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Transcriptome analysis provides a blueprint of coral egg and sperm functions

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Background. Reproductive biology and the evolutionary constraints acting on dispersal stages are poorly understood in many stony coral species. A key piece of missing information is sperm and egg gene expression. This is critical for broadcast spawners, such as our model, the Hawaiian species *Montipora capitata*, because sperm and eggs are exposed to environmental insults during dispersal. Furthermore, parental effects such as transcriptome investment may provide a means for cross- or trans-generational plasticity and be apparent in sperm and egg transcriptome data.

Methods. Here, we analyzed *M. capitata* sperm and egg transcriptomic data to address three questions: (1) Which pathways and functions are actively transcribed in these gametes? (2) How does sperm and egg gene expression differ from adult tissues? (3) Does gene expression differ between these gametes?

Results. We show that egg and sperm appear to have surprisingly similar levels of gene expression and overlapping functional enrichment patterns. These results may reflect similar environmental constraints faced by these motile gametes. We find significant differences in differential expression of egg vs. adult and sperm vs. adult RNA-seq data. Lastly, using gene ontology and KEGG orthology data we show that both egg and sperm have markedly repressed transcription and translation machinery compared to the adult, suggesting a dependence on parental transcripts. We hypothesize that cell motility and calcium ion binding genes may be involved in gamete to gamete recognition in the water column and thus, fertilization.

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Abstract

Background. Reproductive biology and the evolutionary constraints acting on dispersal stages are poorly understood in many stony coral species. A key piece of missing information is sperm and egg gene expression. This is critical for broadcast spawners, such as our model, the Hawaiian species *Montipora capitata*, because sperm and eggs are exposed to environmental insults during dispersal. Furthermore, parental effects such as transcriptome investment may provide a means for cross- or trans-generational plasticity and be apparent in sperm and egg transcriptome data.

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Introduction

Reef-building corals and their photosynthetic dinoflagellate endosymbionts form the structural foundation of complex ecosystems, supporting approximately 25% of marine biodiversity and protecting coastlines from damaging wave energy (Hughes et al. 2017). The rice coral *Montipora capitata* is a dominant reef-builder in the Hawaiian Archipelago that is of interest because of its demonstrated resilience throughout ocean warming events that can lead to loss of algal symbionts (termed "bleaching") and mortality (Grottoli et al. 2006). *M. capitata* has a relatively large genome (ca. 886 Mbp in size) due primarily to high repeat and transposable element content. This genome expansion likely resulted from genetic drift due to small effective population size in the isolated Hawaiian island chain (Shumaker et al. 2019). These features, along with its relatively high tolerance to heat stress and ocean acidification (Gibbin et al. 2015), make *M. capitata* an important model, when compared to more sensitive coral species, for studying the emergence of locally adaptive traits and physiological responses of corals to environmental change.

M. capitata is a broadcast-spawning hermaphrodite that annually releases bundles of sperm and eggs into the water column at roughly three summer intervals, according to the lunar

cycle. Gametes undergo sexual fusion and produce larvae that settle, metamorphose, and develop into the meta-organisms that ultimately build reefs (Padilla-Gamiño et al. 2011; Padilla-Gamiño and Gates 2012). Broadcast-spawning is the most common form of reproduction within Scleractinia (stony corals) and is highly conserved within this order (Baird et al. 2009; Padilla-Gamiño et al. 2011). Unlike brooding corals, *M. capitata* gametes experience direct and prolonged exposure to the marine environment (within bundles, and thereafter upon release at the sea surface) during the several days of development and ~weeks of pelagic larval duration (Concepcion et al. 2012). Thus, these gametes must overcome challenges such as predation, infection by microbial pathogens, and fluctuations in temperature and pH, before taking part in the fertilization process (Baird et al. 2009) and metamorphosing. Despite the significance of gamete survivability in the greater environment and its effect on overall reproductive potential in broadcast-spawning corals, few studies have focused primarily on egg and sperm cells. Investigation of the potential impacts of bleaching on reproduction has not shown significant differences in egg quality or spawning potential between bleached and non-bleached *M. capitata* (Cox 2007) despite impacts on egg algal symbiont populations (Padilla-Gamiño et al. 2013). This resilience may be explained in part by an increase in heterotrophy, offsetting energetic losses due to bleaching (Grottoli et al. 2006), or the timing of gametogenesis relative to the thermal stress. Sperm play an equally important role in determining spawning success, yet little is known about sperm gene expression. However, sperm DNA has been used to generate genome assemblies due to its high seasonal abundance and symbiont-free state, therefore data are readily obtainable for sperm-specific studies (e.g., Putnam et al. 2017; Shumaker et al. 2019).

Here, we analyzed egg and sperm RNA-seq data generated from *M. capitata* colonies located on fringing reefs near the Hawai'i Institute of Marine Biology (HIMB) in Oahu, Hawai'i (for details on related analyses, see Putnam et al. 2017). Three individual egg RNA-seq libraries and three individual sperm RNA-seq libraries (both from ambient colonies) were sequenced on the Illumina platform using the Illumina TruSeq RNA Library Preparation Kit v2. The egg data are publicly available under NCBI BioProject PRJNA616341and the sperm data are publicly available under NCBI BioProject PRJNA339779. We determined which genes are expressed in coral egg and sperm cells and studied their putative functions. We then compared cDNA data from eggs and sperm to that of un-stressed adults generated in a previous project (Shumaker et al. 2019) to determine the degree of differential gene expression (DEG) between gametic and

adult tissues. Finally, we used the expression data to highlight pathways represented by the most differentially expressed genes in egg and sperm and compared gamete-specific functions that account for various known physiological processes in *M. capitata*.

