A History of Multiple Denisovan Introgression Events in Modern Humans

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Published article: https://www.nature.com/articles/s41588-024-01960-y

DOI: https://doi.org/10.1038/s41588-024-01960-y

Abstract

The identification of a new hominin group in the Altai mountains called Denisovans was one of the most exciting discoveries in human evolution in the last decade. Unlike Neanderthal remains, the Denisovan fossil record consists of only a finger bone, jawbone, teeth, and skull fragments. Leveraging the surviving Denisovan segments in Modern Human genomes has uncovered evidence of at least three introgression events from distinct Denisovan populations into modern humans in the past. Each of them presents different levels of relatedness to the sequenced Altai Denisovan, indicating a complex relationship between these sister lineages. Here, we present evidence suggesting that several Denisovan populations, who likely had an extensive geographical range, were adapted to distinct environments and introgressed into modern humans multiple times. We further discuss how archaic variants have been affected by demographic history, negative and positive selection, and close by proposing possible new lines of future research.

Keywords

Denisovans, Introgression, Adaptation, Admixture, Archaic Hominins

Main

In 2010, the first draft of the Neanderthal genome was published[1], and comparisons with modern human genomes revealed that Neanderthal and modern humans had interbred in the past. A few months later, analysis of a genome sequenced from a finger bone excavated in the Denisova cave in the Altai mountains in Siberia revealed that this bone fragment was from a newly discovered hominin group that we now call Denisovans, who also interbred with modern humans[2]. These findings greatly impacted the field as they provided genetic data that could be leveraged to address outstanding questions in human evolutionary biology that fossil and cultural phenotype (i.e. osteological morphology and technocomplexes of stone tools) data alone could not fully resolve. For example, Neanderthals, who have a rich fossil record that had been studied for decades, were deemed and perceived as very different from modern humans, but we did not know whether these phenotypic differences would be mirrored in the genetic data. Access to the Neanderthal genome established their genetic relationship to modern humans and provided evidence that Neanderthals and modern humans interbred[3]. In contrast, Denisovans have a less comprehensive documented fossil record. Identifying this previously unrecognized hominin group, from DNA extracted from a small phalanx, markedly transformed the field of human evolution as it provided a new way to identify ancient remains through genetic analysis alone.

Whereas Neanderthals have an extensive fossil record and several Neanderthal genomes have now been generated[1,4–7], only a single Denisovan genome is available[4], and Denisovans thus remain a more mysterious hominin group. Nonetheless, recent studies have used Denisovan-like DNA in modern humans[8–11], DNA from sediments[12], and newly identified fossils outside the Denisova cave (in Tibet[13] and in Laos[14]) to characterise the Denisovan-like populations that interbred with modern humans.

In this review we aim to discuss recent studies that have identified the existence of several Denisovan populations, who likely admixed with modern humans multiple times. Then, we also provide a summary of the statistical methods that have been used to detect different contributions to modern humans from distinct Denisovan populations, and how relationships between different Denisovan populations are inferred. Moreover, we review how natural selection has impacted archaic variants, focusing on those introgressed from Denisovans. Finally, we propose possible new lines of future research.

Denisovan ancestry in modern humans

Despite not having enough skeletal remains to provide clues on the morphology of Denisovans, we do have genetic data extracted from small fossil remains from Denisovans (see BOX 1 and Supplementary Table 1). These genetic data have been used in several studies to (i) reconstruct the genetic relationships of Denisovan with Neanderthal and modern humans (Fig. 1), (ii) detect and quantify Denisovan ancestry in modern human populations, (iii) leverage surviving Denisovan segments in modern humans to infer multiple interbreeding events between distinct Denisovan populations and modern humans (Fig. 2), iv) describe the evolutionary relationships between different Denisovan populations, (v) identify what genes likely accelerated human adaptations to distinct environments and (vi) identify what genetic variants may be associated with human diseases. Together, this will provide a more comprehensive profile of Denisovan populations, including their interactions with modern humans.

Evidence for multiple Denisovan introgression events with modern humans

The first comparisons of the Denisovan genome with modern human genomes led to some interesting and unexpected results: the Denisovan finger bone was excavated in the Denisova cave in the Altai mountains in Siberia, yet only Papuans – who live far away from the Denisova cave – were inferred to have traces of Denisovan ancestry[4,8]. This was the first indication that Denisovans likely had a large geographical range that spanned Siberia, Southeast Asia, and Oceania. It was later confirmed that East Asians also had some Denisovan ancestry[5,15]. While we now know that East and South-Asian populations, as well as Indigenous American populations, harbour Denisovan ancestry (~0.1%)[9], it pales in comparison to the amount of Denisovan ancestry in Oceania (up to 5%).

Initially, the assumption was that introgression from Denisovans observed in modern humans came from a single introgression event in the past. This was reached owing to the development and use of several methods to detect introgression (summarised in Fig. 3 and Supplementary Table 3). However, in 2018, Browning *et al*.[10] developed a method to detect archaic ancestry in modern humans and visualised the results in an ingenious way (Panel B of Box 2 Figure) that suggested that East Asian populations likely interbred with two distinct Denisovan-like populations. By computing a measure of distance (or match rate, BOX 2) between each inferred archaic fragment identified in an East Asian population and the sequenced Neanderthal or Denisovan genome, they discovered that although most archaic fragments were similar to the sequenced Altai Neanderthals, some surviving archaic fragments were more similar to the sequenced Denisovan than to the Altai Neanderthal and

that within the set of fragments most similar to the sequenced Denisovan, a subset was very similar to the Denisovan (affinity of ~0.8) and a larger set had a moderate affinity (~0.5). Observing two levels of affinity in Denisovan-like fragments in East Asian populations suggests that ancestral populations of East Asians had interbred with two Denisovan-like populations: one closely related to the sequenced Altai Denisovan individual (High-Affinity component, referred to as D0 in Fig. 2), and one from a population more distantly related to the sequenced Altai Denisovan (Moderate-Affinity Component, referred to as D2 in Fig. 2). In contrast, all putative surviving Neanderthal segments had similar levels of affinity to the sequenced Altai Neanderthal, suggesting that either one introgression event had occurred or multiple ones but with closely related Neanderthal populations[10].

Subsequent sequencing of a larger set of individuals from ISEA (Island Southeast Asia) recapitulated the two Denisovan introgressions into East Asians[11], but by using the distribution of match rates, also found evidence for two introgression events into Papuans. This brought the total number of introgression events from Denisovans to three: one from a population closely related to the Altai Denisovan (D0) that is primarily present in East Asians, a second one more distantly related to the Altai Denisovan (Moderate-Affinity Component, D1 in Fig. 2) that is primarily present in Papuans, and a third one (D2) that forms the major part of the Denisovan ancestry in Papuans. The latter is also suggested to have contributed to East Asian populations[10], although these populations have relatively equal and non-negligible contributions from both components (D0 and D2).

A more recent study analysed 462 individuals, of which 317 were newly published, from the Pacific and near Oceania[16]. This study also found evidence for two introgression events into Papuans and two into East Asians, as well as some suggestive evidence for an additional introgression event in populations in the Philippines, which was supported by the finding that the Ayta Magbukon Negritos genomes harbour about 25% more Denisovan ancestry in their genomes than Papuan populations[17]. However, unlike Papuans, the Denisovan ancestry of the Ayta is not associated with Australasian-related ancestry, suggesting that the Ayta have an additional pulse of admixture (D3 in Fig. 2)[17]. This points to at least four distinct Denisovan populations, which suggests that a large geographical region was likely inhabited by a set of diverse Denisovan-like populations each adapted to their local environments. It is worth noting that all studies that proposed more than a single introgression event have used different data sets, as well as different methods to identify Denisovan segments, and measure matching differently between the sequenced Denisovan and the surviving Denisovan segments. It would thus be highly valuable to perform an analysis that includes all samples, the same filtering, processing steps, and methods to identify Denisovan segments. Furthermore, considering what Denisovan segments are shared or private and what is their relationship to the

sequenced Denisovan will help clarify the number of distinct Denisovan populations that introgressed with modern human populations.

Genetic relationships between introgressing Denisovan populations

The finding of several Denisovan introgression events from distinct Denisovan populations raises further questions, including: how closely related are these Denisovan populations to each other, and when did these populations introgressed into modern human populations? To address these questions, the Denisovan introgressed segments in modern human populations have been used to infer the split times between distinct Denisovan populations and the timing of introgression events. For example, the split times of two Denisovan populations (D1 and D2 in Fig. 2) that introgressed with ancestral Papuan populations with the population the sequenced Altai Denisovan belonged to (see BOX 2) have been estimated to around ~363kya for D2 and ~283kya for D1 and the Altai Denisovan population[11]. These estimates suggest that genetic divergences between introgressing Denisovan populations were deep (relative to modern humans). Another study also observed deeply diverged Denisovan lineages and estimated the split time between D2 and the Altai Denisovan population around ~409kya and between D1 and the Altai Denisovan population around ~222kya[16].

