Maternal effects in gene expression of interspecific coral hybrids

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Abstract

Maternal effects have been well documented for offspring morphology and life history traits in plants and terrestrial animals, yet little is known about maternal effects in corals. Further, few studies have explored maternal effects in gene expression. In a previous study, F1 interspecific hybrid and purebred larvae of the coral species *Acropora tenuis* and *A. loripes* were settled and exposed to ambient or elevated temperature and pCO_2 conditions for seven months. At this stage, the hybrid coral recruits from both ocean conditions exhibited strong maternal effects in several fitness traits. We conducted RNA-sequencing on samples from the same experiment and showed that gene expression of the hybrid *Acropora* also showed clear maternal effects. Only 40 genes were differentially expressed between hybrids and their maternal progenitor. In contrast, ˜2000 differentially expressed genes were observed between hybrids and their paternal progenitors, and between the reciprocal F1 hybrids. These results indicate that maternal effects in coral gene expression can be long-lasting. Unlike findings from most short-term stress experiments in corals, no genes were differentially expressed in the hybrid nor purebred offspring after seven months of exposure to elevated temperature and pCO_2 conditions.

Keywords

Maternal effects, interspecific hybrids, gene expression, RNA-sequencing, coral reefs

Introduction

Maternal effects can have a large impact on the fitness of offspring. In plants, maternal effects in seed traits (e.g., seed mass, germination time) and offspring fitness (e.g. growth rates) have been well documented (Donohue, 2009). Maternal age at reproduction is known to affect diapause (i.e., suspended development induced by unfavorable environmental conditions) in offspring of insects (Mousseau & Dingle, 1991), and in amphibians, maternal factors have well known effects in size and rates of development (Warne et al., 2013).

Maternal effects can be the result of the direct effects of the environment on epigenetic marks, genomic imprinting, or maternal provisioning (which is influenced by both environmental and genetic effects). For example, the environment experienced by the mother can affect the expression of genes involved in germination of*Arabidopsis thaliana* offspring (for review, see Donohue, 2009). Genomic imprinting is the epigenetic silencing (e.g., via cytosine methylation or chromatin-mediated processes) of one of the parental chromosomes, leaving only expression from the non-silenced chromosome (Alleman & Doctor, 2000). In the case of maternal effects, only the maternal chromosomes are expressed and this can be transmitted to one or more subsequent generations (Bischoff & Müller-Scharer, 2010). Genomic imprinting has been observed in a few insect species, plants and placental mammals (for review, see Matsuura, 2020; Thamban et al., 2020), but not in egg-laying vertebrates such as birds, monotremes and reptiles by far (Killian et al., 2001; Renfree et al., 2013).

Maternal provisioning is the supply of nutrients, resources and hormones by the mother during seed or egg development (Videvall et al., 2016). For example, the amount of stored nutrient reserves in seeds can significantly influence early seedling growth and development (Slot et al., 2013). Maternal effects can also manifest via the seed coating (which is maternally produced), the endosperm (which is a triploid tissue with two-third of genotype from the maternal parent), and/or via direct maternal effects in dispersal (Donohue, 2009). For instance, flowering time in *Campanula americana* determines whether the progeny will germinate in autumn or spring (Galloway & Etterson, 2007). For many marine larvae, maternal provisioning of lipids is the major source of endogenous energy and this accounts for ˜40% of the metabolic needs of coral larvae (Harii et al., 2010). Maternal provisioning is affected by both the genotype and the environmental conditions experienced by the mother. For example, maternal exposure to hormones can change egg and larval morphology of reef fishes (McCormick, 1999). Maternal effects due to provisioning generally decrease over time (Roach & Wulff, 1987), but can also persist through the entire life cycle of an organism.

When different genotypes are combined to produce F1 (i.e., first generation) hybrids, maternal effects can affect the phenotypes of F1 offspring. Hybridization is the crossing between separate species or between strains/lines/populations within a species. The phenotypes of the F1 offspring may be similar to that of their maternal parents (i.e., maternal effects), intermediate between the parents (i.e., additive effects), similar to that of the dominant parent (i.e., dominance), or different to both parents (i.e., over-dominance or under-dominance) (Chen, 2013; Li et al., 2008; Lippman & Zamir, 2007). For example, environmental conditions experienced by the mother can influence the expression of genes involved of germination in progeny (Donohue, 2009). However, hybrid gene expression studies often only involve hybrids of one direction (Videvall et al., 2016), and hence are unable to distinguish between dominance effects and maternal effects.

For corals, maternal effects in morphology (Willis et al., 2006), survival (Chan et al., 2018; Isomura et al., 2013) and thermal tolerance (Dixon et al., 2015) have been reported. Chan et al. (2018) showed that interspecific hybrids of the corals *Acropora tenuis* and*Acropora loripes* had similar survival and growth to their maternal purebreds, although they exceeded parental performances in some cases. The bacterial and microalgal endosymbiont (Symbiodiniaceae spp.) communities associated with these corals did not differ between the reciprocal hybrids and their maternal and paternal purebreds (Chan et al., 2019). Since these microorganisms carry vital functions to the coral hosts and can contribute to holobiont fitness differences (Blackall et al., 2015; Rosenberg et al., 2007), this finding suggests that the microbial communities were unlikely responsible for the observed holobiont fitness differences, and that these are likely underpinned by coral host genetic and/or non-genetic transgenerational factors.

