15. Studies on Coral Diversity and Biology Using Emerging Cytogenetic and Molecular Approaches

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1. Previous Studies Related to Molecular Analysis of Stony Corals

Stony corals are among the most diverse organisms in the world. They possess high genetic and morphological variation with nearly 1,300 described extant species[1]. Stony corals are the major component of the coral reefs which are formed by pilling up dead hard skeletons on top of another over long period of time. This immense structure provides important ecological goods and services, in which global economic value is estimated to be 375 Billion USD/yr coming from recreation, sea defense services, and food production[2]. However, the world's coral reefs cover has been declining for the past several decades. It is estimated that 20% of the world's coral reefs have been destroyed with no prospects of recovery, and 24% are under imminent risk of collapse[3]. The Great Barrier Reef, the world's most extensive coral reef system, has lost 40% of its cover since 1986[4]. This decline is mainly attributed to coral bleaching brought by increase of the sea surface temperature, as driven by the climate change.

The extent of the environmental threat caused by climate change to stony corals can be understood more by studying the coral biology, physiology, and ecology. Fortunately, molecular sequence data of corals have been become increasingly available as sequencing cost has decreased over the years. These sequence data have become useful to understand more the molecular mechanism of coral responses to environmental stresses. Recent developments in molecular studies of corals have unlocked the fundamental understanding of the biology and development of stony corals. Shinzato and colleagues[5] used eighteen coral genomes of Acroporid species to further understand the development and diversification of today's species. Levy and colleagues[6] have comprehensively elucidated the existence and properties of numerous cell types in the life cycle of a stony coral using single-cell transcriptomics. Transcriptomics and gene expression studies have been broadly used in investigating the susceptibility of corals to bleaching under heat stress. These studies have led to fundamental discoveries in the physiological response and impacts to corals. Although genomic data of several stony coral species have become available, model organisms in this group of animals are absent. This might be due to absence of chromosome-level genomic assembly which is the highest quality of genomic data an organism can have.

Molecular cytogenetics and transcriptomic studies are another set of promising methods in molecular analysis even without chromosome-level genomic data. Despite of this, very few molecular cytogenetic and transcriptomic studies were conducted on stony corals. Earliest studies on stony coral chromosomes were

based on light microscopy, thus most cytogenetic data is limited only on chromosome numbers and structures. In addition, there has been limited understanding on how stony corals can recover on a transcriptomic scale and its implications in conservation efforts.

In this chapter, we will introduce our recent research on stony corals that utilize cytogenetic and molecular approaches, and its potential applications in further biological studies and conservation efforts on scleractinian corals.

2. Molecular Cytogenetics on Corals

2.1 Importance and Application of Molecular Cytogenetics

2.1.1 Karyotyping and Genetic Mapping

Cytogenetics is the branch of genetics that studies the structure of condensed DNA (chromatin, chromosome) within the cell nucleus. The early foundation of cytogenetics is to create the individual's karyotype, which is the process of pairing and ordering all the chromosomes of an organism. Chromosomes were normally prepared from tissue with actively dividing cells containing high number mitotic cells from which condensed forms of nuclear DNA (chromosomes) can be observed. Chromosomes were then stained to be effectively visualized under the light microscope. One popular of which is trypsin treatment followed by Giemsa staining which provides distinct banding patterns, called G-band, to each of the chromosomes. The exact mechanism how the trypsin and Giemsa create the banding patterns is still unknown. Researchers suggested that the heterochromatic regions which are regions of the chromosomes that are less condensed, contain few genes and AT-rich, and are stained more darkly with the Giemsa stain. Giemsa staining produces between 400 and 800 bands distributed among the 23 pairs of human chromosomes. Banding patterns produced by Giemsa have been used until today as markers to give unique location identifier to each known gene of many model organisms.

The generated banding patterns, which should be identical for each chromosome pair, are used to effectively generate the karyotype for an organism with chromosomes of same size. Standard procedures of karyotyping involve pairing the homologous chromosomes and arranged them according to decreasing size except for the sex chromosomes. Chromosomes are then numbered by assigning the starting number 1 to the longest chromosome followed by the rest.

