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LETTER TO THE EDITOR

Denisovans, Melanesians, Europeans, and Neandertals: The Confusion of DNA Assumptions and the Biological Species Concept

Niccolo Caldararo[1]

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Abstract A number of recent articles have appeared on the Denisova fossil remains and attempts to produce DNA sequences from them. One of these recently appeared in *Science* by Vernot et al. (Science 352:235–239, 2016). We would like to advance an alternative interpretation of the data presented. One concerns the problem of contamination/degradation of the determined DNA sequenced. Just as the publication of the first Neandertal sequence included an interpretation that argued that Neandertals had not contributed any genes to modern humans, the Denisovan interpretation has considerable influence on ideas regarding human evolution. The new papers, however, confuse established ideas concerning the nature of species, as well as the use of terms like premodern, Archaic Homo, and *Homo heidelbergensis*. Examination of these problems presents a solution by means of reinterpreting the results. Given the claims for gene transfer among a number of Mid Pleistocene hominids, it may be time to reexamine the idea of anagenesis in hominid evolution.

Keywords Ancient DNA · Neandertals · Denisovans · Sima de los Huesos · Premoderns · Evolution · Speciation · Anagenesis

✉ Niccolo Caldararo
cald@sfsu.edu

[1] Department of Anthropology, San Francisco State University, 1600 Holloway Ave., San Francisco, CA 94132, USA

Introduction

Reading Ann Gibbons' article (2016) on the recent report of an analysis (Vernot et al. 2016) of the ancient DNA of Neandertals, Denisovans and current human populations in Melanesia, Europe and elsewhere brings to mind the 80's tune by DEVO, "Are we not men?" The original article by Vernot et al. (2016) is a tribute to the distance we have come in developing research techniques in the study of ancient DNA. However, the interpretation has significant problems.

When the original Neandertal ancient DNA was first sequenced and a published analysis appeared in *Cell* (Krings et al. 1997), Gabow and Caldararo did a thorough study of the sequence and concluded the following: (1) It was contaminated and therefore the sequence was not entirely authentic and (2) The central assumption that Neandertals could not be related to contemporary humans (due to the number of base pair differences reported) was incorrect. The first problem affected the second to some extent but it was clear that the number of base pair differences they had established as defining species distinction was arbitrary. For example, fully interfertile chimpanzees have more base pair differences.

We published our findings in 2000 (Caldararo and Gabow 2000), including the alignment of the Krings et al. (1997, 1999) data with a number of other sequences of ancient DNA from various sites demonstrating the variations over time in ancient DNA as well as the effects of DNA degradation on sequencing methods. I have also done this with the published Denisova 3 data which appears on the Nature website as a contribution to *Nature Proceedings* with the help of Mike Guthrie. This alignment is made available here as supplementary data for this paper. It does not include sequence data from Denisova 4 or Denisova 8.

While the authors of the reports on these latter two Denisovan sequences interpret the substantial number of base pair differences between the three as evidence of diversity and long residence of Denisovan populations in the area (Sawyer, et al. 2015), they could also be the result of contamination and degradation.

The revised Krings et al.'s (1999) paper was included in our 2000 analysis and added little to our demonstration that the sequence appeared to show that Neandertals varied little from other ancient human DNA and it did not answer our concerns about degradation. Nevertheless, our paper was not referenced and the Krings et al. (1997, 1999) claims for separate species and no transmission of Neandertal DNA to modern humans was sustained and widely cited. Subsequently, Caldararo wrote an article showing that the assumptions behind the statistical claims of species distinction of Neandertals and modern humans could not be supported by reference to inheritance and scientific definitions of species. This paper appeared in the *Linnean* (Caldararo 2003), the official publication of the Linnean Society of London. Our work was validated by the discovery of Neandertal genes in modern humans as reported by Green et al. 2010 and the current work reinforces that finding as does that of Reich et al. (2010) for Denisovans.

Below I review the genetic data regarding different hominids and assess the fossil remains as well as the arguments for DNA significance and the interpretations that have been used to support species designations (in phylogenetic branching scenarios) as opposed to the idea of gradual evolution (anagenesis).

