovan immigrants may have met the indigenous superarchaic population of Eurasia. This suggests a fourth episode of admixture, from superarchaics into neandersovans

▶ On the other hand, some authors prefer a higher mutation rate of 0.5×10^{-9} per year . Under this clock, the lower end of our confidence interval would be a later 1.6 Ma ago. Thus, our results are also consistent with the view that <u>superarchaics entered Eurasia after the earliest remains at Dmanisi.</u>

42. The nature of Neanderthal introgression revealed by 27,566 Icelandic genomes - Laurits Skov, et al., 2020

► Iceland: 270 K population; 200 K are in deCode genetic database

► Unprecedented magnitude of data set; nearly 10% of the entire population, and 286 sub-Saharan Africans in the 1000 Genomes Project

► Found 14.4 million archaic chromosome fragments that were detected in whole-genome sequences from 27,566 Icelanders,

► 56–113 K unique archaic fragments that reside in 38.0–48.2% of the genome.

Skov, 2020

▶ <u>85% of archaic fragments are from Altai or Vindija Neanderthal</u>

▶ <u>3% of fragments from Denisovan</u> origin; 12% of fragments are of unknown origin.

▶ CJV: Note that the study does not give % of D DNA in Icelanders, only % of D fragments; if N DNA is 2%, then D DNA would be <.01%

▶ <u>Vast catalogue of 15 million archaic fragments found in Icelanders but not shared by Africans;</u> 400,000 single-letter sequence variants, that are absent from the African samples

Skov, 2020

▶ Neanderthals had a relatively small total population of 2,000–3,000 individuals

▶ 282 distinct archaic deserts covering 570 Mb; including the entire X chromosome;

    ▶ indicates that non-deleterious archaic variants were more likely to be retained in the human gene pool when they could be uncoupled from deleterious archaic variants by recombination

Skov: Icelanders have D DNA

▶ So how did D genes end up in Islanders' DNA? And when?

▶ Either Neanderthals had children with Denisovans before they met MHs.

  ▶ This would mean that the Neanderthals with whom MHs had children were already hybrids, who transferred both Neanderthal and Denisovan genes to the children

▶ Or MHs met Denisovans long before they met Neanderthals

2019: 8 Denisovan <u>Fossils</u>: Discovery/Publication Dates

1. 2008/2010: Denisova 3: distal fifth finger bone (X-woman), girl

2. 2000/2012: Denisova 4: third molar, male

3. 2010/2015: Denisova 8: upper third molar, male

4. 1984/2017: Denisova 2: deciduous lower molar (milk tooth), girl

5. 2012/2018: Denisova 11: Denny

6. 2014/2019: Denisova 5: Neandertal toe bone: Altai Neanderthal

7. 2016/2019: Denisova 13: parietal bone fragment

8. 1980/2019: Xiahe Mandible

All the Denisovan material fit in a very, very small box.

** <u>Dating</u> of D fossils based on Bayesian Modeling

- Denisova 2:   123-194 Ka, ~170 Ka = D; >; oldest D
- Denisova 8:   106-136 Ka; similar age to D 2, 60 Ka older than D3; *
- Denisova 9:   119-147 Ka = N
- Denisova 5:   90-130 Ka/ nDNA -110-134 Ka, ~120 Ka = Altai N; *
- Denisova 15: 91-130 Ka = N
- Denisova 11: 79-118 Ka/116-141 Ka = Denny; >
- Denisova 4:   55-84 Ka; contemporaneous with D 3;
- Denisova 3:   52-76 Ka; contemporaneous with D 4; <; high coverage D genome
- Denisova 14: 46 Ka; no DNA; most recent

- Some show consistency between optical & sediment aging (*), some older (<), some younger (>); uncertainty of genetic aging or geological repositioning

Name "	Element	Layer	Location	Type	Age	Sex	Discovery/ Publication	First public.
Denisova 2	Deciduous 2nd lower molar	22.1	Main Gallery, Denisova Cave, Russia	Denisovan	194-123 Ka	♀	1984/2017	
Denisova 3 or X Woman	Fifth distal finger phalanx	11.2	East Gallery	Denisovan: nDNA	76-52 Ka	♀	2008/2010	Johannes Krause, et al.
Denisova 4	upper molar	11.1	South Gallery	Denisovan: nDNA	84-55 Ka	♂	2000/2010	
Altai Neanderthal or Denisova 5	proximal toe phalanx	11.4	East Gallery	Neanderthal: nDNA	30–50 Ka[1]	♀	2010/2011	M.B. Mednikova (2011)
Denisova 8[1]	upper 3rd molar	11.4–12	East Gallery	Denisovan: nDNA	136-106 Ka	♂	2010/2015	
Denisova 11 "Denny"	Arm/leg bone fragment	12	East Gallery	Neanderthal/ Denisovan hybrid: nDNA	118-79 Ka	♀	2014	Samantha Brown, et al. (2016)