Both studies[11,16] find that two divergent Denisovan populations introgressed with modern humans, although their estimates for the split times are slightly different, possibly owing to differences in the approaches used and the data sets analysed, including the 317 newly published genomes[16] that were not available in the earlier study[11]. Other differences were in the classification of introgressed segments as Denisovan, the metric used to measure mismatch, as well as the demographic models used to run simulations. This further highlights the need to characterise Denisovan introgressed segments with the same approaches.

Timing of Denisovan populations introgression with modern humans

Several studies have tried to infer the timing of Denisovan introgression events (Fig. 2). Jacobs *et al.* found that the two source populations (D2 and D1) introgressed into ancestral Papuan populations around 46 and 30kya respectively[11]. While Choin *et al.* also found evidence for two Denisovan introgression events into Papuans[16], their estimates of when these populations introgressed with modern humans are different in that, unlike Jacobs *et al.*, they find that the most diverged (from the sequenced Altai Denisovan) lineage (D2) introgressed more recently (25kya) than the lineage (D1, 46kya), which uniquely introgressed into Papuans. Choin *et al.* proposed that the ancestors of Papuan-related groups introgressed first with a Denisovan-like population that is more similar to the Altai Denisovans and subsequently with a more divergent

Denisovan population. The introgression of D1 at 46 kya could have occurred before the colonization of Sahul[18] - the paleocontinent comprising present-day Australia, Tasmania, and New Guinea, along with the land bridges connecting them during the Pleistocene - while the second introgression (around 25 kya from D2) occurred probably after modern humans arrived to Sahul[16].

So far, only one study has provided a direct estimate of when the East-Asian-specific Denisovan lineage (D0) introgressed into ancestral populations of East Asians, which based on tract lengths of Denisovan ancestry in Tibetan individuals at the EPAS1 gene inferred an introgression time of ~48kya[19]. This is consistent with studies of ancient genomes sequenced from 40,000-year-old remains that already exhibited Denisovan ancestry at that time[20].

Choin *et al.* obtained an indirect estimate of the timing of the Denisovan gene flow (D0) into East Asians (~21kya[16]), but this might be too recent, considering that ancient East Asians from around 40kya were already known to have Denisovan ancestry. However, unlike their estimates of when introgression from D1 and D2 happened (using information from introgressed tract lengths), the timing of the D0-introgression event was obtained using fastsimcoal2[21], which uses site frequency spectra to infer parameters of a given demographic model. Choin *et al.* included a Denisovan introgression into East/Southeast Asians in their demographic inference to see how this event affects their estimates of other demographic parameters and not necessarily to provide a direct estimate of Denisovan introgression into East Asians[16].

A second Denisovan lineage that is more diverged from the sequenced Altai Denisovan also contributed to the ancestor populations of East Asians[10], but when this occurred or how closely related it is to the other Denisovan lineages that introgressed into Papuans (D1, D2) remains an open question. However, it has been suggested that D2 might have contributed to both East Asians and Papuans, based on the fact that a subset of introgressed tracts in East Asians and Papuans exhibit similar levels of matching to the sequenced Denisovan[11]. Looking at shared tracts, present in both Papuans and East Asians, might help to determine whether the second Denisovan lineage that introgressed into East Asian populations was more similar to D1 or D2.

Insights from ancient DNA of modern humans

While most of what we know about Denisovans comes from studying present-day humans, a few studies have used ancient DNA to study Denisovan ancestry in ancient humans (Supplementary Table 2). For instance, based on sequenced DNA from a ~34kya female skull cap unearthed from Salkhit valley in Mongolia, introgressed segments in this individual could

be inferred[20], as well as in a ~40kya individual from Tianyuan Cave in China[22], and in two North Siberian individuals[23] (31kya Yana 1, Yana 2). In the Salkhit individual, 18 segments of Denisovan ancestry longer than 0.2cM were identified, inferring that Denisovan ancestry was 7.5% of the Neanderthal ancestry detected, similar to what was observed for the Tianyuan individual (8.1%)[22]. The two Yana individuals have a smaller proportion of Denisovan to Neanderthal ancestry (3.9% and 4.7%)[23], suggesting that the Salkhit and Tianyuan individuals had more Denisovan ancestry than the North Siberian individuals.

Moreover, Denisovan ancestry identified in these early humans was also compared with Denisovan ancestry identified in present-day humans (e.g. other East Asian populations and Papuans) and it was found that these segments derive from the same Denisovan admixture event(s) that contributed to present-day mainland Asians but are distinct from the Denisovan DNA segments in present-day Papuans and Aboriginal Australians[20]. This suggests that the D0 Denisovan lineage (Fig. 2) was one of the contributors of Denisovan ancestry into early humans from East Asia, providing a lower bound of when introgression from this Denisovan lineage into East Asians occurred (~40kya).

Evolution of Denisovan variants in modern humans since the introgression event

Introgression with Denisovans introduced new genetic variation that was either deleterious, neutral or adaptive. Furthermore, since the initial introgression events, Denisovan variants have continued to evolve within modern humans, and been shaped by the demographic history of modern human populations, as well as targeted by natural selection. For example, analyses of human genomes show that Archaic Introgressed Haplotypes (AIH) are not distributed equally[9]; there are both regions with dense archaic haplotypes, as well as deserts of archaic introgression that span regions longer than 10Mb, with an archaic ancestry proportion of less than 0.001[9]. In Oceanians, who harbour one of the largest proportions of Denisovan ancestry, 63 Denisovan deserts have been observed[9]. The presence of AIH is reduced in genomic regions close to functional elements, and the X chromosome is almost empty of both Denisovan and Neanderthal introgression[24–26]. The empirically observed reduction in archaic ancestry near functionally important regions could be explained by a more significant load of weak deleterious alleles in Denisovans and Neanderthals. Simulation studies have shown that, after the introgression event, negative selection quickly removes deleterious archaic variation in the recipient population[26–30]. As Denisovans exhibit lower heterozygosity and lower inferred effective population sizes relative to modern humans[5], Denisovans may have harbour a larger proportion of deleterious variation and after introgression, these variants were subsequently removed by negative selection.

After the removal of deleterious variants by negative selection, demographic factors, such as population expansions, admixtures and population size changes, continued to shape the patterns of Denisovan variation in modern human populations. Indeed, the amount of Denisovan genome that can be recovered from a sample of individuals changes as a function of sample size[31]. By studying patterns within human populations from the 1000 Genomes Project (1000GP)[32], the amount recovered is assumed to be highest in a sample of East Asian individuals because, at the individual level, an East Asian genome has slightly more Denisovan ancestry than a South Asian genome. However, with increased sample size, surprisingly, more of the Denisovan genome can be recovered from a sample of South Asians genomes than an equal-sized sample of East Asian genomes[31]. This means that each additional South Asian genome is more likely to contribute new Denisovan variants than an additional East Asian genome. This is unexpected because we expect most of the variation to be shared amongst human populations, but this is not true for Denisovan variants, as South Asians harbour a large proportion (~45%) of these variants that are not present in East Asian populations[31]. In contrast, Neanderthal variants present in South Asian populations are more likely to be present in other Eurasian populations; only 13.7% of Neanderthal variants in South Asian populations are private. Although a set of South Asian genomes will yield more Denisovan variants (44.7%) than a set of East Asian genomes (27.4%), these Denisovan alleles tend to have lower frequencies in South Asian populations. It has been proposed that this pattern may be explained by the demographic history of these populations, which has been hypothesised as admixtures between a set of ancestral populations with founder events[31]. An alternative explanation could be larger contributions from distinct Denisovan populations in the history of South Asian populations[31].

More recently, over 2700 high-coverage genomes from 18 states in India were sequenced[33] and HMMmix[34] was applied to ~2600 of those, as well as to the 1000GP[32] and Human Genome Diversity Project (HGDP)[35] datasets to detect archaic fragments. By calculating the amount of archaic sequence shared between Indians and other worldwide populations, Oceanians and South Asians (Indians in this study) were found to possess the largest amounts of unique Denisovan sequences[33]. This also held true after downsampling the Indian dataset to a number comparable to the 1000GP and HGDP datasets[33].

Other factors, such as the genomic structure of specific regions of the genome (e.g. exon density, recombination rate) and dominance of deleterious mutations, can also shape patterns of archaic introgression. For instance, archaic haplotypes can increase in frequency under the right evolutionary parameters, even if these haplotypes contain deleterious alleles[36]. One example is heterosis from recessive deleterious variants that are exclusive to a population before introgression[36]. After introgression, hybrid individuals no longer have two copies of the

recessive mutations, and these haplotypes can then increase in frequency in the populations, which could lead to a pattern in genomic data that resembles adaptive introgression. Indeed, recessive deleterious mutations have been shown to raise the false-positive rates of tests for adaptive introgression compared to models without deleterious variants, mainly when the recombination rates are low[36]. However, the heterosis effect is not sufficiently strong to explain the patterns of adaptive introgression in the vast majority of candidate regions in humans. For example, frequency of the Denisovan-like haplotype in the *EPAS1* gene (see below), which has been shown to be adaptively introgressed from Denisovans into Tibetans, cannot be explained by heterosis[36].

