Ancient DNA: A History of Human Paleogenetics, Pt. 2

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Sahelanthropus tchadensis, TM266: Hominin? Or hominoid?

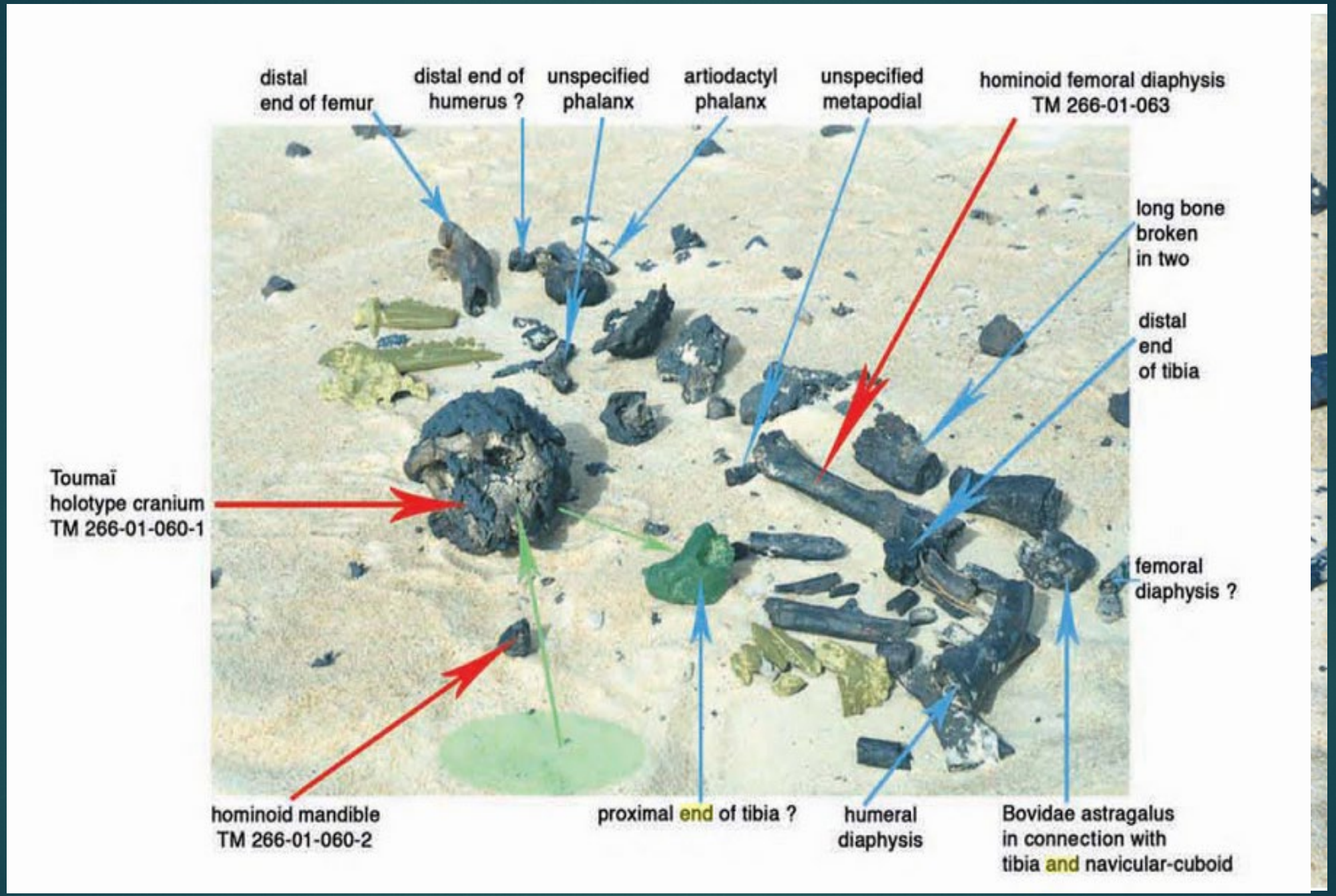
2001: *Sahelanthropus tchadensis*, Chad, 7-6 M

Remarkably complete but distorted cranium & 2 mandibles; no postcranials?

Has been virtually remodeled

Largest hominoid browridge ever discovered

Foramen magnum shape and forward positioning indicate bipedalism



Did camel herders rebury Toumai facing Mecca?

Two contradictory studies: *Sahelanthropus* was and wasn't a hominin.

- Over the years, the undescribed fossil — sometimes dubbed Toumaï's femur, even though it's not clear whether the skull and leg bone belonged to the same individual — became one of palaeoanthropology's worst kept secrets.
- Macchiarelli et al, 2020 : In 2020, a brief description of the femur, based on several days of study done in 2004. Their preliminary analysis concluded that the remains probably did not belong to a species that routinely walked upright.
- In the 2022 Davers paper described the femur, and comes to the opposite conclusion. The team contends that more than a dozen features of the femur suggest that Toumaï's kind walked on two feet, and the ape-like arm bones suggest its species would also have been comfortable clambering in trees.

2023: *Sahelanthropus* is the earliest known knuckle-walking ape. A hominid, not a hominin.

- 2023 Study: found that the distinctive forelimb morphology of the African knuckle-walking apes is present in the forelimb of the roughly 7-million-year-old *Sahelanthropus tchadensis*.

- Ulna is normally a straight bone in the arm. Only chimpanzees and gorillas exhibit robust and forward-curving ulna shafts, which are thought to serve as an adaptation to knuckle-walking. Curvature in the ulna signals more terrestrial quadrupedal postures

Marc Meyer, et al., 2023

Lateral view of ulna of S. tchadensis; normally a straight bone

Knuckle-walking in *Sahelanthropus*? Locomotor inferences from the ulnae of fossil hominins and other hominoids

- Sahelanthropus: Propose 2 theories - (1) that this species represents the earliest known knuckle-walking African ape and was not a hominin, or (2) that even if this species was capable of walking, like chimps and gorillas it was a habitual knuckle-walker.

- While new research from the leg and arm bones of this species strongly refute the idea that Sahelanthropus was an early biped, the new evidence indicating that it was a knuckle-walking Miocene ape shifts it to a uniquely privileged evolutionary position.

S. tchadensis: represents a late Miocene hominid with knuckle-walking adaptations

- Study compared the relative influence of locomotion, taxonomy, and body mass on ulna contours in Homo sapiens (*n* = 22), five species of extant apes (*n* = 33), two Miocene apes (*Hispanopithecus* and *Danuvius*), and 17 fossil hominin specimens including *Sahelanthropus*, *Ardipithecus*, *Australopithecus*, *Paranthropus*, and early *Homo*.
- Ulna shafts significantly correlate with type of locomotion.
- African apes' ulna shafts are more robust and curved than Asian apes and are unlike other terrestrial mammals, curving ventrally rather than dorsally.
- Because this distinctive curvature is absent in orangutans and gibbons, it is likely a function of powerful flexors engaged in wrist and hand stabilization during knuckle-walking, and not an adaptation to climbing or suspensory behavior.

Sahelanthropus = A knuckle-walking hominid

- The *Sahelanthropus tchadensis* fossil differs from other hominins by falling within the knuckle-walking morphology, and thus appear to show forelimb morphology consistent with terrestrial locomotion.

- Along with its associated femur, the TM 266 ulna shaft contours and its deep, keeled elbow joint comprise a suite of traits signaling African ape-like quadrupedalism.

- This study supports the growing body of evidence indicating that *S. tchadensis* was not an obligate biped, but instead represents a late Miocene hominid with knuckle-walking adaptations.

Recommendation

- Let's delete the verbal framework of "primitive" and "archaic" and "modern" and go with "basal" and "derived" in any evolutionary discussion: evolution has no direction.

- My lecture usage of "MH" (modern human) = African *H. sapiens*

Proteomics

- A new biomolecular approach to the identification of ancient material is the analysis of ancient proteins preserved in enamel, dentine and bone from hominin fossils.
- Ancient DNA survives for a limited amount of time, degrading entirely after 0.5 to 2.0 Ma.
- Ancient proteins survive for longer stretches of time than DNA.
- Proteins do not carry nearly as much information as DNA. They only contain about 1% of the maximum information that you could get from a DNA sample

Proteinomics

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

- Ancient proteins preserved in mineralized tissues provide phylogenetically informative amino acid sequences in fossils where no DNA survives, such as demonstrated by the recovery of collagen type I spectra from 3.4 Ma old Camelid bones in the Arctic and 3.8 Ma eggshell proteins in central Africa.

- Ancient proteins therefore provide a biomolecular alternative in areas, time periods, and tissues where ancient DNA does not regularly survive.

Proteins: Not as much info , but for longer time period

- The largest ancient bone proteome published to date contains close to 200 proteins. The total amount of phylogenetically informative positions is therefore drastically reduced in ancient proteomes compared to ancient DNA analysis of entire genomes, but such data can be retrieved from significantly larger periods of time.

- Hominin fossils preserving no, little, or highly contaminated ancient DNA sequences might be amenable to ancient protein analysis.

- In the next 10 years all existing hominin fossils will have proteinomic analysis and phylogenetic description.

Oldest protein analysis

- In 2013, 3.4-million-year-old camel found in the Arctic
- In 2016, researchers reported protein sequences from 3.8-million-year-old ostrich eggshells from Tanzania
- In 2017, a long-necked herbivore dinosaur, *Lufengosaurus*, 195 million years.
- In 2019 another team decoded proteins from a 1.8-million-year-old rhinoceros tooth from Georgia.

Oldest hominin proteins

- 160 Ka Denisovan jawbone and 90 Ka bone - N mom-D dad hybrid Denny
- As for the likely age limit of ancient DNA = 2.6 million years = age limit of the age of permafrost (post a warming period).
- I am waiting for *H. erectus* in deep cold cave. Even better a Neandertal who had trousers on like Otzi.

Genetic variation (in ancient and modern DNA) can be used to understand:

- Demographic (population) histories
- Human migrations
- Ancestral/descendant relationships
- Relationships between communities
- Disease
- Social structure and kinship practices

3 Genetic Ancestral Groups found in modern Europeans

- Present-day western Europeans are genetically related to mixtures of three differing ancestral groups:
  - Mesolithic hunter–gatherers,
  - Anatolian Neolithic farmers,
  - Steppe Yamnaya

Genetic history of migrations

- European genetic history is marked by multiple migrations.

- Anatomically MH HGs were widely distributed in Europe by at least 42-47 Ka.

- The oldest genomic data from a modern human in Europe are from the Oase 1 fossil dated to 37-42 Ka. This individual, who had a direct Neanderthal ancestor in the past four to six generations, was a genetic dead end.

Great Migrations

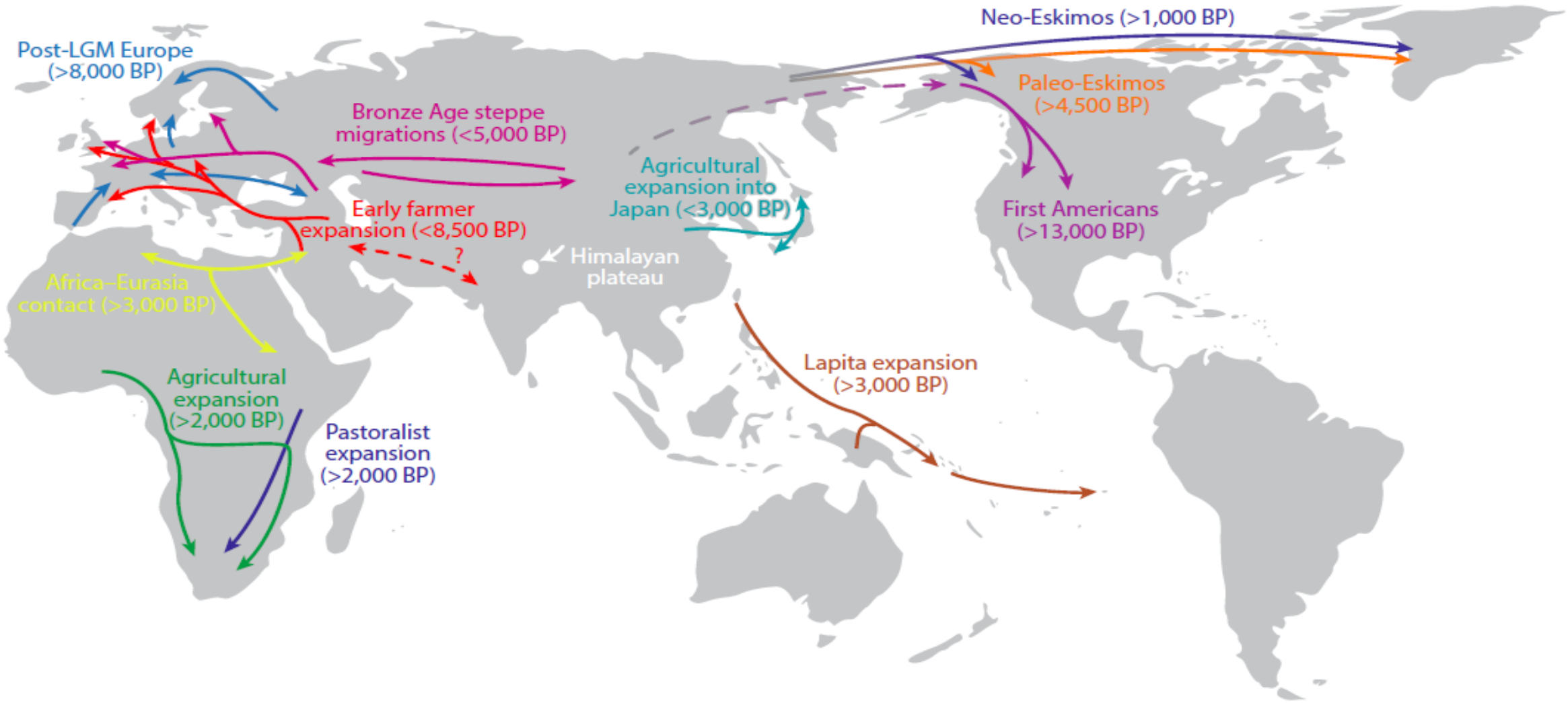


Figure 4

Major Holocene population movements and expansions that have been demonstrated using ancient DNA. Abbreviations: BP, years before present; LGM, Last Glacial Maximum.

Post Glacial HG replacements

- As the Last Glacial Maximum (LGM) came to an end and the ice sheets receded, Europe was repopulated by HGs, from southern European and central Eurasian refugia

- Another transformation may have taken place during a warm period around 15 Ka, replacing the original re-colonizers with a population that would come to form the Mesolithic populations of Europe.

First Farmers

- First Farmers (Anatolian Neolithic group):
  - Starting from the southeast around 9 Ka,
  - the hunter gatherers of Europe were marginalized
  - as a new type of ancestry related to that found in Neolithic northwest Anatolia and, ultimately, to early farming populations of the Levant and northern Iran expanded throughout Europe.

Hunter gatherers then farmers

- This farmer population rapidly reached the extreme edges of Europe, with direct evidence of their presence in Iberia at 7 Ka, in Ireland at 5.1 Ka, and in Scandinavia at 5 Ka.

- This Anatolian Neolithic ancestry was highly genetically differentiated from the hunter–gatherer ancestry of the populations that previously inhabited Europe.

- Across Europe, this Neolithic ancestry's appearance was closely linked to the adoption of an agricultural lifestyle, and it is now clear that this change was driven, at least in part, by the migration.

HG and Farmers merge

- However, the Anatolian Neolithic migrants did not completely replace the hunter–gatherer populations.
- Over the next 4,000 years, the two populations merged,
- By 4.5 Ka, almost all European populations were admixed between these two ancestries, typically with 10–25% hunter–gatherer ancestry.
- This mixture process occurred independently in different parts of Europe, likely driven by local hunter–gatherer populations who lived in close proximity to farming groups.

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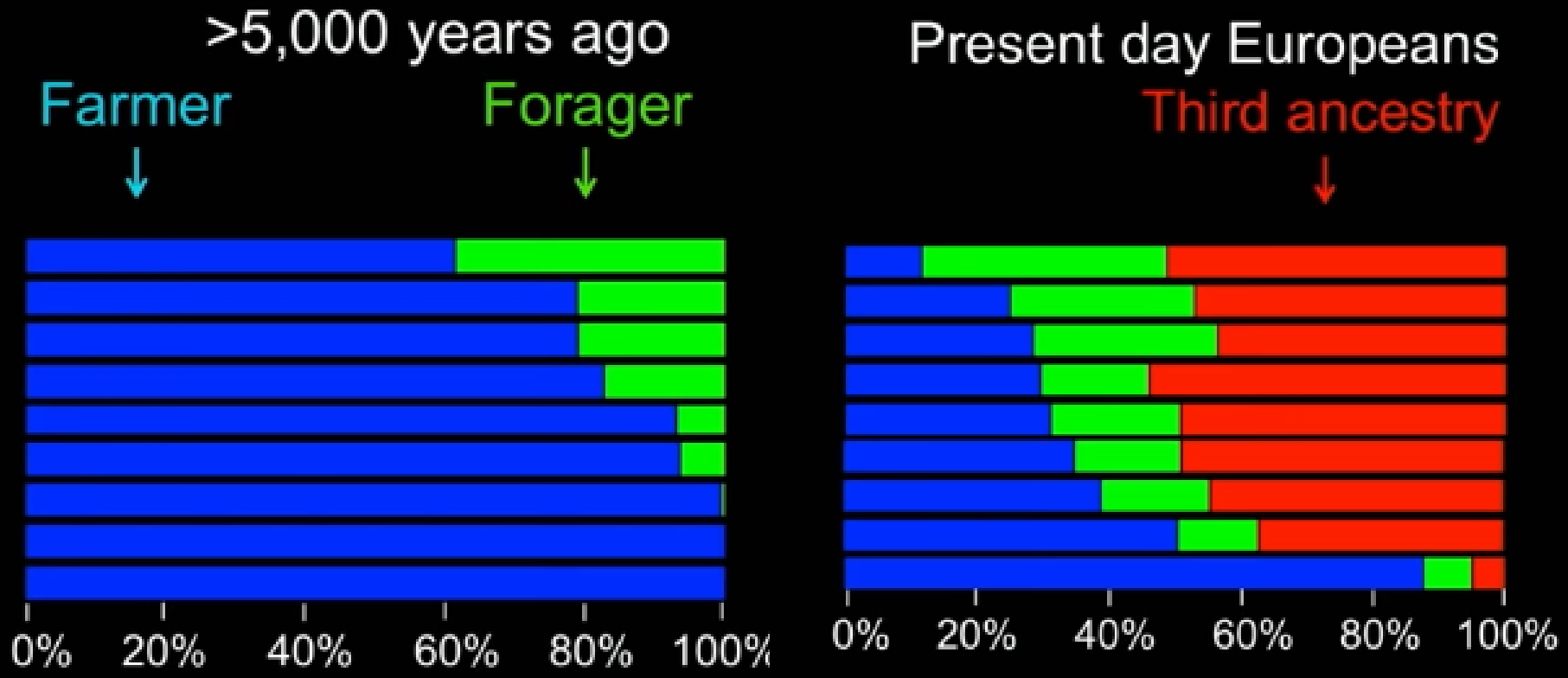
Before 5,000 years ago Europeans were a mixture of two ancestries but today there is a third

Lazaridis et al. *Nature* 2014; Haak et al. *Nature* 2015



Blue = Anatolian farmers

50% of later population ancestry (red) did not exist at 5 Ka

20% was HG in later population

Final merger: Yamnaya migration into Europe

- The next substantial change is closely associated with ancestry that by around 5,000 BP extended over a region of more than 2,000 miles of the Eurasian steppe, including in individuals associated with the Yamnaya cultural complex in eastern Europe

- Steppe ancestry appeared in southeastern Europe by 6,000 BP, northeastern Europe around 5,000 BP, and central Europe at the time of the Corded Ware complex around 4,600 BP.

2014: Ancient human genomes suggest three ancestral populations for present-day Europeans

- Iosif Lazaridis: Sequenced the genomes of a ~7,000-year-old farmer from Germany and eight ~8 Ka hunter-gatherers from Luxembourg and Sweden; and 2345 current humans

- Most present Europeans derive from at least three highly differentiated populations:

Iosif Lazaridis, et al., 2014

Hunter-Gatherers

- 1 - West European Hunter-Gatherers (WHG), who contributed ancestry to all current Europeans but not to Near Easterners

- West European Hunter-Gatherers, arriving 45 Ka, had Europe alone for 30 K years.

- 2023 study: HGs consisted to 8 different genetic groups, not 1 giant Gravettian HG group; final 2 included HGs from refugium Spain and from Italy via the Balkans

Early Farmers

- 2 - Early European Farmers (EEF), who were mainly of Near Eastern origin but also harbored WHG-related ancestry
- Early European Farmers, arriving ~9 K; spread to South & central Europe; gene for light skin spreads
- EEF had ~44% ancestry from a "Basal Eurasian" population that split prior to the diversification of other non-African lineages.

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Ancient North Eurasians -- Yamnaya

- 3 - Ancient North Eurasians related to Upper Paleolithic Siberians, who contributed to both Europeans and Near Easterners; related to Mal'ta child & Native Americans
- Ancient North Eurasians: massive Yamnaya migration arriving 4.5 Ka
  - Corded Ware culture;
  - cattle herders,
  - used wheel and horses;
  - brought Proto-Indo-European language
- Yamnaya went both west to Europe and east to India

Like Europe, India is a genetic admixture

- India: Ancient North Indians (ANI) and Ancient South Indians (ASI) had mixed dramatically in India.
- The result is that everyone in mainland India today is a genetic mix, albeit in different proportions, of ancestry related to West Eurasians, and ancestry more closely related to diverse East Asian and South Asian populations
- No group in India can claim genetic purity.

India

- Reich's group were able to estimate the fraction of West Eurasian (Yamnaya)-related ancestry in each Indian group.
- West Eurasian-related mixture in India ranges from as low as 20 percent to as high as 80 percent.
- No Indian group is unaffected by mixing, neither the highest nor the lowest caste, including the non-Hindu tribal populations living outside the caste system.

Ancestry, Language, Power, and Sexual Dominance

- Groups in India that speak Indo-European languages typically have more ANI ancestry than those speaking Dravidian languages, who have more ASI ancestry.

- The ANI probably spread Indo-European languages, while the ASI spread Dravidian languages.

- The genetic data also hinted at the social status of the ancient ANI (higher social status on average) and ASI (lower social status on average).

All Indians are mixed. No pure ancestry.

Everybody is mixed – no one is "pure"

West Eurasian-related ancestry is 20-80% and is significantly correlated to caste and language

Populations	West Eurasian (ANI%)	Language-family	Traditional Caste / Social group
Madiga	32.0 ± 1.7	Dravidian	Lower caste
Mala	34.3 ± 1.7	Dravidian	Lower caste
Kallar*	37.7 ± 1.8	Dravidian	Tribal
Vysya	37.9 ± 1.8	Dravidian	Middle caste
Chamar*	38.7 ± 1.7	Indo-European	Tribal
Bhil	38.9 ± 1.6	Indo-European	Tribal
Scheduled caste/ tribe*	40.5 ± 1.9	Dravidian	Lower caste
Dushadh*	41.0 ± 1.8	Indo-European	Lower caste
Velama*	43.4 ± 1.7	Dravidian	Upper caste
Dharkar*	47.8 ± 1.5	Indo-European	Nomadic group
Kanjar*	48.2 ± 1.7	Indo-European	Nomadic group
Kshatriya*	54.6 ± 1.6	Indo-European	Upper caste
Kshatriya	60.9 ± 1.3	Indo-European	Upper caste
Brahmin*	61.2 ± 1.4	Indo-European	Upper caste
Brahmin	62.8 ± 1.4	Indo-European	Upper caste
Sindhi+	64.3 ± 1.3	Indo-European	Urban
Kashmiri Pandit	65.2 ± 1.3	Indo-European	Upper caste
Pathan+	70.4 ± 1.2	Indo-European	Urban

West Eurasian ancestry in India is 20-80% and is significantly correlated to caste and language

Upper Caste = Indo-European language

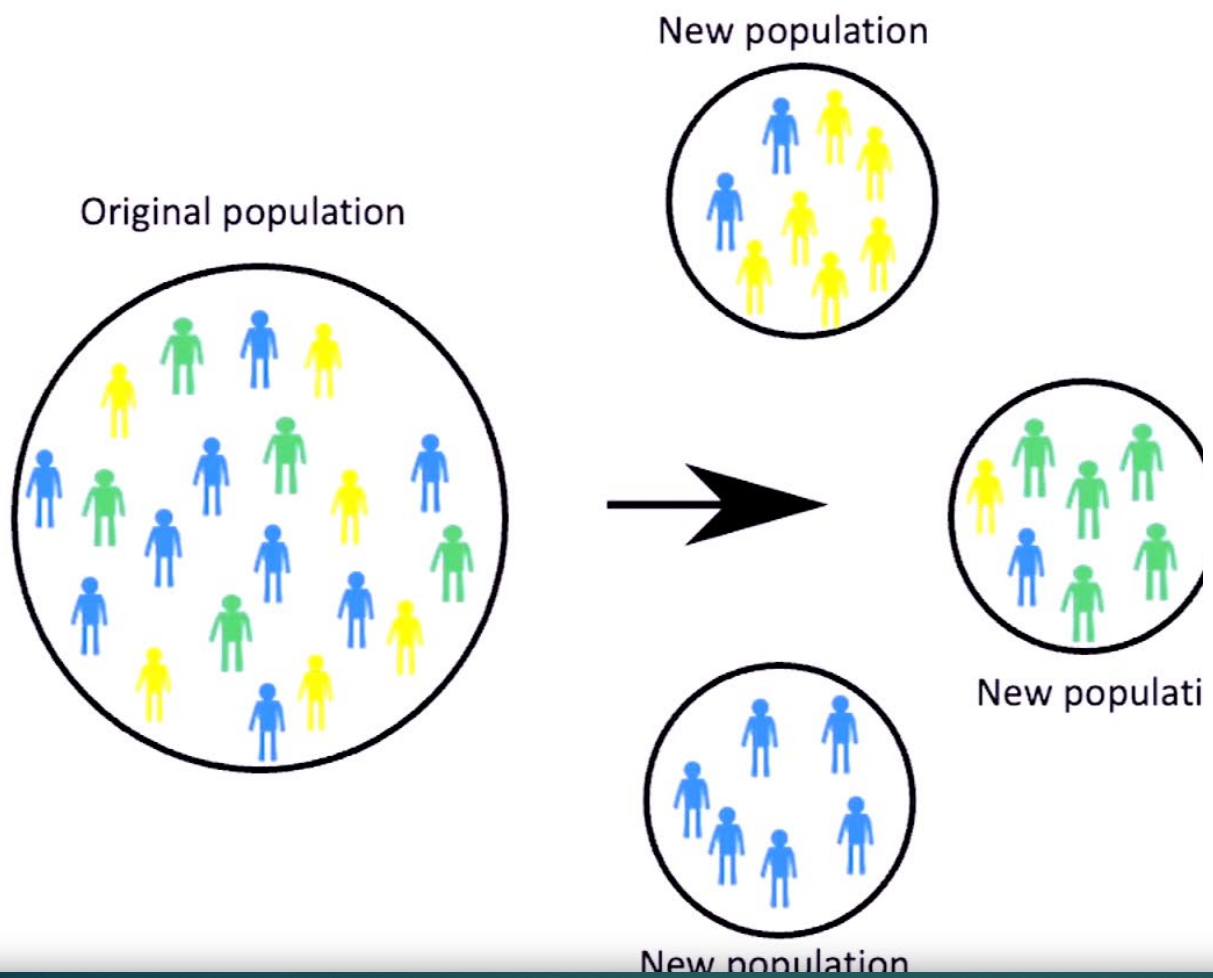
Lower Caste = Dravidian

Political backlash to genetic studies of India, 2014, under PM Modi

Current Indian Prime Minister Modi is known to support a Hindu-majoritarian narrative that rejects a theory stating the Aryan race migrated to India and displaced the country's indigenous population.
The rejection of the Aryan migration theory is something endorsed by the currently ruling Bharatiya Janata Party (BIP).
The archaeologist said they wanted to see how the "mutation and mixing of genes in the Indian population has happened in the last 10,000 years".
"Is Modi government treading Hitler's trail... what does profiling of purity of race signify?" asked Congress leader Sadaf Jafar.

