11. The fate of European Neanderthals: results and perspectives from ancient DNA analyses

D. SERRE
Max-Planck-Institute for Evolutionary Anthropology,
Leipzig D-04103,
Germany

Current address:
Genome Quebec Innovation Center and McGill University,
Montreal,
Canada
david.serre@mail.mcgill.ca

S. PÄÄBO
Max-Planck-Institut für Evolutionäre Anthropologie,
Leipzig D04103,
Germany
paabo@eva.mpg.de

Keywords: mtDNA, genetic diversity, population history, bottleneck, gene flow, coalescence, glaciation

Abstract

Analyses of mitochondrial DNA sequences extracted from several Neanderthal remains have provided new information on their genetic relationship with modern human individuals. However, these results have been interpreted very differently among anthropologists. Here we review these results and present additional data directly addressing the question of genetic continuity among human populations during the Late Pleistocene. An analysis of additional Neanderthal and early modern human remains from Western and Central Europe do not provide any evidence of gene flow between the two groups. We also show that under reasonable assumptions of human demography, these data rule out a major genetic contribution by Neanderthals to the modern human gene pool. Finally, we present preliminary results showing that ancient DNA studies can also contribute to unraveling aspects of Neanderthal demography. Promising avenues of research, such as the investigation of Neanderthal population genetic diversity and organization, as well as analyses of mammal populations contemporary with Neanderthals, could allow us to better understand the dynamics, and perhaps causes, of the demographic changes that occurred in Eurasia during the Late Pleistocene.

Introduction

Most researchers agree that the first hominids evolved in Africa (e.g., Campbell, 1988; Klein, 1989; Lewin, 1999) and that *Homo erectus* left Africa around two million years ago to colonize Europe and Asia as far as Indonesia (e.g., Wolpoff and Caspari, 1997; Gabunia et al., 2000; Oms et al., 2000; Wood and Richmond, 2000; Roebroeks, 2001; Balter and

K. Harvati and T. Harrison (eds.), Neanderthals Revisited: New Approaches and Perspectives, 211–220.

Gibbons, 2002; Vekua et al., 2002). However, the fate of archaic human populations that evolved regionally from this ancestral stock is much debated, especially with regards to a second wave of colonization from Africa around 100 ka. Most notably, attention has been focused on the fate of the Neanderthals,[1] the archaic humans that inhabited Europe and Western Asia during the later part of the Pleistocene (i.e., between 300 ka and 30 ka). Recent ^{14}C-dating confirms that the last Neanderthals could have co-existed with the first modern humans in Europe (Bocquet-Appel and Demars, 2000). However, it is still unclear whether this possible cohabitation influenced the gene pool of the newcomers or if, on the contrary, the Neanderthals went extinct without contributing to the gene pool of early modern humans in Europe.

In 1997, Krings extracted DNA from a humerus of the Neanderthal holotype (Krings et al., 1997). The 379 base pairs (bp) amplified from the hypervariable region of the mitochondrial (mt) genome were different from all modern human DNA sequences. Furthermore, this DNA sequence was too different from the current human sequences observed in the gene pool to be likely to be found in an individual that has not been analyzed yet. Tree reconstructions confirmed these analyses: while all human mtDNA sequences group together with a recent common ancestor,[2] the sequence retrieved from the type specimen of Neanderthal shows a much deeper separation with strong statistical support. This result is often interpreted as compelling evidence for the absence of interbreeding between Neanderthals and modern humans, or even as proof that Neanderthals and modern humans were two different species (e.g., Lindahl, 2000). However, even after the publication of two additional mtDNA sequences, very similar to that of the first individual (Krings et al., 2000; Ovchinnikov et al., 2000), many scenarios are still consistent with the data.