The aim of this study was to test if the phenotypic differences in reciprocal F1 hybrids of the corals *A. tenuis* and *A. loripes* could be linked to patterns of host gene expression. Four offspring groups (i.e., reciprocal F1 hybrids and two parental purebreds) were previously produced via a laboratory cross of *A. tenuis* and *A. loripes* and were exposed to seven months of ambient or elevated temperature and p CO_2conditions (Chan et al., 2018). Using samples from the same experiment, we tested for maternal effects in gene expression, as observed in hybrid survival and growth. In addition, gene expression was examined between temperature/p CO_2 conditions within each offspring group.

Materials and methods

Experimental design and sample collection

Parental coral colonies of *A. tenuis* and *A. loripes* were collected from Trunk Reef (18deg35'S,

146deg80'E), central Great Barrier Reef in November 2015 and crossed in the laboratory to form two F1 hybrid and two parental purebred offspring groups (see Figure 2, Chan et al., 2018 for detailed crossing protocol and experimental design). Briefly, parental colonies were kept and spawned under ambient conditions. Egg-sperm bundles of individual parental colonies were collected and separated with a 100 µm filter. A mixed sperm solution with equal quantity of sperm from each conspecific colony was used to fertilize eggs from the other species in the cross to produce the hybrids, and to fertilize conspecific eggs to produce the purebreds. The abbreviation of the offspring groups throughout this study are: TT (purebred *A. tenuis*),

TL (hybrid), LT (hybrid) and LL (purebred *A. loripes*), where the maternal parent is listed prior to the paternal parent in a genetic cross by convention (Miller et al., 2012). For example, "TL" is a hybrid formed by crossing *A. tenuis* eggs with *A. loripes* sperm.

Embryos were reared to planula stage and settled onto ceramic plugs under ambient conditions five days post-spawning. Settled recruits were randomly and evenly distributed across two treatment conditions: ambient conditions (27ºC and 415 ppm$p$ CO_2) and elevated conditions (ambient +1 °C and 685 ppm p CO_2). There were 12 replicate tanks per treatment and each tank contained 20 ceramic plugs of each of the four offspring group (i.e., each offspring group had 12 x 20 = 240 ceramic plugs per treatment). For the elevated conditions, recruits were ramped at a rate of +2 ºC and +˜50ppm a day until they arrived at the targeted conditions. Given the predicted sea surface temperature (SST) increase in coral reefs ranges from ˜ 1.4 and ˜3.6 °C by the year 2100 (under RCP 2.6 and 8.5 respectively and relative to pre-Industrial period) (Bindoff et al., 2019), an elevated temperature of +1 ºC to present day ambient temperature reflects a realistic scenario that will likely occur in the coming decades. Note that present day SST has already increase by ˜0.9°C since pre-industrial time (Bindoff et al., 2019).

Coral recruits were reared under treatment conditions in filtered seawater for seven months at the National Sea Simulator of the Australian Institute of Marine Science. A microalgal diet supplement was supplied to the corals daily and their fitness traits and associated microbial communities were examined. To mimic the natural environment as closely as possible, the experimental conditions followed diurnal and annual temperature variations of Davies Reef (18.83° S, 147.63° E), which is a reef near the collection sites of the parental colonies. At the end of the seven-month experiment, recruits from three tanks of each treatment were randomly selected for sampling. Due to the small size (and therefore low RNA quantity) of individual recruits, multiple recruits of the same offspring group from the same tank were pooled to form one sample. Each pooled sample contained 30 coral polyps. RNA pooling was considered appropriate as the purpose of this study was to examine population-level rather than individual-level differences (Davies et al., 2016; Kendziorski et al., 2003). Three pooled samples per offspring group per treatment were collected, except only one sample was available for purebred *A. tenuis* (TT) under elevated conditions due to high mortality (Table S1). Samples were snap-frozen in liquid nitrogen and stored at -80 °C until RNA extraction.

RNA extraction

Sample tissues were mechanically disrupted prior to RNA isolation. Approximately 30 acid washed glass beads (Sigma, 710-1180 µm diameter) and 600 µl RLT buffer (Qiagen) were added to each sample. The samples were then subjected to 2 x 40 s cycles of bead beating at 4/s in a fast Prep-245G (MP Biomedicals). Total RNA was isolated from the sample homogenate using Qiagen RNeasy mini kit (including the optional DNase treatment). Total RNA was eluted in 40 µl of RNase free water and 3 µl were visualized on a 1% agarose, 0.5 x TBE gel for quality check. RNA concentration was measured using the Qubit RNA HS Assay (Thermo Fisher Scientific/Invitrogen), with fluorescence analysis on a NanoDrop 3300 Fluorospectrometer (Thermo Fisher Scientific). Between 20.5 and 106 ng total RNA underwent reverse transcription and cDNA was amplified using NuGen's Ovation V2.0 kit (with one cycle amplification). The amplified cDNA was then purified using magnetic beads (Beckman Coulter Agencourt kit) and 1 µl was visualized on a 1% agarose, 0.5 x TBE gel. Purity of sample cDNA was determined by A260/A280 ratios measured with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). cDNA concentration was measured using the Quant-iT PicoGreen dsDNA Assay (Thermo Fisher Scientific/Invitrogen). Sample cDNA concentrations were normalized and 25 µl of 20 ng/µl cDNA were sent to Ramaciotti Centre for Genomics (UNSW, Sydney) for Nextera XT Library Preparation and paired-end sequencing on the Illumina NextSeq500 platform (2 x 75bp). The total RNA concentration and quality, the amount of total RNA that underwent reverse transcription, cDNA concentration and quality, as well as raw reads of each sample are shown in Table S2.

