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Mammalian mitochondrial capture, a tool for rapid screening of DNA preservation in faunal and undiagnostic remains, and its application to Middle Pleistocene specimens from Qesem Cave (Israel)

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ABSTRACT

Faunal skeletal remains from prehistoric sites are often too fragmentary to enable identification at the species level based on morphology, complicating attempts to recover ancient DNA sequences from these remains. We designed a novel approach that enables large-scale screening of undiagnostic remains for the preservation of ancient mitochondrial DNA. Following DNA extraction and DNA library preparations, libraries are enriched for mitochondrial DNA using a set of probes, which cover the entire mitochondrial genomes of 242 mammalian species. We show that the efficiency of mitochondrial enrichment using mammalian capture probes is not substantially lower compared to enrichment using species-specific probes.

Excavations at Qesem Cave (Israel), dated to 420–200 ka, have yielded a large assemblage of skeletal and dental remains, including a small number of hominin teeth. As recent developments in ancient DNA extraction and library preparation methods have enabled the generation of sequencing data from Middle Pleistocene samples, we aimed to investigate the feasibility of recovering ancient DNA molecules from specimens originating in Qesem Cave. The current research was carried out using forty-two faunal remains from six different areas of the cave, as a preliminary study which could then be used to select hominin remains for further genetic analysis. Unfortunately, DNA libraries from Qesem Cave samples yielded few sequences which could be mapped to a mammalian mitochondrial genome. These sequences do not exhibit the patterns of ancient DNA damage expected from endogenous sequences of that age. Thus, we could not detect the presence of ancient endogenous DNA molecules in the faunal remains from Qesem Cave.

1. Introduction

Recent developments in ancient DNA extraction (Dabney et al., 2013) and library preparation methods (Gansauge and Meyer, 2013) have made it feasible to recover genetic material from ancient samples outside of permafrost, as far back as the Middle Pleistocene (Dabney et al., 2013; Meyer et al., 2014). However, the preservation of DNA molecules over time, in terms of both quantity and quality, is highly variable, as they may be affected, among others, by soil temperature, acidity, salinity, exposure to sunlight or availability of free water and oxygen (Lindahl, 1993; Bollongino et al., 2008; Sawyer et al., 2012). In particular, warm climates appear to negatively affect the preservation of ancient DNA molecules (Bollongino et al., 2008; Allentoft et al., 2012). Although 100 mg or less of bone/tooth powder is needed for current DNA extraction techniques (Dabney et al., 2013), sampling remains a destructive process. Thus, ancient DNA preservation is best determined on faunal remains or undiagnostic fragments before sampling hominin fossils, assuming that skeletal remains from similar locations in an archaeological site would have been exposed to similar environmental conditions.

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Screening ancient samples for DNA preservation routinely targets the mitochondrial (mt) genome, a small, circular and non-recombining DNA molecule that is maternally inherited. The occurrence of mtDNA in higher copies per cell, compared to nuclear DNA, increases the chance of ancient DNA recovery (Pääbo et al., 2004). Additionally, the rapid rate of mtDNA evolution enables the identification of discrete organisms based on mitochondrial sequences, which is particularly important when working with bone or teeth fragments of unclear origin. In the past, polymerase chain reaction (PCR) has been used to amplify short stretches of mtDNA that carry distinctive sequence differences among closely related species, for example in the cytochrome b gene (Loreille et al., 1997; Burger et al., 2002). However, this approach is not applicable to a wide range of loosely related taxa. Additionally, PCR-based methods are not sufficiently sensitive to detect the small traces of highly degraded DNA that may be present in very old specimens or in those originating from sites under difficult climatic conditions (Dabney et al., 2013). In contrast, library-based methods enable amplification of all the DNA fragments recovered in a DNA extract. This is achieved by attaching DNA linkers with known sequences to each DNA fragment prior to amplification by PCR. DNA libraries may then be sequenced directly on a high-throughput sequencer, thus recovering sequences from random DNA fragments. This simple strategy is sometimes sufficient to determine the source species of an ancient sample (Blow et al., 2008), but due to the dominance of microbial DNA in most ancient DNA extracts, it is cost-prohibitive for screening large sets of samples. A more cost-effective alternative is enriching DNA libraries for mitochondrial sequences. This is achieved by hybridizing the library to synthetic pieces of DNA ('baits') that carry the sequence of interest. Hybridization capture has successfully been used to generate mtDNA genome sequences even from extremely degraded DNA, such as from bear and hominin remains from Sima de los Huesos (Spain) (Dabney et al., 2013; Meyer et al., 2014). A limitation of hybridization capture is that the selection of appropriate capture baits requires *a priori* knowledge of the species under study.