Adaptive Denisovan haplotypes in human populations

Ancestors of Denisovans and Neanderthals expanded into Eurasia thousands of years before modern human populations. Indeed, by the time modern humans expanded into Eurasia, Denisovans had already adapted to a wide array of environments, from cold temperatures and high altitudes to lowlands and perhaps even humid tropical regions. Therefore, interbreeding between modern humans and Denisovans provided an opportunity to introduce putative adaptive variants to accelerate human adaptations.

Several methods have been developed to scan the genome for detecting adaptive introgression: genomatnn[37], Rd, U, Q95[38], VolcanoFinder[39] and MaLAdapt[40]. Currently, there are fewer examples of adaptive introgression from Denisovans compared to Neanderthals[41,42] (Fig. 4 and Supplementary Table 4). However, as populations that harbour Denisovan ancestry tend to be more underrepresented in genetic studies, it is likely that further instances of adaptation will be identified as the number of sequenced genomes from different geographical locations increases. Below, we provide a description of Denisovan adaptively introgressed genes.

Hypoxia tolerance

In 2014, we investigated the *EPAS1* locus[43], which is related to hypoxia tolerance in Tibetans[44]. *EPAS1* is a transcription factor induced under hypoxic conditions, and it represents the gene with the strongest signal of Tibetan-specific selection. We could show that the source of adaptation was likely to be caused by the introduction of genetic variants from archaic Denisovan-like individuals, specifically those closely related to the Denisovan individual from the Altai Mountains, into the ancestral Tibetan gene pool[43]. More recently, the Tibetan-*EPAS1* haplotype has been shown to originate from the East Asian-specific Denisovan introgression event[19], where it remained selectively neutral for a long time in the population before positive selection occurred; this may be concurrent with the permanent inhabitation of the Tibetan

Plateau after the Last Glacial Maximum[19]. The authors also proposed that this contribution stems from a Denisovan population that was closely related to the Siberian Altai Denisovan, supported by the absence of this signal in samples from Papua New Guinea (PNG), who have been shown to have genetic ancestry from a more divergent Denisovan population[19].

Immunity

Traits related to the immune system have also been frequently described as targets of adaptive introgression. For instance, 126 high-frequency archaic haplotypes have been identified as putative targets of adaptive introgression in geographically diverse populations, with these loci are enriched for immune-related genes (such as *OAS1/2/3*, *TLR1/6/10*, and *TNFAIP3*)[45]. This showed that hybridization between modern and archaic hominins provided an important reservoir of advantageous alleles that enabled adaptation to out-of-Africa environments. *TNFAIP3* was also reported to be adaptively introgressed from Denisova in East ISEA, Papuans, and Baining populations[11].

Another immune gene, *MUC19*, exhibits signals of positive selection in admixed American populations (MXL) from the 1000GP[32], and the beneficial haplotype is Denisovan-like origin. *MUC19* is associated with human gland function, which can be essential in immunity response, particularly its role in mucus formation in tracheal submucosal glands. Like *EPAS1*, Denisovan-like variation in *MUC19* likely facilitated adaptations to new environments (in the Americas) long after introgression, highlighting the evolutionary potential of retaining archaic genetic variation. Interestingly, unlike the Denisovan-like populations that introduced *EPAS1*, the Denisovan population source is more divergent to the Altai Denisovan[46].

A more recent study analysed data from the UK Biobank and Biobank Japan to understand the impact of archaic DNA in the genomes of PNG populations[47]. The authors found four Denisovan-like haplotypes within the Major Histocompatibility Complex (MHC) that showed substantial pleiotropy. These haplotypes, 19 of which are from Denisovan, exhibited significantly higher Fst values (a measure of population differentiation due to genetic structure) compared to non-MHC counterparts, indicating greater genetic diversity in this region. Denisovan-like haplotypes also had higher median and mean Fst levels compared to non-archaic MHC SNPs, a trend not seen in Neanderthal-like haplotypes[47]. Finally, a region containing the immunity-related GBP locus, associated with protective effects against pathogens, has been identified. This shows the highest selection peak among lowlanders from PNG and exhibits sequence similarities to both Denisovan and Altai Neanderthal genomes[48].

Metabolism

Another example of adaptive introgression is related to lipid metabolism in Inuit from Greenland, which have a highly divergent haplotype in the *TBX15/WARS2* region that was probably introduced into the modern human gene pool via introgression with Denisovans[49]. *TBX15* is T-box family transcription factor and a highly pleiotropic gene expressed in multiple tissues at different stages of development; it plays a role in the differentiation of brown and brite adipocytes[50]. These adipocytes produce heat via lipid oxidation when stimulated by cold temperatures, making *TBX15* a strong candidate gene for adaptation to life in the Arctic. The selection affecting the *TBX15/WARS2* haplotype is relatively old, resulting in a high allele frequency in Native American populations and intermediate allele frequencies in East Asia. The introgressed sequence is more closely related to the sequenced Denisovan genome than the Neanderthal ones[49]. The selection signal associated with the *WARS2* gene was also seen in populations from East ISEA[11]. The authors also found another signal related to lipid metabolism adaptation in the *WDFY2* gene, which is involved in endocytosis and adipogenesis suggesting adaptation to different types of diets[11].

Functional consequences of Denisovan introgression

Several examples of positive selection acting on variants introgressed from Denisovans have been presented above, but we still need to learn more about their functional significance.
For instance, Gray *et al.*[51] studied the functional mechanism of noncoding alleles in *EPAS1*, which is responsible for the hypoxia tolerance in Tibetans and is introgressed from Denisovans. They investigated the activity of this gene in different cell types and found that high-altitude alleles disrupt the activity of four *EPAS1* enhancers in one or more cell types.
In the same year, another study[52] examined 56 genomes from Papuan individuals, which had previously been published in 2019[11]. This study aimed to investigate the role of Neanderthal and Denisovan introgressed alleles at the functional level. The authors analysed these variants across various cell types and functional chromatin states, validating earlier findings that archaic SNPs are predominantly located within non-coding sequences[52]. Notably, they identified differences between the Neanderthal and Denisovan ancestries in their patterns of introgression. Specifically, they observed a significant Denisovan-specific enrichment with Cis-Regulatory elements (CREs) that are active within immune cells, suggesting a more pervasive contribution of Denisovan introgression to gene regulatory processes occuring within immune-related cells. In fact, only genes predicted to be regulated by Denisovan SNPs were found to be closely associated with active immune responses[52].
Moreover, Rong and colleagues[53] performed Massively parallel splicing assays (MaPSy) to identify exonic splicing mutations between modern and archaic humans, both Neanderthal and Denisovan. Owing to the use of this technique, stronger splicing effects were found in

archaic humans compared to modern humans, probably due to the fact that purifying selection is more efficient in modern humans because of a larger long-term effective population size. This work also showed that adaptively introgressed variants are enriched in moderate effect splicing variants compared to all introgressed variants[53].

Another study investigated the pattern of alternative splicing in archaic genomes using SpliceAI, a machine-learning algorithm that infers splice-altering variants (SAVs)[54]. This approach showed that Denisovan had the most unique SAVs and also an enrichment for SAVs in genes associated with different skeletal and skeletal muscle system traits. Interestingly, the authors identified two candidate SAVs at the *EPAS1* locus; one variant, rs372272284, is fixed only in the Denisovan and is introgressed in East Asians. This variant has also been shown to be associated with decreased hemoglobin levels in Tibetans[55].

New research directions

In a relatively short time, we have moved from having identified a new hominin group to now having a more detailed history of the now-extinct Denisovans and their impact on modern human populations. However, much more remains to be uncovered about Denisovans, and there are many exciting avenues of research that involve both empirical analyses of genetic data and the development of new methods.

Denisovan ancestry in understudied populations

Studies into how Denisovan ancestry is distributed amongst human populations have focused on Oceanians and ISEA populations mainly because these populations harbour the largest amount of Denisovan ancestry, but investigating Denisovan ancestry in other populations will provide clues to the Denisovan sources that have contributed to the modern human gene pool and how Denisovan variants have continued to evolve in modern humans since their introgression. For example, it was shown that while a single East Asian individual harbours slightly more Denisovan ancestry compared to other Eurasian populations, more of the Denisovan genome can be recovered from a sample (of equal size) of South Asian individuals[31]. South Asian populations also harbour a larger proportion of private Denisovan variation compared to other Eurasian populations[31,33], suggesting that they may have ancestry from another Denisovan source. Therefore, studying patterns of Denisovan introgression specific to these populations may reveal new insights into Denisovans, but it will require the development of better demographic models that reflect features of population-specific demographic histories and gene flow from Denisovans.

