1 **Maternal effects in gene expression of interspecific coral hybrids**

2

3 Wing Yan Chan*†, Jessica Chung‡§, Lesa M. Peplow*, Ary A. Hoffmann‡, Madeleine J. H. van

4 Oppen*†

5 *Australian Institute of Marine Science, Townsville, QLD, Australia, †School of BioSciences,

6 University of Melbourne, VIC, Australia, ‡Bio21 Institute, University of Melbourne, VIC,

7 Australia, §Melbourne Bioinformatics, University of Melbourne, VIC, Australia

8

9 **Corresponding author**

10 Wing Yan Chan

11 Address: School of BioSciences, University of Melbourne, VIC, Australia

12 Email: w.chan@unimelb.edu.au

13

14 **Running title**

15 Coral maternal gene expression effects

16 **Abstract**

17 Maternal effects have been well documented for offspring morphology and life history traits in

18 plants and terrestrial animals, yet little is known about maternal effects in corals. Further, few

19 studies have explored maternal effects in gene expression. In a previous study, F1 interspecific

20 hybrid and purebred larvae of the coral species *Acropora tenuis* and *A. loripes* were settled and

21 exposed to ambient or elevated temperature and pCO_2 conditions for seven months. At this stage,

22 the hybrid coral recruits from both ocean conditions exhibited strong maternal effects in several

23 fitness traits**.** We conducted RNA-sequencing on these corals and showed that gene expression of

24 the hybrid *Acropora* also exhibited clear maternal effects. Only 40 genes were differentially

25 expressed between hybrids and their maternal progenitor. In contrast, ~2000 differentially

26 expressed genes were observed between hybrids and their paternal progenitors, and between the

27 reciprocal F1 hybrids. These results indicate that maternal effects in coral gene expression can be

28 long-lasting. Unlike findings from most short-term stress experiments in corals, no genes were

29 differentially expressed in the hybrid nor purebred offspring after seven months of exposure to

30 elevated temperature and pCO_2 conditions.

31

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-Schärer, 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

3

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.

80

81 For corals, maternal effects in morphology (Willis et al., 2006), survival (Chan et al., 2018;

82 Isomura et al., 2013) and thermal tolerance (Dixon et al., 2015) have been reported. Chan et al.

83 (2018) showed that interspecific hybrids of the corals *Acropora tenuis* and *Acropora loripes* had

84 similar survival and growth to their maternal purebreds, although they exceeded parental

85 performances in some cases. The bacterial and microalgal endosymbiont (Symbiodiniaceae spp.)

86 communities associated with these corals did not differ between the reciprocal hybrids and their

87 maternal and paternal purebreds (Chan et al., 2019). Since these microorganisms carry vital

88 functions to the coral hosts and can contribute to holobiont fitness differences (Blackall et al.,

89 2015; Rosenberg et al., 2007), this finding suggests that the microbial communities were unlikely

90 responsible for the observed holobiont fitness differences, and that these are likely underpinned

91 by coral host genetic and/or non-genetic transgenerational factors.

92

93 The aim of this study was to test if the phenotypic differences in reciprocal F1 hybrids of the

94 corals *A. tenuis* and *A. loripes* could be linked to patterns of host gene expression. Four offspring

95 groups (i.e., reciprocal F1 hybrids and two parental purebreds) were previously produced via a

96 laboratory cross of *A. tenuis* and *A. loripes* and were exposed to seven months of ambient or

97 elevated temperature and pCO$_2$ conditions (Chan et al., 2018). Using samples from the same

98 experiment, we tested for maternal effects in gene expression, as observed in hybrid survival and

99 growth. In addition, gene expression was examined between temperature/pCO$_2$ conditions within

100 each offspring group.