Name "	Element	Layer	Location	Type	Age	Sex	Discovery/ Publication	First public.
Denisova 13	Parietal bone fragment	22	Denisova Cave	Denisovan	~ 285+/-38 Ka		/2019	Bence, V., et al.
Xiahe Mandible	Partial mandible		Baishiya Cave, China	Denisovan	>160 Ka		1980/2019	Chen F. et al , .
Denisova 14	Bone fragment	9.3	Denisova Cave	Homo sp.			2019	Douka, K. *et al.*
Denisova 15	Bone fragment	11.4	Denisova Cave	Neandertal			2019	Douka, K. *et al.*
Denisova 16	Bone fragment	9.1	Denisova Cave	Homo sp.			2019	Douka, K. *et al.*

50. Engraved bones from the archaic hominin site of Lingjing, Henan Province by Z. Li, et al. 2019

▶ Discovery of two engraved bones from the Lingjing site in Henan Province, China, dated to 105–125 Ka.

▶ The carefully engraved nature of the incisions, made on weathered rib fragments, indicates intentional incisions.

▶ Presence of ochre within the incised lines on one specimen.

▶ First evidence for the deliberate use of ochred engravings for symbolic purposes by unknown East Asian Late Pleistocene hominins.
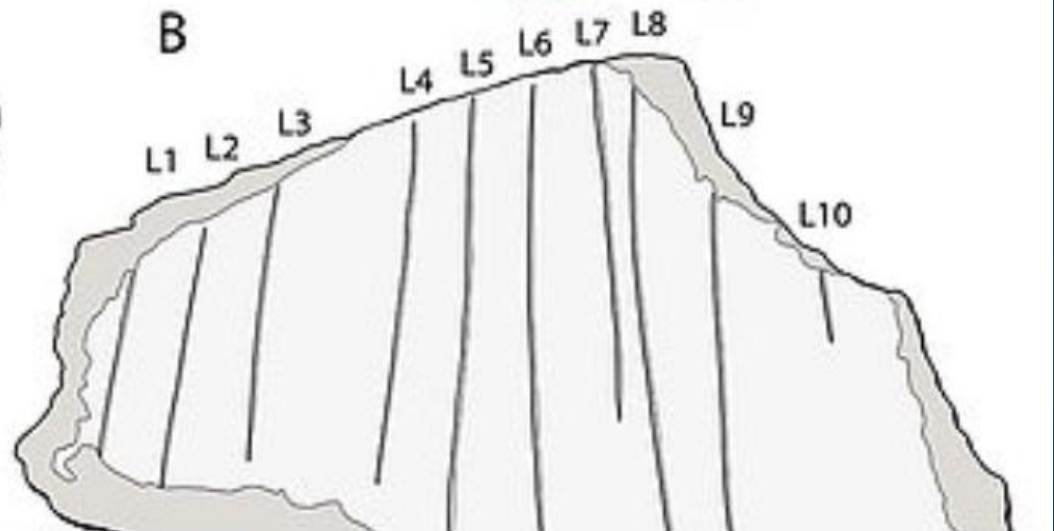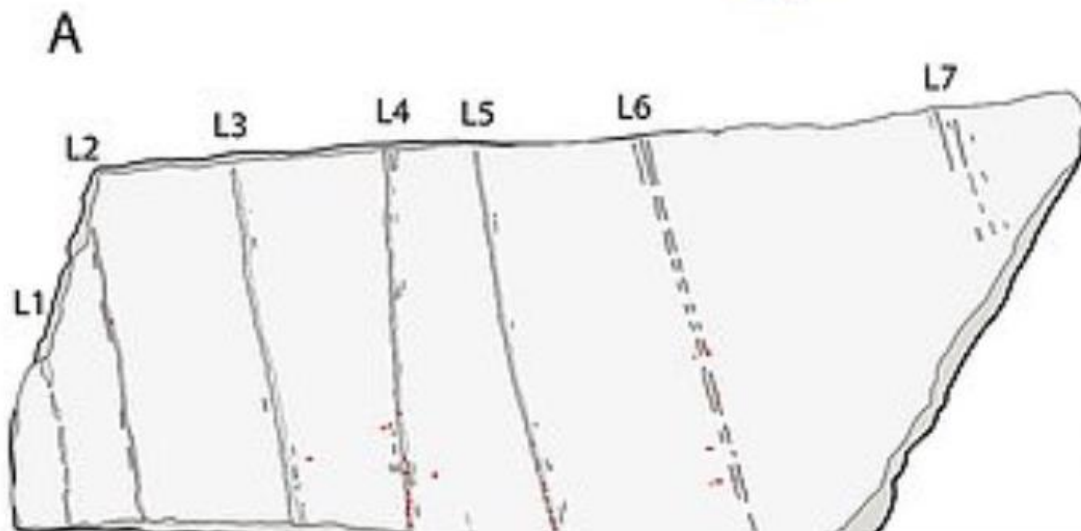
A