Sequence data processing

Quality and adapter trimming were carried out on raw reads using Trimmomatic (Bolger et al., 2014), discarding reads $<$ 50 bp or with an averaged quality score $<$ 20 in a sliding window of five bases. Since

the coral holobiont is associated with high densities of prokaryotes and algal endosymbionts, reads were filtered with the following steps: First, reads were compared to an rRNA database (Silva132_LSU, Silva132_-SSU) and matches (i.e., e-values [?] 10^{-5}) were removed using the program SortMeRNA (Kopylova et al., 2012). Second, reads were compared to the algal endosymbiont genome (genus *Cladocopium,* symC_scaffold_-40.fasta (Shoguchi et al., 2018) and matches were removed using BBDuk (Bushnell, 2020). The remaining reads of each sample are shown in Table S2 and were used to create a *de novo* assembly for the each offspring groups and a combined *de novo* assembly for all four offspring groups using Trinity (Grabherr et al., 2011). Small transcripts of < 400 bp were removed from the assemblies (Kenkel & Bay, 2017), and the longest isoform of each trinity transcript was obtained. Mitochondrial genes were identified running BLASTn against the *A. tenuis*mitochondrial genome (NC_003522.1.fasta, van Oppen et al., 2002) and were retained in the analysis. The remaining transcripts were then identified by BLASTx searches against the most complete coral gene model (*A. digitifera* , GCF_000222465.1_Adig_1.1_protein.faa, Shinzato et al., 2011) and NCBI's nonredundant (nr) protein database, with a e-value cut off [?] 10^{-5}.

Gene names and gene ontologies (GO) of the transcripts were assigned using BLASTx search against UniProt Knowledgebase Swiss-Prot database (The UniProt Consortium, 2015). Duplicate query transcripts were removed. Transcript abundance of the samples was then estimated using RSEM, an alignment-based method (Li & Dewey, 2011). Transcript quantification of the samples was performed by aligning reads using bowtie2 (Langmead & Salzberg, 2012) and estimating abundance with RSEM (Li & Dewey, 2011). For gene expression comparison between hybrids and parental purebreds, we tested estimating transcript abundance using the assembly of purebred *A. loripes* , as well as the combined assembly produced using all offspring groups. The two methods revealed very similar results (Figure S1), and the results presented here are based on transcript abundance estimated using the assembly of purebred *A. loripes* . Due to the small number of samples available for the parental purebred *A. tenuis* (Table S1), a *de novo* assembly was not conducted or tested as a basis for transcript abundance estimate. For gene expression comparison between treatments within an offspring group, the *de novo* assembly of each offspring group was used to estimate transcript abundance. Treatment comparison was not conducted for*A. tenuis* purebreds due to an insufficient number of samples (Table S1).

Statistical analyses

Separate analyses were conducted to compare gene expression between hybrids and parental purebreds, and ambient versus elevated conditions within an offspring group. In addition, a separate analysis was carried out for mitochondrial genes. Transcript abundance of the samples and the BLAST results were analyzed in R and differential expression analysis was performed using the package *limma* (Ritchie et al., 2015). Firstly, only transcripts that were of coral origin were retained, as indicated in the BLAST results. For the mitochondrial analysis, only transcripts that matched with the mitochondrial genome were used. Secondly, transcripts that consistently had zero or very low counts were removed using the edgeR build in function filterByExpr, and scale normalization (TMM) was applied. For Principal Components Analysis (PCA), sample raw counts were transformed into log2-counts per million (log-CPM) to account for library size differences.

A total of four samples were identified to have small library size (three *A. tenuis* purebreds- two under ambient, one under elevated conditions, and one TL hybrid under elevated conditions), and a relative log expression (RLE) plot showed that normalization of these samples was unsuccessful (Gandolfo & Speed, 2018) (Figure S2, Table S2). These samples were excluded from the main manuscript, but their analyses were retained in the Supplemental Information. A heatmap was then used to visualize the 500 most variable genes across samples using the log-CPM expression values with dendrograms computed using Euclidean distances. For the mitochondrial analysis, a PCA and a heatmap were generated using all genes that remained post-filtering.

To fit linear models for comparisons, count data was transformed to log-CPM using the voom function in the limma package. Since no treatment effect was found on gene expression (see Results section), the comparison of hybrids and purebreds combined samples from both treatments. Comparisons were made between: 1) maternal purebred LL and its hybrid LT, 2) paternal purebred LL and its hybrid TL, and 3) between the

reciprocal hybrids LT and TL. The purebred TT (*A. tenuis*) was not included due to a small sample size (n =1, Table S1). Empirical Bayes moderated t-statistics were generated to assess the pairwise comparisons, and p -values were corrected using the Benjamini-Hochberg method (Benjamini & Hochberg, 1995). A gene was considered differentially expressed when $p_{adj} < 0.05$ using the *treat* function in the limma package with a log-fold-change threshold of > 0.2. The list of differentially expressed genes (DEGs) was exported for gene ontology (GO) analyses and visualized using volcano plots (Blighe et al., 2018). The volcano plots and GO analyses focused on the comparison of 1) paternal purebred LL with its hybrid TL, and 2) between the reciprocal hybrid LT and TL only, as these were the pairs with a high number of differentially expressed genes to explore.