There were a multitude of founder events in India

Founder "founding" events



Impact of founder event

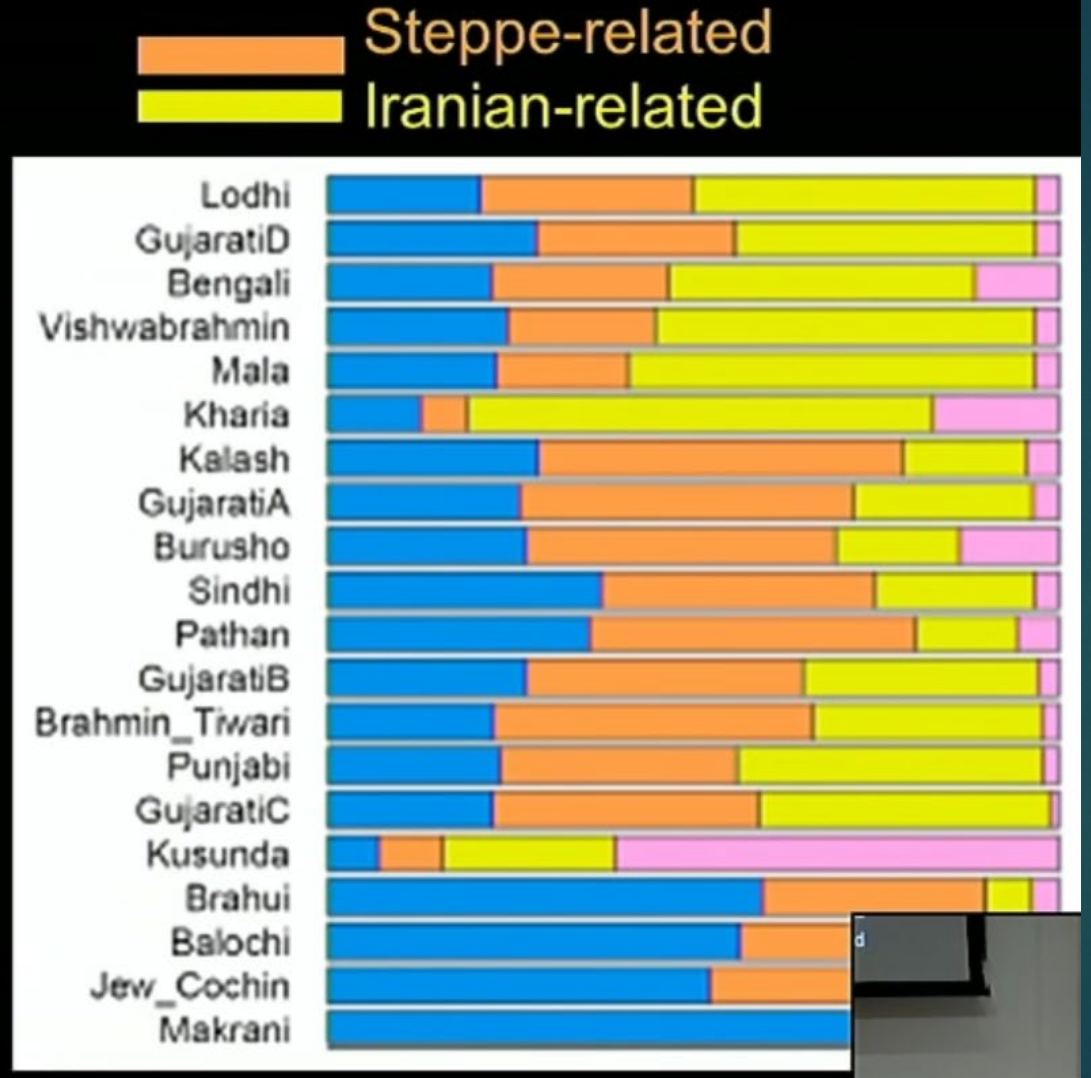
- Leads to lower genetic diversity
- Selection to remove deleterious (disease causing) variants does not work well
- Increased risk of recessive diseases, e.g., Tay Sachs disease in Ashkenazi Jews
- Some examples: Amish, Ashkenazi Jews, Finns, etc.
  - All of India

Bottlenecks

- 30% of Indian groups experienced population bottlenecks as strong or stronger than the ones that occurred among Finns or Ashkenazi Jews.
- Many of the population bottlenecks in India are exceedingly old.
- One of the most striking was in the Vysya of the southern Indian state of Andhra Pradesh, a middle caste group of approximately five million people whose population bottleneck could date to between 3000 to 2000 years ago.

Is there Steppe-Related Ancestry in India?

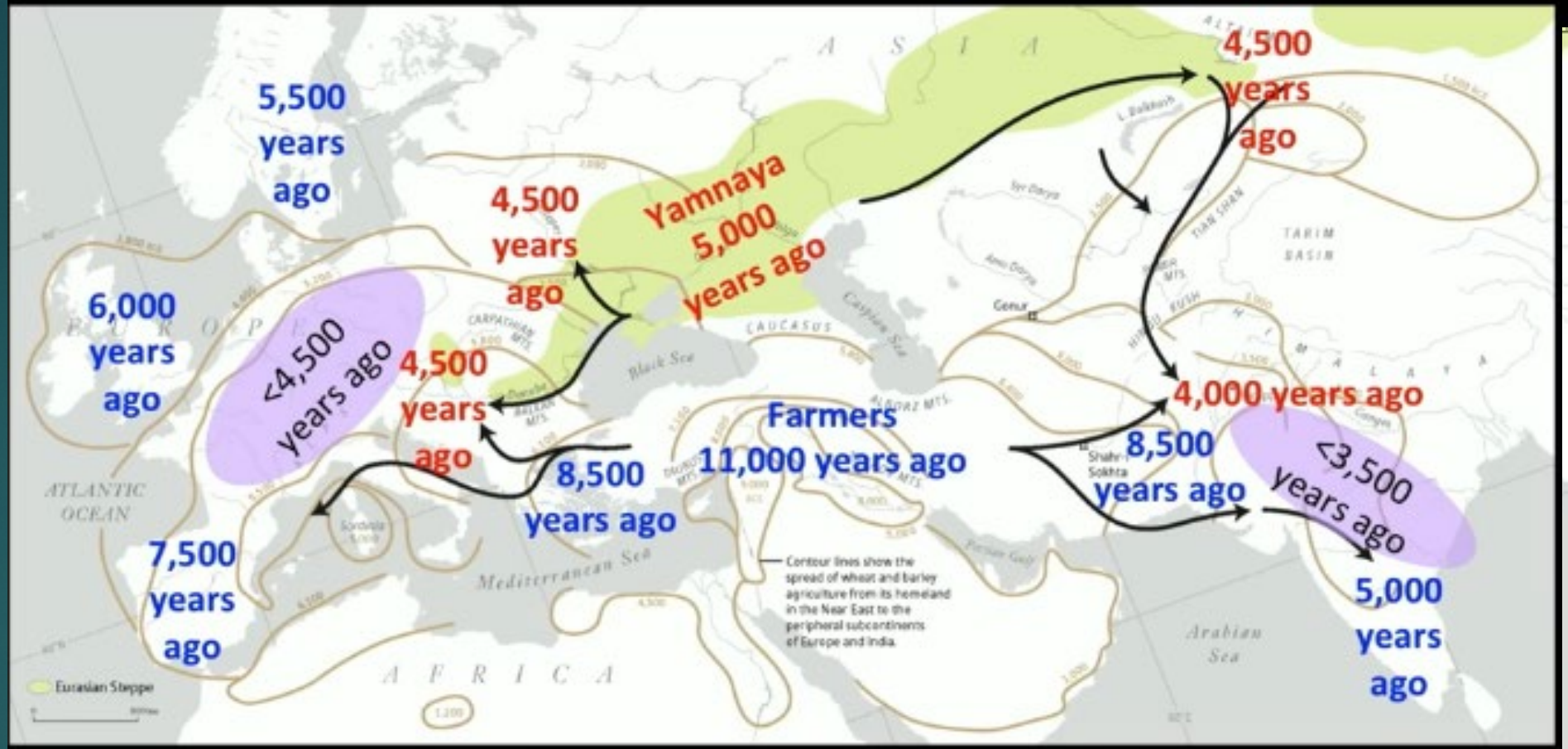
Yes, it is present in almost every Indo-European and Dravidian-speaking population, with higher relative proportions in Indo-European speakers (Lazaridis et al. Nature 2016)



Y chromosome evidence of shared male ancestry betweer
Europe from the time of the Yamnaya (Underhill EJHG 201

Two parallel subcontinents of Eurasia – Europe and India

Haak et al. *Nature* 2015; Narasimhan et al. *bioRxiv* 2018



8,500-5,000 years ago: Mixed populations of farmers and foragers

5,000-3,500 years ago: Spread of Yamnaya ancestry

Today: Mixtures of these mixed populations

Yamnaya Y chromosome predominates

- Maternally inherited mitochondrial DNA sequences changed relatively little when Yamnaya arrived.

- By contrast, between 60 and 90 per cent of men now living in the area can trace their paternally inherited Y chromosome to Yamnaya-related migrants.

- Indigenous males seem to have been marginalized by the new arrivals much more than the women and were unable to have children to the same extent.

Summary: Indian genetic ancestry

- Ancestral North Indians (related to Steppe and Iran) and Ancestral South Indians (indigenous (East Asian and South Asian), no W Eurasian ancestry)
- 4 language groups: Indo Europe, Dravidian, B, Austro?
- 3 sources: steppe, SE Asian, Iranian
- S Indians had 30% Iranian farmer ancestry
- India: steppe pastoralist after 4000 Ka,
- Europe and India poles: 12 Ka agriculture, in near east; 9000 Ka Anatoly farmers to Europe, from Iran to India; Steppe 4000 Ka
- With the Yamnaya comes Indo-European languages

Summary

- Nearly all groups in South Asia are ADMIXED, deriving ancestry from South Asian hunter-gatherers, Iranian farmers and Steppe pastoralists
- Similar patterns are seen in Europe and India suggesting the two continents are united in their history of Iranian and Steppe ancestry
- Following the mixture. India experienced a demographic transformation where mixture across groups become rare, with endogamy prevailing, leading to strong founder effects in many groups
- There are >80 groups with founder events stronger than those seen in Ashkenazi Jews and Finns. both of which have a history of high rales of recessive diseases

Nuclear DNA from two early Neandertals reveals 80,000 years of genetic continuity in Europe

- Neandertals across their entire geographic range from Europe to Central Asia belonged to a single group sharing a most recent common ancestor less than 97 ka ago.

- However, population discontinuity has been observed in Denisova Cave, Russia, further back in time, where the Neandertal component in the genome of a ~90-ka-old Neandertal-Denisovan hybrid offspring shows stronger affinities to late Neandertals in Europe than to the *Altai Neandertal*, another individual found in the same cave.

- Thus, a population replacement likely occurred in the easternmost part of the Neandertal territory between 90 and 120 ka ago.

Stéphane Peyrégne, et al. 2019

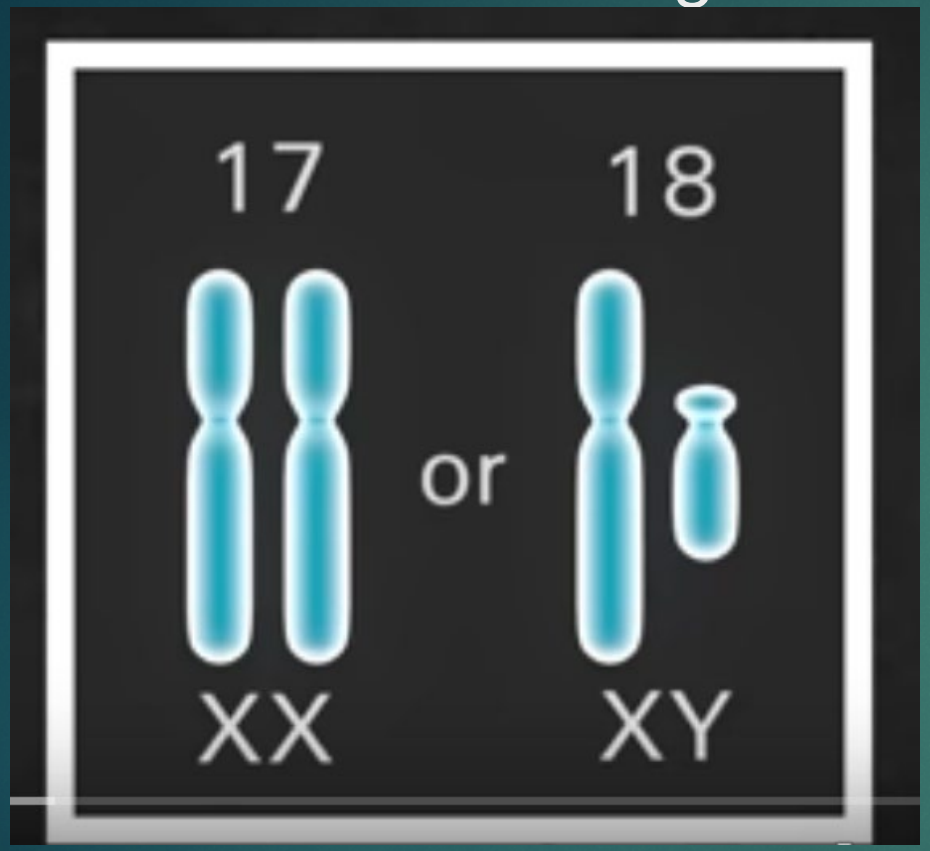
Species problem: no current N DNA in MH gametes

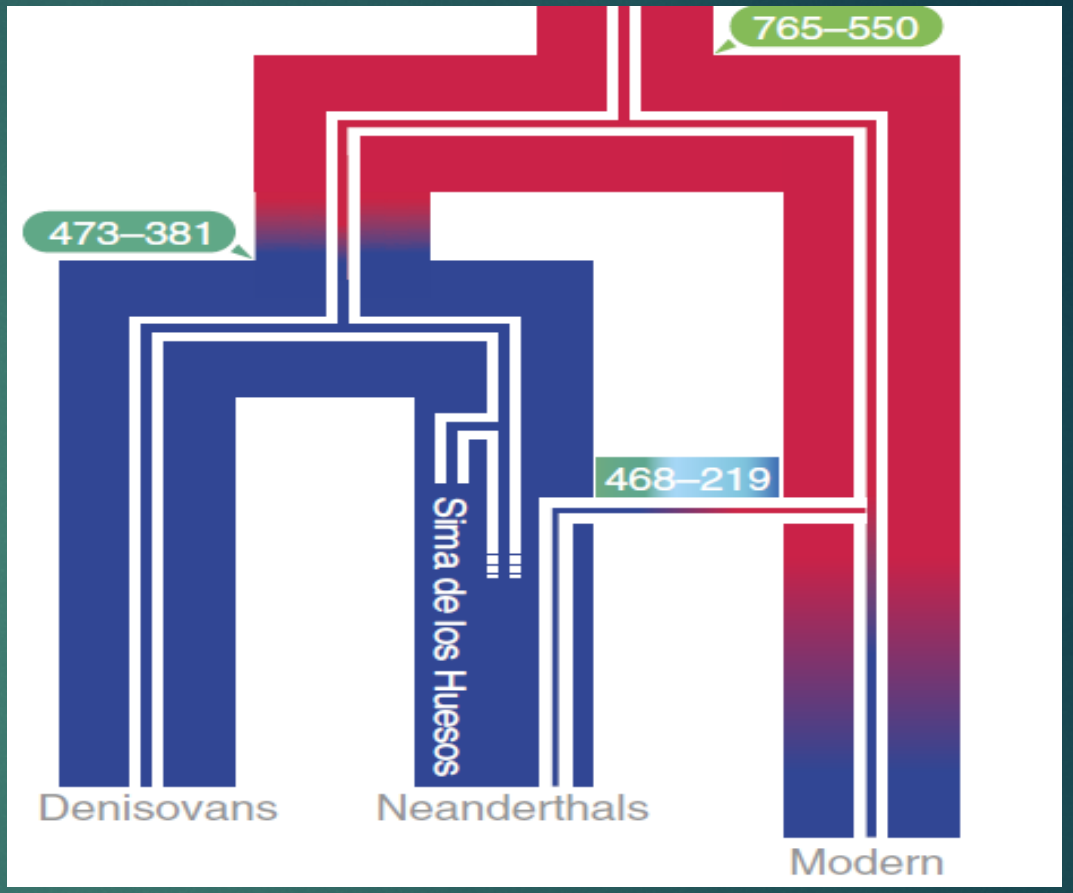
- MH X chromosome is almost devoid of Neanderthal DNA implying male human-Neandertal hybrids may have been infertile.
- The Y-chromosome of male Neanderthals proved to be unviable in hybrids; only the female hybrids proved to be fertile.
- The DNA on the Y chromosome from a male Neandertal who lived at El Sidrón, Spain, 49,000 years ago has not been passed onto modern humans.
- MHs have no Neandertal DNA on their Y chromosomes.

No N Y chromosome

- Neanderthals have long been seen as uber-masculine hunks compared to MHs.
- But a 2020 study finds *Homo sapiens* men essentially emasculated their brawny brethren when they mated with Neanderthal women more than 100,000 years ago. Those unions caused the modern Y chromosomes to sweep through future generations of Neanderthal boys, eventually replacing the Neanderthal Y.
- The best scenario to explain the Y pattern is that early modern human men mated with Neanderthal women more than 100,000 but less than 370,000 years ago. Their sons would have carried the modern human Y chromosome, which is paternally inherited. The modern Y then rapidly spread through their offspring to the small populations of Neanderthals in Europe and Asia, replacing the Neanderthal Y.
- Interestingly, the modern human mates were not ancestors to today's *H. sapiens*—but were likely part of a population that migrated early out of Africa and then went extinct.

Mystery of Neandertal Y chromosome: We met Neanderthals way earlier than we thought



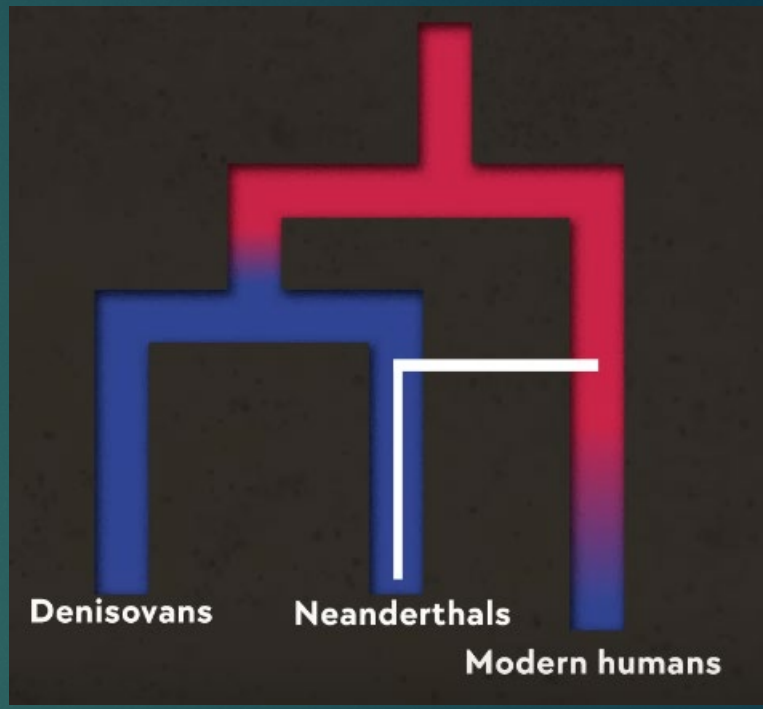


When finally sequenced, Neandertal Y chromosome looks more like MH Y chromosome than to Denisovan Y chromosome: looked like ancient MH Y chromosome

MHs had permanently replaced the Neandertal Y chromosome:

When did MH Y chromosome transfer to Ns happen?

- There have been multiple MH-N interactions prior to 60 Ka
- The African/MH interbreeding with Ns that replaced the Neandertal Y chromosome occurred around 270 Ka
- At least 1 N male with MH Y chromosome had N sons that spread it
- The gene flow resulted in the complete replacement of the original Neandertal Y chromosomes by its early MH counterpart.



Species problem

- This has suggested that female modern humans and male Neandertals were not fully compatible and that male Neandertals may have had problems with sperm production.

- N male and MH female matings likely resulted in infertile male hybrid or fertile female hybrid; we inherited most of our Neanderthal genes through hybrid females
- The El Sidrón Neandertal had mutations in three immune genes, including one that produces antigens that can elicit an immune response in pregnant women, causing them to reject and miscarry male fetuses with those genes.

Species problem

- So even though male Neandertals and female modern humans probably hooked up more than once over the ages, they may have been unable to produce many healthy male babies—and, thus, hastened the extinction of Neandertals.

- Tellingly, 10 of 10 N mitochondrial sequences are outside the current MH range, suggesting that mating of MH males with N females generated nonviable progeny.

Female MHs and N males = viable female hybrid children

- In contrast, progeny of female MHs and N males may have had the opportunity to survive within MH groups, with sufficient mating success rates to allow transmissions of a few alleles valuable to the newcomers, probably related to ecological adaptation.

- A 2016 study presented evidence that Neanderthal males might not have had viable male offspring with AMH females. This could explain why no modern man to date has been found with a Neanderthal Y chromosome.

- So N DNA entered MH genome via female MHs and N males with viable female hybrids

A Partial Review of
15 known introgression events:
from 320 Ka to recent

4 Different N genetic lineages

- 1- El Sidrón, Spain Neanderthals
- 2 - Vindija, Croatia, Neanderthals
- Both display significant rates of gene flow (0.3–2.6%) into MHs
- 3 - Altai Neanderthal, Denisova, Siberia
- 4 – Mezmaiskaya 1, Caucasus Neanderthals
- The Altai Neanderthals separated from the El Sidrón and Vindija Neanderthals at ~110 Ka.

