Taking a crack at the Neandertal mitochondrial genome

The sequencing of the Neandertal DNA is very revealing. First of all, the *FOXP2* gene showed that Neandertals are fully human, since the sequences show the exact same characteristics as in modern man.[1] In addition, it was already shown that in mtDNA of ancient humans the mutation rates are much higher than those calculated based on Darwinian assumptions of putative common ancestry, and that has now been confirmed. Several years ago I noticed that mutations are not just a stochastic phenomenon. Mutation rates are said to be slow. This may be, however, due to an invalid method of comparing biological data, and a result from the Darwinian assumption that all living beings once had a common ancestor. Humans and chimpanzees, for instance, supposedly shared a common genome about six million years ago. This means that the observed mutations in a homologous stretch of DNA of both species were introduced during this period. If we look into the genetic variation within single species, we always find a very high number of genetic polymorphisms; these could be a more accurate measure of mutation rates than the rates deduced from two unrelated species that do not reproduce together. A scientific approach to estimating genetic change should include all polymorphism present in a representative stretch of DNA, so we know how fast that particular DNA sequence changes. If the 'within-species' mutation rate is higher than the 'between-species' rate, the age to the most recent common ancestor will be over-estimated. This has indeed been reported for primates and humans.[2] With the wealth of genetic data that is currently available, we can accurately calculate the mutation rate within species by simply counting the number of polymorphisms in a particular piece of the mitochondrial DNA in several individuals. From this calculated mutation rate we can extrapolate the time to the alleged common ancestor of man and chimpanzee.

I did that for the ancient human mitochondrial DNA presented in the Adcock paper (figure 1).[3] We can calculate the age to the alleged common ancestor of man and chimp using two distinct approaches which give comparable results and which also cast a remarkable light on the supposed 'evolution of man'. Compared to the modern human DNA sequence, 10 mutations can be recognized in the ancient human DNA sequence dated to 62,000 years before present (bp), and 24 mutations are present in the corresponding chimpanzee sequence. Because 10 mutations were generated in 62,000 years, it may take 6,200 years for one mutation to occur. If we assume the molecular clock hypothesis to be right, 24 mutations may have accumulated in roughly 150 thousand years. This extrapolation shows that the common ancestor of humans and chimps lived approximately 150 thousand years bp. Such observations are incomprehensible for the theory of evolution, and I expect they will always be characteristics of interspecies analyses that compare DNA of subpopulations of single species.

That we supposedly share a very recent ancestor with chimpanzees is 'confirmed' by a second approach. Let's focus on specimens labelled LM3 and LM15. They are the oldest and youngest specimens, respectively. LM3 has been dated at 62,000 years, while LM15 is roughly 200,000 years old. Both were isolated from remains retrieved from Lake Mungo. The sequences isolated from the oldest and youngest remains are genetically still closely related—four of the point mutations are shared. Over the years, LM3 accumulated 6 more point mutations in the same region as the original mutations. Because it has been calculated that LM15 and LM3 lived 62,000 years apart it is

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                         00111111111111111222222222222222222222222222233333333333333
                         79001122345668889001223344444555566677888899901112345556688
                         83781269984393499198490413479368923448467803911780715672817   D      Age (kY BP)
Modern Human CRS         ATCCCCTGACTACACTTCTCCTACATGATACACCTCGCACCTCAACTAACCTCTTTTTA   -       0
Ancient Human (LM3)      ....................T.G...........CT.T....T..T......TC....G   10     62
Ancient Human (KS16)     ....................T...................T.............C..C.   4      9-15
Ancient Human (KS13)     .C............T.....T....C.G.................TC.............   7      8-15
Ancient Human (KS8)      ....................T.G.............TG.......C.............   5      8-15
Ancient Human (KS1)      .C............T.....T.........................CG..T.........   6      10
Ancient Human (LM4)      ..................T...........G..............C.............   3      <10
Ancient Human (LM55)     ...........G.......................T.........................   2      <10
Ancient Human (KS9)      .C..................T..............T............C.........G   5      9
Ancient Human (KS7)      ..............T.....T...................T...........C.......   4      8
Ancient Human (LM15)     ....................T.........................T.......C....G   4      0.2



Bonobo                   ......CAT...T..CCTA.TCGA.CACCAA...C.......AG..CCCT..A.CCC..   29     0
Chimpanzee               ....T..ATT.....AA.C.TCGA.CA...A......TG....CG..CT.T.T.C.C..   24     0
Neandertal (FH)          GCTTTT.ATTC.T-.CC.C.T.GT..A...AG.T...T......G.C..T.....C...   27     30+
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Figure 1. Adjusted ancient human mtDNA sequences (from oldest to youngest remains). The number of mutations roughly reflects the age, in particular the outlier LM3, but the incidence of mutations is much higher than expected from interspecies comparison. D = genetic difference with modern human CRS. (From ref. 3).

logical to conclude that the mutations frequency is 1 nucleotide per 10,000 years. A difference in this region of 24 nucleotides, which is the difference between man and chimpanzee, confirms a very young age of the alleged common ancestor—240,000 years bp.