2.1.2 Chromosomal Defects Detection

Using the karyotype, it is easier to identify chromosomal abnormalities that cause several known genetic diseases. The easier to observe are the aneuploidies which are cells having abnormal numbers of chromosomes caused either by loss or duplication of one of the chromosomes. Other abnormalities that can be detected using karyotype are translocations, which happens when the segment of the chromosome breaks and reattaches to a different chromosome. Chromosomal deletion, addition, and substitution are other known chromosomal aberrations that can be observed from the unusual banding patterns from the karyotype. However, to validly identify the aberrations of the chromosomes, it is important to obtain karyotypes from multiple cells to be able to differentiate it from chromosomal defects caused by technical preparation or some inherent structural variation between homologs.

Cytogenetic techniques have become advanced and geared away from conventional techniques. With the

advent of fluorescence microscopy, standard staining techniques can be combined with fluorescent stains and labels which gives better image quality and resolution for karyotyping. Even the specific genes can be labeled using fluorescent probes that can be hybridized to its location in the chromosomes. With this, translocation of not only chromosome portions but also specific genes can be observed. This technique is called fluorescence *in situ* hybridization (FISH).

With the advent of whole genome sequencing, gene mapping along each chromosome can be done *in silico*. A great number of genes from model organisms has been mapped on their chromosomes and this has become very useful to understand more of the organism's genetics and physiology. However, routine detection of aneuploidy using sequencing data is tedious and not economical as of this time. In addition, whole genome data is available only for few organisms and only a portion of them have chromosome-level assembly. Thus, FISH still offers easier approach for molecular cytogenetics even for non-model organisms.

2.1.3 Sex Determination

Aside from cytogenetic mapping, one of the most important milestones of cytogenetics in understanding human biology is the discovery of sex chromosomes. Advancements in molecular cytogenetics allow scientists to understand the chromosomal and molecular mechanisms of sex determination not only in humans but also in wide range of organisms.

2.2 Challenges of Molecular Cytogenetics of Corals

Scarcity of cytogenetic data from stony corals can be attributed to several factors. The first one is the relatively slow growth rate of corals compared with most of animals. This means that they contain actively dividing cells, which are often used in microscopic observation of chromosomes. The second one is their small genome size compared to vertebrates. Initial cytogenetic analysis showed that most stony corals have 28 chromosomes (2n = 28). This resulted that small genome size is distributed to 28 chromosomes, resulting to shorter individual chromosomes compared with species with small genome size but with few chromosome numbers. These short chromosomes cannot produce enough Giemsa banding patterns to be used to distinguish and pair the chromosome pairs. Aside from their size, there is high degree of similarity among chromosome lengths. This also causes difficulty if researcher attempts to create the karyotype based solely on chromosome size.

2.3 Improvements and Recent Findings in Coral Cytogenetic Research

To circumvent the problems of obtaining high numbers of mitotic cells for microscopic observations, cells can be obtained early embryonic cells (1-day old) which contains actively dividing cells. We have been collecting artificially fertilized coral embryos from a coral community in Otsuki, Kochi, Japan. Using chromosomes from coral embryos, molecular cytogenetic study of corals can be conducted. Table 1 shows the number of spawning events from which coral embryos were collected from 2007-2021.

Table 1 Number of spawning events from which coral embryos were collected. The 149 total number is composed by 47 different stony coral species.

	May	**Jun**	**Jul**	**Aug**	**Sep**	**Total**
2007			3	1		4
2009				2		2
2010			1	2		3
2011				1		1
2012			1	8		9
2013			10	4		14
2014		1	16	5		22
2015		1		5		9
2016		6	11	4		21
2017		1	4	11		16
2018	7	6	4	3		20
2019		8	9	1		18
2020			4	3	1	8
2021				2		2
Grand total						**149**

