Génétique et Evolution des populations de Cro-Magnons

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Action " Origine de l'Homme, du Langage et des Langues "

A. FICHE ADMINISTRATIVE

Titre du projet :

Genetics and Evolution of Cro-Magnon populations
Génétique et Evolution des populations de Cro-Magnons

Mots-clés :

Cro-Magnons, ancient DNA, populations genetics

Résumé du projet (10 lignes maximum) :

La culture et le comportement particulier des hommes de Cro-Magnon seraient le reflait de sa capacité à avoir un langage developpé ayant facilité des échanges culturels et génétiques entre les populations. Or, la récupération et l'analyse d'ADN provenant de specimens fossiles permet d'examiner les changements génétiques en temps réel. Le but de ce projet consiste donc à analyser l' ADN extrait de specimens Cro-Magnons venant de différentes régions géographiques, afin de déterminer la diversité génétique dans et entre les populations. Un travail nécessaire et capital concernant les datations et les milieux de preservation des fossiles devra tout d'abord être engagé pour permettre l'analyse moléculaire dans de bonnes conditions.

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B. PROJET SCIENTIFIQUE

Background

The recovery of preserved genetic information from ancient specimens, or ancient DNA, allows genetic change to be examined in real time, providing an opportunity to catch evolution red-handed. Ancient DNA allows, for the first time, genetic events in the past to be recorded, rather than extrapolated from modern populations. While offering many advantages, rhis form of research is not straightforward, as the degradation of DNA over time means only the past 100,000 years or so are accessible, and the trace amounts of DNA preserved in old specimens are very susceptible to contamination. However, these technical difficulties are worth facing as this time period contains many important evolutionary events, particularly in the evolution of humans. In Europe, ancient DNA offers the only means to fully determine what happened when the ancestors of modern Europeans, Cro-Magnons, met and interacted with the existing human inhabitants of Europe, the Neandertals. In addition, the large number of Cro-Magnon specimens provide a unique opportunity to examine genetic diversity in an ancient population of humans.

Human evolution in Europe

The sequencing of Neandertal DNA (Krings et al. 1997, Ward and Stringer 1997, Ovchinnikov et al. 2000) is one of the most important ancient DNA triumphs. The Neandertal mitochondrial DNA (mtDNA) sequences were clearly divergent from the variation observed within modern humans, suggesting that little or no genetic mixing had occurred between the two groups. This was a very important finding, revealing much about how *Homo sapiens* had appeared in Europe, and around the world. However, the conclusions of the Neandertal study were qualified because there are no genetic sequences from Cro-Magnons for comparison, and consequently Neandertal DNA sequences of 30-50,000 years ago were contrasted with DNA from modern human

populations. Obviously, it would be much more valid to compare contemporaneous Neandertal and Cro-Magnon DNA sequences, as it likely that many factors have changed since that time and the modern day. For example, it is possible that climate change during the Last Glacial Maximum (ca. 22-18 Kyr) seriously effected Cro-Magnon populations, decreasing their size and producing bottlenecks which may have removed many older European lineages, including some derived from Neandertals. If this was the case, the genetic diversity of modern Europeans would only have been formed in the Late Pleistocene. Consequently, it is very important to compare DNA sequences from Cro-Magnon specimens dating to before, and after, the LGM. Such comparisons would establish the likelihood that any Neandertal genes existing in Cro-Magnon populations had been lost.

The sophisticated cultural artefacts and behavioural patterns of Cro-Magnon peoples are generally considered to reflect an increased capacity for sophisticated language, and to have facilitated greater cultural and genetic interchange between separate populations, relative to the Neandertals. In fact, differing language capabilities are commonly inferred to be a major component in the genetic barrier between the two groups. These concepts can be examined with DNA extracted from Cro-Magnon specimens from different geographic areas, to determine the diversity within and between populations, and provide estimates of the effective population size. By examining specimens of different ages, it will also be possible to analyse the basis of the large morphological diversity exhibited in Cro-Magnon populations over time, and the extent to which the LGM influenced the genetic make-up of modern Europeans. Some paleoclimatic models suggest northern Europe was completely depopulated during the LGM, and was then re-colonised from the south or east. This idea could be tested by comparing Palaeolithic sequences from north-south and east-west dimensions.

The sequence data will also be used to examine hypotheses about European linguistic history (Sykes 1999, Cavalli-Sforza and Minch 1997). Data from mtDNA of modern European populations has been used to suggest that there were several waves of diversification and colonisation (Sykes 1999). An initial Upper Paleolithic phase (around 50,000 years ago) was followed by a major period of colonisation and diversification in the Late Upper Paleolithic (about 14-11,000 years, after the LGM), followed by a final Neolithic phase (around 8500 years). These phases are characterised by certain mtDNA sequences, and consequently the Cro-Magnon sequences can be used to test this hypothesis.