Two problems limit the range of the conclusions drawn from these studies: first, due to the impossibility of differentiating modern contamination from endogenous DNA sequences, a sample from a Neanderthal individual carrying a sequence similar to that of a current human could be discarded as putative contamination (Nordborg, 1998; Trinkaus, 2001). Second, the absence of early modern human DNA sequences leaves a long time span during which simple demographic processes can lead to the loss of Neanderthal sequences even with a substantial amount of admixture in the past (e.g., Relethford, 1998, 1999, 2001). Thus, the Neanderthal mtDNA could have been swamped by a continuous influx of modern human mtDNA into the Neanderthal gene pool (Enflo et al., 2001), lost by genetic drift (Nordborg, 1998), or by a population replacement much later than the Paleolithic transition, for example during the Neolithic expansion (e.g., Cavalli-Sforza et al., 1993). Here we summarize results that overcome these problems. The paper addresses the question of continuity or replacement between Neanderthals and early modern humans, as well as, more generally, aspects of what happened to the human populations during the transition from the Middle to Upper Paleolithic in Europe.

Looking for Gene Flow Between Neanderthals and Early Modern Humans

Contamination is the major problem of ancient DNA studies dealing with human remains because it is currently impossible to differentiate endogenous DNA sequences from modern contaminants present on the bones and those potentially left by excavators, curators and scientists that handled the bones. It has been shown that most ancient animal remains yield human DNA sequences when sensitive enough amplifications are used (Hofreiter et al., 2001; Wandeler et al., 2003).

This hampers the range of conclusions that can be drawn from studies of Neanderthal mtDNA, since (1) possible evidence of gene flow from modern humans to Neanderthals, such as a Neanderthal specimen yielding only a modern mtDNA sequence, could be considered a contamination artifact and therefore discarded, and (2) there are no conclusive mtDNA sequences from early modern humans that can be compared with Neanderthals. These DNA sequences would be especially informative due to their closer proximity in time to that of Neanderthals than current genetic diversity (Relethford, 1998, 1999, 2001).

However, one can investigate the genetic relationship between Neanderthals and early modern humans by making use of the fact that the Neanderthal mtDNA sequences retrieved so far are distinguishable from all current mtDNA sequences found in the human population. Thus, one way to look for gene flow between Neanderthals and early modern humans is to ask two questions: (1) Do all Neanderthal remains yield a "Neanderthal-like" mtDNA sequence? (2) Do any early modern human remains yield a "Neanderthal-like" mtDNA sequence?

As this approach relies on the presence/absence of a Neanderthal mtDNA sequence it requires some independent criteria to validate that any non-retrieval of Neanderthal mtDNA is effectively due to its absence and not to a lack of preservation of the biomolecules. We used animal remains, for which contamination is easily differentiable from endogenous DNA, to determine which state of biomolecular preservation is correlated with successful retrieval of endogenous DNA. We looked at the preservation of amino acids, the building blocks of the proteins that represent the major biomolecular component of the bone. Analyses of numerous faunal remains showed that using three independent measurements of amino acids preservation (i.e., the total amount of molecules, the ratio of two amino acids, and the chemical preservation of a particular amino acid) we could define strict criteria by which endogenous DNA from animal remains could always be successfully retrieved and amplified (Serre et al., 2004). This method also offers the advantage of being quick and largely non-destructive (less than 10 mg of bone powder is required), thus allowing screening of a large collection of material from which one can later choose only the most promising ones. We screened more than 25 Neanderthal and 40 early modern human remains for amino acid preservation. Five Neanderthal bones and five early modern humans (Table 1) fulfilled our criteria of preservation and therefore must contain retrievable endogenous DNA sequences (Serre et al., 2004; Beauval et al., 2005).