Although new stains and technique modifications have been introduced to enhance the visualization of chromosomes under the light chromosomes, coral chromosomes were best observed using fluorescence microscopy with fluorophores and DAPI as counterstain. We have been detecting the chromosomal location of ribosomal RNA genes, Alu repeats, telomeres in the chromosomes of stony corals from the embryonic cells using FISH. To date, molecular cytogenetic information of five stony coral species from three different families of stony corals have been reported. These are *Acropora solitaryensis* and *Acropora pruinosa* (Acroporidae); *Coelastrea aspera* (*Coelastrea incrustans*); *Platygyra contorta* (Merulinidae); and *Echinophyllia aspera* (Lobophylliidae)[7-12]. In those studies, new cytogenetic evidences were presented, including information regarding chromosome numbers, ribosomal RNA (rRNA) gene loci, the presence of homogeneously staining regions (HSR), and some repeated sequences shared with human satellite DNA. However, a universal chromosomal marker for stony corals was an important discovery for effective karyotyping using FISH. Traditionally, fluorescent probes were prepared from phage, cosmid, BAC, and YAC, all of which contain tens to hundreds of thousands of nucleotides that can be hybridized on the chromosomes. However, due to their immense length, they might contain repetitive elements, in which chromosomal locations are highly varied across individuals and species. This may cause the probe not to hybridize on the target chromosomes when tested to chromosomes of other individuals or species. We have been developing fluorescent probes as chromosome markers using sequence of tandemly repetitive genes such as rRNA, histone, and spliceosomal small nuclear RNA (snRNA) genes. The sequence of these genes was highly conserved, meaning there is little sequence variations across individuals and species, thus the probes can bind to relatively wide range of taxa. In addition, their tandemly repetitive characteristics make them to produce a bright hybridization signal which is important for more accurate and reliable detection.

We developed DNA probes from core histone and snRNA genes that can potentially detect chromosomes of multiple species of stony corals[12]. Sequences of the probes were made publicly available in online databases, so other researchers can use it as chromosome markers for molecular cytogenetics of corals. Although not all

the 28 chromosomes of these corals cannot be detected yet for karyotyping using FISH, this study was a crucial step to discover more of other repetitive genes that can provide a suitable marker for other remaining chromosomes.

2.4 Future Directions and Application of Molecular Cytogenetics in Corals

2.4.1 Biodiversity and Conservation

Karyotyping and molecular cytogenetics on corals have many potential applications. Once sufficient number of stony corals species have been karyotyped, and the chromosomal characteristics are compared (chromosome number, lengths, centromere locations, gene loci), these data can provide insight into how chromosome structure and genome changes over the course of evolution, which may help the stony coral systematics and taxonomy. This is particularly important for this group of animals as the integration of their molecular and morphological characteristics has been a problem in classification. Molecular cytogenetic data may bridge the gap between the two characters as it reflects later level information than molecular sequence data but early information than morphological data. Understanding stony coral evolution can unlock its biodiversity which are vital for conservation and protection strategies of stony corals. Biodiversity is also an important factor in measuring ecosystem-level resilience against environmental stresses.

2.4.2 Artificial Propagation

Another important application of molecular cytogenetic data is that it provides the chromosomal information for development and improvement of new breeds of corals through polyploidy induction. Polyploids are individuals with tripled or quadrupled sets of chromosomes in their cells. Polyploidy induction is already used in some aquaculture facilities to increase the growth rate of fish[13,14], bivalves[15,16] and shrimps[17,18]. These organisms have already established karyological data, unlike stony corals, which makes the technology easier to develop. Since coral nurseries and in-land breeding of corals have become increasingly popular as one of strategies to restore degraded reefs through transplantation, data related to coral chromosomes are prerequisite to evaluate the effectivity of this chromosomal manipulation technique on coral breeding.

3. Gene expression studies on coral bleaching and recovery

3.1 Brief Background on RNAseq Analysis in Stony Corals

The application of transcriptomic analysis (RNAseq) on corals has been key to understanding the impact of anthropogenic environmental disturbance through gene expression analysis of key response genes involved. Physiologically, the negative impacts on corals have been intensively studied, but much remains to be discovered in the processes behind it at the molecular level.

In a study on sediment stress by Bollati et al.[19], RNAseq on ex-situ sediment stress experiments of two coral species under family Merulinidae in Singapore uncovered a shared transcriptomic response to sediment stress. This involved a broad range of genes related to energy metabolism and immune response, specifically for anaerobic glycolysis, hydrogen sulfide metabolism, and pathogen pattern recognition. These results suggest that as sediment stress occurs, several adaptive mechanisms may be upregulated to try to augment effects of hypoxia and reduced symbiont productivity. In a previous study by Poquita-Du et al. [20] on the transcriptomics of sediment stress, this time with heat stress in *Pocillopora acuta*, it was found that sedimentation was

synergistic with heat stress in inducing a complex transcriptomic response. It is speculated that some coral species can adapt to sedimentation stress through metabolic and immunity trade-offs which should be considered in future studies in coral ecology.

