Isotopic dietary analysis and molecular sex identification of adults and juveniles from medieval Great Moravia

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Introduction

Like many complex agricultural societies, medieval European society was strongly patriarchal, with men favored in terms of property rights, political status, and household authority. However, it is unclear whether male dominance in medieval society was manifested in unequal access to food resources between the sexes. In this pilot study, we examine the pattern of sex-related differences in diet through biomolecular analyses of skeletal remains from Kostelisko, a suburban area within the early medieval Great Moravian site of Mikulčice. Mikulčice was a prominent center of Great Moravia, an early Slavic state that existed in the 9th and early 10th centuries AD, and was situated on the lower Morava River valley in the south-eastern corner of what is today the Czech Republic . Previous bioarchaeological studies of skeletal material from Mikulčice have revealed activity differences between males and females (Havelková et al., 2010), as well as health differences according to socioeconomic status (Velemínský et al., 2009). Here we present dietary reconstructions based on bone collagen carbon and nitrogen stable isotope analyses, and we evaluate a new method of molecular sex identification using high resolution melting analysis of ancient DNA.

Materials and Methods

The pilot sample includes bones or bone fragments representing 14 adults and 9 juveniles recovered from the cemetery at Kostelisko, a suburb of the early medieval Great Moravian settlement complex of Mikulčice. The cemetery at Kostelisko is the second largest in the Mikulčice settlement complex, containing over 400 burials, and is thought to have been the burial ground for individuals of relatively high socioeconomic status (Poláček, 2008). The pilot sample is a subset of a larger sample sent to the UAF Laboratory of Biological Anthropology by PV at the Department of Anthropology, National Museum in Prague, where the remains are curated. Osteological age and sex determinations had been made previously by National Museum staff and were available as entries in a skeletal collections database provided by PV.

To avoid the possibility of blurring differences between males and females due to incorrect osteological sex determinations, the adult pilot sample includes only individuals with confident sex determinations, and is equally split between males (n=7) and females (n=7). The juvenile sample includes adolescents aged 15 to 20 (n=4) and children aged 4 to 10 (n=4). A subset of 7 adult individuals was selected for aDNA extraction based on bone preservation quality and bone quantity. In addition, DNA for 10 modern Czechs from the Coriell Institute for Biomedical Research's Human Genetic Cell Repository (sponsored by the National Institute of General Medical Sciences) was analyzed.

Ribs or long bones of the hand and foot were used for collagen and aDNA extraction. Bone fragments were cleaned by abrasion with a sanding drum attached to a rotary tool, then powdered in a ball mill to less than 0.3 mm. (CMH).

Collagen extraction and isotopic analysis. Briefly**,** collagen was extracted (CMH) from powdered bone by demineralization in 0.5 M HCl and treatment with 0.1 M NaOH (details in Halffman, 2009). Bone collagen samples were submitted to the Alaska Stable Isotope Facility for stable isotopic analysis ($\delta^{13}C$ and $\delta^{15}N$) on the Thermo Finnigan Delta-Plus XP continuous flow isotope ratio mass spectrometer, coupled to a Costech Elemental Analyzer.

aDNA extractions. Extractions of ancient DNA (KCH) followed QIAamp® DNA Investigator (QIAgen®) protocols for bones and teeth using a PCR cabinet equipped with UV light for irradiation of work areas between uses.

PCR. For sex determination, a variable area on the amelogenin gene was amplified (X: 106bp; y:112bp). Due to allelic dropout in PCRs using degraded DNA, a secondary sex determination method was employed that targeted a 92bp area of the SRY gene on the Y-chromosome. PCR (10 µl) was conducted (KCH) on the capillary-based LightScanner32® real-time quantitative PCR HRM instrument in the Bioanthropology Lab.

High Resolution Melting (HRM). To genotype individuals, a post-PCR HRM was conducted (KCH) on the capillary-based LightScanner32® real-time quantitative PCR HRM instrument in the Bioanthropology Lab. Amplicons are heated and monitored using an intercalating fluorescent dye LC Green® (Idaho Technology Inc.) that fluoresces brightly when bound to double-stranded DNA. When the melting temperature of the amplicon is reached, double-stranded DNA denatures and fluorescence decreases. The data generated from the continuous real-time detection of the fluorescence signal is plotted against temperature to create a melting curve graph. These data are then used to group samples randomly or as compared to a melting standard.

