The Neandertal mitochondrial genome does not support evolution

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Green *et al.*[1] have recently reported the sequencing of a full-length Neandertal mitochondrial genome. This is not a complete nuclear genome, but only that of one small organelle (the mitochondrion) that exists within all animal cells. From their analysis they concluded, 'Neandertals made no lasting contribution to the modern human mtDNA gene pool.' While this primary conclusion does not necessarily conflict with the creationist position that Neandertals lived after the Flood and are fully human, there are a lot of evolutionary assumptions behind that statement that must be carefully considered. There are actually three separate issues here: is the sequence accurate? Does the sequence prove that Neandertals were a different species? Do the number of variations between Neandertal and modern humans prove that a vast time span separates us?

The accuracy of ancient DNA sequencing

Ancient DNA (aDNA) is problematic. DNA is a long macromolecule that breaks easily, especially between G–T residues where breaks are nearly three times more likely than at other positions.[1,2] In this particular case, the DNA fragments recovered from the Neandertal bone had an average length of only 69.4 bp. That means that thousands of pieces were required to reassemble the 16.5 thousand bp mtDNA genome, and multiple copies of each section are required to correct for the high error rates inherent in sequencing aDNA. Green *et al.* estimated they would need 12-fold coverage to achieve an error-rate of 1 in 10,000. To put that in perspective, the Human Genome Project required only 4–5-fold coverage to complete the draft sequence. The Neandertal mtDNA was completed with a 34.9-fold average coverage, but without a complete modern human mtDNA for comparison, the Neandertal assembly would have been impossible.

Contamination of ancient samples by modern DNA is a constant issue, for the sequencing reactions tend to amplify high-quality modern DNA at the expense of fragmented aDNA. The presence of nuclear copies of the mtDNA is also a concern. The nuclear copies are not exactly identical to the mtDNA and separating the two can be difficult, especially with the short average read length. There are actually four types of mtDNA that the authors had to be concerned about: the fragmented Neandertal mtDNA, low copy number fragmented nuclear copies of Neandertal mtDNA, contaminating modern mtDNA, and low copy number nuclear copies of contaminating modern mtDNA. The authors went to great lengths to address this problem and probably could not have done much more, given the nature of the material.

In ancient DNA, individual DNA residues are chemically altered over time. In particular, frequent deamination of cytosine residues leads to high rates of C–T transitions (and A-G transitions on the complimentary strand).[2] This occurs more often close to the ends of DNA fragments,[3] which is a considerable problem when one considers the small average size of the recovered DNA fragments. The reported Neanderthal mtDNA differs from the standard human mitochondrion (the Revised Cambridge Reference Sequence,[4] or rCRS) by 206 nucleotides (1.2% of the 16,569 nucleotide mitochondrial genome), including 195 transitions and 11 transversions.[5] To put that in perspective, any two modern humans selected at random will differ by an average of about 40 nucleotides, and the most divergent mtDNAs from living humans differ at just over 120 nucleotides.[6] The mutations found in the Neandertal mtDNA are fairly standard. No large indels were found and transversions are uncommon. In fact, the bulk of the differences found between the Neandertal and modern mtDNA are C–T transitions. These are among the most common mutations that occur within living organisms, but it is not clear if they are the result of ancestry or post-mortem alteration of the Neandertal sequence. Many of these mutations might be indicative of errors in the genome assembly that, despite the authors' best efforts, carried through their analysis.

Of particular concern is the discovery of several non-synonymous amino acid changes in protein coding regions of the Neandertal mitochondrial genome, especially that of subunit 2 of the cytochrome c oxidase gene. They claim that this is evidence that purifying selection in the Neandertal mtDNA was reduced probably to due a small population size. This is because these types of substitutions are rare because most are assumed to be detrimental, and because selection breaks down in small populations due to high rates of random shifts in gene frequencies (the fixation rate of new mutations is inversely proportional to population size). But small populations are also at risk due to the high rate of mutation accumulation,[7] which eventually leads to extinction due to 'error catastrophe'. The accumulation of non-synonymous mutations in important genes is evidence for a high mutation rate acting on a small population under threat of extinction. It could also indicate the presence of post-mortem DNA degeneration that their techniques could not discern. If the results are valid, the accumulation of deleterious mutations might help to explain the disappearance of the Neandertals. However, the adaptive significance of the synonymous to non-synonymous ratio

has recently come under fire,[8,9] so we must interpret these findings carefully.

There exists a large body of literature dealing with the pitfalls and assumptions inherent in working with ancient DNA. The authors are aware of this knowledge and did their best to avoid potential problems, but time has been a fickle judge of previous aDNA sequencing efforts. Green *et al.* concluded that this single Neandertal mtDNA 'unequivocally' falls outside the range of modern humans. While this is true at face value, it assumes the sequence is accurate. See Criswell for a detailed discussion on post-mortem DNA decay and problems with current Neandertal mtDNA sequencing efforts.[10]

Were Neandertals a different species?