Majority of coral transcriptomic studies are focused on heat stress and bleaching due to its fast spread and high mortality rate in affected coral reefs. In a 2017 study by Ruiz-Jones, Palumbi[21], their transcriptomic analysis on *Acropora hyacinthus* exposed to tidal heat pulses showed that certain group of genes have a coordinated response to a temperature threshold. This set of genes were found to be involved with unfolded protein response (UPR) in the endoplasmic reticulum. It was in this study that the UPR was identified as the first line of defense in a coral species experiencing heat stress. This was further confirmed in laboratory tank experiments involving acute heat stress exposure, where these genes intensified in expression as bleaching became more severe.

In another study, the long-term recovery of corals in a reef after a bleaching event was monitored through RNAseq analysis. This allowed for the monitoring of the metabolic recovery of the bleached corals, which is key in determining the resilience of coral ecosystems. This study followed the recovery of seven colonies of *A.hyacinthus* bleached in the field[22]. It was found that around 20% of the host transcriptome was differentially expressed, and it remained largely disturbed after 6 months water temperatures have returned and even after 4 months after symbiont populations rebound. These results show the toll of bleaching on the physiology of corals on the molecular level.

3.2 Methodology of RNAseq Analysis Experiments

Many transcriptomic studies on coral bleaching in an aquarium environment have focused on acute to long term responses to heat and bleaching, but not much focus on the recovery after long-term heat stress and bleaching, in terms of symbiont content and of host transcriptome status. We wanted to further explore this through an aquarium tank experiment. Coral nubbins were placed in glass aquariums filled with seawater with adjustable water temperatures (Figure 1). This setup allowed for us to maintain the viability of the nubbins, and to perform visual observations of each sample to track changes in the nubbins.

Figure 1 Aquarium Tank Setup of experimental tanks (A). Bleaching nubbins being monitored (B)

We obtained four coral colonies of *A. hyacinthus* from Nishidomari, Kochi, Japan and colonies were maintained in a tank with running seawater at original sea surface temperature. Nubbins were cut from each colony and mounted to each tile with epoxy, then acclimated at the previous conditions before the experiment was started to minimize the effect of the manipulation stress. The experimental setup of the tanks was composed of three control tanks, and three experimental tanks each containing eighteen nubbins per colony. The environment was maintained with artificial light and seawater circulation, and scheduled heat stress temperatures were assigned to the experimental tanks.

Bleaching period was initiated by a stepwise increase in seawater. Recovery period was then simulated by gradually returning the temperature control conditions. After each stage, Symbiodiniaceae density was measured using a hemocytometer, chlorophyll a (chl a) concentrations were obtained from spectrophotometric measurements, and samples were fixed for total RNA extraction. Symbiodiniaceae physiological parameters were normalized using nubbin surface area measurements obtained from 3D models of nubbins scanned in MicroCT. Total RNA was extracted following TRIzol protocol. The eukaryotic mRNA was used to prepare a cDNA library for Illumina sequencing.

The subsequent steps (Figure 2) were performed in-silico using a 24-core CPU, 64GB RAM, 2TB SSD desktop computer running Linux OS. Quality and length of the sequence reads were verified using the program FastQC[23]. Illumina adapters used for sequencing was trimmed from the reads using Trimmomatic[24]. Sequence reads were mapped to an *A. hyacinthus* reference transcriptome[25] using a script included in the Trinity package[26] to determine expression levels per sample and proportion of reads mapped to the reference transcriptome was also determined.

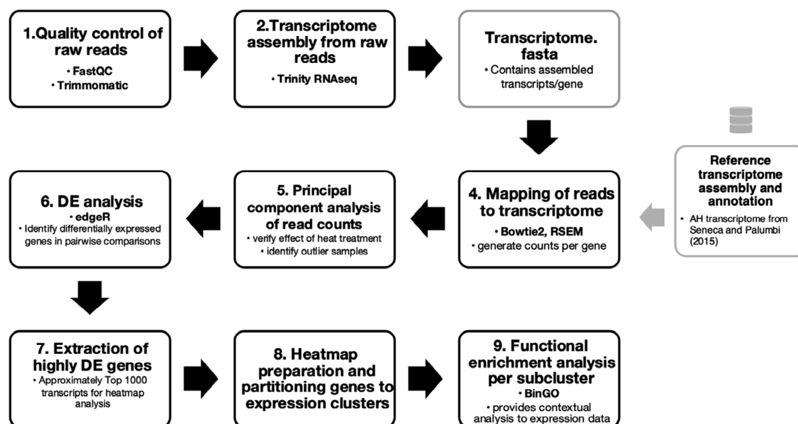

Figure 2 Flow of in-silico analysis for RNAseq data. Ultimately in this study, a reference transcriptome assembly and annotation were used.