Materials & Methods

Sperm sample collection

In the July 2015 spawning period, egg-sperm bundles were collected from five field netted adults of *M. capitata* on the fringing reefs on the west side of Moku o Lo'e under Special Activity Permit 2015–17 from the Hawai'i Department of Aquatic Resources. The egg sperm bundles were collected just after they were released from the adult polyp and floated to the surface of the water. The bundles were placed in 1.5 mL sterile RNase and DNase free microfuge tubes and left for 30 min, with lipid rich buoyant eggs floating to the surface and the denser sperm settling to the bottom of the tubes. The lower sperm fraction was removed by pipetting to a new tube and visually inspecting the liquid for the absence of eggs. The sperm was cleaned by three rinse-and-centrifugation steps, where sperm was rinsed with 0.2 µm filtered seawater and centrifuged at 13,000 rpm for 3 min. The supernatant was removed from the tube and the concentrated sperm was stored at −80 °C.

Sperm RNA extraction and sequencing library preparation

Total RNA was extracted by resuspending each sperm sample in 550 µL of Trizol (ThermoFisher Scientific) (Putnam et al. 2017). These samples were then passed twice through a QiaShredder column (Qiagen, Inc., Hilden, Germany) and transferred to a 1.5 mL microcentrifuge tube. A total of 450 µL of Trizol was added to bring the volume to 1.0 mL. Following a 5 min room temperature incubation, 200 µL of chloroform was added and the sample was vigorously shaken for 15 sec, and then incubated at room temperature for 3 min. The samples were centrifuged for 15 min at 4°C and the upper aqueous layer was transferred to a new 1.5 mL tube, and an equal volume of 70% ethanol was added and gently mixed. Next, the samples were transferred to Qiagen RNeasy mini columns. From here onwards, the Qiagen RNeasy mini protocol was followed, including the optional on-column DNase treatment. Total RNA was eluted in 55 µL of nuclease-free water. Three individual RNA-seq libraries were generated using 200 ng of the total RNA from each sample using the Illumina TruSeq RNA

Library Preparation Kit v2. The libraries were combined in equimolar concentrations and run on a single Illumina MiSeq flowcell using the Illumina MiSeq Reagent Kit v3 (150 cycles, single-end). The genomic and transcriptomic data created for this project are available under NCBI BioProject PRJNA339779 and the three libraries were pooled together and can be found in BioSample: SAMN05607941; Sample name: Mcap_nonBleach_RNA; SRA: SRS1632867.

Transcriptomic and genomic data analysis of sperm

After trimming for quality (parameters shown in Fig. S1) using the CLC Genomics Workbench 8.5.1 (Qiagen, Hilden, Germany), the combined sperm RNA-seq data yielded 29,815,942 high quality reads for assembly. The *M. capitata* genome (Shumaker et al. 2019) and annotations used for the mappings are available at http://cyanophora.rutgers.edu/montipora/. The libraries were individually mapped to the reference genome using CLC Genomics Workbench (count data can be found in Table S1). Only "unique exon reads" counts (i.e., the number of reads that match uniquely to exons, including across exon-exon junctions) were used for downstream analyses.

Egg sample collection

In the June 2018 (6/13/18) spawning period, sperm-egg bundles were collected from ambient conditions in the field on the fringing reefs at the HIMB under Special Activity Permit 2018-50 from the Hawai'i Department of Aquatic Resources. Bundles were brought back to the lab and buoyant eggs were separated from the dense sperm after the bundles broke up. The eggs were subsequently rinsed 3 times with 0.2 µm filtered seawater and then following the removal of the water, replicate tubes of eggs were snap frozen in liquid nitrogen and stored at -80°C.

Egg RNA extraction and sequencing library preparation

Digestion

A total of 30 µl of PK digestion buffer was added to each sample tube, followed by the addition of 5µl Proteinase K. Next, samples were vortexed, spun down, and placed in a Thermomixer for 1 h at 55°C, shaking at 1100 rpm.

RNA extraction

An equal volume (700 µl) of 100% EtOH was added to the 1.5 mL tubes containing the original yellow column flow through which were then vortexed and spun down to mix. A total of 700 µl of the resulting mixture was added to the RNA spin columns where it was centrifuged at 16,000 rcf (g) for 30 sec and the flow through (Zymo kit waste) was subsequently discarded. This step was repeated and then 400 µl DNA/ RNA Wash Buffer was gently added to each RNA column. The samples were centrifuged at 16,000 rcf (g) for 30 sec and flow through (Zymo kit waste) was discarded. Next, 80 µl DNase I treatment master mix (75 µl DNA Digestion buffer x # of samples, 5 µl DNase I x # of samples) was added directly to the filter of the RNA columns and incubated at room temp for 15 min. Then, 400 µl of the DNA/ RNA Prep Buffer was gently added to each column and the mixture was centrifuged at 16,000 rcf (g) for 30 sec flow through (Zymo kit waste) was discarded. The RNA columns were then transferred to new 1.5 mL microcentrifuge tubes and 50 µl of warmed DNase/RNase free water was added to each RNA column by dripping slowly directly on the filter. The samples were incubated at room temperature for 5 min and centrifuged at 16,000 rcf (g) for 30 sec. This step was repeated to obtain a final elution volume of 100 µl, and tubes were stored at -80°C. The cDNA libraries were prepared and sequenced by Genewiz using standard Illumina strand-specific RNA-seq preparation with poly-A selection and then sequenced with the HiSeq instrument using 2 x 150 bp reagents with ~15M raw paired-end reads per sample.

