

# Ancient DNA and Neanderthals Mystery

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**Abstract-** Ancient DNA is DNA isolated from ancient specimens. It can be described as any DNA recovered from biological samples that have not been preserved specifically for later DNA analyses. The analysis of DNA recovered from archaeological and historical skeletal material, mummified tissues, archival collections of non-frozen medical specimens, preserved plant remains, ice and permafrost cores, Holocene plankton in marine and lake sediments, and so on are ancient DNA samples.

Unlike modern genetic analyses, ancient DNA studies are characterized by low quality DNA. This places limits on what analyses can achieve. Furthermore, due to degradation of the DNA molecules, a process which correlates loosely with factors such as time, temperature, and presence of free water, upper limits exist beyond which no DNA is deemed likely to survive. The DNA degrades in an exponential decay process.

**Key words-** Ancient DNA, Genetic Analysis, mtDNA, Neanderthals, Ancestors of Human, Hominin relative, Human fossils

## INTRODUCTION

Ancient DNA is DNA isolated from the ancient specimens [1]. It can be described as any DNA recovered from biological samples that have not been preserved specifically for later DNA analyses. The analysis of DNA recovered from archaeological and historical skeletal material, mummified tissues, archival collections of non-frozen medical specimens, preserved plant remains, ice and permafrost cores, Holocene plankton in marine and lake sediments, and so on are ancient DNA samples.

Unlike modern genetic analyses, ancient DNA studies are characterized by low quality DNA. This places limits on what analyses can achieve. Furthermore, due to degradation of the DNA molecules, a process which correlates loosely with factors such as time, temperature, and presence of free water, upper limits exist beyond which no DNA is deemed likely to survive. Allentoft et al. (2012) tried to calculate this limit by studying the decay mitochondrial and nuclear DNA in Moa bones. The DNA degrades in an exponential decay process. According to their model, mitochondrial DNA is degraded to 1 base pair after 6,830,000 year at  $(-5)^\circ\text{C}$  [2]. Nuclear DNA degrades at least twice as fast as mtDNA. As such, early studies that reported recovery of much older DNA, for example from Cretaceous dinosaur remains, may have stemmed from

contamination of the sample.

The first study of what would come to be called a DNA came in 1984, when Russ Higuchi and colleagues at Berkeley reported that traces of DNA from a museum specimen of the Quagga not only remained in the specimen over 150 years after the death of the individual, but could be extracted and sequenced [3]. Over the next two years, through investigation into natural and artificially mummified specimens, Svante Paabo confirmed that this phenomenon was not limited to relatively recent museum specimens but could apparently be replicated in a range of mummified human samples that dated as far back as several thousand years (Paabo 1985a; Paabo 1985b; Paabo 1986). Nevertheless, the laborious processes that were required at that time to sequence such DNA (through bacterial cloning) were an effective brake on the development of the field of ancient DNA (aDNA). However, with the development of the Polymerase Chain Reaction (PCR) in the late 1980s the field was presented with the ability to rapidly progress [4], [5]. Double primer PCR amplification of a DNA (jumping-PCR) can produce highly skewed and non-authentic sequence artifacts. Multiple primers in nested PCR strategy were used to overcome those shortcomings. Single primer extension (abbr. SEPEX) amplification was introduced in 2007 to address postmortem DNA modification damage [6]. And the PEC was developed in 2007 [7].

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## EXTRACTING THE ANCIENT DNA

### Problems with the extraction of Ancient DNA

A DNA may contain to large number of postmortem mutations, increasing with time. Some regions of polynucleotide are more susceptible to this degradation so sequence data can bypass statistical filters used to check the validity of data [7]. Due to sequencing errors, great caution should be applied to interpretation of population size [8]. Substitutions resulting from deamination cytosine residues are vastly over represented in the ancient DNA sequences. Miscoding of C to T and G to accounts for the majority of errors [9].

### Ancient DNA studies

Despite the problems associated with 'antediluvian' DNA, a wide and ever-increasing range of a DNA sequences have now been published from a range of animal and plant taxa. Tissues examined include artificially or naturally mummified animal remains [10] bone (c.f. Hagelberg et al. 1989; Cooper et al. 1992; Hagelberg et al. 1994) [11], paleofaeces [12], [13] alcohol preserved specimens (junqueira et al. 2002), rodent middens [14], dried plant remains (Goloubinoff et al. 1993, Dumolin-Lapegue et al. 1999) and recently, extractions of animal and plant DNA directly from soil samples [15]. In June 2013, a group of researchers announced that they had sequenced the DNA of a 560-780 thousand year old horse, using material extracted from a leg bone found buried in permafrost in Canada's Yukon Territory [16].

Due to the considerable anthropological, archaeological, and public interest directed toward human remains, it is only natural that they have received a similar amount of attention from the DNA community. Due to their obvious signs of morphological preservation, many studies utilized mummified tissue as a source of ancient human DNA. Examples include both naturally preserved specimens, for examples those preserved in ice, such as the Otzi the Iceman (Handt et al. 1994). Through rapid desiccation, such as high -altitude mummies from Andes (c.f. Paabo 1986; Montiel et al. 2001) as well as various sources of artificially preserved tissue such a chemically treated mummies of ancient Egypt). [17] However, mummified remains are a limited resource, and the majority of human a DNA studies have focused on extracting DNA from two sources that are much more common in the archeological record-bone and teeth. Recently, several other sources have also yielded DNA, including paleofaeces (Poinar at al. 2001) and hair (Baker et al. 2001, Gilbert t al. 20040. Contamination remains a major problem when working on ancient human material.

The use of degraded human samples in a DNA analyses has not been limited to the amplification of human DNA. It is reasonable to assume that for a period of time postmortem, DNA may survive from any microorganisms present in the specimen at death. These include not only pathogens present at the time of death (either the cause of death or long-term infections) but commercials and other associated microbes. Despite several studies that have reported limited preservation of such DNA, for example, the lack of preservation of *Helicobacter pylori* in ethanol - preserved specimens dating to the 18th century [18], over 45 published studies report the successful retrieval of ancient pathogen DNA from samples dating back to over 5,000 years old in humans and as long as 17,000 years ago in other species. As well as the usual sources of mummified tissue, bones and teeth, such studies have also examined a range of other tissue samples, including calcified pleura (Donoghue et al. 1998), tissue embedded in paraffin [19], [20] and formalin-fixed tissue [21].

### Ancient DNA Extractions

The Neanderthal genome project is a collaboration of scientists coordinated by the Max Planck Institute for Evolutionary Anthropology in Germany and 454 Life Science in the United State to sequence the Neanderthal genome.

Founded in July 2006, the project published their result in the May 2010 journal Science detailing an initial draft of the Neanderthal genome based on the analysis of four billion base pairs of Neanderthal DNA. The study determined that some mixture of genes occurred between Neanderthal and anatomically modern humans and presented evidence that elements of their genome remain in that of non-African modern humans [22], [23].

According to preliminary sequences, 99.7% of the base pairs of the modern human and Neanderthal genomes are identical, compared to humans sharing around 98.8% of base pairs with the chimpanzee [24]. (Other studies concerning the commonality between chimps and humans have modified the commonality of 98% to a commonality of only 94 %, showing that the genetic gap between humans and chimps is far larger than originally though.) [25][26].

The researchers recovered ancient DNA of Neanderthal by extracting the DNA from the femur bones of three 38,000-year-old female Neanderthal specimens from Vindija Cave, Croatia, and other bones found in Spain, Russia, and Germany. Only about half a gram of the bone samples (or 21 samples each 50-100 mg [27]) was required for the sequencing, but the project faced many difficulties, including the contamination of the samples by the bacteria

that had colonized the Neanderthal's body and humans who handled the bones at the excavation site and at the laboratory [28].

Additionally, in 2010, the announcement of the discovery and analysis of Mitochondrial DNA (mtDNA) from the Denisova hominin in Siberia revealed that this specimen differs from that of modern humans by 385 bases (nucleotides) in the mtDNA strand out of approximately 16,500, whereas the difference between modern humans and Neanderthal is around 202 bases. In contrast, the difference between chimpanzees and modern humans is approximately 1,462 mtDNA base pairs. Analysis of the specimen's nuclear DNA is under way and is expected to clarify whether the find is a distinct species [29], [30]. Even through the Denisova hominin's mtDNA lineage predates the divergence of Neanderthals, coalescent theory does not preclude a more recent divergence date for her nuclear DNA.

In 2006, two research teams working on the same Neanderthal sample published their result, Richard Green and his team in Nature [31], and Noonan et al. in Science [32]. The results were received with some criticism, mainly surrounding the issue of a possible admixture of Neanderthals into the modern human genome [33]. The speech-related gene FOXP2 with the same mutation as in modern humans was discovered in ancient DNA in the El Sidron wall JD, Kim Sk (October 2007). "Inconsistencies in Neanderthal genomic DNA sequences". PLoS Genet. 3(10): 1862-6. Doi:10.1371/journal.pgen.0030175.PMC 2014787. PMID 17937503.

".....the large fragments are much more similar on average to human DNA. In fact, the large fragment have an estimated human- Neanderthal sequence divergence time that is less than the estimated divergence time between two Hausa (West African) sequence (see Materials and Methods). If true, this would indicate greater similarity between human and Neanderthal than between TWO EXTANT MEMBERS OF THE Hausa population."

Wall and Kim: "As in both Noonan et al. and Green et al., we only use human-specific mutations to calculate sequence divergence times. Neanderthal-specific mutations are excluded because the vast majority of these (90%) are thought to be caused by post-mortem DNA damage."

In recent years, ancient DNA has been used to understand aspects of Neanderthal biology. Both mitochondrial (mtDNA) and nuclear DNA have been extracted from fossils and sequenced. These sequences have provided information about the appearance, speech capability and population structure of Neanderthal as well as their

Phylogenetic relationship with anatomically modern humans.

Neanderthal DNA bears on several debates concerning the origins of modern humans. What was the relationship between Neanderthals and anatomically modern Homo sapiens? Did Neanderthal and anatomically modern humans interbreed? Did Neanderthals contribute to the modern genome? How much? Scientists answer these questions by comparing Neanderthal DNA and mtDNA to that of modern humans.

Ancient DNA has been retrieved and analyzed from Egyptian mummies and from animals such as quaggas, mammoths, moas and marsupial wolves. The first analysis of mitochondrial DNA (mtDNA) from Neanderthals was published in 1997.

The specimen was taken from the first Neanderthal fossil discovered, from Feldhofer Cave, in the Neander Valley, Germany. A small sample of bone was ground up to extract mtDNA.

The Neanderthal mtDNA sequences were substantially different from modern human mtDNA (Krings et al. 1997, 1999). Researchers compared the Neanderthal to modern human and chimpanzee sequences. Most human sequences differ from each other by on average 8.0 substitutions, while the human and chimpanzee sequences differ by about 55.0 substitutions. The Neanderthal and modern human sequence differed by approximately 27.2 substitutions. Using this mtDNA information we found the last common ancestor of Neanderthals and modern humans dates to approximately 550,000 to 690,000 years ago, which is about four times older than the modern human mtDNA pool. This is consistent with the idea that Neanderthals did not contribute substantially to modern human genome.

A second mtDNA sequence, announced in 2000, was derived from a 29,000 year old Neanderthal found in Mezmaiskaya Cave, Russia (Ovchinnikov et al. 2000). Although the Mezmaiskaya Cave sequence was slightly different than the Feldhofer Neanderthal, the two Neanderthal mtDNA sequences were distinct from those of modern humans. These results confirmed the earlier findings that showed that Neanderthals were unlikely to have contributed to the modern human genome. As with the previous study of Neanderthal mtDNA, results were consistent with separation between the Neanderthal and modern human gene pools or with very low amounts of gene flow between the two groups.

Further mtDNA sequence confirmed sequence differences between Neanderthals and modern humans. Researchers

compared Neanderthal mtDNA to that of modern humans from different geographic region. If Neanderthal had interbred with modern humans in Europe, then researchers would have expected to find more similarities between Neanderthals and Europeans than between Neanderthals and other modern humans. However, Neanderthals were equidistant with genetic separation between modern humans and Neanderthals. However, this does not explicitly disprove because interregional gene flow between modern humans could have swamped the Neanderthal contribution to Europeans (Relethford 2001).

Researchers have also studied ancient DNA from anatomically modern Homo sapiens from Europe Dating to the same time period as the Neanderthals. Material from two Paglicci Cave, Italy individuals, dated to 23,000 and 25,000 years old, was sequences. The Paglicci Homo sapiens mtDNA sequences were different from all Neanderthal mtDNA sequences but were within the range of variation for modern human mtDNA sequences (Caramelli et al. 2003). Mitochondrial DNA from the Paglicci specimens as well as other ancient humans fit within the range of modern humans, but the Neanderthals remain consistently genetically distinct. This shows that early anatomically modern Homo sapiens were not very different genetically from current modern humans, but were still different from Neanderthals. Though this evidence does not have disproved the idea of Neanderthal and modern human admixture, it shows that moderns and Neanderthals did not have more genetic similarities during the Pleistocene that were subsequently lost. If interbreeding did occur, Neanderthal mtDNA sequences could have been lost due to genetic drift.

### **Challenges in Extracting Ancient DNA**

Working with ancient DNA is very challenging, both in terms of findings sufficient material to work with after decomposition has occurred, and in terms of eliminating modern human contamination. Distinguishing between modern human and ancient genetic material is particularly difficult when the ancient DNA comes from close relatives of modern humans.

Organisms decompose after death. Water, oxygen and microbes break down DNA. Within 100,000 years, all DNA is destroyed. Ancient DNA tends to be found in small quantities. The DNA that is extracted is generally fragmentary and damaged. Some damage results in changes to the DNA sequence. Cytosine can change to uracil, which is read by copying enzymes as thymine, resulting in a C to transition. Changes from G to A also occur. DNA errors are very common at the ends of molecules.

Contamination by modern DNA is a particularly difficult problem to solve. Labs and chemicals may be contaminated by the DNA of the people working in them, while many fossils have been handled by researchers for years. Contamination is difficult to detect because Neanderthals and humans share much of their genetic material, making some DNA sequences indistinguishable. Researchers have developed ways to analyze the results of ancient DNA sequencing efforts to determine whether contamination is likely and how much has occurred. Analysis of the results and efforts to keep labs and specimens free of modern DNA is very important as some researchers believe that the early studies of Neanderthal DNA included modern contaminants.

## **SEQUENCING OF THE NEANDERTHAL GENOMIC INFORMATION**

### **Sequencing the Complete Neanderthal Mitochondrial Genome**

After successfully sequencing large amounts of DNA and devising strategies to deal with potential contamination, a team led by Svante Paabo from the Max Planck Institute, reported the first complete mtDNA sequence for a Neanderthal (Green et al. 2008). The 0.3 gram sample was taken from a 38,000-year old Neanderthal from Vindija Cave, Croatia. Complete Neanderthal mtDNA sequences give researchers more information about the relationship between modern humans and Neanderthals, as well as information about Neanderthal population size.