L1 L2 L3 L4 L5 L6 L7

B

L1 L2 L3 L4 L5 L6 L7 L8 L9 L10

51. Rare dental trait provides morphological evidence of archaic introgression in Asian fossil record - Shara E. Bailey, et al., 2019

▶ Denisovan *Xiahe jawbone* possesses a 3-rooted lower second molar, dated to 160 Ka

▶ 3-rooted lower molar is rare (less than 3.5% occurrence) in non-Asian *Homo sapiens*. In contrast, its presence in Asian-derived populations can exceed 40% in China and the New World.

▶ Presence of a 3-rooted lower second molar in this 160 Ka fossil hominin suggests greater antiquity for the trait.

3-rooted lower second molar of Xiahe Denisovan mandible

52. Discerning the Origins of the Negritos, First Sundaland People: Deep Divergence and Archaic Admixture T. A. Jinam, et al., 2017

▶ Human presence in Southeast Asia dates back to at least 40 Ka ago, when the current islands formed a continental shelf called Sundaland.

▶ There exist indigenous groups collectively called Negritos in the Philippine Islands, Peninsular Malaysia, and Andaman Islands,

▶ Phylogenetic tree analyses show that Negritos are basal to other East and Southeast Asians, and that they diverged from West Eurasians at least 38 Ka ago.

Jinam, et al., 2017

▶ High traces of Denisovan admixture in the Philippine Negritos, but not in the Malaysian and Andamanese groups, suggesting independent introgression and/or parallel losses involving Denisovan introgressed regions.

▶ The Aeta have the highest proportion of Denisovan ancestry among the four Philippine Negrito groups

▶ Shared genetic loci between all three Negrito groups could be related to skin pigmentation, height, facial morphology and malarial resistance.