Transcriptomic and genomic data analysis of eggs

After trimming for quality (parameters shown in Fig. S1) using the CLC Genomics Workbench 8.5.1 (Qiagen, Hilden, Germany), the combined egg RNA-seq data yielded 125,054,213 high quality paired reads for assembly. The *M. capitata* genome (Shumaker et al. 2019) and annotations used for the mappings are available at http://cyanophora.rutgers.edu/montipora/. The libraries were individually mapped to the reference genome using CLC Genomics Workbench 8.5.1 (QIAGEN, Aarhus Denmark) (count data can be found in Table S2). Only "unique exon read" counts were used for downstream analyses.

Functional Analysis

Read count normalization

Read counts across the three egg and three sperm libraries, as well as three adult ambient temperature, ambient CO_2 treatment [ATAC] RNA-seq runs from Shumaker et al. (2019) used in DEG analysis, were normalized to transcripts per million (TPM). The number of genes expressed at TPM thresholds between 0 and 200 (in increasing increments of 10) were tabulated, and used to determine the proportion of the gene inventory expressed at each threshold. These values were converted to percent of total genes in the genome, and that percentage was plotted on the y-axis vs. the incremental thresholds on the x-axis. Based on the graphs for each of the three datasets (egg, sperm, adult ATAC), there is a steep drop off in the percentage of genes around 100 TPM in both the egg and sperm data, where >100 TPM represents 12.05% (7,620/ 63,227) and 12.31% (7,783/ 63,227) of the total genes, respectively. In the adult read counts, ~12% of all genes corresponds to 60 TPM, however, because this group had many more expressed genes (TPM>0, 58.69%) compared to the egg and sperm (31.98% and 30.27% respectively), the drop-off in percentages of genes above the TPM threshold increments occurs very early in this dataset. Because the adult ATAC data are not relevant to this particular part of the analysis, we arbitrarily chose 100 TPM to be a reasonable threshold for moving forward with the egg and sperm data. Normalized read counts and accompanying graphs are provided in the Supplementary Spreadsheet (Tab 2).

Gene expression in egg and sperm based on gene ontology

To determine which biological, cellular, and molecular functions are most prevalent across the combined egg and combined sperm RNA-seq data, we used the 100 TPM threshold to generate a list of "expressed" genes in both the egg and sperm which consisted of 7,620 and 7,783 genes respectively. The Blast2GO software was used to map GO terms to the *M. capitata* gene inventory and to test for enrichment among the sets of "expressed" genes using Fisher's Exact Test (Götz et al. 2008), using the gene inventory as the reference set.

Using GO terms to organize KEGG pathway data

These sets of "expressed genes" were assigned KEGG Orthology (KO) terms using the KofamScan software (Aramaki et al. 2019), yielding 4,078 genes in the "egg" set with at least one KO term and 3,146 genes in the "sperm" set with at least one KO term. Each set of KOs (egg, sperm) was mapped onto well-studied biological pathways using the "Reconstruct

Pathway" tool (Kanehisa 2017). Next, all KEGG pathways associated with each KO (excluding human-specific pathways) were retrieved and cross-referenced with GO terms of the category "cellular component". This allowed for assignment of each KO term and its associated pathway(s) to a putative location within the cell. The exhaustive list of all KEGG pathway-GO pairs is included in the supplemental spreadsheet (Tabs 4 and 6) and the most common terms relevant to each cellular component were selected as the functional pathways to display in our analysis. This analysis was done to make an overall assessment of which metabolic processes are most frequently expressed in egg and sperm cells based on the normalized read counts for RNA-seq transcript data, and to identify if there are any major differences in overall functionality between each gamete type that can be further investigated.

Differential Gene Expression Analysis

DEG analysis was conducted using the DESeq2 package (Love et al. 2014) using the raw counts as input (parameters shown in Fig. S2). The count matrices and column data used as input can be found in supplementary spreadsheet (Tabs 8 and 9). Differentially expressed genes were identified in contrasts between egg and adult samples, as well as sperm and adult samples. Egg replicates corresponding to individual RNA-seq runs are labeled E1, E2, E3, sperm samples are labeled Ub2, Ub3, Ub4, and ambient control treatment adult samples from Shumaker et al. (2019) are labeled W1, W5, and W7 in all related tables and figures.

Results

Functional Analysis

Fisher's exact test revealed similar functional enrichments among the "expressed" gene sets (TPM >100) in both gamete types. Among the Biological Process and Molecular Function GO terms enriched in the "expressed" genes in the egg samples, the top ten are 'ATP binding', 'GTP binding', 'GTPase activity', 'structural constituent of ribosome', 'chromatin binding', 'microtubule motor activity', 'negative regulation of transcription by RNA polymerase II', 'protein polyubiquitination', 'translation initiation factor activity', and 'ubiquitin ligase activity'. The top ten enriched GO terms for sperm "expressed" vs. all genes are 'ATP binding', 'GTP binding', 'GTPase activity', 'structural constituent of ribosome', 'chromatin binding', 'microtubule binding', 'endonuclease activity', 'microtubule motor activity', 'negative

regulation of transcription by RNA polymerase II', and 'protein polyubiquitylation'. The complete list of the GO terms for each of these enrichment profiles and their dataset of origin are presented in Table 1. These GO lists, their prevalence in the *M. capitata* genome, and degree of enrichment are shown in Figure S3. All GO term enrichments beyond the top 30 shown here were minor and not reported.