Results

Isotopic Analysis

Stable isotope results are found in Figures 1-2. Bone collagen quality indicators fall within accepted limits for all individuals, including %N > 5% and %C > 13%, atomic C:N ratios between 2.9 and 3.6 , and collagen yields above 1% (Ambrose, 1990).

For adult males (n=7), mean $\delta^{13}C$ is −17.8‰ ± 0.4, and mean $\delta^{15}N$ is 10.6‰ ± 0.7. For adult females (n=7), mean $\delta^{13}C$ is −17.8‰ ±0.8, while mean $\delta^{15}N$ is 9.6‰ ± 1.0. There is no mean difference in the carbon stable isotope ratio between the sexes; however, the mean nitrogen stable isotope ratio is 1.0 ‰ higher for males than for females, and this difference is statistically significant (t=2.36, p=0.04).

For adolescent juveniles (n=4), mean $\delta^{13}C$ is −17.9‰ ±0.7, and mean $\delta^{15}N$ is 10.1‰ ± 0.3, while for children (n=4) mean $\delta^{13}C$ is −18.4‰ ±0.4, and mean $\delta^{15}N$ is 9.5‰ ± 0.8. The means for both nitrogen and carbon isotope values are higher in adolescents than in children, but the differences are not statistically significant, possibly due to small sample sizes.
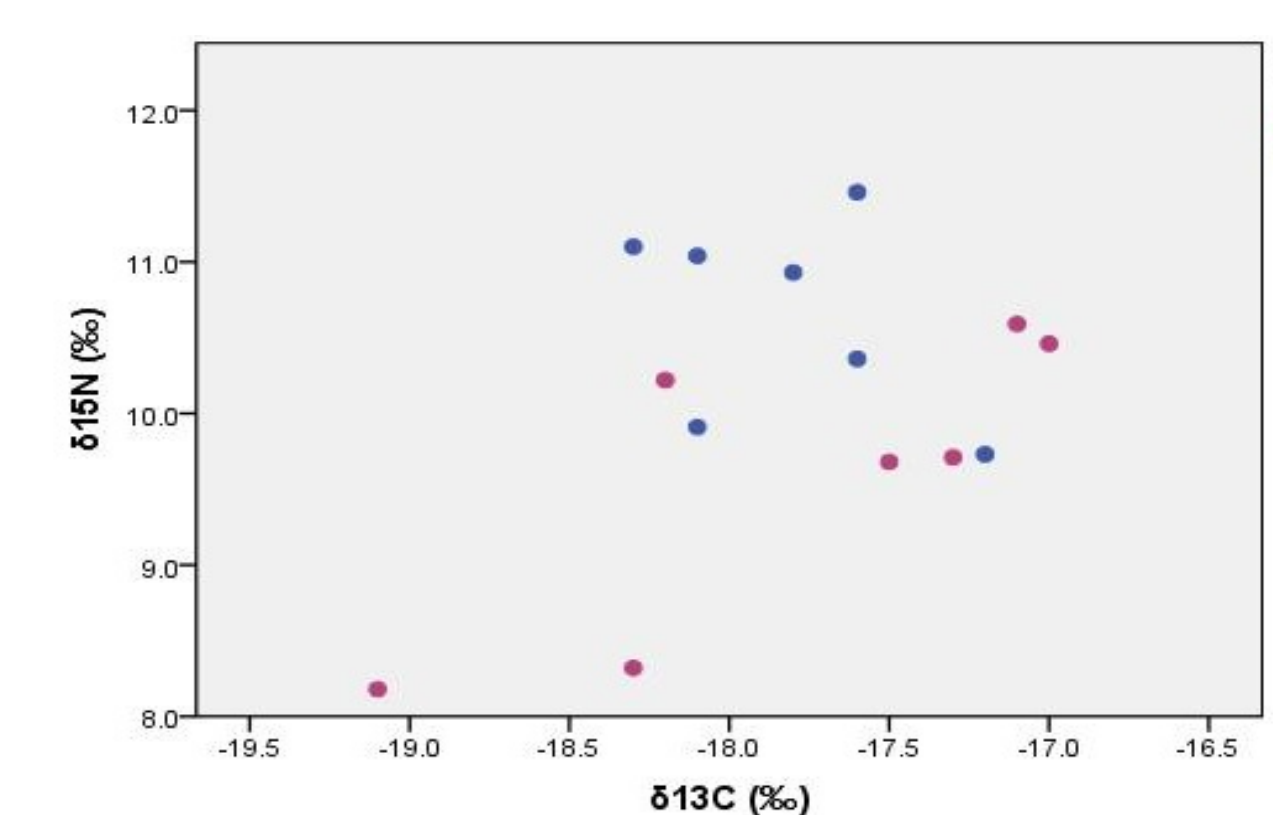
Results cont.