The complete mtDNA sequence shows that Neanderthals were outside the range of modern human mtDNA sequence shows that Neanderthals were outside the range of modern human mtDNA variation. Researchers compared that mtDNA sequence with that of modern humans. They compared sequence changes that resulted in no synonymous amino acid changes with synonymous changes. They found a larger number of no synonymous changes in the Neanderthal lineage, possibly implying that Neanderthals had a small population size with weaker purifying selection (Green et al. 2008).

Map showing range of Neanderthals prior to the entry of Homo sapiens into Europe.

Later, Svante Paabo's Lab sequenced the entire mitochondrial genome of five Neanderthals (Briggs et al. 2009). Sequences came from two individuals from the Neander Valley in Germany, Mezmaiskaya Cave in Russia, El Sidron Cave in Spain and Vindija Cave in Croatia. Though the Neanderthal sample comes from a wide geographic area, the Neanderthal mtDNA sequences were

not particularly genetically diverse. The most divergent Neanderthal sequence came from the Mezmaiskaya Cave Neanderthal from Russia, which the oldest and easternmost specimen. To look at whether age or geographic location contributed to genetic differences, the team sequenced part of the DNA of another Mezmaiskaya Cave Neanderthal that dated to 41,000 years ago. This more recent specimen grouped with the other Neanderthals, possibly showing that age was the cause of the sequence differences (Briggs et al. 2009). Other studies show the existence of eastern, western and southern groups of Neanderthals (Fabre et al. 2009).

On average, Neanderthal mtDNA genome differs from each other by 20.4 bases and is only 1/3 as diverse as modern humans (Briggs et al. 2009). The low diversity might signal a small population size, possibly due to the incursions of modern humans into their range (Briggs et al. 2009). Neanderthal Fossilized Skull from La Ferrassie, France

### **Sequencing the Neanderthal Nuclear Genome**

Recently, there have been efforts to sequence Neanderthal nuclear genes. Two studies, one by Svante Paabo's team and one by Edward Rubin, have sequenced large amount of Neanderthal nuclear DNA using different methods. There results were announced in 2006. Given their success in sequencing some nuclear DNA, both labs launched projects to sequence the entire Neanderthal genome. Nuclear genomic sequences from Neanderthals show differences between modern humans and Neanderthals, and illustrate aspects of Neanderthal biology.

### **One Million Base Pairs of the Neanderthal Sequence**

Svante Paabo team from the Max Planck Institute for Evolutionary Anthropology in Germany announced the sequencing of one million base pairs of nuclear DNA of a Neanderthal specimen in 2006 (Green et al. 2006). After a long search for specimens with a sufficient amount of undamaged DNA to sequence and for the ones with the least evidence of contamination, they focused on Vindija 80, a Neanderthal discovered in Croatia in 1980 that is approximately 38,000 years old.

They estimated that 7.9 % of the changes in human DNA compared with that of the chimpanzee occurred after the split with Neanderthals. They dated the split between the ancestors of modern humans and Neanderthals to 465,000 to 569,000 years ago. They also found that the effective population size of the Neanderthals was small. Their success in sequencing this amount of DNA indicated that a

large-scale project to sequence the Neanderthal genome is possible.

### **Rubin's Neanderthal Nuclear DNA**

Edward Rubin's team from the Lawrence Berkeley National Laboratory in California also sequenced Neanderthal Nuclear DNA (Noonan et al. 2006). They sequenced about 65,000 base pairs from the 38, 000 years old Vindija, Croatia specimen. The technique used here produces a copy of the Neanderthal sequence that can be retained forever, reducing the need for repeated destructive sampling. The DNA is then cloned in bacteria. The average split time between the Neanderthal and modern human populations was around 370,000 years ago. They used the sequence to look at the possibility of interbreeding between Neanderthals and moderns. Admixture would be seen as derived alleles that are found in Neanderthals and in low frequencies among modern humans. They did not detect this in their sample. A simulation to test the Neanderthal Contribution to the human genome found a 0 % chance of Neanderthal input with a 0% to 20% confidence range. With this data, the authors cannot definitely rule out admixture (Noonan et al. 2006).

Some aspects of the two sets of nuclear DNA do not fit together, possibly because of contamination and sequencing errors, especially in the Green et al. (2006) study (Wall and Kim 2007). This has led the researchers to develop new methods of detecting and preventing contamination to ensure that only ancient DNA is being sequenced.

### **A Draft Sequence of the Neanderthal Genome**

In 2010, Svante Paabo's lab announced a draft sequence of the Neanderthal genome (green et al. 2010). This new study has produced evidence consistent with interbreeding between Neanderthals and anatomically modern Homo sapiens and points to aspects of the human genome that may have changed since the split between humans and Neanderthals.

DNA was extracted from three Neanderthal bones from Vindija Cave, Croatia. By comparing sequence from their mtDNA and their nuclear DNA, scientists determined that the three bones came from different individuals, although two of them might be related on their mother's side. The researchers used several methods to ensure that the DNA they were sequencing was derived from the Neanderthal specimens rather than from contamination by modern humans in the lab.

The Neanderthal sequence was compared to those of five modern humans from France, China, Papua New Guinea, as well as Africans from the San and Yoruba groups. Tests indicated that Neanderthals shared more derived alleles with non-African modern humans than with African modern humans. They compared parts of the Neanderthal genome with pairs of modern humans. While the European and Asian pairs had similar amount of derived material compared with the Neanderthal, Neanderthals had more similarities with non-African humans than with Africans. The simplest explanation for these results is gene flow from Neanderthals into modern humans. Gene flow could also have occurred from modern humans into Neanderthals. Interbreeding events between Neanderthals and modern humans might be obscured if the modern human population was large.

Neanderthal has contributed approximately 1% to 4% to the genomes of non-African modern humans. This evidence of interbreeding sheds light on how we think of the expansion of modern humans out of Africa. It refutes the strictest scenario in which anatomically modern humans replaced archaic hominins completely without any interbreeding. However, even with some interbreeding between moderns and archaic hominins, most of our genome still derives from Africa.

The data also point to the time interbreeding might have taken place. Since the Neanderthal DNA was equally related to that of the modern samples from France, China and Papua New Guinea, admixture between moderns and Neanderthals must have occurred before the Eurasian populations split off from each other. Remains of both modern humans and Neanderthals dating to around 100,000 years ago have been found in the Middle East. A few interbreeding events during this period could have produced the result found in this study.

The sequence of our close hominin relative also shows us how humans are unique. Researchers found 78 sequence differences that would have affected proteins in which Neanderthals had the ancestral state and modern humans had a newer, derived state. Five genes had more than one sequence change that affected the protein structure. These proteins include SPAG17, which is involved in the movement of sperm, PCD16, which may be involved in wound healing, TTF1, which is involved in ribosomal gene transcription, and RPTN, which is found in the skin, hair and sweat glands. Scientists do not know the function of the CAN15 protein, which was also one of the differences. Other changes may affect regulatory regions in the human sequence. Some changes are in regions that code for micro RNA molecules that regulate protein manufacture.

## Microcephalin and Archaic Hominins

The microcephalin gene relates to brain size during development. A variant of this, haplogroup D, may have been positively selected for in modern humans – and may also have come from an interbreeding event with an archaic population (Evens et al. 2006). Mutations in microcephalin cause the brain to be 3 to 4 times smaller in size. All of the haplogroup D variants come from a single copy that appeared in modern humans around 37,000 years ago. However, haplogroup D itself came from a lineage that had diverged from the lineage that led to modern humans around 1.1 million years ago. Although there was speculation that the Neanderthals were the source of the microcephalin haplogroup D (Evens et al. 2006), the Neanderthal DNA recently sequenced does not contain the microcephalin haplogroup D (Green et al. 2010).

## BRIEF INTRODUCTION TO NEANDERTHAL

The Neanderthals [34] are an extinct species or subspecies of the genus *Homo* which is closely related to modern humans [35], [36]. They are known from fossils, dating from the Pleistocene period, which have been found in Europe and parts of western and central Asia. The species is named after Neanderthal (“Neander’s Valley”), the location in Germany where it was first discovered.

Neanderthals are classified either as a subspecies of *Homo sapiens* (*Homo sapiens Neanderthalensis*) or as a separate species of the same genus (*Homo Neanderthalensis*) [37]. The First humans with proto- Neanderthal Traits are believed to have existed in Europe as early as 600,000-350,000 years ago [38].

When the Neanderthals went extinct is disputed. Fossils found in the Vindija Cave in Croatia have been dated to between 33,000 and 32,000 years old, and Neanderthal artifacts from Gorham’s Cave in Gibraltar are believed to be less than 30,000 years ago, but a recent study has re-dated fossils at two Spanish sites as 45,000 years old, 10,000 years older than previously thought, and may cast doubt on recent dates at other sites. Cro-Magnon (early-modern-human) skeletal remains showing certain “Neanderthal traits” have been found in Lagar Velho (Portugal) and dated to 24,500 years ago, suggesting that there may have been an extensive admixture of the Cro-Magnon and Neanderthal populations in that region [39].

Several cultural assemblages have been linked to the Neanderthals in Europe. The earlier, the Mousterian stone

tool culture dates to about 300,000 years ago [40]. Late Mousterian artifacts were found in Gorham's Cave on the south-facing coast of Gibraltar [41], [42] other tool cultures associated with the Neanderthals include the Chatelperronian, the Aurignacian, and the Gravettian; their tool assemblages appear to have developed gradually within their populations, rather than being introduced by new population groups arriving in the region [43]. Neanderthal cranial capacity is thought to have been as large as that of modern humans, perhaps larger, indicating that their brain size may have been comparable, or larger, as well. In 2008, a group of scientists created a study using three-dimensional computer-assisted reconstructions of Neanderthal infants based on fossils found in Russia and Syria. The study showed Neanderthal and modern human brains were the same size at birth, but by adulthood, the Neanderthal brain was larger than the modern human brain [44]. They were much stronger than modern humans, having particularly strong arms and hands [45]. Males stood 164-168 cm (65-66 in) and females about 152-156 cm (60-61 in) tall [46]. Genetic evidence published in 2010 suggests that Neanderthals contributed to the DNA of anatomically modern humans, probably through interbreeding between 80,000 and 50,000 years ago with the population of anatomically modern humans who had recently migrated from Africa. According to the study, by the time that population began dispersing across Eurasia, Neanderthals genes constituted as much as 1-4% of its genome [47], [48], [49].

The species is named after the site of its first discovery, about 12 km (7.5 mi) east of Dusseldorf, Germany, in the Feldhofer Cave in the river Dussel's Neander valley named for Joachim Neander, a 17<sup>th</sup>-century German pastor and hymnist. Neander's own name was in turn a Greek translation of the German Neumann (lit. "New man"). Thal is the older spelling of Tal (both with the same pronunciation), the German word for 'Valley' (cognate with English dale) [50], [51], [52].

Neanderthal 1 was known as the "Neanderthal skull" or "Neanderthal cranium" in anthropological literature, and the individual reconstructed on the basis of the skull was occasionally called "the Neanderthal man" [53]. The binomial name *Homo Neanderthalensis*-extending the name "Neanderthal man" from the individual type specimen to the entire species - was first proposed by the Anglo-Irish geologist William King in 1864 and this had priority over the proposal put forward in 1866 by Ernst Haeckel, *Homo stupidus* [54]. The Practice of referring to "the Neanderthals" and "a Neanderthal" emerged in the popular literature of the 1920s [55].

Scientists have debated whether Neanderthals should be classified as *Homo Neanderthalensis* or *Homo sapiens Neanderthalensis*, the latter placing Neanderthals as a subspecies of *H. sapiens* [56], [57]. Some morphological studies support the view that *H. Neanderthalensis* is a separate species and not a subspecies [58]. Others, for example University of Cambridge Professor Paul Mellars, say "no evidence has been found of cultural interaction" [59] and evidence from mitochondrial DNA studies has been interpreted as evidence Neanderthals were not a subspecies of *H. sapiens* [60]. Since species can be defined by reproductive isolation, strong genomic evidence of interbreeding between the two races has led some scientists to incline toward classifying the Neanderthal as a subspecies of *H. sapiens* [61], [62], [63]. But there are documented examples of fertile inter-specific hybridization and introgression, so this is not definitive.

Comparison of the DNA of Neanderthals and *Homo sapiens* suggests that they diverged from a common ancestor between 350,000 and 400,000 years ago. This ancestor not certain, but was probably *Homo Heidelbergensis* (sometimes called *Homo rhodesiensis*). *Heidelbergensis* originated between 800,000 and 1,300,000 years ago, and continued until about 200,000. It ranged over east and South Africa, Europe and west Asia. Between 350,000 and 400,000 years ago the African branch is thought to have started evolving towards modern humans and the European branch towards Neanderthals. Scientist do not agree when Neanderthals can first be recognized in the fossil record, with dates ranging 200,000 and 300,000 years BP [64], [65], [66], [67].

### Neander Valley site

The site of Kleine Feldhofer Grotte where the type specimen was unearthed by miners in the 19<sup>th</sup> century

Neanderthal skulls were first discovered in Engis Caves (Fr), in what is now Belgium (1829) by Philippe-Charles Schmerling and in Forbes' Quarry, Gibraltar 1 (1848), both prior to the type specimen discovery in a limestone quarry of the Neander Valley in Erkrath near Dusseldorf in August 1856, three years before Charles Darwin's *On the Origin of Species* was published [68].

The type specimen, dubbed Neanderthal 1, consisted of a skull cap, two femora, three bones from the right arm, two from the left arm, part of the left ileum, fragments of a scapula, and ribs. The workers who recovered this material originally thought it to be the remains of a bear. They gave the material to amateur naturalist Johann Carl Fuhlrott, who turned the fossils over to anatomist Hermann Schaaffhausen. The Discovery was jointly announced in

1857. To date, the bones of over 400 Neanderthals have been found [69].

Neander Skull found in 1886 in Spy, Belgium. It was Frontal bone of a Neanderthal child from the cave of La Gariguela. Skull from La Chapelle aux Saints also found.

## Neanderthal Fossils

Semi-frontal view of a Neanderthal skull from Gibraltar

1829: Neanderthal skulls were discovered in Engis, in present-day Belgium.

1848: Neanderthal skull Gibraltar 1 found in Forbes' Quarry, Gibraltar called "an ancient human" at the time.

1856: Johann Karl Fuhlrott first recognized the fossil called "Neanderthal man", discovered in Neanderthal, a valley near Mettmann in what is now North Rhine- Westphalia, Germany.

1880: The mandible of a Neanderthal child was found in a secure context and associated with cultural debris, including hearths, Mousterian tools, and bones of extinct animals.

1886: Two nearly perfect skeletons of a man and woman were found at Spy, Belgium at the depth of 16 ft with numerous Mousterian-type implements.

1899: Hundreds of Neanderthal bones were described in stratigraphic position in association with cultural remains and extinct animal bones.