Principal component analysis was performed to verify validity of the reads using a script also included in the Trinity package[26]. Differentially expressed genes were determined using the edgeR package[27] by comparing across the samples and normalizing the changes in expression. This differential expression analysis was then used to generate a heatmap and clustering from the top 1000 DE contigs across the 3 comparisons (Figure 3) were selected for heatmap, clustering, gene ontology analysis based on counts and corrected P. value using the Trinity script[26]. Using BinGO[28] plugin of Cytoscape[29], functional enrichment analysis (FEA)

of the gene ontology biological processes was performed on the heatmap clusters. Following FEA, text mining was performed on the enriched annotations of the clusters to narrow down target pathways for further analysis.

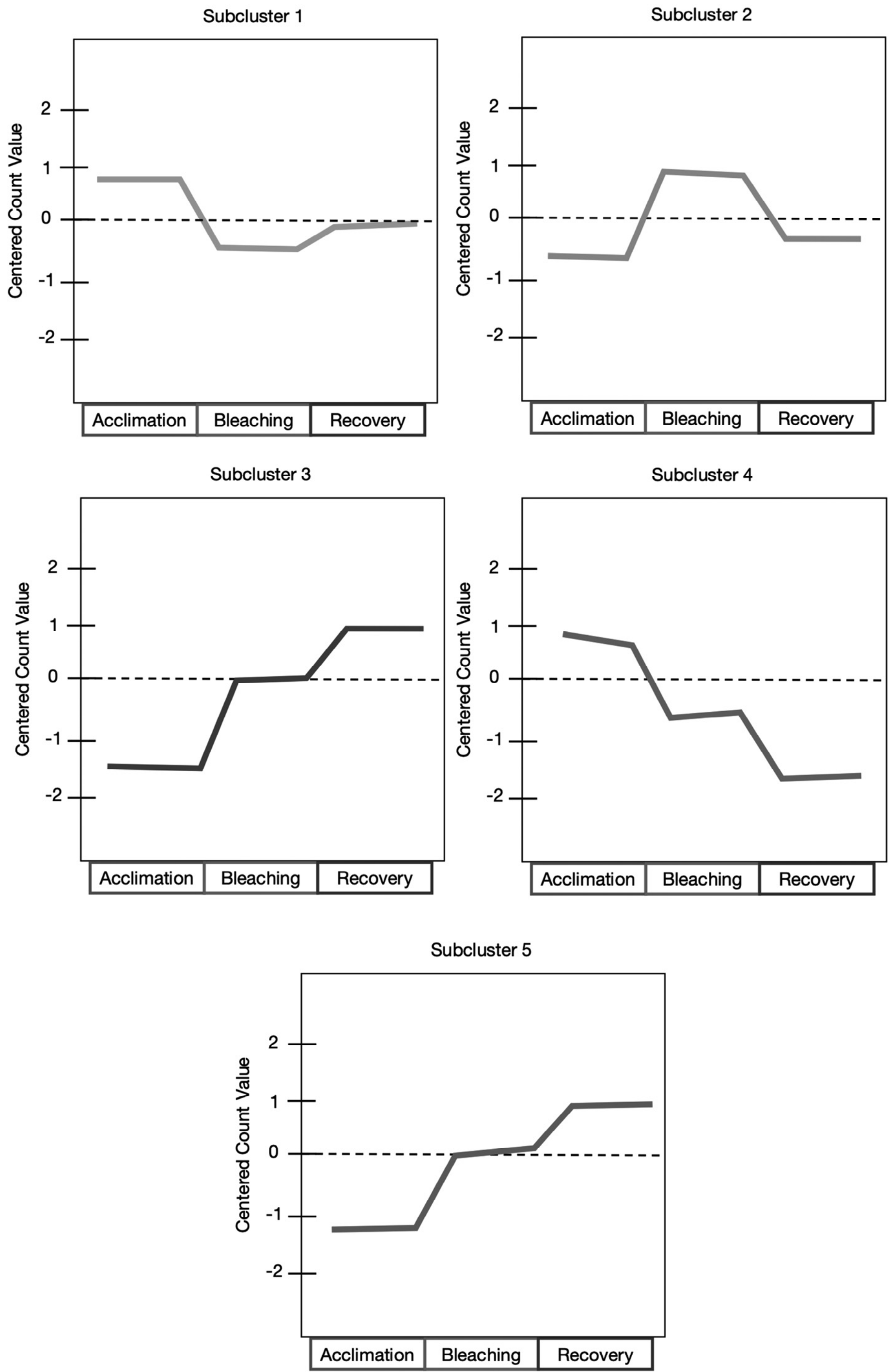

Figure 3 Representative expression patterns of each heatmap subcluster.

3.3 Major Findings from RNAseq Studies of Coral Bleaching and Recovery

The application of heat stress in the experimental nubbins have caused depletion of Symbiodiniaceae populations and its chl *a* concentrations. Furthermore, there was a pattern of continuous decline in both symbiont cell density and chlorophyll a concentration. At the end of the recovery period, the bleached nubbins unexpectedly remained bleached. Physiologically, these nubbins were still considered to be in a bleached state.

The samples' sequence reads had a 21.132% average overall alignment rate to reference transcriptome. Although the alignment rate of the reads seems low, it is anticipated as the coral is a known holobiont possibly containing other associated species included in the sequence reads. These other reads from other species in the transcriptome data were not included using the reference transcriptome for *A. hyacinthus* that has been previously validated. Principal component analysis also revealed that one principal component (PC1) was seen to describe 97.96% of the variation among the samples organized by temperature conditions, which implies that this component is a function of temperature.

DE genes were highest at samples between acclimation and bleaching periods, followed by bleaching versus recovery periods, then the lowest DE genes were in acclimation vs recovery period. The top 1000 DE genes generated a heatmap with five subclusters (Figure 3). In subcluster 1, it was enriched for genes related to cell development, cell differentiation, catecholamine, neurogenesis, response to growth hormones, ROS metabolism, hydrogen peroxide catabolism. In subcluster 2, it was enriched for peptidyl amino acid modification, mitotic cell cycle, cytoskeleton organization, adhesion, protein transport, signaling and gene silencing. For subclusters 3&5, it was enriched for processes related to immune response, gas and ion transport, bioluminescence, maintenance of epithelial structures, mitochondrial ETC, response to UV-B, golgi organization. For subcluster 4, it was only enriched for process relating to membrane lipid catabolic process.

