Genetic and plastic responses in *Daphnia magna*

Comparison of clonal differences and environmental stress induced changes in alternative splicing and gene expression.

**Jouni Kvist**

Institute of Biotechnology, University of Helsinki
Two strains (clones) of *Daphnia magna*  

Xinb3 (Tvärminne, Finland)  
linb1 (Ismaninger, Germany)
Clonal amplification and manipulation

Eliminate effect of genetic variation between experiments

Determine the contribution of genetic background by analyzing different clonal lines
7+1 treatments, 2 strains (clones)

- control
- crowding
- food
  - cyanobacteria non-toxic
  - cyanobacteria toxic
- environmental poisons
  - carbaryl
- parasite
- predation
  - triops
  - fish

Orsini et al. 2016. *Daphnia magna* transcriptome by RNA-Seq across 12 environmental stressors. *Scientific Data*
RNA-seq analysis with two methods

**Bitseq analysis**
- Expression of whole transcripts (multiple transcripts per gene)
- Powerful MCMC prediction of true expression levels
- Alternative splicing $\rightarrow$ relative changes in expression of transcripts
- Confounded by gene expression, SNPs, indels & broken gene models

**KisSplice analysis**
- K-mer analysis without gene models
- Separates indels, SNPs and splicing events
- Differential expression & significance analysis
- Uses only a small fraction of reads in “assembly bubbles” that result from splicing
Treatment induced changes are moderate and specific to strain and stress condition

- Most differential expression (DE) changes are caused by genes with a single transcript
  - 51-92% of observed changes (mean: 74%)

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Fish kairomones alters expression of transcripts, that are be involved in head and tail spine growth and reproduction.
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In Xinb3, fish treatment mostly up-regulates gene expression (unidirectional)

Fish kairomones alters expression of transcripts, that are be involved in head and tail spine growth and reproduction.

In linb1, fish treatment induces bidirectional expression change

Fish kairomones alters expression of transcripts, that are be involved in head and tail spine growth and reproduction.
Shared transcript & genes are rare between clones

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<th>Xinb1</th>
<th>name</th>
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<tr>
<td>Dapma7bEVm012028</td>
<td>1</td>
<td></td>
<td>1 Copper-zinc cu-zn superoxide dismutase. (92%D)</td>
</tr>
<tr>
<td>Dapma7bEVm012028</td>
<td>0</td>
<td>2</td>
<td>2 Copper-zinc cu-zn superoxide dismutase. (92%D)</td>
</tr>
<tr>
<td>Dapma7bEVm009708</td>
<td>3</td>
<td>0</td>
<td>0 Copper-zinc cu-zn superoxide dismutase. (100%N)</td>
</tr>
<tr>
<td>Dapma7bEVm009708</td>
<td>0</td>
<td>1</td>
<td>1 Copper-zinc cu-zn superoxide dismutase. (100%N)</td>
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<tr>
<td>Dapma7bEVm000805</td>
<td>1</td>
<td>0</td>
<td>0 Protease m1 zinc metalloprotease (100%H)</td>
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Shared transcript & genes are rare between clones

Shared gene functions are common

~20% of DE genes have “same” function in both clones
In rare cases we detect the same differentially expressed transcripts in both strains. In both strains “blue” and “green” transcripts are up-regulated in cyanobacteria treatments relative to control.
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The “red” transcript is down-regulated in toxic cyanobacteria treatment.
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The “red” transcript is down-regulated in toxic cyanobacteria treatment.

The basal expression & isoform use is different between the strains.
Differences between strains accounts for majority of variation
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Clone comparisons (control samples)

• 156244  0a: Single SNPs
• 55816   0b: Multiple SNPs (paralogous genes!)
• 53706   1: Alternative Splicing Events
• 242     2: Inexact Tandem Repeats (very few repeats!)
• 54327   3: Short Indels (<3nt) (a lot of indels → paralogs)
• 6990    4: All others (many complex types → paralogs)

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<th>Alternative exon location</th>
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<td>before consensus 5' start</td>
<td>10404</td>
<td>10715</td>
</tr>
<tr>
<td>after consensus 3' end</td>
<td>8305</td>
<td>9606</td>
</tr>
<tr>
<td>within the consensus gene</td>
<td>14146</td>
<td>17503</td>
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Differential expression analysis of splicing event

• KissDE (R-package), based on DESeq

• Difference of read counts per path among conditions

\[
\log \lambda_{ij} = \mu + \alpha_i + \beta_j + (\alpha/\beta)_{ij}
\]

\[
PSI = f = \frac{\#counts_{variant_1}}{\#counts_{variant_1} + \#counts_{variant_2}}
\]

• 74.5% of splicing events in kisssplice are significantly different among strains
  • Adjusted P-value <=0.05
The strains are massively different

- More than 70% of transcripts are differentially expressed (80,187/114,009) between stains
- Most differentially expressed transcripts have fold change $\geq 2x$
  - Up: $34690/40082 = 86.5\%$
  - Down: $33194/40105 = 82.8\%$
- Half of the DE genes (9093/19911 genes) appear differently spliced between the strains
  - Different transcripts of the same gene have opposite expressed
  - A higher in Xinb3, B higher in linb1
Many of the clone specific differences are missed if analyzing only gene expression
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Summary

Stress treatments induce a moderate number of expression changes
  • Single transcript / gene (gene expression) changes
  • DE genes unique to each treatment & different between clones
  • Gene functions of DE genes largely conserved

Differences between strains huge
  • Basal expression differences in alternative transcripts
  • Dominance effect in Xinb3
  • Splicing -> Fuel for adaptation & evolutionary change
Acknowledgements

**Luisa Orsini**, Environmental Genomics Group, University of Birmingham

**Luc De Meester**, Laboratory of Aquatic Ecology, University of Leuven

**Michael Pfrender**, Department of Biological Sciences and Environmental Change Initiative, Galvin Life Science Center

**Mieke Jansen**, Laboratory of Aquatic Ecology, University of Leuven

**Don Gilbert**, Biology Department, Indiana University

**Mikko Frilander**, Institute of Biotechnology, University of Helsinki