We extracted DNA from each of the ten remains and amplified it under two different conditions:

1. an amplification of mtDNA was performed under conditions where modern human and Neanderthal, as well as chimpanzee and gorilla, DNA were successfully amplified. This amplification allowed a wide screening of possible molecules present in the bones. For example, if a bone contained an mtDNA sequence different both from Neanderthal and from modern human

Table 1. Specimen included in the gene flow study

Specimen
Neanderthal remains
Vindija 77 (Vi-77) (Croatia)
Vindija 80 (Vi-80) (Croatia)
Engis 2 (Belgium)
La-Chapelle-aux-Saints (France)
Les-Rochers-de-Villeneuve (RdV 1) (France)
Early modern human remains
Mladeč 25c (Czech Republic)
Mladeč 2 (Czech Republic)
Cro-Magnon (France)
Abri Pataud (France)
La Madeleine (France)

sequences, this "unspecific" amplification could likely detect it.

2. a "Neanderthal-specific" amplification was performed. Under the conditions used, only mtDNA sequences similar to those retrieved from the previously analyzed Neanderthal remains could be amplified while the amplification did not work on modern human mtDNA sequences. This procedure allowed us to "fish out" a Neanderthal mtDNA sequence, even if it was in the presence of a much larger amount of contaminant sequences.

All remains (the five Neanderthals and the five early modern humans) analyzed yielded DNA sequences identical to contemporary human DNA sequences when amplified using the "unspecific" conditions. In 75% of the cases, more than one human mtDNA sequence was amplified from a single bone (Serre et al., 2004). This confirmed previous results that most ancient remains yield human DNA sequences when sensitive enough amplifications are used (Hofreiter et al., 2001; Wandeler et al., 2003). Additionally, all DNA sequences retrieved from the early modern human remains were identical to modern human mtDNA sequences present in DNA sequence database (http://www.ncbi.nlm.nih.gov/Genbank/). Due to ubiquitous contamination in four samples (i.e., those yielding each more than one sequence) and the fact that any DNA sequence amplified can potentially be a contaminant, it is impossible to identify the endogenous mtDNA sequence for any of the early modern human remains. In our view, this shows that it is currently impossible to trust the veracity of any ancient DNA sequence similar to the one found in the modern human gene pool.

By contrast, when the DNA amplification was performed under "Neanderthal-specific" conditions, none of the five early modern human remains yielded an amplification product. Interestingly, all five Neanderthal remains did yield an amplification product and, after sequencing, a short mtDNA sequence fragment was identified that was identical to the corresponding region of one of the four Neanderthals already sequenced (Krings et al., 1997, 2000; Ovchinnikov et al., 2000; Schmitz et al., 2002). Given that the overall state of preservation of the biomolecules is similar, this shows that the Neanderthals formed a homogenous genetic population different from that of early modern humans (Serre et al., 2004; Beauval et al., 2005). This result is supported by the mtDNA sequence of a fragment of 47bp recently retrieved from a Neanderthal from El Sidrón Cave, Spain, that is identical to the sequences from Vindija and Feldhofer 1 (Lalueza-Fox et al., 2005).

Thus, while we applied an unbiased methodology that can detect gene flow between populations, we did not find any evidence of gene flow in either direction. It is important to stress here that some of the samples analyzed in this study have been described as "transitional" between "classical" Neanderthals and early modern humans, such as the Vindija Neanderthals (Smith and Spencer, 1984; Wolpoff, 1999) and the Mladeč individuals (Frayer, 1992; Wolpoff, 1999), so they represent good candidates to reveal potential gene flow.

What is the maximum genetic contribution that might have occurred?

Our analysis of five Neanderthal remains and five early modern humans did not detect any evidence of gene flow. However, given the small sample size one might question the power of this study to detect genetic contribution. In other words, one might want to estimate the level of genetic contribution that can be statistically ruled out given the data. It is important to note that, while the former

results were obtained by straight-forward analyses of the data, estimation of the maximum genetic contribution relies on a theoretical model of what we think is a fair representation of human demographic history: what were the population sizes of Neandertals and early modern humans, when did they meet each other, how long did they interact for, when and how quickly did the modern human population expanded? All these parameters need to be estimated in the model. Therefore, one should keep in mind that any results obtained using this approach are dependant on the assumptions made.