1899: Sand excavation workers found bone fragments on a hill in Krapina, Croatia called Husnjakovo brdo. Local Franciscan friar Dominik Antolkovic requester Dragutin Gorjanovic- Kramberger started to study the remains of bones and teeth that were found there.

1905: During the excavation in Krapina more than 5000 items were found, of which 874 residue of human origin, including bones of prehistoric man and animals, artifacts.

1908: A nearly complete Neanderthal skeleton was discovered in association with Mousterian tools and bones of extinct animals.

1925: Francis Turville-petre finds the 'Galilee Man' or 'Galilee Skull' in the Zuttiyeh Cave in Wadi Amud in The British Mandate of Palestine (now Israel).

1926: Skull fragments of Gibraltar 2, a four-year-old Neanderthal girl, discovered by Dorothy Garrod.

1953-1957: Ralph Solecki uncovered nine Neanderthal skeletons in Shanidar Cave in the Kurdistan region of northern Iraq.

1975: Erik Trinkaus' study of Neanderthal feet confirmed they walked like modern humans.

1987: Thermo luminescence results from Israeli fossils date Neanderthal at Kebara to 60,000 BP and humans at Qafzeh to 90,000 BP. These dates were confirmed by electron spin resonance (ESR) dates for Qafzeh (90,000 BP) and Es Skhul (80,000 BP).

1991: ESR dates showed the Tabun Neanderthal was contemporaneous with modern humans from Skhul and Qafzeh.

1993: A 127,000 years old DNA is found on the child of Sclayn, found in Scladina (fr.), Belgium

1997: Matthias Krings et al. are the first to amplify Neanderthal mitochondrial DNA (mtDNA) using a specimen from Feldhofer grotto in the Neander Valley.

1998: A team led by pre-history archeologist Joao Zilhao discovered an early upper paleolithic human burial in Portugal, at Abrigo do Legar Velho, which provided evidence of early modern humans from the west of the Iberian Peninsula. The remains, a largely complete skeleton of an approximately 4-year-old child, buried with pierced shell and red ochre is dated to ca. 24,500 years BP. The Cranium, mandible, dentition, and postcrania present a mosaic of European early modern human and Neanderthal features [70].

2000: Igor Ovchinnikov, Kirsten Liden, William Goodman et al. retrieved DNA from a Late Neanderthal (29,000 BP) infant from Mezmaiskaya Cave in the Caucasus [71].

2005: The Max Planck Institute for Evolutionary Anthropology launched a project to reconstruct the Neanderthal genome.

2006: The Max Planck Institute for Evolutionary Anthropology announced it planned to work with Connecticut-based 454 Life Sciences to reconstruct the Neanderthal genome.

2009: The Max Planck Institute for Evolutionary Anthropology announced the "first draft" of a complete Neanderthal genome is completed [72].

2010: Comparison of Neanderthal genome with modern humans from Africa and Eurasia shows that 1-4% of

modern non-African human genome might come from the Neanderthals [73], [74].

2010: Discovery of Neanderthal tools far away from the influence of *H. Sapiens* indicate that the species might have been able to create and evolve tools on its own, and therefore be more intelligent than previously thought. Furthermore, it was proposed that the Neanderthals might be more closely related to *Homo sapiens* than previously thought and that may in fact be a sub species of it [75]. Evidence has more recently emerged that these artifacts are probably of *H. sapiens* origin.

2012: Charcoal found next to six paintings of seals in Nerja caves, Malaga, Spain has been dated to between 42,300 and 43,500 years old. The paintings themselves will be dated in 2013, and if their pigment matches the date of the charcoal, they would be the oldest known cave paintings. Jose Luis Sanchidrian at the University of Cordoba, Spain believes the paintings are more likely to have been painted by Neanderthals than early modern humans [76].

2013: A jawbone found in Italy had features intermediate between Neanderthals and *Homo sapiens* suggesting it could be a hybrid the mitochondrial DNA is Neanderthal. [77].

#### **Sites where typical Neanderthal fossils have been found**

Early Neanderthals lived in the Last glacial period for a span of about 100,000 years. Because of the damaging effects the glacial period had on the Neanderthal sites, not much is known about the early species. Countries where their remains are known include most of Europe south of the line of glaciations, roughly along the 50<sup>th</sup> parallel north, including most of Western Europe, including the south coast of Great Britain [78], Central Europe and the Balkans [79], some sites in Ukraine and in western Russia and east of Europe in Siberia to the Altai Mountains and south through the Levant to Indus River. It is estimated that the total Neanderthal population across this habitat range numbered at around 70,000 at its peak [80].

Neanderthal fossils have not been found to date in Africa, but there have been finds rather close to Africa, both on Gibraltar and in the Levant. At some Levantine sites, Neanderthal remains, in fact, date after the same sites were vacated by modern humans. Mammal fossils of the same time period show cold-adapted animals were present alongside these Neanderthals in this region of the Eastern Mediterranean. This implies Neanderthals were better adapted biologically to cold weather than modern humans

and at time displaced them in parts of the Middle East when the climate got cold enough [81].

*Homo sapiens* appears to have been the only human type in the Nile River valley during these periods, and Neanderthals are not known to have ever lived south-west of modern Israel. When further climate change caused warmer temperatures, the Neanderthal range likewise retreated to the north along with the cold-adapted species of mammals. Apparently these weather -induced population shifts took place before modern people secured competitive advantages over the Neanderthal, as these shifts in range took place well over ten thousand years before modern people totally replaced the Neanderthal, despite the recent evidence of some successful interbreeding [82].

There were separate developments in the human line<sup>3</sup>, in other regions such as Southern Africa, that somewhat resembled the European and western/central Asian Neanderthals, but these people were not actually Neanderthals. One such example is Rhodesian man (*Homo rhodesiensis*) who existed long before any classic European Neanderthals, but had a more modern set of teeth, and arguably some *H. rhodesiensis* populations were on the road to modern *H. sapiens*. At any rate, the populations in Europe and West/Central Asia underwent more and more "Neanderthalization" as time went on. There is some argument that *H. rhodesiensis* in general was ancestral to both modern humans and Neanderthals, and that at some point the two populations went their separate ways, but this supposes that *H. rhodesiensis* goes back to around 600,000 years ago.

To date, no intimate connection has been found between these similar archaic people and the Western/Central Eurasian Neanderthals, at least during the same time as classic Eurasian Neanderthals, and *H. rhodesiensis* seems to have lived about 600,000 years ago, long before the time of classic Neanderthals. This said, some researchers think that *H. rhodesiensis* may have lived much later than this period, depending on the method used to date the fossils, leaving this issue open to debate. Some *H. rhodesiensis* features, like the huge brow ridge, may have been caused by convergent evolution. There is no evidence to date that Neanderthals knew how to construct boats or rafts, and the paucity of human fossils in North Africa west of the Nile Valley should be noted.

It appears incorrect, based on present research and known fossil finds, to refer to any fossils outside Europe or Western and Central Asia as true Neanderthals. They had a known range that possibly extended as far east as the Altai Mountains, but not farther to the east or south, and

apparently not into Africa. At any rate, in North-East Africa the land immediately south of the Neanderthal range was possessed by modern humans *Homo sapiens idaltu* or *Homo sapiens*, since at least 160,000 years before the present. 160,000-year old hominid fossils at Jebel Irhoud in Morocco were previously thought to be Neanderthal, but it is now clear that they are early modern humans [83].

Classic Neanderthal fossils have been found over a large area, from northern Germany to Israel and Mediterranean countries like Spain [84] and Italy [85] in the south and from England and Portugal in the West to Uzbekistan in the east. This area probably was not occupied all at the same time. The northern border of their range, in particular, would have contracted frequently with the onset of cold periods. On the other hand, the northern border of their range as represented by fossils may not be the real northern border of the area they occupied, since Middle Paleolithic-looking artifacts have been found even further north, up to 60° N, on the Russian plain [86]. Recent evidence has extended the Neanderthal range by about 1,250 miles (2,010 Km) east into southern Siberia's Altai Mountains [87], [88].

Neanderthal anatomy differed from modern humans in that they had a more robust build and distinctive morphological features, especially on the cranium, which gradually accumulated more derived aspects, particularly in certain isolated geographic regions. Evidence suggests they much stronger than modern humans [89], while they were comparable in height; based on 45 long bones from at most 14 males and 7 females, Neanderthal males averaged 164-168 cm (65-66 in) and females 152-156 cm (60-61 in) tall [90]. Samples of 26 specimens in 2010 found an average weight of 77.6 kg (171 lb) for males and 66.4 kg (146 lb) for females [91]. A 2007 genetic study suggested some Neanderthals may have had hair and light skin color [92]. Neanderthals made advanced tools [93], had a language (the nature of which debated) and lived in complex social groups. The Moldova archaeological site in eastern Ukraine suggests some Neanderthals built dwelling using animal bones. A building was made of mammoth skulls, jaws, tusks and leg bones and had 25 hearths inside [94]. While largely carnivorous [95], [96] and apex predators [97]; new studies indicate Neanderthals had cooked vegetables in their diet [98], [99]. In 2010, a U.S. researcher reported finding cooked vegetable matter in the teeth of a Neanderthal skull. Contracting the earlier belief they were exclusively (or almost exclusively) carnivorous [100] and apex predators [101], [102].

Early investigations concentrated on Mitochondrial DNA (mtDNA), which owing to strictly matrilineal inheritance and subsequent vulnerability to genetic drift is of limited value in evaluating the possibility of interbreeding of Neanderthals with Cro-Magnon people.

In 1997, geneticists were able to extract a short sequence of DNA from Neanderthal bones from 30,000 years ago [103], the extract in of mtDNA from a second specimen was reported in 2000, and showed no sign of modern human descent from Neanderthals [104].

Scientist at the Max Planck Institute for Evolutionary Anthropology started extracting the DNA. In July 2006, the Max Planck institute for Evolutionary Anthropology and 454 Life Sciences announced that they would sequence the Neanderthal genome over the next two years. This genome is expected to be roughly the size of the human genome, three billion base pairs, and share most of its genes. It was hoped the comparison would expand understanding of Neanderthals, as well as the evolution of humans and human brains [105].

Swante Paabo has tested more than 70 Neanderthal specimens. Preliminary DNA sequencing showed from a 38,000 year old bone fragment of a femur found at Vindija Cave, Croatia, in 1980 showed Neanderthals and modern humans share about 99.5% of their DNA. From mtDNA analysis estimates, the two species shared a common ancestor was about 500,000 years ago. An article [106] appearing in the journal Nature has calculated the species diverged about 516,000 years ago, whereas fossil records show time of about 400,000 years ago [107]. A 2007 study pushes the point of divergence back to around 800,000 years ago [108].

Edward Rubin of the Lawrence Berkeley National Laboratory states recent genome testing of Neanderthal suggests human and Neanderthal DNA are some 99.5% to nearly 99.9% identical [109], [110].

On 16 November 2006, Lawrence Berkeley National Laboratory issued a press released suggesting Neanderthals and ancient humans probably did not interbreed [111]. Edward M. Rubin, Director of the U.S. Department of Energy's Lawrence Berkeley National Laboratory and the Joint genome institute (JGI), sequenced a fraction (0.00002) of genome nuclear DNA (nDNA) from a 38,000 year old Vindija Neanderthal femur. They calculated the common ancestor to be about 353,000 years ago, and a complete separation of the ancestors of the species about 188,000 years ago [112].

## NEANDERTHAL GENOME PROJECT

Their results show the genome of modern humans and Neanderthals are at least 99.5% identical, but despite this genetic similarity, and despite the two species having coexisted in the same geographic region for thousands of years, Rubin and his team did not find any evidence of any sufficient crossbreeding between the two. Rubin said, "While unable to definitively conclude that interbreeding between the species of humans did not occur, analysis of the nuclear DNA from the Neanderthal suggests the low likelihood of it having occurred at any appreciable level [113].

In 2008 Richard E. Green et al. from Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany published the full sequence of Neanderthal Mitochondrial DNA (mtDNA) and suggested "Neanderthals had a long findings, James Morgan asserted the mtDNA sequence contained clues that Neanderthal lived in "Small and isolated populations, and probably did not breed with their human neighbors." [114], [115].

In the same publication, It was disclosed by Svante Paabo that in the previous work in the Max Planck institute that "contamination was indeed in issue", and they eventually realized that 11% of their sample was modern human DNA [116], [117]. Since then, more of the preparation work has been done in clean areas and 4-base pair 'tags' have been added to the DNA as soon as it is extracted so the Neanderthal DNA can be identified.

With 3 billion nucleotides sequenced, analysis of about 1/3 showed no sign of admixture between modern humans and Neanderthals, according to Paabo. This concurred with the work of Noonan from two years earlier. The variant of microcephalin, which was suggested to be of Neanderthal origin and responsible for rapid brain growth in humans, not found in Neanderthals. Nor was the MAPT variant, a very old variant found primarily in Europeans [118].

However, an analysis of a first draft of the Neanderthal genome by the same team released in May 2010 indicates interbreeding may have occurred [119], [120]. " Those of us who live outside Africa carry a little Neanderthal DNA in US," said Paabo, who led the study. "The proportion of Neanderthal-inherited genetic material is about 1 to 4 percent. It is a small but very real proportion of ancestry in non-Africans today," says Dr. David Reich of Harvard Medical School, who worked on the study. This research compared the genome of the Neanderthals to five modern humans from China, France, Sub-Saharan Africa, and Papua New Guinea. The finding is that about 1 to 4 percent of the genes of the non-Africans came from Neanderthals, compared to the baseline defined by the two Africans [121].

This indicates a gene flow from Neanderthals to modern humans, i.e., interbreeding between the two populations. Since the three non-African genomes show a similar proportion of Neanderthal sequences, the interbreeding must have occurred early in the migration of modern humans out of Africa, perhaps in the Middle East. No evidence for gene flow in the direction from modern humans to Neanderthals was found. Gene flow modern humans Neanderthals would not be expected if contact occurred between a small colonizing population of modern humans and a much larger resident population of Neanderthals. A very limited amount of interbreeding could explain the findings, if it occurred early enough in the colonization process [122].

While interbreeding is viewed as the most parsimonious interpretation of the genetic discoveries, the authors point out they cannot conclusively rule out an alternative scenario, in which the source population of non-African modern humans was already more closely related to Neanderthals than other Africans were, due to ancient genetic divisions within Africa [123].

Among the genes shown to differ between present -day humans and Neanderthals were RPTN, SPAG17, CAN15, TTF1 and PCD16 [124].

## **EXTINCTION HYPOTHESIS**

### **Neanderthal extinction hypothesis**

Neanderthal fossils found in Vindija Cave in Croatia have been dated to between 32,000 and 33,000 years old [125], and what have been claimed as the last traces of Mousterian culture (Neanderthal artifacts but not bones) have been found in Gorham's Cave on the remote south-facing coast of Gibraltar, dated to less than 30,000 years ago. However, a recent re-examination of Neanderthal bones from two Spanish Neanderthal sites has suggested they were around 45,000 years old, 10,000 years older than previously thought. Prof. Civil Finlayson, who excavated Gorham's Cave, argues that the sites which have been re-dated are highland ones which would have been inhospitable in the approach to an ice age. However bone collagen degrades in the warmer lowland sites where Finlayson thinks Neanderthals would have survived longer and it has yet to be determined whether the re-dating affects other Neanderthal sites with recent dates [126].

