Neanderthal DNA: Not just old but old and cold?

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Environment

Superselective clay for radium uptake

A legacy of the nuclear industry that threatens many areas of the world is contamination by radioactive radium from the solid and liquid wastes left after extracting uranium from its ores. Here we describe a highly charged, synthetic clay that specifically removes radium and immobilizes it at room temperature for safe disposal. This material may prove to be useful in decontaminating drinking water wells and soils of this toxic isotope.

Synthetic cation exchangers have been tailored for decontaminating the environment of radioactive isotopes such as ^{137}Cs and ^{90}Sr (refs 1–3) and immobilizing them through electrostatic bonding[2,3] at room temperature. There is also a need for selective exchangers for the uptake of radium because not only is this isotope extracted into groundwater from uranium wastes but naturally occurring uranium may also contaminate drinking-water wells. Radium must be filtered out of drinking water to protect the populace from cancer and other health risks[4,5].

Two synthetic micas of nominal composition $Na_4Al_4Si_4Mg_6O_{20}F_4 \cdot xH_2O$ ('Na-4-mica') and another two of nominal composition $Na_2Al_2Si_6Mg_6O_{20}F_4 \cdot xH_2O$ ('Na-2-mica') were prepared from sol–gel precursors[3] (gel method) or by using metakaolinite and MgO as precursors[6] (kaolinite method) in sodium fluoride at 890 °C. We compared the uptake properties of several natural montmorillonite clays and of these synthetic clays.

Selective uptake of radium was determined using 0.5 M, 1.0 M or 2.0 M NaCl

Table 1 Radium uptake by different clays

	0.5 M NaCl	1 M NaCl	2 M NaCl
Na-4-mica*	1,034 ± 64	234 ± 15	58 ± 3
Na-4-mica†	856 ± 80	207 ± 7	55 ± 2
Na-2-mica†	787 ± 42	167 ± 4	45 ± 2
Na-2-mica†	544 ± 9	113 ± 4	30 ± 0.5
Montmorillonite, Wyoming	12.4 ± 6.1	4.8 ± 0.3	4.8 ± 0.3
Montmorillonite, Arizona	11.5 ± 4.0	9.6 ± 0.6	7.2 ± 0.1
Montmorillonite, Kunipia	44.4 ± 0.2	16.9 ± 0.3	6.8 ± 0.2

Uptake of radium by different clays from solutions containing high concentrations of NaCl is measured as the distribution coefficient K_d, in ml g^{-1}, defined as the ratio of the amount of radium sorbed per gram of solid to the amount of radium remaining per millilitre of the solution[1–3].
*Synthesized by the kaolinite method; †synthesized by the gel method.

containing $^{226}RaCl_2$ at about 6×10^{-9} M. These solutions have extremely large equivalent ratios of $[Na^+]/[Ra^{2+}]$ (0.6×10^8, 1.2×10^8 and 2.4×10^8 in 0.5, 1.0 and 2.0 M NaCl solutions, respectively). Aliquots of the solution (20 ml) and sorbent powder (20 mg) in a centrifuge tube were equilibrated at room temperature. After 24 h, the solid and solution phases were separated and the ^{226}Ra concentration in solution was determined. The selective uptake of radium is expressed as the distribution coefficient K_d.

Table 1 shows that Na-4-mica is the best of all the clays tested at taking up radium from solutions containing 60 to 240 million times more sodium than radium. This mica removed 95.4 ± 0.3% radium from 0.5 M NaCl solution. Its high charge density and special structure of offset layer stacking means that Na-4-mica prefers to take up fewer hydrated monovalent and divalent ions[3], which accounts for its superselectivity towards radium.

To investigate this selective uptake by Na-4-mica, we determined the equilibrium Na/Ba exchange isotherm using barium as a substitute for radium[7]. The shape of this isotherm[2,3] also reveals the nature of the uptake process[8]. Results shown in Fig. 1 indicate that the mica prefers Ba^{2+} in the region of low concentration (initially steep curve), but then exchange stops (flat curve caused by the collapse of the interlayer space)[3,8]. This type of interlayer collapse occurs in layered clays because of electrostatic bonding[2,3] with ions of low hydration such as Cs^+, Rb^+ and Sr^{2+}.

Calculations from the equilibrium isotherm (Fig. 1) show that about 25% of the available barium enters the Na-4-mica interlayers, corresponding to a capacity of
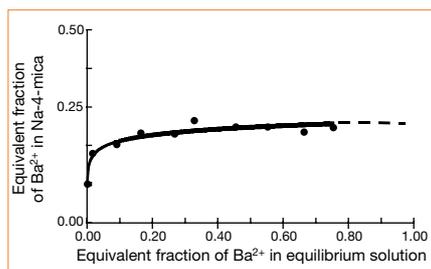

Figure 1 Selective ion-exchange isotherm for Na-4-mica in mixed NaCl/BaCl$_2$ solutions (barium used as a simulant for radium).

115 mequivalents per 100 g, before collapse of the interlayers prevents further exchange. Radium ion, which is less hydrated than barium ion, should not be taken up to the same extent as barium as it is more likely to be trapped (immobilized) in the interlayers after fractional exchange owing to electrostatic and steric factors.

This special superselective mica could be used to help solve not only the radium-separation problem, but also its fixation and safe disposal.

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Neanderthal DNA

Not just old but old and cold?

