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Paleogenomics of Archaic Hominins

Review

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In order to understand the genetic basis for the evolutionary success of modern humans, it is necessary to compare their genetic makeup to that of closely related species. Unfortunately, our closest living relatives, the chimpanzees, are evolutionarily quite distant. With the advent of ancient DNA study and more recently paleogenomics — the study of the genomes of ancient organisms — it has become possible to compare human genomes to those of much more closely related groups. Our closest known relatives are the Neanderthals, which evolved and lived in Europe and Western Asia, from about 600,000 years ago until their disappearance around 30,000 years ago following the expansion of anatomically modern humans into their range. The closely related Denisovans are only known by virtue of their DNA, which has been extracted from bone fragments dating around 30,000 to 50,000 years ago found in a single Siberian cave. Analyses of Neanderthal and Denisovan nuclear and mitochondrial genomes have revealed surprising insights into these archaic humans as well as our own species. The genomes provide a preliminary catalogue of derived amino acids that are specific to all extant modern humans, thus offering insights into the functional differences between the three lineages. In addition, the genomes provide evidence of gene flow between the three lineages after anatomically modern humans left Africa, drastically changing our view of human evolution.

Introduction

Modern humans evolved from earlier species of *Homo* that originated in Africa around 2–2.5 million years ago subsequently migrating into Eurasia at different points in time. While the precise phylogenetic relationships among all these hominin species have remained largely controversial, it seems certain that some of the archaic humans underwent local evolution and adaptation. Neanderthals (*Homo neanderthalensis* or *Homo sapiens neanderthalensis*) were a human lineage — some argue a human species — that evolved principally in Europe and Western Asia (Figure 1), originating from archaic Middle Pleistocene hominin populations that began to exhibit skeletal features characteristic of Neanderthals ca. 400,000 years ago [1]. Usually named *Homo heidelbergensis*, remains of these ancestors include those found at Mauer near Heidelberg (Germany) and Petralona (Greece). In parallel, other hominin populations, often attributed to *H. heidelbergensis* or *H. rhodesiensis* [1] were evolving in Africa ultimately giving rise to modern humans (*H. sapiens*).

Neanderthals display a range of distinctive physical traits at least some of which are likely to have been shaped by isolation and adaptation to dramatic climate fluctuations [2]. Based on their skeletal features, they must have displayed strong, wide, heavily muscled bodies, with shortened distal limb segments, and in comparison to some modern humans, a relatively short stature (average height for males around 1.65 m and weight around 80 kg) [3]. Given that similar body proportions have been observed in other cold-adapted mammals enabling preservation of body heat in cold environments, the Neanderthal body form has been interpreted by some as reflecting a 'hyper-arctic' adaptation [4]. It has also been suggested that the Neanderthals' mid-face projection, including inflated cheek bones and a broad nose, also were adaptations to cold climate. However, this view has recently been challenged as the large nose sinuses characteristic of Neanderthals were found to be atypical for cold-adapted mammals [5]. Regardless of the explanation, however, the fact that across their range the Neanderthals exhibited this distinctive physique implies that their genetic makeup responsible for these traits differed from that of modern humans.

Neanderthal diet has also been the subject of much debate. While isotopic evidence points to an overwhelmingly carnivorous diet, with proteins predominantly obtained from animal sources [6], recent observations of phytoliths and starch grains trapped in Neanderthal dental calculus point to the use of plant resources [7]. Furthermore, one can speculate that their long-term existence in Eurasian latitudes probably involved metabolic and physiological adaptations, and that they required a high calorie intake to sustain their heavily muscled bodies.

