

Denisova Cave, Peștera cu Oase, and Human Divergence in the Late Pleistocene

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ABSTRACT

Krause et al. (*Nature* 464: 894–897) have proposed the existence of a novel human lineage at Denisova Cave based on its divergent mtDNA sequence. Assessment of the Oase 2 early modern human maxillary molars, using the same logic as Krause et al., suggests that its lineage was even more divergent. This exercise highlights the inappropriateness of such an analysis for assessing lineage, or species, distinctiveness based on partial organismal data.

INTRODUCTION

Krause and colleagues (2010) presented the mitochondrial genome (mtDNA) of an (as yet not described or illustrated) purportedly human distal hand phalanx from the little finger (ray 5). The geological age of the specimen is not precisely known, but it appears to derive from the Interpleniglacial (marine isotope stage (MIS) 3) deposits of the Denisova Cave, in the Altai Mountains of southwestern Siberia.

Based on the divergent nature of this mtDNA sequence, relative to both those of extant humans and the modest number known for the Neandertals, they proposed 1) that the mtDNA lineage leading to the Denisova Cave individual was distinct from those of other Late Pleistocene humans; and, 2) that the divergence time of that lineage from the one leading to living humans should be sometime in the Early Pleistocene, ~1,000,000 years BP. Although the mtDNA lineage divergence time is likely to be older than the population splitting time, this should apply similarly both to the ancestral lineage leading to the Denisova Cave individual and to the Neandertal lineage, making the extrapolation to relative population divergence times similar. In addition, even though it was not explicitly stated by them, this interpretation has been taken by others (e.g., Brown 2010) to indicate a new, as yet unnamed, human species.

Given the nature of this claim, it is instructive to examine another possible case of divergent human biology in the Late Pleistocene, the Oase 2 mid-MIS 3 human from the Peștera cu Oase, in the southwestern Carpathians of Romania, dated to approximately the same age as the Denisova Cave phalanx.

THE PEȘTERA CU OASE 2 MAXILLARY THIRD MOLARS

As previously noted (Rougier et al. 2007; Trinkaus et al. 2003a), Oase 2 has exceptional maxillary third molars for a Late Pleistocene human. The teeth are unworn, since they were incompletely formed at the time of death, hav-

ing attained complete crown formation and approximately three-quarters of the root formation. They were therefore unerupted and are securely located in their crypts. There are no apparent developmental or degenerative defects in the M³s, apparent on the surface or radiographically. Although the underlying genetics of dental morphology and size are poorly known, it is likely that they are polygenetic, highly controlled by the individual's genotype, and developmentally stable in the absence of marked environmental stress (Kieser 1990; Scott and Turner 1997). The morphology and dimensions of the Oase 2 M³s may therefore provide some perspective on the genetics of this early modern human.

THE OASE 2 MAXILLARY THIRD MOLARS

The Oase 2 M³s are unusual in their occlusal morphology, in which the occlusal surfaces exhibit at least eleven separate "cusps" or enamel protrusions (Figure 1). Some degree of occlusal complexity is moderately common on human maxillary third molars, but the degree of elaboration of these separate "cusps" of the Oase 2 M³s is unknown among Pleistocene members of the genus *Homo* and, if present among recent humans, is exceedingly rare. Among fossil humans, it is most closely approached by the M³s of OH-5, the type specimen of *Australopithecus boisei* (Tobias 1967).

At the same time, the Oase 2 M³s are exceptionally large. The left M³ has a mesiodistal (MD) diameter of 12.9mm, and the right and left ones have buccolingual (BL) diameters respectively of 14.6mm and 14.9mm. Using the left mesiodistal diameter and the average of the two buccolingual diameters provides a crown "area" (MD x BL) of 184.4mm² (using only the left diameters provides 186.3mm²). The average "area" of the two M³s is 3.43 standard deviations from the mean of a pooled MIS 5 to 3 Neandertal M³ sample (116.2±19.9mm², N=41), which includes the relatively megadont initial MIS 5 Krapina sample. At the same time, it is 3.56 standard deviations from the mean of a pooled MIS 5 to 3 early modern human sample (107.7±21.5mm²,

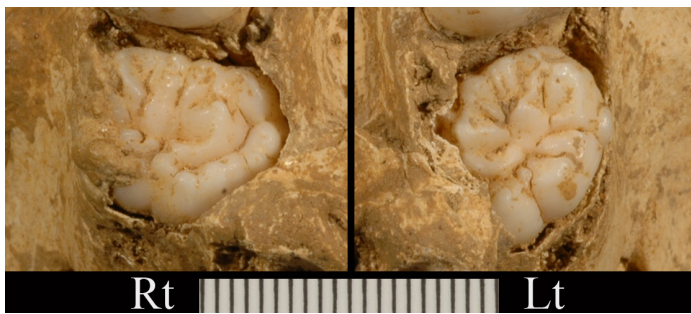


Figure 1. Occlusal views of the Oase 2 maxillary third molars (M^3 s). Scale in mm.