There is one more thing that becomes clear from the Adcock data. The difference between humans and chimpanzees, and humans and bonobos turned out to be 24 and 29 positions respectively, while humans and Neandertals differ at 27 positions. Remarkably, the genetic difference between chimpanzee (*Pan troglodytes*) and its very closely related cousin bonobo (*Pan paniscus*) is 23 nucleotides, while that between chimpanzee and Neandertal is 24 nucleotides. This is all in the same range, meaning that all common ancestors lived around the same time. These data not only show that real mutation rates are much higher than calculated from interspecies comparisons, but they show that something very mysterious is gong on in the mtDNA. The mystery is not so deep, however, and can be solved easily because the mitochondrial DNA of ancient humans also demonstrates that mutations are a non-random phenomenon. In the populations of Lake Mungo and Cow Swamp we find that over and over the same positions mutate independent of age of the sample. Indeed, ordering the samples with respect to age—as I did in figure 1—shows that the number of mutations also does not reflect the calculated ages of the samples. There must be another phenomenon going on in DNA sequences—a phenomenon that introduced the mutations in a non-random fashion.

The Adcock studies are superior to all other studies that compare primate and human mitochondrial chromosomes because sequences in ancient human subspecies contain more information regarding the character of mutations. The 'ancient' mitochondrial sequences provide more exact information about the mutation rate and the position of the mutations. In the ancient individuals, we are able to exactly follow mutations and mutation rates over a more accurate timescale. We do not have to invoke the assumption that humans and chimpanzee have a common ancestor and extrapolate mutation rates from primate data, because we compare sequences within one species. That my 'more accurate' analysis is not compatible with the consensus of neo-Darwinian community—that humans and chimpanzees have a common ancestor around five million years ago—is tale telling. Mutation rates under the Darwinian presupposition of common descent are wrong; real mutation rates are much higher than evolutionists estimate from between-species comparisons. Studies on mice mitochondrial DNA confirm that mutation rates within species tend to be much higher than the between-species rates.[4] The mutation rate paradox is then easily solved, which should result in the rejection of Darwin's idea of common descent. However, that will not happen.

What creation scientists need to address is the actual rate of mtDNA mutations and whether or not these mutations are non random in the sense that 1) the environment is directing or facilitating mtDNA mutations (there is some evidence for this), and 2) whether or not the mutations are a kind of hotspot-mutations.

Mutations occur over and over at the same positions. This indicates that mutations in mitochondrial DNA do not occur randomly. Rather, unknown mechanisms may be responsible for their deliberate introduction—non-random mutations as the result of an external or internal cause. Indeed, it was recently discovered that proteins encoded by mitochondrial DNA 'act like adaptive machines, possessing the ability to control their own evolution.'[5] Proteins that control their own genetic change explain the non-random patterns of observed mutations. This mechanism allows for the rapid diversification in human populations, including Neandertals.

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Robert Carter replies:

Pieter brings up several excellent points. The story of the *FOXP2* gene has been published elsewhere in this journal so I chose not to discuss it in my article. Upon retrospect, I should have included a reference for completeness.

Comparing within-species diversity to among-species diversity as an argument against evolution is an excellent approach and I would like to see much more work done on this. To encourage people to think along these lines, let us consider a few caveats. The first thing to consider is that 'created diversity' is unknown. There are about 12 million single letter variations that have been reported to occur in the human genome, but most of these are very rare. I estimate there are about ~30,000 letter differences shared among all world populations (this number is subject to change as I learn more) and most of these should be part of the initial variation God programmed into Adam and Eve. This assumes a low level of homoplasy (identical mutations occurring in different lineages at different times). Another difficulty is knowing how many of the shared mutations between humans and chimpanzees are due to mutation and how many were initially created.