For most of their history, Neanderthals produced a Middle Paleolithic technology known as Mousterian industry. This technology was not restricted to Neanderthals, as similar stone tools were likely also produced by early modern humans outside of continental Europe [3]. Thus, the recent finding of Mousterian tools near the Arctic Circle [8] does not automatically indicate a Neanderthal presence in the region. Mousterian technology remained largely unchanged for most of the Neanderthal period. However, shortly after the arrival of anatomically modern humans in Europe about 45,000 years ago and before their final extinction 15,000 years later [9], some Neanderthals began to produce so-called transitional industries that showed Upper, as well as Middle Paleolithic characteristics. These industries include among others the Châtelperronian in France, the Szeletian in Central Europe, and the Uluzzian in Italy and have been traditionally interpreted as a sign of the acculturation of the last surviving Neanderthal populations by anatomically modern humans [3]. However, the impression that Neanderthals were merely copying Upper Paleolithic tools may be an over-simplification, and may instead have been the consequence of an independent development by Neanderthals [10]. Alternatively, some of these industries may have been produced by anatomically modern humans [11], and a recent analysis indicates that some teeth found in Uluzzian levels stem in fact from anatomically modern humans [12].

Another controversial issue concerns the cognitive abilities of Neanderthals. Although once thought to be

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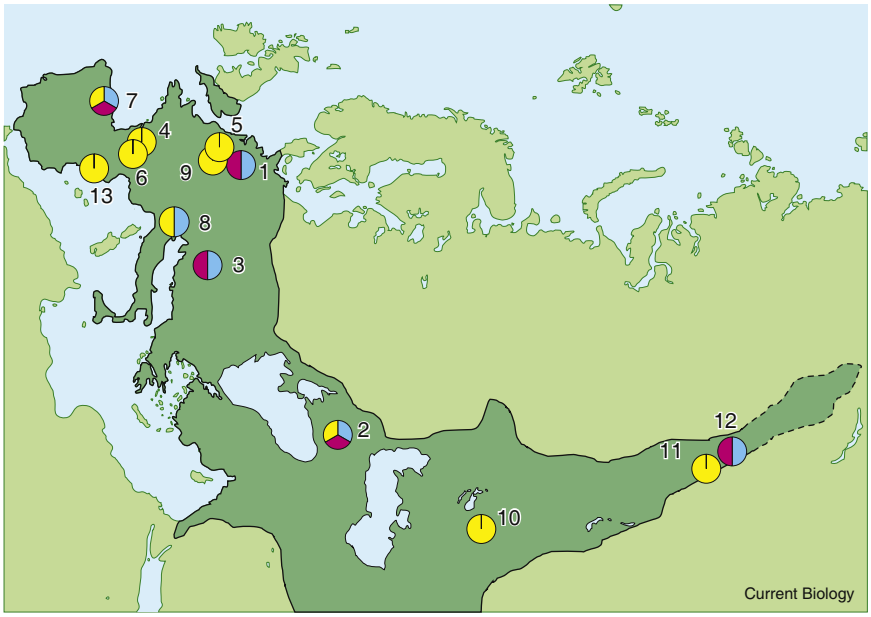

Figure 1. Archaic hominin sites and range in Eurasia.

Archaeological cave sites for Neanderthals (and Denisovans from Denisova cave (12)) from which material for genetic analysis has been retrieved. 1: Feldhofer (Germany), 2: Mezmaiskaya (Russia), 3: Vindija (Croatia), 4: Rochers de Villeneuve (France), 5: Engis (Belgium), 6: La Chapelle-aux-Saints (France), 7: El Sidrón (Spain), 8: Monti Lessini (Italy), 9: Scladina (Belgium), 10: Teshik-Tash (Uzbekistan), 11: Okladnivok (Russia), 12: Denisova (Russia), 13: Cova del Gegant (Spain). The shaded area marks the approximate distribution of Mousterian tools associated with Neanderthals and thus their presumable range.

fundamentally inferior to that of anatomically modern humans, including a lack of language, recent findings that well before the arrival of modern humans in their range Neanderthals have decorated their bodies — showing aspects of modern symbolic behaviour — support the view that they had comparable cognitive abilities to anatomically modern humans [13,14]. However, the attribution of some of these personal ornaments to Neanderthals and the claim of complex symbolic behaviour among them has been challenged by some researchers, who argue that for most of their evolutionary history such evidence is lacking, and that it only seems to appear while anatomically modern humans are dispersing across Europe [15]. Moreover, it has been shown that Neanderthals and anatomically modern humans may have had different phases of brain development after birth, a characteristic that might underlie potential cognitive differences between both groups [16].