4 Different N genetic lineages

- El Sidrón and Vindija Neanderthals are more closely related than the Altai Neanderthal is to the Neanderthals that interbred with MHs about 47-65 Ka.
- Modern humans share more alleles with *Vindija* and *Mezmaiskaya 1* than with the Altai Neanderthal.
- Neanderthal-derived DNA in all non-Africans is more closely related to the genome from the Mezmaiskaya skeleton in the Caucasus than to the Altai or to the Vindija genome
- This shows that the introgression event from Neanderthals into humans likely took place after the split of the lineage of the Altai Neanderthal from that of other Neanderthals, but before the split of the lineage of *Mezmaiskaya 1* and that of other Neanderthals

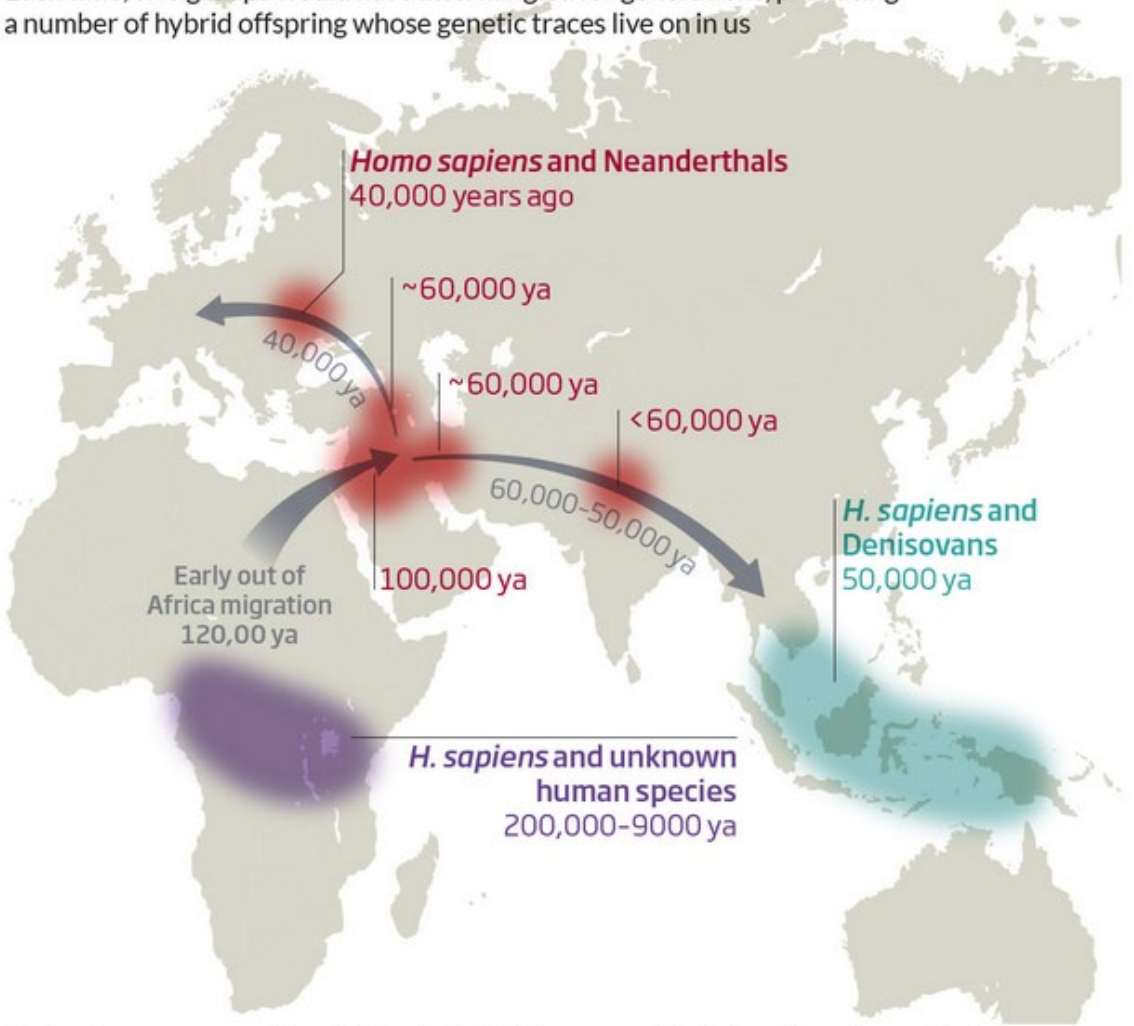
El Sidrón, Spain

- The Spanish site of El Sidrón is thought to be a accumulation of at least 12 Neanderthals including three female and three male adults, three adolescents, two juveniles and one infant.

- Complete and partial mtDNA sequences from all the available individuals suggest that Neanderthals there formed
  - small kinship-structured bands that
  - practiced patrilocal mating behavior (outside women join the group)
  - had relatively long inter-birth intervals (ca 3 years) when compared with modern human populations.

Homo promiscuous

Our ancestors mated with other extinct human species on several occasions. Each time, two groups would have intermingled for generations, producing a number of hybrid offspring whose genetic traces live on in us



Ancient genomes are also yielding insights into our earliest migrations. In particular, a Neanderthal toe bone found in Siberia shows that *Homo sapiens* and Neanderthals interbred 100,000 years ago – 40,000 years before *H. sapiens* was thought to have left Africa. Some early explorer must have ventured out before the main migration. Perhaps one or more small groups went exploring, and met their long-lost cousins in western Asia

Review of 15 known introgression events:

- mtDNA Gene flow from Neanderthals
- mtDNA Gene flow from Denisovans
- 1. The most referenced introgression: 2% N DNA into MH
- 2. N DNA into Ds and vise versa
- 3. Ghost population of Archaic Hominin (*H. erectus*?) into Denisovan DNA – 2-6% of D
- 4. Archaic Asian hominin DNA into MHs
- 5. MH DNA into Ns – 200 Ka: Ns who gave us 2%, already had 3% of MH DNA in their DNA
- 6. N mtDNA inherited from MHs – 270 Ka

Genetic evidence for 15 known introgression events

- 7. MH DNA into nuclear N genome in Middle East ~100 Ka
- 8. MH DNA into Altai Ns ~100 Ka
- 9. Altai N DNA into East Asian MHs ~100 Ka
- 10. N DNA into Ds
- 11. Oase-1: early MH HG with N DNA
- 12. D DNA into MHs
- 13. Archaic hominin into MHs in Africa
- 14. Archaic DNA in the San of South Africa
- 15. Ghost lineages in 4 West African Groups

How to discover a ghost species

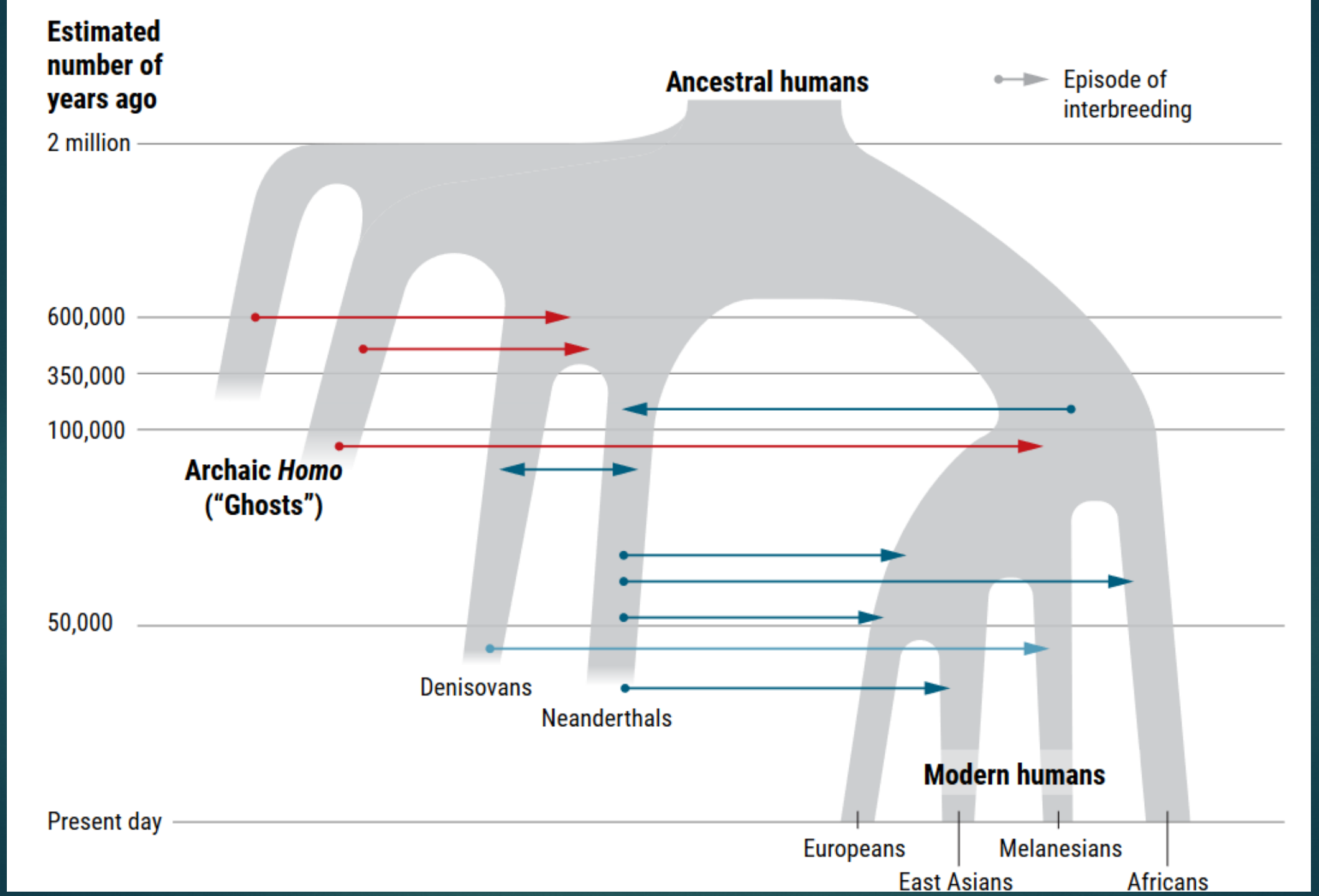
- Ghost Population: Extinct group that explains the current genetic variance in modern population
- Evidence for a previously unknown species of archaic hominin can be found in the genes of a modern-day population
- Historically, archeologists and genetic researchers have required the use of reference DNA sequence in order to compare and draw conclusions from fossilized aDNA.

Ghost populations

- Can now use statistics rather than a reference DNA sequence; computational biologists can identify a ghost population, formed from an extinct relative of humans, to which a modern population can trace a percentage of their genetic ancestry.

- Can identify basepair sections of DNA that are different from MH, N, and D genes, indicating they come from a different ancient relative.

- Can conclude that they come from a heretofore unknown, or ghost, population.


Estimated number of years ago
Ancestral humans
Episode of interbreeding
2 million
600,000
350,000
100,000
Archaic *Homo* ("Ghosts")
50,000
Denisovans
Neanderthals
Modern humans
Present day
Europeans
East Asians
Melanesians
Africans

1997: 1st Neandertal mtDNA

- 1997: mtDNA sequence from Neanderthal-1, the type specimen of *H. "neanderthalensis"*
- N mtDNA fell far outside all MH variation
- 379 base sequence compared to 994 human lineages
- 1997 Conclusion: No N DNA in MHs – no interbreeding
- We have since discovered that there is no N mtDNA in MH gene pool

2008 - mtDNA research: 38,000 Ka Croatian Neandertal

- 38 Ka Croatian Neandertal
- His complete mtDNA has been sequenced (16,000 bp)
- Compared to mtDNA from 53 living humans

- Neandertal mtDNA is not found in modern humans

- Estimate of divergence date between the two mtDNA lineages: 660,000 ± 140,000 ybp

1) **2010: The most referenced introgression: 2% N DNA into MH - N is separate species

- Green et al., 2010: N DNA into MHs – took 3.5 years to sequence
  - Positive evidence for admixture was first published in May 2010.
  - 1 to 4 percent N DNA [later refined to 1.5 to 2.1 %].
- Study concluded that ~20 % of Neanderthal DNA survives in current MHs [now revised to 40+%],

The most famous introgression: 2% N DNA into MH

- Green et al., 2010: First conclusive evidence that humans and Neanderthals mated came from analyzing a draft Croatian Neanderthal genome.
  - Dated to 40-60 Ka
  - N DNA into MH = 1.5-2.1%
  - More N DNA in East Asians than in Europeans
  - Neanderthal genes into out-of-Africa MHs, in Middle East
  - Multiple introgressions from Neanderthals into various modern human populations outside Africa resulting in about 2% Neanderthal DNA in current MHs

N were genetically different

CRS	A	A	T	T	C	C	C	C	G	A	C	T	G	C	A	A	C	T	T	C	A	C	G	C	A	C	-	C	A	T	C	C	G	T	G	G	C
Eve	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	T	C	.	T	G	.	.	.	.	.	-	T	.	C	.	.	.	.	.	.	.
Ne1	G	G	.	C	T	T	T	T	A	T	T	C	.	T	.	C	.	C	C	T	G	T	T	A	G	T	A	T	G	C	T	.	.	C	.	.	T
Ne2	G	G	.	.	.	.	.	.	A	T	T	C	.	T	C	C	.	C	C	T	G	T	A	A	.	T	A	T	G	C	T	.	.	C	.	.	?
Ne3	G	.	.	.	.	.	.	.	A	T	T	.	.	T	C	C	.	C	C	T	G	T	A	A	G	T	A	.	.	C	T	.	A	.	A	A	T
Ne4	G	G	.	.	.	.	.	.	A	T	T	C	.	T	C	C	.	C	C	T	G	T	A	A	G	T	A	T	G	C	T	.	.	C	.	?	?

CRS = Cambridge Reference Sequence for current *Homo sapiens*

Eve = Mitochondrial Eve

- DNA Sequence sample of MH, mEve, & 4 Ns:
  - Clearly, *Homo sapiens* and *Homo Neanderthals* are quite different,
  - whereas the 4 Neanderthals represent a pretty homogeneous group.
- The implication is that Ns are not the ancestors of modern humans.
- A divergence time for the two lines was estimated at 741 to 317 Ka.

2) N DNA into Denisovans and D DNA into Neandertals

- Denisova Cave:
  - Teeth dating to 80 Ka yield DNA from a Denisovan individual – had N DNA
  - Prior study confirmed the existence, via DNA, of a third group of ancient hominins, the Denisovans,
  - that coexisted with Neanderthals and human ancestors.

- There are also indications of early Neanderthal and Denisovan interbreeding

3) Archaic Hominin into Denisovan DNA and into some MHs

- 2011: Archaic hominin DNA constitutes 0.5-8.0% (later 3–6%) of the Denisova genome

- About 15% of these “super-archaic” regions—comprising at least about 4 Mb— were, in turn, introgressed into MHs and exist in modern New Guineans

Waddell et al. 2011.

Denisovan + Ghost species

- Denisovans and a 'ghost' population of hominins:
- The mystery species could be an Asian offshoot of *Homo erectus*, *H. heidelbergensis, Homo floresiensis*
- Likely *H. erectus* given estimated dating of 0.9-1.4 Ma for this archaic introgression

Ewen Callaway

4) Archaic Asian hominin DNA into MHs

- 2004 study led by Daniel Garrigan: DNA sequences from a nonfunctional region of the X chromosome known as RRM2P4.

- Analyses of its reconstructed tree pointed to an origin for the sequence, not in Africa but in East Asia around 1.5 million years ago (*H. erectus*?), implying that the DNA came from an archaic Asian species that intermixed with *H. sapiens*; occurs at frequencies up to 53% in south China; found at less than 1% in African populations

- This ancient lineage is a remnant of introgressive hybridization between expanding anatomically MHs emerging from Africa and archaic populations in Eurasia.

5) MH DNA into Ns – 200 Ka

- 2017, Adam Siepel lab: early interbreeding between MHs and Ns occurred between 300 and 200 KA

- Adam Siepel: around 3% of Neanderthal DNA — and possibly as much as 6% — came from MHs who mated with the Neanderthals more than 200,000 years ago. No convincing evidence that negative selection acted against these regions.

- About 15% of these "super-archaic" regions were, in turn, introgressed into MHs and continue to exist in the genomes of people alive today.

- So Ns who gave us 2%, already had 3% of MH DNA in their DNA

Melissa J. Hubisz, et al., 2017, 2020

3-6% MH DNA into Ns ~200 Ka

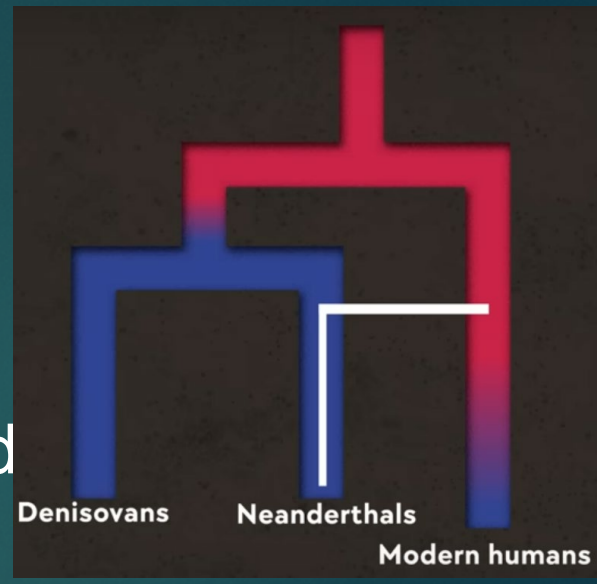
- Study sample: 2 Africans, 2 Neanderthal, 1 Denisovan, and 1 chimpanzee outgroup; a new statistical method, called ARGweaver-D
- Identified 3% of the Neanderthal genome that is introgressed from ancient MHs, and estimate that the gene flow occurred between 200-300kya.
- 3% of MH DNA in both the Altai and Vindija Neanderthal. This number is almost certainly an underestimate, By contrast, only 0.37% of regions are classified as MH into Denisovan.
- Neanderthal genome was likely more influenced by introgression from ancient humans, than non-African MH genomes are by Neanderthal introgression.

6) Some Ns inherited their mtDNA from MHs

- Svante Pääbo in 2016: the "Neandertal" mtDNA actually came from modern humans.
- A Neandertal femur found in 1937 in Hohlenstein-Stadel cave (HST) cave in Germany had inherited modern human mtDNA
- An early MH female mated with a Neandertal male more than 270,000 years ago.
- In time her African mtDNA completely replaced the ancestral Neandertal mtDNA.

Cosimo Posth, et al., 2017

Deeply divergent archaic mitochondrial genome provides lower time boundary for African gene flow into Neanderthals



- Explanation of MH mtDNA in later Ns: very early gene flow from MHs into Ns, from 470 to 220 Ka; then these early MHs died out
- A N femur discovered in Germany had its mtDNA genotyped and it was found that there was introgression from a non-Neanderthal African hominin, either *Homo sapiens* or closely related to us, around 270,000 years ago
- Replacement process: 1 female MH interbreed with 1 N male; she had a hybrid N-MH child who inherited her mtDNA; her female N descendants spread the MH mtDNA to all later Ns, replacing the original D-like mtDNA

Cosimo Posth, et al., 2017

New DNA discoveries in 2017

- So African humans interbred with Neanderthals in the Hohlenstein-Stadel cave (HST) in Germany more than 270,000 years ago.
- This mtDNA from the Hohlenstein-Stadel sample was highly divergent from those of other coexisting Neanderthal groups.
- Late Pleistocene Neandertal mtDNA may have been replaced by more fit African MH mtDNA

Cosimo Posth,, et al., 2017

Femur bone from HST: 124 Ka

MtDNA: Since the bone is 124,000 years old, implies that that Homo sapiens and Neanderthals met and interbred sometime between 470,000 and 220,000 years ago.

A complex history of MH, N, D, + interbreeding: MH DNA into Ns

- Estimated the divergence time between HST N and all other Neanderthals to ~ 270 ka (316–219 ka),

- The MRCA for the Altai N branch was inferred to be ~160 ka (199–125 ka).

- Dating: The three oldest N mtDNAs:
  - Sima, age of 430 Ka; Denisovan like
  - HST with an age of 124 ka (183–62 ka) and
  - Altai Neanderthal with an age of 130 ka (172–88 ka).

HST: N mtDNA

- An early African female mated with a Neandertal male more than 220 Ka.
- All known Neanderthals inherited their mitochondrial DNA from an ancestor who lived before 220 Ka.
- In time her African MH mtDNA completely replaced the ancestral Neandertal mtDNA.
- The evidence suggests that Ns eventually discarded their original mitochondrial DNA for mDNA inherited from this MH mitochondrial Eve.

Hohlenstein-Stadel Neandertal mtDNA

- HST Neandertal's mtDNA was significantly different even from that of proto-Neandertals that date to 430 Ka at Sima de los Huesos in Spain, suggesting that their mtDNA had been completely replaced.

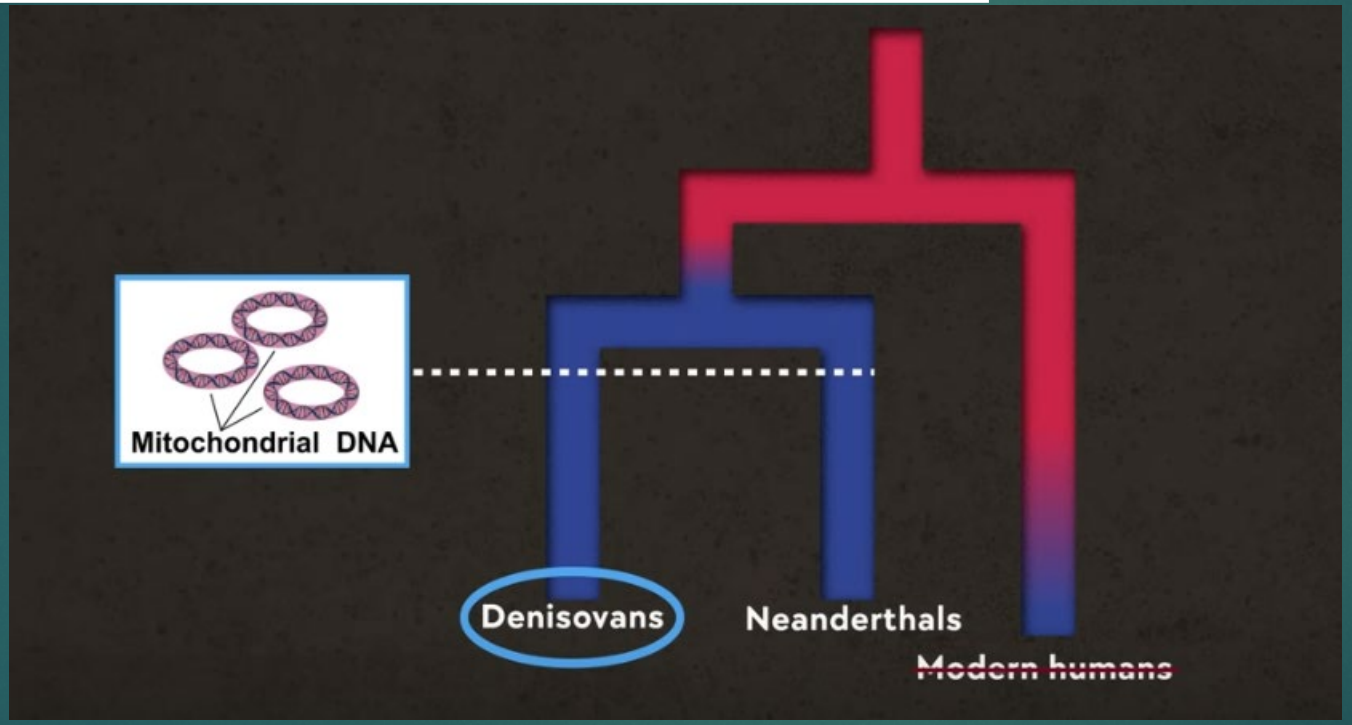
- HST mtDNA split from that of all other Neandertals by at least 220 Ka.

- That's early enough for the new form of mtDNA to have spread among Neandertals and replaced all their mtDNA.

- Conclusion: The mtDNA of these HST Neandertals is not actually from Neandertals, but from an early African human.

Same thing happened with Mitochondrial DNA from MHs to Ns

- Sima de los Huesos Neandertals at 430 Ka: N mtDNA is similar to Denisovan mtDNA



- But in later Ns, N mtDNA is more similar to MH DNA

HST: 1 MH female had a child of male N

- This is evidence that some early members of our own species moved from North Africa into Europe.
-
- Supporting this idea was the discovery of fossils of Homo sapiens in Morocco dating at 315 Ka.
- Somewhere in prehistory, at least one female human from Africa must have carried the child of a male Neanderthal.
- Molecularly dated the split of the HST lineage from other Neanderthal mtDNAs to ~270 ka; to replace the pre-existing Denisovan-like mtDNA (a la Sima de los Huesos N).

But HST mtDNA was eventually replaced by Altai N version

- HST mtDNA was eventually replaced by an Altai N version
- Proposed that the Neanderthal population in western Europe underwent a demographic turnover followed by a subsequent recolonization.
- Under that scenario, the HST lineage would have been largely replaced towards the end of the Neanderthal temporal range by mtDNA descendants on the Altai branch.
- The African introgression hypothesis suggests that Late Pleistocene Neanderthal mtDNAs originated through gene flow from an African source, which took place more than ~270 ka. This N population was a dead end.