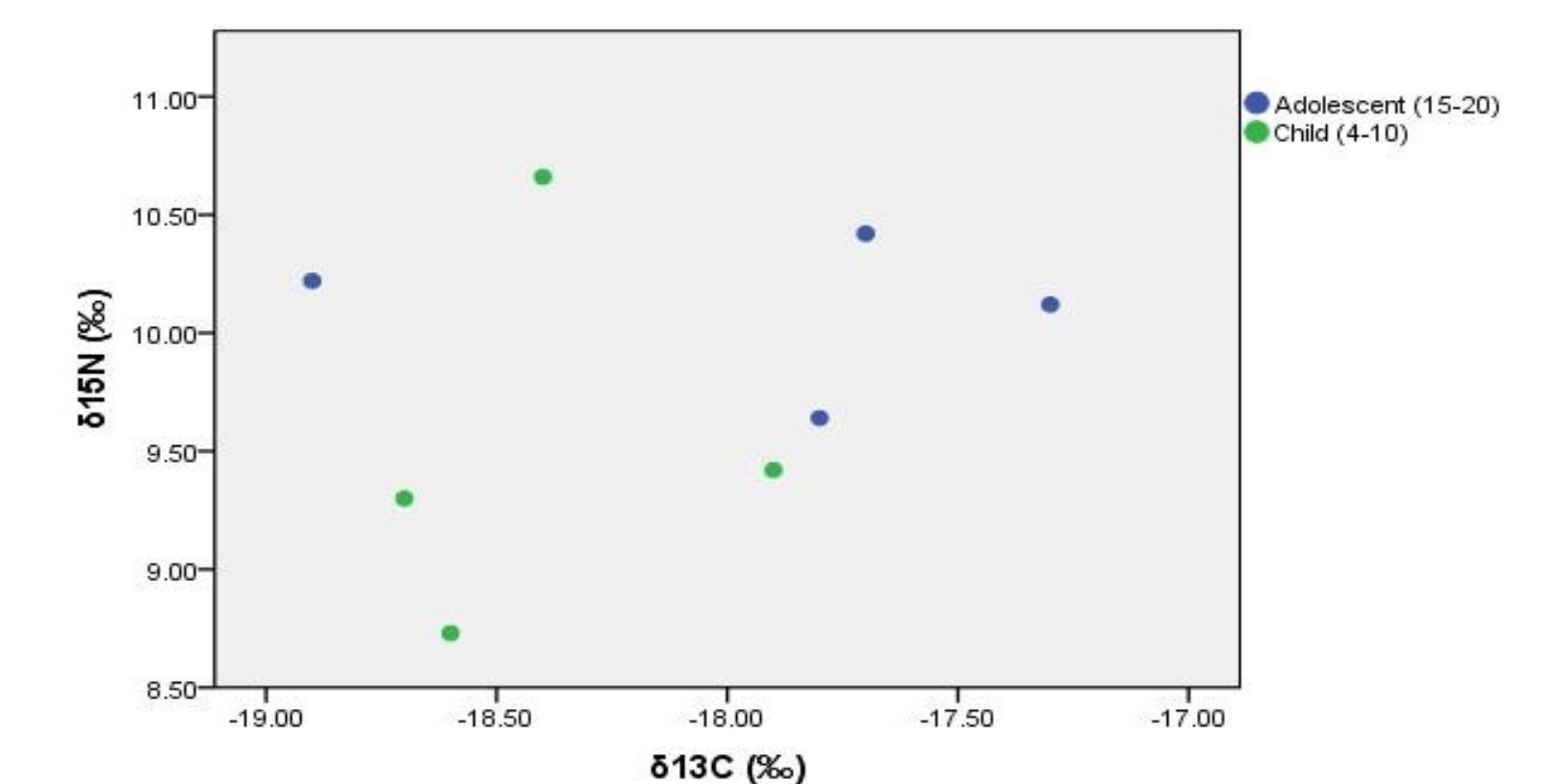*Fig. 1. Adult bone collagen $\delta^{13}C$ & $\delta^{15}N$*