Two different approaches were applied to the GO analyses, including GOseq (Young et al., 2010) and a rank-based GO analysis with adaptive clustering using a Mann-Whitney U (MWU) test (https://github.com/z0on/GO_MWU, Dixon et al., 2015). For GOseq, the analysis was conducted using the list of DEGs and the p- values were corrected with the Benjamini-Hochberg method (Benjamini & Hochberg, 1995). A GO category was considered overrepresented or underrepresented when the p_{adj} was < 0.05 and that the category had > 3 DEGs. For the MWU test, the hierarchical clustering trees utilized the log10-transformed$p$ -values of the DEGs and indicated significantly enriched GO categories by up-regulated (red) or down-regulated (blue), under a false discovery rate of 10%. In addition, differentially expression nuclear genes in GO categories with functions connected to the mitochondrion were identified.

Results

On average, ˜12.5 million raw Illumina reads were obtained per sample. After quality trimming and removal of rRNA and algal endosymbiont components, an average of ˜6.2 million paired reads were retained per sample. The transcriptome of purebred *A. loripes* contained ˜291 k transcripts, and ˜59 k transcripts were left after only retaining the longest isoforms and removal of small transcripts < 400 bp. See Table S3 for details of other transcriptomes used for preliminary analysis and evaluating treatment effect. For a total of ˜35 k transcripts a match of coral origin was found in the NCBI nr database. Following the removal of duplicates and transcripts that consistently had zero or very low counts, 8800 transcripts were retained and used for downstream analyses.

Transcriptome-wide gene expression of the hybrids was similar to that of their maternal purebreds, yet distinct from their paternal purebreds and the reciprocal hybrids (Figures 1-3, S3). Principal component analyses (PCA) showed similar expression patterns of the hybrid LT with its maternal purebred LL under both ambient and elevated conditions (Figure 1). The only exception was one LL purebred sample which showed separation with the others in principle component two (Figure 1). Gene expression of the reciprocal hybrid TL also clustered with its maternal purebred TT (but note that n = 1 for TT), and was separated with hybrid LT and its paternal purebred LL under both treatment conditions (Figure 1). The four samples excluded from the main analyses due to small library sizes also supported the existence of maternal effects (Figure S4-S5). The amount of total RNA and cDNA input, as well as the number of raw reads of the samples showed no specific patterns in the PCA plots, suggesting that the observed maternal patterns of the offspring groups were not driven by these factors (Figure S4). Within an offspring group, gene expression did not differ between ambient and elevated conditions (Figure 1). Maternal patterns were not observed in the PCA plot and heatmap generated using mitochondrial genes only (Figure S6-S7).

Differential expression analysis resulted in only 40 DEGs between the maternal purebred LL and its hybrid LT (Figure 2). In contrast, almost 2000 DEGs were identified between the paternal purebred LL and its hybrid TL, as well as between the reciprocal hybrids LT and TL (Figure 2). Among these ˜2000 DEGs, the hybrid LT and its maternal purebred LL shared 1343 genes that were differentially expressed from the hybrid TL (Figure 2). Maternal effects in gene expression were also evident in the heatmap of the 500 most variable genes across samples (Figure 3). The only exception was one purebred LL sample which clustered away from the other LL samples, and this was the same sample that showed separation in the PCA plot (Figure 1, 3). The heatmap that includes the four samples removed from the main analyses due to small library sizes confirmed the maternal effects observed in the smaller subset of samples (Figure S5).

Among the DEGs with the highest log-fold change (i.e., four DEGs for paternal purebred LL compared to its hybrid TL, and seven DEGs for hybrid LT compared to hybrid TL with LFC > 5), three were shared genes between the two pairs of comparison (Figure S3). Unfortunately, most of these DEGs were annotated as uncharacterized proteins and hence their potential functions were unknown (Table S4). Only one differentially expressed mitochondrial gene (TRINITY_DN76286_c6_g1_i1) was identified between hybrid TL and its paternal purebred LL ($p_{ad}j = 0.03$). No differentially expressed mitochondrial genes were found in all other pairs of comparison.

For gene ontology (GO) analyses using GOseq, GO category "cytosol" (GO: 0005829) was underrepresented in both the comparisons between the paternal purebred LL with its hybrid TL and between the reciprocal hybrids LT and TL, with 90 and 96 DEGs respectively in this category (Table S5). Note that "cytosol" is a very broad GO category and it was comprised of 620 genes in this dataset. In addition, the GO category "membrane" (GO: 0016020) was also underrepresented in the comparison between the paternal purebred LL and its hybrid TL (Table S4). This was also a broad GO category with 255 genes in this dataset, 27 of which were DEGs. In contrast, GO analyses using the MWU test showed no significant GO category was over- or under-represented. However, note that the MWU test omits GO categories that are too broad (i.e., a GO category that contains a large proportion of the total number of genes). For this reason, it was unsurprising that the very broad GO categories "cytosol" and "membrane" that were identified as underrepresented using GOseq were not significant here.

For offspring groups that had different maternal parent species (i.e., between the hybrid TL and its paternal purebred LL, and between the reciprocal hybrids LT and TL), 84-88 DEGs were identified in GO categories with functions connected to the mitochondrion (Table S6). In contrast, no DEGs were found in GO categories linked to the mitochondrion when the offspring groups shared the same maternal parent species (i.e., between the hybrid LT and its maternal purebred LL). The proportion of DEGs over total number of genes was similar between genes in GO categories linked to the mitochondrion and genes in all GO categories (14.9-17.6%, Table S6).