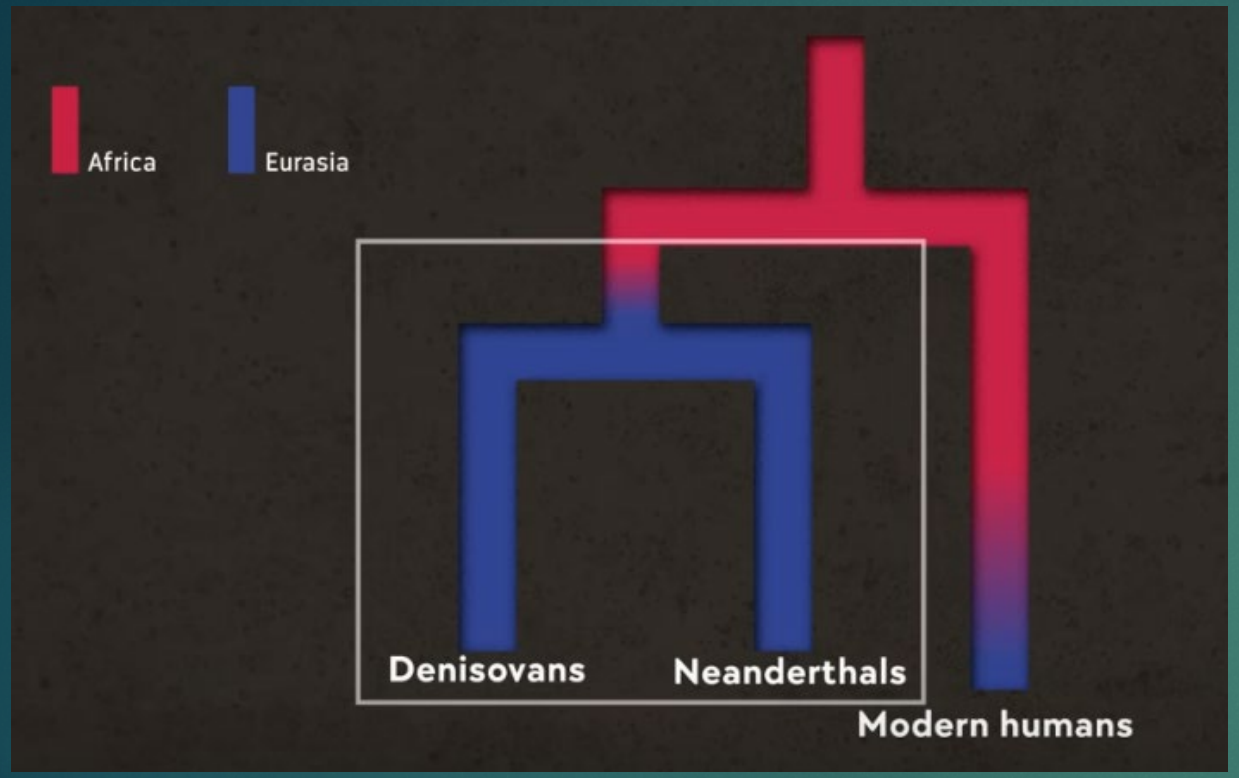
7) MH DNA into nuclear N genome in Middle East ~100 Ka

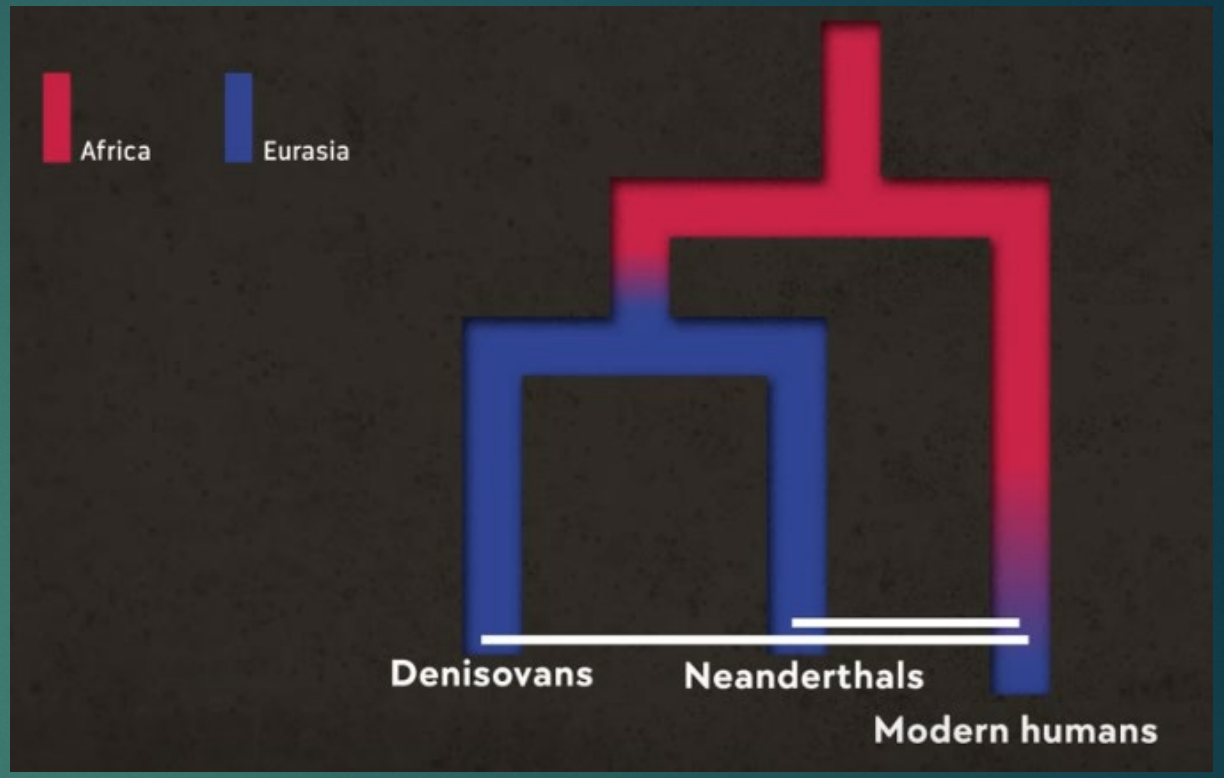
- ~100 Ka: An ancient population of *Homo sapiens* migrated from Africa into Asia. In the Near East they met a population of Neandertals, probably around the Persian Gulf, the Arabian Peninsula or the eastern end of the Mediterranean Sea in Western Asia.

- Then an introgression of MH into N = MH DNA in the genome of a female Neandertal from the Denisova Cave in the Altai Mountains, Siberia; but not in western Ns.

Kahlil et al. (2016).

History of interbreeding





After separation from LCA,
Neanderthal DNA was almost identical to Denisovan DNA because they were each other's closest evolutionary relatives

After 60 Ka, MHs interbreed with Ns and Ds

Divergences & Gene Flow:

- Analysis of Neandertal genome from a cave in the Altai Mountains in Siberia suggests that MH and N diverged 550 to 765 kya.

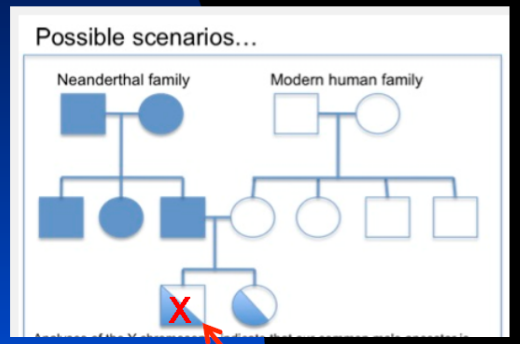
- Denisovan genome gives different date: Neanderthals and Denisovans diverged 381-473 kya.

Martin Kuhlwilm, et al., Nature, 2016

Male MH-Neandertal hybrid male sterility

These results suggest that part of the explanation for reduced Neanderthal ancestry near certain genes is that Neanderthal alleles caused decreased fertility in males when moved to a modern human genetic background.

This suggests that when ancient humans met and mixed with Neanderthals, the two species were at the edge of biological incompatibility



Sterility of male offspring – Haldane's rule operating?

Neandertal versions of genes in the testes, including some needed for sperm function, were also less active than human varieties.

Suggests male human-Neandertal hybrids may have been infertile.

Natural selection in the larger human population started purging those mutations

R.C. McCoy et al. Impacts of Neanderthal-introgressed sequences on the landscape of human gene expression. *Cell*. Vol. 168, February 23, 2017,

8) Altai N into East Asian MHs at 100 Ka

- Altai N DNA into East Asian MHs

- Castellano: humans and the ancestors of the Altai Neanderthals interbred about 100,000 years ago — long before people were thought to have left Africa

9) MH DNA into Altai Ns ~100 Ka

- El Sidrón and Vindija Neanderthals display significant rates of gene flow (0.3–2.6%) into MHs
- El Sidrón and Vindija Neanderthals are more closely related than the Altai Neanderthal is to the Neanderthals that interbred with MHs about 47-65 Ka.
- MH gene flow into Neanderthals mainly took place after the separation of the Altai Neanderthals from the El Sidrón and Vindija Neanderthals that occurred at ~110 Ka.

10) N DNA into Denisovans

- At Denisova: at least 0.5% N genome in D DNA coming from a Neanderthal population more closely related to the Altai Neanderthals

- Denny: The 90 Ka remains of a Denisovan-Neanderthal hybrid in Denisova Cave laid bare the fact that the two groups interbred:
  - Denny = a N mother and D father hybrid

Altai N into D

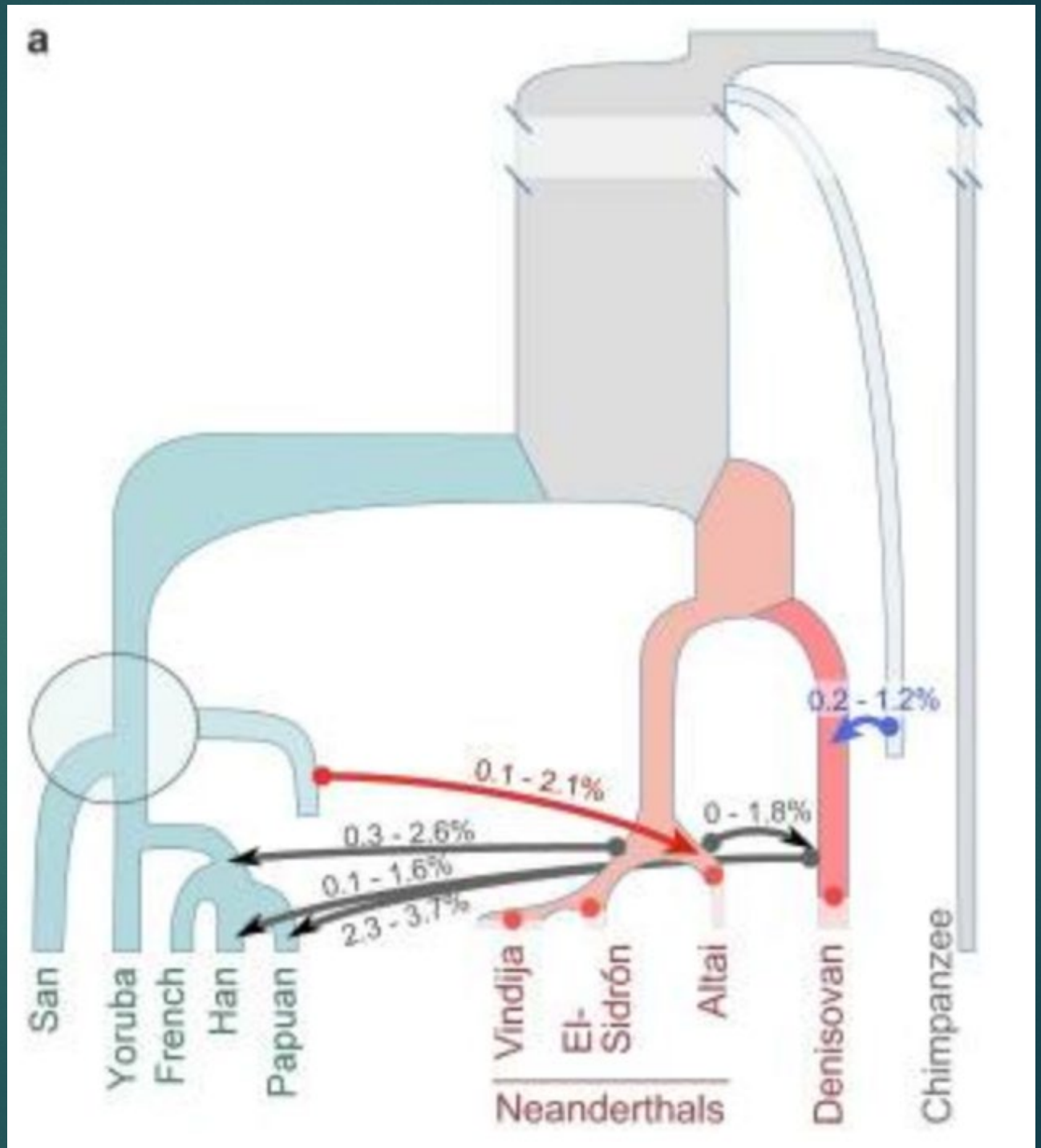
D into Altai N

MH into Altai N

N into MHs

Ns into Han

N into Papuan



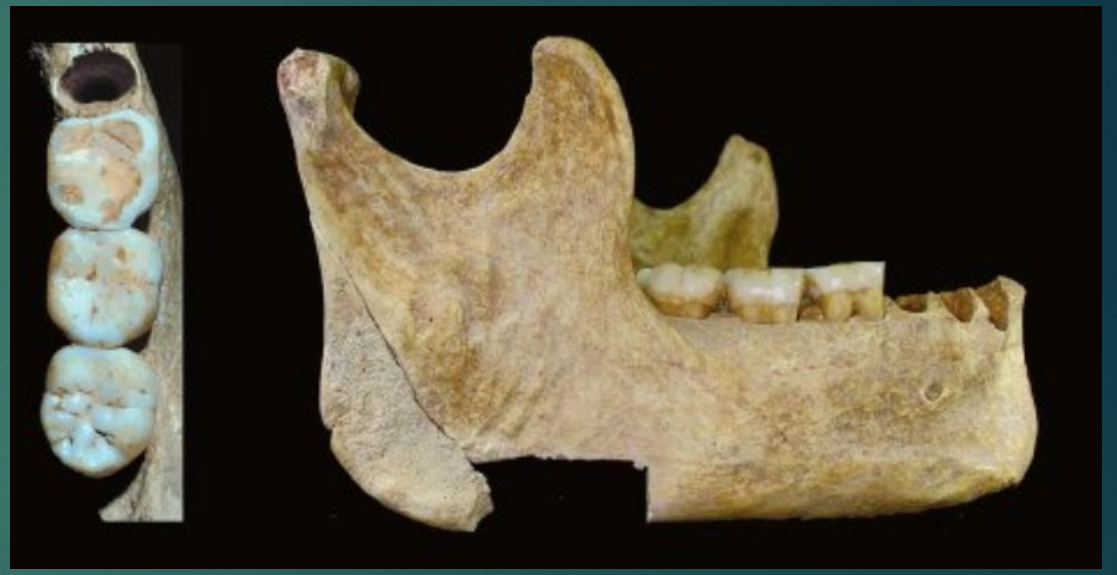
11) Oase-1: early MH HG with N DNA

- 45,000 ya -- evidence of another hybridization in East Europe = The Oase-1 mandible from Romania is one of the oldest European *Homo sapiens* specimens.
- Its MH DNA has an amazing 6 to 9% of Neandertal genome.
- This means he had a full Neandertal ancestor only 4 to 6 generations before him.

Oase 1 & 2 – MH-N hybrid at 45 Ka, with sloping forehead = N

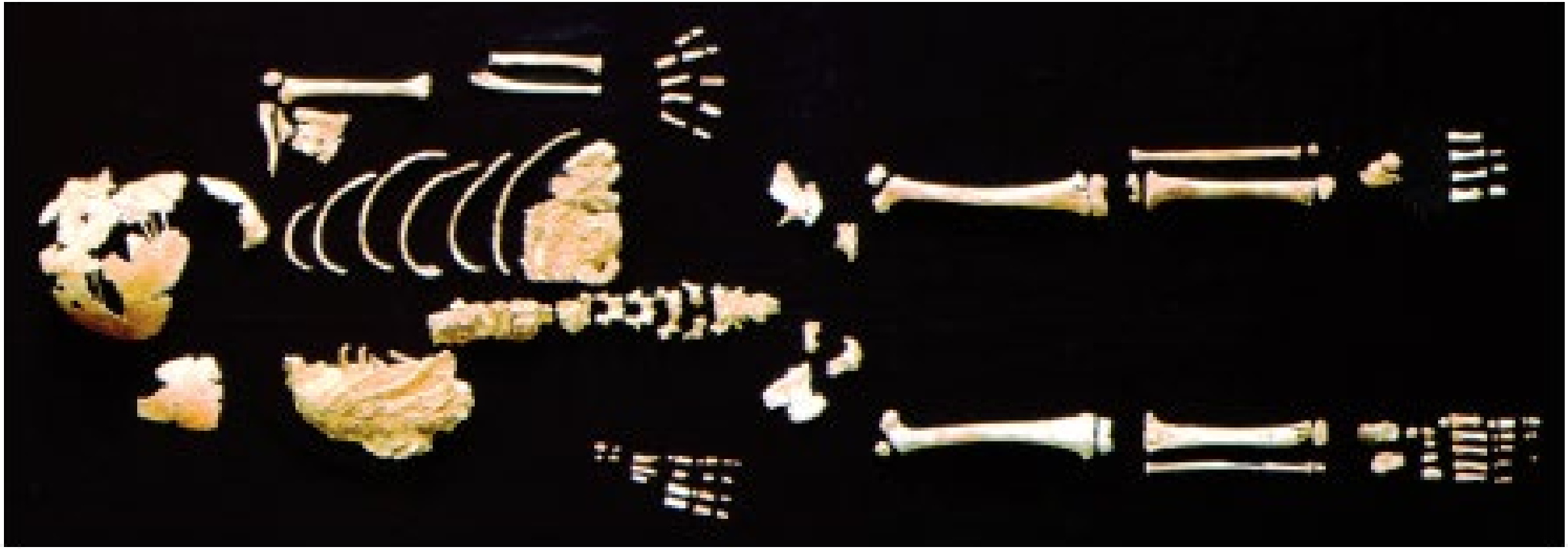
6-9% of the genome is Neanderthal in origin; full N ancestor four to six generations earlier.

Oase 2 – has a chin



But Molars: Smallest to largest molar is not MH
Around 6% of "Oase 2"'s genome is Neanderthal in origin; but 12th chromosome was 50% N

1998: Lagar Velho child skeleton, Portugal: Trinkaus = MH-N hybrid



12) D DNA into MHs

- Denisova introgression resulting in about 2–6% Denisovan DNA in Melanesia ~ 45 Ka (less in South Asia, ~0.2%)

- Denisovans once lived all across Asia, giving them ample opportunity to interbreed with MHs there. There were at least 2 or 3 D lineages (N & S)

- Flow of Southern Denisovan DNA into Papuans: People from Papua New Guinea and elsewhere in Oceania carry fragments of Denisovan DNA, as do East Asians

(Skoglund and Jackobsson 2011),

13) Archaic hominin into MHs in Africa

- Sub-Saharan archaic hominin into African MHs (MUC7 in saliva)
- A salivary antibacterial mucin (divergent *MUC7* haplotype) likely originated in an unknown African hominin population and introgressed into ancestors of modern Africans.
- A ghost population in current Africans

Duo Xu, et al., 2017

14) Archaic DNA in the San of South Africa

- Gene flow from archaics into African MHs, the San, ~2%
- The greatest genetic diversity today is among the Khoe-San peoples of southern Africa.
- The deepest split between human populations that still exists, points to the San as the stem population of all living people. The San are descendants of the earliest diversification event in the history of all humans. Stone-age hunter-gatherers from South Africa diverged from other modern-day populations >260,000 years
- It reflects a deep history of diversity among African populations

(Hammer et al. 2011).

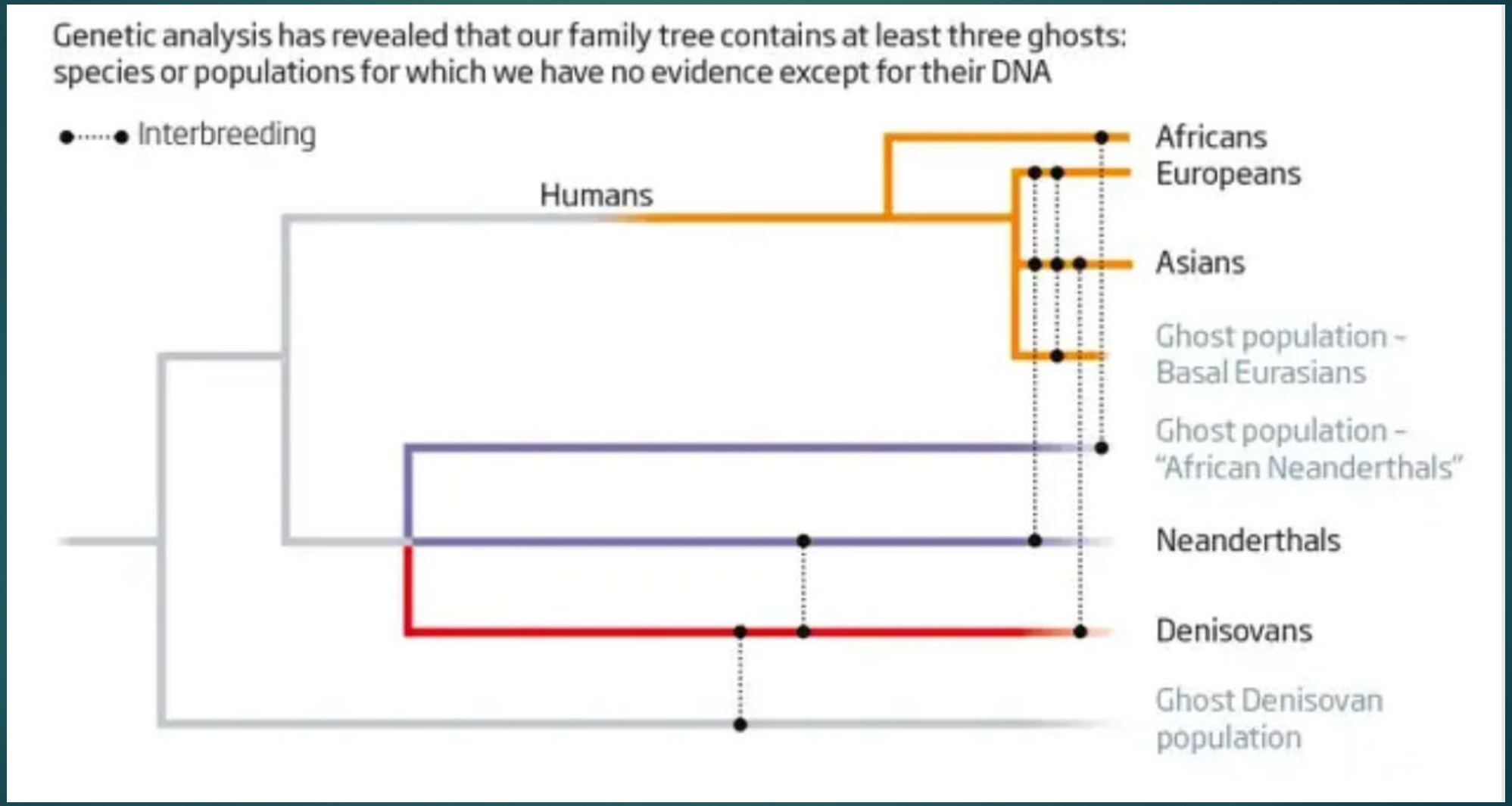
15) Ghost lineage in 4 West African Groups

- Four West African groups — Yoruba in southwestern Nigeria, Esan in southern Nigeria, Gambians in western Gambia, and Mende in Sierra Leone

- Derive 2% to 19% of their DNA from an archaic ghost lineage. Ghost lineage diverged from the ancestors of Neanderthals and modern humans up to 1.02 Ma and interbred with the ancestors of modern West Africans from 124 Ka up to the present day.

- A number of previous studies have found evidence for deeply diverged lineages contributing genetic ancestry to the Pygmy (and Yoruba populations).

Mystery ancestors in *H. sapiens*



Ghost species

- The idea that our ancestors hybridized with other hominins was once dismissed. Now it was starting to look as though they would mate with anything vaguely human.
  - Hominin species living recently with us in Africa
  - Neanderthals, Denisovans
  - Ancient Near Easterners: ~12,000 and 1,400 bc, from Natufian hunter–gatherers to Bronze Age farmers.
  - The earliest populations of the Near East derived around half their ancestry from a 'Basal Eurasian' lineage that had little if any Neanderthal admixture and that separated from other non-African lineages before their separation from each other. The most-likely explanation is that soon after MH migration circa 60 Ka, a group of humans became isolated while the rest bumped into and mated with Ns

Ghost species via statistics rather than DNA from bones

- It is possible to spot signs of extinct populations in the DNA of modern humans, simply by using advanced statistics.
- No ancient African hominin has had its genome sequenced,
- By looking at mutation patterns in modern populations, it is possible to spot segments that don't match the usual *H. sapiens* pattern. These are presumed to come from populations that evolved separately from our own species for thousands of years before mating with humans.
- Statistical modelling can then produce estimates of when the two groups mated and how different the other population was from our ancestors.