In parallel to Neanderthals in Eurasia, a previously unknown type of hominin, the so-called Denisovans named after the Denisova cave in Siberia, were probably inhabiting parts of continental Asia. Because the existence of this group has only recently been inferred by DNA analyses of fragments of bone, nothing is known at present about the morphology or culture of Denisovans [17].

Over the last 15 years, developments in ancient DNA techniques have revolutionised our understanding of recent hominin paleontology (Box 1). Until recently, the most significant discovery in this regard was the 1997 publication of short fragments of mitochondrial DNA (mtDNA) from the Neanderthal holotype [18]. At the time — and indeed even only a few years ago — it would have been unimaginable that this emerging field would one day culminate in the publication of entire ancient genomes. Given the challenges of ancient DNA research, such as degradation of the DNA to short error-containing molecules, and contamination with exogenous sources of DNA [19–22], it was simply inconceivable that the molecular biology techniques available to study it — PCR and Sanger sequencing — could ever generate enough data to reconstruct a nuclear genome (Box 1). But, thanks to the revolution in sequencing technology [23], 2010 saw the publication of not just one, but two extinct hominin genome drafts, that of the Neanderthals [24], and the mysterious Denisovans [17].

By providing enormous amounts of more objective data, human paleogenomics has the potential to settle long-lasting debates that originated from the incompleteness of the archaeological and paleontological record. At the same time, it can help establish a more realistic view on the complexity of human evolution.

Neanderthal Mitochondrial DNA: Demography and Population Genetics

Neanderthals occupied a large geographic range (Figure 1), from the Altai mountains to the Iberian Peninsula, and from the Middle East to Britain. Calculations that couple density parameters of modern hunter-gatherer populations with this range distribution provide estimates of a maximum number of between 140,000 and 350,000 Neanderthals in Europe at any one time [25]. Irrespective of the actual figures, a recent analysis of Neanderthal and anatomically modern humans archaeological site density in south-western France suggests that the latter outnumbered Neanderthals ten to one [26]. It is thus increasingly clear that knowledge of Neanderthal demography is crucial for understanding both their evolution, and their extinction.

As genetic diversity relates to demographic history, analysis of genetic markers, in particular mtDNA, from multiple Neanderthal individuals provides a new way to explore Neanderthal demography. To date, 20 partial and 7 complete mtDNA sequences have been published that correspond to 27 Neanderthal individuals from 12 archaeological sites [18,27–38] (Figure 1). About twice the number of specimens have been tested but not yielded PCR-amplifiable DNA [24,30]. In light of the fact that there are a total of only ca. 400 partial Neanderthal specimens known [3], it is thus clear that the number of Neanderthal mtDNA sequences available for study will ultimately be very limited.

Despite consisting of only 378 base pairs (bp) of mtDNA from a single individual, the first Neanderthal mtDNA sequence already provided sufficient phylogenetic signal to clearly indicate that Neanderthals were a sister group to anatomically modern humans [18]. Furthermore, the data

Box 1

Landmarks of ancient hominin paleogenomics.

1997
First Neanderthal mitochondrial DNA sequence [18].
2000
Second and third Neanderthal mitochondrial DNA sequences [27,28].
2006
One million nucleotides from the Neanderthal genome sequenced in two studies [66,67].
2007
One of the 2006 studies shown to be significantly contaminated with modern human DNA [68].
Neanderthal *FOXP2* gene identical to that of modern humans [40].
Neanderthals found to have red hair [41].
mtDNA retrieved from Siberian and Central Asian Neanderthals [35].
2008
First complete Neanderthal mitochondrial genome [39].
Neanderthals found to be O blood group [42].
2009
Five more complete Neanderthal mitochondrial genomes [36].
2010
Neanderthal nuclear genome draft [24].
Denisovan complete mitochondrial genome and nuclear genome draft [17,57].
2011
mtDNA from a Neanderthal family group of 12 individuals [37].
Detection of ancient hominin gene flow in modern human genomes worldwide [63,64].

provided no evidence of interbreeding between Neanderthals and anatomically modern humans at a level sufficient to result in Neanderthal mtDNA introgression into the modern human gene pool. This notion was confirmed as short mtDNA sequences from additional Neanderthals were sequenced [18,27–38], leading many to conclude that interbreeding between Neanderthals and modern humans was rare, if not absent.