Studying Denisovan variation in humans from other geographical regions might provide further evidence that Denisovan introgression likely facilitated adaptations from standing Denisovan introgression because modern humans continued to expand and occupy new environments after introgression happened (e.g. *EPAS1* and *MUC19*).

Neanderthal populations also had some Denisovan ancestry, providing evidence of Denisovan gene flow into Neanderthals[56,57]. For example, fine-scale analysis of the adaptively introgressed gene *MUC19*, suggests that it was late Neanderthals who introduced the haplotype into Eurasian populations, and Neanderthals themselves may have acquired it from Denisovans[46]. As more Neanderthal and Denisovan genomes are sequenced, it will be possible to characterize the extent, timing and impact of these admixture events. It has also been proposed that a more divergent archaic population introgressed into Denisovans, and modern humans may have inherited some genetic ancestry from this ghost archaic population by interbreeding with Denisovans. Identifying traces of a ghost archaic introgression in modern humans will be needed to support these hypotheses.

Development of new methodologies

An important research avenue is the development of new methods to jointly infer archaic ancestry from multiple archaic populations (e.g. Denisovans, Neanderthals, and ghost archaic populations) in modern humans. Most methods use one of the archaic genomes to detect ancestry from a single archaic population, but jointly inferring ancestry from several archaic populations will improve the identification of segments originating from Denisovans or Neanderthals. This is important because many analyses of the genetic history of Denisovans depend on correctly identifying a segment as being from Denisovans and not Neanderthals. However, studies make several arbitrary filters and post-processing steps[10,11,16] to make these distinctions, and thus more standardised approaches are needed. We also need to improve our understanding of the expected genetic relationships between inferred Denisovan segments (in modern humans) and the sequenced Denisovan individual. For example, a recent study found that most of the archaic segments inferred in a set of Papuan individuals have lower sequence divergence to Neanderthals than the sequenced Denisovan[47]. This finding is striking because Papuans presumably have more genetic ancestry from Denisovans (~4%) than Neanderthals (~2%), so we would expect a larger number of the introgressed segments to be closer to the sequenced Denisovan. One interpretation is that the actual Denisovan donor is not closely related to the sequenced Denisovan individual, but we need to gain a better understanding of the genetic relationship between an actual Denisovan donors and both Neanderthal and Denisovan individuals, for whom we have genomic data.

Moreover, in places like the Americas, a large proportion of present-day genomes have traces of recent admixture events with Europeans and Africans, and developing methods that can jointly infer archaic ancestry, as well as recent genetic ancestry from European, Indigenous Americans and African populations, will enable better estimates of introgression in these admixed populations. Deconvoluting Denisovan and recent modern human genetic ancestry will also enable us to study whether Denisovan ancestry in the Americas originated from an Indigenous American or European ancestor and to study the genetic relationship of Denisovan segments in genomes from the Americas to the Denisovan segments identified in other populations.

Another exciting research avenue is integrating and incorporating ancient genomes from Eurasia and the Americas, which may provide a more accurate representation of how Denisovan genetic ancestry evolved in humans over the last 40,000 years. One challenge is that most ancient genomes are only sequenced at low coverage leading to a large amount of missing data; therefore, developing methods that can account for missing data will be useful to better infer archaic segments in ancient genomes better. Utilising ancient genomes will be informative with regard to genetic drift, timing, and magnitude of both introgression and natural selection. Determining these parameters will enable us to perform fine-scale analyses to characterise the evolutionary histories of adaptively introgressed regions, and to specify the mode of natural selection to answer the question of whether Denisovan variants were immediately beneficial or if positive selection acted on introgressed Denisovan variants.

Recently, imputation of missing genotypes in ancient genomes using reference panels composed of contemporary human genomes has been shown to be a promising strategy[58]. Whether we can indeed infer Denisovan ancestry in imputed genomes is an open question, and benchmarking studies to quantify this are necessary. Creating new software tools that can be used to study and test methods to detect introgression is also needed. For example, tools that can rapidly identify introgressed segments (and what donor they come from) in simulated data under complex demographic models will be useful for standardisation as this will help test new methods. Current tools to study the effects of phasing and imputation of ancient DNA[59] should also be extended to incorporate archaic humans. Now that we have access to so many contemporary human genomes (that harbor Denisovan genetic ancestry), we can use them as reference panels to test how much of the Denisovan genome we can phase and impute. Phasing Denisovans and Neanderthals might also help identify regions of the genome where Denisovans have Neanderthal ancestry and vice versa[46].

Integrating genetic and archaeological data

Currently, we only have access to a single Denisovan genome, and obtaining more DNA from Denisovans, from populations not closely related to the Altai Denisovan, would help solidify and expand our inferences from surviving Denisovan segments in modern humans and could provide further evidence of Denisovan presence in diverse environments and a wide geographical range. For example, Denisovan DNA obtained from sediments in the Baishiya Karst Cave, showed that Denisovans had occupied high-altitude plateaus for a really long time (at least from ~160 to ~45 kya)[13], suggesting their geographical range extended well beyond Siberia and that they may have been adapted to live at high altitude. Denisovan genomes would also help to characterise the genetic diversity and genetic continuity of Denisovan populations, such as how often they interbred with Neanderthals (or other ghost archaic populations), and infer evolutionary parameters including the timing of introgression and divergence times between distinct Denisovan populations.

Such access to additional Denisovan genomes may come from museum collections and archaeological field research. The Denisovan fossil record remains extremely sparse, and museum collections may already house unidentified fossil remains that could in fact be from Denisovans. Although genetically, Denisovans are closest to Neanderthals, it is unclear whether this genetic similarity translates to shared morphological features. Based on a mandible and few molars, it has been suggested that fossils from Xuchang, Xujiayao or Tam Ngu Hao 2 may be of Denisovan origin[14,60,61]. We also need to establish more field excavations of archeological sites, which may yield more Denisovan fossil remains and other artifacts that will complement and contextualize insights from genetic data.

Concluding remarks

In summary, in order to construct a comprehensive history of who Denisovans were, their relationships to other hominins, and their impact on modern human populations will require integrating multiple types of data, developing new methods and fostering interdisciplinary collaboration with archaeologists, museum curators, anthropologists, evolutionary computational and molecular biologists.

Acknowledgements

L.O. and E.H.S. are supported by the European Research Council (ERC), Horizon 2018 Starting Grant ERC-2018-STG, no. 804994.

Author contributions

E.H.S conceived the manuscript. L.O. and E.H.S wrote the manuscript.

Competing interests

The authors declare no competing interests.

Figures

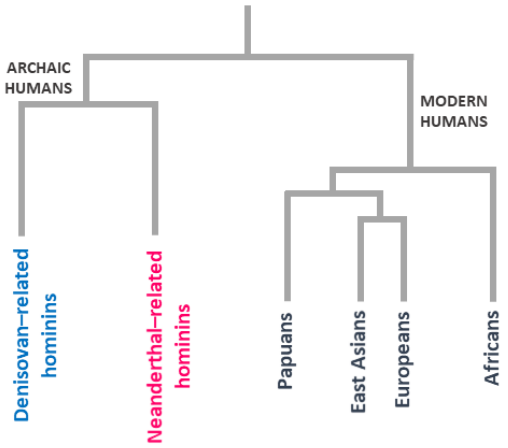


Fig. 1: Archaic and modern human phylogenetic tree. Simplistic representation of the phylogenetic relationships between Archaic hominins and modern humans based on autosomal DNA data.