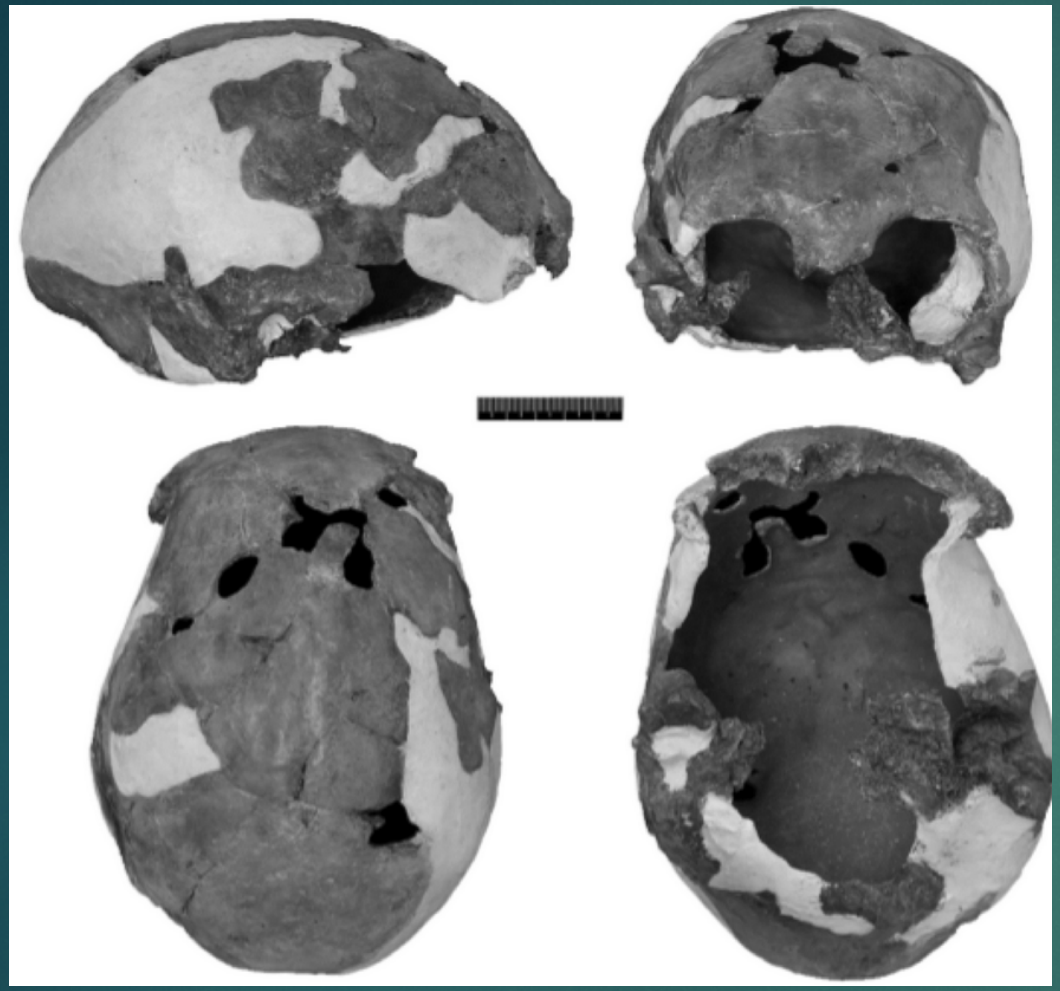
Ghost species via statistics rather than DNA from bones

- Sort of like an "African Neanderthal": Baka hunter-gatherers from Cameroon, and the Hadza and Sandawe from Tanzania.

- Within these genomes, they have found stretches of DNA that appear to come from another hominin species, that lived alongside MHs in Africa.

- Because this DNA is found only in the descendants of African people – not in any Eurasians – the ghost species must have interbred with *H. sapiens* after the out-of-Africa migration 60,000 years ago.

- In fact, by the team's calculations, this probably happened within the past 30,000 years.



- Iwo Eleru, Nigeria, skull: outside the range of modern human variability; dated to ~11.7–16.3 ka
- low and elongated cranial shape
- received trait from archaic introgression?

African ghost lineages

- Chris Stringer: “My bet is that *Homo heidelbergensis* is the introgressor”. Broken Hill skull now at 300 Ka

- Alternatively, the ghosts may have been a subpopulation of *H. sapiens* that, like the Basal Eurasians, was isolated from other populations for long enough that its members’ DNA acquired different markers.

- African multiregionalism: It could be the case that 100,000 years ago or more, there are different populations of anatomically modern humans in different parts of Africa. And maybe at some point they mix with each other, while some populations died out.

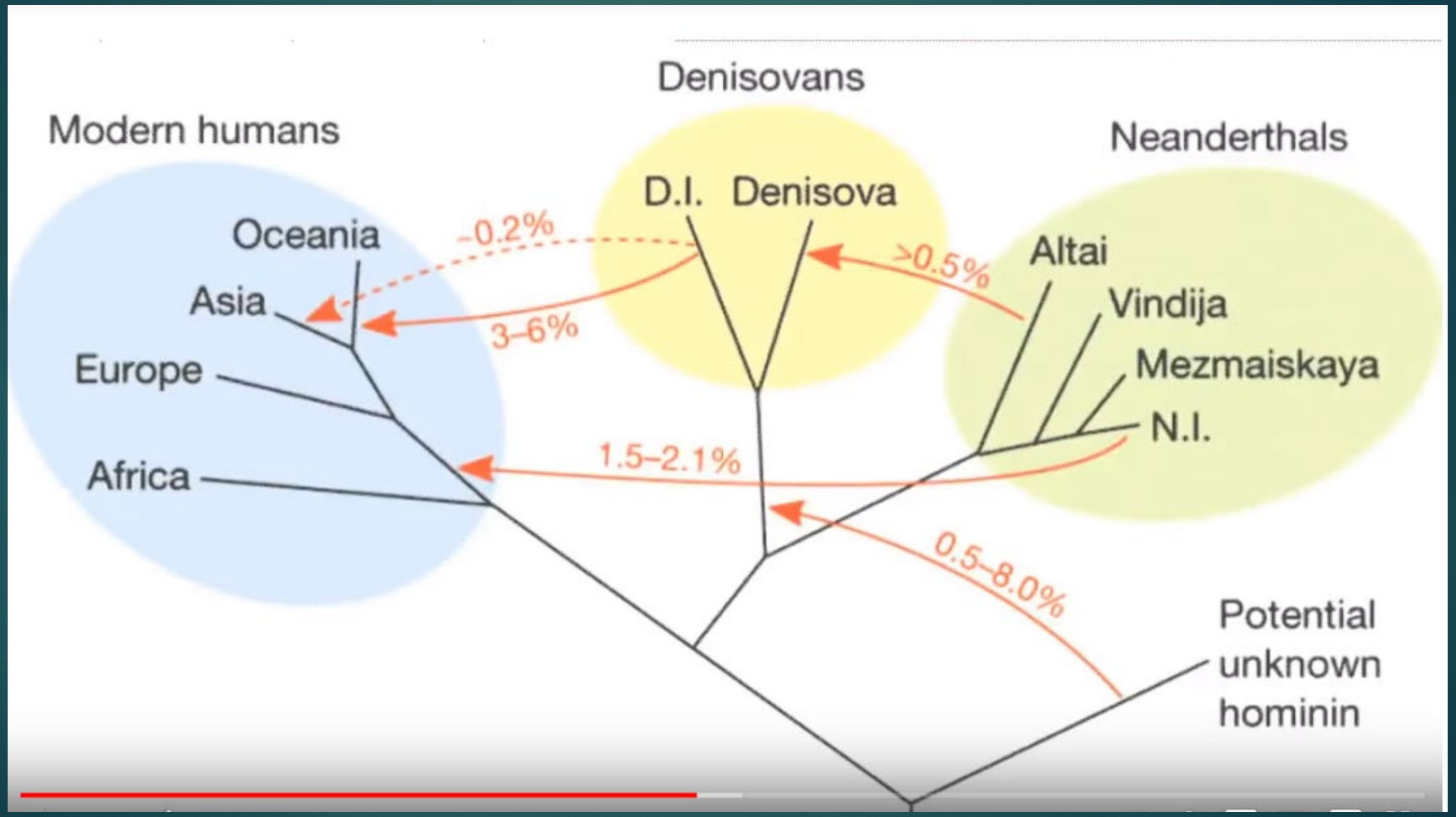
How did the KhoeSan (and Africans) get N DNA

Not so isolated: Khoisan tribes have European DNA
Ariadne Van Zandbergen/Alamy

N DNA in KhoeSan & Yorba

- The KhoeSan tribes of southern Africa are hunter-gatherers and pastoralists who speak unique click languages; and have oldest DNA on planet.
- A subset of the KhoeSan, known as the Khoe-Kwadi speakers, arrived in southern Africa from east Africa around 2200 years ago.
- The proportion of Eurasian DNA was highest in Khoe-Kwadi tribes, who have up to 14 per cent of western Eurasian ancestry, including N DNA. About .03% N DNA
- East African tribes from which the Khoe-Kwadi descended, had up to 50 per cent Eurasian DNA.
- Neanderthal traces have also been found in the Yoruba

We now know who is related to who; and that hominins hooked up with everyone they met



N functional genetic effects in MHs today
via N genetic introgression into MHs

N DNA in MHs

- Neanderthal-derived DNA has been found in the genomes of most or possibly all contemporary populations, varying noticeably by region.

- Neandertal DNA:
  - 1.8–2.6% of modern genomes for people outside Sub-Saharan Africa,
  - up to 0.3% for those in Africa.
  - Above are averages for whole MH genome; some specific genomic areas are 62% N DNA

Functional effects of Neanderthal DNA in MHs

- Neandertal DNA in MHs has had both negative and positive effects (adaptive introgression), with some resultant modern phenotypical results

- Most Neandertal DNA in Modern Humans was removed via natural selection (removal of alleles that are harmful)

- There are also Neandertal “deserts” in Modern Human DNA, esp. in X chromosome & testes

Functional effects of Neanderthal DNA in MHs

- No Neandertal mtDNA or Y-DNA exists in current Modern Humans
- But there has been retention of some beneficial Neandertal & Denisovan DNA
- Important Note: There is N DNA in current MHS, i.e. some N DNA is associated with smoking behavior in current MHs.
- But the original function that this still existing N DNA played in Ns when they were alive is unknown

Quick summary of known functional effects of N DNA:

- Most N variants are associated with diseases (Type 2 Diabetes, Lupus, Crohn's, Obesity, Depression risk, Covid)
- But there are also beneficial functional effects of N immunity receptors
- Hypersensitive N immunity that produced protection from *H. pylori*, also produces more allergies in us
- Highest genetic risk factor for Covid 19 is N, but also protection vs HIV.
- N protection against RNA viruses (West Nile, Hep C, Covid 19 viruses),

Did Neandertals survive?

Russian boxer
Nicholai Valuev

7 feet tall; 300 lbs
15 years as boxer

A Russian politician;
Drafted in 2022 into
Russian army

More Eastern N DNA? Higham says no

- Why would eastern Asians inherit slightly more Neanderthal DNA? In 2020 the answer to this conundrum came from new research on modern human DNA in Africa, which takes account of the fact that there is a small amount of Neanderthal DNA in many living Africans derived from the movement of Western MHs.

- When this DNA is taken into account and subtracted from the analysis, the proportion of Neanderthal DNA in the east and west of Eurasia is much more similar.

Hunter-gatherer admixture facilitated natural selection in Neolithic European farmers

- Genome-wide DNA from 677 individuals spanning Mesolithic and Neolithic Europe.
- The region around the pigmentation-associated gene SLC24A5 shows the greatest overrepresentation of Neolithic local ancestry in the genome.
- In contrast, we find the greatest overrepresentation of Mesolithic ancestry across the major histocompatibility complex, an immunity region.
- Study extends previous results that highlight immune function and pigmentation as targets of adaptation in more recent populations to selection processes in the Stone Age.

Tom Davy, et al., 2023

Quick summary of known functional effects of N DNA in current MHs:

- Pigmentation (pale skin that burned, lighter skin, UV protection)
- Longer skull morphology
- More sensitive pain perception
- Reduced pharmacological response to certain medications
- Denisovan example: EPAS1 - anti-hypoxia – high altitude adaptation gene in both Ds and modern Tibetans

What parts of modern genome are similar to N's?

- ABO blood group – ABO gene with 3 variants
  - N had A and O alleles (no B yet)– shared these with MH from LCA

- FoxP2 present in both N and MH - from LCA

- HAR1 (Human Accelerated Region – difference from chimps) - not present in primates; Denisovans have 2 differences not present in MH; 1 difference from N; HAR1 evolved during differentiation of these 3 groups; mostly regulatory regions, related to brain development genes

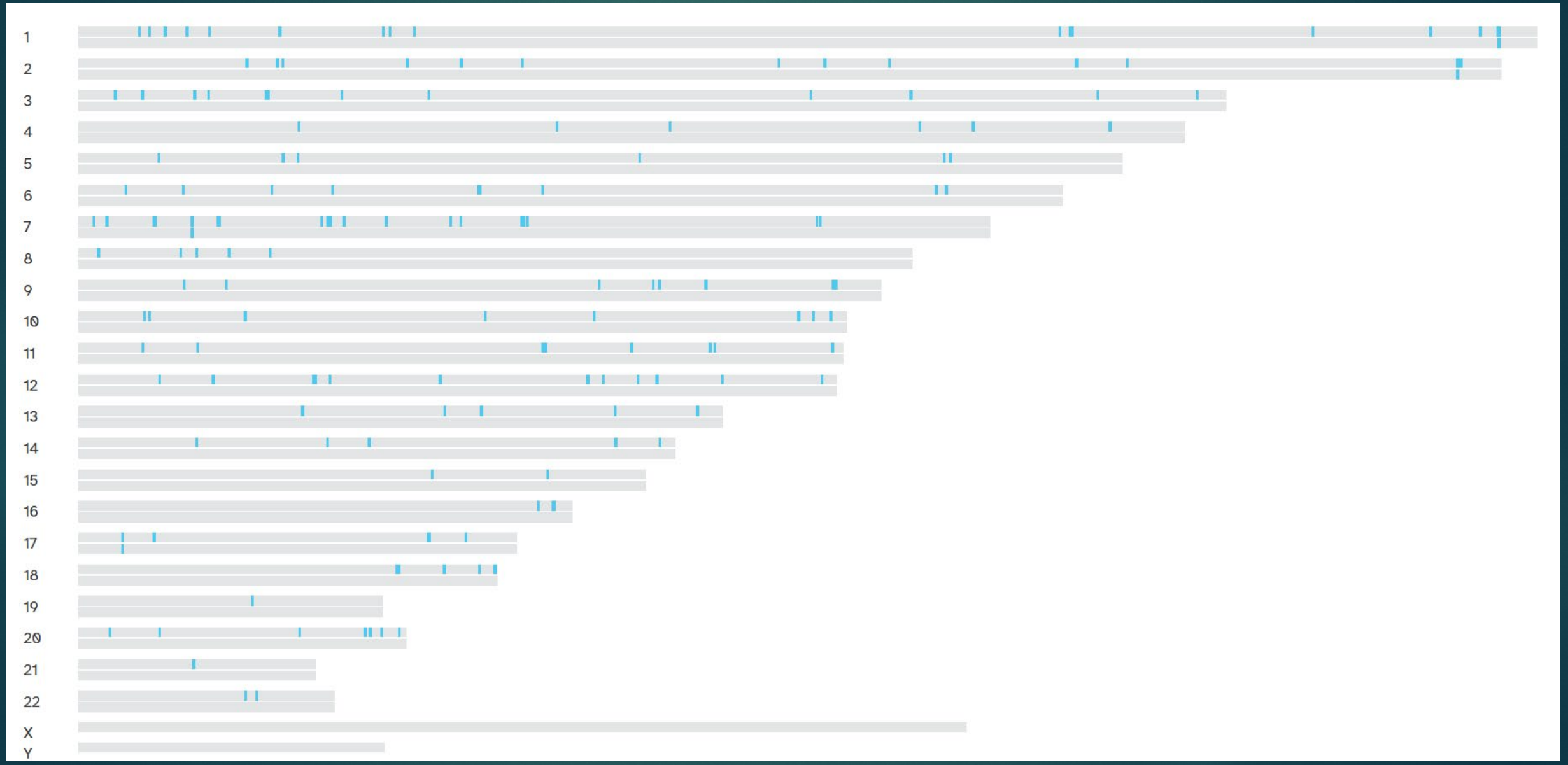
N & D DNA in MHs

- Low traces of Denisovan-derived ancestry have been found in mainland Asia, with an elevated Denisovan ancestry in South Asian populations.
- Mainland Asian and Native American populations have a 0.2% Denisovan contribution
- Melanesians (New Guinea, Fiji, etc.) have up to 6% of Denisovan DNA

23andMe: I am not very Neandertal

- **Charles's Neanderthal variants =** 223.
- This is less than 94% of 23andMe customers.
- My mother = 268; My brother = 240

Charlie’s 223 N variants in his chromosomes



Charlie's N variants

- rs62405860: C: having difficulty discarding rarely-used stuff x 2
- rs4849721 :T: Less back hair
- rs11213819: T: Less likely to sneeze after eating dark chocolate x 2
- rs12458349: G: Slightly less straight hair
- rs7544462: C: MEAF6 : Height: Not 1 inch shorter Height
- rs1877547: A: LPP: Height: Not 1 inch taller Height
- rs74606019: C: more mosquito bite itching
- rs12912713: C: better sprinter vs. distance runner
- rs4849721: T: sweat more during a workout x 2

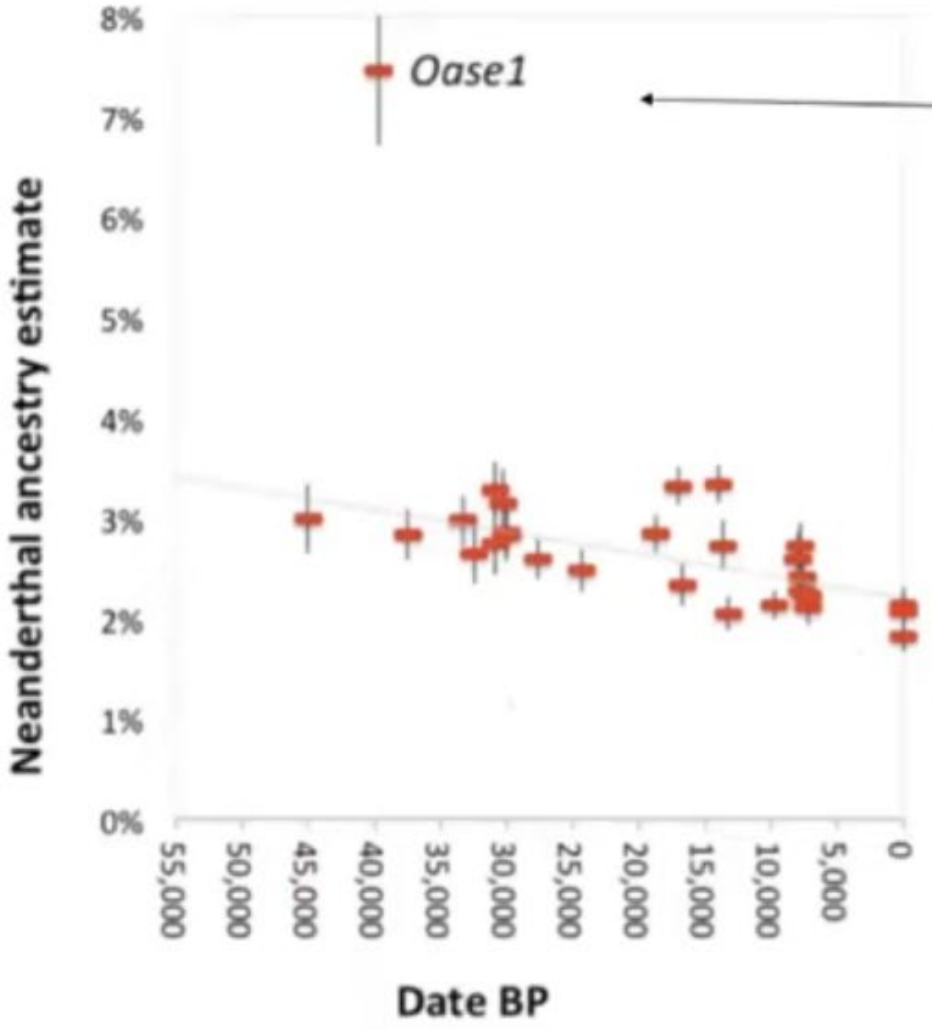
N DNA: not in coding genes, but in regulatory regions

- Study asked whether Neanderthal sequences make any contribution to gene expression variability.
- The answer was a resounding yes.
- Rotival et al.: found a strong depletion of Neanderthal variants in coding portions of genes, and a slight enrichment of the archaic sequences in regulatory regions

Negative selection of N DNA has been effective

- We now have only 1-2% N DNA, not 10% present in 45 Ka. Negative selection vs. N DNA occurred in MHs
- N DNA is not located in areas that are now functionally important for survival in MHs
- Neandertal variants don't usually fall within genes, instead regulate DNA by influencing where, when and how strongly genes are activated.

Most Neanderthal DNA in Humans Experienced Negative Selection



Outlier, that is not a direct ancestor of living humans

Fraction of Neanderthal DNA in relevant human genome samples has *decreased* over time

Fu et al. 2016

Fast N allele depletion in MHs

- Initially it seemed that selection over time reduced our Neanderthal ancestry from higher levels close to the date of introgression down to the 1.1–2.5 per cent we have today.

- Recent work has suggested that this reduction in fact happened quite rapidly, within ten to twenty generations of interbreeding.

- Following this, the proportion of Neanderthal DNA in non-African populations has stayed at a very similar level. M. Petr, et al 2019

Negative selection happened relatively quickly

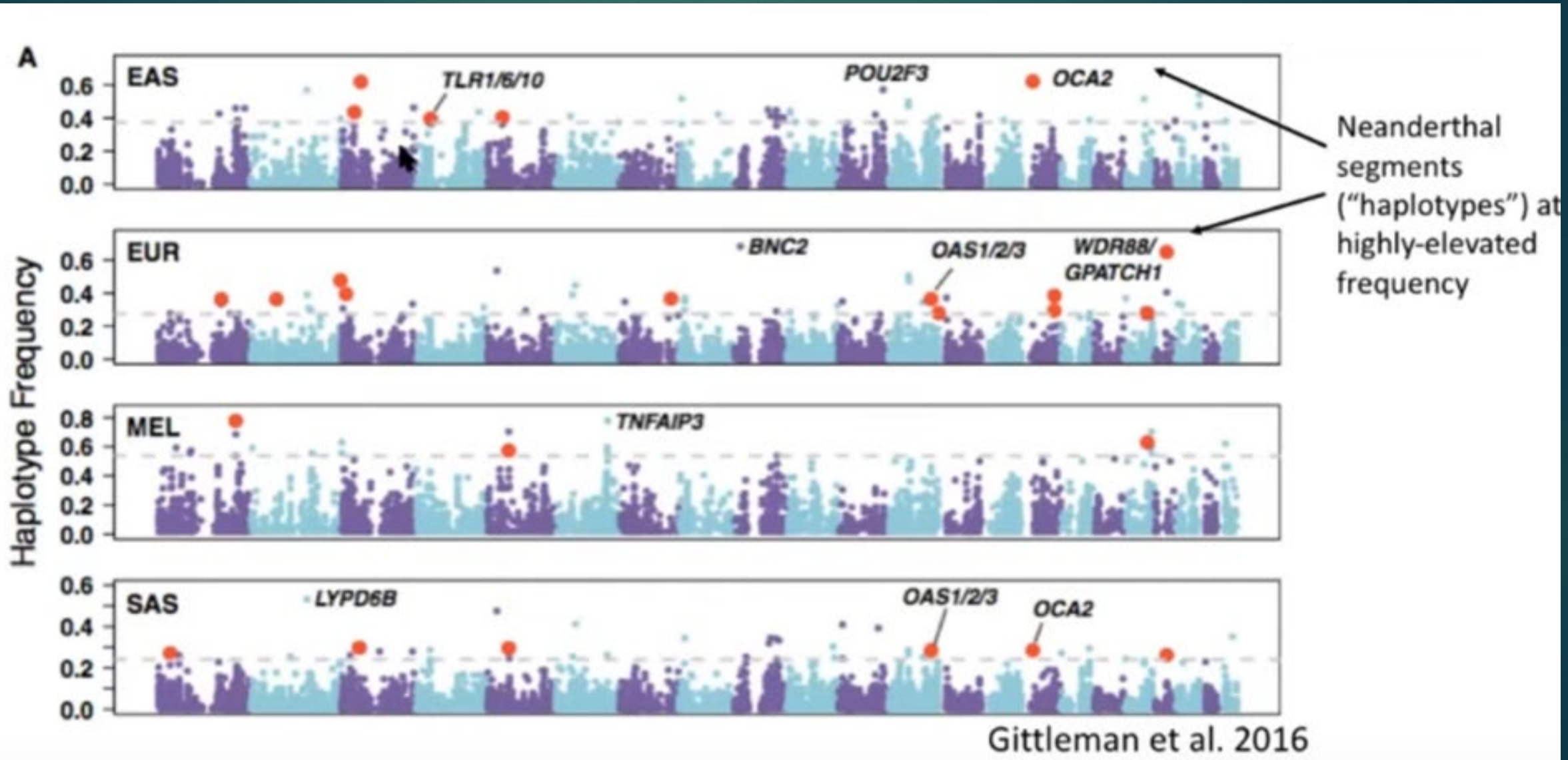
- Petr, M. et al., 2019: Reevaluation of Neandertal ancestry in modern human genomes indicates that overall levels of Neandertal ancestry in Europe have not significantly decreased over the past 45,000 years, and that previous observations of continuous Neandertal ancestry decline were likely an artifact of unaccounted-for gene flow increasing allele sharing between West Eurasian and African populations.
- Found evidence of:
  - selection against Neandertal DNA in the genome-wide distribution of Neandertal ancestry
  - N ancestry depleted in promoter and other noncoding conserved DNA more strongly than in protein-coding sequence,
  - raising the possibility that Neandertals may have differed more from modern humans in their regulatory variants than in their protein-coding sequences, and that regulatory variation may provide a richer template for selection to act upon.