We decided to use the simplest model possible (to account for the small data set we have) and to work under the assumption that the human population is panmictic (i.e., random mating) and of constant size through time. We estimated, using this model (Tavare, 1984), that the current mtDNA gene pool had only between four and seven ancestors at 20–30 ka. This shows the limitations of using only current diversity to obtain insights about the mtDNA gene pool in the late Pleistocene. In fact, the five early modern human individuals analyzed here provide almost as much information about the mtDNA gene pool of modern humans in the late Pleistocene as would the sequencing of mtDNA sequence from all now-living humans. They also add information that could not be obtained by studying additional now-living individuals. The mtDNA ancestry of current humans is already intensively explored with respect to deep divergences, so that additional major lineages are unlikely to be discovered (Sykes, 2001). Given that all Neanderthal bones analyzed yield mtDNA sequences that are similar to each other and absent in the five early modern humans analyzed, as well as in all modern humans, we can exclude (at 95% confidence) any Neanderthal contribution to the modern human gene pool greater than 25% (Serre et al., 2004). This might seem a rather uninformative result, but it is in fact a major improvement. When Neanderthal mtDNA sequences are considered alone, only a scenario of random-mating population comprising both Neanderthals and modern humans can be excluded (Nordborg, 1998). Thus, even using a conservative model of population history we can exclude a large Neanderthal contribution to the modern human gene pool.

If we consider a more realistic scenario where the spread of modern humans (before and during their migration out of Africa and subsequent colonization of western Eurasia) was accompanied by a population growth, we can exclude a smaller Neanderthal contribution. However, the importance of the contribution that can be excluded depends critically on when and how the expansion occurred. For example, Currat and Excoffier (2004) recently estimated that under a much more complex scenario, in which an expanding modern human population spread progressively in Europe and competed with the less numerous Neanderthals, the maximal genetic contribution compatible with the data is smaller than 0.1%.

Can Ancient DNA Studies Tell us What Happened to the Neanderthals During the Middle to Upper Paleolithic Transition?

The genetic data collected so far support a scenario of no major interbreeding between the two human populations in the Late Pleistocene. Leaving aside discussions of species/sub-species status and interbreeding capacity/incapacity, we can still try to understand why Neanderthals disappeared during the transition from Middle to Upper Paleolithic. Two avenues of research are promising for this purpose: (1) the analyses of genetic diversity within Neanderthals that can lead to a greater understanding of their demographic history; and (2) the investigations of potential demographic changes in animal populations contemporary with the Neanderthals to obtain a more global understanding of the environment and its influences.

By comparing the Neanderthal mtDNA sequences of the four individuals with the most complete genetic information, we find that the Neanderthals carry a genetic diversity for the mtDNA similar to that of the current human population and approximately 5 times smaller than that of the African great apes (Krings et al., 2000; Schmitz et al., 2002). We have shown that this low diversity within Neanderthals is not an artifact, since all well-enough preserved remains yield very similar sequences (Serre et al., 2004). One commonly proposed explanation for the reduced genetic diversity in humans relative to our closest living relatives is that gorillas and chimpanzees have always lived in the African rainforest, which was not drastically modified by climatic changes (e.g., Lahr and Foley, 1998). The African great apes may, therefore, have maintained a stable population over a long period of time and accumulated a large genetic diversity. In contrast, human populations expanding in open environments were more exposed to climatic fluctuations and likely underwent a series of drastic reductions in population size followed by expansions (e.g., Takahata, 1994; Lahr and Foley, 1998; Reich and Goldstein, 1998; Zietkiewicz et al., 1998; Adams et al., 2000). The preliminary data concerning the Neanderthal population show the same general trend, and suggest a rather unstable population history. Additionally, it is interesting to note that the mtDNA sequence retrieved from the second individual of Feldhofer, Germany (Schmitz et al., 2002) carries three differences from the type specimen mtDNA sequence (Krings et al., 1997) while carrying only one difference from the Croatian Neanderthal mtDNA sequence (Krings et al., 2000). This suggests that no strong geographical clustering of mtDNA sequences was present in Neanderthals, at least in western and central Europe. It is clear that more individuals are needed in order to arrive at more definitive conclusions about the geographic organization of the Neanderthal mtDNA gene pool, but it is interesting that this preliminary observation contrasts with the picture given by some paleoanthropologists who present Neanderthals as having strong cultural or behavioral differences correlated with their geographical origins (e.g., Bahn, 1998; d'Errico et al., 1998; Stringer et al., 2000). In this context, one can note that all Neanderthal DNA analyzed so far dates from the early to middle part (~59–35 ka) of the MIS 3 interstadial. An interesting working hypothesis would be that the Neanderthals of the Saalian glaciation (MIS 6, ~195–128 ka) consisted of a metapopulation with strong phylogeographical structure, and that the MIS 3 Neanderthal population is the result of post-glacial expansion of only one, or a few, surviving local population(s).