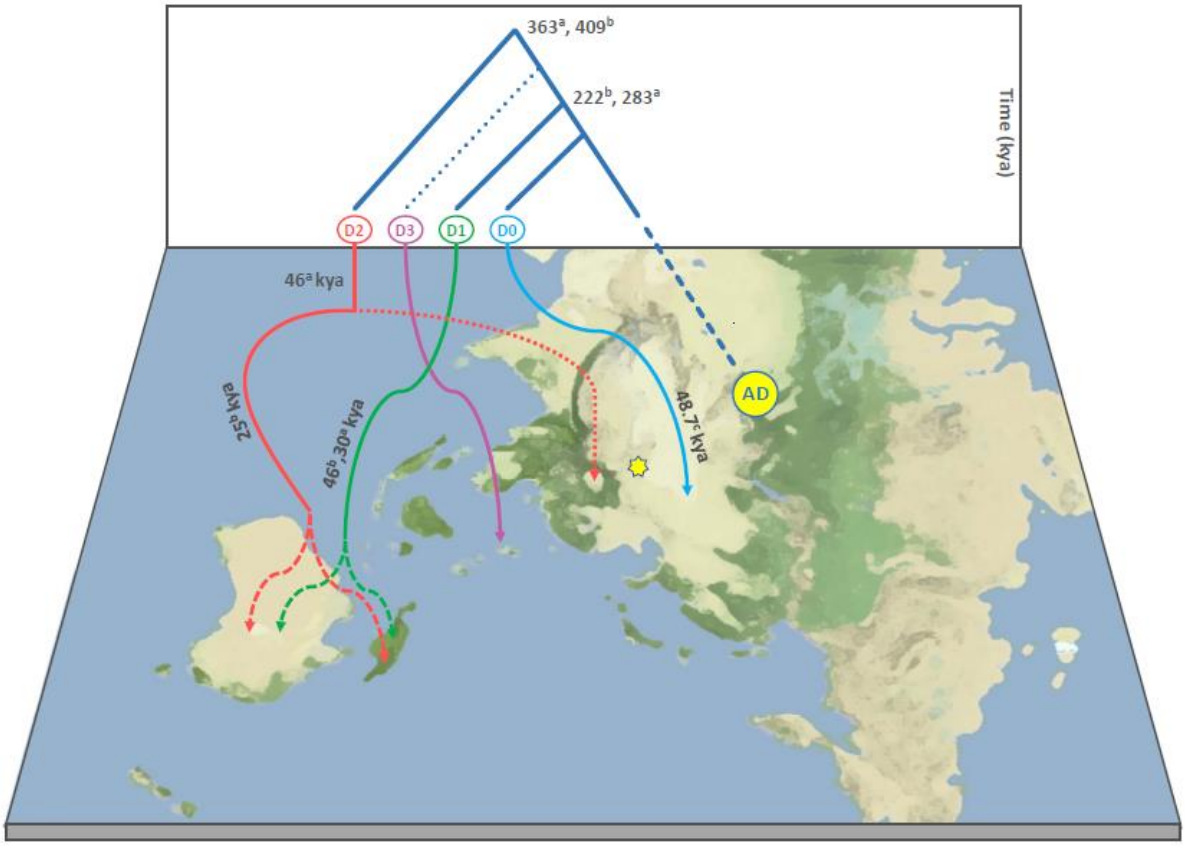


Fig. 2: Overview of the distinct Denisovan populations that introgressed into modern Humans. AD marks the Altai Denisova cave, the source of the Denisovan fossil record. The yellow star marks Baishiya Karst Cave on the Tibetan Plateau, where Denisovan DNA was

found in late Pleistocene sediments. Times on the tree indicate the split period of the different Denisovan populations, whereas times on the arrows display when that Denisova population introgressed into Modern Humans as inferred from previous studies (a: Jacobs *et al.*[11], b: Choin *et al.*[16], c: Zhang *et al.*[19]). All the estimates were inferred using track length. The arrows indicate in which populations and areas a particular Denisovan population has been found to have introgressed. A red dotted line is used to indicate an uncertain connection, such as the introgression date of D2 in East Asia. A blue dotted line is used to indicate the split between the ancestors of D3 and Altai Denisova. Additionally, dashed lines represent the presumed final geographic locations of the spread of D1 and D2 into Papua New Guinea and Australia. The map has been taken from ggmap[62].

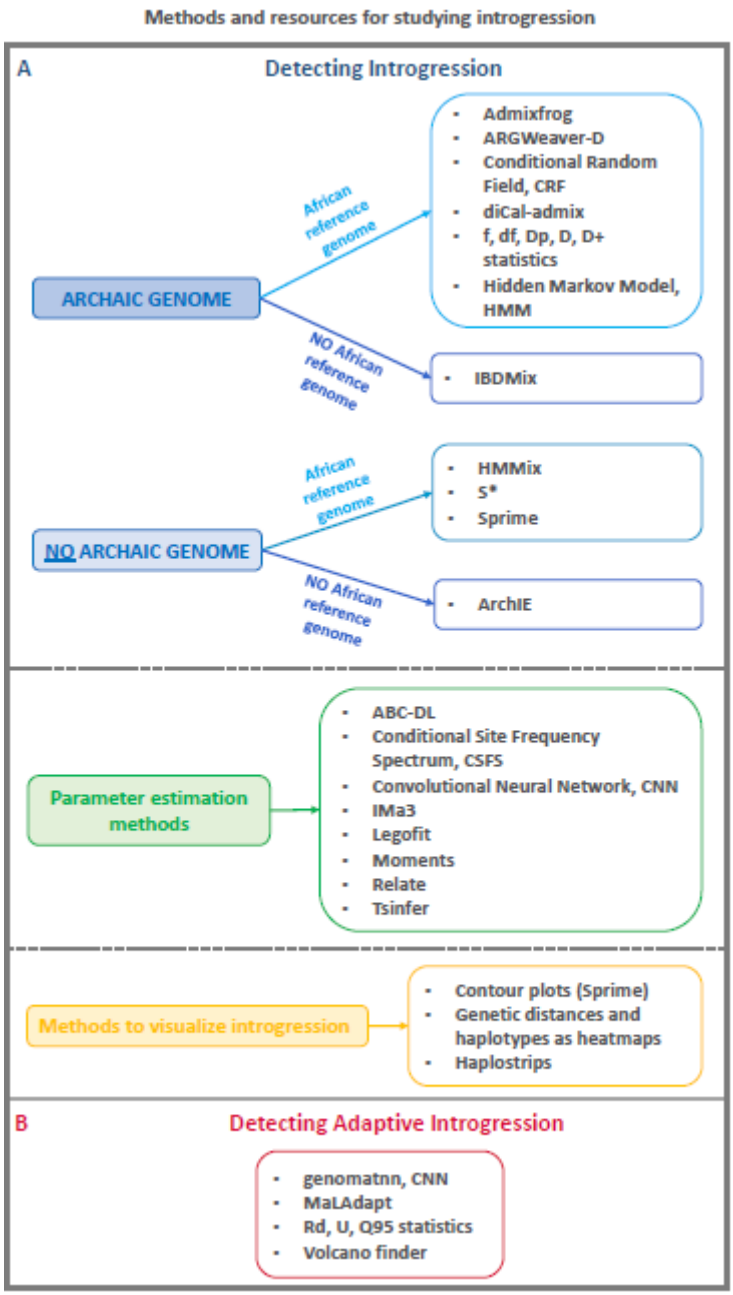


Fig. 3: Methods and resources to study introgression. Shown here is an overview of the main available approaches for the detection of introgression events and adaptive introgression. Some of these need an archaic genome as a reference and/or an unadmixed reference genome, such as an African genome, whereas other do not require either either. Other methods are employed to estimate the parameters and then used to detect introgression. Citations and links for each method are presented in Supplementary Table 3.

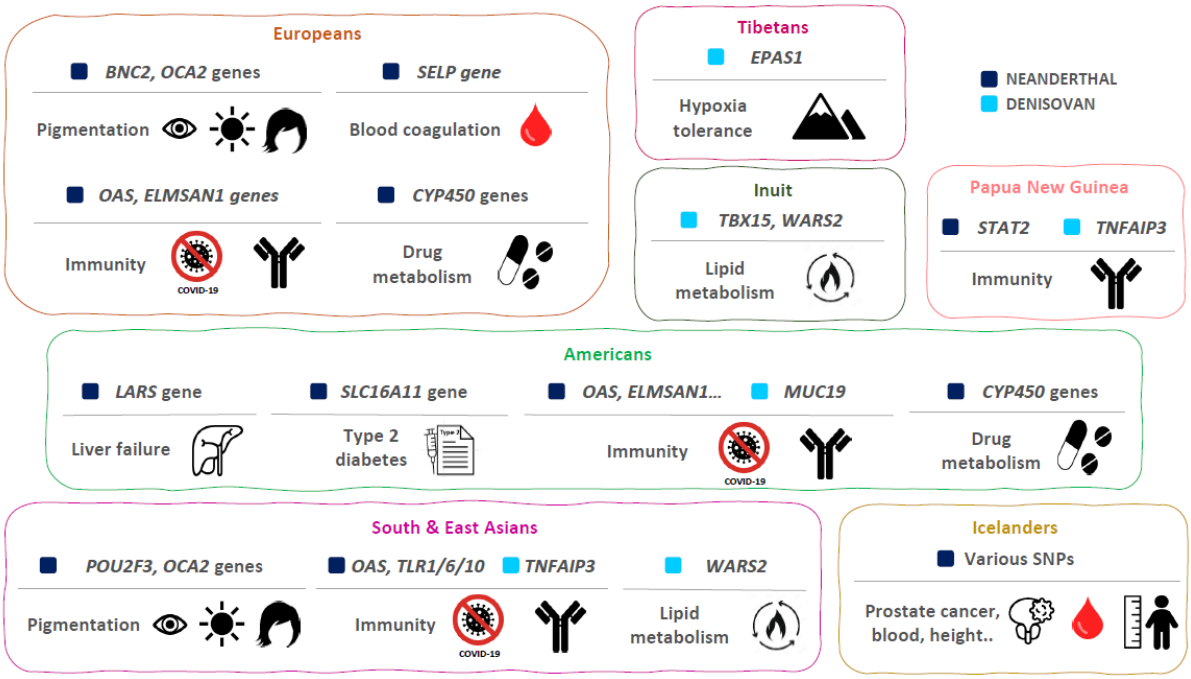


Fig. 4: Archaic traits adaptively introgressed in modern populations. A selection of the most important traits that have been identified as adaptively introgressed from Archaic Humans is subdivided by the main population group in which they have been described. This figure shows traits adaptively introgressed from Denisovans and Neanderthals to provide a more comprehensive summary, while in the text, we focus only on the Denisovan ones. Specific citations can be found in Supplementary Table 4.

