

COMPARISON OF MODERN HUMAN AND NEANDERTHAL DNA

**Igor V. Ovchinnikov^{1,2,3}, Anders Götherström⁴, Galina P. Romanova⁵,
Vitaliy M. Kharitonov⁶, Kerstin Lidén⁴, William Goodwin¹**

¹ *Human Identification Centre, University of Glasgow, Glasgow G12 8QQ, Scotland, UK*

² *Institute of Gerontology, Moscow, Russia*

³ *Department of Medicine, Columbia University, New York, New York 10032 USA*

⁴ *Archaeological Research Laboratory, Stockholm University, 106 91 Stockholm, Sweden*

⁵ *Institute of Archaeology, Moscow 117036, Russia*

⁶ *Institute and Museum of Anthropology, Moscow State University, Moscow 103009, Russia*

Neanderthals occupied large areas of Eurasia from about 200,000 years ago until their relatively rapid replacement by modern humans around 28,000-30,000 years ago. Many anthropologists believe that modern humans originated in Africa and entered Europe around 40,000 years ago [1]. The replacement theory hypothesizes that modern humans arriving from Africa out competed the Neanderthals, causing their numbers to reduce and for them eventually to become extinct. Another possibility is that the Neanderthals and modern humans interbred to produce a hybrid population that in time became the modern Europeans, in which case the Neanderthals were absorbed rather than replaced.

Since the development of molecular biology techniques a number of studies have been carried out using both modern and ancient DNA, in particular using the maternally inherited mitochondrial DNA (mtDNA) in an attempt to address the replacement versus absorption theories. Studies of modern European mtDNA found that the differences in mtDNA were very small which indicated that there was no archaic Neanderthal DNA in the current European mtDNA pool [2] and that all the mtDNA within the European pool had a relatively recent common origin. Direct evidence that the Neanderthals did not contribute to mtDNA gene pool of modern humans came when a study described the retrieval of DNA from the Neanderthal, recovered from Feldhofer Cave in the Neander Valley in western Germany [3]. These conclusions were based on the molecular analysis of a single specimen that left some people doubting the source of the DNA and gave no information on the levels of diversity within the Neanderthal gene pool; therefore the analysis of additional samples was highly desirable.

Excavations between 1987-1997 of the Mezmaiskaya Cave, which is located within the northern Caucasus in southern Russia 1,310 m above sea level revealed the remains of a Neanderthal infant, aged between a fetus of seven months and a new born of 2 months [4].

Small pieces of bone fragments were used to analyze the preservation of the macromolecules within the sample in order to determine the level of diagenetic modification of fossil bones of the Mezmaiskaya infant before using more of the bone material to isolate DNA. The relatively high level of preserved collagen-type debris was within the values recovered from previously analysed prehistoric samples that had displayed good preservation [5]. The high collagen yield made it possible to radiocarbon date, by accelerator dating in the Uppsala laboratory, the Neanderthal infant to $29,195 \pm 965$ years B.P. (before present) [5]. Therefore, the remains

belonged to one of the latest Neanderthals found so far from a Neanderthal refuge located on the eastern most edge of Europe in the Caucasus.

After the sample had been shown to be well preserved, mtDNA analysis was undertaken on the rib sample. Two sections of the rib were independently analyzed in two separate laboratories. A total 345 bp of hypervariable region I (HVRI) of mtDNA was determined from two overlapping PCR fragments with lengths of 232 and 256 bp. Forty PCR amplification cycles produced sufficient product to enable both direct DNA sequencing and also cloning of PCR fragments followed by DNA sequencing. The preservation of 256 bp long DNA fragments in the bone demonstrated a positive correlation with the collagen content and the skeletal morphology [5].

The Neanderthal DNA sequences were compared to the modern human mtDNA. The Mezmaiskaya Neanderthal differed to the Cambridge reference sequence [6] by 22 substitutions and one insertion; in comparison with the Feldhofer Neanderthal there were 12 substitutions. There was therefore a 3.48% divergence between the two Neanderthals from geographically distant populations separated geographically by over 2,500 km and probably by thousands of years in time [5].

Further comparisons were undertaken with the Neanderthal DNA and modern human DNA. The differences between the Neanderthal and 300 Caucasians, 300 east Asians and 300 sub Saharan Africans were calculated to be 25.45 ± 3.27 , 23.27 ± 4.06 and 23.09 ± 2.86 , respectively. This demonstrated that the Neanderthals were no more related to any one of the modern racial groups [5].

Phylogenetic analysis arranged the data to show the evolutionary relationship of the different DNA sequences by was performed. When the Neanderthal DNA was compared to 5,846 modern human DNA sequences it was sufficiently different to be considered a different group. This indicates that the two Neanderthal sequences are closely related to each other and that the 5,846 modern human DNA sequences are closely related to each other but that the Neanderthal mtDNA is in a different cluster than all modern human sequences. Together these analyses of Neanderthal DNA provide support for the hypothesis of no or a very low gene flow between the Neanderthals and modern humans and points to the Neanderthals existence as a separate branch of hominid evolution [5].

Using the mtDNA as a molecular clock it was possible to estimate firstly the age of the most recent common ancestor of the mtDNA of the eastern and western Neanderthals to be from 151,000 to 352,000 years. This "molecular time" coincides with the time of emergence of the Neanderthal lineage in the palaeontological records. Secondly, the divergence of modern human and Neanderthal mtDNA was estimated to be between 365,000 and 853,000 years ago when the common ancestor of the Neanderthals and modern humans lived [5].

The excellent preservation of this specimen leads to the potential of analyzing the entire Neanderthal mitochondrial genome and fragments of nuclear Neanderthal DNA and also suggested that some other Neanderthal samples may be amenable to molecular analyses. This has been born out with the successful analysis of a third Neanderthal found in Croatia [7]. In order to obtain a more complete picture of the relationship of Neanderthals to modern humans,

local Neanderthal populations especially from the regions where they may have co-existed with early modern humans should be analyzed.

References

1. Stringer CB Mackie R. African Exodus. In: The origin of modern humanity; 1996 Cape, London.
2. Richards M *et al.* Paleolithic and neolithic lineages in the European mitochondrial gene pool. *A. J. Hum. Genet.* 1996; 59: 185-203.
3. Krings M, Stone A, Schmitz RW, Krainitzki H, Stoneking M, Pääbo S., Neandertal DNA sequence and the origin of modern humans. *Cell* 1997; 90: 19-30.
4. Golovanova LL, Hoffecker JF, Kharitonov VM, Romanova GP. Mezmaiskaya Cave: A Neanderthal occupation in the Northern Caucasus. *Cur. Anthropol.* 1999; 40: 77-86.
5. Ovchinnikov IV, Götherström A, Romanova GP, Kharitonov VM, Lidén K, Goodwin W. Molecular analysis of Neanderthal DNA from the northern Caucasus. *Nature* 2000; 404: 490-493.
6. Anderson S. *et al.* Sequence and organization of the human mitochondrial genome. *Nature* 1981; 290: 457-474.
7. Krings M *et al.*. A view of Neandertal genetic diversity. *Nature Genet.* 2000; 26: 144-146.