Another promising approach to better understand the history of Neanderthal and early modern human populations is to analyze faunal remains contemporary with these populations. Ancient DNA analyses of animal remains are far easier and more efficient than those of human remains because: (1) many more samples are available for analyses; and (2) contamination is not an issue.[3] In a recent pilot study we analyzed remains from cave bears, cave hyenas, and brown bears across Europe, all dated to ~70–30 ka (Hofreiter et al., 2004). In none of these data sets were we able to detect a correlation between the mtDNA sequence carried by an individual and its geographical origin (sometimes this is referred to as phylogeographic structure). This finding is striking when compared to current genetic diversity data: most species living today in Europe show a strong correlation with the mtDNA gene pool organized in two or three clades found almost exclusively in Western Europe, Eastern Europe or Southern Europe (e.g., Taberlet et al., 1998; Avise, 2000; Hewitt, 2000). This organization of current genetic diversity is believed to be the result of glacial periods when many species survived only in a few ice-free refugia (the Iberian Peninsula, the Balkans, and Italy) and

spread from there across Europe at the end of the glaciation. Interestingly, while the time of the setting of this phylogeographic structure is believed to date to early in the Pleistocene (e.g., Hewitt, 2000), we find no evidence of such organization in the three species we looked at. We concluded that the setting of this phylogeographic structure possibly occurred just a couple of tens of thousands of years ago (Hofreiter et al., 2004). It will be interesting to see if this result holds when more species contemporary with the Neanderthals are analyzed. This preliminary result might indicate that many species underwent major demographic rearrangements around the time that Neanderthals became extinct. This observation is of particular interest as any event that affected the environment so drastically must have affected the human populations as well, if not directly, at least through the changes of the dietary resource availability. An understanding of the dynamics of animal populations in the Pleistocene might therefore lead to major breakthroughs in our understanding of Neanderthal extinction.

Conclusion

We have shown here that genetic analyses of Neanderthal and early modern human remains can provide information about the relationship and dynamics of these two populations. Neanderthals, at least those living in the last interglacial period, constitute a homogenous genetic population different from the early modern humans that followed them in Europe. Recent analyses of Paleolithic human remains found no evidence of gene flow between the two populations in either direction, and we can show that, if any, the genetic contribution from the Neanderthals to the modern human gene pool must have been limited. We are also beginning to obtain some information concerning the demography of the Neanderthals. Their low genetic diversity relative to that of the African great apes, and similar to that of current humans, suggests major demographic changes during the Late Pleistocene. The geographic homogeneity of the gene pool of the Neanderthals investigated so far, strikingly contrasts with their apparent cultural diversity and requires further investigation. Preliminary analyses of faunal remains contemporary with the Neanderthal suggest that major demographic changes occurred in Europe around the time when Neanderthals became extinct. Further investigations in this direction might lead to a better understanding of the context in which this disappearance occurred and perhaps to its causes. Analyses of the DNA molecules preserved in Pleistocene human bones are tedious and, unfortunately, still require the destruction of a small amount of material. Nonetheless, these analyses provide information that cannot be obtained by looking at the current genetic diversity or through morphological/archeological studies. Eight years after the publication of the first Neanderthal mtDNA sequence we have shifted the research focus towards understanding of the Neanderthal population history, and we are only beginning to reveal this fascinating period of human evolution. The conclusions are still limited, but future analyses of additional individuals will allow us to verify (or contradict) our preliminary results and offer an exciting challenge for the coming years.