Using GO terms to organize KEGG pathway data

After indexing KO terms by their corresponding Cellular Component GO terms, the top KEGG pathway for selected parts of the cell were identified, excluding those that are human/ mammal-specific or disease-related. Each KO term was counted for each cellular component and the most frequent KEGG terms before a significant drop in quantity were included in Figure 1. The complete list of all of the raw data used in this analysis can be found in the supplemental spreadsheet (Tabs 5 and 7). These results further support the Biological Process and Molecular Function GO data shown above by confirming the similarity between the egg and sperm with respect to which pathways are most prominently expressed and where they are active within the cell. There are no major differences in which pathways were assigned to each cellular component, however there are some differences in the frequency of KO terms assigned to Cellular Component GO terms, shown by the order in which they appear in the figure and their KO assignment frequencies denoted in the spreadsheet. The functional implications of these differences will be discussed further below.

Differential gene expression analysis

From a broader perspective, and perhaps surprisingly, *M. capitata* egg and sperm appear to have similar patterns of gene expression and share functional enrichment patterns. However, in order to gain a more in-depth understanding of how each of these cells function, how those functions compare with gene expression in adult *M. capitata* tissue, and how egg and sperm compare to each other on a more specific level, it is crucial to statistically test for differential gene expression. As expected, given the divergent different tissue types analyzed, principle components analysis (PCA) shows strong differentiation in gene expression patterns between gametic and adult libraries (Fig. S4).

More specifically, with respect to the DEG analysis of egg vs. adult ATAC RNA-seq data, DESeq2 identified 13,890 transcripts that were significantly differentially expressed (defined in this study as FDR-adjusted p-value < 0.05), of which 4,487 were up-regulated and 9,403 down-regulated. With respect to the differential expression analysis of sperm vs. adult ATAC RNA-seq data, DESeq2 identified 9,717 transcripts that were significantly differentially expressed (defined in this study as FDR-adjusted p-value < 0.05), of which 2,985 were up-regulated and 6,732 down-regulated. Log2-fold change estimates (L2FC), all FDR-adjusted p-values, putative annotations, blastx hits and percent identity (PID), KO terms, and GO terms are provided for all of these genes the Supplemental spreadsheet (Tabs 10 and 11). The top ten DEGs that had BLAST hits for egg up- and down-regulated and sperm up- and down-regulated are shown in Tables S3 and S4 with all of their differential expression and BLAST statistics and the full lists of DEGS and accompanying annotations are in Supplemental spreadsheet (Tab 3). It is difficult to use these data to gain specific insights because many of the DEGs lack blast hits, and those that do, most are to predicted, hypothetical, or uncharacterized proteins in recently sequenced corals. This trend is illustrated in Figure 2 that provides a snapshot of the degree of similarity between the egg and sperm DEG lists as well as the limitations associated with DEGs that have poor annotation.

To understand some of the basic functions of the egg and sperm transcriptomes, four sets of DEGs were used as the test sets for separate GO-enrichment analyses (Fisher's Exact Test) against the reference set of all genes in the *M. capitata* genome (Blast2GO OmicsBox 1.1.164; Götz et al. 2008): (1) egg up-regulated genes (l2fc >1.5; 3,645 DEGs), (2) egg down-regulated genes (l2fc <-1.5; 8,760 DEGs), (3) sperm up-regulated genes (l2fc >1.5; 2,795 genes), and (4) sperm down-regulated genes (l2fc <-1.5; 6,606). The top enriched GO terms for egg up-regulated genes are "ATP binding", "phosphatase activity", "positive regulation of biosynthetic process", "protein serine/threonine kinase activity", and "GTP binding". The top enriched GO terms for egg down-regulated genes are "signaling receptor activity", "regulation of transcription by RNA polymerase II", "lipid metabolic process", "organonitrogen compound catabolic process", and "regulation of signaling". The top enriched GO terms for sperm up-regulated genes are "purine nucleotide binding", "purine ribonucleoside triphosphate binding", "enzyme binding", "positive regulation of RNA metabolic process", and "regulation of localization". The top enriched GO terms for sperm down-regulated genes are "positive regulation of cellular metabolic process",

306 "regulation of transcription by RNA polymerase II", "carboxylic acid metabolic process",
307 "regulation of cellular protein metabolic process", and "animal organ development". The full
308 datasets are represented by the bar charts of combined Biological Process and Molecular
309 Function "most specific" enriched GO terms in Figure S5.
310 To further resolve the enrichment and DEG data, separate lists of up-regulated (l2fc >1.5)
311 and down-regulated (l2fc <-1.5) genes (same sets as used above for Fisher's Exact Test) were
312 combined for each of the egg and sperm, indexed with their KO terms retrieved from Kofamscan
313 (Aramaki et al. 2019), and then uploaded into the KEGG "Reconstruct Pathway" tool (Kanehisa
314 2017). For every pathway, the up- and down-regulated genes were noted and based on trends
315 seen across egg and sperm data in the above enrichment analysis, connections between KEGG
316 pathway activity and known physiological mechanisms of egg and sperm data were made. One
317 major finding from pathway analysis is that nearly every ribosomal protein in both the egg and
318 sperm datasets is down-regulated (Fig. S6).
319

