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Ancient DNA: Would the Real Neandertal Please Stand up?

Dispatch

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Mitochondrial DNA sequences recovered from eight Neandertal specimens cannot be detected in either early fossil Europeans or in modern populations. This indicates that, if Neandertals made any genetic contribution at all to modern humans, it must have been limited, though the extent of the contribution cannot be resolved at present.

The genetic affinities of the earliest modern humans of Europe and the earlier homonid occupants of the area, the Neandertals, has remained a hotly debated topic since the discovery of the extraordinarily robust skull cap and limb bones in the Neander Valley in 1856. While it is impossible to rule out a surreptitious coupling of the two groups in the more than 10,000 years they apparently co-occupied Europe, recent research and population genetic theory suggest that any genetic interchange was limited. This issue is central to the two main theories of modern human origins: the replacement model, where modern humans rapidly replaced archaic forms, such as Neandertals, as they began to spread from Africa through Eurasia and the rest of the world sometime around 100,000 years ago [1]; and the multi-regional model, where genetic exchange or even continuity exists between archaic and modern humans [2,3]. Two years ago, a review [4] reported that characteristic mitochondrial DNA (mtDNA) sequences retrieved from remains of four Neandertals are absent from modern human populations. It remained possible, however, that these sequences had been present in early modern humans, but had been lost through genetic drift or the continuous influx of modern human DNA in the intervening 28,000 years since Neandertals became extinct.

The difficulty in testing these ideas using ancient DNA is that most ancient human remains are contaminated with modern human DNA, which deeply penetrates bone and teeth samples during the washing and routine handling that takes place after excavation. This modern DNA will either out-compete authentic ancient sequences in PCR reactions, or recombine with them to produce artificial, but authentic looking genetic sequences [5,6]. Consequently, even when strict criteria for authenticating ancient DNA results are followed (Table 1), it can be impossible to determine the authenticity of results [7] such as a recent study of early modern human mtDNA [8,9].

The approach taken recently by Serre *et al.* [10] avoided this problem by searching only for the presence of Neandertal mtDNA sequences in both early modern human and Neandertal fossils, while ignoring modern human sequences because they are potentially contaminants. Four additional Neandertal specimens tested positive, but Neandertal sequences could not be detected in five early modern human fossils with biochemical preservation consistent with DNA survival from the Czech Republic and France. This appears to confirm that sequences characteristic to Neandertal remains were not widespread in early modern humans.

While this is an encouraging result, it depends on the human fossils actually containing any preserved ancient DNA at all. Serre *et al.* [10] used biochemical measures of protein diagenesis in the fossil human and Neandertal bones to indirectly support DNA survival, but this association is imprecise, and the threshold levels associated with DNA preservation have changed appreciably since first proposed [11]. Several of the human fossils are near the threshold levels, while others are left unexamined because they fall slightly outside, even though the first DNA-yielding Neandertal specimen also fails this test [12]. Furthermore, none of the Neandertal sequences were independently replicated, a key criterion for ancient DNA research because it is extremely liable to intra-laboratory contamination [13]. This is of particular importance because the very short Neandertal sequences obtained by Serre *et al.* [10] are all identical to others previously amplified in their laboratory.

Given an apparent lack of Neandertal mtDNA in the five early human fossils, Serre *et al.* [10] examined the maximum Neandertal genetic contribution to early modern human populations that can be excluded. Using coalescent theory, and assuming a constant effective population size of 10,000, the mtDNA of all modern humans can be traced back to just four to seven ancestral lineages around 20,000–30,000 years

Table 1. Standard criteria for the authentication of ancient DNA results (from [13]).

Criteria for ancient DNA authentication	Serre *et al.* [10]	Caramelli *et al.* [8]
1. Complete physical isolation of work area	•	•
2. Multiple control reactions	•	•
3. Appropriate molecular behaviour	•	•
4. Reproducibility within laboratory	•	•
5. Cloning of PCR products	•	•
6. Replication in independent laboratory	x	•
7. Biochemical preservation of specimen	•	•
8. Quantification of DNA template	x	•
9. Examine DNA in animal bones at sites	•	•

Even when all requirements are met it does not guarantee authenticity. For example, the Caramelli *et al.* study [8] included independent replication, but the fossil human sequences were identical to modern humans and may reflect sample contamination [9,10].