BOXES

BOX 1. Insights from Denisovan remains

Here, we provide a brief summary of Denisovan remains. For a more comprehensive review, please refer to Douka et al. (2019)[63] and Peyregne et al. (2024)[64]. The Altai mountains in southern Siberia represent one of the most significant areas of the world's Middle and Upper Paleolithic cultural history. The Denisova cave is one of the most well-known caves in that region, mainly because of the finding of a phalanx fragment in 2008. This fossil, named Denisova 3, was discovered in layer 11 together with other specimens and various stone tools.

From its mitochondrial and nuclear DNA analysis, a previously unknown archaic hominin group was identified and named Denisovan[2]. Since this, the cave has been subjected to many excavations. It contains a rich stratigraphic record allowing archaeologists to find multiple remains belonging to archaic hominins (Supplementary Table 1). It is the only site where the presence of both Denisovans and Neanderthals has been determined based on DNA recovered from both fossils and cave sediments in several layers. In addition, the presence of early modern humans has recently been confirmed at the site based on mitochondrial DNA (mtDNA) recovered from sediments[12,65].

Moreover, in 2018 the genome extracted from a bone fragment belonging to a female found in layer 12.3 of the East Chamber of Denisova cave, Denisova 11[66], was analysed and showed that proportionally her alleles matched equally to the sequenced Neanderthal and Denisova genomes suggesting that she was a first generation hybrid[67]. As Denisova 11 carries a Neanderthal mtDNA, it is inferred that the mother was closely related to the sequenced Neanderthal. The father was a Denisovan bearing some traces of Neanderthal ancestry in his genome, suggesting possible admixture between Neanderthal and Denisovan populations in the past. This discovery suggests that mixing between Late Pleistocene hominin groups could have been common in regions of geographical overlap.

More recently, five new hominin bones were identified in the Denisova cave, and the mtDNA of four of them was analysed[65]. Three were found in layer 15 of the cave (Denisova 19, 20 and 21) and carry Denisova mtDNA, while the remaining was found in layer 12 and carries Neanderthal mtDNA (Denisova 17). Using BEAST, the authors estimated that the molecular age of the mtDNA of Denisova 17 is ~134 ka. The molecular age of Denisova 19, 20 and 21 have been estimated to be ~187 ka, making them the oldest Denisovans currently reported[65].

The presence of Denisovan hominins was recently confirmed in another cave, the Baishiya Karst Cave (BKC) in China, a limestone cave located on the north-eastern edge of the Tibetan Plateau. A mandible, dated at least 160 thousand years old, was found and identified as of Denisovan origin based on ancient protein analysis[68]. In a more recent study, the authors retrieved mitochondrial DNAs from sediments from different cave layers and reconstructed phylogenetic trees using previously published mtDNAs from the Denisova cave and the individual from Sima de Los Huesos[13] (an ancient hominin living approximately 430kya in Spain). They showed that Denisovans probably occupied BKC at ~100 and ~60 ka, confirming that they were widely distributed in Asia during that period[13]. These data provide direct proof of Denisovans inhabiting regions outside the Altai Mountains.

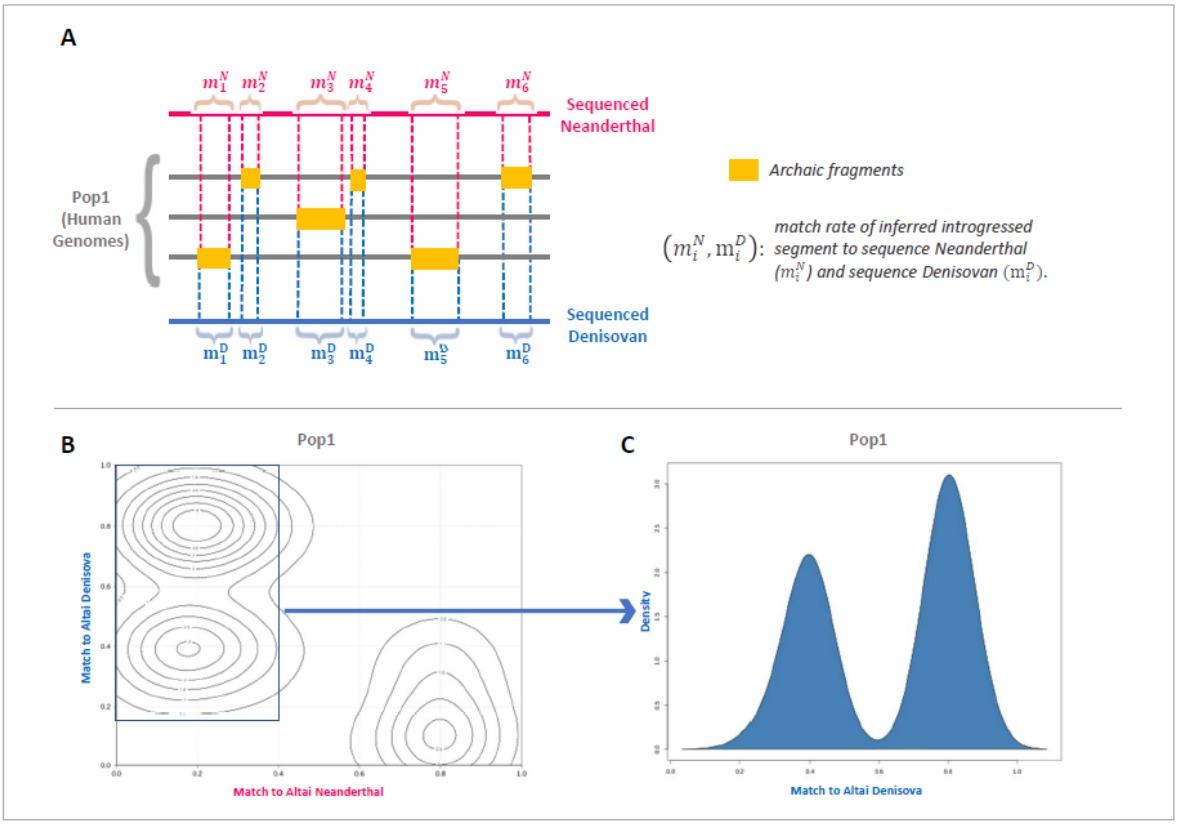
Since the original Denisova discovery, various fossils have been classified as potential Denisovans based on morphological characteristics but, unfortunately, without molecular evidence. Fossils from Xuchang or Xujiayao[60] exhibit a similar jaw morphology to the Xiahe mandible. Similarly, a specimen from Laos (Tam Ngu Hao 2)[14] shares dental morphology

resembling that of the Xiahe specimen, leading to the hypothesis that they belong to the same taxon and could potentially be identified as Denisovans.

BOX 2. Using match rate distributions to detect the number of introgression events and the number of distinct Denisovan populations

There are several methods to infer introgressed segments in modern humans (see Fig. 3 and Supplementary Table 3). Some of these methods use an archaic reference genome, and some can identify archaic segments without an archaic reference genome (e.g Browning *et al.*[10], Skov *et al.*[25]). For methods that do not use an archaic reference, one can gain information on the origin of an introgressed segment by comparing it to the available sequenced archaic humans. For instance, Browning *et al.* detected archaic segments in human genomes, and for each inferred segment they computed the match rate to the sequenced Altai Denisova (m^D) and Altai Neanderthal (m^N, see panel A of the box figure)[10]. The data reported in the box figure are made up to give an example. Visualising the joint distribution of m^D and m^N (see panel B) reveals information about how closely matched the inferred introgressed segments are to the sequenced Denisovan and Neanderthal segments. We do not expect the inferred segments to be identical to the Denisovan ($m^D \neq 1$) nor the Neanderthal ($mN \neq 1$) because these sequenced archaic humans are not likely actual members of the populations that interbred with modern humans in the past. However, a few studies[10,11] have now shown that most introgressed segments in Eurasian populations have a high affinity to Neanderthal ($mN \approx 0.8$), suggesting that most surviving introgressed segments likely entered humans through interbreeding with Neanderthals. Some populations such as East Asians do show some traces from more than one Denisovan population. Specifically, the contour plot (panel B) shows that some segments have low affinity to Neanderthal ($mN < 0.4$) and high affinity to Denisovan ($mD > 0.5$), and plotting the distribution of match rates (to the sequenced Denisovan) for those segments shows that the match rate distribution to Denisovans is bimodal (panel C). This suggests that some segments come from a Denisovan population that is more closely related to the sequenced Denisovan (segments with $mD \approx 0.8$), while others come from a Denisovan population that is more distantly related to the sequenced Denisovan (segments with $mD \approx 0.4$).