Identification of suitable specimens

The degradation of DNA over time is controlled by temperature, and the availability of oxygen and water (Lindahl 1993). These parameters can be modelled, and if the appropriate activation energy for DNA deterioration is used, it is possible to calculate a 'thermal age' for a preservation site (Collins *et al.* submitted). The thermal age indicates how long a specimen would have to be held at constant 10°C to produce similar amounts of damage, and can be used to predict where DNA preservation will be likely. An example is shown below based on a survey of Neandertal sites in Europe.

Thermal ages were calculated for a series of Holocene fossils and for Pleistocene sites for which we had reliable palaeoclimatic reconstructions (Fig. 1). When aDNA retrieval is compared to thermal age, no site older than the Neander Valley (Feldhofer) specimen (16.3 $kyr_{10°C}$) has been successful (Krings *et al*. 1997). The material from Mezmaiskaya (Ovchinnikov et al. 2000), although at a lower latitude than Feldhofer, has a younger thermal age, because of its altitude (1.3 km). Failure to amplify DNA from two sites less than 163$kyr_{10°C}$ demonstrates that thermal age is not the only factor that prevents aDNA amplification.

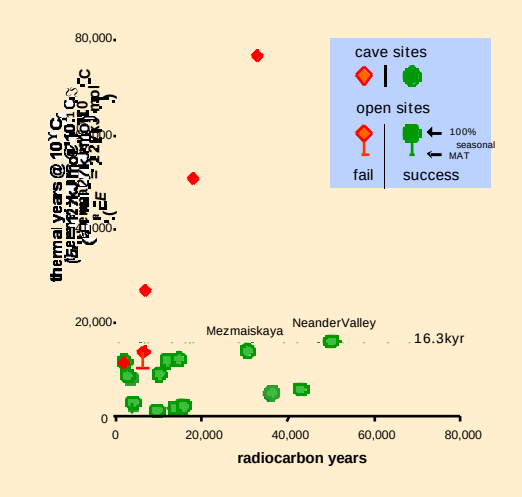

Figure 1. Success with PCR amplification is related to thermal age. When a comparison is made of between sites (A.C. unpublished data), Neanderthal 1 (Feldhofer) represents the current limit for successful PCR amplification. Thermal age of Mezmaiskaya is conservative as it assumes a constant temperature of 5.7°C due to lack of detailed palaeoclimatic information.

A similar example, examining collagen survival in bone is modelled below (activation energy E_a = 174 kJ mol^{-1}). Figure 2a shows that collagen yield is poorly predicted (most sites have less than the theoretical maximum) indicating that other parameters at the sites are less than optimal (eg microbial attack, mineral alteration). In contrast, Figure 2b shows that when the limit of survival is modelled, the thermal age is highly predictive.

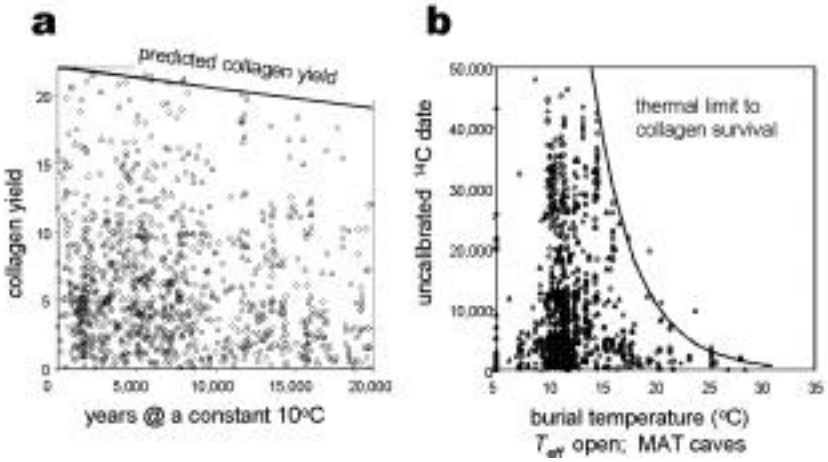

Fig 2a) The yield of collagen is typically lower than is predicted from the model, but b) the model does successfully identify the limit of survival, which decreases with increasing burial temperature. The thermal history of burials differ for bones from open (♉) and cave (♉) sites. The former uses a Mean Annual Temperature (MAT), and the latter an estimate of effective temperature based upon seasonal temperature fluctuations at 0.5 - 1 m depth.