Comparing and Alignment of Sequences

The Altai (Denisova Cave) fossil mtDNA sequence was purported to be so different from anatomically modern humans (AMH or contemporary *Homo sapiens sapiens*) that it was suggested to be a different species, yet the bone material it was drawn from seems to have the physiological landmarks of modern human species designation (Krause et al. 2010). This is difficult to determine given the small fragment of a juvenile manual phalanx. Nuclear DNA (nDNA) was drawn from a molar tooth and DNA analysis indicated both bone samples were from the same population but it was initially claimed, and continues to be held, that the relationship of this population to modern humans is separated by over half a million years as well as from Neandertals (Callaway 2013). Degradation of nDNA is a concern (Cooper et al. 2001; Caldararo and Gabow 2000; Yao et al. 2004), especially given the drastically different contamination estimates given for the Denisovan 4 and 8 (Sawyer et al. 2015). Further, it is claimed that present-day Melanesians share genetic material with the Denisova population. What significance this has is unclear especially regarding theories of the evolution of modern humans and warrants further study of the sample sequence. Examination of the published sequence found that the alignment of segments in the mtDNA hypervariable regions could be aligned with that of anatomically modern humans if one introduced an insert at a position found in Neandertals. Some other points of interest arise from a reconsideration of the sequences for other published samples and Neandertals from the same perspective. The implications for systematics and human phylogenetic analysis are considerable.

The publication of a mtDNA sequence by Krause et al. (2010) produced a proposal by the authors that the differences between this sequence, that of modern humans and Neandertal sequences indicated that the Denisova individual was probably derived from an unknown hominid population that shared its last common ancestor with anatomically modern humans and Neandertals before 1.0 MYA; others placed the date at 700,000 or 800,000 (Callaway 2013). The reason for this speculation was the great number of base pair differences between the Denisova sample and AMH and Neandertal samples. Alignment of retrieved sequences from PCR products and the amplification process can produce problems and yet some strange features can be found. For example, if one attempts to align the Sima sequence from 16021 with that of the Anderson reference sample you find substantial disagreement, but if one aligns Sima with Kostenki 14 beginning at 16022 to the Anderson at 16021 you find 5 differences for Sima and 1 for Kostenki 14, though the Sima sequence is missing 32 bases (using GenBank sequence FK683087 for Sima *Homo heidelbergensis* and FN600416.1 for Kostenki 14).

While there is significant evidence of degradation present in the reported sequence which parallels degraded mtDNA as Caldararo and Gabow (2000) argued in our *Ancient Biomolecules*, paper and Caldararo (2004) extended in a later analysis, some sequences do align with the published samples. Hawks and Wolpoff (2001a, b) noted the contaminated sequences of modern DNA in some of the cloned Feldhofer DNA. Contamination is also a problem for various site exposure sources, bacteria, fungi, or from laboratory sources.

My analysis in the current paper leads to the conclusion, however, that the Denisova sample was significantly degraded and the resulting sequence up to 16193 contains corrupted mtDNA. After 16193, if one reads into the sequence a break as appears in the Krings et al.'s 1997 paper, as an insert of a cytosine between 16193 and 16194 the sequence aligns as human given human reference samples in GenBank and presented by Caldararo and Gabow (2000) and Krings et al. 1997. We note this insert in the Caldararo and Gabow (2000) paper where we align

several modern reference sequences with several ancient sequences both identified from Neandertal material (Feldhofer consensus) and early moderns. There is another insert near 16262, 16263, and 16264 in the Krings et al.'s (1997) sequence for Feldhofer but this does not appear in the Denisova sequence. If one adds the Denisova Cave sequence to our alignment (see Supplementary data[1]) and begin the reading from the Neandertal insert, we find that the Denisova sequence then fits the reference sequence of Anderson and that used by Krings et al. (1997) with only a few base pair variations (18). The same is not true when one reaches 16264. There is also an insert between 16263 and 16264 in the Neandertal sequence but if one reads ignoring the Neandertal insert using the Denisova data, the reading is nearly identical to the contemporary modern human sequence.

This changes the nature of the Denisova sequence and makes it appear to reflect a combination of Neandertal sequences and AMH sequences consistent with the recent analysis of the Neandertal genome by Green et al. (2010). In the entire sequence from 16020 to 16409, the adjusted Denisova sequence agrees with the Neandertal sequence of Krings et al. (1997), 17 times where both do not agree with the Anderson sequence. But the Neandertal sequence differs from the Denisova sequence but agrees with the Anderson sequence in other 124 locations, but of these 113 are found up stream of the C insert between location 16193 and 16194. Of the other ancient mtDNA sequences, we provide in our 2000 chart, Betty et al. (1996) agrees 19 times with Denisova where variations occur from the Anderson sequence, Horai and Hayasaka (1990) 17 times, Handt et al. (1996) eight times and Handt et al. (1994) 4 times. The Betty sample is from Australian Aborigines and the Horai and Hayasaka from Japan. However, if one compares the Kostenki 14 sample (FN600416.1 GenBank) with the Sima (FK683087.1 GenBank), one finds considerable agreement, as opposed to most other samples. This is strange, as one is an early modern human (about 36,000 B.P.) from Russia and the other designated *Homo heidelbergensis* from Spain (Meyers et al. 2014). Degradation of the Sima sample and contamination were noted in the report, but the similarity in the sequences is curious.