101

102 **Materials and methods**

Experimental design and sample collection

Parental coral colonies of *A. tenuis* and *A. loripes* were collected from Trunk Reef (18°35′S, 146°80′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 (27°C and 415 ppm pCO$_2$) and elevated conditions (ambient +1 °C and 685 ppm pCO$_2$). 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 °C 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

6

126 et al., 2019), an elevated temperature of +1 ℃ to present day ambient temperature reflects a

127 realistic scenario that will likely occur in the coming decades. Note that present day SST has

128 already increase by ~0.9°C since pre-industrial time (Bindoff et al., 2019).

129

130 Coral recruits were reared under treatment conditions in filtered seawater for seven months at the

131 National Sea Simulator of the Australian Institute of Marine Science. A microalgal diet

132 supplement was supplied to the corals daily and their fitness traits and associated microbial

133 communities were examined. To mimic the natural environment as closely as possible, the

134 experimental conditions followed diurnal and annual temperature variations of Davies Reef

135 (18.83° S, 147.63° E), which is a reef near the collection sites of the parental colonies. At the end

136 of the seven-month experiment, recruits from three tanks of each treatment were randomly

137 selected for sampling. Due to the small size (and therefore low RNA quantity) of individual

138 recruits, multiple recruits of the same offspring group from the same tank were pooled to form

139 one sample. Each pooled sample contained 30 coral polyps. RNA pooling was considered

140 appropriate as the purpose of this study was to examine population-level rather than individual-

141 level differences (Davies et al., 2016; Kendziorski et al., 2003). Three pooled samples per

142 offspring group per treatment were collected, except only one sample was available for purebred

143 *A. tenuis* (TT) under elevated conditions due to high mortality (Table S1). Samples were snap-

144 frozen in liquid nitrogen and stored at -80 °C until RNA extraction.

145

146 ***RNA extraction***

147 Sample tissues were mechanically disrupted prior to RNA isolation. Approximately 30 acid

148 washed glass beads (Sigma, 710-1180 µm diameter) and 600 µl RLT buffer (Qiagen) were added

7

149 to each sample. The samples were then subjected to 2 x 40 s cycles of bead beating at 4/s in a fast

150 Prep-245G (MP Biomedicals). Total RNA was isolated from the sample homogenate using

151 Qiagen RNeasy mini kit (including the optional DNase treatment). Total RNA was eluted in 40

152 µl of RNase free water and 3 µl were visualized on a 1% agarose, 0.5 x TBE gel for quality

153 check. RNA concentration was measured using the Qubit RNA HS Assay (Thermo Fisher

154 Scientific/Invitrogen), with fluorescence analysis on a NanoDrop 3300 Fluorospectrometer

155 (Thermo Fisher Scientific). Between 20.5 and 106 ng total RNA underwent reverse transcription

156 and cDNA was amplified using NuGen's Ovation V2.0 kit (with one cycle amplification). The

157 amplified cDNA was then purified using magnetic beads (Beckman Coulter Agencourt kit) and 1

158 µl was visualized on a 1% agarose, 0.5 x TBE gel. Purity of sample cDNA was determined by

159 A260/A280 ratios measured with a NanoDrop 2000 spectrophotometer (Thermo Fisher

160 Scientific). cDNA concentration was measured using the Quant-iT PicoGreen dsDNA Assay

161 (Thermo Fisher Scientific/Invitrogen). Sample cDNA concentrations were normalized and 25 µl

162 of 20 ng/µl cDNA were sent to Ramaciotti Centre for Genomics (UNSW, Sydney) for Nextera

163 XT Library Preparation and paired-end sequencing on the Illumina NextSeq500 platform (2 x

164 75bp). The total RNA concentration and quality, the amount of total RNA that underwent reverse

165 transcription, cDNA concentration and quality, as well as raw reads of each sample are shown in

166 Table S2.

167

168 *Sequence data processing*

169 Quality and adapter trimming were carried out on raw reads using Trimmomatic (Bolger et al.,

170 2014), discarding reads < 50 bp or with an averaged quality score < 20 in a sliding window of