The successful retrieval of ancient DNA from two geographically dispersed Neanderthal skeletons[1,2] has fuelled a demand for more Neanderthal DNA sequences for analysis. However, these exceptionally well-preserved specimens were geologically young and the mean annual temperature of their cave sites low, so the survival of this ancient DNA could have been due to unusually favourable conditions. Here we calculate the thermal history of a range of Holocene and Pleistocene bones whose DNA quality has been tested and find that in only very few sites with Neanderthal remains is the preservation of

brief communications

DNA likely to match the quality of that from the skeleton found at Mezmaiskaya Cave[2]. We recommend that any additional Neanderthal destined for destructive analysis should be carefully selected, taking into account its integrated thermal history.

We calculated the likely deterioration of DNA for a series of Holocene fossils and at Pleistocene sites[1–8] for which we had reliable regional palaeoclimatic reconstructions[9–11]. We assumed that depurination is the principal pathway of DNA decomposition in most environments[12] (activation energy, $E_a = 127$ kJ mol^{-1}) and have taken account of altitude and the integrated regional palaeotemperature data in the calculations. For each sample, the expected deterioration in DNA by depurination alone is expressed in terms of its 'thermal age', which is the number of years required, at a constant 10 °C, to produce the degradation calculated from its thermal history.

Figure 1 shows that bones with thermal ages greater than that of the Feldhofer Neanderthal[1] (17,000 years at 10 °C) failed to yield DNA. The material from Mezmaiskaya[2], although this is at a lower latitude than Feldhofer[1], was cooler because of its altitude (1.3 km; ref. 2) and so has a lower thermal age. High altitude is a common factor in low-latitude sites that have yielded ancient DNA[6,7] and is anticipated from thermal age analysis. The failure to amplify DNA samples in the polymerase chain reaction from specimens from two sites whose thermal ages are both younger than 17,000 years at 10 °C demonstrates that thermal age is not the only factor that preserves DNA quality for successful amplification. Nevertheless, our analysis indicates that the original Feldhofer Neanderthal DNA sequence[1] represents the current technical limit for retrieval.

When we subjected other Neanderthal sites from northwestern Europe to the same analysis, only nine (of 39) cave sites are thermally younger than Feldhofer, and none strikingly so (see table in supplementary information). Despite the importance of gaining further insight into Neanderthal genetics, our results reveal that careful consideration is necessary before embarking on the destructive analysis of remains from all but a handful of sites.

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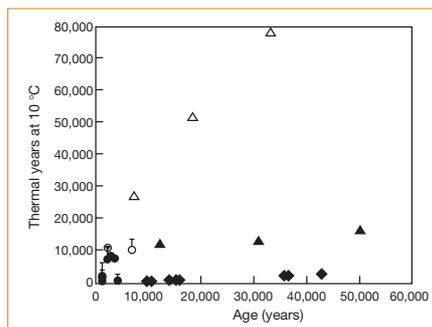
Figure 1 The success of amplifying ancient DNA by polymerase chain reaction[1–8] is related to its thermal age — the thermal age of the original Neanderthal DNA amplification[1] represents the limit using current techniques. Filled symbols represent sites where ancient DNA has survived, clear symbols sites where it has not: diamonds, permafrost sites; triangles, cave sites; and circles, open sites. Thermal ages of open sites are calculated using mean annual air temperatures, with the upper range representing the effect of temperature fluctuation (estimated from monthly averages). The relation between air temperature and permafrost surface temperature is difficult to predict[13], but these problems are largely offset because the rate of depurination is apparently weakly temperature-sensitive in frozen samples[14]. Methodological details are available from the authors.

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Ovchinnikov et al. *reply* — Smith *et al.* have shown an interesting correlation between the thermal age of Pleistocene–Holocene fossils and ancient DNA retrieval and advocate using this criterion to assess the merit of subjecting ancient bones to destructive analysis, particularly any scientifically valuable bones of Neanderthals and anatomically pre-modern humans. The post-mortem DNA quality is, of course, dependent on the surrounding temperature, but — as the authors point out — many other important factors influence fossil DNA preservation, such as air and soil humidity, soil pH, phosphorus content of the soil, average temperature in different earth layers, and microbial-mediated decay, which also have to be taken into account.

The complex interaction of several factors is illustrated by differential preservation of skeletons and bones from the same site, and even between different parts of the same skeleton or mummy. In addition, some specimens recovered from permafrost or glacial conditions after present-day thawing have shown variable degrees of mitochondrial DNA preservation (for example, in the Tyrolean Ice Man[1]) or they contained no recoverable mitochondrial DNA (as discovered in the Altai Princess and Warrior mummies; I.V.O. and W.G., manuscript in preparation), illustrating that low temperatures alone are not sufficient to preserve all specimens.

Relying too heavily on temperature alone could lead to important specimens being excluded from DNA analysis. The Feldhofer Neanderthal is an outlier based on this model, which would predict that the possibility of recovering fossil DNA from this specimen would be very remote. Obtaining a sample of the Feldhofer Neanderthal for destructive analysis would presumably have been even more difficult if too much significance had been placed on such a model at the time the work was carried out.

Another example for which the thermal age may not be an accurate guide to the state of preservation is the Neanderthal recovered from the Marillac Cave in France. As this has a thermal age of 30,539 years, the model would predict that there should be very little chance of fossil DNA remaining. However, the collagen yield is comparable to that of the Mezmaiskaya Neanderthal, which had very good DNA preservation[2,3].

In conclusion, we agree that there is a real correlation between DNA survival and thermal history, but the accuracy to which the survival of DNA can be predicted is limited. Important specimens could be overlooked if too much emphasis is placed on the thermal age alone.

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