

Research article

The development of visual and chemical methods for predicting the likelihood of obtaining a DNA profile from degraded bone samples

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Abstract

Gross morphology, histology and nitrogen content were examined in bone samples from individuals that had been buried for approximately 12 years in Kuwait and Iraq. The results indicate that the gross morphology and histology are useful indicators of DNA survival. Nitrogen content did not show a significant correlation with DNA preservation.

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1. Introduction

Many forensic cases involve the identification of skeletal remains. Predicting whether a bone sample can be successfully analysed would be valuable when planning and managing the large-scale analysis of skeletal remains. Unfortunately this is difficult as the relationship between the burial environment and DNA preservation is complicated.

In this study, we have compared three diagenetic parameters with DNA recovery. These methods have been used previously with ancient DNA samples to assess the likelihood of obtaining endogenous DNA [1,2]. The bone samples are from some of the 605 Kuwaiti victims of the first Gulf war (1990–1991) and were buried for approximately 12 years before they were recovered.

2. Materials and methods

In total, 50 bone samples were collected from 5 mass graves; three in Iraq at Karbala, Al-Amarah and Al-Samawah; two in Kuwait at Al-Solibekhat and Sabhan. A combination of low power and stereo microscopy were used for morphological assessment. For histological examination, bone samples were decalcified and sectioned and then stained with hematoxylin and eosin. The chemical composition of the bone samples was assessed using CHN (carbon, hydrogen and nitrogen) microanalysis.

For DNA extraction, bone samples were cleaned using abrasion, 10% bleach and detergent followed by UV irradiation. Samples were then powdered under liquid nitrogen, 3 ml of extraction buffer (0.5 M EDTA, 20 µg/ml Proteinase K, 0.5% Triton X-100) was added to 0.2 g bone powder and the extractions were incubated at 56 °C overnight. After organic extraction with phenol:chloroform:isoamyl alcohol (25:24:1) the aqueous phase was concentrated and washed using an Amicon YM-30 filter [2]. PCR amplifications were carried out using the AmpF ℓ STR[®] SGM Plus[®] kit (30 cycles).

3. Results and discussion

Samples with high levels of surface pitting and displaying cracks, along with poor histology, did not yield DNA that could be profiled using the AmpF ℓ STR[®] SGM Plus[®] kit (Fig. 1). There were clear differences in preservation between different sites that individuals were recovered from, for example, Karbala yielding samples which displayed good morphological/histological preservation—these could be successfully profiled. In contrast, sites in Kuwait, and in particular Sabhan, contained bone material with poor morphological/histological preservation that could not be profiled. Despite the clear morphological variation in the preservation at different sites the nitrogen content did not vary significantly (Fig. 2).

Morphological and histological assessment of the bone could be used in this scenario to indicate whether DNA would be recovered from a particular sample; however, based on these

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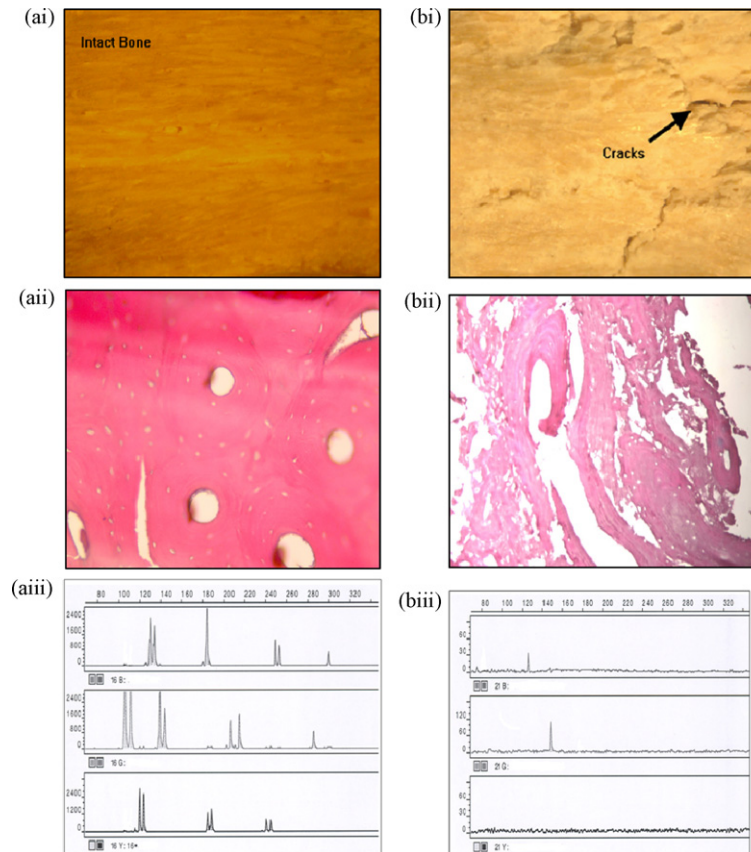


Fig. 1. (i) Gross morphology (100×), (ii) histology (400×) and (iii) SGM Plus® DNA profile for a representative femur sample from (a) Karbala and (b) Sabhan.

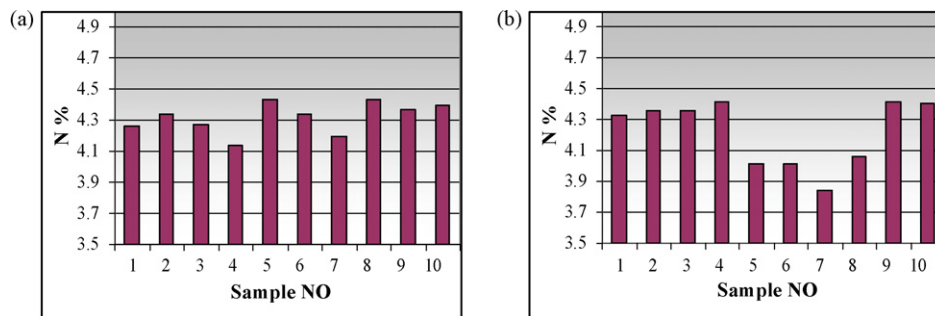


Fig. 2. The nitrogen content from samples recovered from (a) Karbala and (b) Sabhan. Ten samples relating to five individuals were tested from each site; odd numbers represent a femur and even numbers a humerus.

results the nitrogen content is a poor predictor of molecular preservation.

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Conflict of interest

None.

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