$N=32$), combining western Eurasian samples from the Middle Paleolithic, the Early Upper Paleolithic (Aurignacian), and the Mid Upper Paleolithic (Gravettian); inclusion in the sample of the few known African Middle Paleolithic M^3 s would change the values little. To my knowledge, there are no maxillary third molars securely attributed to the genus *Homo* (*H. erectus sensu lato* and later) that are as large or larger than the Oase 2 molars. The Oase 2 M^3 “area” is, however, matched by the *Australopithecus afarensis* mean (180.0mm^2) provided by Wood (1991); it is exceeded by the mean values for other species of *Australopithecus*.

If the Oase 2 maxillary third molars had been found in isolation in the bone bed of the Peștera cu Oase which yielded the remains, it would have been difficult to subsume them morphologically and/or morphometrically within the human populations known from western Eurasia (or the Old World) in MIS 3 (or most of the Pleistocene). By the criteria that are commonly used to identify novel paleontological species—a morphological pattern distinct from other closely-related species—it would have been natural to propose a new species of the genus *Homo* for the teeth, with a divergent lineage leading to it. In other words, they could easily have been taken to indicate the presence of an additional, hitherto undocumented, lineage of humans in the southwestern Carpathians of MIS 3.

However, the Oase 2 maxillary molars were not found in isolation, but securely in their crypts, unerupted, exposed only on their occlusal surfaces, and otherwise accessible through CT scanning (see Figure 1). The remainder of the largely complete Oase 2 cranium is, and has always been, considered to be that of an early modern human, securely attributable to *H. sapiens sensu stricto* (Rougier et al. 2007; Trinkaus et al. 2003a). It exhibits a suite of uniquely derived characteristics of extant humanity in its facial, supraorbital, temporal, and occipital external morphology (Figure 2). For this reason, despite the exceptional nature of the Oase 2 dentition, explanations for the morphological pattern evident in the specimen have been sought in the population processes of Late Pleistocene early modern and late archaic humans, and not in lineage/species level distinctions between it and the temporally and geographically adjacent available human samples (see also Bailey et al. 2009; Crevecoeur et al. 2009; Trinkaus 2007; Zilhão et al. 2007).

ESTIMATING DIVERGENCE TIMES

In addition to suggesting lineage distinction for the Denisova Cave phalanx, Krause and colleagues (2010) inferred an approximate time of divergence of its ancestral lineage from that of extant humans. They estimated the Denisova Cave mtDNA sequence to be approximately twice as divergent from living human mtDNA as is the average Neandertal mtDNA sequence. Assuming a mean Neandertal-modern human lineage divergence estimate of $\sim 466,000$ years BP and a linear “molecular clock,” they roughly estimated the divergence of the Denisova Cave lineage from the extant human one at approximately one million years BP, fully acknowledging the approximate nature of such an estimate. A similar exercise can be done with the dimensions of the Oase 2 M^3 s.

If one compares the “area” of the Oase 2 M^3 s to the means for the other samples (Table 1), it is apparent that the differences between the Oase 2 M^3 dimensions and those of the comparative samples are substantial, with respect to both the Late Pleistocene samples (see above) and especially the recent (late Holocene) samples. Following the logic of Krause et al. (2010), one can then compare the degree of divergence of the Oase 2 value from the recent human values to the degree of divergence of the mean Neandertal value from those same recent human values (see Table 1). From this comparison, the Oase 2 – recent human divergence is nine times as great as the Neandertal–early modern human difference and more than four times as great as the Neandertal–recent human divergence.

Again following the logic and assumptions of Krause and colleagues, if one assumes a Neandertal–recent human divergence of $\sim 466,000$ years (which assumes no gene flow between the lineages and may well be a minimum in any case), one can translate the differences in M^3 “areas” into divergence times assuming a similar “morphological (or phenotypic) clock.” With respect to Neandertal–early modern human divergence, this provides a completely unrealistic early Pliocene divergence of the Oase 2 lineage ~ 4.2 million years BP, long before the generally accepted origin of the genus *Homo* sometime between 2.0 and 2.5 ma BP (cf., Wood 2010). Using the more directly appropriate Neandertal–recent human degrees of divergence provides a more modest, but still pronounced, Oase 2–recent human divergence time between 2.0 and 2.2 ma BP. More ancient estimates for the divergence age of the Neandertal and modern human lineages (600,000 or 700,000 years BP) would only push these Oase 2–recent human “morphological clock” divergence times back further in the Pliocene.