### **Possible scenarios for the extinction of the Neanderthals**

Neanderthals were a separate species from modern humans, and became extinct (because of climate change or interaction with humans) and were replaced by modern humans moving into their habitat beginning around 80,000 years ago [127]. Competition with humans probably contributed to Neanderthal extinction [128], [129]. Jared Diamond has suggested a scenario of violent conflict and displacement [130].

Neanderthals were a contemporary subspecies that bred with modern humans and disappeared through absorption (interbreeding theory).

A companion ignimbrite volcanic super-eruption around 40,000 years ago, followed by a second one a few thousand years later, has been hypothesized as having contributed to the demise for the Neanderthal, based on evidence from Mezmaiskaya cave in the Caucasus Mountains of Southern Russia. Cave is radiocarbon dated to be about 29,000 years BP and therefore from one of the latest living Neanderthal individuals. The sequence shows 3.48% divergence from the Feldhofer Neanderthal. Phylogenetic analysis places the two Neanderthals from the Caucasus and western Germany together in a clad that is distinct from modern humans, suggesting that their mtDNA types have not contributed to the modern human mtDNA pool [131].

mtDNA-based simulation of modern human expansion in Europe starting 1600 generations ago for Neanderthal range in light grey [132].

As Paul Jordan notes: "A natural sympathy for the underdog and the disadvantaged lends a sad poignancy to the fate of the Neanderthal folk, however it came about. "Jordan, though, does say that there was perhaps interbreeding to some extent, but that population that remained totally Neanderthal were probably out – competed and marginalized to extinction by the Aurignacians [133].

### **Climate change**

About 55,000 years ago, the weather began to fluctuate wildly from extreme cold conditions to mild cold and back in a matter of few decades. Neanderthal bodies were well suited for survival in a cold climate – their barrel chests and stocky limbs stored body heat better than Cro-Magnons. However, the rapid fluctuations of weather caused ecological changes to which the Neanderthals could not adapt; familiar plants and animals would be replaced by completely different ones within a lifetime. Neanderthals' ambush techniques would have failed as grasslands replaced trees. A large number of Neanderthals would

have died during these fluctuations, which peaked about 30,000 years ago [134].

Studies on Neanderthal body structures have shown that they needed more energy to survive than any other species of hominid. Their energy need were up to 100-350 calories more per day comparing to projected anatomically modern human males weighing 68.5 kg (151.0 lbs) and females 59.2 kg (130.5 lbs) [135]. When food became scarce, this difference may have played a major role in the Neanderthals' extinction [136].

## **COEXISTENCE WITH HOMO SAPIENS**

### **Genome globe current event**

This section's factual accuracy may be compromised due to out-of-date information. Please update this article to reflect recent events or newly available information. (May 2012)

There is no longer certainly regarding the identity of the humans who produced the Aurignacian culture, even though the presumed westward spread of anatomically modern humans (AMHs) across Europe is still based on the controversial first dates of the Aurignacian. Currently, the oldest European anatomically modern Homo sapiens is represented by a robust modern-human mandible discovered at Pesteră cu Oase (Southwest Romania), dated to 34,000-36,000 years ago. Human skeletal remains from the German site of Vogelherd, so far regarded as the best association between anatomically Homo sapiens and Aurignacian culture, were revealed to represent intrusive Neolithic burials anatomically into the Aurignacian levels and subsequently all the key Vogelherd fossils are now dated to 3,900-5,000 years ago instead. As for now, the expansion of the first anatomically modern humans into Europe cannot be located by diagnostic and well-dated AMH fossils "West of the Iron Gates of the Danube" before 32,000 years ago [137].

### **Reconstruction of a Neanderthal man and woman from the Neanderthal Museum**

Consequently, the exact nature of biological and culture interaction between Neanderthals and other human groups between 50,000 and 30,000 years ago has been contested [138]. A new proposal strives to resolve the issue by proposing the Gravetians rather than the Aurignacians as anatomically modern humans who contributed to the Eurasian genetic pool after 30,000 years ago [139]. Correspondingly, the human skull fragment found at the Elbe River bank at Hahnofersand near Hamburg was once radiocarbon-dated to 36,000 years ago and seen as possible

evidence for the intermixing of Neanderthals and anatomically modern humans. It is now dated to the more recent Mesolithic [140].

### **Interbreeding hypotheses**

Chris Stringer's hypothesis of the family tree of genus *Homo* published 2012 in *Nature*. *Homo floresiensis* originated in an unknown location from unknown ancestors and reached remote parts of Indonesia. *Homo erectus* spread from Africa to western Asia, then East Asia and Indonesia; its presence in Europe uncertain, but it gave rise to *Homo antecessor*, found in Spain. *Homo Heidelbergensis* originated from *Homo erectus* in an unknown location and dispersed across Africa, southern Asia and southern Europe (other scientists interpret fossils, here named *Heidelbergensis*, as late *erectus*). *Homo sapiens* spread from Africa to western Asia and then to Europe and southern Asia. Eventually reaches Australia and the Americas. In addition to Neanderthals and Denisovans a third gene flow in Africa is indicated at the right [141].

An alternative to extinction is that Neanderthals were absorbed into the Cro-Magnon population by interbreeding. This would be counter to strict versions of the Recent African Origin, since it would imply that at least part of the genome of Europeans would descend from Neanderthals.

Hans Petter Steensby, while strongly emphasizing that all modern humans are of mixed origins, proposed the interbreeding hypothesis in 1907, in the article *Race studies in Denmark* [142]. He held that this would best fit current observation, and attacked the by then widespread idea that Neanderthals were ape-like or inferior.

The most vocal proponent of the hybridization hypothesis is Erik Trinkaus of Washington University [143]. Trinkaus claims various fossils as products of hybridized populations. Including the "child of Large Velho", a skeleton found at Large Velho in Portugal dated to about 24,000 years ago [144]. In a 2006 publication co-authored by Trinkaus, the fossils found in 1952 in the cave of Pesteria Muierii, Romania, are likewise claimed as descendants of previously hybridized populations [145].

Genetic research has confirmed that some admixture took place [146]. The genomes of all non-African include portions that are of Neanderthal origin [147], [148] due to interbreeding between Neanderthals and the ancestors of Eurasians in Northern Africa or the Middle East prior to their spread. Rather than absorption of the Neanderthal population, this gene flow appears to have been of limited duration and limited extent. An estimated 1 to 4 percent of

the DNA in Europeans and Asians (French, Chinese and Papua probands) is non-modern, and shared with ancient Neanderthal DNA rather than with Sub-Saharan Africans (Yorba and San probands) [149]. Nonetheless, more recent genetic studies seem to suggest that modern humans may have mated with "at least two groups" of ancient humans: Neanderthals and Denisovans [150].

While modern humans share some nuclear DNA with the extinct Neanderthals, the two species do not share any mitochondrial DNA [151], which in primates is always maternally transmitted. This observation has prompted the hypothesis that whereas female humans interbreeding with male Neanderthals were able to generate fertile offspring, the progeny of female Neanderthals who mated with male humans were either rare, absent or sterile [151]. However, some researchers have argued that there is evidence of possible interbreeding between female Neanderthals and male modern humans [152], [153].

### **The Ferrassie skull**

Neanderthal 1: Initial Neanderthal specimen found during an archaeological dig in August 1856 which was discovered in a limestone quarry at the Feldhofer grotto in Neanderthal, Germany. The find consisted of a skull cap, two femora, the three right arm bones, two of the left arm bones, ilium and fragments of a scapula and ribs.

La Chapelle-aux Saints 1: Called the Old Man, a fossilized skull discovered in La Chapelle-aux-Saints, France, by A. and J. Bouyssonie, and L. Bardon in 1909. Characteristics include a low vaulted cranium and large brow ridge typical of Neanderthals. Estimated to be about 60,000 years old, the specimen was severely arthritic and had lost all his teeth, with evidence of healing. For him to have lived on would have required that someone process his food for him, one of the earliest examples of Neanderthal altruism (similar to Shanidar I.)

La Ferrassie : A fossilized skull discovered in La Ferrassie, France, by R. Captain in 1909. It is estimated to be 70,000 years old. Its characteristics include a large occipital bun, low-vaulted cranium and heavily worn teeth.

Le Moustier: A fossilized skull discovered in 1909, at the archaeological site in Peyzac-le-Moustier, Dordogne, France. The Mousterian tool culture is named after Le Moustier. The skull, estimated to be less than 45,000 years old, includes a large nasal cavity and a somewhat less developed brow ridge and occipital bun as might be expected in a juvenile.

### **Type Specimen, Neanderthal 1**

Shanidar 1: Found in the Zagros Mountains in (Iraqi Kurdistan); a total of nine skeletons found believed to have lived in the Middle Paleolithic. One of the nine remains was missing part of its right arm; theorized to have been broken off or amputated. The find is also significant because it shows that stone tools were present among this tribe's culture. One was buried with flowers, showing that some type of burial ceremony may have occurred.

### **Bones with Neanderthal traits in chronological order (Sorted by time)**

Mixed with H. Heidelbergensis traits

>350ka: Sima de los Huesos c. 500:350 ka ago [154], [155].

350-200 ka: Pontnewydd 225 ka ago.

200-135ka: Atapuerca [156], vertesszollos, Ehringsdorf, Casal de Pazzi, Biache, La Chaise, Mountain, Prince, Lazaret, Fotechevade

### **Typical H. neanderthalensis traits**

135-45 ka: krapina, Saccopastore skulls, Malarnaud, Altamura, Ganovce, Denisova, Okladnikov Altai, Pech de I Aze Tabun 120ka -100+-5ka, [114] Qafzeh 100, Shanidar 1 to 9 80-60ka, La Ferrassie 1 70 ka, Kebara 60 ka, Regourdou, Mt. Circeo, Combe Grenal, Erd 50ka, La Chapelle-aux Saints 1 60ka, Amud 1 53 +-8 ka [156], [157] Teshik-Tash.

45-35ka : Le Moustier 45 ka, Feldhofer 42 ka, La Quina, I Horus, Hortus, Kulna, Sipka, Saint Cesaire, Bacho Kiro, El Castillo, Bnolas, Arcy-sur-Cure. [158].

<35 ka: Chatelperron, Figueria Brava, Zafaraya 3

0ka [159], Vogelherd 3 [160], Vindija 32,400 +-800 B.P [160]. (Vi-208 31,390+-220, vi-207 32,400 +-1,800 14C B.P.) [160]. Velika Pecina,

### **Homo sapiens with some Neanderthal-like archaic traits**

<35 pester cu Oase 35 ka, Mladec 31 ka, Pester Muierii 30ka (n/s) [161], Lapedo Child 24.5 ka

Early artistic reconstructions mostly presented Neanderthals as beastly creatures, emphasizing hairiness and rough, dark complexion [162]. More recent reconstructions acknowledge that due to the lineage evolution in European latitude there is reason to believe that Neanderthals were fair-skinned probably with no more facial hair than modern man. Also, archeological evidence exists indicating that they may have communicated by speech, used tools and engaged in artistic endeavors.

Reconstructions of Neanderthal men, women and children have become much more intelligent-looking and pleasing to the modern eye [163], [164].

### **Neanderthal Language & Speech**

A recent paper, authored by Dutch scientists at the Max Planck Institute of Psycholinguistics, argues that not only did Neanderthals and modern humans interact and interbreed – but they also likely shared some elements of speech and language.

In fact, this new research claims that modern language and speech date back to the most recent ancestor we shared with the Neanderthals, Homo heidelbergensis. And it's even possible that the language we speak today retain some elements of Neanderthal language.

“We suggest that if Neanderthals had something like modern speech and language, and that we did interact with them, then maybe modern languages have some trace of that language” Dr. Dan Dediu, psycholinguist and one of the study's lead authors, told The Huffington Post.

By pointing to ancient DNA and new archeological discoveries, the linguistic suggests that language developed through a gradual Darwin process of both biological and culture evolution—rather than, as another popular theory states, through one or just a few random genetic mutations.

If this new theory is correct, the team's findings could push back the origins of modern language by 10 times what was previously thought.

While many believe that modern language began around 50,000 years ago, the paper names a period about a million years ago as the beginning of modern language—sometime between the emergence of our genus, Homo sapiens (around 1.8 million year ago), and the emergence of Homo heidelbergensis.

“I think the most important point we're trying to make is that modern language is old, which means that there has been a lot of time for language to become changed and shaped by culture and biology, “Dediu said. “It didn't happen overnight.”

The paper, published online on July 5 in the journal *Frontiers in Language Sciences*, comes on the heels of the Neanderthal Genome Project—a 2010 collaboration of scientist at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany who found that ancient Neanderthals and modern humans shared some DNA.

## NEANDERTHAL EVOLUTION

According to new research conducted by two Dutch scientists, Neanderthals had language and speech capabilities much like modern day humans.

Humans may be even closer to their Neanderthal cousins that they previously thought. According to new research, the extinct human species had language and speech capabilities much like modern-day humans.

Dutch researchers Dan Dediu and Stephen C. Levinson argue in a paper that modern speech dates back to the Neanderthal and Denisovans, human species that died out roughly half a million years ago, according to a statement. If so, modern language could be 1 million years old, not 10,000 to 50,000 years old as widely believed by the scientific community.

The two researchers from the Max Planck Institute in the Neanderthals drew their conclusion based on their interpretation of DNA evidence, palaeoanthropological and archaeological discoveries surrounding Neanderthals. One piece of evidence that may shed light on the theory is the genetic evidence that modern humans interacted with Neanderthals and Denisovans in Africa. In the same way humans carry some of their genes, perhaps African and non-African languages have Neanderthal origins.

One of the genes modern humans and Neanderthals share in common is FOXP2, gene associated with language. While some variants are linked to language dysfunction in modern humans and some believe that Neanderthals were incapable of producing vowel the vowel sounds "a", "e", and "u" – that many not be the case. New anatomical research shows that Neanderthals had the same genetic sequence as modern humans, making them capable of speech, the New York Times reports.

While theories surrounding the origin of speech vary from the belief that language arose with creativity and self-awareness to stone technology from 2 million years ago - scientists have relied on computer models of the human vocal tract to determine how different species produced sound, BBC reports.

"I estimated what a Neanderthal's vocal tract would look like if it had a human-like vocal tract as well as a chimp-like vocal tract. You can input the basic shapes into a computer program, which return the acoustical properties for the vowel 'e'" Franklin Yates of George Washington University told the BBC.

According to Yates, Neanderthals had deeper voices than modern humans.

"Their voices would have been very deep compared to our own. They would have had an entirely different octave range. So if Barry White had sung in a choir of Neanderthals, he would have been the tenor," Yates said.