Discussion

Maternal effects in coral fitness are reflected in gene expression patterns

Maternal effects in recruit survival and size previously reported for *A. loripes* x *A. tenuis* hybrid corals (Table 1) were consistent with their gene expression patterns. At the time when the corals were sampled for gene expression analyses, the hybrid LT and its maternal purebred LL had higher survival compared to the hybrid TL and its maternal purebred TT under both ambient and elevated conditions. Although the corals did not differ in size at seven months of age, maternal effects in size were evident by one year of age (Table 1). Maternal effects have previously been reported for other Indo-Pacific *Acropora* hybrid corals obtained via laboratory crossing. These include effects in: 1) morphology of interspecific hybrids from an *A. pulchra* x *A. millepora* cross (Willis et al., 2006), 2) survival of interspecific hybrid larvae from an *A. florida* x*A. intermedia* cross (Isomura et al., 2013), and 3) thermal tolerance of intraspecific *A. millepora* hybrid larvae from a higher and lower latitude population. In contrast, paternal effects were found in morphology of natural interspecific hybrids of *A. palmata* and *A. cervicornis* from the Caribbean (Vollmer & Palumbi, 2002), and additive effects in survival (i.e., hybrid survival was intermediate between the parental offspring) were observed in experimentally produced intraspecific hybrids of *A. millepora*from a higher and lower latitude cross (van Oppen et al., 2014).

While a few studies have reported maternal effects in coral fitness and morphology, little is known about maternal effects in gene expression. In addition to the coral host, the host-associated microbiome can also have an impact on host gene expression (Barfield et al., 2018; Buerger et al., 2020; Helmkampf et al., 2019). In our study, however, the bacterial and microalgal endosymbiont communities of the corals were similar at the time of sampling (Table 1). The consistency between host gene expression and phenotypic results thus suggests that maternal host-related factors were likely the drivers behind the observed fitness differences. A large number of differentially expressed genes (˜2000 DEGs) were found when comparing offspring groups

that had different maternal parent species (i.e., between the hybrid TL and its paternal purebred LL, and between the reciprocal hybrids), but not when the groups shared the same maternal parent species (i.e., only 40 DEGs between the hybrid LT and its maternal purebred LL). Maternal effects were evident in these corals based on PCA, heatmap and volcano plots. While a statistical comparison cannot be made back to the parental purebred TT due to small sample size, gene expression of hybrid TL was similar to the only TT sample tested based on PCA and the heatmap was indicative of maternal effects. The four samples omitted from the main analyses because of their small library sizes also supported the presence of maternal effects, although inferences drawn from these samples should be taken with caution.

In our study, however, no mitochondrial genes were differentially expressed and PCA and heatmap of mitochondrial genes did not show maternal patterns. In other words, evidence of maternal gene expression patterns was only found in the nuclear genes, but not in the mitochondrial genes or via mito-nuclear crosstalk in this study (although note that only seven mitochondrial genes were available for comparison post-filtering).

Several studies have reported maternal effects in gene expression including in a perennial herb (Videvall et al., 2016), coral (Dixon et al., 2015), pipefish (Beemelmanns & Roth, 2016) and stickleback (Metzger & Schulte, 2016; Mommer & Bell, 2014; Shama et al., 2016), and maternal environments have also been demonstrated to affect DNA methylation of sea urchin (Strader et al., 2020). Videvall et al. (2016) showed that gene expression patterns were distinct between parental populations of 12-week-old seedling of the perennial herb*Arabidopsis lyrata* , and expression in intraspecific hybrids was frequently more similar to that of the maternal than paternal population. Only 15 DEGs were found between the hybrid produced in one direction and its maternal population, yet $>$ 8800 DEGs were found when compared to its paternal population (Videvall et al., 2016). Interestingly, maternal effects were weaker in the hybrid cross of the other direction, with 334 and 661 DEGs observed when compared to its maternal and paternal population respectively (Videvall et al., 2016). Only one previous study has examined maternal effects in coral hybrid gene expression and only coral larvae were studied. Consistent with our findings, Dixon et al. (2015) showed that gene expression of intraspecific *A. millepora* hybrid larvae was similar to that of their maternal population (i.e., up to 2,000 genes in hybrids followed the expression patterns of the maternal population). In these studies (Dixon et al., 2015 and Videvall et al., 2016) however, maternal effects were examined in early life stages only (i.e., 12-week-old seedling and 6-day-old larvae). Our results show that maternal effects can continue to influence gene expression of hybrid corals up to the age of at least seven months, indicating the potential long-term nature of maternal effects.

While differences in gene expression patterns were obvious between reciprocal hybrids as well as between the hybrid TL and its paternal purebred, it was unclear what pathways and mechanisms were linked to these differences and underpinned observed phenotypic differences (Chan et al., 2018). Gene ontology (GO) analyses revealed underrepresentation of a very broad GO category, "cytosol", in both pairs of comparison. It is also possible that maternal provisioning had long-lasting effects in offspring (that were seven months old) and was responsible for the phenotypic and gene expression differences (i.e., poorly provisioned offspring may exhibit pervasive differences in transcription). Future studies on maternal effects in corals will benefit from quantifying differences in maternal provisioning between the parental species, such as lipid/protein content of eggs and early larvae.

In contrast, clear pathways involved in maternal effects were observed in the intraspecific *A. millepora* hybrid larvae (Dixon et al., 2015). Analyses of cellular component categories of tolerance-associated genes (i.e., genes for which expression levels prior to stress predicted the probability of larval survival under stress) showed enrichment of nuclear-encoded mitochondrial membrane components in hybrid coral larvae whose parents come from a warmer latitude (Dixon et al., 2015). The most upregulated GO categories were energy production and conversion, and encompassed mitochondrial proteins, suggesting mitochondrial protein variation in larvae may have contributed to maternal effects in thermal tolerance (Dixon et al., 2015).

