Variation in the genetic response to high temperature in *Montastraea faveolata* from the Florida Keys & Mexico

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Many threats currently face coral reefs

- **Overfishing & Extractive resource use**
- **Pollution & Coastal Development**
- **Climate Change:**
  - Rising sea level
  - Ocean Acidification
  - Rising Sea Surface Temperatures (SST)

This situation is exacerbated in Caribbean reefs by declining juvenile recruitment.

- Combined with reduced growth, and increased mortality this is leading to population declines.
- Successful sexual reproduction is necessary for recovery and persistence of these ecosystems.

Can corals adapt to rapid climate change?

The answer depends upon the adaptive potential inherent to coral populations!

Con:

• Corals currently live at or near their thermal maximum throughout much of their range
• Local adaptation will be hindered by long distance migration

Pro:

• Corals have the ability to switch symbiont types
• There is Evidence for local adaptation in several species:
  • small scale population structure has been observed in multiple coral species
Each member of the holobiont contributes to fitness

- It is important to understand the contribution of different symbiont types
- Coral larvae allow investigation of the host response in isolation
Larvae are critical for coral survival:

• By maintaining genetic connectivity among populations

• Dispersing larvae are the only way corals can escape unsuitable habitat and exploit new ones
Gametes from multiple parents from two locations were collected:

Study sites are linked by regional currents

No geographical populations structure was detected
Temperatures in Mexico reach higher extremes than Florida.

Annual means (05 - 08) differ by ~2° C.

Summer highs average 33 in Mexico and 31 in Florida.

Florida has a greater thermal range with lower winter lows.
Crosses included offspring from a minimum of 3 parents from each site

- Collect gametes from parents at both populations
- Pool sperm and eggs to generate batch crosses
- Raise larvae at 2 temperatures
  - 27 and 30 in Florida
  - 27.5 and 32.5 in Mexico
- Need larval tissue to avoid genetic material from the algal symbiont!
Do corals differ in their response to thermal stress depending on the location where they live?

1. Transcription profiles will reflect location specific variation in thermal stress response

2. Thermal stress response will include differential expression of genes for previously identified stress markers
   1. Heat shock proteins
   2. Oxidative stress genes

3. DEGs relating to cell structuring and development will be observed
Higher temperatures resulted in more malformed larvae.

Transcription profiling is a powerful tool for observing physiological variation even in the absence of obvious external differences.

Developmental differences between temperatures were not observed at 24 or 48 hours.

<table>
<thead>
<tr>
<th>Location</th>
<th>Age</th>
<th>Temperature</th>
<th>Irregular Embryos</th>
<th>Normal Embryos as Invaginated</th>
<th>Normal Embryos as Gatrula</th>
</tr>
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<tbody>
<tr>
<td>Florida</td>
<td>22</td>
<td>27</td>
<td>6</td>
<td>96</td>
<td>0</td>
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<td></td>
<td>22</td>
<td>30</td>
<td>7</td>
<td>98</td>
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<td></td>
<td>46</td>
<td>27</td>
<td>0</td>
<td>3</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>30</td>
<td>50</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Mexico</td>
<td>21.5</td>
<td>27.5</td>
<td>4</td>
<td>94</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>21.5</td>
<td>31.5</td>
<td>8</td>
<td>99</td>
<td>0</td>
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<td></td>
<td>28</td>
<td>27.5</td>
<td>9</td>
<td>100</td>
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</tr>
<tr>
<td></td>
<td>28</td>
<td>31.5</td>
<td>20</td>
<td>0</td>
<td>100</td>
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<td></td>
<td>50.5</td>
<td>27.5</td>
<td>11</td>
<td>0</td>
<td>99</td>
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<td></td>
<td>50.5</td>
<td>31.5</td>
<td>4</td>
<td>0</td>
<td>100</td>
</tr>
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</table>
There is a strong geographical component to the response of larvae to thermal stress
Response to thermal stress has both conserved and site specific components.

At high temperatures there is little overlap in DEGs. By 48 hours ~25% are shared, but still many are population specific.
Our ability to interpret the function of these differences is limited by a lack of annotation.

Only a small fraction of the genome is represented on the array.

Even less (~20%) is functionally annotated.
Conclusions:

1. There is a strong **geographical component** to the response of coral larvae to thermal stress

   1. Management efforts at one location may not give the same results in the other

2. Application of thermal stress leads to a **conserved response** across populations

3. Understanding the function of DEG’s requires better annotation of cnidarian genomes and consideration of gene function at specific life stages
The *A. palmata* transcriptome provides a comprehensive set of ESTs with which to survey gene expression.

<table>
<thead>
<tr>
<th></th>
<th>N sequences</th>
<th>total length [Mb]</th>
<th>Avg length (sd) [bp]</th>
<th>Depth of coverage (max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>raw reads</td>
<td>964,519</td>
<td>384</td>
<td>398 (118)</td>
<td></td>
</tr>
<tr>
<td>trimmed reads</td>
<td>741,271</td>
<td>320.5</td>
<td>432 (64)</td>
<td></td>
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<tr>
<td>contigs</td>
<td>42,630</td>
<td>44</td>
<td>1034 (624)</td>
<td>5.6 (315)</td>
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<tr>
<td>&gt;1 kb</td>
<td>16,274</td>
<td></td>
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<tr>
<td># annotated</td>
<td>29,413</td>
<td></td>
<td></td>
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<tr>
<td>Singletons</td>
<td>45,390</td>
<td>20</td>
<td>441 (95)</td>
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<tr>
<td># annotated</td>
<td>16,848</td>
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</table>
Coverage of the *A. palmata* transcriptome is comparable to the *N. vectensis* genome.
A 135K feature microarray will enable more detailed surveys of gene expression

Nimblegen 12-plex slides will be used to profile gene expression patterns in A. palmata adults and larvae

Tests for interspecific hybridization will be performed using A. cervicornis

• 2 probes per contig
• 1 probe per singleton
• Enriched for stress & calcification related transcripts

http://ddlab.sci.univr.it/FunctionalGenomics/facility.html
Thank You!