The sequencing of multiple Neanderthal individuals, in particular the sequencing of six complete mtDNA genomes [36,39], also allowed population genetic inferences to be made. These included an estimate of a female effective population size of less than 3,500 (about one-third of that of modern humans at the time) and an age of the mtDNA's most recent common ancestor (about 110,000 years ago), that is about half the age estimated for modern humans' mtDNAs [36]. Furthermore, the distribution of this genetic diversity in space and time provided further insights into Neanderthals: first, the presence of identical mtDNA genomes in two spatially quite separated specimens from Vindija (Croatia) and Feldhofer (Germany) was best explained by assuming that the Neanderthal population was very small, never numbering more than a few thousands in a very large area [36]; second, the finding of identical mtDNA genomes in two individuals from Vindija (Vi33.16 and Vi33.26) separated by about 6000 years suggested long-term continuity of maternally-related individuals in the same place [24].

A more recent study [37] of mtDNA of Neanderthals from the Spanish site of El Sidrón (Figure 2) — a synchronic accumulation of 12 Neanderthals including female and male adults, adolescents, juveniles and one infant — focussed on the kinship relationships within a group of Neanderthals. These data suggest that Neanderthals formed small kinship-structured bands that practised patrilocal mating behaviour, and had relatively long inter-birth intervals (ca. three years) compared to anatomically modern humans [37]. In addition to providing intriguing anthropological insights — similar features have also been described in modern hunter-gatherer groups — such information may help in choosing demographic parameters when generating future models of Neanderthal populations.

Overall, mtDNA analyses suggest that Neanderthal populations exhibited little clear phylogeographic structure, despite their wide geographic range [36]. However, partial mtDNA sequences from the oldest Neanderthal specimens (such as Scladina and Mezmaiskaya 1) [34,36] (Figure 1) are more divergent, suggesting that some population bottlenecks and/or local extinctions have taken place along the Neanderthals' evolutionary history. Therefore, there are several limitations to the mtDNA data: first, complete mtDNA genomes only exist from what may in fact be a genetically fairly homogenous group of the last Neanderthals. Second, the mtDNA genome is a single genetic marker, representing only part of the Neanderthals' genetic history, and carries little functionally-relevant information, which is precisely why the publication of the first nuclear DNA genome sequences was so eagerly anticipated.

The Neanderthal Nuclear Genome: Genes with Fixed Differences

Having a Neanderthal genome draft is an impressive technical achievement that allows us to start answering many evolutionary questions. Some key adaptations of Neanderthals are impossible to ascertain from morphology or archaeology alone, but can nevertheless be explored from their genes. In the years immediately prior to the publication of the draft nuclear genome, several such genes were retrieved [40–43], providing a new, genetic perspective on the Neanderthal phenotype. For example, *FOXP2*, a gene putatively associated with the human ability to speak, was found to be identical in Neanderthals and anatomically modern humans [40]. This was argued to potentially have endowed Neanderthals with language abilities comparable to our own. Furthermore, analysis of the pigmentation gene MC1R provided evidence that some Neanderthals were red-haired and had fair skin [41], traits that are similarly hard to infer from the fossil record.

Given the potential power of such analyses, one of the first uses once the Neanderthal genome draft was completed (Box 2) was to identify genes that changed specifically in modern humans, but not Neanderthals. This was done by screening for amino acid changes that are derived, i.e. different from our closest living relative, the chimpanzee, and fixed in all modern humans, but are putatively ancestral in Neanderthals (i.e. identical to the chimpanzee) [24,44]. These analyses yielded between 78 and 83 genes that differ on the amino acid level between the two hominin groups. This list comprises genes associated with various functions, including metabolism, cranial development, pigmentation, skin physiology, cognition and even sperm movement.