Regarding the Adcock[1] paper, I have a few difficulties in accepting the

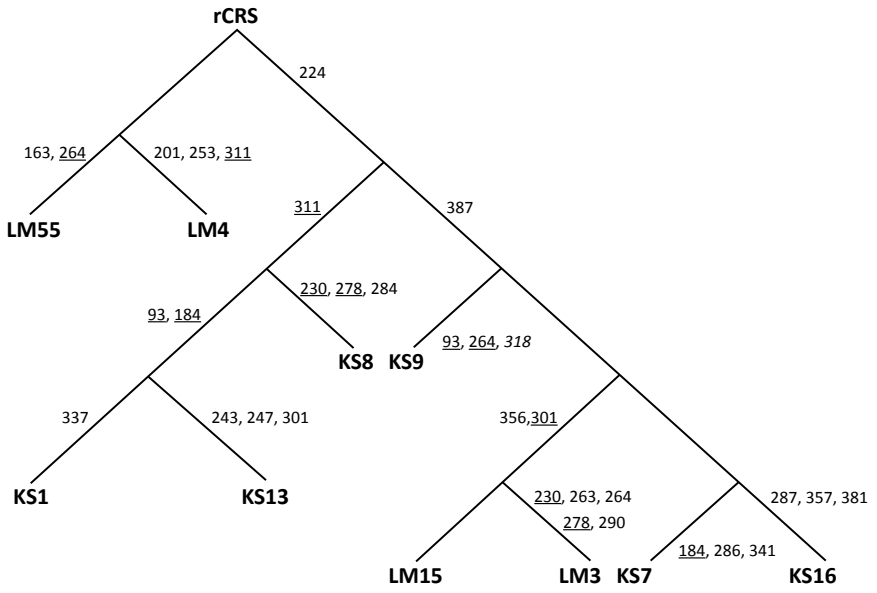

Figure 1. One of many possible phylogenetic trees based on the Adcock data. The Revised Cambridge Reference Sequence (rCRS)6 is used as an arbitrary root. The mutations that lead to each specimen (labeled at the termini) are given along each branch. Since most mutations are transitions, they are reported as a simple number, where the number represents the nucleotide position in the rCRS. To determine the nucleotide changed, start at the rCRS nucleotide and change A to G, C to T, or T to C (no G to A transitions were found). The single A to C transversion (in the branch leading to KS9) is in italics. Recurrent mutations (homoplasies) are underlined. For clarity, only one of many possible trees is illustrated and the multiple possible paths to each specimen are not illustrated.

data as-is. As I briefly discussed in my paper, ancient DNA is problematic. It is very difficult to recreate an accurate DNA sequence from the fragmentary genetic remains found in very old samples, due to the small size of the remaining pieces and post-mortem chemical alteration of the nucleotides, and any sequence from a study more than a few years old is probably not accurate enough to use. In this case, only a small part of the mitochondrial genome is being reported on, but there is evidence that the data are not accurate.

I have created a simple tree plot, by hand, to demonstrate a significant number of problems with the data (figure 1). Starting with what I felt was the most discriminatory mutation (at position 224), and allowing for no back-mutations (they would be expected to be rare), I simply illustrated the mutations (each listed as a number along the various branches) in the Adcock data and show how they separate the various sequences. For comparison, I also created a network phylogeny (figure 2) using a common evolutionary methodology.[2,3] Adcock discussed creating several such trees, but none was shown in the text of the main paper and no supplementary information is available on the website. Figure 1 is similar to Figure 2, but simplified by leaving out the recursive networks. From these two trees, it is evident that there is no way to determine either the ancestral sequence or the genealogical relatedness of the individuals. This would not be the case with more reliable data.

I did not attempt any (evolutionary) statistical tests on either of these trees for it is quite obvious that a significant amount of homoplasy exists in the dataset. This is due to some combination of three possible processes. First, it may be indicative of a high level of recurrent mutations appearing in different lineages. While possible, it is not likely, for this would be orders of magnitude higher than what is found in the phylogeny of modern human mtDNA.[4] Second, it may be due to a high level of mitochondrial recombination. This is also possible, but again not likely. Recent support for mitochondrial recombination has been produced,[5] but it is not thought to occur very often. It would also have to include a significant amount of paternal input, which again goes against theory. And this level of recombination would make a historical reconstruction of this kind impossible. Third, and more likely, the reported sequences may be riddled with errors due to post-mortem chemical DNA degradation. DNA decay reduces the signal-to-noise ratio, where the signal is the original DNA sequence and the noise is due to the errors caused by post-mortem decay. In this case, the noise is so high that any statistical test would yield a very low confidence for any given tree. And this is only a small portion of the mitochondrial genome. With this level of homoplasy, a network phylogeny of the complete genomes would resemble a bird's nest.