Negative selection during 1st few hundred generations

- Furthermore, simulations suggest that negative selection against introgression is expected to have the strongest impact on genome-wide Neandertal ancestry during the first few hundred generations,

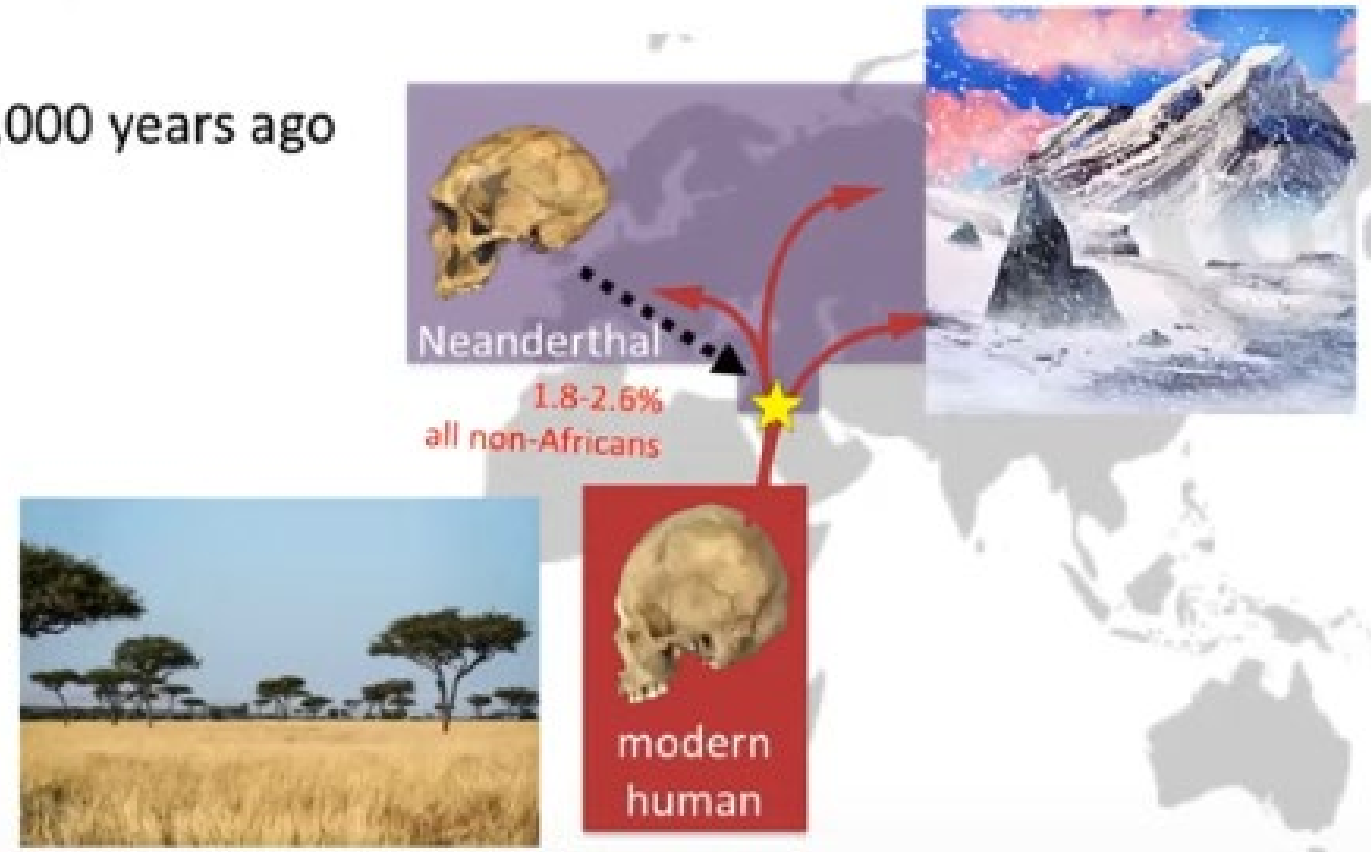
- Therefore, any long-term decline in overall N ancestry proportions in MHs over time are likely to be the result of forces other than negative selection, for example gene flow admixture with one or more other populations.

Some Introgressed Neanderthal DNA Shows Evidence of Positive Selection



Modern Humans May Have Acquired Eurasian Adaptations from Interbreeding with Archaic Hominins

~55,000 years ago



Movement of people

Gene Flow or "introgression"

- Colder Climate
- Decreased Daylight
- Novel Food Sources
- Novel Immune Stimuli

Modified from Dr. Bridget Alex

Repeated Evidence of Neanderthal Adaptive Contribution to Modern Human Immune System

Genomic Signatures of Selective Pressures and Introgression from Archaic Hominins at Human Innate Immunity Genes — AJHG, 2016

Matthieu Deschamps, Guillaume Laval, Maud Fagny, Yuval Itan, Laurent Abel, Jean-Laurent Casanova, Etienne Patin, and Lluis Quintana-Murci

Genetic Adaptation and Neandertal Admixture Shaped the Immune System of Human Populations — Cell, 2016

Hélène Quach, Maxime Rotival, Julien Pothlichet, Yong-Hwee Eddie Loh, Michael Dannemann, Nora Zidane, Guillaume Laval, Etienne Patin, Christine Harmant, Marie Lopez, Matthieu Deschamps, Nadia Naffakh, Darragh Duffy, Anja Coen, Geert Leroux-Roels, Frederic Clément, Anne Boland, Jean-François Deleuze, Janet Kelso, Matthew L. Albert, and Lluis Quintana-Murci

Introgression of Neandertal- and Denisovan-like Haplotypes Contributes to Adaptive Variation in Human Toll-like Receptors — AJHG, 2016

Michael Dannemann, Aida M. Andrés, and Janet Kelso

Archaic Hominin Admixture Facilitated Adaptation to Out-of-Africa Environments — Current Biology, 2016

Rachel M. Gittelman, Joshua G. Schraiber, Benjamin Vernot, Carmen Mikacenic, Mark M. Wurfel, and Joshua M. Akey

Genetic Ancestry and Natural Selection Drive Population Differences in Immune Responses to Pathogens — Cell, 2016

Yohann Nédélec, Joaquin Sanz, Golshid Baharian, Zachary A. Szpiech, Alain Pacis, Anne Dumaine, Jean-Christophe Grenier, Andrew Freiman, Aaron J. Sams, Steven Hebert, Ariane Pagé Sabourin, Francesca Luca, Ran Blekhman, Ryan D. Hernandez, Roger Pique-Regi, Jenny Tung, Vania Yotova, and Luis B. Barreiro

Evidence that RNA Viruses Drove Adaptive Introgression between Neanderthals and Modern Humans — Cell, 2018

David Enard and Dmitri A. Petrov

Understanding the specific phenotypes produced by Neanderthal introgression could inform human evolution and health

N introgression

- Neanderthal introgression contributed genetic variants that were selected in local populations and contribute to important immune phenotypes that continue to affect our biology today.

- New technologies exist with potential to:
  - Make cell-type specific genome-wide maps of regulatory activity
    Connect genetic variation to regulatory effects
  - Broad Institute creating such maps

N DNA is regulatory

- MH protein coding gene regions are depleted of N DNA
- N DNA is in regulatory regions
- N DNA is most depleted in promotors (which initiate transcription of that gene) and in highly conserved coding regions, which are important to survival

N and H sapiens: Not a good mix

- The DNA of *Homo sapiens* and Neanderthals didn't mix very well. Long, long stretches of human DNA have no Neanderthal gene input at all.
- This indicates that genetic modifications in these regions proved negative for survival. For example, the MH FOXP2 gene for motor coordination and language and speech production has no Neanderthal input.
- Neanderthal input is entirely absent from large sections ("deserts") of human DNA.

Unique MH DNA & N DNA deserts

- Human male X chromosomes are particularly lacking in Neanderthal input, meaning there's a good chance that male children of a Human-Neanderthal unions had lower fertility than average. It was MH women hybrids who passed on N DNA

- Most Neanderthal genes survive in *H. sapiens* in regions of non-coding DNA. The regions that are most important for function—the protein-coding genes—are depleted of Neanderthal DNA.

- Areas that are strongly depleted of archaic ancestry can help identify which functional regions contribute to the uniqueness of some modern human traits.

Negative selection = Genomic Deserts with no N DNA

- N deserts in MH DNA: Mostly non-coding regions located preferentially near protein-coding genes,
- Evolutionarily conserved genes MHs involved in brain development, including FOXP2
- Neanderthal ancestry has been selected against in these conserved biological pathways, such as RNA processing
- Most-pronounced on the X chromosome and testes -- potentially relevant for N male hybrid sterility.
- The most plausible explanation for this loss of archaic DNA is negative selection

Neandertal DNA in Modern Humans

- Upside of N DNA: helped our ancestors survive in prehistoric Europe.
- Neanderthals are believed to have lived out of Africa long enough to adapt to the unique European ecosystem, dietary, and pathogenic landscapes found at higher latitudes.
- When humans migrated into Eurasia, they encountered unfamiliar hazards and pathogens. By mating with Neanderthals, MHs gave their offspring needed immunities, defenses and advantages.
- N immune system-related genes, conferred adaptive advantages against infectious microorganisms in new environments.

Negative Selection

- No evidence of Neanderthal mitochondrial DNA has been found in modern humans. This suggests that successful Neanderthal admixture = Neanderthal males and modern human females

- There is a presence of large genomic regions in MHs with strongly reduced Neanderthal DNA due to negative selection, partly caused by hybrid male infertility.

- These large regions of low Neanderthal DNA were most-pronounced on the X chromosome and testes

Neandertal functional contribution to modern humans?

Is it currently genetically functional?

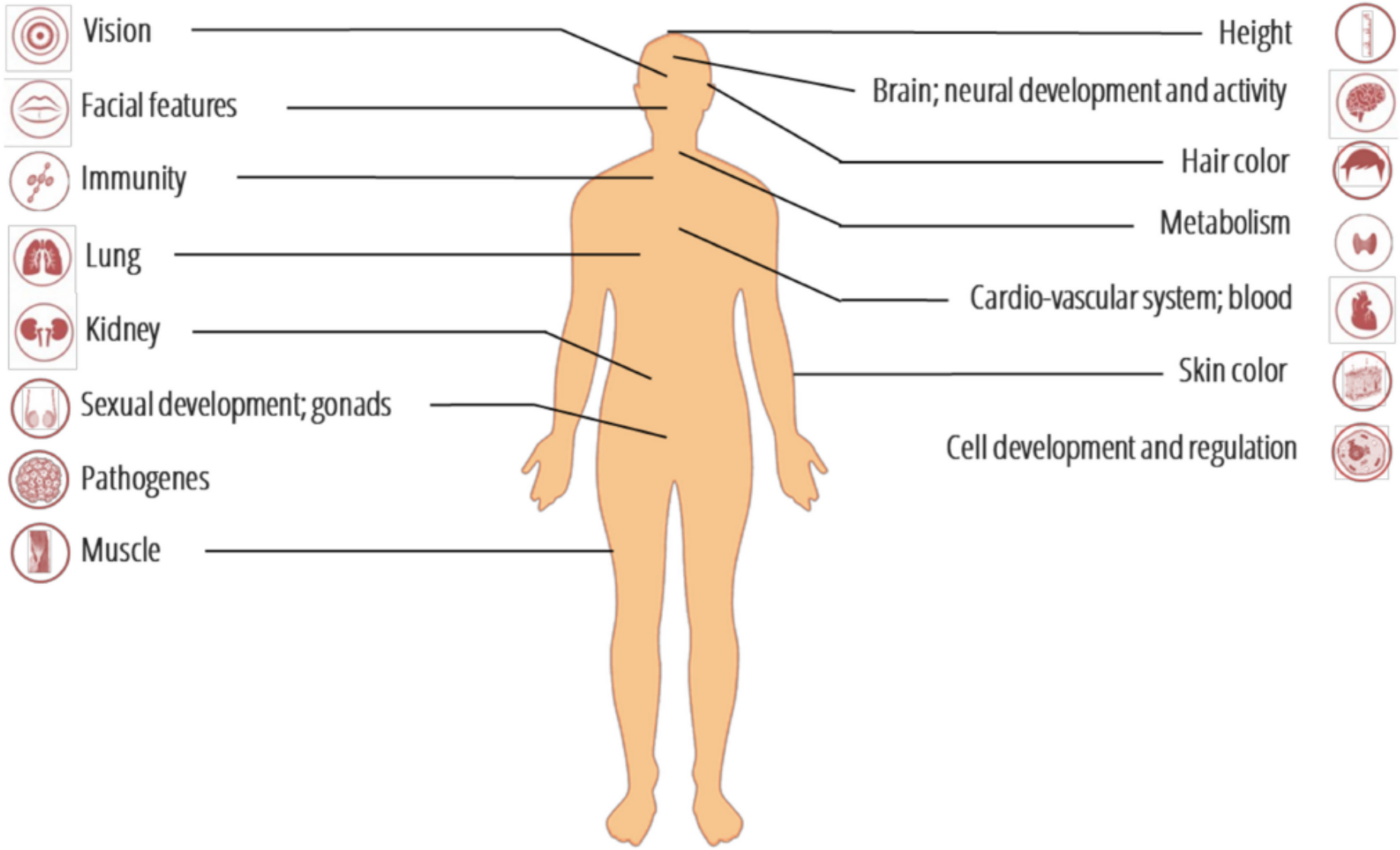


Figure 2. The modern human organs and systems affected by introgressed variants from ancient genomes (see Table S1 for details).

Candidates for adaptive introgression of N genes

- Immunity genes
- Pigmentation
- Keratin
- Metabolism
- Height
- Denisovan - Altitude adaptation

N pigmentation alleles

- Relationship between Neanderthal-derived alleles and the phenotypes in the UKB (500 K people health data).

- 50+% of the N alleles that initially registered as significant were related to hair and skin biology.

- Previous studies had obtained evidence purporting to link Neanderthals with red hair, but no link between Neanderthal DNA and red hair could be found in the UKB genome data, suggesting that perhaps it was rare or at very low frequency amongst Neanderthals.

Tom Higham

N Pigmentation: lighter skin

- Neanderthal DNA was found in over 60 per cent of people near a gene called BNC2, and that individuals in the UKB who carried this Neanderthal DNA reported high incidences of childhood sunburn and poor tanning ability typical of those with fair skin.

- Interestingly, other N segments in the UKB individuals were associated with more olive skin tones, suggesting that there may have been variation amongst the wider Neanderthal population in skin and hair color.

Adaptation through Introgression: adaptation to new European environment, pathogen defense

- Chrom 1: Denisovan in Native Americans/Siberians: *TBX15/WARS2* Body fat distribution
- Chrom 2: Denisovan in Tibetans (80%): *EPAS1* High altitude low oxygen adaptation
- Chrom 3: Neandertal in East Asians 50%: *HYAL2.* UV radiation response
- Chrom 4: Neandertal/Denisovan: >50%: *TLR6-1-10.* Innate immunity

Peaks of archaic ancestry

- Chrom 9: Neandertal: Europeans 70%: *BNC2* Skin pigmentation. only 2% carried it
- Chrom 11: Neandertal: East Asians 66%: *POU2F3* Kératinocyte differentiation
- Chrom 12: Neandertal: Papuans 54%: *STAT2:* Innate immunity

Immunity: Toll-like receptors

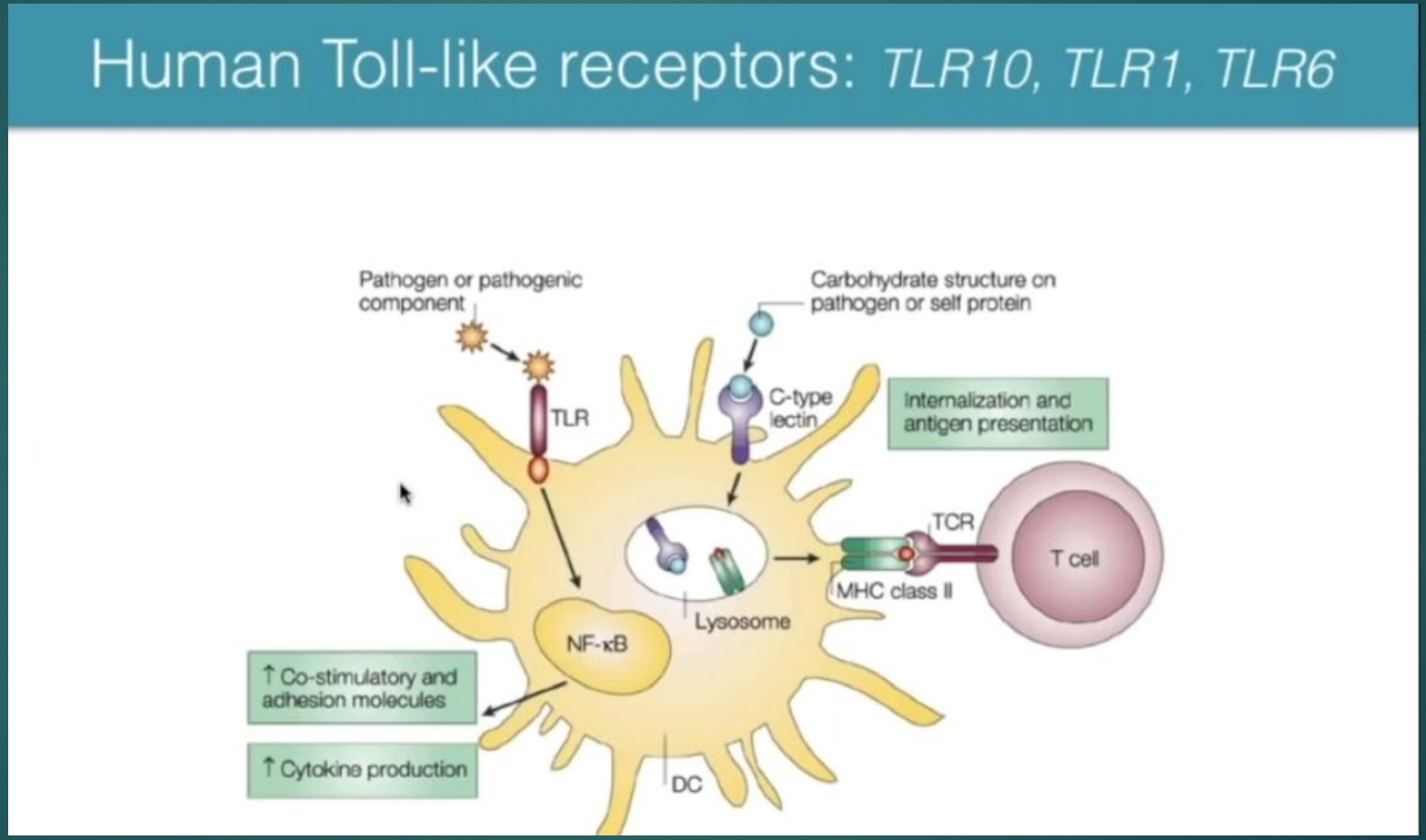
- Pathogens are one of the strongest selective forces out there. New lands have novel infectious diseases for which newcomers have no immunity.

- Janet Kelso et al. identified a large stretch of Neanderthal DNA — 143,000 DNA base-pairs long. Spanning three different genes that are part of the innate immune system.

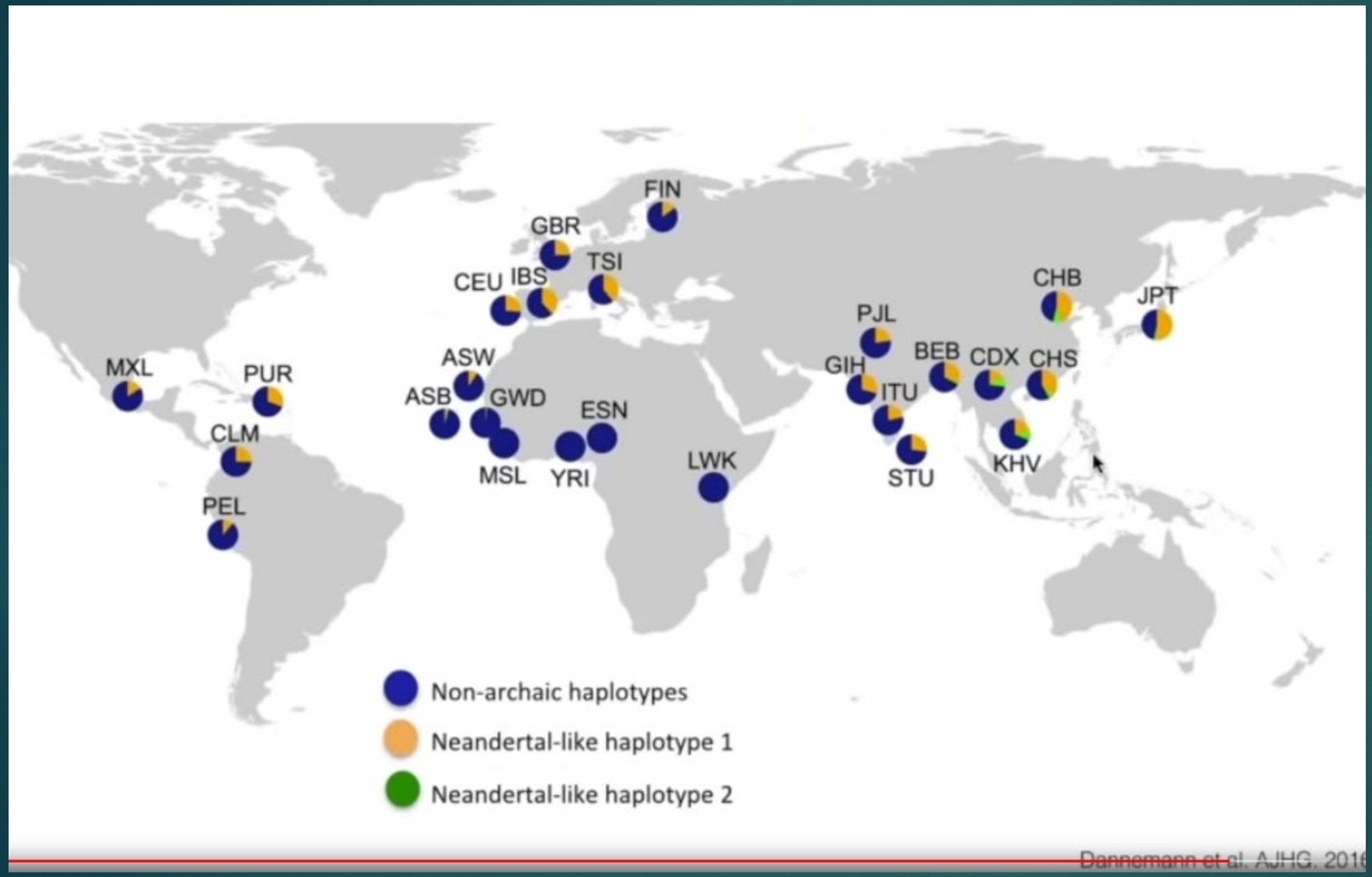
- These genes produce proteins called toll-like receptors, which help immune cells detect foreign invaders and trigger the immune system to attack.

- At least three of the variants appear to have come from archaic humans — two from Neanderthals and one from Denisovans.

N innate immune variants: inflammatory antimicrobial responses – first line of defense vs bacterial pathogens



Geographic distribution of N introgressed *TLR* haplotypes



N immune variants that identify invaders and trigger adaptive immune response

N variant has spread worldwide

Neandertal Immunity Genes

- MH inherited some immunity genes (Toll-like receptor genes *TLR1*, *TLR6* and *TLR10)* from Ns

- Spending a night with a Neandertal produced a big benefit: got thousands of years of genetic adaptation.

K. Harris and R. Nielsen, 2015

The progesterone receptor

- The Neandertal progesterone variant (A, V660L) is associated with preterm births
  - but
  - is also protective against miscarriage and
  - results in more live births...

- Doctors now use increased progesterone in women with preterm birth risk

Increased fertility for women with N progesterone gene

- The hormone progesterone is important for preparing the uterine lining for egg implantation and for maintaining the early stages of pregnancy.

- 30% of European women inherited the PGR receptor for progesterone from Neanderthals -- a gene variant associated with increased fertility, fewer bleedings during early pregnancy, fewer miscarriages, and having more children.

- N = 244 K women: 29 % carry 1 copy of the Neandertal receptor and 3 % have 2 copies. These women produce more progesterone receptors in their cells

Hugo Zeberg , Janet Kelso, and Svante Pääbo, 2020

Poison-antidote model of adaptive introgression

- Long, frequent—and more adaptive—segments of Neanderthal ancestry in modern humans are enriched for proteins that interact with viruses.
- These proteins which interacted specifically with RNA viruses were more likely to belong to introgressed N segments in modern Europeans.
- Retained segments of Neanderthal ancestry can be used to detect the occurrence history of ancient Neandertal epidemics.

Virus exchange between Ns and MHs

- Virus exchange between Ns and MHs: A 2018 study concluded that interbreeding between Neanderthals and modern humans led initially to the
  - exposure of each species to unfamiliar viruses.
  - Later on, the exchange of genes also granted resistance to those viruses.