171 five bases. Since the coral holobiont is associated with high densities of prokaryotes and algal

8

172   endosymbionts, reads were filtered with the following steps: First, reads were compared to an

173   rRNA database (Silva132_LSU, Silva132_SSU) and matches (i.e., e-values $\leq 10^{-5}$) were removed

174   using the program SortMeRNA (Kopylova et al., 2012). Second, reads were compared to the

175   algal endosymbiont genome (genus *Cladocopium*, symC_scaffold_40.fasta (Shoguchi et al.,

176   2018) and matches were removed using BBDuk (Bushnell, 2020). The remaining reads of each

177   sample are shown in Table S2 and were used to create a *de novo* assembly for the each offspring

178   groups and a combined *de novo* assembly for all four offspring groups using Trinity (Grabherr et

179   al., 2011). Small transcripts of < 400 bp were removed from the assemblies (Kenkel & Bay,

180   2017), and the longest isoform of each trinity transcript was obtained. Mitochondrial genes were

181   identified running BLASTn against the *A. tenuis* mitochondrial genome (NC_003522.1.fasta, van

182   Oppen et al., 2002) and were retained in the analysis. The remaining transcripts were then

183   identified by BLASTx searches against the most complete coral gene model (*A. digitifera*,

184   GCF_000222465.1_Adig_1.1_protein.faa, Shinzato et al., 2011) and NCBI's nonredundant (nr)

185   protein database, with a e-value cut off $\leq 10^{-5}$.

186

187   Gene names and gene ontologies (GO) of the transcripts were assigned using BLASTx search

188   against UniProt Knowledgebase Swiss-Prot database (The UniProt Consortium, 2015). Duplicate

189   query transcripts were removed. Transcript abundance of the samples was then estimated using

190   RSEM, an alignment-based method (Li & Dewey, 2011). Transcript quantification of the samples

191   was performed by aligning reads using bowtie2 (Langmead & Salzberg, 2012) and estimating

192   abundance with RSEM (Li & Dewey, 2011). For gene expression comparison between hybrids

193   and parental purebreds, we tested estimating transcript abundance using the assembly of purebred

194   *A. loripes*, as well as the combined assembly produced using all offspring groups. The two

195 methods revealed very similar results (Figure S1), and the results presented here are based on

196 transcript abundance estimated using the assembly of purebred *A. loripes*. Due to the small

197 number of samples available for the parental purebred *A. tenuis* (Table S1), a *de novo* assembly

198 was not conducted or tested as a basis for transcript abundance estimate. For gene expression

199 comparison between treatments within an offspring group, the *de novo* assembly of each

200 offspring group was used to estimate transcript abundance. Treatment comparison was not

201 conducted for *A. tenuis* purebreds due to an insufficient number of samples (Table S1).

202

203 ***Statistical analyses***

204 Separate analyses were conducted to compare gene expression between hybrids and parental

205 purebreds, and ambient versus elevated conditions within an offspring group. In addition, a

206 separate analysis was carried out for mitochondrial genes. Transcript abundance of the samples

207 and the BLAST results were analyzed in R and differential expression analysis was performed

208 using the package *limma* (Ritchie et al., 2015). Firstly, only transcripts that were of coral origin

209 were retained, as indicated in the BLAST results. For the mitochondrial analysis, only transcripts

210 that matched with the mitochondrial genome were used. Secondly, transcripts that consistently

211 had zero or very low counts were removed using the edgeR build in function filterByExpr, and

212 scale normalization (TMM) was applied. For Principal Components Analysis (PCA), sample raw

213 counts were transformed into log2-counts per million (log-CPM) to account for library size

214 differences.