320 ## Discussion

321 On the most general level, the egg and sperm of *M. capitata* each differ considerably in their
322 gene expression when compared with RNA-seq data from adult cells but differ much less when
323 compared with each other. The *M. capitata* genome contains 63,227 genes, of which 20,220
324 (31.98%) in the egg and 19,140 (30.27%) in the sperm are expressed (i.e., based on our chosen
325 threshold of TPM >100), when compared with 37,105 (58.69%) in the adult. Because gametes
326 are specialized cells with the purpose of uniting and producing an embryo, it is not surprising
327 that their transcriptomes are more streamlined and specialized. This is seen in our data in the
328 down regulation of ribosomal proteins as well as in the raw RNA-seq data *via* TPM distributions.
329 What is surprising, however, is the degree of similarity these data show between egg and sperm
330 functional capacity. In addition to their mutual down-regulation of transcription and translation,
331 the most highly expressed genes in both egg and sperm datasets share the same core functions at
332 similar levels (Table 1 and Fig. 1). Although most DEGs lack unambiguous annotations, DEGs
333 between the egg and ambient adult samples and sperm and ambient adult samples are very
334 similar (Fig. 2).
335

336 *Translation*

One of the starkest differences to emerge from the DEG analysis is the marked down-regulation of nearly every ribosomal protein-encoding gene in both the egg and sperm datasets when compared with the adult (Fig. S6). This trend has been documented extensively in human and mammalian sperm where it has been found that sperm are "translationally silent"; i.e., cytoplasmic ribosomal assembly and thus activity is not fully functional when the sperm reaches maturity and nuclear-encoded transcripts may be primarily translated on mitochondrial ribosomes (Gur and Breitbart 2006; Zhao et al. 2009; Amaral et al. 2014). This trend has also been documented in the non-coral egg literature as well with studies of *Xenopus* eggs (Arne et al. 2014) and in mice oocytes, where ribosomal protein expression is repressed during late stage oocyte development (Taylor and Pikó 1992). As highly specialized cells with much lower overall gene expression compared to cells of the adult tissue, it is not surprising for ribosomal protein genes and thus, translation to be down-regulated in both the sperm and egg. However, this topic needs to be further studied to determine whether this phenomenon in sperm is due solely to its role as a gamete or because the cytoplasmic ribosomes may be translationally inactive.

Fertilization

It is well-documented that chemical signals are secreted by egg cells to attract sperm. Whether this process is utilized to guide sperm to an egg within the body of an individual organism or the broader environment depends on the system being studied. Regardless, there are two major components to this process: secretion of the chemical attractant by the egg, and taxis initiated by chemical receptors, and made physically possible by motile cilia/ the flagellum of the sperm. In mammalian sperm, for example, motile cilia and the sperm flagellum develop in a similar fashion, have the same axoneme structure, and are virtually identical (Clermont et al. 1993; Avidor-Reiss and Leroux 2015; Wachten et al. 2017). The evolution of compartmentalized sperm cilia (*via* primary cytosolic ciliogenesis) is necessary for the flagellar movement that propels the sperm cell through its environment to the egg. This is thought to be a universal process in metazoans because it is present in both protostomes (*Drosophila*) and deuterostomes (mammals, humans), as well as in basal metazoans (corals, sponges) (Avidor-Reiss and Leroux 2015). In broadcast-spawning corals like *M. digitata* (a relative of *M. capitata*), eggs play a significant role in the regulation of sperm activity and the fertilization success, including sperm signaling and stimulation of flagellar motility (Coll et al. 1994; Morita et al. 2006). The signals

produced by the egg must be species-specific to prevent hybridization during mass spawning events where many species release gametes into the water column at the same time. In organisms like ascidians and echinoids, Ca^{2+} has been shown to induce sperm flagellar motility (Yoshida et al. 2003; Morita et al. 2006). A similar effect has been shown experimentally in corals in *Acropora* species although this alone is insufficient to explain the species-specific nature of sperm and egg union (Morita et al. 2006). It is difficult to find evidence of these complex processes in the summary data of *M. capitata* discussed above due to the large number of genes lacking annotations. However, upon taking a closer look at the GO data used for Figure 1, for "motile cilium", 79 genes are associated with this GO term, 38 are present in the sperm with TPM counts >100, and two of those (g63277 and g9762) are also linked to the GO term "calcium ion binding". Neither of these genes were assigned a KEGG annotation which is why the data for flagellar and motile cilia cellular components are scarce for this analysis. Both of these genes have the same top blastx hit: XP_015777656.1 PREDICTED: uncharacterized protein LOC107355583 isoform X1 in *Acropora digitifera*. Both of these genes have transcripts that are significantly up-regulated in the sperm vs. adult DESeq2 data and egg vs. adult data. Based on these results, it is possible these genes play a key role in communication associated with the fertilization process. Differential expression and BLAST annotation statistics for these two genes are presented in Figure S7. These genes are potential targets for CRISPR/Cas9 based gene knockdowns (recently developed for Scleractinia; Cleves et al. 2018, 2020) to explore coral reproductive biology.