Let us assume the Neandertal mtDNA sequence is accurate. Even then, comparing a single Neandertal to a representative sample of modern humans is not highly informative. It may be that Neandertals were a unique side branch of modern humans with limited genetic diversity due to inbreeding. Alternatively, it may be that Neandertals were a highly heterogeneous group with a rich genetic heritage that encompasses modern humans. I suspect the former is true, but we will have to wait for additional Neandertal sequences to become available before we can make strong conclusions.

It is entirely possible that Neandertals accumulated mutations very rapidly in the years after the Flood. Based on this single sample, Neandertals have many mutations not seen in any modern human. Even so, this Neandertal sequence is closer to modern humans than many living chimps are to one another! Diversity within living chimpanzees is three- to four-fold higher than within the modern human population,[11,12] even though chimpanzees are descended from a single pre-Flood pair and thus should have less genetic diversity than humans. This is evidence for a chimpanzee genome in rapid decline and might indicate some degree of entropy was acting on the Neandertal genome.

Coalescence theory[13] predicts that living populations should be descended from only a small fraction of the ancestral population. The Recent African Origins Theory[14] originally postulated that all people alive today descend from a single female ('Mitochondrial Eve') living in Africa about 200,000 years ago (the estimated date varies from author to author). This does not mean that she was the *only* female alive at that time, but that the lineages of every living person coalesce in this single person. The theory has since been expanded to include 'Y Chromosome Adam'.[15] Coalescence has been demonstrated in the Icelandic population, where only 6.6% of the females and 10% of the males alive between 1698–1742 are, respectively, the ancestors of 62% of females and 71% of the males alive today.[16] Coalescence might be a general phenomenon in all populations, acting like a funnel to channel genetic diversity from a limited pool of ancestors. It seems there has been a loss of variation within the English population over the past 1,000 years[17] due to disease and other factors. If processes like this have existed throughout human history, we should not expect modern humans and Neandertals to share the same 'mutations'.

Photo from <www.wikipedia.com>

Neandertal reconstruction

Coalescence in small populations might occur several times in its history. One founder might be the mitochondrial ancestor of the entire population, fixing those founder mutations. If the population remains small, a second founder event could occur, adding the mutations that have accumulated in a later individual to the pool of fixed mutations. This is a concern for the captive maintenance of endangered species in zoos and becomes evident in various breeds of domestic animals when they display characteristic debilitating mutations. Small populations drift rapidly and this is what may have happened to Neandertals, allowing for the rapid accumulation of new mutations.

Green *et al.* made explicit the standard assumption that mtDNA is only maternally inherited and that mitochondrial recombination does not occur. They then conclude that Neandertals made no lasting contribution to the modern human mtDNA gene pool. Although these two assumptions have been argued back and forth for several years in the literature, the latest evidence seems to indicate that they may in fact be incorrect.[18] If evidence for mitochondrial recombination continues to accumulate, this conclusion will need to be re-evaluated, for it might then be possible that *parts* of the Neandertal mitochondrial genome are present in modern humans. Green *et al.* found one mutation in a modern human that is found nowhere else but in Neandertal and they attributed this to a reversion back to the Neandertal/ancestral state. The correct conclusion is probably that the mutation appeared twice in two separate lineages, but it is an interesting observation.

Is this evidence of great age?

Green *et al.* date the divergence of the modern human and Neandertal lineages to 660,000 ± 140,000 years bp. In order to do this, they outwardly stated they based this

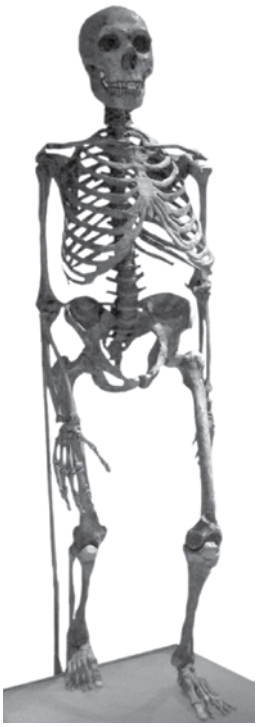


Photo by Claire Houck <www.wikipedia.com>

Neandertal skeleton

on the assumption of a molecular clock, on an assumed human-chimpanzee split 6–8 million years ago, and on the Standard Neutral Model of evolution.[19] The neutral model makes the assumption that mutation accumulation in mitochondria occurs without natural selection and that the genetic (and cultural) factors that control mutation rates do not vary across the human population or over time. But since we do not know current mutation rates, since we do not know historic mutation rates, and since the assumptions behind the Standard Neutral Model are all questionable,[20] we must conclude that the degree of relatedness of this single Neandertal specimen to modern humans is unknown at this time.