215

216 A total of four samples were identified to have small library size (three *A. tenuis* purebreds- two

217 under ambient, one under elevated conditions, and one TL hybrid under elevated conditions), and

10

218   a relative log expression (RLE) plot showed that normalization of these samples was

219   unsuccessful (Gandolfo & Speed, 2018) (Figure S2, Table S2). These samples were excluded

220   from the main manuscript, but their analyses were retained in the Supplemental Information. A

221   heatmap was then used to visualize the 500 most variable genes across samples using the log-

222   CPM expression values with dendrograms computed using Euclidean distances. For the

223   mitochondrial analysis, a PCA and a heatmap were generated using all genes that remained post-

224   filtering.

225

226   To fit linear models for comparisons, count data was transformed to log-CPM using the voom

227   function in the limma package. Since no treatment effect was found on gene expression (see

228   Results section), the comparison of hybrids and purebreds combined samples from both

229   treatments. Comparisons were made between: 1) maternal purebred LL and its hybrid LT, 2)

230   paternal purebred LL and its hybrid TL, and 3) between the reciprocal hybrids LT and TL. The

231   purebred TT (*A. tenuis*) was not included due to a small sample size (n =1, Table S1). Empirical

232   Bayes moderated t-statistics were generated to assess the pairwise comparisons, and *p*-values

233   were corrected using the Benjamini-Hochberg method (Benjamini & Hochberg, 1995). A gene

234   was considered differentially expressed when $p_{adj} < 0.05$ using the *treat* function in the limma

235   package with a log-fold-change threshold of > 0.2. The list of differentially expressed genes

236   (DEGs) was exported for gene ontology (GO) analyses and visualized using volcano plots

237   (Blighe et al., 2018). The volcano plots and GO analyses focused on the comparison of 1)

238   paternal purebred LL with its hybrid TL, and 2) between the reciprocal hybrid LT and TL only,

239   as these were the pairs with a high number of differentially expressed genes to explore.

240

Two different approaches were applied to the GO analyses, including GOseq (Young et al., 2010)

242 and a rank-based GO analysis with adaptive clustering using a Mann-Whitney U (MWU) test

243 (https://github.com/z0on/GO_MWU, Dixon et al., 2015). For GOseq, the analysis was conducted

244 using the list of DEGs and the *p*-values were corrected with the Benjamini-Hochberg method

245 (Benjamini & Hochberg, 1995). A GO category was considered overrepresented or

246 underrepresented when the p_{adj} was < 0.05 and that the category had > 3 DEGs. For the MWU

247 test, the hierarchical clustering trees utilized the log10-transformed *p*-values of the DEGs and

248 indicated significantly enriched GO categories by up-regulated (red) or down-regulated (blue),

249 under a false discovery rate of 10%. In addition, differentially expression nuclear genes in GO

250 categories with functions connected to the mitochondrion were identified.

251

252 **Results**

253 On average, ~12.5 million raw Illumina reads were obtained per sample. After quality trimming

254 and removal of rRNA and algal endosymbiont components, an average of ~6.2 million paired

255 reads were retained per sample. The transcriptome of purebred *A. loripes* contained ~291 k

256 transcripts, and ~59 k transcripts were left after only retaining the longest isoforms and removal

257 of small transcripts < 400 bp. See Table S3 for details of other transcriptomes used for

258 preliminary analysis and evaluating treatment effect. For a total of ~35 k transcripts a match of

259 coral origin was found in the NCBI nr database. Following the removal of duplicates and

260 transcripts that consistently had zero or very low counts, 8800 transcripts were retained and used

261 for downstream analyses.

262

263 Transcriptome-wide gene expression of the hybrids was similar to that of their maternal

264 purebreds, yet distinct from their paternal purebreds and the reciprocal hybrids (Figures 1-3, S3).