Fig. 2. Juvenile bone collagen $\delta^{13}C$ & $\delta^{15}N$

Molecular Sex Identification

SRY. The Moravian samples did not amplify so no melt data were generated. As expected, modern Czech female samples did not amplify and modern Czech male samples amplified (Figure 3). Modern male Czech amplicons melted between 87-89°C (Figure 4) forming one homogenous group.
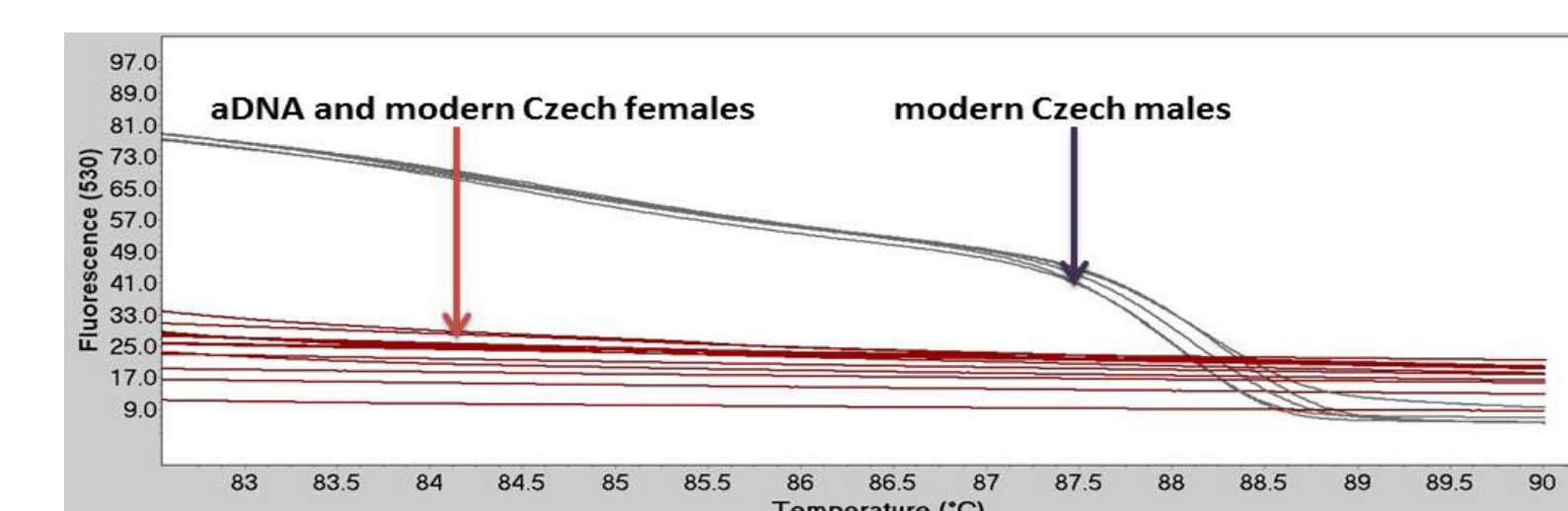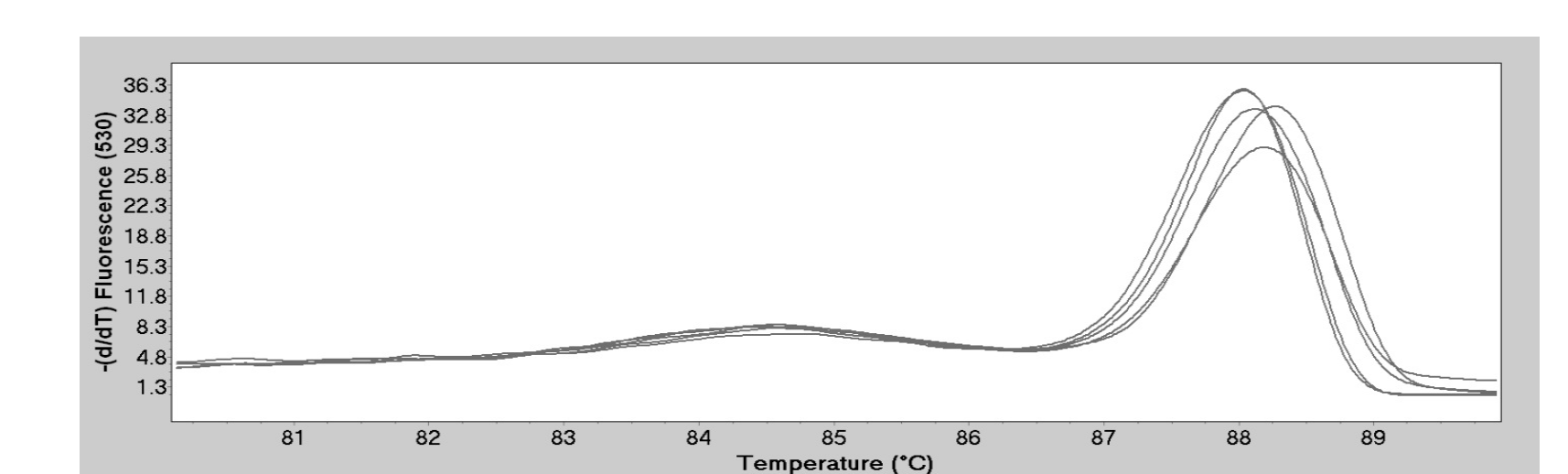