The specific MCR1 mutation in Neanderthals has not found in modern humans (or occurs extremely rarely in modern humans). This indicates that the two mutations for red hair and pale skin occurred independently and does not support the idea of gene flow between Neanderthals and modern humans. Pale skin may have been advantageous to Neanderthals living in Europe because of the ability to synthesize vitamin D.

### Neanderthals, Language and FOXP2

The FOXP2 gene is involved in speech and language (Lai et al.2001). Changes in the FOXP2 gene sequence led to problems with speech, oral and facial muscle control in modern humans with a mutation in the gene. It impairs language function. Modern humans and Neanderthals share two changes in FOXP2 compared with the sequence in chimpanzees (Krause et al. 2007). Neanderthals may also have their own unique derived characteristics in the FOXP2 gene that were not tested for in this study.

The human FOXP2 gene is on a haplotype that was subject to a strong selective sweep. A haplotype is a set of alleles that are inherited together on the same chromosome. The researchers then tried to determine how the FOXP2 variant came to be found in both Neanderthals and modern humans. One scenario is that it could have been transferred between species via gene flow. The researchers do not think this is likely since there is no evidence indicating that gene flow has occurred. Another possibility is that the derived FOXP2 was present in the ancestor of both anatomically modern Homo sapiens and Neanderthals with the selective sweep that made it prevalent occurring after the divergence between the groups. A third scenario, which the authors think is most likely, is that the changes and selective sweep occurred before the divergence between the populations.

The gene that produces the ABO blood system is polymorphic in humans. Various selection factors may favor different alleles, leading to the maintenance of distinct blood groups in modern human populations. Though chimpanzees also have different blood groups, they are not the same as human blood types. While the mutation that causes the human B blood group arose around 3.5 Ma, the O group mutation dates to around 1.15 Ma. Lalueza-Fox and colleagues (2008) tested whether

Neanderthals had the O blood group. They found that two Neanderthal specimens from Spain probably had the O blood type, though there is the possibility that they were OA or OB. Though the O allele was likely to have already appeared before the split between humans and Neanderthals, it could also have arisen in the Neanderthal genome via gene from modern humans.

### **Bitter Taste Perception and Neanderthals**

Like some modern humans, some Neanderthals were able to taste bitter substances. Some items that taste bitter may be toxic in large quantities so that ability to taste bitter substances may have protected hominins from accidental poisoning. Some of these bitter chemicals are found in vegetables. For instance, humans vary in their ability to perceive a bitter substance similar to that found in Brussels sprouts, broccoli and cabbage.

The ability to taste bitter substances is controlled by a gene, TAS2R38. Some individuals are able to taste bitter substances, while others have a different version of the gene that does not allow them to taste bitter items. Possession of two copies of alleles associated with tasting bitter substances gives the individual greater perception of bitter tastes than the heterozygous state, in which individuals have one tasting allele and one non-tasting allele. Two copies of a non-tasting allele lead to inability to taste bitter substances.

A Neanderthal from El Sidron, Spain was sequenced for the TAS2R38 gene. They found that this individual was heterozygous and thus was able to perceive bitter taste, although not as strongly as a homozygous individual with two copies of the tasting allele would be able to (Lalueza-Fox et al. 2009). Since the Neanderthal sequenced was heterozygous, the two alleles (tasting and non-tasting) were probably both present in the common ancestor of Neanderthals and modern humans. Though chimpanzees also vary in their ability to taste bitterness, their abilities are controlled by different alleles than those found in humans, indicating that non-tasting alleles evolved separately in the hominin lineage.

The comparison also pointed out regions that might have been under positive selection in modern humans. Though some of the genomic areas that may have been positively selected for in modern humans may have coded for structural or regulatory regions, others may have been associated with energy metabolism, cognitive development and the morphology of the head and upper body.

## **THE RELATION BETWEEN MODERN HUMANS AND NEANDERTHALS**

The relationship between modern humans and archaic hominins, particularly Neanderthals, has been the subject of much debate. While the idea that modern humans originated in Africa and spread out to other parts of the world (Out of Africa) is widely accepted, several scenarios have been proposed to account for the replacement of archaic hominin populations. Under strict replacement, modern humans did not interbreed with the archaic populations as they expanded their geographic range. In less strict scenarios, admixture between the populations occurred, but in small amounts, with the bulk of modern human ancestry tied to Africa. The multiregional hypothesis holds that hominin populations in Eurasia and Africa were held together by gene flow. Fossil and genetic evidence supports an African origin for *Homo sapiens*.

Mitochondrial DNA shows differences between Neanderthals and modern humans. Neanderthal mtDNA also differed from that of anatomically modern *Homo sapiens* from the same time period. Proponents of multiregional and admixture models argue that these results are consistent with African origin for modern *Homo sapiens*, but do not explicitly rule out admixture between modern humans and archaic populations (Templeton 2007, Relethford 2008). Neanderthal genetic sequences introduced into the human genome may have been subsequently lost through genetic drift (Relethford 2001), while similarities between modern Europeans and Neanderthals, which would be expected if Neanderthals and modern humans interbred while in Europe, could have been lost due to gene flow between modern humans from different regions.

Various analyses have examined the amount of Neanderthal contribution to modern human mtDNA. One analysis was unable to find positive evidence for interbreeding, but could not rule out a small genetic contribution (Serre et al. 2004). Other researchers (Plagnol and Wall 2006, Wall et al. 2009) looked at the pattern of variation in modern human DNA to determine whether modern humans mixed with more ancient populations. Their recent models are consistent with 14% archaic-modern admixture in European and American populations, and 1.5% admixture in East Asian populations. Nested clad Phylogenetic analysis shows evidence of three expansions out of Africa at 1.9 Ma, 650,000 years, and 130,000 years, which is consistent with the admixture between ancient and modern populations rather than complete replacement (Templeton 2002, 2005, 2007). Other researchers contend that factors such as

population structure within Africa could have preserved old haplotypes and produced the pattern found in the nested clade analysis (Satta and Takahata 2002).

Though it is difficult to prove or quantify admixture, small amounts of interbreeding were supported by a variety of analysis. However, the substantial differences between Neanderthal and modern human mtDNA is consistent with large-scale replacement and some amount of interbreeding between modern and archaic populations. Interbreeding between archaic and moderns may have involved different species of archaic hominins, including populations in Africa, Asia and Europe.

The draft sequence of the Neanderthal genome provides more evidence that interbreeding between Neanderthals and modern humans may have occurred. It showed more similarities between non-African modern humans and Neanderthals than between African modern humans and Neanderthals. This difference between regions is consistent with interbreeding between Neanderthals and the ancestors of Eurasian modern humans before they branched off into regional groups. Approximately 1 to 4% of non-African modern human DNA is shared with Neanderthals.

### Red-Headed Neanderthals

Ancient DNA has been used to show aspects of Neanderthal appearance. A fragment of the gene for the melanocortin 1 receptor (MRC1) was sequenced using DNA from two Neanderthal specimens from Spain and Italy, El sidron 1252 and Monte Lessini (Lalueza-Fox et al. 2007). Neanderthals had a mutation in this receptor gene that has not been found in modern humans. The mutation changes an amino acid, making the resulting protein less efficient. Modern humans have other MCR1 variants that are also less active resulting in red hair and pale skin. The less active Neanderthal mutation probably also resulted in red hair and pale skin, as in modern humans. The 1253 and 1351c specimens [165], suggesting Neanderthals might have shared some basic language capabilities with modern humans [166].

Svante Paabo, director of the Department of Genetics at the Max Planck Institute for Evolutionary Anthropology and head of its Neanderthal genome project.

In 2006, Richard Green's team had used a then new sequencing technique developed by 454 Life Sciences that amplifies single molecules for characterization and obtained over a quarter of million unique short sequences ("reads"). The technique delivers randomly located reads,

so that sequences of interest, e.g. genes that differ between modern humans and Neanderthals, show up at random as well. However, this form of direct sequencing destroys the original sample so to obtain new reads more sample must be destructively sequenced [167].

Noonan et al., led by Edward Rubin, used a different technique, one in which the Neanderthal DNA is inserted into bacteria, which make multiple copies of a single fragment. They demonstrated that Neanderthal genomic sequences can be recovered using a metagenomic library-based approach. The entire DNA in the sample is "immortalized" into metagenomic libraries. A DNA fragment is selected, and then propagated in microbes. The Neanderthal DNA can be sequenced or specific sequences can be studied [168].

Overall, their results were remarkably similar. One group suggested there was a hint of mixing between human and Neanderthal genomes, while the other found none, but both teams recognized that the data set was not large enough to give a definitive answer [169].

The publication by Noonan et al. revealed Neanderthal DNA sequences matching chimpanzee DNA, but not modern human DNA, at multiple locations, thus enabling the first accurate calculation of the date of the most recent common ancestor of *H. sapiens* and *H. Neanderthalensis*. The research team estimates the most recent common ancestor of their *H. Neanderthalensis* samples and their *H. sapiens* reference sequence lived 706, 000 years ago (divergence time), estimating the separation of the human and Neanderthal ancestral populations to 370,000 years ago (split time) [170]. Earlier mitochondrial DNA research led by geneticist Svante Paabo in 1997 had indicated present day *Homo sapiens* and Neanderthals mtDNA split into separate lineages approximately 500,000 years ago. Green et al. calculated a divergence time of 516,000 years ago and do not indicate a split, while they claim the average divergence time between alleles within humans is thus 459,000 years with a 95% confidence interval between 419,000 and 498,000 years. These two dates (~500k) were calculated with assumption on non-selective pressure. If positive selection forced mtDNA changes then the split time may be shorter. In this study the team stated: "Neanderthal genetic difference to humans must therefore be interpreted within the context of human diversity." On the other hand, Noonan et al. found no evidence of Neanderthal admixture to the modern human genome, but they did not preclude an admixture of up to 20% with a ca certainty better than 95%, and hence did not claim to present a definite answer to the question.

In February 2009, the Max Planck Institute's team led by Paabo announced that they had completed the first draft of the Neanderthal genome [171]. An early analysis of the data suggested in "the genome of Neanderthals, a human species driven to extinction" "no significant trace of Neanderthal genes in modern humans" [172]. New results suggested that some adult Neanderthals were lactose intolerant. 15 On the question of potentially cloning a Neanderthal, Paabo commented, "Starting from the DNA extracted from a fossil, it is and will remain impossible" [172].

In May 2010 the project released a draft of their report on the sequenced Neanderthal genome. Contradicting the results discovered while examining mitochondrial DNA, they demonstrated a range of genetic contribution to non-African modern humans ranging from 1% to 4%. From their Homo sapiens samples in Eurasia (French, Han Chinese & Papuan) the authors state that it is likely that interbreeding occurred in the Levant before Homo sapiens migrate into Europe [173]. However, this finding is disputed because of the paucity of archeological evidence supporting their statement. The fossil evidence does not conclusively place Neanderthals and modern humans in close proximity at this time and place [174].

Previously, in 1999, a report was made of a rib fragment from the partial skeleton of a Neanderthal infant found in the Mezmaiskaya cave in the northwestern foothills of the Caucasus Mountains that was radiocarbon-dated to 29,195. Previously, in 1999, a report was made of a rib fragment from the partial skeleton of a Neanderthal infant found in the Mezmaiskaya cave in the northwestern foothills of the Caucasus Mountains that was radiocarbon-dated to 29,195 + 965 B.P., and therefore belonging to the latest lived Neanderthals. Ancient DNA was recovered for a mtDNA sequence showing 3.48% divergence from that of the Feldhofer Neanderthal, some 2,500 km to the west in Germany. Phylogenetic analysis placed the two in a clad distinct from modern humans suggesting that their mtDNA types have not contributed to the modern human mtDNA pool [175].

### Criticism

This section has multiple issues. Please help improve it or discuss these issues on the talk page.

This article's Criticism or Controversy section may compromise the article's neutral point of view of the subject. (November 2010)

An editor has expressed a concern that this article lends undue weight to certain ideas, incidents, controversies or

matters relative to the article subject as a whole. (April 2012)

A 2007 review of the data by Wall and Kim 22 reanalyzes the data obtained from the published papers of Noonan et al. 12 and Green et al., 11 and it holds that the results are inconsistent with each other. The review proposes serious problems with the data quality in one of the studies, possibly due to modern human DNA contaminants and/or a high rate of sequencing errors. The reanalysis confirmed both results to the Human-Neanderthal DNA Sequence Divergence Time (common ancestor), that is 706 kya (thousands of years ago) to the Noonan et al. analysis and 516 kya to the Green et al. analysis. The modern European-Neanderthal population split time was estimated at 35 kya for Green et al. data, and 325 kya for the Noonan et al. data. Before, no split time was estimated by the Green et al. study, and according to Wall and Kim the split time originally estimated by Noonan et al. was even higher: 440 kya (Noonan et al. paper mentions 370 kya) [176]. While Noonan et al. were unable to definitively conclude that interbreeding between the two species of humans did not occur, they proclaim little likelihood of it having occurred at any appreciable level [177]. The study opts for a 0% contribution of Neanderthal DNA to the modern European gene pool, based on the 95% confidence interval that indicates a margin between 0% and 20% contribution. The reanalyses of Wall and Kim yielded interbreeding margin between 0% and 39% to the data of Noonan et al., and margins between 81% and 100% to the data of Green et al. These vastly inconsistent results could only be reconciled by assuming a very recent split time between the two populations of 60 kya or less. However, such a recent split time would not be consistent with the estimate modern European-Neanderthal population split time from the Noonan et al. data.

The key assumption of Noonan et al [178], [179] is that the 38,000 years of fossilization suffered by the Neanderthal DNA should have the genome analysis focus on ancient DNA fragments of about 50 to 70 base pairs in length.[clarification needed] Green et al. do not make such an assumption; they generalized towards the exclusion of modern human nuclear DNA contamination by finding little evidence of modern human DNA contamination. Such mitochondrial tends to remain preserved longer than nuclear DNA [180]. However, wall and Kim noted a length dependence of the results, having the small fragments pointing to a divergence time [clarification needed] similar to the result of Noonan et al. and the large fragments much more similar on average to modern human DNA – even to the extent of indicating an estimated human-Neanderthal sequence divergence time that is less than the estimated

divergence time of two extant members of one referenced population in west Africa [181].

Although wall and Kim hold modern human contamination to be size-biased, since Neanderthal DNA would be expected to have a tendency to be degraded into short fragments, they noted that length dependence of the result means that alignment issues alone are unlikely to be a sufficient explanation, since longer fragments would be easier to align and thus the data from longer fragments should be more accurate. Still they mark this as a signal of potential contamination in the data of Green et al. No similar signal of potential contamination was found in the data of Noonan et al.

Contamination in the data of Green et al. should have decreased the Neanderthal-specific sequence divergence in this study. Since this is not the case, the assumption of contamination also would indicate a higher sequencing error rate in the Green et al. data, since sequence errors would look the same as Neanderthal-specific mutations. These Neanderthal-specific mutations already were considered prone to error due to post-mortem DNA damage in both studies, and were excluded from the result [181].