As we have observed, the transcriptome response of the coral host was more coordinated with the temperature conditions of the aquarium tank. The transcriptome response in the bleaching period showed a high proportion of genes were affected by heat stress, specifically among the highly DE genes. Within this were processes that suggest an increase in organization and repair processes during heat stress. At the recovery period, there was the return in expression of some processes such as cell development and growth hormone response. There was also a continuing upregulation, which started from the bleaching period, with processes involving immune response, epithelial structure maintenance, and mitochondrial electron chain transport. At this period, it could be seen that the coral host has responded to the return of the temperature conditions, as most genes in the clusters have returned to acclimation levels. However, a complete recovery of the transcriptome was not evident. This demonstrates that some recovery-associated processes could be long-term responses to heat-stress and may also be expressed as a response to damage. We speculate that at this state during the recovery period, if these nubbins can survive for a longer period, symbiont recovery may be seen. Our findings add to previous findings[25] that some responses to heat stress are reversible, as well as that corals have mechanisms in place to adapt (to a degree) to harsh environmental conditions.

3.4 Prospects and Future Application of Gene Expression Studies in Corals

3.4.1. Uncovering Response Mechanisms to Heat Stress in Corals from Different Regions

In a study by Lee et al. in 2018[30], corals from the temperate region of the Kuroshio region were found to have a potential to acclimate to seasonal fluctuations in sea surface temperatures. By collecting corals during opposing seasons and subjecting these to acute cold and heat stress in an aquarium tank, they were able to

create a profile of the transcriptome in these corals to learn about the capacity of these corals in adapting to changing environmental conditions. Colonies sampled during the summer were not bleached under either thermal or cold stress, while those sampled during winter season were bleached from thermal stress. Cold stress caused summer season corals to upregulate actin-related protein and serine/threonine protein kinase, while the transcriptome of the winter season corals was not affected. Thermal stress in summer season corals did not seem to affect the coral transcriptome, while winter season corals saw an upregulation of phosphoenolpyruvate carboxykinase. These results suggest that corals can acclimate to seasonal changes in seawater temperature by frontloading gene products that would have been expressed in response to temperature stress, which may contribute to bleaching resilience among these corals and possibly in other corals from the region as well.