The recognition of the Neandertal inserts in the Denisova sequence changes the reading considerably and indicates that the sequence before the insert at 16194, perhaps ending at 16181 is corrupt either from degradation, contamination, or during preparation for sequencing. There may be another explanation for the lack of sequence alignment before that location and the substantial agreement after it. Nevertheless, this finding argues for *Homo sapiens* (or Archaic?) status of the Denisova sample and against a new species designation as suggested from the original analysis (Krause et al. 2010) and is maintained by Vernot et al. (2016). Variations in mtDNA in populations, and their significance, given the natural history of mitochondria, have been noted by Ballard and Whitlock (2004) and they caution their use to build phylogenetic relationships. The production of algorithms to assess the relevance of gaps in alignment, deletions, insertions, etc., has resulted from a number of philosophical assumptions about their placement and frequency and length (Needleman and Wunsch 1970; Sellers 1974; Forster et al. 2001) with varied results using control region mtDNA data and RFLP analysis. Historically, these included a variety of indexes, including similarity indexes, distance indexes, and others (Wen-Hsiung and Dan 1991). Understanding the meaning in evolution of such alignment differences depends on the assumptions behind the indexes rather than how the gaps have affected selection or how they reflect mechanisms of the chemistry of molecular evolution or contamination and degradation processes.

Interpretation of Sequences and Fossils

Perhaps the agreement of algorithms has become more important than the biology of the organisms. In the same way the mathematics and philosophy (assumptions) of the Ptolemaic Geocentric system retarded the development of astronomy, we are seeing a focus on algorithms that supersedes analysis of the molecular biology of the gene in evolutionary context. Leslie White (1949) produced one of the most concise commentaries showing how mathematics is dependent on cultural assumptions, but Keynes (1921) reminded economists in his work on statistics that probability is often mistaken for reality. I discussed this regarding various ideas of the species concept and ancient mtDNA in my article in The Linnean (Caldararo 2003). Current models of speciation and admixture are based on a number of assumptions and vary in results (Frantz et al. 2014). There are a number of definitions of inserts, mtDNA sequences transferred to the nDNA and mutations in sequences of both nDNA and mtDNA, the latter can move the frame and can be either silent or damaging to replication of proteins. This latter case is the situation with the Neandertal insert. One has to keep in mind that the published sequences are of varied quality (Carter 2007). However, inserts are not unusual in the human mitochondrial genome, reference to the MITOMAP database produces many locations for inserts and deletions (http://www.mitomap.org/bin/view.pl/MITOMAP/PolymorphismsControl). Mutations in the D-loop area are also associated with some diseases as in cancers (Lee et al. 2005) and in aging, though recent surveys have shown that cumulative levels of base

[1] http://precedings.nature.com/documents/5360/version/3.

substitutions in mtDNA can be very low (Shokolenko et al. 2009).

Some of the philosophical aspects of phylogenetic tree building are discussed my article in 2002 (Caldararo 2002) and by Caldararo and Guthrie (1998) regarding constructing programs especially using Neutral Theory (Kimura 1983). But also this is significant concerning the nature of the mitochondrial genome and its inheritance and relating variations in the Y chromosome to nDNA as well as interpretations of the Mungo sequence published in 2001 (Adcock et al. 2001). In the correction published in 2002 by Adcock et al., we find the Mungo (LM3) sequence aligns with some positions in those of Tyrol, Hori, AU/HK, Neandertal, and Ventana Cave, yet the relation of this group to contemporary Australian Native populations remains controversial as the sequence was regarded as being highly contaminated (Cooper et al. 2001). The points raised in these papers demonstrate that the philosophical basis of gene sequence variation is neither clearly integrated between these three sources of information and variation, nor is it defined concerning what variations mean to the species status of individuals possessing these variations. Other critics of these assumptions have questioned the veracity of molecular clocks in general (Schwartz and Maresca 2006). As Caldararo and Gabow (2000) demonstrated in an analysis of chimpanzee DNA and the reported Feldhofer mtDNA, the variations between interfertile chimpanzees were greater than those reported between modern humans and Neandertals. So, the idea that Neandertals should be considered a different species seems illogical. But as Haller (1970) noted over 40 years ago looking back on 200 years of attempts to understand human variation, "The term 'species' plagued anthropologists."