Conclusions

Our findings suggest that coral egg and sperm should not be thought of as cells that are highly differentiated with respect to functional capabilities. Rather, as motile cells released into the water column and subjected to the same environment prior to fertilization, these morphologically divergent cell types nonetheless share a conserved gene expression pattern and thus, may be under similar functional constraints. To this end, it would be of interest to investigate egg and sperm transcriptomics in brooding corals (i.e., these species do not release gametes into the water column prior to fertilization) and to compare with the results of our study.

In addition to the major finding of largely shared expression profiles, the data also provide insights into which genes may play key roles in the fertilization process. By being

associated with cell motility and calcium ion binding, two genes (g63277 and g9762) emerge from our dataset as possible candidates for future experimental studies on how eggs and sperm recognize each other in the water column despite the presence of many other gametes from other organisms. In summary, we present here the first study of the transcriptome of coral sperm and eggs and reach many interesting conclusions that pave the way for future multi-omics and genetics investigations on this topic (Cleves et al. 2020), particularly in the context of anthropogenic climate change influences on the marine environment.

Acknowledgements

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Figure legends

Figure 1

Sperm and egg overall functions.

(A) Schematic image of an *M. capitata* sperm cell depicting cellular structures and organelles associated with GO terms and their accompanying KEGG pathways determined from KO terms. (B) Schematic image of an *M. capitata* egg cell depicting cellular structures and organelles associated with GO terms and their accompanying KEGG pathways determined from KO terms. Both images created with Biorender.com.

Figure 2

Differentially expressed genes in sperm and eggs.

(A) Venn diagram showing the top ten up-regulated DEGs in egg only (left), sperm only (right), and shared (center). (B) Venn diagram showing the top ten down-regulated DEGs in egg only (left), sperm only (right), and shared (center). * ^ # indicate the top BLAST annotation is to a "predicted", "hypothetical", or "uncharacterized" protein, respectively.

Table legends

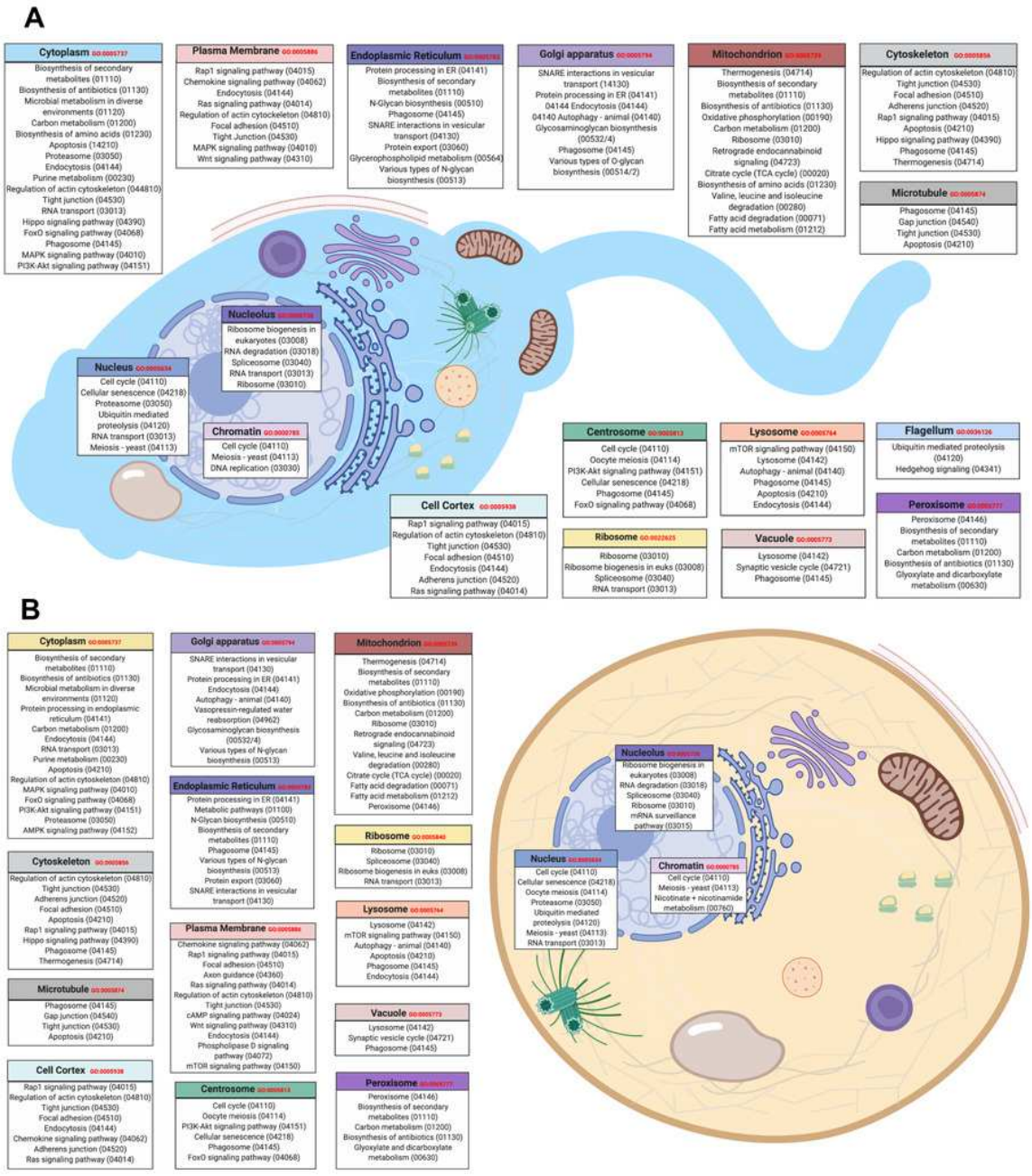
Table 1. Listing of enriched GO terms found in the egg, sperm, and egg and sperm "expressed" gene sets.