Fig. 3. SRY melting curves

Fig. 4. SRY melting peaks

Amelogenin. All samples amplified (Figure 5) but the melt data for the Moravian samples did not fit the internal standards (modern male and female Czechs).. Melt peaks for modern males and females were at the expected temperatures (Figure 6). The modern female Czech amelogenin amplicons melted with one peak at 82°C. The modern male Czech DNA amplified and amelogenin amplicons melted with two peaks (75-76°C and 82°C).
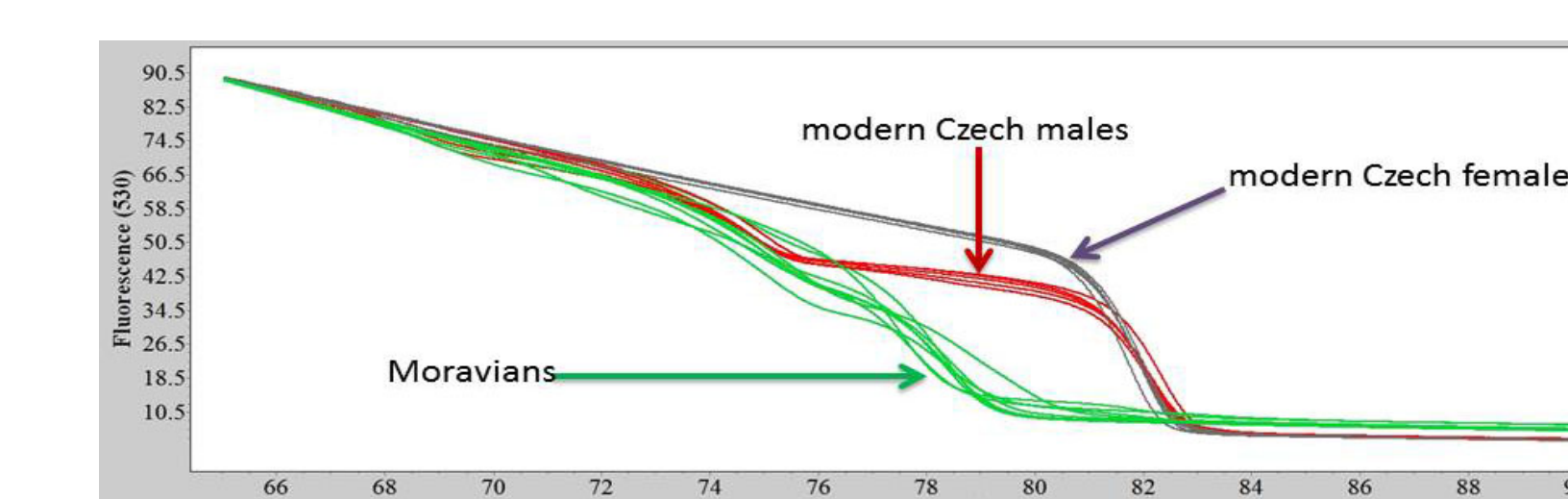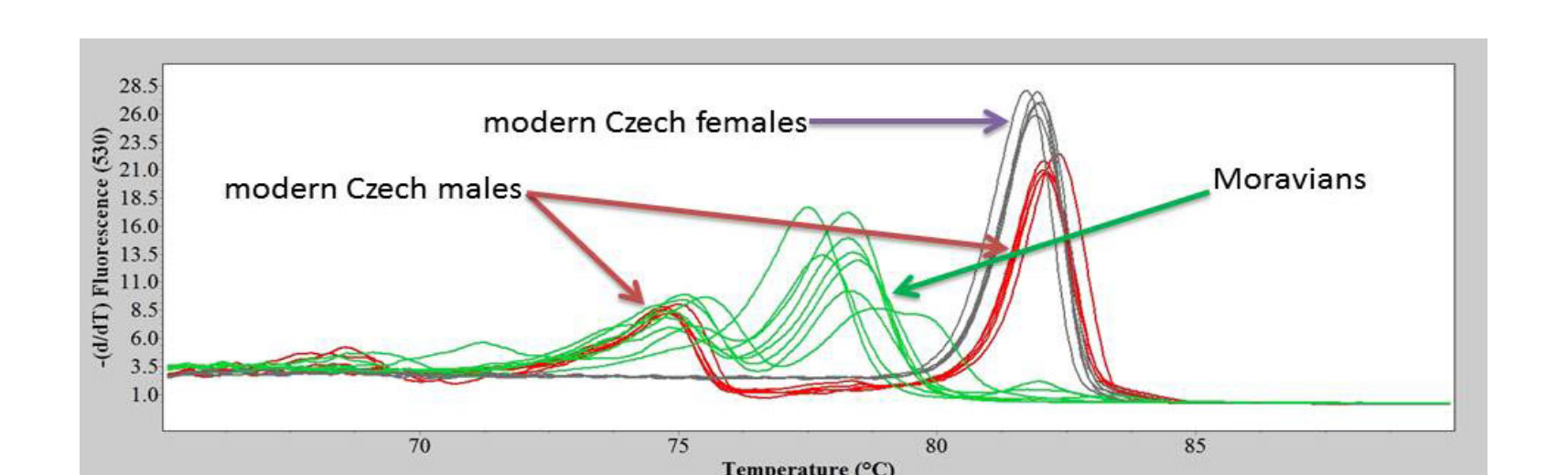