265 Principal component analyses (PCA) showed similar expression patterns of the hybrid LT with

266 its maternal purebred LL under both ambient and elevated conditions (Figure 1). The only

267 exception was one LL purebred sample which showed separation with the others in principle

268 component two (Figure 1). Gene expression of the reciprocal hybrid TL also clustered with its

269 maternal purebred TT (but note that n = 1 for TT), and was separated with hybrid LT and its

270 paternal purebred LL under both treatment conditions (Figure 1). The four samples excluded

271 from the main analyses due to small library sizes also supported the existence of maternal effects

272 (Figure S4-S5). The amount of total RNA and cDNA input, as well as the number of raw reads of

273 the samples showed no specific patterns in the PCA plots, suggesting that the observed maternal

274 patterns of the offspring groups were not driven by these factors (Figure S4). Within an offspring

275 group, gene expression did not differ between ambient and elevated conditions (Figure 1).

276 Maternal patterns were not observed in the PCA plot and heatmap generated using mitochondrial

277 genes only (Figure S6-S7).

278

279 Differential expression analysis resulted in only 40 DEGs between the maternal purebred LL and

280 its hybrid LT (Figure 2). In contrast, almost 2000 DEGs were identified between the paternal

281 purebred LL and its hybrid TL, as well as between the reciprocal hybrids LT and TL (Figure 2).

282 Among these ~2000 DEGs, the hybrid LT and its maternal purebred LL shared 1343 genes that

283 were differentially expressed from the hybrid TL (Figure 2). Maternal effects in gene expression

284 were also evident in the heatmap of the 500 most variable genes across samples (Figure 3). The

285 only exception was one purebred LL sample which clustered away from the other LL samples,

286 and this was the same sample that showed separation in the PCA plot (Figure 1, 3). The heatmap

287 that includes the four samples removed from the main analyses due to small library sizes

288 confirmed the maternal effects observed in the smaller subset of samples (Figure S5).

289

290 Among the DEGs with the highest log-fold change (i.e., four DEGs for paternal purebred LL

291 compared to its hybrid TL, and seven DEGs for hybrid LT compared to hybrid TL with LFC >

292 5), three were shared genes between the two pairs of comparison (Figure S3). Unfortunately,

293 most of these DEGs were annotated as uncharacterized proteins and hence their potential

294 functions were unknown (Table S4). Only one differentially expressed mitochondrial gene

295 (TRINITY_DN76286_c6_g1_i1) was identified between hybrid TL and its paternal purebred LL

296 (p_{adj} = 0.03). No differentially expressed mitochondrial genes were found in all other pairs of

297 comparison.

298

299 For gene ontology (GO) analyses using GOseq, GO category "cytosol" (GO: 0005829) was

300 underrepresented in both the comparisons between the paternal purebred LL with its hybrid TL

301 and between the reciprocal hybrids LT and TL, with 90 and 96 DEGs respectively in this

302 category (Table S5). Note that "cytosol" is a very broad GO category and it was comprised of

303 620 genes in this dataset. In addition, the GO category "membrane" (GO: 0016020) was also

304 underrepresented in the comparison between the paternal purebred LL and its hybrid TL (Table

305 S4). This was also a broad GO category with 255 genes in this dataset, 27 of which were DEGs.

306 In contrast, GO analyses using the MWU test showed no significant GO category was over- or

307 under-represented. However, note that the MWU test omits GO categories that are too broad (i.e.,

308 a GO category that contains a large proportion of the total number of genes). For this reason, it

14

309 was unsurprising that the very broad GO categories "cytosol" and "membrane" that were

310 identified as underrepresented using GOseq were not significant here.