Rather I look at the variations as similar to those reported for wolves, coyotes, and dogs, all of which are interfertile (Wayne 1993; Rutledge et al. 2010). One wonders how different Neandertal DNA would be compared with a sequence of a surviving sample from a population dated to that of the Feldhofer sample but from a anatomically similar skeleton to that of Cro Magnon. The Kostenki 14 sample would seem to provide that comparison as compared with that of Sima, yet the similarity in the sequences is confounding, is it due to contamination, to procedures or to interpretation? Or are they authentic, if so it would seem to argue for considerable association, but then how many differences equal species designation? We have to keep in mind that 1 in 200 individuals alive today are asymptomatic carriers of a pathogenic mtDNA mutation (Cree et al. 2009) and that variations in ancient samples must be considered in theoretically robust ideas of both inheritance and population genetics.

And here we return in another paper (Reich et al. 2010) reporting on the sequencing of nDNA from samples of human bone from the same Denisovan site. The authors remark on the "exceptional preservation" of the Denisova nDNA and note that it is above 70 % compared with Neandertal at less than 5 %. The length of the sequences is also unusual at 58 bp over Neandertal of less than 50pb. We should question the authenticity of this sample amplification as it seems too good and essentially argues for a near stasis of degradation over the period since burial. Had this sample come from a site that was unusual in its preservation or location, there might be reason to accept the results, but this was not the case (Vorobieva et al. 2011). This reminds one of the criticisms of the Mungo Lake sequence, that it was too well preserved, that cold stable environments are necessary for preservation, yet preservation of organic materials and animal tissue are found in desert environments that are stable and to not suffer cycling (Kahle and Caldararo 1986; Caldararo 1994).

However, other sites where Neandertal remains were found are in the same general area. This study (Reich et al. 2010) combined findings from an earlier sequencing of the Neandertal nuclear DNA which argued that present-day humans share common ancestors with Neandertals about 800,000 years ago and that a population split occurred between premodern populations leading to modern humans and Neandertals took place at about 270,000–440,000 years ago. It also asserted that Neandertals shared more genetic variants with present-day humans in Eurasia than with present-day humans in Africa. Applying this interpretation to their sequence from Denisova, Reich et al. (2010) argue that they found the Denisova Cave population contributed between 4 and 6 % of its genetic material to the genomes of present-day Melanesians.

Degradation and Authenticity

Degradation of Neandertal mtDNA samples demonstrated "drastically" different levels of contamination as reported by Green et al. (2006). In some widely separated sites where samples were found (France, Russia and Uzbekistan), only "around 1 %" of the mtDNA displayed Neandertal-like sequences. Of course, if one has interpreted a degraded sample sequence as "Neandertal-like," then one is simply searching for degradation parallels and not authentic species sequences. One sample from Croatia and one from Spain contained around 5–75 % Neandertal-like sequences according to Green et al. (2006).

In another paper by Green et al. 2010, the authors report on a draft sequencing of the Neandertal genome. The results show "more shared genetic variants with present-

day humans in Eurasia than with present-day humans in sub-Sahara Africa." These two conclusions confound current theories of modern human evolution. The Out of Africa/Replacement theory espoused by Stringer and Andrews (1988) applies the perspective that modern humans evolved in South Africa and migrated north eventually replacing all other premodern hominids, with no gene flow or contribution to present-day human populations from premoderns elsewhere. The Regional Continuity theory of Wolpoff and Caspari (1997) presents the idea of geographic populations in Europe, Central Asia, North and South Asia and Australia and Africa all evolving from premodern status to anatomically modern human status as a widespread single species maintained by gene flow. Disagreements continued as to these scenarios and how to test the theories (Brauer et al. 2004; Hawks et al. 2000; Hawks and Wolpoff 2001a). A third alternative that has become popular in recent years is a combination of Out of Africa, with waves of migration or gene flow over the past 2 million years and comprehensive interbreeding patterned by alternating periods of isolation (Brauer 1989; Hawks and Wolpoff 2001a, b; Templeton 2005). A more complex situation is emerging from an analysis of a recent skull from Central Africa dated about 13,000 (Harvati et al. 2011). This skull is more robust than we would assume from its late date and seems to reflect on a greater degree of persistence of "pre-modern" traits. mtDNA sequences from this skull would be most interesting.

Regarding the genetic information from Denisova, Henry Harpending was quoted as saying that his group had a number of hints that there was something else in the Melanesian genome, "…an admixture from some other group," he said. "To discover that it was from this particular group suggests that it was pretty widespread in Asia." (Maugh 2010).

Denisovans, Sima de los Huesos and Archaic Homo or Premoderns

At first blush it would appear that the Reich et al. 2010 conclusion would support the third theory of African waves of migration and interbreeding. However, the analysis that Templeton (2005) produced does not provide for the complete isolation of Melanesian populations as proposed by Reich et al. 2010. Rather, the Reich et al. 2010 proposal and the illustration in Vernot et al. (2016) could inadvertently give life to an earlier theory of human evolution of the polygenesis proponent, Carlton Coon (1962). Coon argued that hominids had evolved to the *Homo erectus* grade all over the old world and then evolved in situ into perceived geographically distinct races today. This did not preclude gene flow at times, but was not necessarily considered by Coon to be significant. Perhaps it is unfair to describe Coon as a polygenist, but the idea of waves of humanity moving across the planet producing a variety of local penetration of genes complicates and contradicts such ideas of simple species designations. Yet such a restricted regionally limited view of traits is contradicted by fossil evidence, for example, where Weidenreich (1938-9) shows that crania with modern Melanesian traits can be found in the Upper Paleolithic of Northern China and a Lower Neolithic site (Lang-Cuom) as well as a Mesolithic site (Pam-Pong) in what was called Indochina in his day. But his main point in the article was to show the great variation of hominin crania from Europe to Asia from the Middle Pleistocene to the Neolithic, specifically with traits argued by some to be "European" or "Asian" found across the regions. Modification of this "regional view," referred to as the Regional Continuity theory, have appeared in recent years including work by Brace (1967) and Wolpoff et al. (2001). The two approaches are significantly different, the one by Coon to explain the existence of contemporary races, and that by Wolpoff and his associates to account for the great diversity and continuity of hominins over the past million years.

However, this has been a problem throughout the 20th century as the same fossils have often been called *Homo erectus*, Neandertal, and *Homo heidelbergensis* over time confusing their status (Conroy 2005; Wood and Baker 2011). As Pearson (2001) demonstrates from a comprehensive analysis of the postcranial remains of the past two million years, what we find is a tremendous amount of variation. As he states, from an analysis of the African remains where we should find the earliest traces of modern human anatomy and a clear development into contemporary form, the following:

"In Africa, the few human postcranial fossils postdating H. erectus exhibit a striking amount of morphological diversity and include specimens that do not differ substantially from modern humans as well as bones that are distinctly not modern." This does not support Coon's (1962) theory of the origin of contemporary human races, yet does create problems for theories that are based on a full blown appearance of modern physiology in one place replacing all others. It does support the conclusion that the diversity of humans has been great in the past and yet what we conceive of as human races must have changed over time and been both varied and not limited to specific areas. In fact, as Pearson (2001) notes, defining a human physiology from contemporary variation is also a problem.

The distribution of Neandertal genes in Europe as well in Asia presents, but especially in Melanesia, another opportunity for interpretation. Since Neandertals have long been considered to have been limited to Europe and the Middle East with minor extension into western Eurasia, the appearance of Neandertal genes in Melanesia is significant

and contradicts this concept, rather it supports an older view of a Neandertaloid stage in human evolution, one associated with the concept of anagenesis, or the maintenance of one widely spread species evolving into stages of evolutionary phases as described by Brace (1967). The evidence of interbreeding between modern humans and Neandertals has accumulated in the past 5 years (Vernot and Akey 2014) and the rate of Neandertal transmission has grown to as much as 7.3 % (Lohse and Frantz 2014).

The issue of the nature of population variants and the contributions of haplotypes needs to be clarified to not only determine the species status of Neandertals and the Denisova remains, but how we also consider population diversity in general. An example of this appears in the Green et al.'s (2010) study where a Yoruba individual has a divergence estimate to the human genome sequence about 14 % greater than previous estimates for an African American individual and the heterozygosity measured in another Yoruba individual. Such individual variation needs to be considered in understanding the comparability of ancient DNA samples from populations dating tens of thousands of years ago. We are ignorant of the population diversity of the past and the role sedentary behavior had played in forming present human diversity, especially under the pressure of epidemic diseases (Caldararo 1996; Caldararo and Gabow 2000; Fabrega 1997). In this regard, by analyzing HLA haplotypes, Abi-Rached et al. (2011) have identified adaptive introgression from Archaic to modern humans using data from the Denisova aDNA.