We will identify Cro-Magnon sites likely to contain DNA using thermal age calculations, as well as data from an on-going EU funded project on Bone Deterioration. The EU project measures parameters characteristic of the diagenetic state of the organic and mineral components of bone. We will measure nitrogen content (%N), histological preservation, bone porosity, mineral alteration, crystallinity, and carbonate/phosphate ratios of bones from Cro-Magnon sites in order to identify favourable conditions for DNA preservation. We will attempt to confirm our predictions by amplifying DNA from associated faunal remains at the sites before trying to get DNA from Cro-Magnon material. In this way we can avoid unnecessary destructive sampling of these valuable specimens.

An important component of the analyses will be an accurate date estimate, which will be obtained using carbon isotope data at the Oxford Radiocarbon Unit. This data will be essential for

the analysis of changes in genetic diversity through time, as well as for the calculation of thermal ages. We will attempt to extract DNA from 10-20 specimens that have recently been carbon-dated by Dr Pettitt, and for which we are likely to gain permission to sample. Further samples will be identified in due course.

Pre-LGM	
Sunghir, Russia	3 individuals (include. double burial)
Brno II, Czech Republic	1 individual
Dolni Vestonice 1 & 2, Czech Republic	Several dozen
Pavlov, Czech Republic	Several dozen
Vogelherd (Stetten), Germany	3 individuals
Grimaldi caves, Italy	ca. 10 individuals
Kent's Cavern, England	1 individual
Goat's Hole, Paviland, Wales	1 individual
Numerous sites around France	About 1 dozen
Post-LGM	
Gough's Cave, England	4 individuals
Sun Hole, England	1 individual
Bruniquel, France	6 individuals
Oberkassel, Germany	2 individuals
Arene Candide, Italy	4 individuals

Ancient DNA techniques

Contamination prevention

Ancient DNA research is particularly difficult because the extremely low concentrations of surviving genetic material are very easily swamped by contamination from modern molecular work (Hänni et al. 1994, Cooper 1997, Cooper *et al.* 1997). This problem is compounded in the case of ancient human studies, where the contamination provided by archaeologists and museum curators handling the bones is likely to generate sequences closely related to the authentic DNA. Consequently, it is very important that ancient DNA research is performed to the utmost technical standards - such as complete physical isolation of the laboratory, independent replication of the results, and use of supporting biochemical and genetic data (Cooper 1997). Our project will make use of the new Ancient Biomolecules Centre (ABC), recently established in Oxford as the centre for ancient DNA research in the UK, and a world leading research facility. The ABC consists of two research facilities - an Ancient DNA Facility (ADF) located in the Oxford University Natural History Museum, and a Molecular Analysis Facility (MAF) situated in the physically remote Zoology building. The ADF is located in an area free of any modern molecular research, minimising the risk of contamination and permitting technically demanding research such as that involving ancient humans. In addition, to further confirm their authenticity, we will independently replicate successful results in the Ancient DNA Laboratory at the Université Claude Bernard. These laboratories feature physically isolation in areas where no molecular work is carried out, positive air pressure systems, and other high-tech anti-contamination measures. This generates a very high degree of isolation in an area with no background level of amplified DNA - and creates new levels of experimental opportunities for ancient DNA research.

Small samples (about 0.5g) from long bones or teeth will be obtained for analysis using a temporary sterile work site created at the museums housing the specimens, or if possible at the

ABC in Oxford. HPLC analysis of amino acid racemisation and concentration will be performed in the ABC, and isotopic analysis at the RLAHA. Other studies of bone chemistry (porosity, crystallinity, mineral alteration) will be carried out at Newcastle University.

Methodology

The potentially contaminated outer surface of hard samples will be removed using a shot-blaster in the positive air pressure laboratory in the ADF. DNA will be extracted using an organic solvent based approach (Cooper 1993, 1997), and stored at –20'C in the ADF, where PCR reactions will be prepared. PCR reactions will use wax barriers or immobilised polymerases to minimise non-specific extension of primers or template, and will use standard techniques for the amplification of ancient DNA (Cooper 1993, 1997, Leonard et al. 2000). The prepared PCR reactions will be transferred to the MAF, where the actual PCR reaction will be performed, to ensure the amplified DNA never enters the ADF. All work will be performed with stringent precautions to avoid contamination, such as multiple controls at each step, and regular PCR tests on swabs taken from laboratory surfaces (Pääbo 1989, Handt et al. 1994, Cooper 1997a, Krings et al. 1997, Cooper and Wayne 1998). Work areas and equipment will be UV-irradiated each night for several hours, and bleached or acid-washed before use. In addition to wearing disposable paper overalls and breathing masks, workers will not be permitted to enter the ADF if they have previously been in an area of molecular biological research on a given day to reduce the risk of transporting PCR products. PCR products will be visualised using gel electrophoresis, re-amplified, purified and sequenced (Cooper 1997) using dye-labelled terminator mixes and an ABI 377. Cloning experiments will be used on representative PCR products to examine the underlying sequence diversity - generally due to errors during amplification due to the damaged ancient DNA template (Krings et al. 1997). We will use PCR primers specific to rapidly evolving sequences of either humans or fauna that define a fragment of between 50 and 200 bp.