Egg "expressed" enriched GO terms	Enriched GO terms shared by egg and sperm "expressed" gene sets	Sperm "expressed" enriched GO terms
Guanyl-nucleotide exchange factor activity	ATP binding	Microtubule binding
Phosphoric diester hydrolase activity	GTP binding	Endonuclease activity
Meiotic cell cycle	GTPase activity	Ubiquitin protein ligase binding
Cysteine-type endopeptidase activity	Structural constituent of ribosome	Translational elongation
Protein processing	Chromatin binding	SNARE binding
DNA-templated transcription, elongation	Microtubule motor activity	Protein secretion
	Protein polyubiquitination	Protein localization to plasma membrane
	Translation initiation factor activity	RNA helicase activity
	Ubiquitin protein ligase activity	RNA methyltransferase activity
	Negative regulation of transcription by RNA polymerase II	
	Thiol-dependent ubiquitin-specific protease activity	
	Rab protein signal transduction	
	Unfolded protein binding	
	Single-stranded DNA binding	
	Actin filament binding	
	Magnesium ion binding	
	Protein heterodimerization activity	
	GTPase activator activity	
	Rab GTPase binding	
	Peptidyl-serine phosphorylation	
	RNA helicase activity	
	NAD binding	

Figure 1

Sperm and egg overall functions.