Denisovan DNA in Southeast Asia

Denisovan ancestry (in percent) for various populations

"tr" denotes trace percentage, close to zero
data from Jinam et al. (2017) doi:10.1093/gbe/evx118
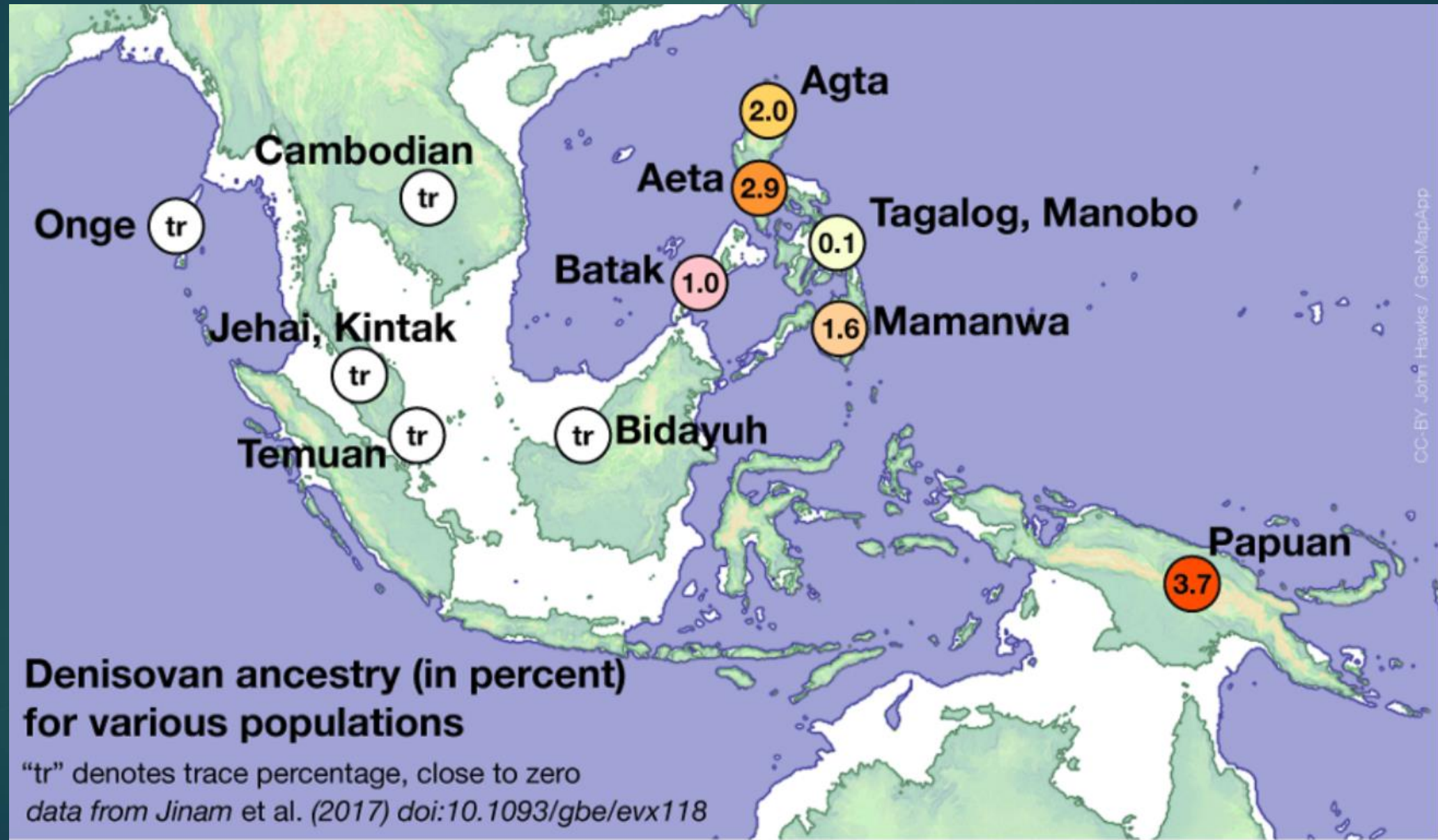
52. A genomic history of Aboriginal Australia – A. Malaspinas, et al., 2016

▶ During most of the last 100 Ka, Australia, Tasmania and New Guinea formed a single continent, Sahul, separated from Sunda (the continental landmass including mainland and western island Southeast Asia) by a series of deep oceanic troughs

▶ Colonization of Sahul is thought to have required at least 8–10 sea crossings between islands. Sahul was settled by 47–55 Ka, at the time of earliest MHs in Sunda.

▶ Both Aboriginal Australian and Papuan ancestors admixed with Neanderthals and Denisovans.

▶ Increased desertification of Australia during the last glacial maximum 19–27 Ka affected the number and density of human populations. Desert groups were hypothesized to withstand sub-zero night temperatures without showing increase in metabolic rates.

Malaspinas, 2016

▶ There was <u>a bottleneck in the ancestral Australo-Papuan population ~ 50 kya.</u>

▶ An admixture estimate of <u>~ 4.0%</u>, suggest that Denisovan/Australo-Papuan admixture took place ~ 44 kya

▶ Suggest that the time since Neanderthal admixture was about 11% greater than the time since Denisovan admixture:

  ▶ earlier Neanderthal admixture (55–84 kya).

  ▶ later Denisovan admixture (31–50 kya) versus

54. Mapping gene flow between ancient hominins through demography-aware inference of the ancestral recombination graph - Melissa J. Hubisz, et al., 2020

▶ New modeling method, called ARGweaver-D, that samples ancestral recombination graphs conditional on a generic demographic model, including population divergence times, size changes, and migration events; powerful for identifying introgressed regions

▶ 3% N DNA is from ancient MHs, occurred between 200-300 kya.

▶ 1% of the Denisovan genome was introgressed from a highly diverged, superarchaic ancestor.