While such analyses ultimately try to characterise what makes anatomically modern humans different from their

Figure 2. Caving at El Sidrón.

In El Sidrón cave in Asturias (Spain), the remains of twelve Neanderthal individuals synchronically accumulated and dated to about 49,000 years ago are being excavated. Insert: El Sidrón SD 1253 bone sample, one of the best preserved Neanderthal bones for paleogenetic analysis. (Images: El Sidrón research team.)

ancestors and relatives, a similar undertaking, i.e. identifying Neanderthal-specific changes, is much harder to achieve because of the low coverage of the genome draft. At the 1.3-fold coverage currently obtained, if a particular Neanderthal sequence contains an ancestral nucleotide in a position where modern humans have a fixed difference, it is unlikely that this read could derive from an undetected chimpanzee contamination in the laboratory, *post mortem* damage or sequencing error. However, it is much more difficult to validate positions where modern humans carry a fixed ancestral variant and Neanderthals a derived one. In this case, while a Neanderthal sequence might harbour a novel variant, the change could also be due to DNA damage or a sequencing error. Given that damage and sequencing errors tend to be template-specific, increasing genome coverage — for example to the 20-fold achieved with the third ancient genome, that of a 4,500 year old Greenlander [45] — will render it possible to track genes that have been modified and fixed only in the Neanderthal lineage. The analysis of segregating loci in Neanderthals suffers from a similar shortcoming. With the current low coverage, it is impossible to distinguish random damage and/or contamination background from true heterozygosity.

Apart from the around 80 genes with changes in their coding sequence, additional changes in gene regulation can be assumed to account for the phenotypic differences between Neanderthals and modern humans. Therefore, there is a whole level of genomic complexity that requires exploration, including segmental duplications, regulatory regions and microRNAs. This may in the future be addressed through higher sequence coverage, in particular in regions suspected to be important for genome architecture. Targeted capture sequencing or other enrichment methods can be useful for this purpose; alternatively, repeated genotyping of a particular genomic segment is equivalent to an increased coverage [36,44,46]. For instance, in a pre-genome analysis of the ABO blood group gene, five different amplifications were generated from two El Sidrón specimens [42]; even in the unlikely scenario that each PCR reaction started from a single DNA template, this is equivalent to 10x coverage in that particular segment.

Finally, some or many of the genes in the list may have little significance for understanding key metabolic, cognitive, physiological and morphological evolutionary differences between Neanderthals and modern humans. The functional relevance of all the genetic changes will have to be investigated. Ultimately, our knowledge of the differences between Neanderthals and us will depend on how well we understand the phenotypic effects of these genetic changes in living organisms.

Gene Flow between Neanderthals and Modern Humans?

One of the big questions in hominin evolution concerns the disappearance of the Neanderthals, and in particular if their demise was related to encounters with anatomically modern humans. The 'Out of Africa' hypothesis argues that they were totally replaced by our ancestors without mixing with them, while the alternative multiregionalist hypothesis proposes a long-term biological continuity between archaic forms and modern humans in all continents, not only in Europe. Although a few Upper Paleolithic hominin skeletal remains have been suggested to exhibit evidence of admixture between modern humans and late Neanderthals, notably that of a child from Lagar Velho in Portugal [47], the current consensus is that Neanderthal morphological traits disappeared from the fossil record around 30,000 years ago, along with the Middle Palaeolithic tools and the transitional industries. Furthermore, for more than a decade, repeated analyses of Neanderthal mtDNA sequences failed to provide any evidence that gene flow took place. Therefore, a particularly unexpected outcome of the Neanderthal genome analysis was evidence of a significant degree of gene flow with anatomically modern humans, most likely populations that had already left Africa [24]. This was determined by the existence of almost identical chromosomal regions shared between Neanderthals and non-African modern humans that were clearly different in sub-Saharan Africans.

The genomic signal of this interbreeding, which presumably occurred in the Near or Middle East between ca. 50,000 and 80,000 years ago (but see [48] for alternative possibilities such as back to Africa migration after an earlier admixture with Neanderthals, ca. 100,000 years ago), is preserved in chromosomal regions of low recombination

Box 2

Sequencing the Neanderthal genome.