The difference in GO associated patterns between these two studies may be due to 1) the parental populations chosen for hybridization, 2) the symbiotic/aposymbiotic nature of the corals and 3) the life stage of the corals. Parental populations of the same species from different latitudes were selected in Dixon et al. (2015), whereas

parental populations of two different species from the same reef were chosen for this study. The differences in parental thermal regimes in Dixon et al. (2015) may lead to clearer maternal effects in thermal stress-related GO categories. Moreover, gene expression responses of aposymbiotic larvae in Dixon et al. (2015) were likely different from coral recruits (in this study) that were associated with a high density of microalgal endosymbionts. The effects of maternal provisioning on gene expression is also likely to be stronger in early larvae than in seven-month-old recruits. Hence, the contrasting results of the two studies are unsurprising. Further, mitochondrial genes may not show maternal patterns if maternal provisioning was responsible for the phenotypic maternal patterns observed in these corals.

Gene expression was unaffected by long-term exposure to elevated temperature and pCO_2 conditions

Elevated temperature and p CO_2 conditions had a negative impact on survival and size of the corals used in this study (Table 1), yet gene expression within an offspring group did not differ between ambient and elevated conditions (Figure 1). Nevertheless, gene expression changes under short-term acute stress are commonly found in coral. This often involves the regulation of genes encoding heat shock proteins, ion transport, apoptosis, immune responses and/or oxidative stress (Barshis et al., 2013; Desalvo et al., 2008; Meyer et al., 2011; Ruiz-Jones & Palumbi, 2017). The absence of DEGs in corals under ambient versus elevated conditions was unexpected and may be due to the relatively mild and long-term nature of the treatments. The elevated conditions of this study (ambient +1 °C, 685 ppm p CO_2) were relatively mild compared to many other longer-term studies (e.g., ambient +7 and + 12 ºC, Maor-Landaw et al., 2017; 856-3880 ppm$p$ CO_2, Vidal-Dupiol et al., 2013). In addition, gene expression responses of corals under long-term stress have been shown to differ from those under short-term stress. Despite significant differences in CO_2 concentration under control and natural CO_2 seep sites (i.e., ˜355 versus 998 ppm), only 61 DEGs were found in *A. millepora* from the two sites (Kenkel et al., 2017). Similarly, the expression of calcification-related genes changed significantly in *A. millepora* subjected to short-term (i.e., 3 days) high p CO_2 exposure (Moya et al., 2012, 2015), but far fewer DEGs were found as exposure time increased (Moya et al., 2015; Rocker et al., 2015). Since cellular stress gene expression responses can be transient (Kültz, 2003), certain expression changes may only be detectable during the initial exposure and therefore fewer differentially expressed genes are generally found in long-term studies.

Conclusions and future studies

This study showed that maternal effects manifested as gene expression differences in interspecific hybrids of the coral *A.tenuis* and *A. loripes* . We also showed that maternal effects can persist to at least seven months of age in coral and were likely responsible for the phenotypes of F1 hybrids. However, the pathways and mechanisms responsible for the phenotypic differences were unknown and exposure to elevated temperature and$p$ CO_2 conditions did not result in differential coral gene expression. Although the composition of bacterial and microalgal endosymbiont communities of these corals was similar under ambient and elevated conditions and between hybrids and purebreds, these microbes may have expressed different genes and contributed to holobiont phenotypic differences. Future studies will benefit from examining the gene expression of these microbial communities alongside the host. Other less studied members of the coral holobiont, such as viruses and fungi (that were not examined), may also have contributed to coral survival and size differences between offspring groups and treatment conditions. Further, post-transcriptional and epigenetic regulation (e.g., DNA methylation) may have varied between treatments and hybrid and purebreds and may have resulted in phenotypic differences (Dimond et al., 2017). Future studies should consider adopting a multi-omics approach and assessing other members of the coral-associated microbiome to explore other mechanisms that underpin the phenotype of the coral holobiont.

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Author's ontribution. W.Y.C., M.J.H.O., L.P. and A.H. designed the study. W.Y.C. and

L.P. conducted the experiment. L.P. carried out the laboratory work. J.C., W.Y.C. and A.H. undertook bioinformatic and statistical analyses. W.Y.C. and M.J.H.O. wrote much of the manuscript and all authors contributed to the final edited version of the manuscript.

Data Availability Statement

Raw sequences are available in GenBank (SRR12695232 to SRR12695253, project accession no.: PRJNA665083) and the R scripts for statistical analyses are available as Appendix S1.

Data Citation

[dataset]Chan, W. Y., Chung J., Peplow, L., Hoffmann, A. A., & van Oppen, M. J. H. (2020). Maternal effects in gene expression of interspecific coral hybrids. GenBank: PRJNA665083; SRR12695232-SRR12695253.