3.4.2 Application of Gene Expression Studies in Biosurveillance

The development of tools to track bleaching in corals is important in forecasting bleaching events and monitor the recovery progress of corals. In a review in 2016 by Solayan[31], candidate biomarkers for early detection of bleaching in corals were identified. They also found several studies since 1995 that utilize these biomarkers; however, no proper monitoring protocol has been established and applied for widespread monitoring. There was also a lack of bleaching monitoring studies using biomarkers on temperate region corals in either the review or in a literature search at the time of writing this manuscript. Omics based approaches have yielded many data points that have yet to be aggregated and compared in a meta-analytical approach. The insights that can be gathered from this analysis could be used to develop tools useful to give new perspectives in coral biology studies. The development of such biomarker monitoring tool and combining it with locally relevant transcriptome response data, and meteorological data would aid in the prediction of bleaching events and its severity, as well as estimating potential coral recovery.

References

1 Cairns, S. D. 1999. Species richness of recent Scleractinia. Atoll Research Bulletin, 459, 1-46. https://doi.org/10.5479/si.00775630.459.1.

2 Costanza, R. et al., 1997. The value of the world's ecosystem services and natural capital. Nature, 387, 253-260. https://doi.org/10.1038/387253a0

3 Wilkinson, C. 2012. Status of coral reefs of the world: summary of threats and remedial action. In Coral Reef Conservation (3-39). Cambridge University Press. https://doi.org/10.1017/cbo9780511804472.003

4 Bellwood, D. R. et al., 2004. Confronting the coral reef crisis. In Nature (Vol. 429, Issue 6994, 827-833). Nature Publishing Group. https://doi.org/10.1038/nature02691

5 Shinzato, C. et al., 2020. Eighteen coral genomes reveal the evolutionary origin of Acropora strategies to accommodate environmental changes. Molecular Biology and Evolution, 38, 16-30. https://doi.org/10.1093/molbev/msaa216

6 Levy, S. et al., 2021. A stony coral cell atlas illuminates the molecular and cellular basis of coral symbiosis, calcification, and immunity. Cell, 184. https://doi.org/10.1016/j.cell.2021.04.005

7 Taguchi, T. et al., 2013. Detection of characteristic heterochromatin distribution, highly amplified rRNA genes and presence of the human satellite III DNA motif in the scleractinian coral Echinophyllia aspera