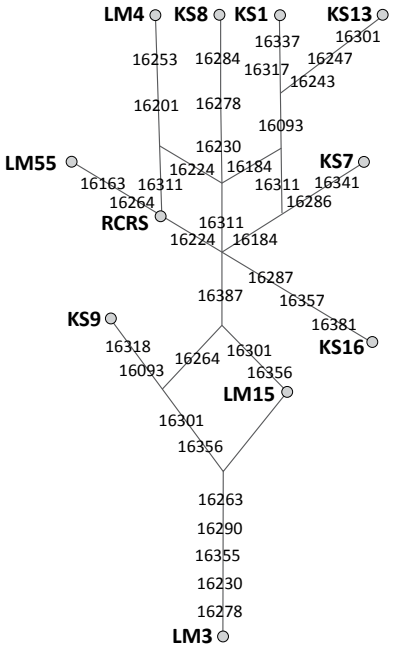

Figure 2. 'Reduced Median Network' of the ancient Australian sequence dataset of Adcock. The tree was calculated with Network 4.5023 (available at <www.fluxus-engineering.com/sharenet.htm>) using default parameters. Considerable homoplasy is evident in the dataset, as seen in the recursive network in the tree branches and the number of times specific mutations are labeled.

Some of the sequences, especially LM3 (the 'outlier'), appear to be worse than others. Of note is the fact that LM3 is currently only found as a copy on chromosome 11. Is it truly a mtDNA sequence, or is it an artifact of contamination either from modern human DNA or from the LM3 nuclear genome? Others, such as LM15, appear to have less degeneration. Note that LM15 is only 4 mutations away from the rCRS (my arbitrary root for the tree in figure 1), while LM3 has an additional 5 mutations, 3 of them homoplasies.

In summary, Pieter raises some excellent points. I would encourage him, and others, to continue to pursue this line of reasoning. I would only caution against trusting ancient DNA data, especially anything produced by any study more than a few years old. A revolution in ancient DNA is brewing and it would be a shame to base our conclusions on older work, much of which will probably be invalidated.

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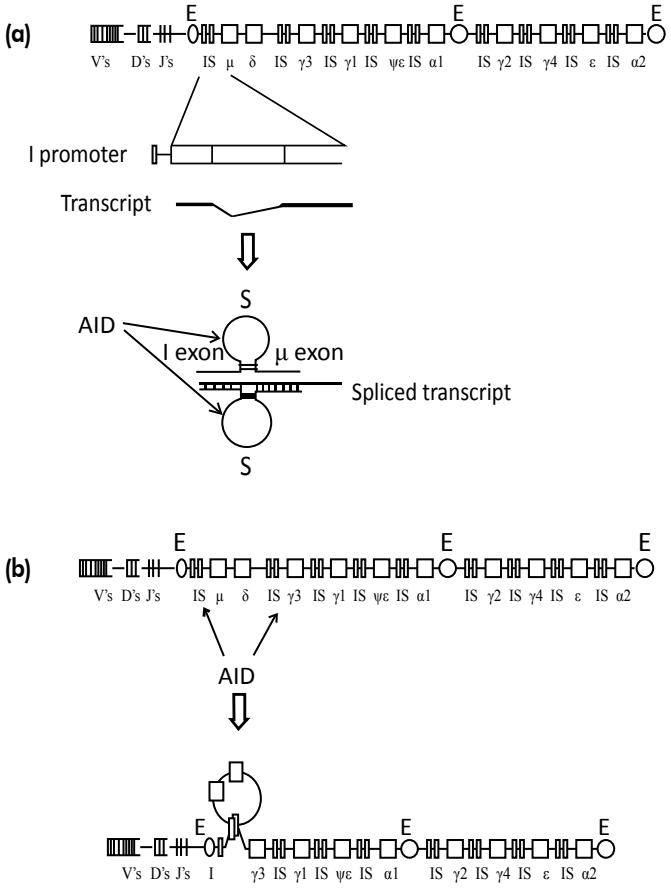
Errata

Journal of Creation **22**(3)

Liu, Y., The immunoglobulin heavy chain gene family and gene duplication. On p. 19, in figure 2, every 'IS' was incorrectly represented as 'Iδ'. The correct figure should be as follows:



Borger, P., Evidence for the design of life: part 2—Baranomes. On p. 73, in figure 2, left panel, the line running towards *Saccharomyces mik* is missing. The correct figure should be as follows: