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Brief Communication: Paleoanthropology and the Population Genetics of Ancient Genes

John Hawks[1]* and Milford H. Wolpoff[2]

[1]*Department of Anthropology, University of Utah, Salt Lake City, Utah 84112*
[2]*Paleoanthropology Laboratory, Department of Anthropology, University of Michigan, Ann Arbor, Michigan 48109-1382*

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ABSTRACT The Mezmaiskaya cave mtDNA is similar in many ways to the Feldhofer cave Neandertal sequence and the more recently obtained Vindija cave sequence. If we accept the contention that the Mezmaiskaya cave specimen is a Neandertal infant, its mtDNA provides no new information about the fate of the European Neandertals. However, there is reason to believe that the Mezmaiskaya cave infant is not a Neandertal, and this places its importance in another light, because it delimits the possible hypotheses of Neandertal and recent human genetic relationships. One possibility is a that the pattern found in ancient mtDNA results from the replacement of an isolated gene pool (Neandertals) by one of its contemporaries (modern humans). A second possibility is natural selection expressed as the substitution of an advantageous mtDNA variant within a single large species, including both Neandertals and modern humans. The geologic, archaeological, and dating evidence shows the Mezmaiskaya cave infant to be a burial from a level even more recent than the Upper Paleolithic preserved at the site, and its anatomy does not contradict the assessment that the Mezmaiskaya cave infant is not a Neandertal. Therefore, the second pattern can be favored over the first. Am J Phys Anthropol 114:269–272, 2001. © 2001 Wiley-Liss, Inc.

The interpretation of ancient mitochondrial DNA (mtDNA) sequences obtained from fossil humans has been rightly viewed as a complex undertaking, one that is illustrated well by the recovery of an mtDNA segment from the 29,000-year-old Mezmaiskaya cave infant (Ovchinnikov et al., 2000). This partial first hypervariable region (HVR-1) sequence is 12 base pairs different from that recovered from the Feldhofer cave Neandertal specimen, and like it, both different from living Europeans and not related more to them than to other extant populations. The similarities are shared with a more recently recovered sequence from Vindija cave, which adds to our knowledge of the European Neandertal sample, but was not available for comparison at the time of the Mezmaiskaya analysis. The difference between the Mezmaiskaya cave and Feldhofer cave fossils discussed by Ovchinnikov et al. (2000), and with the closely related Vindija fossil (Krings et al., 2000), suggests a level of variation among these Late Pleistocene specimens not greater than that found in recent humans, but on a separate mtDNA clade.

The Mezmaiskaya findings were interpreted by Ovchinnikov et al. (2000) to "provide no evidence for the multiregional hypothesis" (p. 490). Other interpretations have gone further. According to Höss (2000), the results also "argue against the idea that modern Europeans are at least partially of Neanderthal origin" (p. 454). However, the relevance of genetic data to this issue has proved to be difficult to evaluate, because such data are often consistent with several hypotheses about the origins of the living people who make up our comparative genetic sample. In the case of the Mezmaiskaya specimen more than most, our interpretations must be weighed not only against the genetic theory that addresses evolutionary questions of relationship, but also against the archaeological context from which the specimen was recovered. Here we suggest a different way of interpreting the new information that is more in line with other known facts about mtDNA evolution, as well as resolving certain discrepancies that now exist in the Caucasus burial data.

WHAT IS THE MEZMAISKAYA CAVE INFANT?

There is one clear case in which the mtDNA sequence from Mezmaiskaya could address the question of Neandertal isolation. If the specimen were shown to be post-Neandertal, then it would be clear evidence of the persistence of Neandertal-like mtDNA sequences in later populations, falsifying the hypothesis of Neandertal isolation. This possibility is raised by the archeological circumstances and the description of poorly defined layers where the burial was recovered (Golovanova et al., 1999).

*Correspondence to: John Hawks, Department of Anthropology, University of Utah, Salt Lake City, UT 84112.
E-mail: john.hawks@anthro.utah.edu

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It was assumed in the interpretations cited above that both of the ancient HVR-1 sequences recovered at that time were Neandertals (Höss, 2000; Ovchinnikov et al., 2000). However, bone from the Mezmaiskaya cave infant's skeleton was directly dated to only 29,000 BP (Ovchinnikov et al., 2000). This is a date that is out of sequence with the other dates from the site (Golovanova et al., 1999), a discrepancy which is explained by "the incorrect identification of the poorly defined layers in this area of the cave" (Ovchinnikov et al., 2000, p. 491), and by the assumption that the dates reported for the animal bones from better-defined layers were incorrect. But "poorly defined layers" near both the existing ground surface and the original cave entrance lead to a serious contextual problem with the specimen. The skeleton was recovered from layer 3, a Mousterian layer that is otherwise AMS-dated from animal bone to >45,000 BP (Golovanova et al., 1999), which is not unexpected for the Middle Paleolithic of the region. The exceptional preservation and orientation of this very delicate skeleton support the claim that the infant was intentionally buried. Yet no burial pit was found, and there were no grave goods (perhaps, as the authors suggest, because the sediments were disturbed subsequently); this is important, because it means that there are no direct cultural affiliations. At the front of the cave where the burial was found in the Mousterian of level 3, this Mousterian is directly below the earliest Upper Paleolithic layer, dated by AMS on wood charcoal to just over 32,000 BP, a date also not unexpected for the Upper Paleolithic of the region. So why should an AMS date on this skeleton be 29,000, even younger than the Upper Paleolithic layer overlying the Mousterian layer in which it was found? One possibility is to accept the age of the skeleton and ignore the other dates, as suggested by Ovchinnikov et al. (2000). But all these radiocarbon dates would be consistent if the burial intruded from above the Mousterian of layer 3.

In this context, it is important to look extremely carefully at the morphology of the specimen to evaluate whether, in the absence of any cultural affiliation, it can be identified as Neandertal. The age of the specimen is in a range within which many claim Neandertals are particularly difficult to distinguish from other human populations, including modern ones (Creed-Miles et al., 1996). It seems the only ostensible reason to assume it is a Mousterian burial is the absence of Upper Paleolithic grave goods. But after the troubling misidentification of the Starosele remains, where a Neolithic infant was buried in a Mousterian layer (Marks et al., 1997) and both identified and analyzed as a Mousterian individual (Ullrich, 1955), human paleontologists must exercise great caution in these identifications.

Anatomically, a Neandertal affiliation for the Mezmaiskaya specimen is doubtful. The only Neandertal morphologies identified in the specimen are a large paramastoid, an oval foramen magnum, and short distal limbs (Golovanova et al., 1999), although in no case are these quantified. Lagar Velho, from the Upper Paleolithic of Portugal, long postdates the Neandertals of Western Europe, but has most of these features (Duarte et al., 1999). This does not necessarily mean that these geographically distant children were two members of the same population, but it is certainly credible that they are results of the same evolutionary process. The one conclusion compatible with all the known facts, and the only one contradicted by none, is that the Mezmaiskaya cave infant is a later burial, from the same time as or later than the Upper Paleolithic at the site, that is intrusive into an older Mousterian layer. This is justified by the descriptions of the site and its published profile, fits with the anatomical features, and would explain the dating discrepancies.

HOW CAN WE ACCOUNT FOR ANCIENT mtDNA VARIATION?

It is now known that the observed mtDNA sequence variation in recent humans is lower than that in Late Pleistocene humans; the known sample is preserved in the Mezmaiskaya sequence, in the Feldhofer 1 and Vindija sequences, and in the ancestors of recent human sequences. This provides clear evidence of nonneutral evolution that has otherwise been confirmed in living humans by the frequency spectrum of mtDNA variation (Merriwether et al., 1991). Ovchinnikov et al. (2000) assert that the pattern of evolution of ancient mtDNA was the replacement of an isolated Neandertal gene pool by one of its contemporaries (our ancestors). But the data may equally reflect the substitution of an advantageous mtDNA variant within a single large species, including both Neandertals and other ancestors. Either of these evolutionary hypotheses would predict the observed results. The first hypothesis is inconsistent with a hypothesis of regional continuity for Late Pleistocene European evolution; the second is not. The two hypotheses also differ as to the level of gene flow among Neandertals and their ancient contemporaries: the first requires that level to be zero; the second requires it to be nonzero.

Is there any other way to determine which of these two hypotheses is correct? Unfortunately, the observed sequence variation of Neandertals provides no test of the level of gene flow among ancient human groups. With a larger sample of Neandertal mtDNA or even nuclear DNA, we might attempt such a test, but such efforts are hampered by the continuing problem of contamination. This is because of the suspicion that while any sequence taken from a Neandertal fossil that looks like recent humans may be genuine, it may instead be contaminated. Though analysis of ancient DNA can use many methods to confirm the possibility that ancient DNA sequences have survived in a specimen, the ultimate proof of the ancient origin of any DNA recovered from such a specimen remains the sequence itself. In some cases, DNA contaminants are obvious, especially when they are exotic sequences

with laboratory origins. In other cases, in a field where ancient Europeans may have been measured by paleoanthropologists of European descent, had tissue extracted by European laboratory technicians, and may or may not have European-like sequences of their own, contamination is difficult to detect. For example, contaminant sequences from recent Europeans were found in a minority of cloned DNA reproduced from the Feldhofer specimen (Krings et al., 1997). These were readily identified as contaminants because of the simultaneous presence of the very different endogenous DNA sequences in the samples, but without the chance preservation of these sequences, the short contaminant DNA might have been accepted as part of the reconstructed Feldhofer sequence. Where genuine ancient sequences are present and are clearly different from recent humans, the problem can be visually corrected, but if the genuine ancient sequence were similar to recent humans, the possibility that it partially or completely represents contaminants would be difficult to exclude. This problem is an inevitable artifact of the cloning and PCR methods used at present to recover ancient DNA, and until it is circumvented, no valid estimate of ancient gene flow can be obtained.

However, the identification of the Mezmaiskaya specimen as post-Neandertal implicates a within-species process of selection to explain the divergence of these ancient sequences from recent humans. There is abundant evidence from the mtDNA of living humans to suggest that a recent substitution of a favorable mtDNA variant has occurred. Human mtDNA is not in mutation-drift equilibrium (Merriwether et al., 1991), which means that it is still in the process of accumulating mutational variation from a recent time when variation was more limited. Though this past restriction may have arisen from a small population size, most nuclear DNA studied thus far is in equilibrium (Hey, 1997; Templeton, 1997), and excludes the hypothesis of either a recent population size bottleneck or a long history of small population size (Hawks et al., 2000). The most parsimonious explanation for the discrepancy among these genetic systems is that mtDNA and some nonrecombining segments of nuclear DNA have been influenced by selection, likely because selection on any small part of them is the same as selection on the whole (Kim and Stephan, 2000; Nachman et al., 1998). The linkage of genes on these segments is not broken up by recombination (Templeton et al., 1995), and levels of diversity are correlated with cross-over rates in *Drosophila* and humans (Przeworski et al., 2000). We can then expect that the variation in mtDNA reflects principally the time span since the last selection event and secondarily the cumulative effects of purifying selection against deleterious mutations across the mitochondrial genome (Fay and Wu, 2000; Wise et al., 1998). In humans, these effects may have been very pronounced, because evolutionary changes in the brain, life span, and energy expenditure would have created new selective pressures on mitochondrial genes that were important all across the human range, and these anatomical and behavioral changes occurred worldwide.

A significant illustration of this selection is that the level of divergence between the ancient sequences referenced in this study and recent human sequences is much less than that present between chimpanzees of different subspecies, even though these ancient humans occupied a far greater geographic range (the actual difference in diversity may be even greater, since the substitution of favorable mtDNA variants may also explain the unusual pattern of mtDNA variation among chimpanzee subspecies) (Wise et al., 1997). The most credible explanation for this low level of mtDNA diversity even in ancient humans is that the ancient diversity we are sampling within Neandertals and other Late Pleistocene humans is the relic of many ancient episodes of selection. We should not assume that a unique event led to the origins of human mtDNA variation; it is likely that selective events have altered the pattern of human mtDNA variation, and possibly that of other human nonrecombining genes, many times during the past million or more years.

The hypothesis that a recent selective substitution reduced human mtDNA variation leads to the prediction that a sample of mtDNA from before the sweep should be equally related to all mtDNA lines today, since the diversity of these lines evolved after the Neandertals existed. The Mezmaiskaya cave specimen is compatible with this prediction. It further supports the contention that human mtDNA probably underwent selective events many times, because a previous event would then account for the close relationship between these Pleistocene Europeans. Just as for many anatomical traits, the range of variation of recent humans may be quite different than in humans living 30,000 years ago or more, because the human population has not remained static, but has been subject to selection. Therefore it is ironic but true that for all its advantages, the study of ancient DNA imposes on geneticists the same set of quandaries with which we paleoanthropologists have always dealt: very small samples and the question of what variation is due to selection and what variation is due to drift. In fact, because our samples are much larger, and we have a better idea of where selection acts on human anatomy, we may well be better off.

CONCLUSIONS

Despite the evidence for recent selection on mtDNA within one human species, the issue of whether selection among ancient isolated gene pools or selection within a single ancient gene pool (Wise et al., 1998) is responsible for the observed pattern of ancient mtDNA variation must ultimately be settled with reference to nuclear genes. Many anatomical studies indicate that individual elements of Ne-

andertal anatomy are still found in recent and living European populations (Frayer, 1993; Mann et al., 1991; Szilvássy et al., 1987; Wolpoff and Caspari, 1996), and were much more prevalent before the Neolithic, which argues against a hypothesis of Neandertal isolation. The hypothesis of isolation has also been addressed by molecular geneticists, who have found no evidence for isolation of ancient human groups (Templeton, 1998). Though many critics of the model have described multiregional evolution in less general terms than its proponents, its key prediction is the evolution of modernity in more than one region. Multiregional evolution could be correct even if all Neandertals became extinct without descendents, because it does not require evolution without replacement in *every* region (Relethford 1998, 1999; Wolpoff, 1998a; Wolpoff et al., 2000). However, the hypothesis of Neandertal extinction does not appear to have empirical support. In the estimation of the contribution of Neandertals to later Europeans, the Mezmaiskaya infant takes an important place in the post-Neandertal population, because a few elements of its skeletal anatomy and its mtDNA sequence appear to reflect Neandertal admixture with populations entering Europe during the interstadial. Under these circumstances, we find its mtDNA sequence to be perhaps the most important single piece of evidence yet found to address the issue of Late Pleistocene human evolution.

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