Fig. 5 Amelogenin melting curves

Fig. 6. Amelogenin melting peaks

Conclusions

Dietary Reconstruction. Bone collagen carbon and nitrogen isotope signatures for the Kostelisko human remains are consistent with a largely terrestrial diet that included both plant-derived and animal-derived protein. Human $\delta^{15}N$ values are elevated over regional herbivore values, which cluster around 6.0-7.0‰ (Hakenbeck et al., 2010; Reitsema et al., 2010). suggesting that a substantial proportion of human dietary protein was from animals. Human $\delta^{13}C$ values are also elevated over those of regional domesticated herbivores, which cluster around −21.5‰. This suggests consumption of the C4 plant millet, or animals foddered on millet (Murray and Schoeninger, 1988).

Sex Differences in Diet. The mean nitrogen stable isotope ratio is 1‰ higher in males than in females, which may indicate important dietary differences. Assuming that there is a 4‰ spread between an all-plant and an all-animal protein diet, every 1‰ increase in bone collagen $\delta^{15}N$ represents a 25% increase in the animal contribution to total dietary protein. The magnitude of the difference in mean $\delta^{15}N$ between males and females in the Kostelisko sample thus suggests that males consumed considerably larger proportions of meat and/or dairy, although alternative explanations for variation in bone isotopic signatures between the sexes (e.g., metabolic differences) (Schurr and Powell, 2005) cannot be discounted with the current data.

Molecular Sex Identification. The HRM genotyping method is rapid and accurate. The amplicon lengths are short enough to capture even fragmentary and degraded DNA. But, as with all aDNA studies, the success of genotyping lies in a sufficient starting copy number of DNA. The samples used for this study are over 1000 years old and stored under variable conditions throughout the years. Future work will focus on more sensitive extraction methods to increase starting copy number.

References

See Appendix 1 (handout) for a complete list of references cited.