The draft Neanderthal nuclear genome was generated to 1.3-fold coverage, principally using three bone samples derived from three different females (Vi33.16, Vi33.25 and Vi33.26) that were excavated from the Vindija site in Croatia [24]. Additional sequences were obtained from El Sidrón 1253 (Figure 2), Mezmaiskaya 1 and Feldhofer 1 (0.1%, 2% and 0.1% of the genome, respectively) [24]. Given that numerous genomes of modern humans can be sequenced in parallel at much higher coverage, in a single run of a second-generation sequencer, the scale of this achievement is not always apparent to those from outside the paleogenomic field. The principal challenges are inefficiency and contamination. First, given that the data were largely generated through Roche FLX and Illumina shotgun sequencing of samples in which most (ca. 96%) of the DNA is derived from environmental soil microorganisms, the majority of raw sequence produced was not relevant. A second challenge is the presence of contaminant modern human DNA in the specimens. Although in the final draft genome the estimate of modern human contamination is less than 1% [24] (based on the mtDNA sequences produced, the residual presence of Y-chromosome sequences, and autosomal heterogeneity), up to 80% of the first million nucleotides published in 2006 [66] appear to derive from modern human DNA that entered the Neanderthal library at the commercial sequencing facility in which the initial data were generated [68]. The effect of such contamination was an overestimate of the proportion of Neanderthal DNA in the modern human genome, implying relatively high levels of interbreeding — something contradictory to the findings of a parallel publication [67]. As a result of this early pitfall, more stringent measures were taken to guarantee the quality of the later data, and as such, it is less likely that the current 1.3x draft suffers similar problems.

and encompasses about 2.5% (ranging between 1 and 4%) of the human genome [24]. Strikingly, the Neanderthal contribution is equally present in all non-African genomes studied (French, Chinese and Papuan), which suggests the introgression must have happened prior to the expansion of anatomically modern humans from the Middle/Near East into the ancestral populations that are thought to have subsequently given rise to the modern European, and Asian/Australasian populations (Figure 3). With the future availability of more modern human genomes from different areas of the globe, the potential gene flow can be explored in more detail, both in different geographic areas and at a finer scale in the genome. Additional modern human genomic regions apparently introgressed from Neanderthals, Denisovans and also putative archaic African hominins have been recently described [49–51]. Simulations on admixture models suggest that the gene flow observed is compatible with a very low rate of interbreeding (<2%) between Neanderthals and expanding anatomically modern human populations [52]; similar figures can be probably extrapolated to putative contacts between anatomically modern humans and other archaic hominins elsewhere.

The nuclear genomic data also contribute to a subject of much debate among paleontologists, namely that of the nature and timing of the encounter between Neanderthals and anatomically modern humans as the latter entered Europe at least 45,000 years ago [12,53]. The fact that at present the handful of modern European genomes analysed appropriately do not show signs of gene flow above the levels observed in other Eurasian genomes is intriguing, considering evidence of several thousand years of coexistence before the final Neanderthal extinction. While some have argued that extensive meeting of the two groups was unlikely [54,55], there are alternative explanations that could explain the genomic observations. First, interbreeding may have left no genetic trace today due to different demographic dynamics of the two groups — not least, Neanderthals were probably heavily outnumbered by anatomically modern humans. Alternatively, hybrids of putative contact may have disappeared during the still largely unknown Aurignacian–Gravettian transition due to new migrations into Europe. One future way to potentially solve this issue will be through sequencing of genomes from anatomically modern human samples that date to the Upper Palaeolithic [56], although this approach will face substantial contamination issues.

The recent analysis of the Neanderthal genome has shifted the debate on the origin of our species, from the previous extreme models to less popular so-called assimilation models, which support a recent out-of-Africa spread with partial local replacements (although usually suggesting rather high levels of admixture).