To infer when other Denisovan populations split from the Denisovan population the Altai Denisovan belonged to, a few studies have leveraged the observed distribution of match rates to Denisovan. For example, under a demographic model for Papuans[11,16], the match rate distribution can be simulated and then fit to the observed distribution to estimate the parameters of the split time. Actual estimates of when different Denisovan lineages split from the Altai Denisovan lineage are shown in Fig. 2.


A
Sequenced Neanderthal
Pop1 (Human Genomes)
Archaic fragments
match rate of inferred introgressed segment to sequence Neanderthal and sequence Denisovan.
Sequenced Denisovan
B
Pop1
Match to Altai Denisova
Match to Altai Neanderthal
C
Pop1
Density
Match to Altai Denisova

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Supplementary material

Individual ID	Location	Cave	Hominin	Date (kya)	DNA	Type	Publication	Ref.
Baishiya Karst Cave	China	Baishiya Karst Cave	Denisova	> 160	mtDNA	Sediments	Zhang et al. 2020	1
Chagyrskaya8	Russia	Chagyrskaya Cave	Neanderthal	~80	WGS HighCov	Phalanx	Mafessoni et al. 2020	2
Denisova 11	Russia	Denisova cave	N-D	79.3–118.1	WGS	Long bone	Slon et al. 2018	3
Denisova 17	Russia	Denisova cave	Neanderthal	134.1–150.6	mtDNA	bone fragment	Brown et al. 2022	4
Denisova 19	Russia	Denisova cave	Denisova	187.0–216.9	mtDNA	bone fragment	Brown et al. 2022	4
Denisova 2	Russia	Denisova cave	Denisova	122.7–194.4	WGS	Tooth	Slon et al. 2017	5
Denisova 20	Russia	Denisova cave	Denisova	187.0–216.9	mtDNA	bone fragment	Brown et al. 2022	4
Denisova 21	Russia	Denisova cave	Denisova	187.0–216.9	mtDNA	bone fragment	Brown et al. 2022	4
Denisova 3	Russia	Denisova cave	Denisova	51.6-76.2	WGS HighCov	Phalanx	Meyer et al. 2012	6
Denisova 4	Russia	Denisova cave	Denisova	55.2–84.1	WGS	Tooth	Sawyer et al. 2015	7
Denisova 5	Russia	Denisova cave	Neanderthal	120-130	WGS HighCov	Phalanx	Prufer et al. 2014	8
Denisova 8	Russia	Denisova cave	Denisova	105.6–136.4	WGS	Tooth	Sawyer et al. 2015	7
ElSidron1253	Spain	El Sidron, Asturias	Neanderthal	~49	WGS	Bone	Green et al. 2010	9
Feldhofer1	Germany	Feldhofer Cave	Neanderthal	~42	WGS	Bone	Green et al. 2010	9
GoyetQ305_4	Belgium	Troisième Caverne	Neanderthal	43-45	mtDNA	Tibia	Rougier et al. 2016	10
GoyetQ305_7	Belgium	Troisième Caverne	Neanderthal	Unknown	mtDNA	Tibia	Rougier et al. 2016	10
GoyetQ374a_1	Belgium	Troisième Caverne	Neanderthal	Unknown	mtDNA	Tibia	Rougier et al. 2016	10
GoyetQ56_1	Belgium	Troisième Caverne	Neanderthal	43-42	WGS	Femur	Hajdinjak et al. 2018	11
GoyetQ57_1	Belgium	Troisième Caverne	Neanderthal	43-45	mtDNA	Tibia	Rougier et al. 2016	10
GoyetQ57_2	Belgium	Troisième Caverne	Neanderthal	40-41	mtDNA	Femur	Rougier et al. 2016	10
GoyetQ57_3	Belgium	Troisième Caverne	Neanderthal	42-43	mtDNA	Tibia	Rougier et al. 2016	10
LesCottesZ4_1514	France	Les Cottes Cave	Neanderthal	42-43	WGS	Tooth	Hajdinjak et al. 2018	11
Mezmaiskaya1	Russia	Mezmaiskaya Cave	Neanderthal	60-70	WGS	Skeleton	Hajdinjak et al. 2018	11
Mezmaiskaya2	Russia	Mezmaiskaya Cave	Neanderthal	43-44	WGS	Skull fragment	Hajdinjak et al. 2018	11
Okladnikov2	Russia	Okladnikov Cave	Neanderthal	30-40	mtDNA	Bone	Krause et al. 2007	12
Sima de los Huesos	Spain	Sima de los Huesos cave	Sima de los Huesos	~430	WGS	Bone	Meyer et al. 2014	13
Spy94a	Belgium	Spy Cave	Neanderthal	37-39	WGS	Tooth	Hajdinjak et al. 2018	11
Vi33.16	Croatia	Vindija Cave	Neanderthal	~38	WGS	Bone	Green et al. 2010	9
Vi33.25	Croatia	Vindija Cave	Neanderthal	Unknown	WGS	Bone	Green et al. 2010	9
Vi33.26	Croatia	Vindija Cave	Neanderthal	~44	WGS	Bone	Green et al. 2010	9
Vindija 33.19	Croatia	Vindija Cave	Neanderthal	~50	WGS HighCov	Bone	Prufer et al. 2017	14
Vindija 87	Croatia	Vindija Cave	Neanderthal	>44	WGS	Bone fragment	Hajdinjak et al. 2018	11

Supplementary Table 1. List of sequenced archaic individuals. Kya stands for kylo years ago.

Individual ID	Country	Location	Time Period	Date (kya)	DNA	Type	Publication	Ref.
AR33K	China	Amur River Basin	UP	33	Genome-wide	Bone fragment	Mao et al. 2021	16
Bacho Kiro AA7-738	Bulgaria	Bacho Kiro Cave	IUP	43	Genome-wide	Bone fragment	Hajdinjak et al. 2021	14
Bacho Kiro BB7-240	Bulgaria	Bacho Kiro Cave	IUP	44	Genome-wide	Bone fragment	Hajdinjak et al. 2021	14
Bacho Kiro BK-1653	Bulgaria	Bacho Kiro Cave	UPa	35	Genome-wide	Bone fragment	Hajdinjak et al. 2021	14
Bacho Kiro CC7- 2289	Bulgaria	Bacho Kiro Cave	IUP	43	Genome-wide	Bone fragment	Hajdinjak et al. 2021	14
Bacho Kiro CC7-335	Bulgaria	Bacho Kiro Cave	IUP	45	Genome-wide	Bone fragment	Hajdinjak et al. 2021	14
Cioclovina1	Romania	Peştera Cioclovina Uscată	UP	32	Genome-wide	Cranium	Fu et al. 2016	17
GoyetQ116-1	Belgium	Troisième Caverne	UPa	35	Genome-wide	Humerus	Fu et al. 2016	17
GoyetQ376-19	Belgium	Troisième Caverne	UP	27	Genome-wide	Humerus	Fu et al. 2016	17
GoyetQ53-1	Belgium	Troisième Caverne	UP	28	Genome-wide	Fibula	Fu et al. 2016	17
GoyetQ56-16	Belgium	Troisième Caverne	UP	26	Genome-wide	Fibula	Fu et al. 2016	17
Kostenki12	Russia	Volkovskaya	UPa	32	Genome-wide	Cranium	Fu et al. 2016	17
Kostenki14	Russia	Markina Gora	UPa	38	WGS	Tibia	Seguin-Orlando et al. 2014	18
Krems1-1	Austria	Krems-Wachtberg	UP	29	Genome-wide	Petrous	Teschler-Nicola et al. 2020	19
Krems1-2	Austria	Krems-Wachtberg	UP	29	Genome-wide	Petrous	Teschler-Nicola et al. 2020	19
KremsWA3	Austria	Krems-Wachtberg	UP	29	Genome-wide	Cranium	Teschler-Nicola et al. 2020	19
Les Cottes Z4-1514	France	Les Cottes	IUP/UPa	41	WGS	Tooth	Hajdinjak et al. 2018	11
Mal'ta	Russia	Mal'ta	UP	24	WGS	Humerus	Raghavan et al. 2014	20
Muierii2	Romania	Peştera Muierilor	UP	32-33	Genome-wide	Temporal	Fu et al. 2016	17
Oase1	Romania	Peştera cu Oase	IUP/UPa	40	WGS	Mandible	Fu et al. 2015	21
Ostuni1	Italy	Apulia, Ostuni	UP	25	Genome-wide	Long bone	Fu et al. 2016	17
Ostuni2	Italy	Apulia, Ostuni	UP	27	Genome-wide	Long bone	Fu et al. 2016	17
Paglicci108	Italy	Apulia, Paglicci	UP	26	Genome-wide	Phalanx	Fu et al. 2016	17
Paglicci133	Italy	Apulia, Paglicci	UP	33	Genome-wide	Tooth	Fu et al. 2016	17
Pavlov1	Czech Republic	Dolni Vestonice	UP	28	Genome-wide	Femur	Fu et al. 2016	17
PM1	Baia de Fier, Peştera Muierii	Romania	UP	32	Genome-wide	tooth	Svensson et al. 2021	22
Salkhit	Mongolia	Khentii Province, Norovlin County, Salkhit Valley	UP	34	WGS	Skull cap	Massilani et al. 2020	23
Spy 94a	Belgium	Spy Cave	IUP/UPa	39	WGS	Molar	Hajdinjak et al. 2018	11
Sunghir1	Russia	Sunghir	UP	32	WGS	Tooth, bone	Sikora et al. 2017	24
Sunghir2	Russia	Sunghir	UP	32	WGS	Tooth, bone	Sikora et al. 2017	24
Sunghir3	Russia	Sunghir	UP	34	WGS	Tooth, bone	Sikora et al. 2017	24
Sunghir4	Russia	Sunghir	UP	32	WGS	Bone fragment	Sikora et al. 2017	24
Tianyuan	China	Tianyuan	IUPa	40	Genome-wide	Femur, tibia	Fu et al. 2013	25
Ust'Ishim	Russia	Ust'-Ishim, Siberia	IUPa	45	WGS	Femur	Fu et al. 2014	26
Vestonice13	Czech Republic	Dolni Vestonice	UP	31	Genome-wide	Femur	Fu et al. 2016	17