It seems, from analysis of the Denisova mtDNA sequence that the Reich et al. 2010 conclusion is unnecessary and in error. A simpler explanation is arrived at by the Neandertal insert concept we have proposed which leaves us with an early modern population in the process of the Out of Africa wave theory of Hawkes, Wolpoff and Templeton. I am suspicious of the nDNA sequence Reich et al. have produced as nuclear DNA is notoriously more liable to degradation than mtDNA (Caldararo and Gabow 2000). While I commend the authors of these different studies of Neandertal DNA and the Denisova samples their efforts to eliminate contamination, the sequences do not appear without considerable difficulty to assure authenticity.

What is also interesting is that the Genbank Neandertal Feldhofer sequence (FM865407.1) differs substantially from the clones published in the original *Cell* article by Krings et al. (1997). In the original paper, the various clones are shown aligned together with the Anderson reference sample in Figs. 4 and 5 of their paper beginning in Fig. 4 at 16,022 in Fig. 4 and running to 16,401 in Fig. 5 with the Neandertal consensus of the clones from the different laboratories at the bottom. For example, an "A" appears at position 16,037 in the Anderson reference sample but a consensus of the clones for the Neandertal finds a "G" at this site. No other differences are seen in the sequence for the Feldhofer as consensus before this site except in two clones at position 16,036. In the GenBank sequence for Feldhofer, I find 10 differences listed. These identical differences are found in the GenBank sequences for Neandertal isolates from Mezmaiskaya 1, Vindija 33.25, and Sidron 1351e. It is difficult to explain this situation and I will not venture an explanation but would hope that one is available from the laboratories involved. It may be that the sequences are contaminated as has been found to be true of many of the non-Primate archived sequences discovered by Mark Longo and his colleagues (Longo et al. 2011; Phillips 2011). Forster (2003) in an earlier paper detailed problems with human mtDNA published data, reinforced by the work of Yao et al. 2004.

Paleospecies, Phylogenetics, and Hybrids

It often appears that the state of molecular phylogenetics is like morphological genetics before Huxley's "new systematics" in the 1930s. It seems in the condition molecular studies of viruses was before the concept of "quasi-species" was introduced. I discussed this issue in a review of a book on the evolution of HIV in the *American Journal of Human Biology* in (Caldararo 2001). We see the same passion to name each difference in a sequence as a species as in Darwin's day a new species was named with every difference in morphology. Stephen J. Gould's discussion of this regarding Darwin's redefinition of von Baer's work on embryology and recapitulation is worth revisiting (Gould 1977). It does, however, seem clear that assumptions of human diversity in the past as in the present are in need of revision. Work by Fraumene et al. 2006 shows much more diversity in a population in Sardinia than would be expected given present assumptions of human diversity. Since present phylogenetic analysis is based on these assumptions (Frantz et al. 2014), new paradigms of the role of diversity should be explored. Our best assessment at present, keeping in mind the problems in species designation mentioned above, is that the idea of separate species in the Middle Pleistocene is the problem if we have considerable gene flow and local adaptation to climate. This brings to mind the description of the Narmada hominid by Kennedy et al. (1991) where we find traits in one specimen in India that are common in a variety of other areas, Kabwe, Dali, Arago, Steinheim, Ngandong, Sangiran, Zhoukoudian, etc. If we theorize a widespread interfertile species, the explanation seems less contradictory.

The problem with paleospecies is magnified by our lack of information on fertility. Clifford Jolly (e.g., Lewin 1989) often made reference in this regard to the fact that *Papio anubis* and *Papio hamadryas,* with overlapping ranges in

Ethiopia, often interbreed producing fertile offspring. Factors affecting the range of fertility in hybrids and gene flow among dispersed hominid populations with substantial phenotypic variability render our concepts of species untenable in many cases. In an earlier paper, Gabow and Caldararo referred to the canid data for examples (Caldararo and Gabow 2000).