Denisovans and Assimilation Models with Low Admixture Levels

Shortly after the Neanderthal genome, a second hominin draft genome, that of the so-called 'Denisovan', was released. What made this even more remarkable is that the existence of this new Asian hominin lineage was not anticipated from paleontology and thus it was discovered from genetics alone. The story commenced earlier in 2010, when a publication described a complete mtDNA genome sequence [57] from a morphologically undiagnostic finger bone found in the Denisova cave in southern Siberia that was more divergent than the known diversity of modern human and Neanderthal mitochondrial genomes together. Based on these data alone, however, plus stratigraphic disturbance problems with regard to the bone's dating (30,000 to 50,000 years ago), plus the fact that the divergent mtDNA did not necessarily imply that the bone was from a new hominin species, further conclusions could not be drawn without nuclear DNA data. Consequently, a 1.9-fold coverage draft genome was generated from the same sample [24]. With a stated endogenous DNA content of ca. 70%, the microbial contamination load in this sample was much lower than that in other temperate-preserved samples so far studied, including both other Neanderthals and a molar tooth from the same site, and rivals that of frozen preserved mammoth bone [58] or less permeable materials such as hair [48,59,60]. The Denisova tooth, which subsequent mtDNA genome sequencing confirmed as belonging to

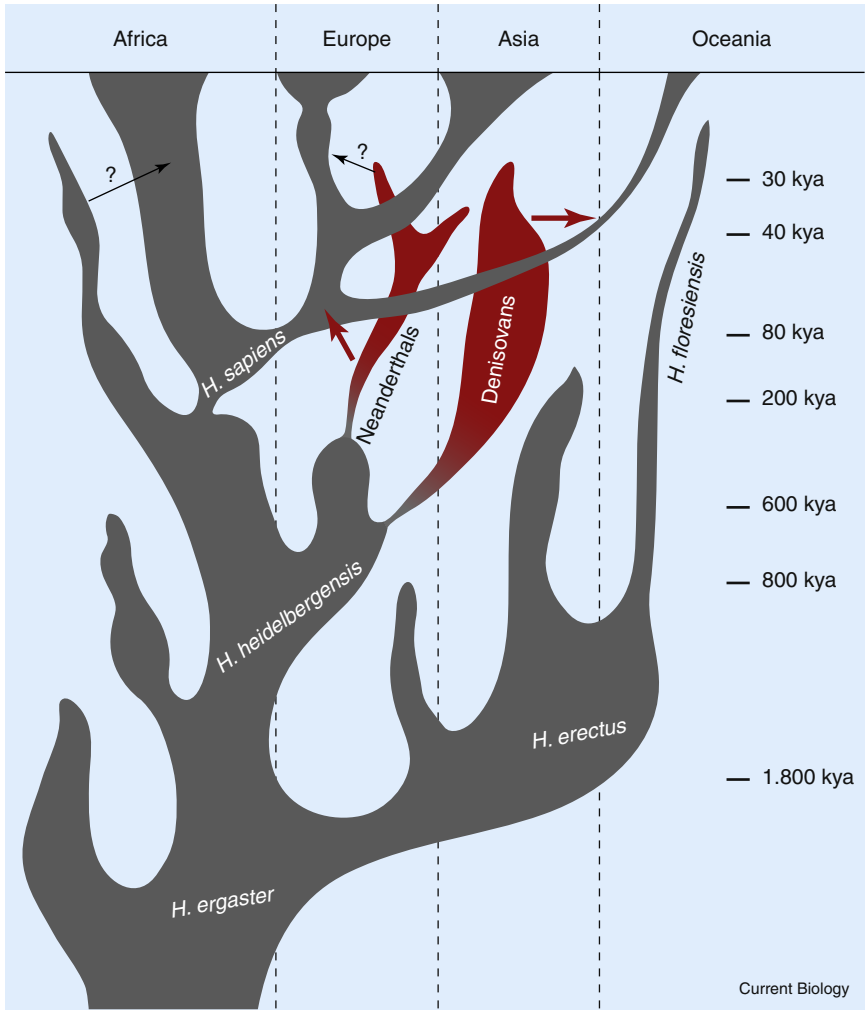

Figure 3. Hypothetical evolutionary relationships among modern human populations, Neanderthals and Denisovans as inferred from genomic data.

