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Y-Chromosome-Specific DNA Amplified in Ancient Human Bone

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The discovery of genetically determined characters from past populations and individuals has always been a focus of interest within modern physical anthropology. The interest in paleoserology [1] as well as in discrete morphological traits [2] was due to interest in the dynamics of population genetics and kinship.

The development of the polymerase chain reaction (PCR) [3] and its high sensitivity [4,5] now provides the possibility to obtain genetic information directly on a molecular level even from ancient source material [6,7]. The extraction and amplification of mitochondrial DNA from archaeological bone were first reported by Hagelberg [8], Horai et al. [9], and Hänni et al. [10]. Most recently, the successful amplification of HLA genes from the 7500-year-old Windover brain has been reported [11].

Here, we report on the enzyme-directed amplification of human Y-chromosome-specific sequences from ancient bone material. This method allows one to identify the sex of skeletal remains on the DNA level.

The bone samples for this investigation came from individuals which had been determined as male by means of morphological and metrical traits. After staining thin sections of compact bone the samples with the most promising histomorphology, i.e., stained nucleic material (Fig. 1), were chosen for further work. Two of the males (SB 419 and SB 510) were buried on a site belonging to a 17th/18th century Dutch whaler station on Spitsbergen/Norway [12]. The other two male individuals (Mü 330 and Mü 530) were buried in a graveyard for the high clerus in medieval North Rhine Westphalia [13]. The samples were compact parts of the diaphysis of either femur or humerus. The outer surfaces of the samples were removed to avoid recent contamination. After placing each sample in liquid nitrogen for 5 min the bones were powdered. Samples of about 0.5 g of bone powder each were mixed with 1 ml of 0.5 *M* EDTA, pH 8 and 10 μl proteinsase K (stock 20 mg/ml). After 5–20 days with constantly shaking at room temperature, the bone solutions were heated to 95 °C for 10 min. DNA extraction and the first steps of purification then basically followed laboratory routines given in [14]. Phenol-chloroform-isoamylalcohol extraction was carried out twice, the chloroform-isoamylalcohol step once. DNA was precipitated with ethanol and ammonium actetate at room temperature for 1h. The pellet was washed with ethanol and resolved in 100 μl 1 × TE buffer. Further purification with the Gene Clean kit (Dianova) was performed with 50 μl of the DNA solutions. After this final procedure only high-molecular-weight DNA was left. Additions showing blue fluorescence in UV light were no longer detectable. They often pass through phenol extraction, coprecipitate with bone DNA and inhibit the Taq polymerase. All decalcification, extraction, and purification steps were accompanied by positive and negative controls. Positive controls were samples of modern bone of female and male individuals from the dissection room. The DNAs of these bones

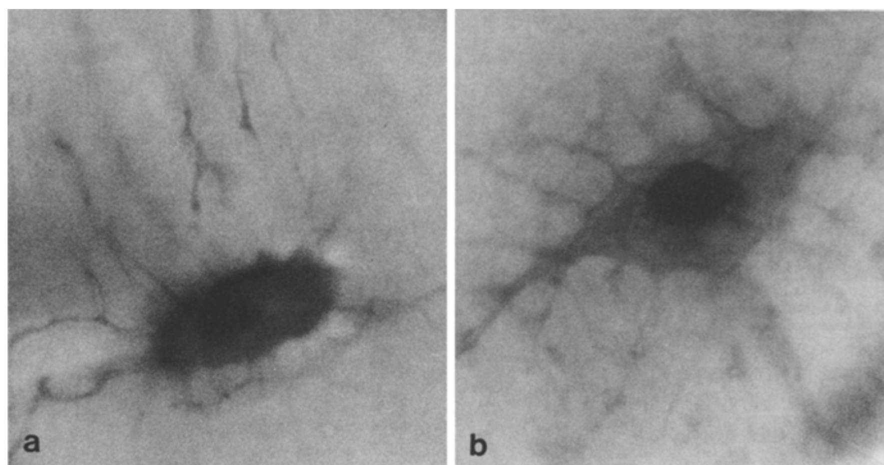

Fig. 1 a) Thin section (100 μm) of femur compacta of a male individual (SB 419) from the burial site of a 17th/18th century Dutch whaler station. The osteocyte shows dark stained nucleic material. b) in comparison the thin section of a modern bone from the dissection room with principally the same staining behavior and cell features. Both thin sections are fuchsin-stained, magnification 4000 ×

already revealed results with Y-specific primers [15]. Negative controls were samples which had undergone the whole treatment but did not contain bone powder.