Analysis of the mitochondrial control region

The mtDNA control region will be our primary target because of the rapid evolutionary rate, large comparative database in modern humans, and existing Neandertal sequences. Most ancient DNA projects focus on mtDNA because it is commonly preserved in ancient samples in comparison with nuclear DNA (presumably due to the high copy number of mtDNA in living tissues). A further benefit is the sensitivity of mtDNA to fluctuations in population size due to the maternal mode of inheritance (Avise 1994). We will also use competitive PCR assays to determine the concentration of mtDNA in the ancient specimens as a further check of authenticity (Krings et al 1997). Animal bones associated with Cro-Magnons will be used as a control to examine the authenticity of the sequences, and detect contamination.

Sequence comparison

The resulting sequences will be compared to existing databases of human genetic variation, to examine the genetic continuity of European populations over the past 30,000 years, and to interpret past cultural and environmental events. Data from the different Cro-Magnon specimens will be used to examine the amount of genetic interchange between populations, and to estimate their effective population sizes. The data will be contrasted to the Neandertals, and used to create phylogenetic trees demonstrating the relationship between the two groups during the Upper Paleolithic. Changes in genetic patterns throughout this period will be related to the use of language and the appearance of other cultural behaviours such as art.

Responsibilities

The members of the group include experts in human evolution, radiocarbon dating, ancient DNA, and biomolecular preservation. Tasks will be divided amongst the group as follows;

Alan Cooper. Sample collection, DNA analysis at Oxford, sequence analysis
Paul Pettitt. Assistance with sample collection, AMS radiocarbon-dating, isotope studies.
Chris Stringer. Assistance with sample collection, anthropological data/interpretation.
Catherine Hänni. Assistance with sample collection, DNA analysis in Lyons.
Matthew Collins. Biochemical and physical analyses of samples. Calculation of thermal age of Cro-Magnon sites.
Ryk Ward. Sequence and population genetics analysis.

References:

Avise J (1994) Molecular markers, Natural History and Evolution. Chapman and Hall, New York

Cavalli-Sforza LL and Minch E (1997) Paleolithic and Neolithic lineages in the European mitochondrial gene pool. *Am J Hum Genet* **61**:247-251

Collins M *et al.* (submitted) Neandertal DNA; Not just old but old and cold

Cooper A *et al.* (1997) Neandertal genetics. *Science*, **277**:1022-1025

Cooper, A (1997) Ancient DNA - How do you really know when you have it? *Am J Hum Genet*, **60**:1001-1002.

Handt O, Höss M, Krings M, Pääbo S (1994) Ancient DNA: methodological challenges. *Experientia* **50**:524-529

Hänni C. *et al.*(1994) Tracking the origins of the cave bear using mt DNA sequences. *Proc. Nat. Acad. Sci*. **91**: 12336-12340

Krings M *et al.* (1997) Neandertal DNA sequence and the origin of modern humans. *Cell,* **90**:19-30.

Leonard J, Wayne RK and Cooper A (2000) Population genetics of Ice Age brown bears. *Proc. Natl Acad Sci USA* **97**:1651-1654

Lindahl T (1993) Instability and decay of the primary structure of DNA. *Nature* **362**:709-715

Ovchinnikov I *et al.* (2000) Molecular analysis of Neandertal DNA from the Northern Caucasus. *Nature* **404**: 490-493.

Pääbo S (1989) Ancient DNA: Extraction, characterization, molecular cloning, and enzymatic amplification. *Proc Natl Acad Sci USA* **86**: 1939-1943

Sykes B. (1999) The molecular genetics of European ancestry, *Phil. Trans. Roy, Soc. Lon.* B **354**: 131-138

Ward R, Stringer C (1997) A molecular handle on the Neandertals. *Nature* **388**:225-226.

Wayne RK, Leonard J, Cooper A (1999) Ancient DNA: Full of sound and fury. *Ann. Rev. Ec*