Notes

1. Throughout this paper we use the term "human population" to describe both Neanderthals and early modern humans. All the results presented here deal with the population history of "modern humans" and "Neanderthals" and can be explained by demographic processes that do not necessitate reproductive isolation or any other biological criterion that can be used to define species.
2. The concept of genetic ancestry, as used throughout this paper, is not identical to the popular meaning of ancestry. In its most common meaning, the ancestors of a particular individual are his/her parents, the

parents of this individual's parents and so on. As a consequence, the number of ancestors increases continuously when one looks back in time, at least during the first generations. In contrast, if one considers a short fragment of a DNA molecule in an individual, it is inherited from only one of her/his parents, who has also inherited it from only one parent. Therefore, the number of genetic ancestors does not increase with the number of generations. Additionally, as one looks back in time, two now-living individuals will have inherited the fragment of DNA considered from a common ancestor in the *n*th generation. Working from this definition of genetic ancestry, only this last individual will be a genetic ancestor of the two now-living individuals in the *n*th generation, while all other individuals will not be (despite the fact that they are all ancestors per the popular meaning). Thus, the number of genetic ancestors decreases when one looks back in time as more and more individuals have common ancestors until, eventually, a single most recent common ancestor (MRCA) remains. It is worth noting here that this MRCA (sometimes referred to as "eve" for the mitochondrial DNA) is not an isolated individual, but the particular member of a large population that carries the fragment of DNA present in all now-living individuals (who can harbor different DNA sequences due to the accumulation of mutations).

3. It is trivial to differentiate a human DNA sequence from that of non-human animal and, additionally, animal DNA contamination is unlikely if standard laboratory procedures are followed.

References

Adams, E.J., Cooper, S., Thomson, G., Parham, P., 2000. Common chimpanzees have greater diversity than humans at two of the three highly polymorphic MHC class I genes. *Immunogenetics* 51, 410–424.

Avise, J.C., 2000. *Phylogeography*. Harvard University Press, Cambridge, MA.

Bahn, P.G., 1998. Neanderthals emancipated. *Nature* 394, 719–721.

Balter, M., Gibbons, A., 2002. Were "little people" the first to venture out of Africa? *Science* 297, 26–27.

Beauval, C., Maureille, B., Lacrampe-Cuyaubere, F., Serre, D., Peressinotto, D., Bordes, J.G., Cochard, D., Couchoud, I., Dubrasquet, D., Laroulandie, V., Lenoble, A., Mallye, J.B., Pasty, S., Primault, J., Rohland, N., Pääbo, S., Trinkaus, E., 2005. A late Neandertal femur from Les Rochers-de-Villeneuve, France. *Proc. Natl. Acad. Sci. U.S.A*. 102, 7085–7090.

Bocquet-Appel, J.-P., Demars, P.Y., 2000. Neanderthal contraction and modern human colonization of Europe. *Antiquity 74*, 544–552.

Campbell, B.C., 1988. *Humankind Emerging*. Scott, Foresman and Company, Glenview, Boston, London.

Cavalli-Sforza, L.L., Menozzi, P., Piazza, A., 1993. Demic expansions and human evolution. *Science* 259, 639–646.