Vestonice14	Czech Republic	Dolni Vestonice	UP	31	Genome-wide	Femur	Fu et al. 2016	17
Vestonice15	Czech Republic	Dolni Vestonice	UP	31	Genome-wide	Femur	Fu et al. 2016	17
Vestonice16	Czech Republic	Dolni Vestonice	UP	30	Genome-wide	Femur	Fu et al. 2016	17
Vestonice43	Czech Republic	Dolni Vestonice	UP	30	Genome-wide	Femur	Fu et al. 2016	17
Yana1	Russia	Siberia	UP	32	WGS High-Cov	Tooth	Sikora et al. 2019	27
Yana2	Russia	Siberia	UP	32	WGS	Tooth	Sikora et al. 2019	27
Zlatý Kůň	Czech Republic	Koněprusy cave	Szeletian/IUPa	>45	Genome-wide	Skull	Prüfer et al. 2021	28

Supplementary Table 2. List of early human genomes available. Kya stands for kylo years ago. UP: Upper Paleolithic, IUP: Initial Upper Paleolithic.

Detecting Introgression	**Publication**	**Ref.**	**Link**
Admixfrog	Peter 2020	29	https://github.com/BenjaminPeter/admixfrog
ARGWeaver-D	Hubisz et al. 2020	30	https://github.com/CshlSiepelLab/argweaver
CRF	Sankararaman et al. 2014, 2016	31,32	https://www.sciencedirect.com/science/article/pii/S0960982216302470?via%3Dihub#bib24
diCal-admix	Steinrucken et al. 2018	33	http://dical-admix.sourceforge.net/
f stats	Martin et al. 2014	34	https://uqrmaie1.github.io/admixtools/articles/admixtools.html
df stats	Pfeifer and Kapan 2019	35	
Dp stats	Hamlin et al. 2020	36	
D stat	Green et al. 2010	9	https://uqrmaie1.github.io/admixtools/articles/admixtools.html
D+ stat	Lopez Fang et al. 2022	37	
HMM	Prufer et al. 2014	8	
HMM	Bergstrom et al. 2020	38	
HMM	Seguin-Orlando et al. 2014	18	
IBDMix	Chen et al. 2020	39	https://github.com/PrincetonUniversity/IBDmix
HMM	Skov et al. 2018	40	https://github.com/LauritsSkov/Introgression-detection/
S*	Vernot and Akey 2014, Vernot et al. 2016	41,42	https://github.com/bvernot/freezing-archer
Sprime	Browning et al. 2018	43	https://github.com/browning-lab/sprime
Archie	Durvasula and Sankararaman 2019	44	https://github.com/sriramlab/ArchIE
Parameters estimation	**Publication**	**Ref.**	**Link**
ABC-DL	Mondal et al. 2019	45	https://github.com/oscarlao/ABC_DL
CSFS	Durvasula and Sankararaman	46	http://web.cs.ucla.edu/~sriram/software.html
CNN	Flagel et al. 2019	47	https://github.com/flag0010/pop_gen_cnn
IMa3	Hey et al. 2018	48	https://github.com/jodyhey/IMa3
Legofit	Rogers 2019	49	https://github.com/alanrogers/legofit
Moments	Ragsdale and Gravel 2019	50	https://moments.readthedocs.io/en/latest/index.html
Visualising Introgression	**Publication**	**Ref.**	**Link**
Contour plots	Browning et al. 2018	43	https://github.com/browning-lab/sprime
Heatmaps			
Haplostrips	Marnetto and Huerta-Sanchez 2017	51	https://bitbucket.org/dmarnetto/haplostrips/src/master/
Adaptive introgression	**Publication**	**Ref.**	**Link**
genomatnn	Gower et al. 2021	52	https://github.com/grahamgower/genomatnn
MaLAadapt	Zhang et al. 2022 biorxiv	53	https://www.biorxiv.org/content/10.1101/2022.05.16.491756v1.full.pdf
Rd, U, Q95	Racimo and Marnetto et al. 2017	54	https://academic.oup.com/mbe/article/34/2/296/2633371#61701351
Volcano finder	Setter et al. 2020	55	http://degiorgiogroup.fau.edu/vf.html

Supplementary Table 3. Methods and resources to study introgression.

Gene	Trait	Archaic	Population	Publication	Ref.
OAS1/2/3	Immunity (SARS-Cov-2)	Neanderthal	Americans	Zeberg and Pääbo 2021	55
LARS	Liver failure	Neanderthal	Americans	Racimo, Marnetto et al. 2017	54
SLC16A11	Type 2 diabetes	Neanderthal	Americans	Racimo, Marnetto et al. 2017	54
ELMSAN1	Immunity	Neanderthal	Americans	Jagoda et al. 2022	56
CYP450	Drug metabolism	Neanderthal	Americans	Wroblewski et al. 2021	57
MUC19	Immunity	Denisova	Americans	Witt et al. 2023	58
BNC2	Pigmentation	Neanderthal	Europeans	Jacobs et al. 2013	59
OCA2	Pigmentation	Neanderthal	Europeans	Gittelman et al. 2016	60
OAS1/2/3	Immunity	Neanderthal	Europeans	Mendez et al. 2013	61
ELMSAN1	Immunity	Neanderthal	Europeans	Jagoda et al. 2022	56
OAS1/2/3	Immunity (SARS-Cov-2)	Neanderthal	Europeans	Zeberg and Pääbo 2021	55
SELP	Blood coagulation	Neanderthal	Europeans	Simonti et al 2016	62
CYP450	Drug metabolism	Neanderthal	Europeans	Wroblewski et al. 2021	57
Various SNPs	Prostate cancer, blood…	Neanderthal	Icelanders	Skov et al. 2020	63
TBX15	Lipid metabolism	Denisova	Inuit	Racimo et al. 2017	64
WARS2	Lipid metabolism	Denisova	Inuit	Racimo et al. 2017	64
STAT2	Immunity	Neanderthal	Papua New Guinea	Mendez et al. 2012	65
TNFAIP3	Immunity	Denisova	Papua New Guinea	Gittelman et al. 2016	60
OAS1/2/3	Immunity (SARS-Cov-2)	Neanderthal	South & East Asians	Zeberg and Pääbo 2021	55
OCA2	Pigmentation	Neanderthal	South & East Asians	Gittelman et al. 2016	60
POU2F3	Pigmentation	Neanderthal	South & East Asians	Vernot and Akey 2014	41
TLR1/6/10	Immunity	Neanderthal	South & East Asians	Gittelman et al. 2016	60
OAS1/2/3	Immunity	Neanderthal	South & East Asians	Gittelman et al. 2016	60
WARS2	Lipid metabolism	Denisova	South & East Asians	Jacobs et al. 2019	66
TNFAIP3	Immunity	Denisova	South & East Asians	Jacobs et al. 2019	66
EPAS1	Hypoxia tolerance	Denisova	Tibetans	Huerta-Sanchez et al. 2014	67

Supplementary Table 4. Main archaic traits adaptively introgressed in modern populations.

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