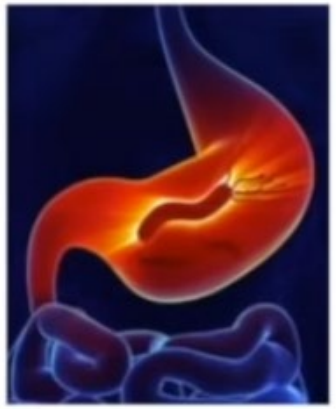
And the other way…Infections from MHs to Ns

- Tropical infections were likely to have passed from humans to Neanderthals –
  - such as tapeworm, tuberculosis, stomach ulcers and types of herpes --
  - chronic diseases that would have weakened the Neanderthals (= 1 theory of N demise)

- *Helicobacter pylori*, a bacterium that causes stomach ulcers, as a prime candidate for a disease that humans may have passed to Neanderthals
- Herpes simplex 2

Charlotte Houldcroft, 2016

Evolutionary tradeoffs: enhanced innate immunity surveillance, increased reactivity to pathogens and to nonpathogenic allergens



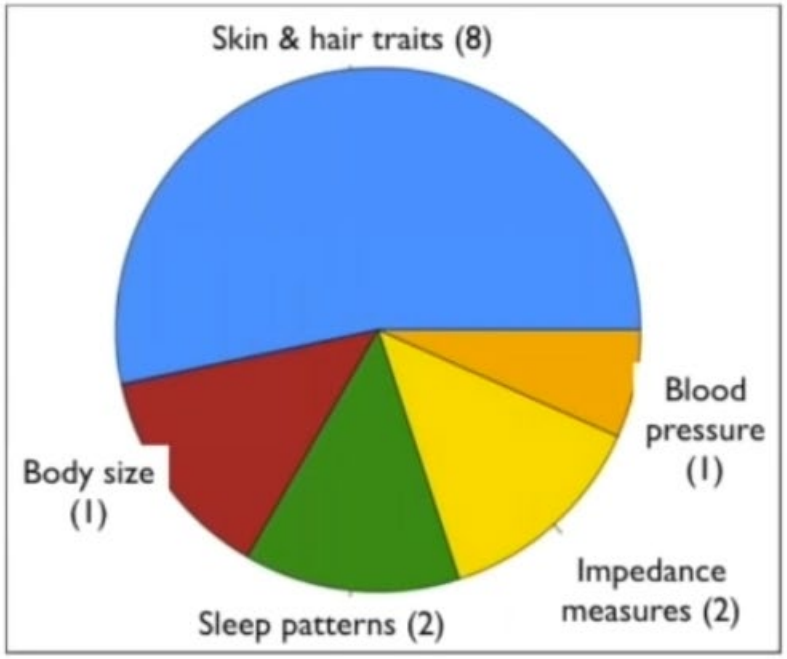
Introgressed alleles associated with

- reduced *H. pylori* infection
- increased allergic susceptibility

Modern Males Lack Neanderthal Y Chromosome Genes

- All the first analyzed N genomes were female. We only have female N sexual DNA
- No information on N's Y chromosome
- Neanderthal Y chromosome DNA has never been observed in any human sample ever tested
- If females consistently miscarried male babies carrying Neanderthal Y chromosomes, that would explain its absence in modern humans. Male fetuses conceived through sex with Neanderthal males would have miscarried.

Traits associated with introgressed N variants



Phenotype	Overlapping gene(s)	Association P-value
Natural hair color	*SPIRE2, TCF25, MC1R, TUBB3, FANCA*	4E-202
Skin color	*RUNX2*	4E-30
Ease of skin tanning	*BNC2*	2E-22
Natural hair color	*SLC24A4*	5E-21
Skin color	*BNC2**	2E-14
Comparative height at age 10	*ZNF536*	4E-14
Pulse rate, automated reading	*GJA1**	6E-14
Morning/evening person	*ASB1*	4E-10
Skin color	*CHORDC1**	6E-10
Impedance of leg (left)	*ADAMTSL3, GOLGA6L4*	1E-09
Childhood sunburn occasions	*BNC2*	1E-09
Sitting height	*PBLD*	2E-09
Natural hair color	*EXOC2*	3E-09
Daytime dozing / sleeping	*EXOC6*	4E-09
Impedance of leg (right)	*ADAMTSL3, GOLGA6L4*	6E-09

50% of N variants = Skin & hair traits, esp. pigmentation

N variants and MH phenotype traits: skin, hair

Phenotype	Overlapping gene(s)
Natural hair color	*SPIRE2. TCF25. UC1R TUBQ3. FANCA*
Skin color	*RUNX2*
Skin tanning	*BNC2*
Natural hair color	*SIC 24 A 4*
Skin color	*BNC2-*
Height at age 10	*ZNF536*
Pulse rate,	*GJAV*
Morning/evening person	*ASB1*

Phenotype	Overlapping gene(s)
Skin color	*CHORDCV*
Impedance of leg (left)	*ADAMTSL3, GOLGA6L4*
Childhood sunburn occasions	*BNC2*
Sitting height	*PBLD*
Natural hair color	*EXOC2*
Daytime dozing / sleeping	*EXOC6*
Impedance of leg (right)	*ADAMTSL3, GOLGA6L4*

N gene alleles in MH genome: Conditions associated with N alleles

- Variation in interleukin-18 levels
- Innate immunity genes (Toll-like receptor (TLR) genes--TLR1, TLR6, and TLR10)
- Variation in optic disc size
- FOXP2 (language)

B. Vernot & J. Akey, 2014

Negative selection from N sex genes

- X chromosome is significantly depleted of Neanderthal sequence, with an approximate 5-fold reduction in Neanderthal ancestry compared to the autosomes (other 22 chromosomes), suggesting reduced fitness in male hybrid offspring (and perhaps male hybrid sterility).

- Testes-specific genes are also significantly depleted of Neanderthal DNA, further supporting the inference of reduced fitness in male hybrid offspring.

- 2016 research indicates some Neanderthal males might not have had viable male offspring with some AMH females. This could explain the reason why no modern man has a Neanderthal Y chromosome.

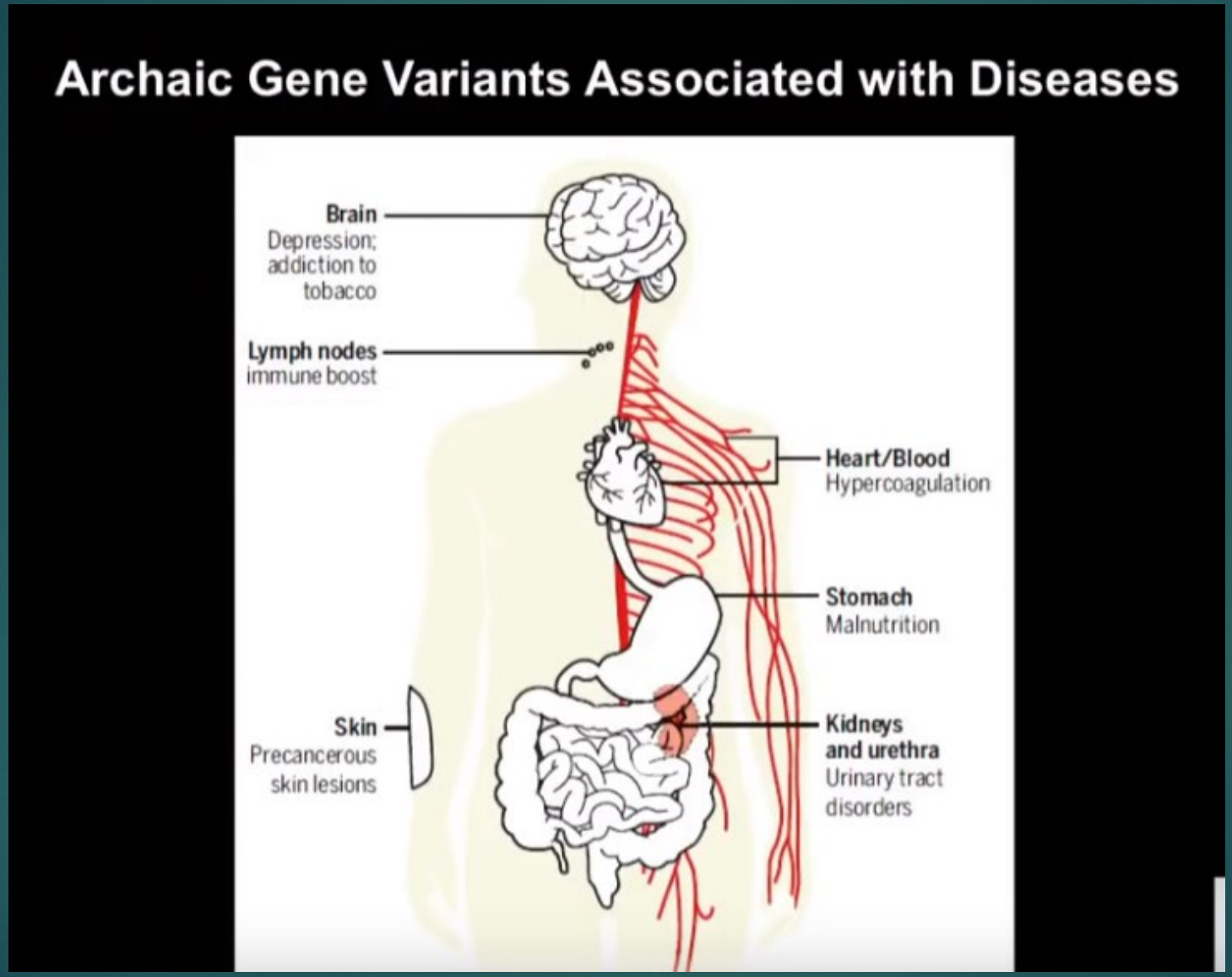
Alternate theory: N fitness

- Hypothesize that stronger purifying selection on the X chromosome as well as matings between Neanderthal males and human females could account for the reduced level of Neanderthal-derived ancestry seen on that chromosome.

- Selection against deleterious alleles, rather than hybrid incompatibilities, most likely accounts for patterns of Neanderthal ancestry in modern human populations.

- Jurich *et al*. and Harris and Kelley estimate an average F1 hybrid individual would carry somewhere between an astonishing 40 to 94% reduction in fitness compared to modern humans.

Most N variants associated with diseases



Neandertal DNA is slightly detrimental to modern humans, making some people more prone to certain diseases,

- Increased disease risk associated with Neandertal alleles:
  - Lupus
  - Primary biliary cirrhosis
  - Crohn's disease (2 alleles)
  - Type 2 diabetes
  - Covid-19
  - More allergies
  - Cystic fibrosis lung function
  - Cholesterol levels

B. Vernot and J. M. Akey, Science; Sankaraman et al., Nature, 2014; Corinne N. Simonti, et al., 2016

The phenotypic legacy of admixture between modern humans and Neanderthals

- Skin lesions resulting from sun exposure (actinic keratosis)
- Obesity
- Seborrheic keratosis
- Acute upper respiratory infections

Corinne N. Simonti, et al., 2016

N genes and disease

- Human papillomavirus HPV16 strain from N or D
- Both increased and decreased risk for depression (1-2%).
- Trait for faster blood clotting. In the modern world, however, this trait means greater risk for stroke and pregnancy complications.
- Lower risk of schizophrenia
- Response to antipsychotic drugs

Simonti et al., 2016; Rajiv C. McCoy, et al., 2017

N Gene association

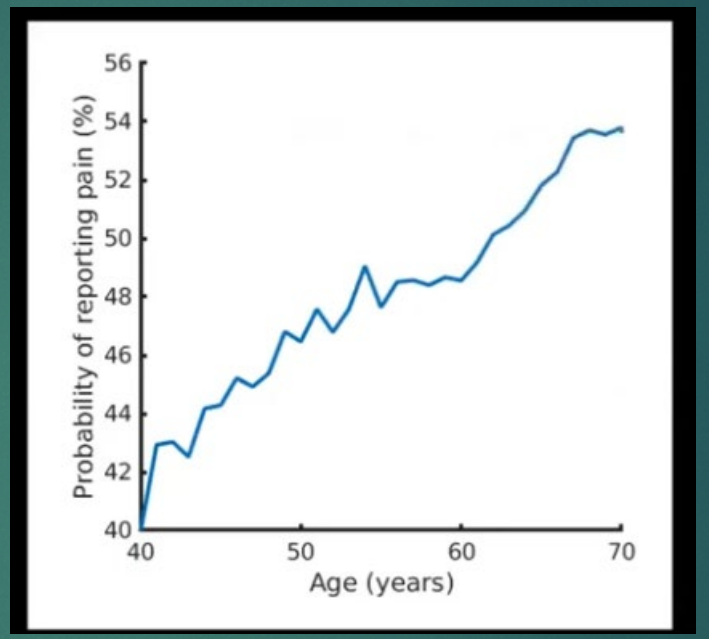
- Slightly increased height on average
- Accumulation of belly fat
- Risk of developing eating disorders
- Rheumatoid arthritis
- Genital warts
- Slightly increased risk of heart attack
- More corns and callouses
- More bladder pain and incontinence

UK BioBank: 0.4% carry N variant: Neanderthal sodium channel increases pain sensitivity in present-day humans

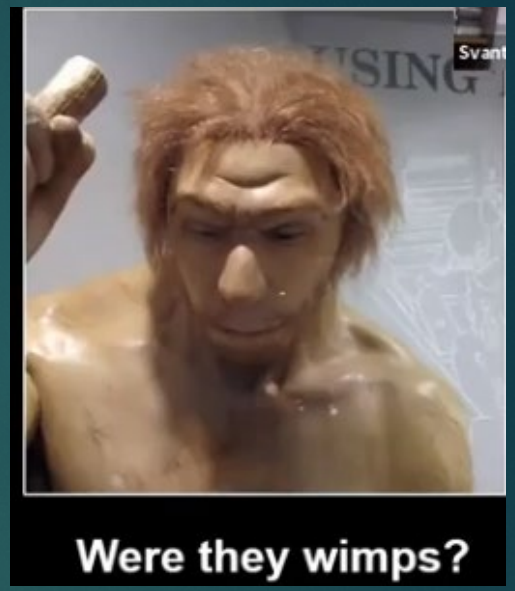
UK BioBank

362,944 individual, 1,337 (0.4%) carry the Neandertal allele

Specific pain phenotypes
Chest pain due to walking ceases when standing still
Knee pain for 3 months
Facial pains for 3 months
Neck/shoulder pain for 3 months
General pain for 3 months
Chest pain or discomfort
Back pain for 3 months
Chest pain or discomfort when walking uphill or hurrying
Chest pain or discomfort walking normally
Leg pain when walking normally
Leg pain on walking
Stomach/abdominal pain for 3 months
Leg pain when standing still or sitting
Hip pain for 3 months
Chest pain felt outside physical activity
Leg pain when walking uphill or hurrying
Leg pain in calf/calves
Leg pain when walking ever disappears while walking
Chest pain felt during physical activity

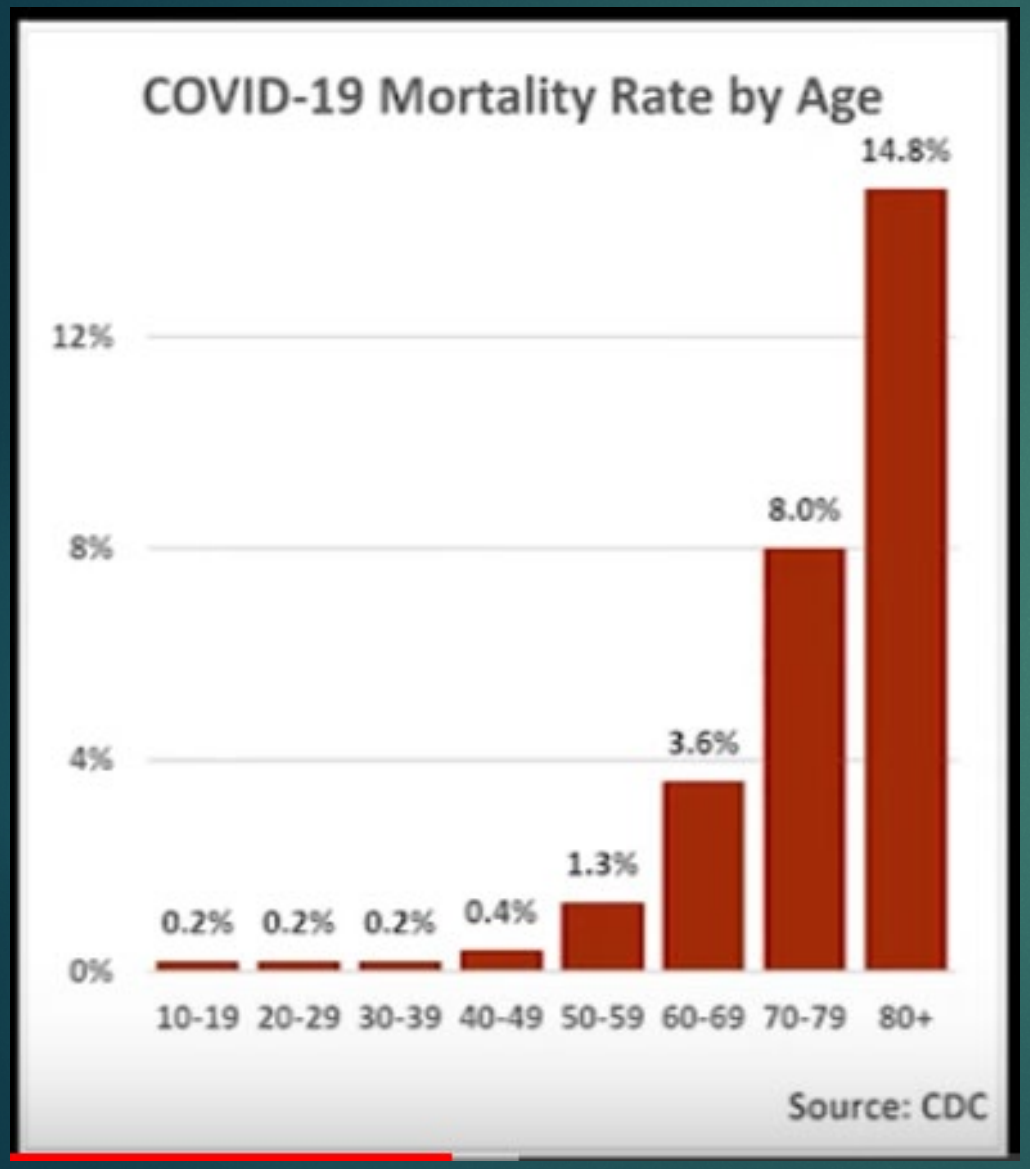


More pain reports with age



Those who carry N variant report more pain;
equivalent of 8 years older in pain report

Covid Pandemic: 7 Million dead

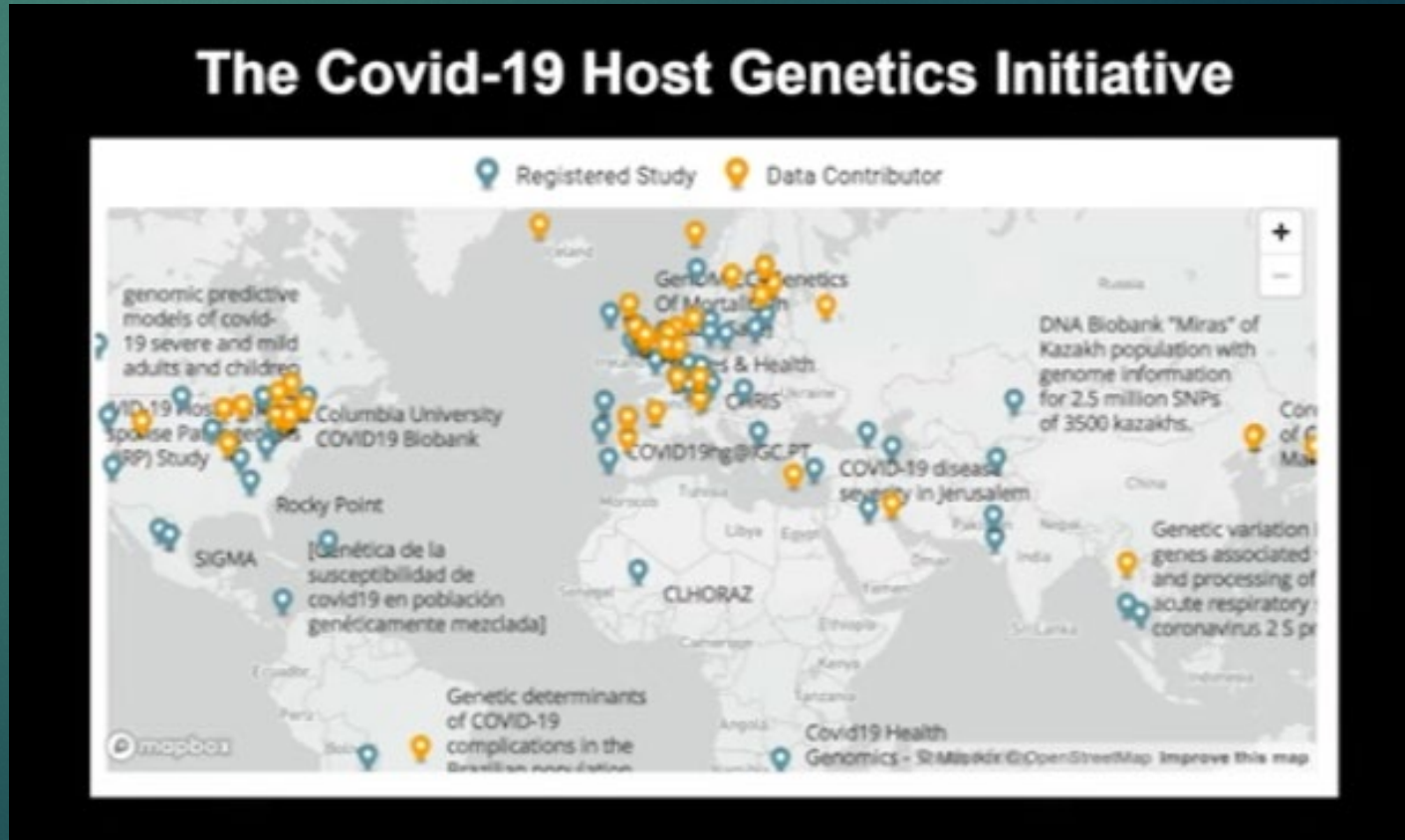


Main risk factors;

Old age

Male sex

Diabetes



N OAS haplotype: detected RNA viruses

- The ancestral Neandertal OAS locus variants may thus have been advantageous to modern humans throughout Eurasia, perhaps due to one or many prior N epidemics involving RNA viruses,

- Neandertal haplotype has been found to be protective for at least three RNA viruses (West Nile virus, hepatitis C virus, SARS-CoV).

- A Neandertal haplotype on chromosome 12 is protective for severe disease in the current SARS-CoV-2 pandemic.
  - present in populations in Eurasia and the Americas at carrier frequencies that reach 50%.

