Homework 3 | Csenge Petak | 04/10/2020

**Background** *Acartia tonsa* is a wildly distributed abundant zooplankton with large population sizes. This species is greatly important from an ecological point of view; it forms a crucial link between primary producers and fish in the food chain and it has an essential part in biochemical cycling. Due to its vast worldwide distribution, individuals of this species can experience varying levels of environmental conditions like temperature, salinity and pH (chen 2008). Due to ongoing climate change conditions oceans are absorbing more and more atmospheric CO2 that causes world-wide water acidification. If CO2 emission remains unchanged it is predicted that by 2100 global average ocean pH levels could fall to 7.67 (hofmann). Global ocean temperatures are also predicted to increase, at least by ~4oC by 2100 (laffoley). Given the large population size, short generation time and widespread nature of this copepod species adaptation to these changed environmental conditions is likely. However, it is important to understand the extent of evolvability of this species given its ecological role. Also, by investigating the molecular mechanisms of adaptation in this useful model organism, we can more deeply understand the underpinnings of adaptation to rapid environmental change in general. In this study, we focused on how epigenetics mechanisms, specifically DNA methylation, can facilitate adaptation under different temperature and dissolved CO2 conditions.

Individuals collected from wild populations were kept in common garden conditions for 3 generations. The copepods were then separated into four treatment groups: ambient (18oC, 400 ppm), high dissolved CO2 concentration (2000 ppm), high temperature (22oC), and finally both high CO2 concentration and temperature. The temperature and CO2 concentration chosen for the ambient condition were shown to be ideal for *A. tonsa* reproduction and survival, while these were found to be negatively influenced by high temperature and CO2 concentration conditions chosen for this experiment (peck -T, cripps co2). Of each treatment there were four replicates with ~3,000-5,000 individuals, which were reared under treatment conditions for 25 generations. DNA was extracted at generation 0 from individuals in the ambient treatment group, and from individuals from all treatment groups after 25 generations for reduced representation bisulfite sequencing (BS-Seq). As a reference to check the efficiency of the bisulfite conversion *E.coli* (unmethylated) DNA was added.

**Bioinformatics Pipeline**

**Trimming and Mapping** Quality check and trimming with Trimmomatic (bolger) was done for us. Reads were aligned to the *Acartia tonsa* reference genome (tue) using the flexible and time-efficient tool Bismark, which maps the BS-Seq reads while simultaneously performing methylation calling in a single step.

As reference transcriptomefor *P. rubens* doesn’t exist, cleaned reads from each sample were mapped to a reference transcriptome of a closely related species, Norway spruce (*P. abies*). Salmon, a “wickedly fast” command line tool was used to simultaneously map reads to the reference transcriptome and quantify the abundance of these mapped reads [5]. For this program to run, first we had to index the reference transcriptome using Salmon’s indexing tool. The minimum length for a valid match was set to 31. We used the Selective alignment feature of the tool (set by --validateMappings), which enables a more sensitive scheme to be used and thus enhances quantification accuracy. Mapping to the reference containing only exons yielded low quality mapping, thus we decided to include 3’UTRs as well. This resulted in a much higher mapping quality, on average 52% (compared to 32.4% in the ASC population).

**Differential analysis of count data (DESeq2)** We used DESeq2, an R package that uses negative binomial distribution to tests for differential expression. This tool is excellent at accounting for small replicate numbers, large dynamic ranges and outliers and enables the users to calculate the strength, not just the presence of differential expression [6]. DESeq2 takes the counts data matrix as an input (matrix containing counts data from each sample in each gene), which was generated using the tximport R package [7]. Before running the program, we filtered the counts data matrix to contain only genes that have at least 1 read per sample on average. While this excludes gene from the analysis with very low levels of expression, it also cleans the data from potential noise and significantly increases the strength of the statistics as there are less variables to account for (number of genes to test decreased from 66408 to 23887). After the analysis, count data of the most significantly differentially expressed genes was visually inspected to confirm the pattern.