In summary, Wall and Kim consider a model with 78% contamination more likely than a model with no contamination and 94% admixture. A 2009 Green paper addresses some of the criticism [182].

## CONCLUSION

What if you could trace your ancestry back to around 5,000 years ago? Researchers were able to do just that in a fascinating new DNA study, which found a direct genetic link between the ancient remains of Native Americans and their living relatives.

"It's very exciting to be able to have scientific proof that corroborates what our ancestors have been telling us for generations," study co-author and participant Jocelyn Mitchell said in a written statement. "It's very amazing how fast technology is moving to be able to prove this kind of link with our past."

In the study, U.S. and Canadian researchers used mitochondrial genomes (mitogenomes) sequencing to analyze DNA inherited exclusively through mothers. Looking at the mitogenomes is cheap, easier to sequence than nuclear DNA, and skirts around the problem that European men mixed with Native American women.

The researchers collected DNA from 60 living indigenous people belonging to the Tsimshian, Haida and Nisga'a tribes in the northern coast of British Columbia. The tribes' oral histories and archaeological sites indicate they have lived in the region for generations, which made them good candidates for tracing their lineage back so many years.

Complete mitogenomes were extracted from the remains of four Mid-Holocene individuals found in British Columbia's Lucy Islands and Dodge Island, and then that information was compared to the DNA of the study participants.

What was found? The research team discovered one of the living individuals carried this same "mitogenomic signature" as a young adult female who lived on Dodge Island 2,500 years ago – which also matched the mitogenomes of the remains of a woman who lived in the Lucy Islands 5,500 years ago. Wow.

Three other living participants had mitogenomes that linked back to the remains of another individual found on Dodge Island, who may have lived around 5,000 years ago.

This is the beginning of the golden era for ancient DNA research because we can do so much now that we couldn't do a few years ago because of advances in sequencing technologies," study co-author Dr. Ripan Malhi, an anthropology professor at the University of Illinois and Institute for Genomic Biology professor, said in a written statement. "We're just starting to get an idea of the mitogenomic diversity in the Americas, in the living individuals as well as the ancient individuals." The new study was published online on June 7, 2013 in the journal PLoS ONE.

## Mitochondrial DNA is in our blood!

Paleo-DNA Laboratory is the leader in human mitochondrial DNA analysis. We were one of the first Canadian laboratories to develop mitochondrial DNA analysis techniques. We were the first laboratory to offer a DNA training course emphasizing mitochondrial DNA analysis. Several biotech companies, DNA service and forensic labs have extensively used the Paleo-DNA Laboratory as the source for staff training and new employee recruitment.

We are the preferred outsourcing partner for mitochondrial DNA analysis for one of the largest DNA service companies in the United States. Our lab analyzes samples from various partner companies and institutions from around the world. Our DNA laboratory is specifically designed to accommodate low-copy number or degraded samples. Each sample is considered unique and is analyzed

as such. Our commitment to contamination control and high standards emphasizes the reliability of results produced at the Paleo-DNA Laboratory. If you need timely, accurate results why not go where the world's largest companies go?

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## REFERENCE

- [1] Green RE, Krause J, Briggs AW, et al. (May 2010). "A draft sequence of the Neanderthal genome". *Science* 328 (5979) : 710-22. Doi : 10.1126/science.1188021. PMID 20448178.
- [2] The Neanderthal in Us
- [3] Than, Ker (6 May 2010). "Neanderthals, Humans Interbred-First Solid DNA Evidence". National Geographic Society. Retrieved 9 May 2010.
- [4] Cohen, Jon (29 June 2007). "Relative Differences : The Myth of 1%". AAAS.
- [5] Humans and Chimps: Close But Not That Close." *Scientific American*. 2006-12-19. Retrieved 2006-12-20.
- [6] Scientists Decode Majority of Neanderthal Man's Genome." *Deutsche Welle*. 13 February 2009.
- [7] Green RE, Krause J, Briggs AW, et al. (May 2010). "A draft sequence of the Neanderthal genome". *Science* 328 (5979) : 710-22. Doi:10.1126/science.1188021. PMID 20448178.
- [8] Mc Groarty, Patrick (12 February 2009). "Team in Germany maps Neanderthal genome." *The Associated Press*.
- [9] Brown, David (March 25, 2010). "DNA from bone shows new human forerunner, and raises array of questions." *Washington Post*
- [10] Krause J, Fu Q, GoodJM et al. (April 2010). "The complete mitochondrial DNA genome of an unknown hominin from southern Siberia". *Nature* 464 (7290) : 894-7 doi : 10.1038/nature08976. PMID 20336068
- [11] Green, Richard E.; et al. 916 November 2006). "Analysis of one million base pairs of Neanderthal DNA." *Nature* 444 (7117): 330-336 doi : 10.1038/nature05336. PMID17108958.
- [12] Noonan, James P.; et al. (17 November 2006). "Sequencing and Analysis of Neanderthal Genomic DNA."
- [13] *Science* 314 (5802) : 1113-1118. Doi:10.1126/science.1131412. PMC 2583069, PMID17110569.
- [14] Timmer, John (2006-11-17). Welcome to Neanderthal genomics."
- [15] Krause J, Lalueza-Fox C, Orlando L, et al. (November 2007). "The derived FOXP2 variant of modern humans was shared with Neanderthals". *Curr. Biol.* 17 (21) : 1908-12. Doi:10.1016/j.cub.2007.10.008 PMID 17949978.
- [16] Inman, Mason (12 February 2009). Neanderthal Genome "First Draft" Unveiled". *National Geographic News*.
- [17] Lynn Yarris (15 November 2006). Neanderthal Genome sequencing Yields surprising results and opens a New Door to future studies. "Lawrence Berkley National Laboratory. Retrieved 2009-02-16.
- [18] Noonan et al. (2006) : "Our analyses suggest that on average the Neanderthal genomic sequence we obtained and the reference human genome sequence share a most recent common ancestor ~706,000 years ago, and that the human and Neanderthal ancestral populations split ~370,000 years ago, before the emergence of anatomically modern humans."
- [19] Wade, Nicholas (12 February 2009). "Scientists in Germany Draft Neanderthal Genome." *The New York Times*. Retrieved 20 May 2010.
- [20] Igor V. Ovchinnikov; Anders Gotherstrom, Galina P. Romanova, Vital M. Kharitonov, Kerstin Liden & William Goodwin (30 March 2000). "Molecular analysis of Neanderthal DNA from the northern Caucasus". *Letters to Nature*. *Nature* 404. Pp 490-493 doi:10.1038/35006625. Retrieved 13 March 2011.
- [21] Briggs, A.W., Good, J.M., Green R.E., Krause, J. Maricic, T., Stenzel, U., Lalueza-Fox, C., Rudn, P., Brajkovi, D., Kuan, Gui, I., Schmitz, R., Doronichev, V.B., Golovanova, L.V., De la Rasilla, M., Fortea, J., Rosas, A., Paabo, S., 2009. Targeted retrieval and analysis of five Neanderthal mtDNA genomes. *Science* 325 : 318-321.
- [22] Brown T.A., 2010 stranger from Siberia. *Nature* 464:838-839.
- [23] Callaway, Ewen 2009. First draft of Neanderthal genome is unveiled. *New scientists* 12 Feb, 2009.
- [24] Caramelli, D., Lalueza-Fox, C., Vernesi, C., Lari, M., Casoli, A., Mallegni, F., Chairelli, B., Dupanlop, I., Bertranpetit, J., Barbujani, G., Bartorelle, G., 2003. Evidence for a genetic discontinuity between Neanderthals and 24,000 year old anatomically modern Europeans. *Proceedings of the National Academy of Sciences* 100:6593-6597.
- [25] Caramelli, D. Milani L., Vai, Modi A Pacholi, E. Girardi, M. Pilli, E., Lari, M. Lippi, B. Ronchitelli, A. Mallegni, F. Casoli, A, Bartorelle, G. Barbujani, G. 2008, A 28,000 years old Cro-Magnon mtDNA sequence differ from all potentiality contaminating modern sequence. *PLoS One* 3(7):e2700
- [26] Coop, G, Bullaughe, K. Luca, F. Przeworski, M. 2008 the timing of selection at the human FOXP2 Gene. *Molecular Biology and Evolution* 25(7):1257-1259
- [27] Currat, M. Excoffier, L. 2004, modern humans did not admit with Neanderthals during their range expansion into Europe. *PLoS Biology* 2:e421.
- [28] Dalton R., 2006 Neanderthal DNA yields to genome foray. *Nature* 441:260-261.
- [29] Dalton R., 2006. Neanderthal genome sees first light. *Nature* 444:254
- [30] Evans, P.D. Mekel-Bobrov, N., Vallender, E.J Hudson, R.R Lahn, B.T. 2006. Evidence that the adaptive allele of the brain size gene microcephalin introgressed into Homo sapiens from an archaic Homo Lineage. *Proceedings of the National Academy of Sciences* 103(48) : 18178-18183
- [31] Fabre, V., Condeani, S, Degioanni, A., 2009 Genetic evidence of geographical groups among Neanderthals. *PLoS One* 4(4):e5151
- [32] Green R.E., J. Krause, Briggs, A.W., Marcic, T., Stenzel, U., Kircher, M., Patterson, N. Firtz, M., Hansen, N. Durand, E.Y., Malaspina, A-S, Jensen, J.D., Marques-Bonet, T., Alkan, C., Prufer, K., Meyer, Meyer, M., Burbano, H.A., Good, J.M., Schultz, R., Aximu-Petri, A., Butthof, A., Hober, B., Hoffner, B., Siegemund, M., Weihmann, A., Nusbaun, C.,

- Lander, E.S., Russ, C., Novod, N., Affourtit, J., Egholm, M., Verna, C., Rudan, P., Brajkovic, D., Kucan, Z., Gusic, I., Doronichev, v.b., Golovanova, L.V., Lalueza-Fox, C., de la Rasilla, M., Forste, J., Rosas, A., Schmitz, R.W., Eichler, E.E., Falush, D., Birney, E., Mullikan, J.C., Slatkin, M., Neilsen, R., Kelso, J., Lachmann, M., Reich, D., Paabo, S., 2010. A draft sequence of the Neanderthal genome. *Science* 328:710-722.
- [33] Green R.E., Krause, J., Ptak, S.E., Briggs, A.W., Ronan, M.T., Simons, J.F., Du, L., Egholm, M., Rothberg J.M., Paunovic, M., Paabo, S., 2006. Analysis of One million base pairs of Neanderthal DNA. *Nature* 444:330-336.
- [34] Green, R.E., Malaspina, A.-S. Krause, J., Briggs, A., Johnson, P., Uhler, C., Meyer, M., Good, J., Maricic, T., Stenzel, 2008. A complete Neanderthal mitochondrial genome sequence determined by high-throughput sequencing. *Cell* 4: 416-426.
- [35] Hofreiter, M., Serre, D., Poinar, H.N., Kuch, M., Paabo, S., 2001. Ancient DNA. *Nature Reviews* 2: 353-359.
- [36] Holden, C., 2006. It's Neanderthal Time. *Science* 313 : 279.
- Krause, J., Lalueza-Fox, C., Orlando, L., Enard, W., Green, R.E., Burbano, H.A., Hublin, J.-J., Hanni, C., Forste, J., de la Rasilla, M., Bertranpetit, J., Rosas, A., Paabo, S., 2007. The derived FOXP2 variant of modern humans was shared with
- [37] Neandertals. *Current Biology* 17: 1908-1912. Krings, M., Stone, A., Schmitz, R.W., Krainitzki, H., Stoneking, M., Paabo, S., 1997. Neanderthal DNA Sequences and the origin of modern humans. *Cell* 90 : 19-30.
- [38] Krings, M., Geisert, H., Schmitz, R.W., Krainitzki, H., Paabo, S., 1999. DNA Sequence of the mitochondrial hypervariable region II from the Neanderthal type specimen. *Proceedings of the National Academy of Sciences USA* 96 : 5581-5585.
- [39] Lalueza-Fox, C., Gigli, E., de la Rasilla, M., Forste, J., Rosas, A., Bertranpetit, J., Krause, J., 2008. Genetic characterization of the ABO blood group in Neandertals. *BMC Evolutionary Biology* 8: 342.
- [40] Lalueza-Fox, C., E. Gigli, E., de la Rasilla, M., Forste, J., Rosas, A., 2009. Bitter taste perception in Neandertals through the analysis of the TAS2R38 gene. *Biology Letters* 5: 809 – 811.
- [41] Lalueza-Fox, C., Rompler, H., Caramelli, D., Staubert, C., Catalano, G., Hughes, D., Rohland, N., Pilli, E., Longo, L., Condemi, S., de la Rasilla, M., Forste, J., Rosas, A., Stoneking, M., Schoneberg, T., Bertranpetit, J., Hofreiter, M., 2007. Melanocortin 1 receptor allele suggests varying pigmentation among Neandertals. *Science* 318: 1453 – 1455.
- [42] Mackelprang, R., Rubin, E.M., 2008. New tricks with old bones. *Science* 312: 221-212.
- [43] Noonan, J.P., Coop, G., Kudaravalli, S., Smith, D., Krause, J., Alessi, J., Chen, F., Platt, D., Paabo, S., Pritchard, J.K., Rubin, E.M., 2006. Sequencing and analysis of Neanderthal genomic DNA. *Science* 314: 1113-1118.
- [44] Ovchinnikov, I.V., Gotherstrom, A., Romanova, G.P., Kharitonov, V.M., Liden, K., Goodwin, W., 2000. Molecular analysis of Neanderthal DNA from the northern Caucasus. *Nature* 404: 490-493.
- [45] Pennisi, E., 2009. Sequencing Neanderthal mitochondrial genomes by the half-dozen. *Science* 325: 252. Plagnol, V., Wall, J.D., 2006. Possible ancestral structure in human populations. *PLoS Genetics* 2(7): 0972-0979.
- [46] Relethford, J.H., 2001. Absence of regional affinities of Neanderthal DNA with living humans does not reject multiregional evolution. *American journal of Physical Anthropology* 115: 95-98. Relethford, J.H., 2008. Genetic evidence and the modern human origins debate. *Heredity* 100: 555-563.
- [47] Satta, Y., Takahata, N., 2002. Out of Africa with regional interbreeding? modern human origins. *Bio Essays* 24: 871-875.
- [48] Schmitz, R., Serre, D., Bobani, G., Feine, S., Hillgruber, F., Krainitzki, H., Paabo, S., Smith, F.H., 2002. The Neanderthal type site revisited : interdisciplinary investigations of skeletal remains from the Neander valley, Germany. *Proceedings of the National Academy of Sciences* 99(20): 13342-13347.
- [49] Serre, D., Langaney, A., Chech, M., Teschler-Nicola, M., Paunovic, M., Mennecier, P., Hofreiter, M., Possnert, G., Paabo, S., 2004. No evidence of Neanderthal mtDNA contribution to early modern humans. *PLoS Biology* 2: 0313-0317.
- [50] Templeton, A., 2002. Out of Africa again and again. *Nature* 416: 45-51.
- [51] Templeton, A., 2005. Haplotype trees and modern human origins. *Yearbook of Physical Anthropology* 48: 33-59.
- [52] Templeton, A., 2007. Genetics and recent human evolution. *Evolution* 61(7) : 1507-1519.
- [53] Wall, J.D., Kim, S.K., 2007. Inconsistencies in Neanderthal genomic DNA sequences. *PLoS Genetics* 3(10): e175.
- [54] Neanderthal. (2012). Dictionary.com. Retrieved September 7, 2012, from link.
- [55] Colin P.T. Baillie ; University of California, Berkeley. " Neandertals : Unique from Humans, or Uniquely Human ?". Berkeley.edu.
- [56] Smithsonian Institute National Museum of American History. " Ancient DNA and Neanderthals ". si.edu.
- [57] Tattersall I, Schwartz JH (June 1999). " Hominids and hybrids : The place of Neanderthals in human evolution ". *Proceedings of the National Academy of Sciences* 96 (13): 7117-9. Bibcode : 1999PNAS...96.7117T. doi:10.1073/pnas.96.13.7117. PMC 33580. PMID 10377375. Retrieved 17 May 2009. " Thus, although many students of human evolution have lately begun to look favourably on the view that these distinctive hominids merit species recognition in their own right as *H. Neanderthalensis* (e.g., refs. 4 and 5), at least as many still regard them as no more than a variant of our own species, *H.sapiens*."
- [58] J.L. Bischoff et al. (2003). " The Sima de los Huesos Hominids Date to Beyond U/Th Equilibrium (>350 kyr) and perhaps to 400 – 500 kyr. : New Radiometric Dates ". *J.Archaol.Sci.* 30(30): 275. doi: 10.1006/jasc.2002.0834.
- [59] Duarte C, Mauricio J, Pettitt PB, Souto P, Trinkaus E, van der Plicht H, Zilhao J (June 1999). "The early Upper paleolithic human skeleton from the Abrigo do Lagar Velho (Portugal) and modern human emergence in Iberia". *Proceedings of the National Academy of Sciences* 96 (13): 7604-9. Bibcode : 1999PNAS...96.7604D. doi: 10.1073/pnas.96.13.7604. PMC 22133. PMID 10377462. Retrieved 16 May 2009.
- [60] Skinner, A., Blackwell, R. Long, M.R. Seronie-vivien, A.-M. Tiller and J. Blickstein; New ESR dates for a new bonebearing layer at Pradayrol, Lot, France; *Paleoanthropology Society* March 28, 2007
- [61] Finlayson, C.; Pacheco, F.; Rodriguez-Vidal, J.; Fa, D.; Gutierrez-Lopez, J.; Santiago-Perez, A.; Finlayson, G.; Allue, E.; Baena, Preysler, J.; Caceres, I.; Carrion, J.; Fernandez, J.;

