Project plan for degree project for the master program in bioinformatics (**1MB830**)

Thermal plasticity and adaptation along a latitudinal gradient in moor frog (*Rana arvalis*)

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Background

Phenotypic plasticity plays an important role in how organisms respond to environmental variation, and temperature is one of the main drivers of developmental and physiological differences in many species. In *Rana arvalis*, previous common garden experiments have shown that tadpoles from northern populations develop faster than those from southern populations, and that this difference becomes more pronounced at higher temperatures, highlighting the importance of understanding how temperature driven variation arises.

While developmental and life history responses to temperature have been well studied in this species, the molecular basis underlying these responses is still not fully understood. Transcriptomic approaches offer a way to examine which genes respond to temperature and which genes differ consistently among populations. In this project, liver RNA from tadpoles originating from nine populations along a latitudinal gradient from Latvia and Kalmar to Norrbotten was sequenced after rearing individuals at two temperature treatments in a common garden experiment. This dataset provides the foundation for examining the genomic basis of thermal adaptation and plasticity along the latitudinal gradient.

Purpose

The purpose of this project is to analyze transcriptomic responses to temperature and population origin in *Rana arvalis* in order to better understand how thermal plasticity and thermal adaptation are reflected at the genomic level. By examining gene expression and gene co-expression network patterns across populations, the project also contributes to understanding how these responses may have evolved along the latitudinal gradient. This work is relevant for predicting how natural populations might respond to ongoing climate change, and it supports ecological and conservation perspectives by clarifying how developmental responses to temperature are regulated at the genomic level.

Project goal and delimitations

The overarching objective of the project is to understand the genomic basis of thermal adaptation and plasticity along a latitudinal gradient in *Rana arvalis*. To achieve this, the project will compare both temperature treatments and population origins by conducting a reference-based differential gene expression analysis using already available RNA-seq reads from the populations and a reference genome that will be available during the project. The project will continue by performing gene co-expression network analysis to identify groups of genes with coordinated expression patterns that may underlie shared regulatory or

functional responses. Further analyses include identification and expression analysis of transposable elements. Additionally, if time allows, analyses such as exploring alternative splicing patterns, differential exon usage, and SNP calling may also be performed.

Methods and schedule

The project will follow a sequential workflow. Raw RNA-seq reads will be aligned to the reference genome using STAR, and gene counts obtained with featureCounts will be used in DESeq2 for the differential expression analysis to identify genes associated with temperature treatment and population origin. Differential co-expression network analysis will be carried out using WGCNA to detect clusters of genes that are co-expressed and functionally related. Detection and expression analysis of transposable elements will be performed using the TE-Seq pipeline. Finally, if time allows, further analyses such as examining alternative splicing and SNP calling will be explored.

- Week 1 & 2: Literature review.
- Week 3 & 4: Map raw reads to the reference genome and obtain gene counts.
- **Week 5: Submission of revised project plan.**
- Week 5 - 10: Differential gene expression analysis and gene co-expression network analysis.
- **Week 10: Half-time presentation.**
- Week 11 - 13: Detection and expression analysis of transposable elements.
- Week 14 & 15: Extra analyses (Alternative splicing and SNP calling).
- Week 16 - 19: Write and send a report.
- **Week 20: Final presentation.**