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Figures

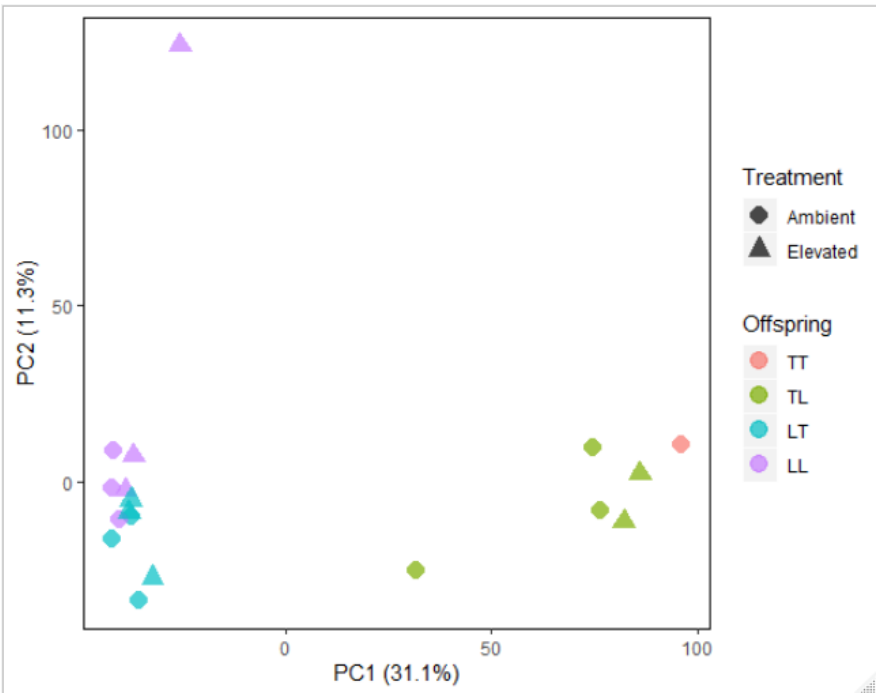

Figure 1. Principal component analyses of the offspring groups using normalized counts (i.e., log-CPM) of the 8,880 genes retained post filtering. The maternal parent is listed prior to the paternal parent for the abbreviation of the offspring groups, where "T" is *A. tenuis* and "L" is *A. loripes* .

(a)

	LL vs TL	LL vs LT	LT vs TL
Up regulated	1346	34	1323
Down regulated	605	6	659
Total differentially expressed	1951	40	1982
Not significant	6929	8840	6898

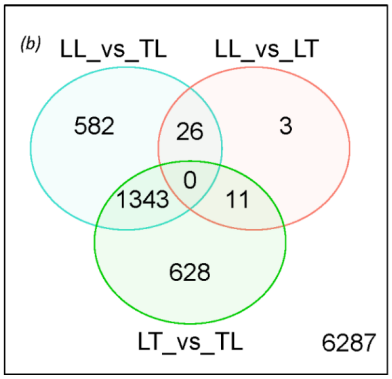

Figure 2. (*a*) The number of up or down regulated genes between the pairs of offspring groups ($p_{adj} < 0.05$ when tested with a log-fold-change threshold > 0.2). (*b*) Venn diagram showing the number of differentially expressed genes (DEGs) between the pairs of offspring groups. The overlapping space between the circles indicates the number DEGs in both pairs of comparison. The abbreviation of the offspring groups is that the first letter indicates maternal parent and the second letter the paternal parent, where "T" is *A. tenuis* and "L" is *A. loripes* .

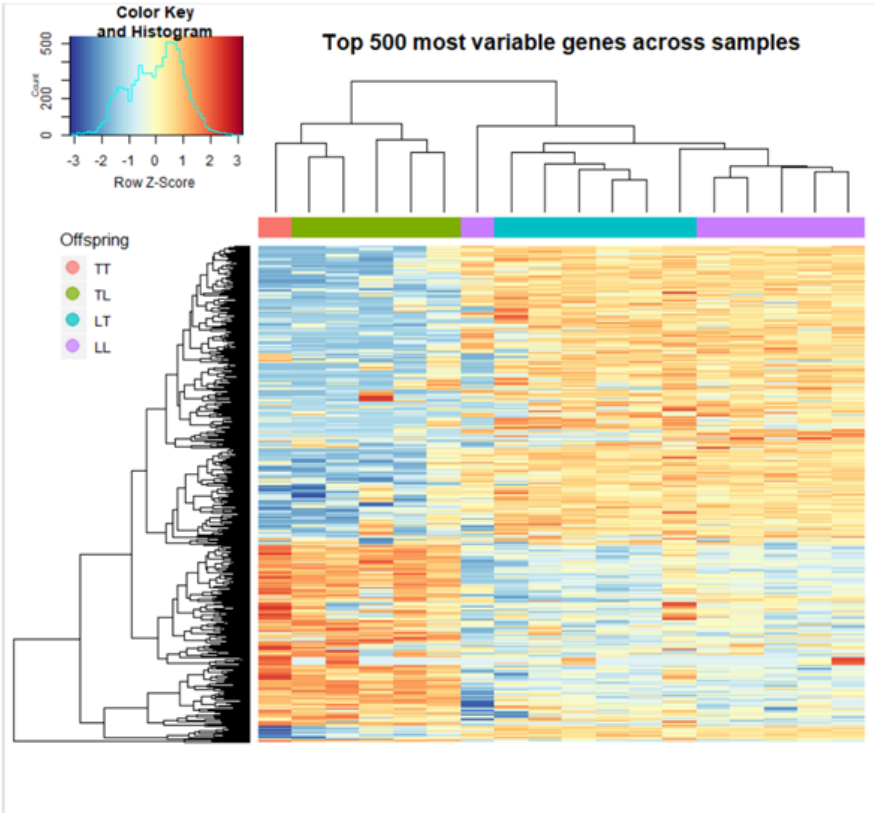

Figure 3. Heatmap of the 500 most variable genes across samples using the log-CPM expression values with dendrograms computed using Euclidean distances. "T" refers to *A. tenuis* and "L" refers to *A. loripes* in the offspring group abbreviation, and the maternal parent is listed prior to the paternal parent.

Table 1. A summary of the key conclusions from previous works on the phenotypes and microbiome of the corals of this study.