Currat, M., Excoffier, L., 2004. Modern humans did not admix with Neanderthals during their range expansion into Europe. *PLoS Biol*. 2, 2264–2274.

d'Errico, F., Zilhão, J., Julien, M., Baffier, D., Pelegrn, J., 1998. Neanderthal acculturation in Western Europe? A critical review of the evidence and its interpretation. *Curr. Anthropol*. 39, S1–S44.

Enflo, P., Hawkes, K., Wolpoff, M., 2001. A simple reason why Neanderthal ancestry can be consistent with current DNA information. *Am. J. Phys. Anthropol*. 114, 62.

Frayer, D.W., 1992. Evolution at the European edge: Neanderthal and Upper Paleolithic relationships. *Prehist. Europeenne* 2, 9–69.

Gabunia, L., Vekua, A., Lordkipanidze, D., Swisher, C.C. III, Ferring, R., Justus, A., Nioradze, M., Tvalchrelidze, M., Antón, S.C., Bosinski, G., Joris, O., Lumley, M.A., Majsuradze, G., Mouskhelishvili, A., 2000. Earliest Pleistocene hominid cranial remains from Dmanisi, Republic of Georgia: taxonomy, geological setting, and age. *Science* 288, 1019–1025.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. *Nature* 405, 907–913.

Hofreiter, M., Serre, D., Poinar, H.N., Kuch, M., Pääbo, S., 2001. Ancient DNA. *Nat. Rev. Gene*. 2, 353–359.

Hofreiter, M., Serre, D., Rohland, N., Rabeder, G., Nagel , D., Conard, N., Munzel, S., Pääbo, S., 2004. Lack of phylogeography in European mammals before the last glaciation. *Proc. Natl. Acad. Sci. U.S.A*. 101, 12963–12968.

Klein, R.G., 1989. *The Human Career: Human Biological and Cultural Origins*. University of Chicago Press, Chicago.

Krings, M., Capelli, C., Tschentscher, F., Geisert, H., Meyer, S., von Haeseler, A., Grossschmidt, K., Possnert, G., Paunovic, M., Pääbo, S., 2000. A view of Neandertal genetic diversity. *Nat. Genet*. 26, 144–146.

Krings, M., Stone, A., Schmitz, R.W., Krainitzki, H., Stoneking, M., Pääbo, S., 1997. Neandertal

DNA sequences and the origin of modern humans. *Cell* 90, 19–30.

Lahr, M.M., Foley, R.A., 1998. Towards a theory of modern human origins: geography, demography, and diversity in recent human evolution. *Am. J. Phys. Anthropol*. Suppl. 27, 137–176

Lalueza-Fox, C., Sampietro, M.L., Caramelli, D., Puder, Y., Lari, M., Calafell, F., Martínez-Maza, C., Bastir, M., Fortea, J., Rasilla, M.D., Bertranpetit, J., Rosas, A., 2005. Neandertal evolutionary genetics: mitochondrial DNA data from the Iberian Peninsula. *Mol. Biol. Evol*. 22, 1077–1081.

Lewin, R., 1999. *Human Evolution: An Illustrated Introduction*. Blackwell Science, London.

Lindahl, T. 2000. Fossil DNA. *Curr. Biol*. 10, R616.

Nordborg, M., 1998. On the probability of Neanderthal ancestry. *Am. J. Hum. Genet*. 63, 1237–1240.

Oms, O., Pares, J.M., Martínez-Navarro, B., Agusti, J., Toro, I., Martinez-Fernandez, G., Turq, A., 2000. Early human occupation of Western Europe: paleomagnetic dates for two Paleolithic sites in Spain. *Proc. Natl. Acad. Sci. U.S.A*. 97, 10666–10670.