Baums Lab: Iliana Baums, Meghann Devlin-Durante, Katey Glunt, Jennifer Boulay, Dannise Ruiz, John Parkinson, Dennis Xu

Medina Lab: Michael De Salvo, Chris Voolstra, Julia Schnetzer Erika Diaz, Collin Closek, Shini Sunagawa, Monica Medina

NOAA: Margaret Miller, Abel Valdivia

Secore, CARMABI

NSF

NSF Graduate Research Fellowship Program
Sequencing results also identify a wealth of new molecular markers
Even with limited annotation, enrichment of key functions is observed:

<table>
<thead>
<tr>
<th>Common to both populations</th>
<th>Unique to Florida</th>
<th>Unique to Mexico</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Up</strong></td>
<td><strong>Unique to Florida</strong></td>
<td><strong>Unique to Mexico</strong></td>
</tr>
<tr>
<td>cell proliferation, growth, development</td>
<td>autophagy, protein degradation</td>
<td>degradation</td>
</tr>
<tr>
<td>cell structure, motility</td>
<td>cytoskeleton, cell adhesion</td>
<td>DNA</td>
</tr>
<tr>
<td>cytoskeleton, cell adhesion</td>
<td>DNA repair</td>
<td>ER, ion binding, transport</td>
</tr>
<tr>
<td>lipid binding/metabolism</td>
<td>iron transport</td>
<td>lipid binding</td>
</tr>
<tr>
<td>response to stress</td>
<td>lipid binding/metabolism</td>
<td>metabolism</td>
</tr>
<tr>
<td>transcription, transcription regulation</td>
<td>metabolism</td>
<td>protein binding</td>
</tr>
<tr>
<td></td>
<td>ribosome, translation</td>
<td>response to oxidative stress</td>
</tr>
<tr>
<td></td>
<td>transcription regulation, development</td>
<td>transcription regulation</td>
</tr>
<tr>
<td><strong>Down</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apoptosis</td>
<td>cell adhesion, development</td>
<td>cell growth, development</td>
</tr>
<tr>
<td>cell proliferation, growth, development</td>
<td>cell growth, development</td>
<td>cell structure</td>
</tr>
<tr>
<td>cytoskeleton, cell adhesion</td>
<td>cell structure, motility</td>
<td>degradation</td>
</tr>
<tr>
<td>DNA</td>
<td>degradation</td>
<td>energy metabolism</td>
</tr>
<tr>
<td>electron transport, oxidative phosphorylation</td>
<td>protein biosynthesis</td>
<td>ER, iron ion binding, transport</td>
</tr>
<tr>
<td>metabolism</td>
<td>protein degradation</td>
<td>metabolism</td>
</tr>
<tr>
<td>response to oxidative stress</td>
<td>protein biosynthesis/folding/transport</td>
<td>protein</td>
</tr>
<tr>
<td>response to stress</td>
<td>response to oxidative stress</td>
<td>response to oxidative stress</td>
</tr>
<tr>
<td>RNA, mRNA modification</td>
<td>RNA binding</td>
<td>response to stress</td>
</tr>
<tr>
<td>signaling</td>
<td>ribosome, translation</td>
<td>ribosome, translation</td>
</tr>
<tr>
<td>translation, ribosomes, protein biosynthesis</td>
<td></td>
<td>transcription, apoptosis</td>
</tr>
</tbody>
</table>

- Common to both populations
- Unique to Florida
- Unique to Mexico

*Note: The table with red circles highlights specific functions that are unique to Florida and Mexico.*
Several genes related to heat and oxidative stress response are downregulated:
Heat shock proteins blast to *N. vectensis* genome with high homology:

**Hsp 90 a: AOSC617**

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**Down-regulation of Hsp90 could change cell cycle distribution and increase drug sensitivity of tumor cells**

The consequences of expressing hsp70 in Drosophila cells at normal temperatures.

J H Feder, J M Rossi, J Solomon, et al.

*Genes Dev.* 1992 6: 1402-1413

Access the most recent version at doi: 10.1101/gad.6.8.1402
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Image Source: The World Resources Institute
All 3 main effects influence gene expression profiles:

M – Mexico (green)
F – Florida (blue)
1 – day 1
2 – day 2
m – avg temp. (black)
h – high temp. (red)
Do corals differ in their response to thermal stress depending on the location where they live?

- Gamete bundles collected from Mexico and Florida
- Gametes pooled in batches and allowed to fertilize 1 hour
- Fertilized eggs distributed into aquaria at 2 treatment temperatures (mean & high)
- RNA was extracted from samples at 24 & 48 hours of development
- 3 replicates of each sample used to interrogate 1300 feature microarray

Microarrays were run for 2 time-points, at 2 temperatures, from both locations.