Trait	**Key conclusions**	**Reference**
Survival (7 months)	Hybrid LT and its maternal purebred LL survived better (7-23%) than hybrid TL and its maternal purebred TT (36-49%) under both ambient and elevated conditions. Only purebred TT had significantly poorer survival under elevated (7%) than ambient conditions (13%).	Chan et al., 2018
Size (7 months)	Offspring groups were not different in size under both ambient and elevated conditions. Elevated temperature and pCO_2 conditions resulted in smaller size of all purebred and hybrid offspring groups.	Chan et al., 2018

Trait	Key conclusions	Reference
Size (1 year)	Hybrid LT and its maternal purebred LL grew bigger (290-366 mm^2) than hybrid TL (47mm^2). Purebred TT had no survivors.	Chan et al., 2018
Bacterial community (7 months)	Offspring groups were not associated with different bacterial communities as determined with 16S rRNA gene metabarcoding.	Chan et al., 2019
Microalgal symbiont community (7 months)	Offspring groups were not associated with different microalgal symbiont communities as determined with ITS2 metabarcoding.	Chan et al., 2019

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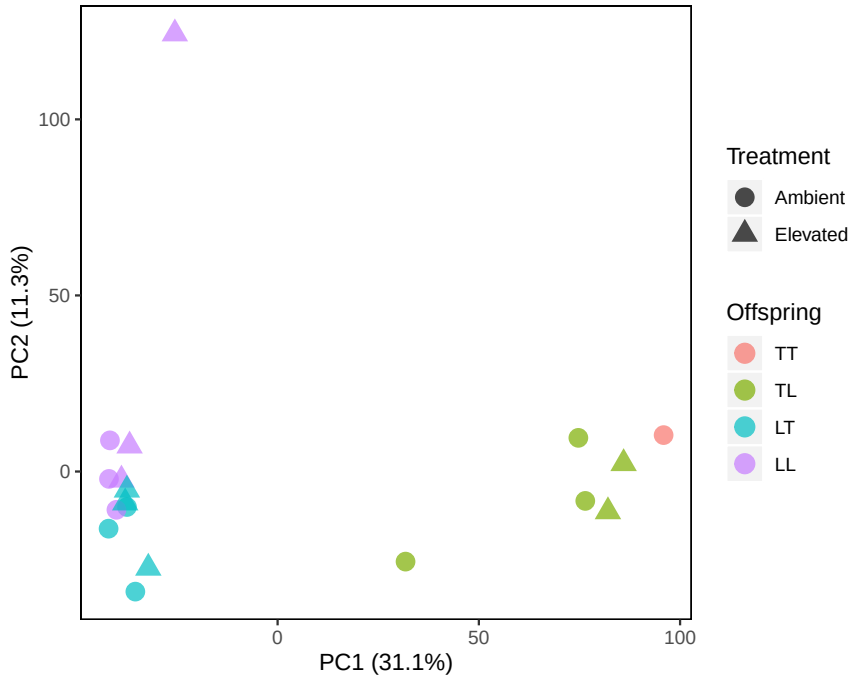

(a)

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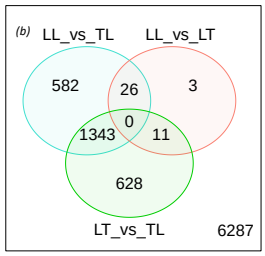

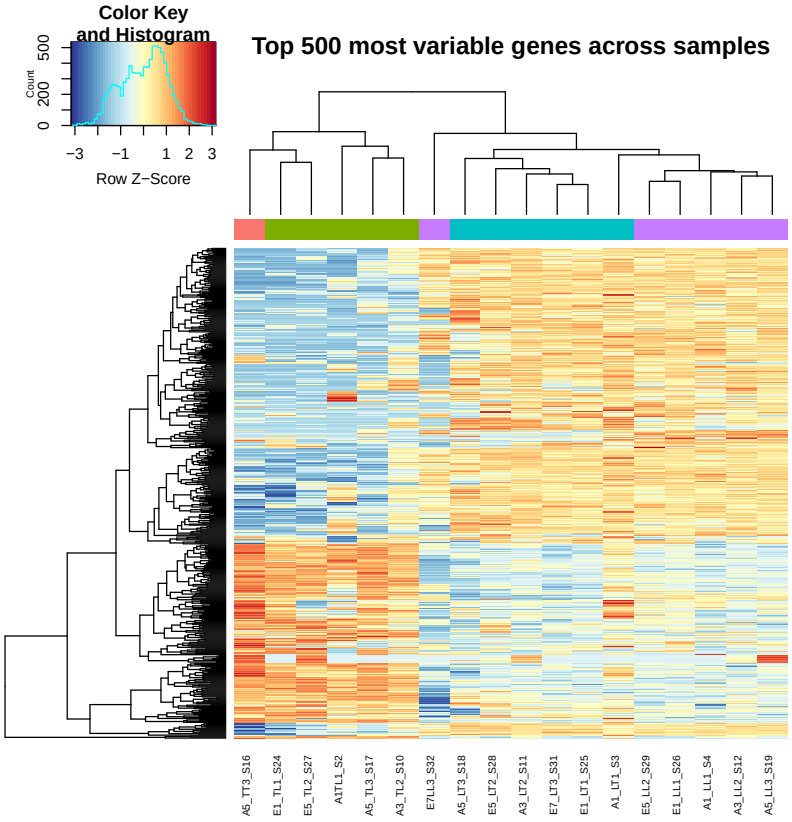

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