Figure 1 shows four examples of hominid evolution, the scenario at the bottom right is redrawn from a chart on the website the American Museum of Natural History and displays the ideas of branching phylogenetic process with numerous hominin species becoming extinct over the past 7 million years. This includes extinct lineages of *Homo ergaster*, *Homo erectus*, and Neandertals. The bottom left scenario is adapted from Tobias (1965) which is a form of anagenesis that I have modified to appear more like that described by Nelson and Jermain (1979). The top left is drawn from descriptions appearing in Coon's 1962 book. The top right scenario is from the Vernot et al. (2016) paper and illustrates not only their idea of gene transmission

Fig. 1 Anagenesis, monogenesis, and cladistics

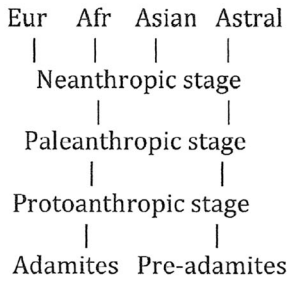

Figure 1a Polygenist Scheme

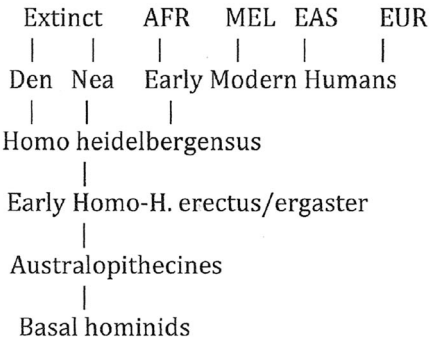

Figure 1d Vernot, et al. 2016

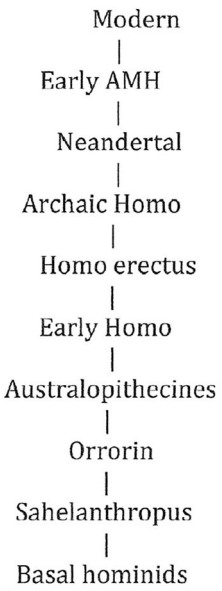

Figure 1b Anagenesis

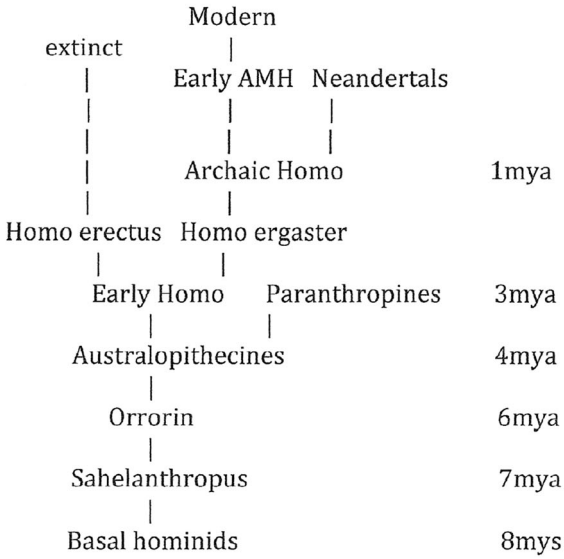

Figure 1c Cladistic Scheme (cerca 2000)

Figure 1 Redrawn from Tobias (1965), Leakey (1960) Coon (1962) Tattersall (as reproduced by the American Museum of Natural History, 2016). Figure 1d is derived from Vernot, et al., 2016 which appears without time references but inducates an ancient division between contemporary human populations identified as European, East Asian, Melanesian and African, with the separation for Africans as the oldest.

(intromission) from matings of Neandertals and Denisovans, but also indicates that these two populations become extinct, thus treating them as separate species.

What is most remarkable about the article by Ms. Gibbons (2016) (especially quotes from various scientists on the new results) are the references to Neandertals mating with Melanesians, or Denisovans with Europeans and Neandertals. While we know now that these matings occurred, it is distracting to call the contemporary Melanesians as participants, given that these events occurred thousands of years ago, this reference is to a place and a time as well as a living people who are certainly different from those who may have lived in the area at that time.

The African findings of Vernot et al. (2016) are curious, and while the authors report Neandertal genes in some contemporary African populations (Luhya and Gambians) but not others, this could be an artifact of the structure of the sampling process they are drawing from and its composition. It seems odd for them to dismiss Neandertal genes in these populations due to supposed contact with European colonials given that, for example, the Luhya (formerly known as the Bantu Kavirondo) are rather isolated in Western Kenya and had a hostile relationship with the British (Wagner 1949; Ehert 1974). Why should Neandertal genes appear in them and not surrounding peoples, Kikuyu for example, given colonial contact?