▶ About 15% of these "super-archaic" regions—comprising at least about 4Mb—were, in turn, introgressed into modern humans and continue to exist in the genomes of people alive today.

Hubisz, et al., 2020

▶ Prior studies: These events include gene flow/introgression between:

  ▶ Neanderthals and Denisovans (Nea↔Den)

  ▶ Ns and ancient MHs who left Africa over 100 Ka ago (Hum→Nea)

  ▶ "Super-archaic" hominin (~ *Homo erectus*) and Denisovans (Sup→Den)

  ▶ Other "Super-archaic" hominins and various MH groups in Africa (Sup→Afr)

▶ Average coalescence time (time to ancestral gene) for introgressed lineages

  ▶ between Vindija N and Papuan is 262 Ka;

  ▶ for Altai N and Papuan is 326 Ka, and

  ▶ for Denisovan and Papuan is 396 Ka.

▶ 0.5% introgression from the Neanderthal into current African individuals (back-migration into Africa from Europe)

Hubisz, et al., 2020

► Hum→Nea: ~3% in both the Altai and Vindija Neanderthal. This number is almost certainly an underestimate.

► Hum→Den: only ~0.37% of regions.

► Depletion on the X chromosome of archaic introgression into humans.

► High coverage of Hum→Nea introgression on the X chromosome for both the Altai and Vindija samples.

Hubisz, et al., 2020: Human introgression into Ns

► Substantial proportion of the Neanderthal genome consists of regions introgressed from ancient MHs.

► While we identified only 3% of the Neanderthal genome as introgressed, the true fraction may be ~7% .

► Hum→Nea gene flow occurred roughly between 200 and 300 kya

► Instead, our timeline suggests an earlier MH migration, occurring at least 200 Ka.

Hubisz, et al., 2020

▶ Most Neanderthal mtDNA is introgressed from ancient humans.

▶ A recent study bounded this introgression event at ≥ 270 kya, (recently analyzed Hohlenstein-Stadel MH femur sample)

▶ Recent discoveries of ancient fossils with human characteristics outside of Africa, including a 180 kya jawbone from Misliya Cave in Israel, and a 210 kya fossil from Apidima Cave in southern Greece with human features.

Hubisz, et al., 2020

▶ These findings suggest that early modern humans were present on the Eurasian continent at roughly the time at which Hum→Nea gene flow is estimated to have occurred.

▶ These early migrating humans later went extinct, leaving a genetic trace only in introgressed segments in Neanderthals.

Hubisz, et al., 2020

▶ Question of the possible functional impact of the Hum→Nea introgression.

▶ The strongest remaining pieces of evidence for negative selection against Nea→Hum introgression are the depletion on the X chromosome and in several other genomic deserts.

▶ But for the Hum→Nea event, we see no depletion on the X, and while there were too few samples to detect deserts across Neanderthals, we did confirm that previously identified Nea→Hum deserts are not depleted for Hum→Nea introgression.

N and D DNA

▶ Ns: 2 % of their DNA to MHs outside Africa

▶ Ds: 0.2 % to Asian and Native American people

▶ But the geographic pattern of that legacy was odd.

▶ D DNA did not show up significantly in Russia or China; but did find it in the genomes of New Guineans, other people from islands in Melanesia, and Australian Aborigines.

▶ On average genomes of these latter 3 groups are about 5 % Denisovan.

▶ Negritos in the Philippines have as much as 2.5 %.

Origins

▶ Pääbo and his MPI group: a scenario to explain what might have occurred:

▶ Sometime before 500 Ka ago, probably in Africa, the ancestor (H. heidelbergensis?) of MHs split off from the lineage that would give rise to Neanderthals and Denisovans.

▶ While our ancestors stayed in Africa, the common ancestor of Neanderthals and Denisovans migrated out.

▶ Those two lineages later diverged, with the Neanderthals initially moving west into Europe and the Denisovans spreading east.

Denisovan interbreeding in SE Asia

▶ MHs interbreed with Ns in Middle East and Central Asia.

▶ According to evidence presented by <u>David Reich</u>, this mixing most likely occurred between <u>67-46 Ka ago</u>.

▶ One population of MHs then continued east into Southeast Asia, where, sometime around 40 Ka ago, they encountered Denisovans.

▶ The MHs interbred with them as well and then moved into Australasia, carrying Denisovan DNA.