Red arrows mark genetic evidence of interbreeding among different hominin populations. Black arrows mark suggested or possible additional gene flow. The complexity of the uncovered population interrelationships helps to explain uncertainties in the attribution of hominin fossils to different species.

a second, closely related individual, has a more conventional [36] endogenous DNA content of only 0.17% [24].

The analysis of the genetic data yielded intriguing insights. First, while both Denisovan mtDNA sequences represent individual archaic hominin lineages, the Denisovan nuclear genome appears less divergent, forming a sister group with Neanderthals [24]. Second, the data suggest gene flow between Denisovans and current Melanesians (Figure 3), 4.5% of whose genomes appear to derive from Denisovans or related hominins [24]. Given that there is no evidence to suggest that the ancestors of modern Melanesians went near Siberia, a more plausible explanation for this observation is that Denisovans, or close relatives, were present in South-East Asia at the time that the Melanesian's ancestors entered the region. With this huge geographic range, it can be expected that Denisovans will exhibit larger genomic diversity than Neanderthals, just because the populations could be larger. In agreement with this, the yet unpublished finding of a second Denisovan tooth showed that their mtDNA has more variation than seven Neanderthal mtDNAs [61]. Unfortunately, with only fragmentary and non-diagnostic morphological remains found to date, we are as yet unable to reconstruct what Denisovans may have looked like. One suggestion is that archaic Chinese hominins that are problematic to attribute taxonomically, such as the 300,000 year-old Dali skull, could be related to these Denisovans [62]. If so, in light of the nuclear DNA relationship between the two lineages, it might be hypothesised that Neanderthals and Denisovans represent two co-evolving lineages in Western Eurasia and Asia, respectively. Intriguingly, the analysis of a toe bone recently found in Denisova shows that Neanderthals were also present in the cave, probably after the Denisovans [61].

The sequencing of further ancient and modern genomes from Asia and Australasia is shedding more light on the Denisovan affinities and their contribution to the modern human gene pool [63,64]. Furthermore, if anything, the Denisova genome has demonstrated the value of undertaking pilot genetic analyses of ancient remains, regardless of their morphological attribution or stratigraphic context. More evolutionary surprises may be out there and even a small bone flake may contain a significant tale.

Future Directions

The ancient hominin genomes of Neanderthals and Denisovans have demonstrated that conventional models of human evolution based on genetic analyses of modern data are over-simplified. While the out of Africa migration of anatomically modern humans continues to play a dominant role in the origin of modern Eurasians, it is clear that successive episodes of hybridisation with archaic hominins have played a role in this process. Considering the discrepancy of the nuclear and mtDNA data in Denisovans [24,57], it is possible that these kinds of processes have also taken place in previous hominin migrations.

In the future, hominin paleogenomics will likely move in several directions: the investigation of genomic diversity by analysing other samples, the increase of the coverage in genomic regions of interest in the samples analysed, and the undertaking of functional studies, such as comparing the function of modern human and Neanderthal gene variants in mouse models. With regard to increasing the dataset, although nuclear DNA sequences (whether shotgun or PCR targeted) have been recovered to date from only 5 of the known Neanderthal archaeological sites (El Sidrón,

Feldhofer, Mezmaiskaya, Monti Lessini and Vindija) [24,40–44], the power of modern sequencing techniques to study DNA molecules at the level of tens of base pairs in size suggests that ultimately nuclear DNA analyses could be possible on some other samples that have yielded mtDNA to date — at least in those in which modern human contamination is minimal or can be controlled during excavation [65].

A further exciting prospect is whether other extinct hominins will be found to contain DNA suitable for genomic analyses. While fossils attributed to *Homo floresiensis* are within the time range of DNA retrieval, but come from a warm and damp environment that is unfavourable for DNA preservation, and fossils attributed to *Homo erectus* might be too old, other hominins, such as *H. heildelbergensis* in Europe, or similar forms in Asia, might be suitable for ancient DNA analysis. Palaeogenomics, a discipline that has begun to revolutionise the study of human evolution, has not yet reached its temporal and geographic temporal limits.

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