Ovchinnikov, I.V., Gotherstrom, A., Romanova, G.P., Kharitonov, V.M., Liden, K., Goodwin, W., 2000. Molecular analysis of Neanderthal DNA from the northern Caucasus. *Nature* 404, 490–493

Reich, D.E., Goldstein, D.B., 1998. Genetic evidence for a Paleolithic human population expansion in Africa. *Proc. Natl. Acad. Sci. U.S.A*. 95, 8119–8123.

Relethford, J.H., 1998. Genetics of modern human origins and diversity. *Ann. Rev. Anthropol*. 27, 1–23.

Relethford, J.H., 1999. Models, predictions, and the fossil record of modern human origins. *Evol. Anthropol*. 8, 7–10.

Relethford, J.H., 2001. Absence of regional affinities of Neandertal DNA with living humans does not reject multiregional evolution. *Am. J. Phys. Anthropol*. 115, 95–98.

Roebroeks, W., 2001. Hominid behaviour and the earliest occupation of Europe: an exploration. *J. Hum. Evol*. 41, 437–461.

Schmitz, R.W., Serre, D., Bonani, G., Feine, S., Hillgruber, F., Krainitzki, H., Pääbo, S., Smith, F.H., 2002. The Neandertal type site revisited: interdisciplinary investigations of skeletal remains from the Neander Valley, Germany. *Proc. Natl. Acad. Sci. U.S.A*. 99, 13342–13347

Serre, D., Langaney, A., Chech, M., Teschler-Nicola, M., Paunovic, M., Mennecier, P., Hofreiter, M., Possnert, G.G., Pääbo, S., 2004. No evidence of Neandertal mtDNA contribution to early modern humans. *PLoS Biol.2*, 313–317.

Smith, F.H., Spencer, F. (Eds.), 1984. *The Origins of Modern Humans: A World Survey of the Fossil Evidence*. Alan R. Liss, New York.

Stringer, C., Barton, N.H., Finlayson, C. (Eds.), 2000. *Neanderthals on the Edge*. Oxbow Books, Oxford.

Sykes, B., 2001. *The Seven Daughters of Eve*. W. W. Norton, New York.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.G., Cosson, J.F., 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Mol. Ecol*. 7, 453–464.

Takahata, N., 1994. Repeated failures that led to the eventual success in human evolution. *Mol. Biol. Evol*. 11, 803–805.

Tavare, S., 1984. Line-of-descent and genealogical processes, and their applications in population genetics models. *Theor. Popul. Biol*. 26, 119–164.

Trinkaus, E., 2001. The Neandertal paradox. In: Finlayson, C. (Ed.), *Neanderthals and Modern Humans in Late Pleistocene Eurasia*. The Gibraltar Museum, Gibraltar.

Vekua, A., Lordkipanidze, D., Rightmire, G.P., Agusti, J., Ferring, R., Maisuradze, G., Mouskhelishvili, A., Nioradze, M., De León, M.P., Tappen, M., Tvalchrelidze, M., Zollikofer, C., 2002. A new skull of early *Homo* from Dmanisi, Georgia. *Science* 297, 85–89.

Wandeler, P., Smith, S., Morin, P.A., Pettifor, R.A., Funk, S.M., 2003. Patterns of nuclear DNA degeneration over time – a case study in historic teeth samples. *Mol. Ecol*. 12, 1087–1093.

Wolpoff, M., Caspari, R., 1997. *Race and Human Evolution*. Simon & Schuster, New York.

Wolpoff, M.H., 1999. The systematics of *Homo. Science* 284, 1774–1775.

Wood, B., Richmond, B.G., 2000. Human evolution: taxonomy and paleobiology. *J. Anat*. 197 (Pt 1), 19–60.

Zietkiewicz, E., Yotova, V., Jarnik, M., Korab-Laskowska, M., Kidd, K.K., Modiano, D., Scozzari, R., Stoneking, M., Tishkoff, S., Batzer, M., Labuda, D., 1998. Genetic structure of the ancestral population of modern humans. *J. Mol. Evol*. 47, 146–155.