They make an interesting admission, 'However, if the estimated date of the divergence between humans and chimpanzees, or current assumptions about how the mtDNA evolves, were found to be incorrect, the estimates in calendar years of the divergence of the Neandertal and human mtDNAs would need to be revised.' They also admit that 'the evolutionary dates are clearly dependent on many tenuous assumptions'.

There are several particular issues that pertain to the age of this fossil that I would like to discuss.

Mutation rates are unknown

Most mutation rate estimates we see in the scientific literature are biased downwards because of the assumption of deep-time evolution. This is a problem for two reasons. First, they may be calculating divergence times for two species that were created separately (e.g. chimps and humans) and are thus not technically comparable. Second, divergence rate calculations are calibrated by comparing them to imagined past events. For example, if humans and chimps are X% different, to calculate mutation rates, they divide X by 6–8 million (the number of years since we supposedly diverged from chimps). The timing of the split between modern humans and Neandertals is based on divergence rate calculated from the assumed human-chimp divergence time. Mutation rates based on genealogy are much higher than those based on phylogeny[20] and are probably much more realistic. Recent studies have shown that *measurable* mutation rates are much higher than either the phylogenetic or even the genealogical methods predict.[21]

Neutral theory does not allow for mutations in DNA polymerase or in anything that affects DNA copying or repair to occur in only a single subpopulation. That would destroy the very notion of a molecular clock, for then mutations would not be expected to accumulate evenly across the board. But we can measure the fidelity of DNA polymerases, including the human mitochondrial DNA polymerase,[22] and we know that mutations in DNA polymerases can elevate error rates in human mitochondria.[23]

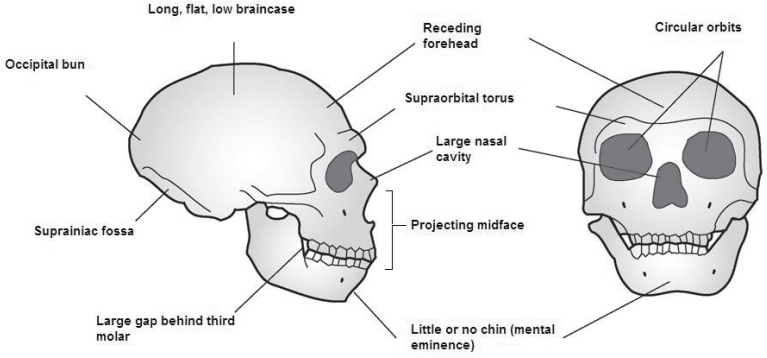
Rapid mutation in harsh environments

Bruce Ames, a member of the prestigious US National Academy of Science, has suggested that genetic damage can be directly linked to poor nutrition.[24] According to the theory, when under starvation conditions, the body has to decide which systems to keep working and which to shut down. This genetic 'triage' mechanism would keep an organism alive, but at the expense of less-than-critical cellular operations like DNA repair.

It has been suggested by several creationists that the Neandertal population lived in Europe under less-than-ideal conditions and was subjected to nutrient limitations, specifically vitamin D deficiency due to the perpetually cloudy weather during the post-Flood Ice Age. Add a harsh environment and poor nutrition to a small inbreeding population and you have an instant recipe for the rapid accumulation of mutations in any human population.

Predetermined mutation pathways?

One of the assumptions behind neutral theory is that all mutations are independent and random. It is probably a mistake to believe that mutations occur at random and that they do not interact, however, for any mutation can only occur in the context of the surrounding genetic information. One mutation may be excluded by another (because the



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Neandertal cranial anatomy

combination might be deadly) or may lead to a series of other mutations (because some mutation sets may be excluded by specific individual mutations). Evidence for this kind of mutation interaction is limited, but it does exist.[21] If some mutations lead to others, we should not expect two separate lineages to follow the same mutational pathway. This is especially true if a mutation affects the fidelity of the DNA copying mechanism.

Conclusions

While evolutionists (including theistic evolutionists) and 'progressive creationists' will probably be trumpeting this new paper as evidence that Neandertals and modern humans are two distinct species, I believe their conclusions are premature. As I have briefly outlined above, there is a lot we do not know about the science of modern genetics. And there are factors like coalescence, rapid genetic drift, and genetic triage in small isolated populations that can potentially explain the findings. In any case, modern chimps can differ more from each other in their mtDNA than modern humans differ from this Neandertal specimen, so beware of anyone who claims Neandertals are a separate species based on genetic differences.

When we approach evidence like this, we need to be skeptical, we need to understand the theory that led to the conclusions and we need to question the assumptions behind the theory. If we do these three things, we need not be afraid that Neandertal Man will in some way fall outside the biblical creation model.

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