- Gleed-Owen,Cp; Jimenez,Espejo,Fj; Lopez,P; Lopez,Seaz,Ja; riquelme,Cantal,Ja; Sanchez,Marco,A; Guzman,Fg; Brown,K; Fuentes,N; Valarino,Ca; Villalpando,A; stringer,Cb; Martinez,Ruiz,F; Sakamoto,T (October 2006). "Late survival of Neanderthals at the southernmost extreme of Europe". *Nature* 443 (7113): 850-3. Bibcode : 2006Nature.443..850F.doi: 10.1038/nature05195. ISSN 0028-0836. PMID 16971951.
- [62] Outside Europe, Mousterian tools were made by both Neanderthals and early modern Homo sapiens. (Donald Johanson & Blake Edgar (2006) *From Lucy to Language*, Simon & Schuster, p. 272)
- [63] R.G.Bednarik (2011). "The Expulsion of Eve". *Developments in Primatology* : 25-55
- [64] Neanderthal brain Size at birth sheds Light in Human Evolution". *National Geographic*. 2008-09-09. Retrieved 2009-09-19
- [65] Helmuth H (1998). "Body height, body mass and surface area of the Neanderthals". *Zeitschrift Fur Morphologie Und Anthropologie* 82 (1): 1-12. PMID 9850627.
- [66] Cochran, Gregory ; Harpending, Henry (2009). *The 10,000 year explosion : How Civilization Accelerated Human Evolution* . New York : Basic Books . "Logically, if admixture occurred at all, it had to happen somewhere in Neanderthal – occupied territory , which means Europe and Western Asia."
- [67] Richard E. Green et al (2010). "A Draft Sequence of the Neanderthal Genome". *Science* 328(5979):710–722.Bibcode: 2010Sci...328..710G. doi : 10.1126/science. 1188021. PMID 20448178.
- [68] Rincon, Paul. "Neanderthal genes survive in us". BBC News (BBC). Retrieved 2010-05-07.
- [69] Howell, F.Clark . "The Evolutionary Significance of Variation and Varieties of 'Neanderthal' Man". *The Quarterly Review of Biology*, Vol.32, No.4 (Dec.1957),pp.330-347.
- [70] Foley, Tim. Talkorigins Archive. "Neandertal or Neandertal ?". 2005.
- [71] Inter alia, Vogt, Karl C. & al. "Lectures on man : his place in creation, and in the history of the earth", publications of the Anthropological Society of London, p.302 & 473. Longman, Green, Longman, & Roberts (1864).
- [72] Inter alia, *Boys' Life*, p. 18. January 1924.
- [73] *The Oxford illustrated Dictionary*. Great Britain : Oxford University Press. 1976 [1975]. P.564. "(tahl)"
- [74] "Neanderthal adjective – definition in British English Dictionary & Thesaurus – Cambridge Dictionary Online".
- [75] *Dictionary*. Cambridge .org. 2013-01-08. Retrieved 2013-01-22.
- [76] Free online dictionary definitions for learners of English – Oxford Advanced Learner's Dictionary
- [77] "Neanderthal I Define Neanderthal at Dictionary. Com". *Dictionary. reference. Com*". Retrieved 2013-01-22.
- [78] Kurten , Bjorn (10 October 1995). *Dance of the Tiger : A Novel of the Ice Age*. University of California Press. pp.xxi. ISBN 0 – 520 – 20277 – 5. Retrieved 9 May 2012.
- [79] "....And Etymology". Carl J. Pollet. *Science News*, vol. 140, No. 12 (Sep. 21, 1991), p.191. published by : Society for Science & the Public. Article Stable URL : <http://www.jstor.org/stable/3975867>. Access date : 9 May 2012.
- [80] "The early Upper Paleolithic human skeleton from the Abrigo do Lager Velho (Portugal) and modern human emergence in Lberia". Pnas.org. 1999 – 06 – 22. Retrieved 2013 – 01 – 22.
- [81] Harvati k, Frost SR, McNulty KP (February 2004). "Neanderthal taxonomy reconsidered : Implications of 3D primate models of intra –and interspecific difference". *Proceedings of the National Academy of Science* 101 (5): 1147 – 52. Bibcode :2004PNAS..101.1147H.doi: 10.1073/pnas.0308085100. PMC 337021. PMID 14745010.
- [82] "Modern humans, Neanderthals shared earth for 1,000 years". ABC News (Australia). 1 September 2005. Retrieved 19 September 2006.
- [83] Hedges SB (December 2000). "Human evolution. A start for population genomics". *Nature* 408 (6813): 652-3.doi : 10.1038/35047193. PMID 11130051.
- [84] "Homo heidelbergensis : Evolutionary Tree". Smithsonian National Museum of Natural History. Retrieved 17 March 2013.
- [85] Stringer, Chris. "The Ancient Human Occupation of Britain". Natural History Museum, London. Retrieved 17 March 2013.
- [86] Stringer , Chris (2011). *The Origin of our Species*. Penguin. Pp. 26 – 29, 202.
- [87] Johansson, Donald ; Edger, Blake (2006). *From Lucy to Language*. Simon & Schuster . p.38. Isbn 978 – 0 – 7432 – 8064 – 8.
- [88] "Homo neanderthalensis". Smithsonian Institution . Retrieved 18 May 2009.
- [89] "New Evidence On The Role Of Climate In Neanderthal Extinction". *Science Daily*.
- [90] Krings, M; Stone, A; Schmitz, Rw; Krainitzki, H; Stoneking, M; Paabo , S (1997). "Neandertal DNA sequences and the origin of modern humans". *Cell* 90 (1): 19-30. Doi:10.1016 / S0092 – 8674 (00) 80310 – 4. ISSN 0092-8674. PMID 230299.
- [91] Duarte et al. "The early Upper aleolithic human skeleton from the Abrigo do Lagar Velho (Portugal) and modern human emergence in the Iberian eninsula". *Proceedings of the National Academy of Sciences of the United of America (PNAS)* 96 (13) : 7604 – 609.
- [92] Ovchinnikov, Iv; Gotherstrom , A; Romanova, Gp; Kharitonov, Vm ; Liden , K; Goodwin, W (March 2000).
- [93] "Molecular analysis of Neanderthal DNA from the northern Caucasus". *Nature* 404 (6777) : 490 – 3.doi : 10.1038 / 35006625. ISSN 0028 – 0836. PMID 10761915.
- [94] Morgan , James (2009). "Neanderthals distinct from us". BBC News. [1] [dead link]
- [95] Benqazzi, S.; Douka, K.; Fornai, C.; Bauer, C.C.; Kullmer, O.; Svoboda, J.L.; Pap,I., Mallegni,F.et al.(2011). "Early dispersal of modern humans in Europe and implications for Neanderthal behaviour". *Nature* 479 (7374) : 525 – 528.doi : 10.1038 / Nature 10617. PMID 22048311.edit
- [96] Fergal Macerlean (2012). "First Neanderthal cave paintings discovered in Spain". *New Scientist*.
- [97] Viegas, Jennifer (27 March 2013). "First Love Child of Human, Neanderthal Found". *Discovery News*. Retrieved 11 April 2013.
- [98] Dargie, Richard(2007). *A History of Britain*. London : Arcturus.
- [99] Ancient tooth provides evidence of Neanderthal movement" (Press release). Durham University. 11 February 2008. Retrieved 18 May 2009.
- [100] O'neill, Dennis. " Evolution of Modern Humans : Neanderthals", Palomar College, June 10, 2011, accessed August 21, 2011.

- [101] Jordan, P.(2001) Neanderthal : Neanderthal Man and the story of Human Origins. The History Press .
- [102] Arsuage, J.L; Gracia, A; Martinez, I; Bermudez de castro, J.M; Rosas, A; Villaverde, V; Fumanal, M.P (1989). " The human remains from Cova Negra (Valencia, Spain) and their place in European Pleistocene human evolution". *Journal of Human Evolution* 19: 55- 92.doi: 10.1016 / 0047 – 2484 (89) 90023 – 7.
- [103] Mallegni, F.,Piperno, M., and Segre, A (1987). " Human remains of Homo sapiens neanderthalensis from the Pleistocene deposit of sants Croce Cave, Bisceglie (Apulia), Italy". *American Journal of Physical Anthropology* 72 (4):
- [104] Pavlov P, Roebroeks W, Svendsen Ji (2004). " The Pleistocene colonization of northeastern Europe : a report on recent research". *Journal of Human Evolution* 47 (1-2) : 3 – 17.doi : 10.1016 / j.jhevol.2004.05.002. PMID 15288521.
- [105] Wade, Nicholas ( 2 October 2007). " Fossil DNA Expands Neanderthal Range". *The New York Times*. Retrieved 18 May 2009.
- [106] Ravilious, Kate (1 October 2007). " Neandertals Ranged Much Father East Than Thought". *National Geographic Society*.
- [107] "Neanderthal". *BBC*. Retrieved 18 May 2009.
- [108] Helmuth H (1998). " Body height, body mass and surface area of the Neanderthals". *Zeitschrift Fur Morphologie und Anthropologie* 82 (1): 1 – 12.
- [109] Froehle, Andrew W; Churchill, Steven E (2009). " Energetic Competition Between Neandertals and Anatomically Modern Humans". *Paleo Anthropology* : 96 – 116.
- [110] Laleuzq – Fox, Carles; Holger Rompler et al. (2007 – 10 – 25). " A Melanocortin 1 Receptor Allele Suggests Varying Pigmentation Among Neanderthals". *Science* 318 (5855) :1453 – 5.doi: 10.1126 / science.1147417. PMID 17962522.;see also Rincon, Paul (25 October 2007). "Neanderthals were flame – haired". *BBC News*. Retrieved 25 October 2007.
- [111] Moskvitch, katia (2010 – 09 – 24). "Neanderthals were able to 'develop their own tools". *BBC News (BBC)*. Retrieved 2010 – 10 – 01.
- [112] Gray, Richard (December 18, 2011). "Neanderthals built homes with mammoth bones". *Telegraph.co.uk*.
- [113] Richards MP, Pettitt PB, Trinkaus E, Smith FH, Paunovic M, Karavanic I ( June 2000). "Neanderthal diet at Vindija and Neanderthal predation : The evidence from stable isotopes". *Proceedings of the National Academy of Sciences* 97 (13) : 7663 – 6.
- [114] Bocherens H, Drucker DG, Billiou D, Patou-Mathis M, Vandermeersch B. (July 2005). "Isotopic evidence for diet and subsistence pattern of the Saint-Cesaire I Neanderthal : review and use of a multi-source mixing model." *Hum Evol.* 49 (1) : 71 – 87.doi : 10.1016 / j.jhevol.2005.03.003. PMID 15869783.
- [115] Ghosh, Pallab. " Neanderthals cooked and ate vegetables." *BBC News*. December 27, 2010.
- [116] Henry, A.G.; Brooks,A.S.; Piperno, D.R. (2010). " Microfossils in calculus demonstrate consumption of plants and cooked foods in Neanderthal diets (Shanidar III, Iraq; Spy 1 and II, Belgium)". *Proceedings of the National Academy of Sciences* 108 (2) : 486 – 491.
- [117] Brown, Cynthia Stokes. *Big History*. New York, NY : The New Press, 2008. Print.
- [118] Moulson, geir; Associated Press ( 20 July 2006). " Neanderthal genome project launches". *MSNBC*. Retrieved 22 August 2006.
- [119] Green Re, Krause J, Ptak SE, et al. ( November 2006). "Analysis of one million base pairs of Neanderthal DNA". *Nature* 444 (7117) : 330 – 6. Bibcode : 2006 Nature. 444..330G.doi: 10.1038 / nature 05336. PMID 17108958.
- [120] Wade, Nicholas ( 15 November 2006). " New Machine sheds Light on DNA of Neanderthals." *The new York Times*. Retrieved 18 May 2009.
- [121] Pennisi E (May 2007). "ancient DNA. No sex please, we're Neandertals". *Science* 316 (5827) : 967.doi: 10.1126 / science. 316.5827.967a. PMID 17510332.
- [122] "Neanderthal bone gives DNA clues". *CNN*. Associated Press. 16 November 2006. Archived from the original on 18 November 2006. Retrieved 18 May 2009.
- [123] Than, Ker; *Live Science* ( 15 November 2006). "Scientists decode Neanderthal genes". *MSNBC*. Retrieved 18 May 2009.
- [124] Neanderthal Genome Sequencing yields Surprising results And Opens A New Door to future studies" (Pressrelease). *Lawrence Berkeley National Laboratory*. 16 November 2006. Retrieved 31 May 2009.
- [125] Hayes, Jacqui ( 15 November 2006). "DNA find deepens Neanderthal mystery". *Cosmos*. Retrieved 18 May 2009.
- [126] Green , Re; Malaspinas, As; Krause, J;Briggs, Aw; Johnson, Pl;Uhler, C;Meyer, M;Good, Jm;Maricic, T;Stenzel, U;Prufer, K;Siebauer, M;Burbano, Ha; Ronan, M;Rothberg, Jm; Egholm, M; Rudan, P; Brajkovic, D; Kucan, Z; Gusic, I; Wikstrom, M;Laakkonen, L; Kelso, J; Slatkin, M; Paabo, S (August 2008). " A Complete mitochondrial genome sequence determined by high – throughput sequencing". *Cell* 134 (3) : 416 – 26. Doi: 10.1016 / j.cell.2008.06.021. ISSN 0092 – 8674. PMC 2602844. PMID 18692465.
- [127] Evans PD, Mekel – Bobrov N, Vallender EJ, Hudson RR, Lahn Bt (November 2006). " Evidence that the adaptive allele of the brain size gene microcephalin introgressed into Homo sapiens from an archaic Homo lineage". *Proceedings of the National Academy of Sciences* 103 (48) : 18178 – 83. Bibcode : 2006PNAS..10318178E. Doi: 10.1073 / pnas.0606966103. PMC 1635020. PMID 17090677.
- [128] Evans PD, Gilbert SL, Mekel-Bobrov N, Vallender EJ, Anderson JR, Vaez – Azizi LM, Tishkoff SA, Hudson RR, Lahn BT (September 2005). "Microcephalin, agene regulating brain size, continues to evolve adaptively in humans". *Science* 309 (5741): 1717 – 20. Bibcode 2005Sci...309. 1717E.doi: 10.1126 /science.1113722. PMID 16151009.
- [129] Elizabeth Pennisi ( 2009). " NEANDERTAL GENOMICS : Tales of a Prehistoric Human Genome". *Science* 323 (5916) : 866 – 871.
- [130] Green RE, Briggs AW, Krause J, Prufer K, Burbano HA, Siebauer M, Iachmann M, Paabo S. (2009). "The Neandertal genome and ancient DNA authenticity". *EMBO J* 28 (17) : 2494 – 502.
- [131] Higham, Tom et al (2006). "Revised direct radiocarbon dating of the Vindija G1 Upper Paleolithic Neandertals". *Proceedings of the National Academy of Sciences of the United States of America*.
- [132] Callaway, Ewen ( 4 February 2013). " Neanderthal settlements point to earlier extinction". *Nature News*.
- [133] First genocide of human beings occurred 30,000 years ago". *Pravda*. 24 October 2007. Retrieved 18 May 2009.
- [134] McKie, Robin ( 17 May 2009). "How Neanderthals met a grisly fate : devoured by humans". *The Observer ( London)*. Retrieved 18 May 2009.