But N DNA is double edged sword

Risk for Covid

Protection from Covid

Article | Published: 30 September 2020

The major genetic risk factor for severe COVID-19 is inherited from Neanderthals

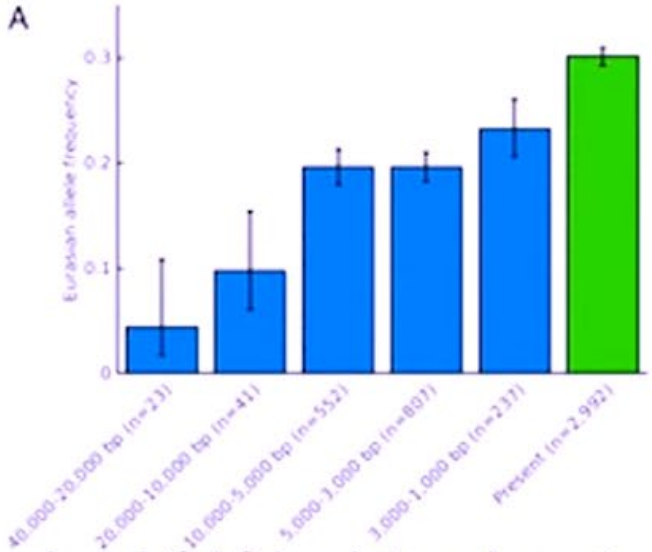
Hugo Zeberg & Svante Pääbo

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

Neanderthal-derived risk variant increases the risk of hospitalization by 60%

A genomic region associated with protection against severe COVID-19 is inherited from Neandertals

Hugo Zeberg and Svante Pääbo



Neandertal *OAS* haplotype is protective against severe COVID-19

N variants: both Positive Selection and Disease Associations

- N variant in Europeans & Melanesians may facilitate immune response to Flu
- N variant provides decreased risk of gastrointestinal diseases in Asian populations
- N variant leads to increased activation of the Toll Like Receptor immunity pathway and contributing to allergy and asthma risk in Europeans and East Asians

nature

https://doi.org/10.1038/s41586-020-2818-3

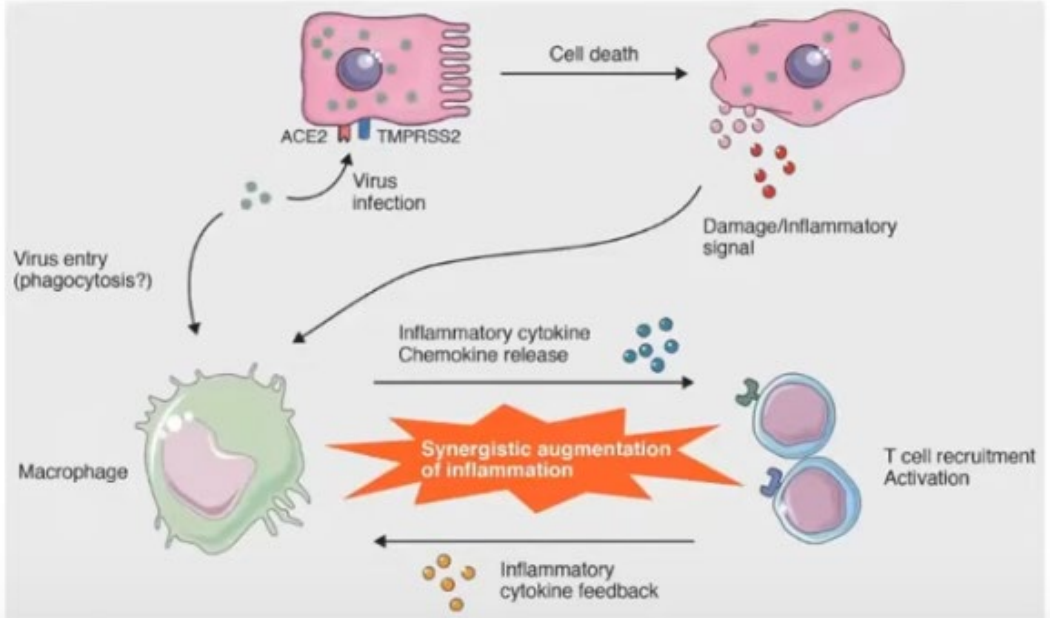
Accelerated Article Preview

The major genetic risk factor for severe COVID-19 is inherited from Neanderthals

Zeberg and Paabo 2020

CCR1 and CCR5 contribute to COVID-19 induced "cytokine storm"

Cytokine = immune messenger molecule
Chemokine = type of cytokine



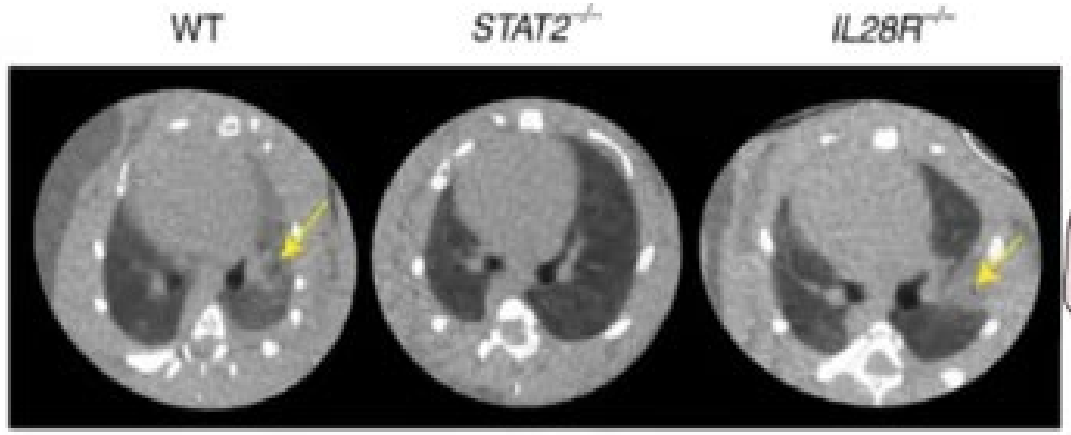
(Ryo Otsuka, Ken-ichiro Seino. Inflammation and Regeneration. August 6, 2020)

N Introgression alters CCR1/CCR5 regulation increasing the COVID-19 cytokine storm

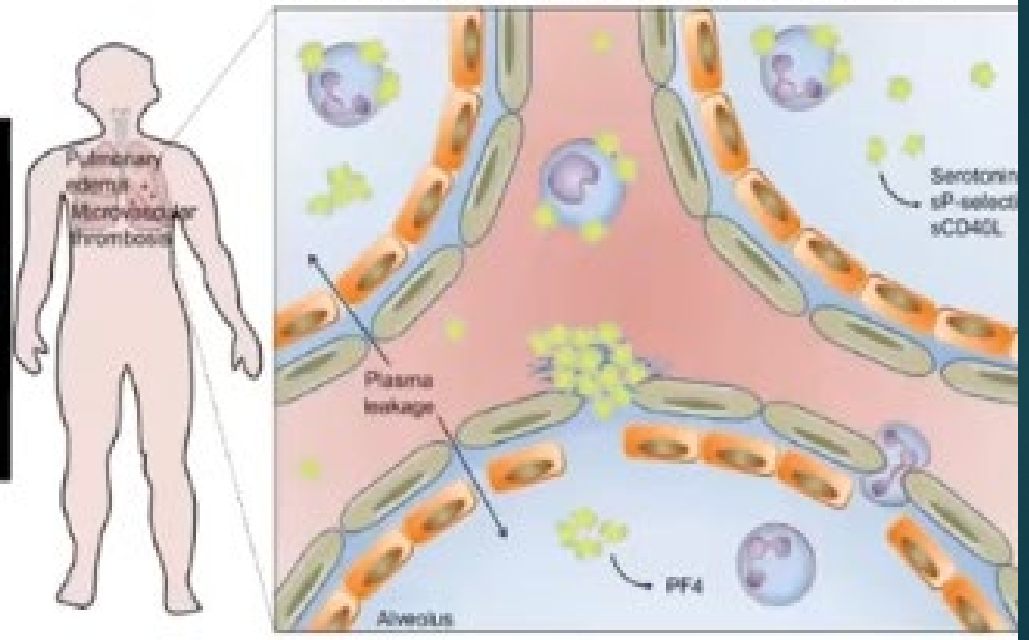
Adaptive Hypothesis = N allele increases Covid 19 Risk: Neandertal Rs80317430 decreases STAT2 expression which reduces risk of hyper-5W2 mediated disease complications including platelet- induced lung damage

Hyper-expression of STAT2 Leads to Severe Consequences

Exuberant innate response by *STAT2* drives SARS-CoV-2-induced lung pathology in hamsters. (Boudewijns et al. 2020)



Platelet–fibrin clot deposits build up in lungs during flu infection and contribute to pneumonia and lung injury (Hottz et al. 2018)



N DNA and Covid-19

- N genomic regions associated both with increased risk for and also protection against severe COVID-19

- The major genetic risk factor associated with becoming severely ill with COVID-19 when infected by severe acute respiratory syndrome SARS-CoV-2 is inherited from Neandertals.

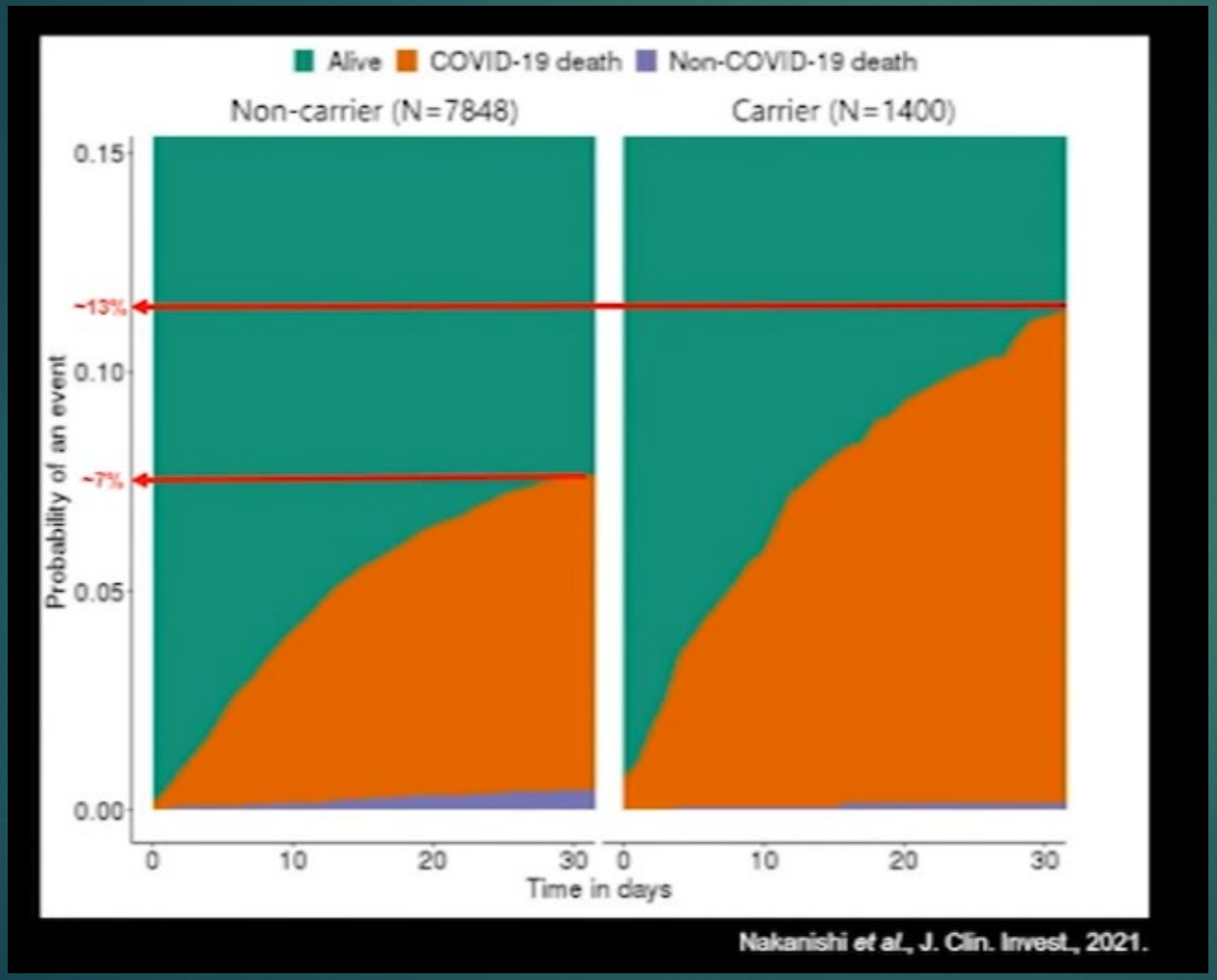
Hugo Zeberg and Svante Pääbo, 2022

Covid-19

- After age, N variant is strongest risk factor for severe response to COVID-19 = 10% of deaths
- A Neandertal haplotype in a region on chromosome 3 is associated with becoming critically ill upon infection with Covid 19. Produces higher susceptibility to respiratory failure
- Each copy of this haplotype approximately doubles the risk of its carriers requiring intensive care.
- You can now check for C bp at rs10490770 at 23andMe, Family Finder, and Ancestry raw data files, or directly on 23andMe using Browse Raw Data.

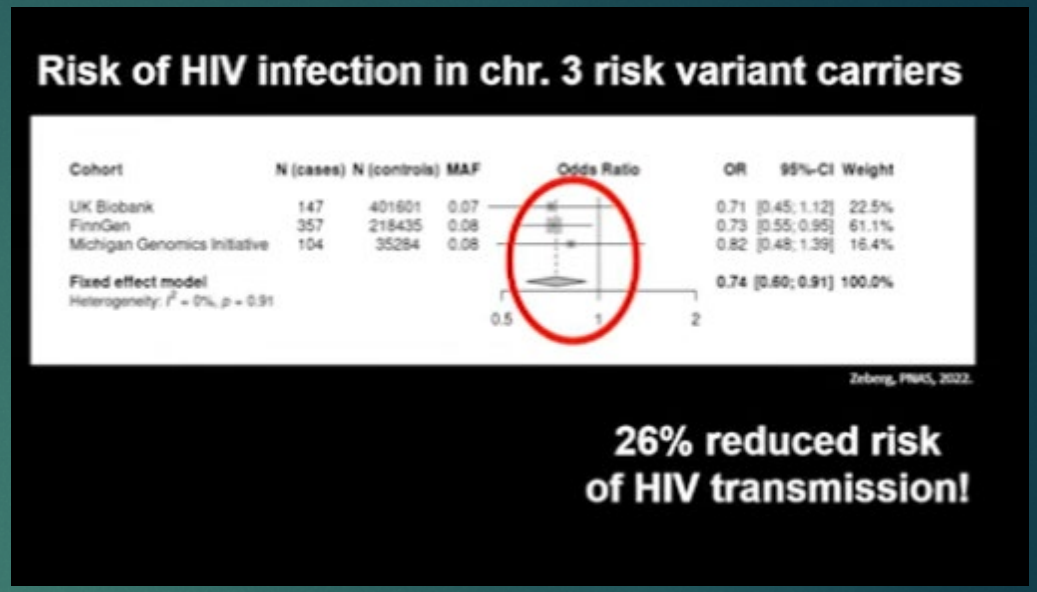
N Covid variant (rs35044562): positively selected for only in SE Asia, where it had some unknown positive effect; 50% of carriers in S Asia (India); 0% in East Asia

Major common genetic risk factor for COVID-19 of severity and risk of dying mortality in under 60 age: Non-carriers: 7% risk of dying; N carriers: 13% risk of dying; = 1.1 M deaths (N's revenge?)



N genetic risk factor (chromosome 3 locus tagged by rs10490770): higher susceptibility to respiratory failure

CCR5 N variant on Chromosome 3 reduces risk of HIV



The chromosome 3 locus
·Decreased risk for HIV infection

Chromosome 3:
~100% increased risk per allele
Chromosome 12:
- 26% decreased risk per allele

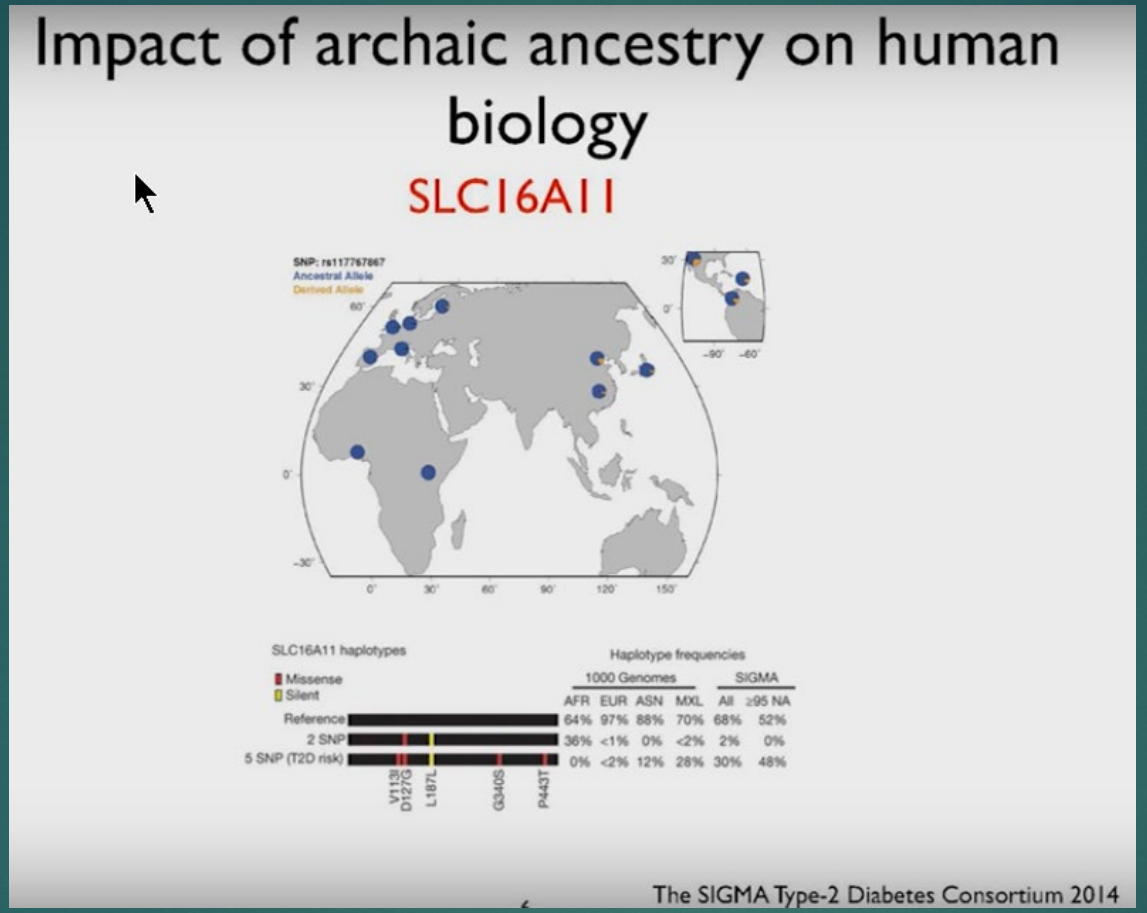
N and Diabetes

- N allele and Diabetes:
  - Mayas in Mexico, some Native Americans, and about 25% of Asians
  - retain an allele from Neandertals that boosts their risk for Type 2 diabetes.

- Genes involved in fat synthesis (lipid catabolism, the breakdown of fats to release energy) held more than three times as many Neandertal sequences

- N Risk variant for Type 2 Diabetes: lipid transportation; Today, though, these fatty acids are also implicated in diseases that are part of the so-called "metabolic syndrome"

Type II DM in Mexican Americans: GWA study



Unique variance in SLC16A11 gene with unique distribution (high frequency in Americas, largely absent in Africa, and low frequency everywhere else; the DM variant originated in Ns

Lipid catabolism

- Don't see the same alleles in the Denisovans
- In Ns, good during starvation; better energy conservation; but bad if a lot of food available; so likely a N adaptation to starvation
- Neanderthal DNA at various sites in the genome influences obesity and malnutrition, pointing to potential metabolic effects

N allele variant: skull shape

- A large study of 4,468 living Europeans showed that variants introduced from Neanderthals near two genes did indeed have a subtle influence on the globular shape of their skulls.
- Elongated vs round skulls: two Neanderthal gene variants linked to slightly less globular head shape in living people.
- MH infants start life with elongated skulls, somewhat like Neanderthals.
- It's only when the modern human brain nearly doubles in size in the first year of life that the skull becomes globular

N Chronotypes

- Do you know people who describe themselves as a ‘morning person’? Perhaps you are more of an ‘evening person’, or perhaps something in between. We call these categories ‘chronotypes’ and, it turns out, these have a strong genetic basis. There are four categories in the UKB datasets: ‘definitely an evening person’, ‘more an evening than a morning person’, ‘more a morning person than an evening person’ and ‘definitely a morning person’.
- The UKB comparison revealed that Neanderthal alleles associated with genes ASB1 and EXOC6 are strongly associated with these preferences. What is interesting is that there is a significant association between latitude and these chronotypes – the further you are from the equator the greater the chance you have the Neanderthal allele at ASB1. Your preference for being a morning person appears to be increased by having the Neanderthal allele variant.

Chronotype

- Chronotype is linked with daylight exposure. Given that Neanderthals had adapted and lived for more than 200,000 years in the northern parts of Europe and Eurasia, it seems reasonable to expect adaptation to lower UV levels and sunlight duration in them compared with modern humans ultimately coming out of Africa.
- There is physical evidence to support this as well. If we plot the size of the eye orbit and eye volume of Neanderthals and compare them with contemporary modern humans living in Africa and lower latitudes, we find a significant difference.
- Neanderthals had larger eye sockets, probably to compensate for lower light levels in northern latitudes and the long periods of winter darkness.

N variants in MH phenotypes

- Neanderthal variants are associated with
  - Loneliness and feelings of isolation,
  - low mood,
  - frequency of being unenthusiastic or disinterested
  - variation in smoking behavior.

- Like chronotypes, the degree of light exposure is a key factor in these mood-related behaviors, hence the Neanderthal link.
- Determining how the effects seen in present-day people might once have affected Neanderthals themselves is one of our crucial challenges. i.e. Neanderthals did not smoke (but were exposed to lots of smoke).

Depression

- Neanderthal variants linked to risks for depression and addiction
- Light might be a unifying factor, with both changes in day-length patterns, lesser light, and UV exposure reductions as they moved to more-northern latitudes.
- The Neandertal variants most strongly associated with depression were located near circadian clock genes
- Since it is unlikely that Neanderthals experienced such disturbances to their natural sleep cycles, they may never have expressed this gene, but in modern, this gene is expressed more frequently.

Kelso and Dannemann, 2017

Neanderthal Introgression Shaped Human Circadian Traits

- The Eurasian environments where Neanderthals and Denisovans were located at higher latitudes with more variable photoperiods than the landscape where AMH evolved before leaving Africa.

- Study identified lineage-specific genetic variation in circadian genes, their promoters, and flanking distal regulatory elements.
- Many N introgressed alleles have strong associations with chronotype.

- Strikingly, the strongest introgressed effects on chronotype increases morningness, which is consistent with adaptations to high latitude in other species.

K. Velazquez-Arcelay, et al., 2023

Neanderthal effects drug metabolism

- Some N variants cause less efficient drug elimination.
- Notably reduced metabolism of:
  - warfarin,
  - phenytoin,
  - statins,
  - ibuprofen,
  - leading to potential toxicity at otherwise therapeutic doses.
- Therapeutic doses can be toxic for carriers of the Neanderthal gene variants

Another N functional consequence in MHs: Longer Ibuprofen effect

N variant is cause of 9-hour, instead of 2-hour, effect on Ibuprofen:

The presence of the N allele *CYP2C8*3* was found to influence the pharmacokinetics of (R)-ibuprofen in a gene–dose effect manner = 400 mg normally lasts for 2 hours; But in some lasts for 9 hours because liver does not degrade it

Neanderthal Variant: Decreased absorption of warfarin; higher doses may be toxic; need lower dosing

"Neandertal" *CYP2C9*2*

Recommended daily warfarin doses (mg/day)

*CYP2C9*1/*1*	*CYP2C9*2/*2*
5–7	3–4

Adapted from Johnson *et al.* (2011) *Clin Pharmacol Ther.* 90(4):625-9.

De-extincting potential N antibiotics

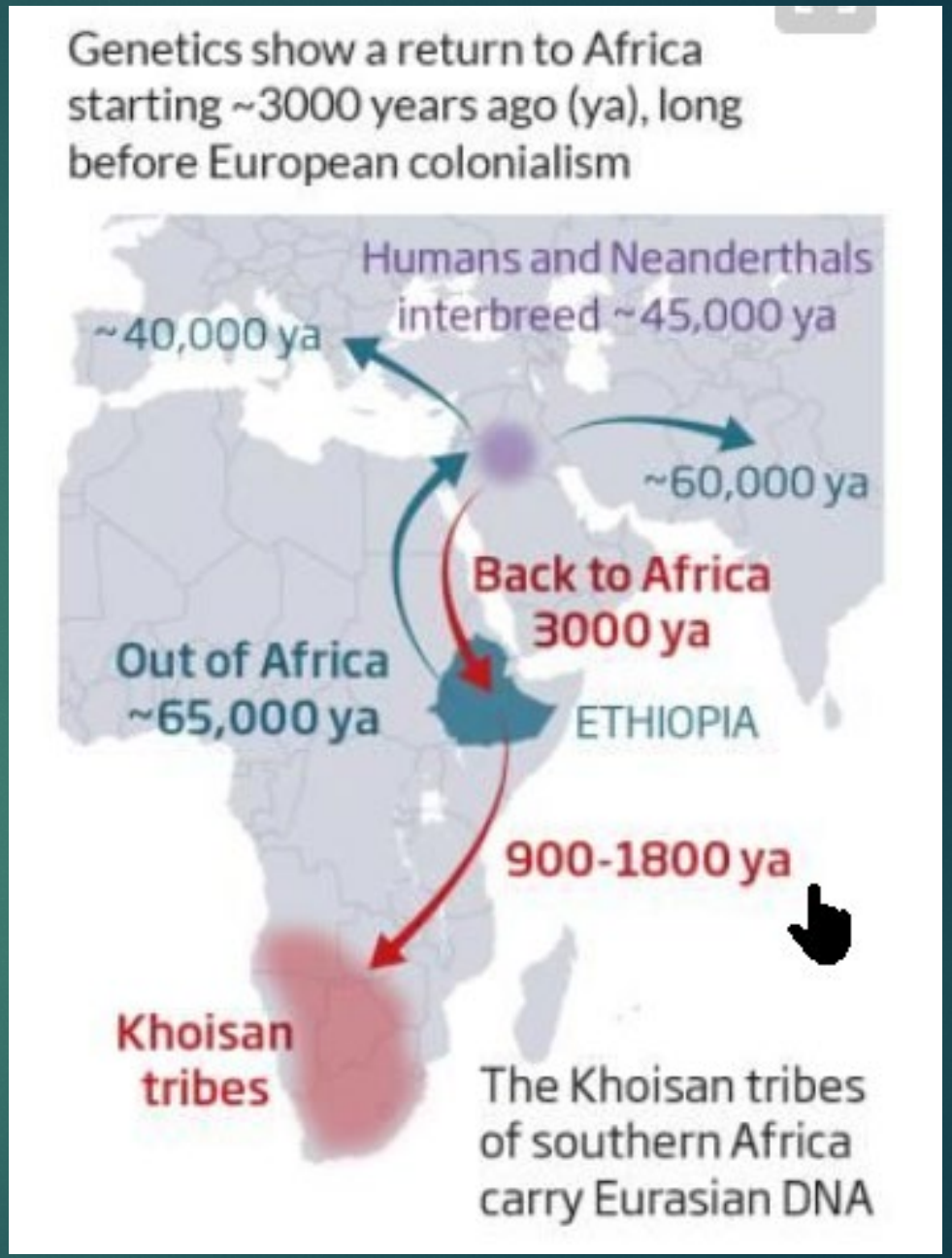
- By adding 69 N molecules to Petri dishes containing a range of bacteria, study discovered six previously unknown antibiotic ones.

- Three of them came from Neanderthals and three from Denisovans, and each worked against at least one species of bacterium. These included *Escherichia coli*, *Pseudomonas aeruginosa* – which causes lung and blood infections in hospitals – and *Acinetobacter baumannii*, which infects people with suppressed immune systems.

- The antimicrobial peptides are non-toxic, comparably potent to other antimicrobial peptides and had good effectiveness in a mouse model,

Some Africans have N DNA:

- "All modern non-African humans have 1-2%"
- But ~ 3000 ya MHs with Neandertal DNA returned to Africa
- Today there is N DNA (.03%) in some Khoisan tribes of S Africa



Kay Prüfer, et al., 2014

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