

SEX DETERMINATION IN ANCIENT BONES

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Summary

Using the PCR based amelogenin system anthropological results could be proved and be completed: the 'Velké Pavlovice grave' contains the skeletons of two adults (female and male) and six children (one female, five males). The anthropological theory of a family grave could not be refuted.

Introduction

The remains of eight individuals of the Early Bronze Age Veterov culture found at Velké Pavlovice (distr. Breclav, Moravia, CR) were subjected to a PCR-based sex determination. The skeletal material represents two adult individuals and six children from two to ten years of age (Fig.1). To verify the hypothesis that this group of individuals represent a proper family, at first the sex of the individuals was determined.

Material and Methods

Sample Pretreatment: Since samples probably have been touched during excavation or previous investigations it is necessary to decontaminate the sample properly. Irradiation with shortwave UV light (254 nm) induces covalent bindings of opposite thymine bases and prevents therefore double-stranded DNA from melting during PCR.

DNA-Extraction: A small piece of bone is ground to a fine powder in a bone-mill. About 500 mg of the bone meal is transferred into a microtube and mixed with 1 ml extraction buffer (0.3M Sodium-acetate, 10 mM Tris-HCl, 1 mM EDTA, 1% SDS, pH 7.8) and 0,5 mg proteinase K. This mixture is incubated overnight at 55°C in a shaking water bath or in an incubator. Periodical vortexing should be performed to achieve an optimal intermix.

DNA-Isolation: After incubation two 340 µl aliquots of the supernatant are transferred into sterile microtubes. After addition of 100 µl 5 M sodium perchlorate the DNA-isolation was carried out according to the protocol of the Nucleon DNA Extraktion Kit (Nucleon, Scotlab).

DNA-Amplification: Amplification is performed using the GenePrint STR System DC4080 (Amelogenin) (Promega) according to the technical manual. To obtain the recommended concentration of DNA, it was diluted in the ratio of one to four with 10mM Tris-HCl, pH 8,7.

Electrophoresis: The electrophoresis is carried out as a semidry, ultrathin horizontal polyacrylamide-gel electrophoresis. The following protocol is for the preparation of a polyacrylamide gel with the dimensions of 26 cm x 20 cm x 800 µm (w x h x thickness): 30% Acryl-PDA, 8,7 ml; Aqua bidest., 21,0 ml; CHES, 4,0 ml; 500 mM Tris-Formiat, 6,6 ml; 10 % APS, 300µl; TEMED, 15,5 µl. 2% Agaroseplugs in 2 x Tris-Borat-Buffer, pH 9,0 stored in 2 x Tris-Borat-Buffer/0,01% Bromphenolblue serve as reservoir for the buffer. 4 µl of the DNA samples were applied on small pieces of filter paper. Electrophoresis was carried out on a cooling plate (15°C) for 1,5 h at 5 W, 40 mA, 1000V, 1 h at 10 W, 40 mA, 1000 V and ca. 1 h (until bromphenolblue reaches the anode) at 15 W, 40 mA , 1000 V. Silver staining was performed according to Budowle et al. (1991).

Results and Discussion

The traditional prehistoric kinship analysis represents an attempt to establish the similarity or dissimilarity of individuals on the basis of external skeletal variants. In principle it is a polysymptomatic estimation of similarity based on morphognostic characteristics like anthropological (non-genetic) paternity testing (Knussmann, 1988). For morphognostic traits the exact processes of transmission are more or less unknown. although there is agreement on the facts that genetic factors play a role in their development and that environmental factors modify their expression. Morphognostic characteristics can therefore not be used for genetic analysis s.s. but for establishing phenotypical similarities between individuals. In the case of „Velké Pavlovice“ the skeletons partly are in too bad a state to get definite results from the anthropometric sex analysis, especially the hypothetical mother. Nevertheless, a family (father, mother and six children) is suggested. Typing the sex of the children - by anthropological methods - was impossible (Alt et al., in press).

The results of the genetic sex determination (amelogenin) proved the results of the anthropological investigation: the skeleton of the hypothetical "mother" is female. In addition the children's skeletons were typed: five male and one female remains. These results represent the importance of DNA-PCR analyis for the investigation of prehistoric bones. Further investigations on the Velké Pavlovice material - kinship analysis using STR systems - are planed.

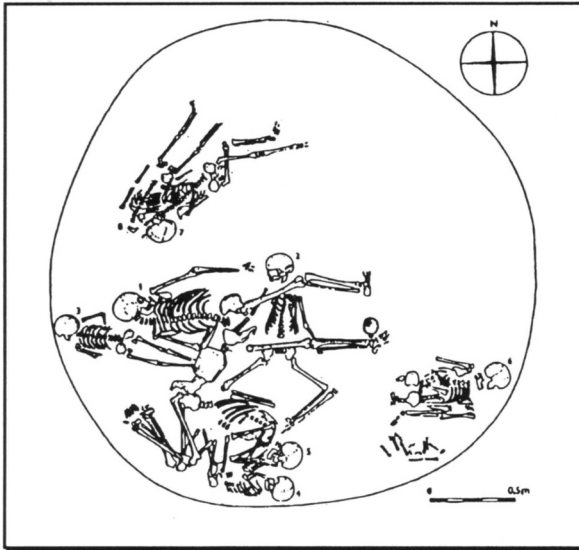


Fig.1 The ‘family burial’ from Velké Pavlovce.

References

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