Ellis and Solander 1788. Chromosome Science, 16, 33-38. https://doi.org/10.11352/scr.16.33
8 Taguchi, T. et al., 2014. Molecular cytogenetic analysis of the scleractinian coral Acropora solitaryensis veron & Wallace 1984. Zoological Science, 31, 89-94. https://doi.org/10.2108/zsj.31.89
9 Taguchi, T. et al., 2016. Identification of homogeneously staining regions by G-banding and chromosome microdissection, and FISH marker selection using human Alu sequence primers in a scleractinian coral *Coelastrea aspera* Verrill, 1866 (Cnidaria). Comparative Cytogenetics, 10, 61-75. https://doi.org/10.3897/CompCytogen.v10i1.5699
10 Taguchi, T. et al., 2017. Molecular cytogenetic analysis and isolation of a 5S rRNA-related marker in the scleractinian coral *Platygyra contorta* Veron 1990 (Hexacorallia, Anthozoa, Cnidaria). Cytologia, 82, 205-212. https://doi.org/10.1508/cytologia.82.205
11 Taguchi, T. et al., 2020. Karyotypic mosaicism and molecular cytogenetic markers in the scleractinian coral *Acropora pruinosa* Brook, 1982 (Hexacorallia, Anthozoa, Cnidaria). Coral Reefs, 39, 1415-1425. https://doi.org/10.1007/s00338-020-01975-x
12 Vacarizas, J. et al., 2021. Cytogenetic markers using single-sequence probes reveal chromosomal locations of tandemly repetitive genes in scleractinian coral *Acropora pruinosa*. Scientific Reports, 11, 11326. https://doi.org/10.1038/s41598-021-90580-1
13 Okomoda, V. T. et al., 2020. First Report on Successful Triploidy Induction in *Clarias gariepinus* (Burchell, 1822) Using Electroporation. Scientific Reports 2020 10: 1, 10, 1-7. https: //doi. org/10. 1038/s41598-020-59389-2
14 Pradeep, P. J. et al., 2012. Effects of triploidy induction on growth and masculinization of red tilapia [*Oreochromis mossambicus* (Peters, 1852) × Oreochromis niloticus (Linnaeus, 1758)]. Aquaculture, 344-349, 181-187. https://doi.org/10.1016/J.AQUACULTURE.2012.03.006
15 Yang, H., Guo, X. 2006. Polyploid induction by heat shock-induced meiosis and mitosis inhibition in the dwarf surfclam, Mulinia lateralis Say. Aquaculture, 252, 171-182. https://doi.org/10.1016/J.AQUACULTURE.2005.07.017
16 Yang, H. et al., 2000. Triploid and Tetraploid Zhikong Scallop, Chlamys farreri Jones et Preston, Produced by Inhibiting Polar Body I. Marine Biotechnology 2000 2: 5, 2, 466-475. https: //doi. org/10. 1007/PL00021688
17 Coman, F. E. et al., 2008. The effects of triploidy on Penaeus (Marsupenaeus) japonicus (Bate) survival, growth and gender when compared to diploid siblings. Aquaculture, 276, 50-59. https://doi.org/10.1016/J.AQUACULTURE.2008.01.031
18 Foote, A. R. et al., 2012. Tetraploid inductions of Penaeus monodon using cold shock. Aquaculture International, 20, 1003-1007. https://doi.org/10.1007/S10499-012-9524-6/TABLES/1
19 Bollati, E. et al., 2021. Untangling the molecular basis of coral response to sedimentation. Molecular Ecology, 31(3), 884-901. https://doi.org/10.1111/mec.16263
20 Poquita-Du, R. C. et al., 2019. Short term exposure to heat and sediment triggers changes in coral gene expression and photo-physiological performance. Frontiers in Marine Science, 6. https: //doi. org/10. 3389/fmars.2019.00121
21 Ruiz-Jones, L. J., Palumbi, S. R. 2017. Tidal heat pulses on a reef trigger a fine-tuned transcriptional response in corals to maintain homeostasis. Science Advances, 3. https://doi.org/10.1126/sciadv.1601298
22 Thomas, L., Palumbi, S. R. 2017. The genomics of recovery from coral bleaching. Proceedings of the Royal Society B: Biological Sciences, 284, 20171790. https://doi.org/10.1098/rspb.2017.1790

23 Andrews, S. 2010. FastQC: A Quality Control Tool for High Throughput Sequence Data [Online]. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

24 Bolger, A. M. et al., 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics, 30, 2114-2120. https://doi.org/10.1093/bioinformatics/btu170

25 Seneca, F. O., Palumbi, S. R. 2015. The role of transcriptome resilience in resistance of corals to bleaching. Molecular Ecology, 24, 1467-1484. https://doi.org/10.1111/mec.13125

26 Haas, B. J. et al., 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity Platform for reference generation and analysis. Nature Protocols, 8, 1494-1512. https://doi.org/10.1038/nprot.2013.084

27 Robinson, M. D. et al., 2009. Edger: A bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics, 26, 139-140. https://doi.org/10.1093/bioinformatics/btp616

28 Maere, S. et al., 2005. Bingo: A Cytoscape plugin to assess overrepresentation of gene ontology categories in Biological Networks. Bioinformatics, 21, 3448-3449. https://doi.org/10.1093/bioinformatics/bti551

29 Shannon, P. et al., 2003. Cytoscape: A software environment for integrated models of Biomolecular Interaction Networks. Genome Research, 13, 2498-2504. https://doi.org/10.1101/gr.1239303

30 Lee, S. T. et al., 2018. Transcriptomic response in Acropora muricata under acute temperature stress follows preconditioned seasonal temperature fluctuations. BMC Research Notes, 11. https://doi.org/10.1186/s13104-018-3230-z

31 Solayan, A. 2016. Biomonitoring of coral bleaching - a glimpse on biomarkers for the early detection of oxidative damages in corals. Invertebrates - Experimental Models in Toxicity Screening. https://doi.org/10.5772/61831