Genetic Analysis of Historical Human Remains –
What ancient Bones can tell if asked the right Questions using optimized Methodology…

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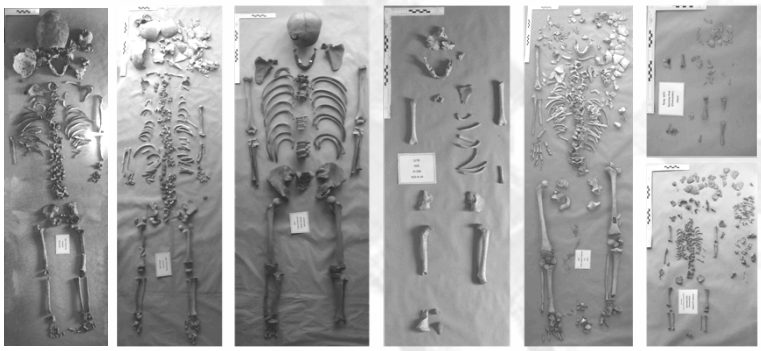
Ancient DNA analysis of historical human remains explores similar questions, utilizing similar and frequently the same methodology as applied in the forensic human identification context, while doing so under extreme conditions regarding DNA content, degree of degradation and presence of inhibitors. Consequently, improvements of methods and procedures in one of the areas will inform the other and vice versa. Our current Ancient DNA based research utilizes historical skeletal human remains from the collection of the Museum of Glouceste to investigate a variety of methodological questions in both the osteological as well as the DNA analysis context.

In the forensic context, the ability to sex skeletal remains reliably is of utmost importance in order to ascertain the identity of an individual. Contrary to the assumption that sex determination in case of sub-adults skeletal remains it is impossible or unreliable (Cardoso & Saunders 2008, Wilson et al. 2008), it is indeed possible to sex juvenile skeletal remains and findings regarding sexual dimorphisms in the sub-adult skeleton have been published for more than a century (Thomson 1899, Boucher 1957, Sundick 1977). A variety of methods to determine the sex of immature human skeletal remains reliably have been published and subsequently tested by experienced anthropologists (e.g. Hunt 1990, Mittler and Sheridan 1992, Scheuer 2002, Sutter 2003, Wilson et al. 2008, Cardoso & Saunders 2008).

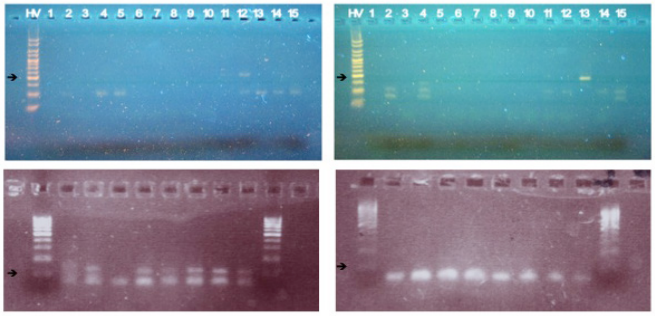


Sub-adult skeletons selected for the study (infans I to juvenis). Left to right: GF B5 and GF B6 (16th cent.), BM A8206 (roman), 5/78 HSR A22-A-14, T 1871 4th Century (top) and T 1871 (bottom).

To evaluate the reliability of these methods when applied by researchers with limited experience in osteological analysis, six previously published methods assessing different skeletal areas (Boucher 1957, Black 1978, Weaver 1980, Schutkowski 1993, Loth & Henneberg 2001, Rogers 2009) were tested in use by students with initial osteological training. The selected methods were utilized to determine the sex of seven sub-adult skeletal individuals from the Gloucester Museum collection. Results of the osteological analysis were compared to molecular sex determination based on amplifications of both Amelogenin (Sullivan et al. 1993) and SRY (Santos et al. 1998).

