DETERMINING THE SEX OF ANCIENT HUMAN REMAINS USING NEW PRIMERS TO AMPLIFY X AND Y AMELOGENIN ALLELES

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Introduction

Men and women had each a different social status in past populations. Archaeologists have always been interested in determining the gender of discovered skeletons from a certain society as it might aid them in the process of illustrating the social relations that were manifested between the members of said society. Sex attribution based merely on the anthropometric study of a skeleton may fail if the skeleton is not complete or belongs to an infant. In order to overcome these inconveniences a molecular method was developed.

Anthropometric estimation of sex

Estimating the sex of human remains found in archaeological sites requires the anthropometric analysis of the skull and the pelvis (Figure 1, 2.) and even though standardized methods are usually applied in the process, the accuracy of the estimation may be influenced by the observer's subjectivity [1].

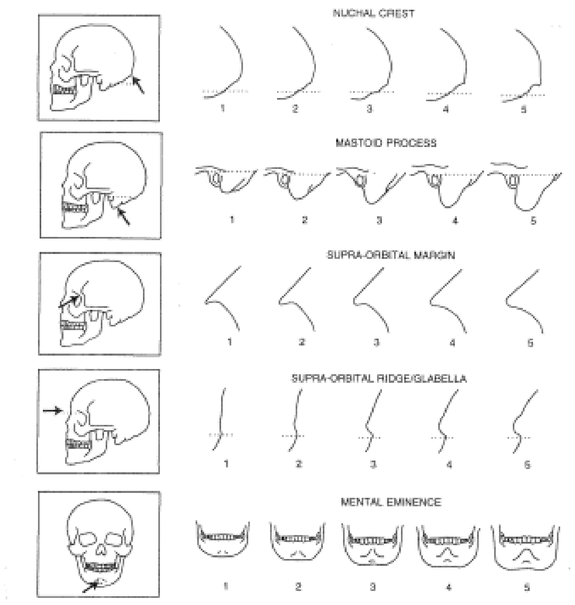

Figure 1. Sex differences remarked after skull examination. Males tend to have larger and more robust heads (right) than females (left) [1].

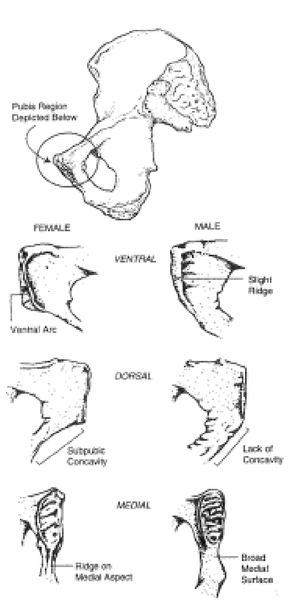

Figure 2. Sex differences in subpubic region [1].

Molecular sex attribution

Amelogenin is a major dental enamel-forming protein found in mammals. The amelogenin gene is a single copy gene, homologues of which are located on Xp22.1-Xp22.3 and Yp11.2 [2]. Their location on sex chromosomes inspired Faerman to design three primers in order to amplify segments with different length on both AMELX and AMELY (Figure 3.).

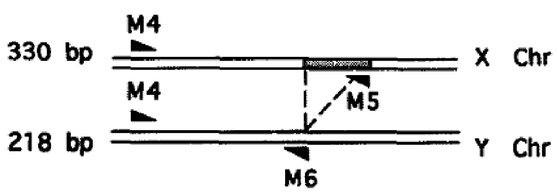

Figure 3. Primers designed by Faerman for amplyfing AMELX and AMELY [3].

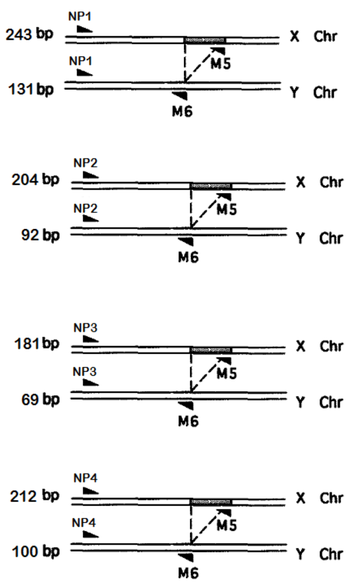

Figure 4. New designed primers. They are considerably shorter.

Because the length of segments obtained using Faerman's primers is too long when ancient DNA is used as template new primers were designed in order to increase the success of amplification (Figure 4.).

Acknowledgement

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The new designed primers were first tested on modern DNA in order to verify their specificity. The third primer (NP3) showed best results in amplifying specific DNA template consequently being used on ancient DNA template (Figure 5.).

Figure 5. Specific modern DNA amplification using new primers. 1. Marker 50 bp. 2. Female DNA amplification using NP1 as forward primer. 3. Male DNA amplification using NP1 as forward primer. 4. Female DNA amplification using NP2 as forward primer. 5. Male DNA amplification using NP2 as forward primer. 6. Female DNA amplification using NP3 as forward primer. 7. Male DNA amplification using NP3 as forward primer. 8. Female DNA amplification using NP4 as forward primer. 9. Female DNA amplification using NP4 as forward primer.

Previously selected primer (NP3) was used to amplify DNA extracted from medieval (Xth century) human remains found in south-eastern part of Romania (Constanţa) (Figure 6.).

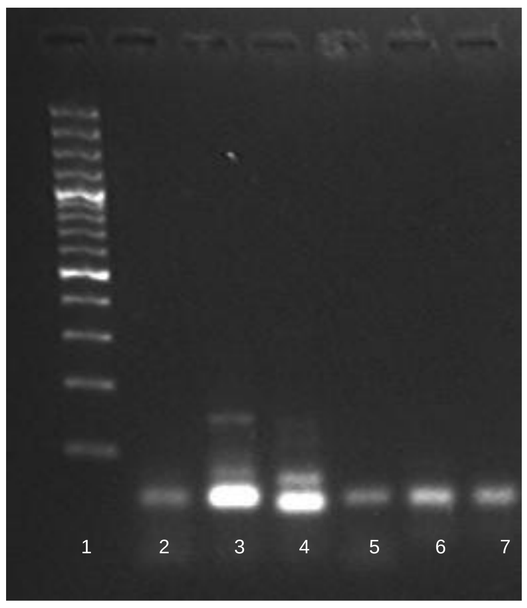

Figure 6. Specific ancient DNA amplification using NP3. 1. Marker 100 bp. 2.Male DNA 3. Female DNA . 4. Male DNA. 5. Female DNA. 6. Negative PCR control. 7. Negative PCR control.

Conclusions

- the new primers amplify shorter fragments which seems to suit better when working with ancient DNA;
- the success of amplification depends mainly on the quality of extracted DNA;
- primers tend to form primer-dimers when little DNA template is present;

References

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