We here describe a novel approach, 'mammalian mitochondrial capture', which enables large-scale screening of faunal and undiagnostic remains for ancient DNA preservation. For this purpose, we use a bait pool that includes mtDNA genome sequences from 242 mammalian species, including most species (or close relatives thereof) found in typical Middle and Late Pleistocene sites. We demonstrate the efficiency of mammalian mitochondrial capture on cave bear samples previously known to contain DNA, and use this approach to determine the state of DNA preservation in the Middle Pleistocene remains from Qesem Cave.

Qesem Cave is a karst chamber located in a hilly limestone terrain approximately 12 kms from the modern city of Tel-Aviv (Israel). Hominin occupation of the cave ranged between 420 and 200 thousand years (ka) ago (Barkai et al., 2003; Gopher et al., 2010; Mercier et al., 2013), as attested by the discovery of several hominin teeth (Hershkovitz et al., 2011), lithic artefacts pertaining to the Acheulo-Yabrudian Cultural Complex (Barkai and Gopher, 2013), evidence for use of fire (Karkanas et al., 2007; Barkai and Gopher, 2013; Shahack-Gross et al., 2014), and thousands of faunal skeletal and dental remains (Stiner et al., 2009, 2011; Blasco et al., 2014). The sediments at the site consist mostly of dark-colored layers of clay, most probably originating from infiltrating terra rossa (Karkanas et al., 2007). The average annual temperature in the region is approximately 20 °C. The average precipitation is ~500 mm per year, although annual precipitation can vary drastically.

Morphological analysis of the hominin dental remains from Qesem Cave revealed their similarity to the later Skhul/Qafzeh Early Anatomically Modern Humans, albeit with some Neanderthal-like traits (Hershkovitz et al., 2011), bringing forth several evolutionary scenarios accounting for these anatomical traits. Genetic data from the dental remains would allow to further investigate the identity of the Qesem Cave hominins, as well as to elucidate their relationship to other archaic hominins for which DNA sequences have already been generated (e.g., Meyer et al., 2012, 2014; Prüfer et al., 2014). The abundance of faunal remains in Qesem Cave offers the opportunity to study ancient DNA preservation in different areas of the cave. This preliminary study could then be used to select hominin remains for further genetic analysis, thus diminishing destructive sampling of these valuable fossils. Moreover, many of the faunal remains from Qesem Cave are too fragmentary to enable identification at the species level based on their morphology (Stiner et al., 2009; Blasco et al., 2014). Identification of species based on their genetic material would help shed further light on the environment and hunting patterns of the Qesem Cave hominins.

2. Materials and methods

2.1. Materials

Forty-two faunal remains from six areas in Qesem Cave were selected for this study (Table 1). The sampling areas chosen are squares in which at similar elevations below datum, a well-preserved hominin tooth was discovered. Prior to their excavation, samples from squares I/12 and J/15 were covered by 10–50 cm of sediments. Specimens found in square M/13 were covered by between 0.5 m and 1 m of sediments, however, due to their vicinity to the section, they could have been exposed for a long time prior to their removal from the site. Samples from square G/22 were covered by approximately 1.5 m of sediments, and samples from E/6-E/8 were uncovered below 3–4 m of rocks and sediments. When possible, the faunal remains were identified by species, however for approximately half of our sample, the specimens were too fragmentary to confidently identify the source organism.

Furthermore, a 44 ka-old cave bear bone from Gamsulzen (Austria) and a ~430 ka cave bear bone from Sima de los Huesos (Spain), both of which had yielded mitochondrial sequences in previous studies (Krause et al., 2008; Dabney et al., 2013), were used to test the efficiency of the mammalian mitochondrial capture probe set designed for mitochondrial enrichment of unidentified faunal remains.

2.2. DNA extraction and library preparation

A sample of between 25 mg and 51 mg was removed from each specimen using a sterile dentistry drill. DNA was extracted as described in Dabney et al. (2013) and eluted in 50 μl of TET (10 mM Tris–HCl, 1 mM EDTA, 0.05% Tween-20, pH 8.0). 15 μl of each DNA extract was converted into a single-stranded DNA library (Gansauge and Meyer, 2013). The number of DNA molecules in each library was determined either by quantitative PCR (Stratagene MX3005P, Agilent Technologies), using 1 μl of a 20-fold dilution of each library as template for a Maxima SYBR Green (Fermentas) assay with primers IS7 and IS8 (Meyer and Kircher, 2010); or using digital droplet PCR (BioRad QX 200), using 1 μl of a 5000-fold dilution of each library as template for an EvaGreen (BioRad) assay with the above-mentioned primers. Each library was barcoded with a pair of unique indexes (Kircher et al., 2012) and amplified using AccuPrime Pfx DNA polymerase (Life Technologies) (Dabney and Meyer, 2012). Amplification products were purified using a MinElute PCR purification kit (Qiagen). The resulting amplified libraries were quantified on a NanoDrop ND-1000 (NanoDrop Technologies) photospectrometer.