For DNA analysis ca. 1g of bone from rib- or long bone fragments was sampled, surfaces were removed, the sample decontaminated by UV irradiation and ground to a fine powder. Previous attempts at analysis of DNA isolated from this material (Deutschmann & Schmerer 2012) utilized a less hazardous modified Chelex-based protocol (Schmerer in prep.) with extraction from 0.1g bone powder after a 70h decalcification in 0.5M EDTA (pH 8.3), following a Chelex protocol with subsequent alcoholic precipitation of the DNA. Initial amplifications showed the presence of remaining inhibitors in extracts from the historical remains (Deutschmann & Schmerer 2012), which is a common problem when amplifying ancient DNA (Höss & Pääbo 1993, Schmerer et al. 1999). In the first phase, ribs were sampled whenever possible to minimize invasiveness of sampling. To improve outcomes, the second phase utilizes 0.3g of samples with higher content of compact bone.

The extraction was optimized in the current phase of the study by including a replacement of the EDTA solution after 24h and a decalcification for 96h (Schmerer 2003a), followed by phenol chloroform extraction (Schmerer et al 1999, Schmerer 2003a) and subsequent additional purification by silica column-based extraction (Omega Biotek 2013) of the aquatic phase of the chloroform step. This optimized protocol resulted in successful removal of the comparatively high content of inhibitory substances in this material, as demonstrated by un-inhibited amplification of the resulting extracts.



Amelogenin and SRY PCR products. 3.5% Agarose gel, EtBr (above) and Midori Green stained (below). Arrows indicate the 100bp in each of the size standards used.

Acknowledgements: We like to thank the Gloucester Museum and Art Gallery for kindly providing us with the historical skeletal remains analysed here. We also thank the students who participated in the first phase of the study (Kelly Cheshire, Frauke MN Deutschmann, Renu Gogna, Izabella Jankowska, Faye Parker, Steven Smith, Julie Whitmore).

References: **Black TK (1978)** Am J Phys Anthrop 48: 77-82, **Boucher BJ (1957)** Am J Phys Anthrop 15(4): 581-600, **Cardoso HFV & Saunders SR (2008)** Forensic Sci Int 178: 24-29, **Deutschmann FMN, Schmerer WM (2012)** Sexing sub-adult human remains with osteological and DNA analysis based methodology. Poster presentation. FIRN Midlands Regional Student Forensic Science Conference, April 20, 2012, Staffordshire University, **Höss M & Pääbo S (1993)** Nucleic Acids Res 21(16): 3913-3914, **Hunt DR (1990)** J Forensic Sci 35(4): 881-885, **Loth SR & Henneberg M (2001)** Am J Phys Anthrop 115(2): 179-186, **Mittler DM & Sheridan SG (1992)** J Forensic Sci 37(4): 1068-1075, **Omega Biotek (2013)** E.Z.N.A.® Blood DNA Mini Kit. Manual Revision May 2013, **Rogers TL (2009)** Am J Phys Anthrop 140: 148-154, **Santos FR, Pandya A & Tyler-Smith C (1998)** Nature Genetics 18(2): 103, **Scheuer L (2002)** Am J Phys Anthrop 119(2): 189-191, **Schmerer WM, Hummel S & Herrmann B (1999)** Electrophoresis 20(8): 1712-1716, **Schmerer WM** (in prep.), **Schmerer WM (2003a)** Methods Mol Biol 206: 57-61, **Schutkowski H (1993)** Am J Phys Anthrop 90(2): 199-205, **Sullivan KM, Mannucci A, Kimpton CP & Gill P (1993)** BioTechniques 15: 637-641, **Sundick RI (1977)** J Forensic Sci 22(1): 141-144, **Sutter RC (2003)** J Forensic Sci 48(5): 927-935, **Thomson A (1899)** J Anat Physiol 33(3): 359-380, **Weaver DS (1980)** Am J Phys Anthrop 52(2): 191-196, **Wilson LA, Macleod N & Humphrey LT (2008)** J Forensic Sci 53(2): 269-278.