For PCR a set of primers was used which matched a human Y-specific 3.4-kb repeat sequence [16]. The primers amplified a 154-bp sequence spanning an Eco RI site (fragment size 102 and 52 bp). Two subsequent PCR runs were carried out with 60 (40 + 20) cycles in total (Fig. 2). The amplifications were reproducible for all individuals. For the Spitsbergen whalers amplification was also possible with samples available from earlier, ca. 9-month-old DNA extractions which have now been purified with the Gene Clean kit (Dianova).

In order to verify the identity of the PCR products, they were digested with the restriction enzyme Eco RI. All fragments were cut to the expected sizes (Fig. 3).

With these first results a method is described to determine the sex of ancient skeletal material by means of molecular genetic methods. This might represent a breakthrough to basic sociobiological information in historic and prehistoric populations. Especially for sexing subadult and infant individuals, it provides a promising and helpful perspective. Moreover, practical application may be possible in forensic medicine. Before becoming a routine procedure in the named fields, the risks of contamination due to excavation and laboratory work need further attention.

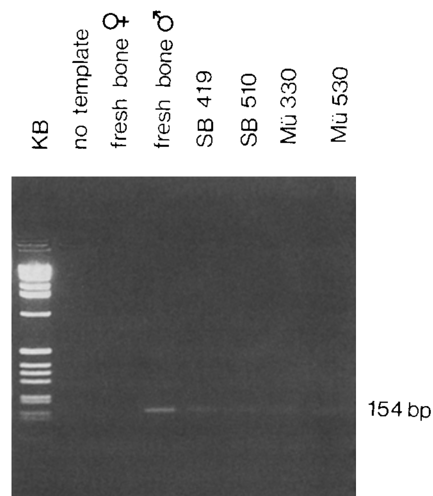

Fig. 2. The PRC products (10 μl) were run on a 2 % agarose gel and stained with ethidium bromide. The first lane is the negative no-template control. The second and third lanes show the results of the positive controls, no product results in the female or the 154-bp fragment in the male fresh bone sample. Lanes four to seven show the 154-bp fragment in the ancient bone samples (SB = Spitsbergen 17th/18th century and Mü = medieval North Rhine Westphalia). All four individuals were also determined as male by means of morphological and metric criteria. The molecular weight standard is the l-KB ladder (Gibco/BRL). The first 50 μl reaction mix contained 10 m*M* Tris-HCl pH 8.3, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 175 μ*M* each dNTP (Boehringer), 10 p*M* each primer and 1 % of the DNA extracted. Before adding 2.5 U of Taq polymerase (Gibco/BRL) and starting the cycling, the mixes were heated to 94 °C for 5 min. Cycling parameters were 1'30'' 94 °C, 20'' 55 °C, 10'' 72 °C in a Thermal Cycler (Perkin Elmer Cetus), 40 cycles. The second reaction mix had the same buffer and dNTP concentrations, but only 5 p*M* each primer and 1 U Taq polymerase. 10 % (5 μl) reaction products of the first PCR were added. Cycling parameters were the same, but only 20 cycles

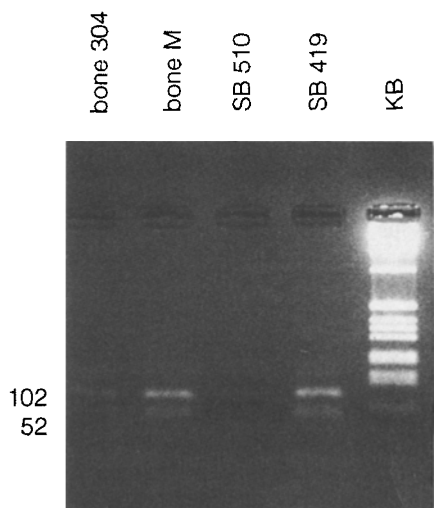

Fig 3. Digestion of the human Y-chromosome-specific 154-bp fragment with the restriction endonuclease Eco RI (Boehringer). All PCR products were cut by Eco RI to the expected sizes of 52 and 102 bp. 15 μl amplified DNA was incubated with 8 U Eco RI and 1 × H-buffer in a total volume of 20 μl at 37 °C for 1

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