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Observed lineages at time of mixing	Maximum Neandertal contribution	
	One locus	Two loci
4	53%	31%
5	45%	26%
6	39%	22%
7	35%	19%
8	31%	17%
9	28%	15%
10	26%	14%
25	11%	6%
50	6%	3%
100	3%	1%

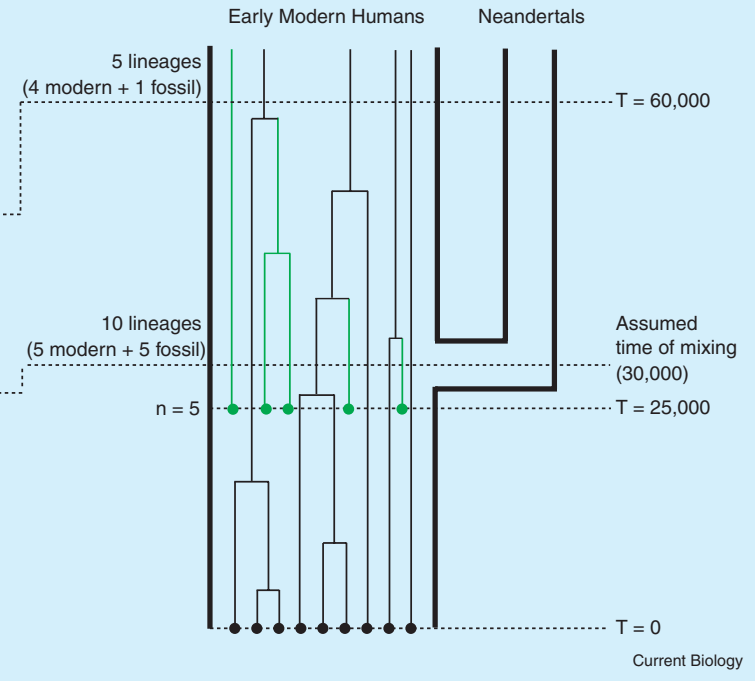

Figure 1. An example genealogy and table of exclusion probabilities.
Coalescent theory indicates that all mtDNA lineages from modern humans (black) can be traced back to four to seven ancestral sequences around 20,000–30,000 years ago. In contrast, the five inferred fossil human sequences (green) are likely to still be distinct lineages 5,000 years earlier, at the assumed time of mixing. The maximum Neandertal genetic contribution (%) that can be excluded is given for differing numbers of non-Neandertal lineages (both fossil and inferred modern sequences) observed at the time of mixing (from [10]).

ago. As the five early modern human fossils must also have contained mtDNA lineages, and these are unlikely to exactly match the ancestral lineages, around 10 non-Neandertal mtDNA lineages can now be inferred for the early modern human population around 25,000 years ago (if the human fossils are also assumed to be around that age). Under these circumstances, and if the mixing of Neandertal and early modern humans occurred slightly earlier (around 30,000 years ago), then a contribution larger than 26% can be excluded at the 5% level (Figure 1).

As Serre *et al.* [10] note, however, the results are dependent on a number of assumptions. For example, if some of the human fossils did not actually contain any ancient DNA, the loss of a data point reduces the rejection power of the analysis (Figure 1). Furthermore, the proximity of the early modern human sequences to the mixing event has a large effect on their resolving power. Under the above population parameters, all five early fossil human sequences might well survive as distinct lineages 5,000 years earlier, but far fewer would be expected by, say 60,000 years ago, most having coalesced with ancestors of modern humans (Figure 1). Therefore, if the mixing event was earlier or the early human fossils younger, the maximum reasonable Neandertal contribution could be substantially larger. Interestingly, coalescent theory indicates that certain levels of Neandertal genetic contribution can be rejected from modern human sequences alone [14]. In fact, the five modern lineages inferred at 30,000 years ago allow a Neandertal contribution of more than 45% to be rejected (Figure 1).