- [135] Hortola P, Martinez – Navarro B (2012). “ The Quaternary megafaunal extinction and the fate of Neanderthals : An integrative working hypothesis”. *Quaternary* (in press).doi : 10.1016 / j.quaint.2012.02.037.
- [136] Diamond, Jared M. (1992). *The third chimpanzee : the evolution and future of the human animal*. New York City:Harper Collins.p. 52. ISBN 0-06-098403-1. OCLC 60088352.
- [137] Liubov Vitaliena Golovanova; Vladimir Borisovich Doronichev; Naomi Elancia Cleghorn; Marianna Alekseevna Koulkova; Tatiana Valentinovna Sapelko; M. Steven Shackley (2010; 51 (5): 655). “Volcanoes Wiped out Neanderthals, New Study Suggests” (news release). *Science Daily* ( University of Chicago Press Journals). *Journal Current Anthropology*.doi: 10.1086 / 656185. “ Significance of Ecological Factors in the Middle to Upper Paleolithic Transition”.
- [138] Bruce Bower (October 23, 2010). “Neandertals blasted out of existence, archaeologists propose”. *Science News* Vol.178#9.p.12. Retrieved March 3, 2011. “Modern humans may have thrived thanks to geographic luck, not wits”.
- [139] Currat, Mathias; Excoffier, Laurent (December 2004). “Modern Humans Did Not Admix With Neanderthals during Their Range Expansion into Europe”. *PLoS Biology*. Retrieved January 2010.
- [140] “The Mysterious Downfall of the Neandertals”, *Scientific American*, August 2009. ISSN 1545-0031
- [141] Conard, NJ; Grootes, Pm; Smith, Fh (July 2004). “ Unexpectedly recent dates for human remains from Vogelherd”. *Nature* 430 (6996): 198 – 201. Bibcode : 2004Natur. 430.198C.doi: 10.1038 / nature 02690. ISSN 0028 – 0836. PMID 15241412.
- [142] Soficaru A, Dobos A, Trinkaus E (November 2006). “ Early modern humans from the Pesteria Muierii, Baia de Fier, Romania”. *Proceedings of the National Academy of Sciences* 103 (46) : 17196 – 201.
- [143] Finlayson C, Carrion JS (2007). “ Rapid ecological turnover and its impact on Neanderthal and other human populations”. *Trends in Ecology & Evolution* ( Personal Edition) 22 (4) : 213 – 22.
- [144] Terberger, Thomas ( 2006). “ From the First Humans to the Mesolithic Hunters in the Northern German Lowlands – Current Results and Trends”. In Keld Moller Hansen and Kristoffer Buck Pedersen . *Across the Western Baltic*.
- [145] Vordingborg, Denmark : Sydsjællands Museum.pp. 23 – 56. ISBN 87 – 983097-5-7. Retrieved 17 May 2009.
- [146] Chris Stringer : Comment : What makes a modern human. In : *Nature*. Vol.485, No.7396, 2012, pp.33 – 35 (here p.34), doi : 10.1038 / 485033a
- [147] Michael F. Hammer et al. : genetic evidence for archaic admixture in Africa. In : *PNAS*. Band 108, Nr.37, 2011, S.15123 – 15128, doi : 10.1073 / pnas. 1109300108
- [148] [http://img.kb.dk/tidsskriftdk/pdf/gto/gto\\_0019-PDF/gto\\_0019\\_67206.pdf](http://img.kb.dk/tidsskriftdk/pdf/gto/gto_0019-PDF/gto_0019_67206.pdf)
- [149] Dan Jones: *The Neanderthal Within.*, *New scientist* 193.2007, H.2593 (3 March), 28 – 32. *Modern Humans, Neanderthals May have Interbred; Humans and Neanderthals interbred*
- [150] Andrei soficaru u.a.: *Early modern humans from Pesteria muierii, Baia de Fier, Romania*. In: *Proceedings of the National academy of Sciences*. Washington 2006.
- [151] “Cousins of Neanderthals left DNA in Africa, Scientists Report”. *The New York Times*. July 26, 2012.
- [152] Yotova, vania; Jean-Francois Lefebvre, Claudia Moreau, Elias Gbeha, Kristine Hovhannesian, Stephane Bourgeois, Sandra Be’darida, Luisa Azevedo, Antonio Amorim, Tamara Sarkisian, Patrice Hodonou Avogbe, Nicodeme chabi , Mamoudou hama Dicko, Emile Sabiba Kou’ Santa Amouzou, Ambaliou Sanni, June Roberts-Thomson, Barry Boettcher, Rodney J. Scott, and damian Labuda (July 2011). “ An X-linked haplotype of the neandertal origin is present among all non-African populations”. *Molecular Biology and Evolution*.
- [153] All Non – Africans Part Neanderthal, genetics Confirm : *Discovery News*
- [154] R.E.Green et al. (2010). “ A Draft Sequence of the Neandertal Genome”. *Science* 328 (5979) : 710 – 722.
- [155] Bibcode : 2010Sci...328..710G.doi :10.1126 / science.1188021. PMID 20448178.
- [156] Mitchell, Alanna (January 30, 2012). “DNA Turning Human Story into a Tell – All”. *NYTimes*. Retrieved January 31,2012.
- [157] Krings M, Stone A, Schmitz RW, Krainitzki H, Stoneking M, Paabo S. (1997). “Neandertal DNA sequences and the origin of modern humans”. *Cell* 90: 19-30.
- [158] Paul H.Mason, Roger V. Short (2011). “Neanderthal – human Hybrids.” *Hypothesis* 9 :e1.
- [159] Viegas, Jennifer (27 March 2013). *First Love Child of Human, Neanderthal Found*. *Discovery*.
- [160] Condemi, Silvana et al (27 March 2013). *Possible Interbreeding in Late Italian Neanderthals ? New data from the Mezzena Jaw (Monti Lessini, Verona, Italy)*. *PLOS ONE*.doi : 10.1371 / journal.pone.0059781.
- [161] Bischoff, J; Shamp, Donald D.;Aramburu, Arantza; Arsuaga, Juan Luis ; Carbonell, Eudald; Bermudez De Castro, J.M.2003). “The sima de los Huesos Hominids Data to Beyond U / Th equilibrium (>350kyr) and perhaps to 400 – 500kyr: EW Radiometric Dates”. *Journal of archaeological Science* 30(3) : 275.doi : 10.1006/jasc.2002.0834.
- [162] Arsuagee JL, Martinez I, Gracia A, Lorenzo C(1997). “The sima de los Huesos crania (Sierra de Atapuerca, Spain). A comparative study”. *Journal of Human Evolution* 33 (2-3):219 – 81.doi:10.1006 / jhev.1997.0133. PMID 9300343.
- [163] Kreger,C.david. “Homo neanderthalensis”. *Archaeologyinfo.com*. Retrieved 16 May 2009.
- [164] Mcdermott, F; Grun, R; Stringer, Cb; hawkesworth, Cj (May 1993). “ Mass – spectrometric U-series dates for Israeli eandertal / early modern hominid sites”. *Nature* 363 (6426): 252-5. Bibcode : 1993Nature.363..252M.doi :10.1038 / 363252a0. ISSN 0028-0836. PMID 8387643.
- [165] Rink, W. Jack, H.P.Schwarcz, H.K.Lee, J.Rees-Jones, R.Rabinovich & E. Hovers (August 2002). “ Electron spin onance (ESR) and thermal ionization mass spectrometric (TIMS) 230Th/234U dating of teeth in middle paleolithic ers at Amud Cave, Israel”. *Geoarchaeology* 16 (6) : 701 – 707. Doi : 10.1002 / gea.1017.
- [166] Alladas,Helene, N.Merciera, L. Frogeta, E. Hoversb, W.H.Kimbeld & T.Rak (March 1999). “TL Dates the Neanderthal Site of the Amud Cave, Israel”. *Journal of Archaeological Science* 26 (3): 259 – 268.
- [167] B Rincon, Paul (13September 2006). “ neanderthals’ ‘last rock refuge”. *BBC News*. Retrieved 18 May 2009.
- [168] Onard, Nj;Grootes, Pm;Smith, Fh (July 2004). “ Unexpectedly recent dates for human remains from Vogelherd”. *Ure* 430 (6996): 198-201. Bibcode : 2004Natur.430..198C.doi:10.1038 /nature02690. ISSN 0028-0836. PMID 41412.

- [169][168] O Higham T, Ramsey CB, Karavanic I, Smith FH, Trinkaus E (January 2006).
- [170] "Revised direct radiocarbon dating of vindija G1 Upper Paleolithic Neandertals". Proceedings of the National Academy of Sciences 103 (3) : 553-7. Bibcode:2006PNAS..103..553H.doi: 10.1073/pnas.0510005103. PMC 1334669. PMID 16407102.
- [171] Yes, Jacqui (2 November 2006). Humans and Neanderthals interbred". Cosmos. Retrieved 17 may 2009.
- [172] Bioinformatics and Functional Genomics By Jonathan Pevsner ISBN 978 - 0 - 470-08585-1
- [173] Allentift ME, Collins M, Harker D, Haile J, Oskam CL, hale ML, Campos PF, Samaniego JA, Gilbert MTP, Willerslev E, Zhang G, Scofield RP, Holdaway RN, Bunce M (2012). "The half-life of DNA in bone : measuring decay kinetics in 158 dated fossils". Proc. R.Soc.B 279 : 4724 - 33.
- [174] Higuchi R, Bowman B, Freiberger M, Ryder OA, Wilson AC (1984). "DNA sequences from the quagga, an extinct member of the horse family". Nature 312 (5991) : 282-4.
- [175] Mullis KB, Faloona FA (1987). "Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction". Meth.Enzymol. methods in Enzymology 155 : 335 - 50. Doi:10.1016 / 0076 - 6879(87)55023 - 6. ISBN 978 -0 -12 - 182056 -5. PMID 3431465.
- [176] Saiki RK, Gelfand DH, Stoffel S et al. (January 1988). "Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase". Science 239 (4839) : 487 - 91.Bibcode : 1988Sci...239..487S. doi:10.1126/science.24448875. PMID 24448875.
- [177] Brotherton, P; Endicott, P; Sanchez, Ji; Beaumont, M; Barnett, R; Austin, J; Cooper, A (2007). "Novel high - resolution characterization of ancient DNA reveals C>U-type base modification events as the sole cause of post-mortem miscoding lesions". Nucleic Acids Research 35 (17): 5717 - 28.
- [178] <http://www.454genomics.net/downloads/news-events/geneticanalysisfromancientdna.pdf>
- [179] Johnson, PI; Slatkin, M (jan 2008). "Accounting for bias from sequencing error in population genetic estimates" (Free full text). Molecular Biology and Evolution 25 (1) : 199-206. Doi:10.1093/molbev/msm239. ISSN 0737-4038. PMID 17981928.
- [180] Briggs, Aw; Stenzel, U; Johnson, PI; Green, Re; Kelso, J; Prufer, K; Meyer, M; Krause, J; Ronan, Mt et al. (Sep 2007). "Patterns of damage in genomic DNA sequences from a Neandertal". Proceedings of the National Academy of Sciences of the United States of America 104(37) : 14616 - 21.
- [181] Woodward SR, Weyand NJ, Bunnell M (November 1994). "DNA sequence from Cretaceous period bone fragments". Science 266 (5188): 1229 - 32. Bibcode : 1994Sci...266.1229W.doi:10.1126/science.7973705. PMID 7973705.
- [182] Vreeland RH, Rosenzweig WD, Powers DW (October 2000). "Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal". Nature 407 (6806):897-900.doi:10.1038/35038060. PMID 11057666.