On the other hand, given the physiological similarities of some Archaic Homo who in the past were classified as Neandertaloid (Montagu 1962), like the Kabwe cranium or Omo I and II (which differ significantly in "archaic vs modern traits" yet come from the same strata, (Mcdougall et al. 2005). Why should we not expect such genes in Africa due to migration or hybridization? In fact, this reanalysis continues today with what to include in *Homo heidelbergensis* (Stringer 2012). Classifying premodern and especially Neandertal traits, as noted above, has been problematic (Pearson 2001). This problem is magnified in the Melanesian interpretation, as one wonders the logic of such a dramatic variance among the populations of modern humans located in close contact as shown in the Vernot et al. 2016, Fig. 1d, redrawn here as Fig. 1d. While we lack any idea of who the Denisovans were or if they were a subpopulation of "pre" Neandertals or other Archaic Homo, it seems incredible that so many variants could have been produced across Asia at a distance in time from the Denisovans to the present.

Conclusions

In the Vernot et al.'s 2016 paper as well as most others on the subject, modern humans are treated as contemporaries of Neandertals and Denisovans, though the latter are drawn to indicate they become extinct (see Vernot et al. 2016 chart). This was an effort to identify "deleted Neandertal sequences" due to selection where contemporary populations were compared to putative Neandertal individuals instead of including ancient DNA from human samples. However, in the Caldararo and Gabow (2000) and Caldararo and Guthrie (2012) papers, the Neandertal and Denisovan sequences were bracketed by the use of ancient human DNA sequences to put the variations in context of time so that evolution and degradation could be assessed. Just as Meyers et al. (2014) had difficulty placing the ancestors of the Denisovans and Sima de los Huesos due to their inability to conceive of anything but a special overlap with Neandertal ancestors, the problem here is a continuing concept of species rather than populations. If we consider populations of premoderns living at the time of the people of Sima de los Huesos, having a variety of haplotypes as we find in today's modern populations, then the movement of peoples and genes over a distance of Spain to Siberia and a time frame of several hundred thousand years is less daunting. The special overlap disappears.

It is also unclear where these events occurred. I am sure the authors do not know. It is also clear that to say there was mating between Europeans and Denisovans or Neandertals not only implies that the individuals involved were members of the same species, according to the Biological Species Concept, but that they also produced viable offspring. But those individuals involved are hardly the same people as today's Europeans, or Melanesians, given thousands of years of environmental selection, migrations, invasions, disease selection, and war. It does suggest long periods of hybridization between widely spread populations whether we call them *Homo sapiens neandertalensis* or *Homo heidelbergensis* or *Homo sapiens denisova* or altai or Archaic Homo or *Homo sapiens.*

It also seems premature to conclude that there are "deserts" (Vernot et al. 2016) where no Archaic Homo genes appear in modern humans given the recent "discovery" of such genes, certainly given the earlier claims by Krings et al. (1997, 1999) that none were present at all. The authors seem to acknowledge this fact. New technologies or more precise methods of characterizing Archaic genes (or whatever we shall call them, "Premodern?") may produce new discoveries in the future. But this brings up a related point. The entire discussion of "Neandertal" or "Denisovan" genes is not based on population genetics, but on, for Neandertals, a small number of samples and for Denisovans perhaps only fragments of three individuals. The lack of a population basis of known values of variation places attempts to characterize genetic diversity of the two groups in considerable question. While the statistical methods Vernot et al. (2016) use to this end, without the physical evidence is logical, it is based on assumptions that

may not be realistic. The development of the Cambridge Reference Sequence (CRS) has gone far to elucidate and demonstrate the variability of human DNA, and a systematic analysis of all the sequences of ancient DNA (aDNA) should be undertaken to replicate and characterize the development of that variability over time.

While we generally find Neandertals classed in our taxonomic charts as *Homo neandertalensis*, we might want to consider changing that to *Homo sapiens neandertalensis* and something more inclusive for the Denisovans. This implies that they (or populations descendant from them) were interfertile with ancient modern Homo sapiens who came Out of Africa after 200,000 B.P. A bit of anagenesis is perhaps appropriate here, for it remains questionable whether the Denisovan signal is authentic, in whole or in part and what the amount of divergence, should it be verified in the future with less degraded samples, can mean. Speculation of potential Neandertal sequence association with contemporary disease is useful but should be discussed with caution. Association of factors with disease states or pathology, in medicine is not equivalent to causation, reference to Koch's Postulates is still useful today. Yet they have been modified as the nature of disease agency has become more detailed by scientific advances in the biology of disease, especially regarding viruses and prions. These have included those by Evans, Rivers, and Heubner (Fredricks and Relman 1996). While many disease states are associated with Mendelian inheritance, others are the result of complex etiologies and a variety of factors that require careful epidemiological work.

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