**BIOL 722 Project Proposal – Natassja Brien**

**Research problem**. Methylation of cytosine residues in DNA plays an important role in gene expression and regulation, and methylation analysis is a growing subfield in ancient DNA (aDNA) research. Although many tools exist to analyse patterns of methylation in DNA generally, such as methylKit in R (Akalin et al., 2012), these tools are not easily applicable to aDNA. One of the main differences between modern and ancient DNA regarding methylation patterns is the natural deamination of cytosine residues over time. In living organisms, this type of damage is repaired by cellular mechanisms (Clark et al., 2006), but in ancient DNA this damage accumulates. Methylated cytosines become thymines, and unmethylated cytosines become uracils, causing confusion after sequencing when thymines in reads could represent a) originally methylated cytosines, b) originally unmethylated cytosines that were converted to uracils then paired with adenines during amplification, or c) original thymines. Distinguishing between these three cases is a key goal of any methylation analysis in aDNA. Although previous aDNA methylation analysis has focused on global or regional methylation levels (Gokhman et al., 2014; Pedersen et al., 2014; Schmidt et al., 2021), site-specific methylation analysis is a new area.

**Research questions and interests**. The research questions are as follows: 1) What tools exist for methylation analyses in aDNA? 2) Which of these tools, if any, can provide methylation status information at a single-base pair resolution, given a set of specific loci? 3) What are the differences in the outputs of each of these tools, and how would that affect the choice of tool for different research goals? My specific interest is a set of 95 loci that are informative about chronological age (Gopalan et al., 2019; Reppe et al., 2017), so one of the goals of this project is to determine whether any of these existing tools can help me determine methylation at a set of given loci.

**Data and methods**. There are a few tools that are specifically designed to analyse methylation in ancient DNA: epiPALEOMIX (Hanghøj et al., 2016), and an updated tool, DamMet (Hanghøj et al., 2019). RoAM, an earlier tool, was developed for use in MatLab and is only applicable to ancient hominins (Hanghøj et al., 2019). Since my research is not at a point where I have sequence data, I will generate simulated aDNA sequence data with gargammel (Renaud et al., 2017), which generates sequences from a reference and induces characteristic aDNA damage, including natural deamination of methylated and unmethylated cytosines (Hanghøj et al., 2019). The human genome GRCh38 (hg38) will be used as a reference sequence. Then, sequences will be analyzed using both DamMet and epiPALEOMIX and specific C:T ratios will be extracted for the ninety-five loci of interest. Results from both tools will be compared.

**Applications**. Methylation analysis in aDNA is a small but growing field, with only a few groups specifically investigating (Gokhman et al., 2016, 2017; Harney et al., 2021; Poullet & Orlando, 2020; Seguin-Orlando et al., 2015). However, methylation analysis has already been used to reconstruct methylation maps of ancient hominin species (Gokhman et al., 2014), make inferences about past environments (Gokhman et al., 2017), and could be used to learn more about people who lived in the past and their experiences (Gopalan et al., 2019; Schmidt et al., 2021).

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