While a convincing template for future work, the Serre *et al.* [10] study demonstrates the limited power of coalescent models when a few sample points from a single genetic marker are used. We should also remember that a Neandertal male contribution to early modern humans would not be recorded in the maternally inherited mtDNA sequences. But it is perhaps too soon to conclude that definitive knowledge of the Neandertal contribution to the modern human gene pool will not be possible [10]. Future methodological developments are likely to allow nuclear loci to be retrieved from Neandertal fossils, and if non-human sequences are obtained from an independent locus the exclusion probabilities would multiply, substantially increasing the power (Figure 1). In the short term, recent advances in sequencing fossil proteins [15] and DNA retrieval from ancient sediments [16] provide a means to access much larger numbers of early modern human and Neandertal molecular sequences through time and space. Together these new avenues promise to reveal whether Neandertals were indeed the original ‘bit of rough’.

References

1. Stringer, C.B., and Andrews, P. (1998). Genetic and fossil evidence for the origin of modern humans. Science *239*, 1263–1268.
2. Hawks, J.D., and Wolpoff, M.H. (2001). The accretion model of Neandertal evolution. Evol. Int. J. Org. Evol. *55*, 1474–1485.
3. Templeton, A. (2002). Out of Africa again and again. Nature *416*, 45–51.
4. Schmitz, R.W., Serre, D., Bonani, G., Feine, S., Hillgruber, F., Krainitzki, H., Pääbo, S., and Smith, F.H. (2002). The Neandertal type site revisited: interdisciplinary investigations of skeletal remains from the Neander Valley, Germany. Proc. Natl. Acad. Sci. USA *99*, 13342–13347.

5. Pääbo, S., Higuchi, R.G., and Wilson, A.C. (1989). Ancient DNA and the polymerase chain reaction. J. Biol. Chem. *264*, 9709–9712.
6. Gilbert, M.T.P., Willerslev, E., Hansen, A.J., Barnes, I., Rudbeck, L., Lynnerup, N., and Cooper, A. (2003). Characterisation of genetic miscoding lesions caused by post-mortem damage. Am. J. Hum. Genet. *72*, 48–61.
7. Cooper, A. (1997). Ancient DNA - how do you really know when you have it? Am. J. Hum. Genet. *60*, 1001–1002.
8. Caramelli, D., Lalueza–Fox, C., Vernesi, C., Lari, M., Casoli, A., Mallegni, F., Chiarelli, B., Dupanloup, I., Bertranpetit, J., Barbujani, G., et al. (2003). Evidence for a genetic discontinuity between Neandertals and 24,000–year–old anatomically modern Europeans. Proc. Natl. Acad. Sci. USA *100*, 6593–6597.
9. Abbott, A. (2003). Anthropologists cast doubt on human DNA evidence. Nature *423*, 468.
10. Serre, D., Langaney, A., Chech, M., Teschler-Nicola, M., Paunovic, M., Mennecier, P., Hofreiter, M., Possnert, G., and Pääbo, S. (2004). No evidence of Neandertal mtDNA contribution to early modern humans. PLOS Biol. *2*, 313–317.
11. Poinar, H.N., Höss, M., Bada, J.L., and Pääbo, S. (1996). Amino acid racemization and the preservation of ancient DNA. Science *272*, 864–866.
12. Krings, M., Stone, A., Schmitz, R.W., Krainitzki, H., Stoneking, M., and Pääbo, S. (1997). Neandertal DNA sequences and the origin of modern humans. Cell *90*, 19–30.
13. Cooper, A., and Poinar, H.N. (2001). Ancient DNA: do it right or not at all. Science *18*, 1139.
14. Nordborg, M. (1998). On the probability of Neandertal ancestry. Am. J. Hum. Genet. *63*, 1237–1240.
15. Nielsen-Marsh, C.M., Ostrom, P.H., Gandhi, H., Shapiro, B., Cooper, A., Hauschka, P.V., and Collins, M.J. (2002). Sequence preservation of osteocalcin protein and mitochondrial DNA in bison bones older than 55 ka. Geology *30*, 1099–1102.
16. Willerslev, E., Hansen, A.J., Brand, T.B., Binladen, J., Gilbert, M.T.P., Shapiro, B., Bunce, M., Wiuf, C., Gilichinsky, D.A., and Cooper, A. (2003). Diverse plant and animal DNA from Holocene and Pleistocene sedimentary records. Science *300*, 792–795.
17. Tavare, S. (1984). Line-of-descent and genealogical processes, and their applications in population genetics models. Theor. Popul. Biol. *26*, 119–264.