SOME THOUGHTS

This exercise is based on the logic that was used in the paper by Krause and colleagues to interpret the unusual mtDNA sequence that they derived from the Denisova Cave phalanx, applied to the best direct reflections of the genotype present in the morphology of fossil human remains, dental occlusal morphology and crown dimensions. If one only had the Oase 2 M^3 s from that Late Pleistocene individual and if one followed the logic of Krause *et alia*, one would



Figure 2. Left lateral view of the Oase 2 cranium. Scale bar: 10 cm.

come to a conclusion similar to theirs, that there were indeed multiple undescribed human lineages with deep Pleistocene (or even Pliocene) lineage divergence times in many locales of the Late Pleistocene Old World. However, there are issues that make the above analysis of the Oase 2 M³s largely invalid, which also apply to the Denisova Cave mtDNA analysis.

First and foremost, both “molecular clocks” and “morphological clocks” assume that the cumulative rate of change is relatively constant over the time period involved, an assumption that requires that the traits in question be selectively neutral. It is for this reason in particular that “morphological clocks,” which assess aspects of the phenotype which are minimally under stabilizing selection and may be under varying directional selection, rarely if ever work.

It is unlikely that the dental traits in question are strictly selectively neutral, being expressed in a functioning portion of the phenotype. Yet, the discrete traits of occlusal morphology are routinely used as though they are essentially neutral (cf., Scott and Turner 1997; Bailey 2006), and both absolute and relative dental crown dimensions are routinely employed, especially in human paleontology, to assess the affinities of specimens/samples. Moreover, pat-

terns of molar dimensions in both recent and Pleistocene *Homo* show considerable within and between sample variation, only some of which patterns in time and space (cf., Kieser 1990; Trinkaus 2002; Wood 1991).

At the same time, although mtDNA sequences present very different kinds of data than teeth and analyses of them have frequently emphasized the purported selective neutrality of its non-coding regions, there is growing evidence of selection acting upon mtDNA in humans (cf., Hawks 2006; Mishmar et al. 2003). The validity of applying “molecular clocks” to it is therefore questionable.

The second issue is that neither analysis, of the Denisova Cave mtDNA or of the Oase 2 molars here, views the variation in terms of population variation. Both appear as outliers, although the Interpleniglacial (MIS 3) human mtDNA variation of eastern Eurasia is unknown [the one specimen, from Okladnikov Cave (Krause et al. 2007), does not provide a variance], and the western Eurasian Early Upper Paleolithic M³ comparative sample size is five.

Furthermore, unlike the Denisova Cave mtDNA sequence, the Oase 2 M³s do not exist in a biological vacuum. And it is that biological context of the Oase 2 M³s (its cranium), as well as the associated Oase 1 mandible with its similar combination of derived modern human and archaic

Table 1. For OASE 2, LATE PLEISTOCENE SAMPLES, AND RECENT HUMANS:

1) average maxillary third molar “areas” (length x breadth, in mm²), 2) absolute differences between the average Oase 2 M³ “area” and the average M³ “areas” of the comparative samples, in mm², 3) absolute differences between the average Neandertal M³ “area” and those of the recent human samples; and, 4) the difference in M³ “area” between the Oase 2 M³s and early and recent human samples divided by the difference between the Neandertal mean M³ “area” and the early and recent modern human means. The last is provided as a measure of the divergence of the Oase 2 M³ “area” scaled against the divergence of Neandertal M³ “area.”

	1	2	3	4
	Average M ³ “Area”	Oase 2 M ³ “Area” versus:	Neandertal M ³ “Area” versus:	Oase 2 / Neandertal M ³ “Area” Differences
Oase 2	184.4			
Neandertals	116.2	68.2		
Early Modern Humans	107.7	76.7	8.5	9.02
Recent Humans: Means^a	97.4	87.0	18.8	4.63
Recent Humans: Individuals^b	95.7	88.7	20.5	4.33

^a The average of the means (N=78) provided by Kieser (1990) for global recent human native samples.

^b The average of the individuals (N=3,121) for the same 78 recent human native samples provided by Kieser (1990).

features (including large M₃s) (Trinkaus et al. 2003b), combined with the issues of selective neutrality, population frameworks, and “molecular/morphological clocks,” that makes the above exercise of comparative M³ morphology and especially the inference of divergence times for Oase 2 rather silly. We should view the Denisova Cave mtDNA results in the same light.

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