**Results** When all data is considered (~ climate + treatment + day), PCA results showed that the data is clustered along PC2 such that day 10 samples are different from sample at other time points. While climate of origin doesn’t seem to have an effect, the heat+drought treatment is separated by PC2 as well (overlaps with day 10). Thus, we decided to analyse data from day 10 and look at the effect of treatment and climate in that subset of the data. Despite this decision, I decided to include an interesting observation from using the whole dataset (design ~ climate + treatment + climate:treatment). Comparing the effect of climate of origin across all treatments, one gene was found to be significantly differentially regulated, **Class VII chitinase (**p = 4.97e-10). This gene’s expression was much higher in individuals from the hot and dry climate (15.96 log fold change). Interestingly, there was a significant interaction of the effect of climate and treatment on the expression of this gene: it was only upregulated in individuals from the hot and dry climate under the heat+drought treatment (p = 1.585e-8).

In the dataset including data only from day 10, PCA showed a similar pattern as before; climate of origin didn’t seem to have an effect while PC1 (32% variance) clustered samples from the heat+drought treatment. The heatmap reinforced this observation (Figure 1a). When comparing **climates of origin** across treatments, again, one gene was significantly differentially expressed: MA\_129323g0010, which was downregulated in individuals from the hot and dry climate (p=0.011, foldchange = -21.55). Unfortunately, this gene is labelled as unknown on the Congenie database and using NCBI BLAST of the CDS didn’t result in any hit either. When comparing the samples from the **heat+drought** **treatment** to the control, 257 gene were significantly upregulated and 330 were significantly downregulated in heat+drought. When comparing **heat treatment** to control instead, there were only 6 genes that were differentially regulated – 4 up and 2 down compared to control. Remarkably, 3 out of the 4 gene upregulated in heat treatment were genes encoding heat shock proteins (two KDa class I heat shock -like, one KDa class IV heat shock -like). The upregulation of these genes was independent of climate of origin and fascinatingly only the class I heat shock proteins were also upregulated in the heat+drought treatment, making them the only two gene in the dataset that overlapped between treatments (Figure 1b). There were **no significant interactions** between climate and treatment in this reduced dataset.

**Conclusion** The results of our data analysis clearly showed that the effect of treatment on gene expression was much larger than the effect of climate of origin (PCA, heatmap and number of genes differentially expressed when single factor is considered). We found only one gene, a chitinase, that was differentially expressed in individuals from different climates of origin. Chitinases were found to be upregulated in response to fungal infection and drought in *P. abies* [8] and in a highly drought tolerant wild tomato but less so in a susceptible species of the same genus [9]. Since individuals from the hot and dry climate upregulated this gene in the heat+drought treatment much more than individuals from the cold and wet climate in the same treatment, this could be a signature of local adaptation. However, this should be further investigated and overall there was little evidence for the effect of climate of origin. There was a much stronger effect of treatment, and heat+drought had much bigger effect than heat alone. Interestingly, only two genes were differentially regulated in response to both heat and heat+drought, which is similar to what was found in *Populus simonii* were the number of overlappingheat-/drought-responsive genes is also small [10]. These two proteins were heat shock proteins belonging to the same class. Upregulation of heat shock proteins under drought stress was observed in *Pinus halepensis*, thus drought stress alone can result in this response[11]. In a future study, it would be useful to increase the sample size to pick up small but potentially important differences based on climate of origin. Also, drought stress response can be very different in different tissue types [12], so separately analysing them could be more informative.

1. **b)**

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Figure 1: a) Heatmap of counts data of genes in control (C), heat (H) and heat+drought (D) treatments in individuals from coldwet (CW) and hotdry (HD) climates. There is a clear significant effect of the heat+drought treatment. In contrast, when testing the effect of heat alone or climate, there is not clear pattern. b) Expression of one of the KDa class I heat shock-like proteins in treatments and climates as mentioned in a). Climate of origin has no effect on expression, and it is upregulated in both heat and heat+drought treatments. Both a) and b) are of day 10.

**References**

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