Where is the evidence of Denisovans

► This scenario might explain why the only evidence so far that the Denisovans even existed is fossils from a cave in Siberia and a 5 percent stake in the genomes of people living today in far away Southeast Asia.

► But If the Denisovans were so widespread, why was there so little trace of them in the genomes of Han Chinese or of any other Asian people between Siberia and Melanesia?

► Why had they left no mark in the archaeological record—no distinctive tools, say?

► Who were they really? What did they look like?

Denisovans in SE Asia

▶ Denisovans' DNA legacy still runs through native populations in Asia, the Americas, and the Oceanic islands.

▶ Between 1.9 and 3.4 percent of all present-day Melanesians' genes can be traced to Denisovans.

Denisovans appear to have crossed the Wallace Line

Use of watercraft
or flotation method

Denisovans

► <u>Wallacea</u> = group of mainly <u>Indonesian</u> islands separated by deep-water <u>straits</u> from the <u>Asian</u> and <u>Australian</u> <u>continental shelves</u>.

► Based on the modern distribution of Denisova DNA, Denisovans may have crossed the <u>Wallace Line</u>, with <u>Wallacea</u> serving as their last <u>refugium</u>.

► Mainland Asians and Native Americans had around 0.2% D DNA ancestry.

Summary:
The Denisovans
2010-2020

Our Current Knowledge about Denisovans

▶ Most Neanderthal fossil remains have been found across western Eurasia

▶ Denisovans bones have only been discovered in Denisova Cave, and China

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

Complete Denisovan Fossil Record in 2020: 15 items
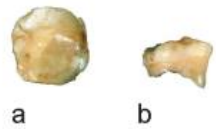
Current Knowledge about Denisovans

▶ Use of new methodologies:

   ▶ PCR cloning of ancient DNA fragments

   ▶ FINDER project: shift thru 40 K bones via collagen protein testing for phylogenetic identification

   ▶ ZooMS

   ▶ Methylation based prediction of D skeleton

   ▶ Optical analysis of sediment grains for dating of sedimentary layers

   ▶ Ability to identify mtDNA in only sediments

Our Current Knowledge about Denisovans as of 2020

▶ Discovery of Denisovan mtDNA and nDNA in a finger bone at Denisova Cave, Siberia

▶ Genetic <u>sister clade to Ns;</u> Everyone with Denisovan ancestry has some amount of Neanderthal ancestry

▶ <u>Divergence</u> between ancestral populations
  ▶ <u>Ns & Ds from MHs</u> occurred ~770-550 Ka
  ▶ N and D populations separated 470-380 Ka

▶ Ds had small population and low genetic diversity

▶ An Eastern population

Current Knowledge about Denisovans

▶ D genome is more similar to N genome than to MH genome

▶ Parts of D nuclear DNA and all of mitochondrial DNA are very divergent from MH and N DNA

▶ Mitochondrial DNA: Living humans have a common ancestor

  ▶ 200K with MHs

  ▶ 500-700K with Ns

  ▶ over 1 Ma for Ds

K. Prüfer, 2013: multiple 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%

Superarchaic
 to D = 0.5-8.0%
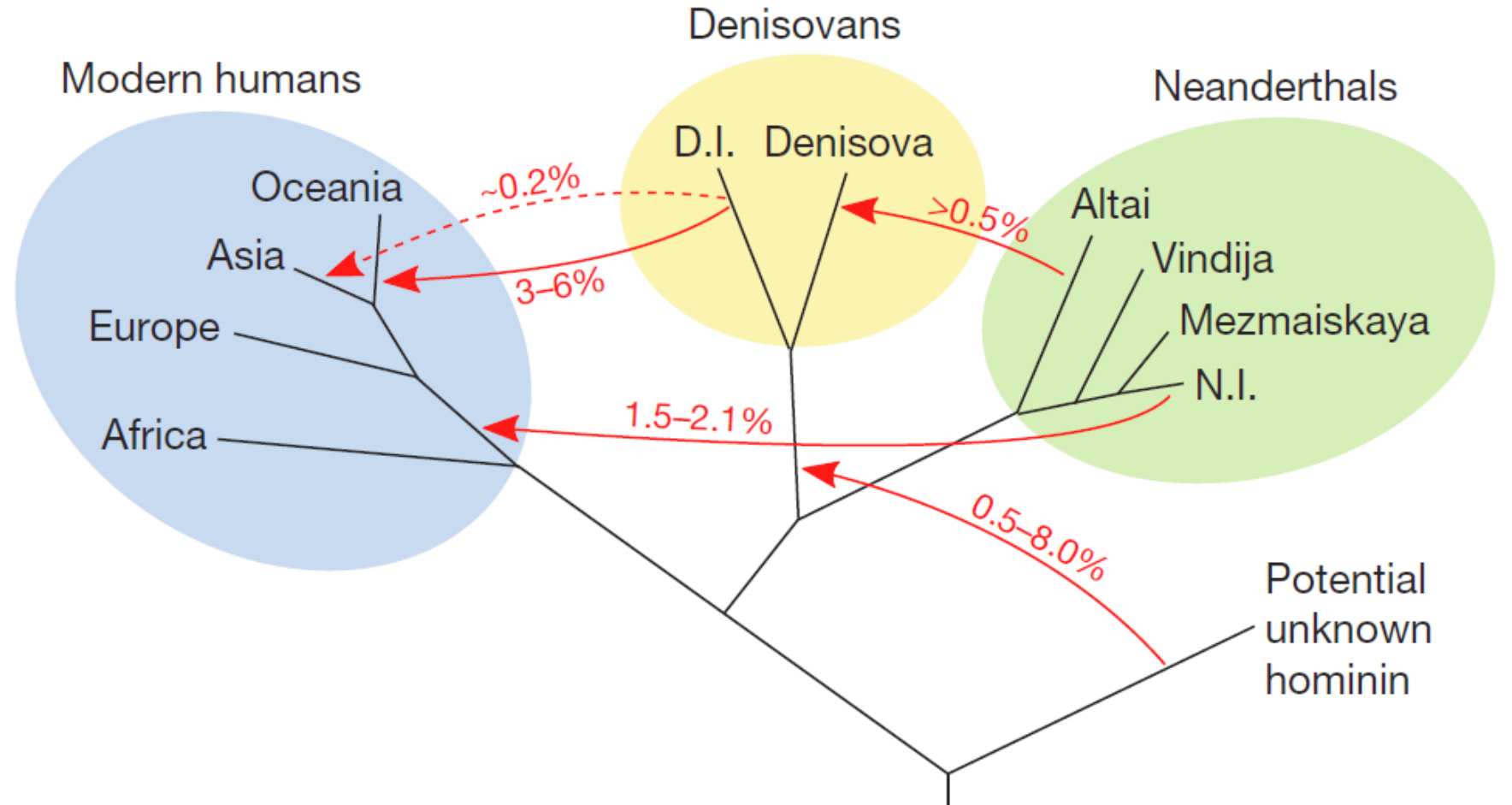
Figure 8 | A possible model of gene flow events in the Late Pleistocene. The

Our Current Knowledge about Denisovans

▶ <u>Europeans</u> have N but not D DNA, except for Iceland

▶ <u>Denisovan's admixtures with MHs were more recent than those of Ns</u>

▶ Ds have 17% N DNA; and 6-8% superarchaic lineage (*H. erectus*?)

▶ Ds interbreed with MHs of SE Asia: D DNA in Australian Aboriginals (5%); Highland, New Guinea (5%); Melanesia, New Britain, New Caledonia; Eastern Indonesia; Polynesia; in Mainland Asia (>1-.2%)

▶ D DNA has been found in Australian Aborigines, East Asians, Papua New Guineans, Tibetans, Native Americans, Peruvians and <u>Icelanders</u>

Our Current Knowledge about Denisovans

▶ Melanesians, N Guineans, Australian Aboriginals carry

  ▶ 2-3% N DNA

  ▶ 4–6% D DNA

▶ Mainland Asians and Native Americans have

  ▶ 0.2% D DNA

  ▶ ~0.2% of E Asian DNA, rising up to 0.3-0.6% in parts of S Asia.

▶ Papuan genome: ~7% ancient DNA:

  ▶ 2.5 % N

  ▶ later ~4.8 % D DNA

Current Knowledge about Denisovans

▶ Later African MH migration covered up much of this SE Asian D DNA (like a palimpsest, skin used for multiple layers of writing); multiple remixtures

▶ Denny, first verified ancient hybrid: Neandertal mother and a Denisovan father. Dated to 79-118 Ka ago.

D genetic characteristics

▶ The Denisovan genome from Denisova Cave has variants of genes which, in modern humans, are associated with:

  ▶ dark skin, brown hair, and brown eyes.

  ▶ variant region around the EPAS1 gene that in Tibetans assists with <u>adaptation to low oxygen levels</u> at high elevation; and genes for surviving the cold

  ▶ WARS2 and TBX15 loci which affect <u>body-fat distribution</u> in the Inuit.

D genetic characteristics 2

▶ Shared functional genes with Ns: Half of the HLA alleles of modern Eurasians were shown to represent archaic HLA haplotypes (N & D)

▶ In Papuans, introgressed Neanderthal alleles are highest in frequency in genes expressed in the brain, whereas Denisovan alleles have highest frequency in genes expressed in bones and other tissue.

Ghost populations: only genetic evidence, no bones

▶ **Discovery of "ghost lineages"** only found in <u>genomes</u> that indicate <u>unknown ancient genetic populations for whom we do not have fossil bones</u>

▶ **Ghost populations**:

  ▶ in African pygmies

  ▶ in Southern Africans

  ▶ ancient African populations mixing with each other to a slight degree

  ▶ .5 to 8% ghost population in D genome

Current Knowledge about Denisovans

▶ Altai population of Denisovans is characterized by

  ▶ low nuclear genome diversity,

  ▶ consistent with a prolonged small population size

▶ Identification of 3 Denisovan genetic lineages;

  ▶ D0 – Siberia

  ▶ D1 & D2 in Papua New Guinea

Denisovan Anatomy

▶ Almost nothing is known of the precise anatomical features of the Denisovans

▶ The only physical remains discovered so far are <u>a finger bone, three teeth, long bone fragments, a partial jawbone, and a parietal bone skull fragment</u>.

  ▶ The finger bone is within the modern human range of variation for women,

  ▶ The massive, robust molars which are more similar to those of Ns.

  ▶ The third molar is outside of the range of any *Homo* species except <u>*H. habilis*</u> and <u>*H. rudolfensis*</u>, and is more like those of <u>australopithecines</u>.

  ▶ The second molar is larger than those of MHs and Ns, and is more similar to those of <u>*H. erectus*</u> and *H. habilis*.

Denisovan Anatomy

▶ Like Neanderthals, the mandible had a retromolar gap, and the front teeth were flattened; but Denisovans lacked a high mandibular body, and the mandibular symphysis at the midline of the jaw was more receding. No chin.

▶ The parietal bone has thickness reminiscent of *H. erectus*.

▶ A facial reconstruction has been generated by comparing methylation at individual genetic loci associated with facial structure.

▶ This analysis suggested that Denisovans, much like Neanderthals, had a long, broad, and projecting face; larger nose; sloping forehead; protruding jaw; elongated and flattened skull; and wide chest and hips. Denisovan tooth row was longer than that of Ns and AMHs.

Current Knowledge about Denisovans

▶ Study of sediments: in Denisova Cave contains evidence of cave use by

  ▶ humans and other animals, including hyenas, bears and wolves;

  ▶ but scant evidence for the use of fire.

▶ Human activity was intermittent over the past three glacial–interglacial cycles (>300 Ka to ~20 Ka).

▶ The stone artefact assemblages indicate long-term hominin presence at the site during both warm climates and cold conditions.

▶ Hyenas are most likely the main accumulating agents of the faunal remains, given the dominance of their coprolites in the cave sediments.

Current Knowledge about Denisovans

▶ Icelandic study of 27,566 Icelanders genomes:

   ▶ contains N and D DNA; amount of archaic variants in modern genome = 2% archaic variants in non-Africans;

      ▶ Archaic genetic fragments: Vindija Neanderthal (51%), Altai Neanderthal (13%), Denisovan (3%);

   ▶ Raises the possibility that there were Denisovan-like groups west of the Altai mountains;

   ▶ Archaic variants for association with 271 phenotypes; only 5 significant; conclude that N variants in modern humans have little phenotypic consequences

   ▶ 282 distinct archaic deserts, covering 25% of MH genome; the X chromosome is devoid of archaic introgression

   ▶ Neanderthal children had older mothers and younger fathers

Our Current Knowledge about Denisovans

► Denisovans went extinct after the N extinction;

► Now considered the most recent extinct relatives of MHs.

► Possibly between 30 to 15 Ka

Denisova

▶ The most important message of the Denisova story is that no fossil is unimportant. Even the tiniest fragment that seems completely useless, like a fragment of a finger bone, can open up a completely new world of human evolution to us.

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