(A) Schematic image of an *M. capitata* sperm cell depicting cellular structures and organelles associated with GO terms and their accompanying KEGG pathways determined from KO terms. (B) Schematic image of an *M. capitata* egg cell depicting cellular structures and organelles associated with GO terms and their accompanying KEGG pathways determined from KO terms. Both images created with Biorender.com.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Cytoplasm GO:0005737
Biosynthesis of secondary metabolites (01110)
Biosynthesis of antibiotics (01130)
Microbial metabolism in diverse environments (01120)
Carbon metabolism (01200)
Biosynthesis of amino acids (01230)
Apoptosis (14210)
Proteasome (03050)
Endocytosis (04144)
Purine metabolism (00230)
Regulation of actin cytoskeleton (044810)
Tight junction (04530)
RNA transport (03013)
Hippo signaling pathway (04390)
FoxO signaling pathway (04068)
Phagosome (04145)
MAPK signaling pathway (04010)
PI3K-Akt signaling pathway (04151)
Plasma Membrane GO:0005886
Rap1 signaling pathway (04015)
Chemokine signaling pathway (04062)
Endocytosis (04144)
Ras signaling pathway (04014)
Regulation of actin cytoskeleton (04810)
Focal adhesion (04510)
Tight Junction (04530)
MAPK signaling pathway (04010)
Wnt signaling pathway (04310)
Endoplasmic Reticulum GO:0005783
Protein processing in ER (04141)
Biosynthesis of secondary metabolites (01110)
N-Glycan biosynthesis (00510)
Phagosome (04145)
SNARE interactions in vesicular transport (04130)
Protein export (03060)
Glycerophospholipid metabolism (00564)
Various types of N-glycan biosynthesis (00513)
Golgi apparatus GO:0005794
SNARE interactions in vesicular transport (14130)
Protein processing in ER (04141)
04144 Endocytosis (04144)
04140 Autophagy - animal (04140)
Glycosaminoglycan biosynthesis (00532/4)
Phagosome (04145)
Various types of O-glycan biosynthesis (00514/2)
Mitochondrion GO:0005739
Thermogenesis (04714)
Biosynthesis of secondary metabolites (01110)
Biosynthesis of antibiotics (01130)
Oxidative phosphorylation (00190)
Carbon metabolism (01200)
Ribosome (03010)
Retrograde endocannabinoid signaling (04723)
Citrate cycle (TCA cycle) (00020)
Biosynthesis of amino acids (01230)
Valine, leucine and isoleucine degradation (00280)
Fatty acid degradation (00071)
Fatty acid metabolism (01212)
Cytoskeleton GO:0005856
Regulation of actin cytoskeleton (04810)
Tight junction (04530)
Focal adhesion (04510)
Adherens junction (04520)
Rap1 signaling pathway (04015)
Apoptosis (04210)
Hippo signaling pathway (04390)
Phagosome (04145)
Thermogenesis (04714)
Microtubule GO:0005874
Phagosome (04145)
Gap junction (04540)
Tight junction (04530)
Apoptosis (04210)
Nucleolus GO:0005730
Ribosome biogenesis in eukaryotes (03008)
RNA degradation (03018)
Spliceosome (03040)
RNA transport (03013)
Ribosome (03010)
Nucleus GO:0005634
Cell cycle (04110)
Cellular senescence (04218)
Proteasome (03050)
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RNA transport (03013)
Meiosis - yeast (04113)
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Cellular senescence (04218)
Phagosome (04145)
FoxO signaling pathway (04068)
Lysosome GO:0005764
mTOR signaling pathway (04150)
Lysosome (04142)
Autophagy - animal (04140)
Phagosome (04145)
Apoptosis (04210)
Endocytosis (04144)
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Ubiquitin mediated proteolysis (04120)
Hedgehog signaling (04341)
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Rap1 signaling pathway (04015)
Regulation of actin cytoskeleton (04810)
Tight junction (04530)
Focal adhesion (04510)
Endocytosis (04144)
Adherens junction (04520)
Ras signaling pathway (04014)
Ribosome GO:0022625
Ribosome (03010)
Ribosome biogenesis in euks (03008)
Spliceosome (03040)
RNA transport (03013)
Vacuole GO:0005773
Lysosome (04142)
Synaptic vesicle cycle (04721)
Phagosome (04145)
Peroxisome GO:0005777
Peroxisome (04146)
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Carbon metabolism (01200)
Biosynthesis of antibiotics (01130)
Glyoxylate and dicarboxylate metabolism (00630)
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Biosynthesis of secondary metabolites (01110)
Biosynthesis of antibiotics (01130)
Microbial metabolism in diverse environments (01120)
Protein processing in endoplasmic reticulum (04141)
Carbon metabolism (01200)
Endocytosis (04144)
RNA transport (03013)
Purine metabolism (00230)
Apoptosis (04210)
Regulation of actin cytoskeleton (04810)
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Tight junction (04530)
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Rap1 signaling pathway (04015)
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Phagosome (04145)
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Gap junction (04540)
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Cell Cortex GO:0005938
Rap1 signaling pathway (04015)
Regulation of actin cytoskeleton (04810)
Tight junction (04530)
Focal adhesion (04510)
Endocytosis (04144)
Chemokine signaling pathway (04062)
Adherens junction (04520)
Ras signaling pathway (04014)
Golgi apparatus GO:0005794
SNARE interactions in vesicular transport (04130)
Protein processing in ER (04141)
Endocytosis (04144)
Autophagy - animal (04140)
Vasopressin-regulated water reabsorption (04962)
Glycosaminoglycan biosynthesis (00532/4)
Various types of N-glycan biosynthesis (00513)
Endoplasmic Reticulum GO:0005783
Protein processing in ER (04141)
Metabolic pathways (01100)
N-Glycan biosynthesis (00510)
Biosynthesis of secondary metabolites (01110)
Phagosome (04145)
Various types of N-glycan biosynthesis (00513)
Protein export (03060)
SNARE interactions in vesicular transport (04130)
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Focal adhesion (04510)
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Thermogenesis (04714)
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Retrograde endocannabinoid signaling (04723)
Valine, leucine and isoleucine degradation (00280)
Citrate cycle (TCA cycle) (00020)
Fatty acid degradation (00071)
Fatty acid metabolism (01212)
Peroxisome (04146)
Ribosome GO:0005840
Ribosome (03010)
Spliceosome (03040)
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Lysosome (04142)
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Phagosome (04145)
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Peroxisome (04146)
Biosynthesis of secondary metabolites (01110)
Carbon metabolism (01200)
Biosynthesis of antibiotics (01130)
Glyoxylate and dicarboxylate metabolism (00630)
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Ribosome biogenesis in eukaryotes (03008)
RNA degradation (03018)
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RNA transport (03013)
Chromatin GO:0000785
Cell cycle (04110)
Meiosis - yeast (04113)
Nicotinate + nicotinamide metabolism (00760)

Figure 2

Differentially expressed genes in sperm and eggs.

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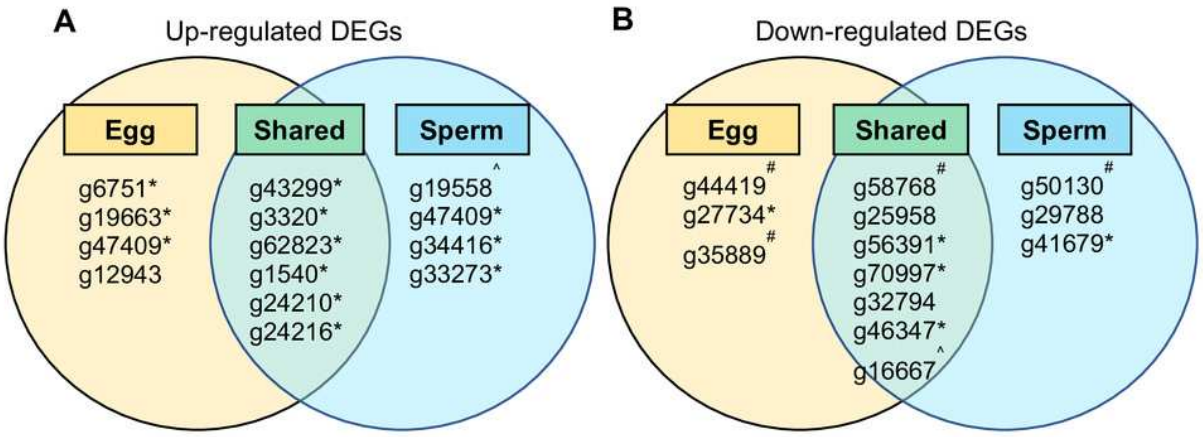
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