311

312 For offspring groups that had different maternal parent species (i.e., between the hybrid TL and

313 its paternal purebred LL, and between the reciprocal hybrids LT and TL), 84-88 DEGs were

314 identified in GO categories with functions connected to the mitochondrion (Table S6). In

315 contrast, no DEGs were found in GO categories linked to the mitochondrion when the offspring

316 groups shared the same maternal parent species (i.e., between the hybrid LT and its maternal

317 purebred LL). The proportion of DEGs over total number of genes was similar between genes in

318 GO categories linked to the mitochondrion and genes in all GO categories (14.9-17.6%, Table

319 S6).

320

321 **Discussion**

322 ***Maternal effects in coral fitness are reflected in gene expression patterns***

323 Maternal effects in recruit survival and size previously reported for *A. loripes* x *A. tenuis* hybrid

324 corals (Table 1) were consistent with their gene expression patterns. At the time when the corals

325 were sampled for gene expression analyses, the hybrid LT and its maternal purebred LL had

326 higher survival compared to the hybrid TL and its maternal purebred TT under both ambient and

327 elevated conditions. Although the corals did not differ in size at seven months of age, maternal

328 effects in size were evident by one year of age (Table 1). Maternal effects have previously been

329 reported for other Indo-Pacific *Acropora* hybrid corals obtained via laboratory crossing. These

330 include effects in: 1) morphology of interspecific hybrids from an *A. pulchra* x *A. millepora cross*

331 *(Willis et al., 2006), 2)* survival of interspecific hybrid larvae from an *A. florida* x *A. intermedia*

15

332 cross (Isomura et al., 2013), and 3) thermal tolerance of intraspecific *A. millepora* hybrid larvae

333 from a higher and lower latitude population. In contrast, *paternal effects were found in*

334 *morphology of natural interspecific hybrids of A. palmata and A. cervicornis from the Caribbean*

335 *(Vollmer & Palumbi, 2002), and additive effects in survival (i.e., hybrid survival was*

336 *intermediate between the parental offspring) were observed in experimentally produced*

337 *intraspecific hybrids of A. millepora from a higher and lower latitude cross (van Oppen et al.,*

338 *2014).*

339

340 *While a few studies have reported maternal effects in coral fitness and morphology, little is*

341 *known about maternal effects in gene expression. In addition to the coral host, the host-*

342 *associated microbiome can also have an impact on host gene expression (Barfield et al., 2018;*

343 *Buerger et al., 2020; Helmkampf et al., 2019). In our study, however,* the bacterial and microalgal

344 endosymbiont communities of the corals were similar at the time of sampling (Table 1). The

345 consistency between host gene expression and phenotypic results thus suggests that maternal

346 host-related factors were likely the drivers behind the observed fitness differences. A large

347 number of differentially expressed genes (~2000 DEGs) were found when comparing offspring

348 groups that had different maternal parent species (i.e., between the hybrid TL and its paternal

349 purebred LL, and between the reciprocal hybrids), but not when the groups shared the same

350 maternal parent species (i.e., only 40 DEGs between the hybrid LT and its maternal purebred

351 LL). Maternal effects were evident in these corals based on PCA, heatmap and volcano plots.

352 While a statistical comparison cannot be made back to the parental purebred TT due to small

353 sample size, gene expression of hybrid TL was similar to the only TT sample tested based on

354 PCA and the heatmap was indicative of maternal effects. The four samples omitted from the main

16

355 analyses because of their small library sizes also supported the presence of maternal effects,

356 although inferences drawn from these samples should be taken with caution.

357

358 In our study, however, no mitochondrial genes were differentially expressed and PCA and

359 heatmap of mitochondrial genes did not show maternal patterns. In other words, evidence of

360 maternal gene expression patterns was only found in the nuclear genes, but not in the

361 mitochondrial genes or via mito-nuclear crosstalk in this study (although note that only seven

362 mitochondrial genes were available for comparison post-filtering).

363

364 Several studies have reported maternal effects in gene expression including in a perennial herb

365 (Videvall et al., 2016), coral (Dixon et al., 2015), pipefish (Beemelmanns & Roth, 2016) and

366 stickleback (Metzger & Schulte, 2016; Mommer & Bell, 2014; Shama et al., 2016), and maternal

367 environments have also been demonstrated to affect DNA methylation of sea urchin (Strader et

368 al., 2020). Videvall et al. (2016) showed that gene expression patterns were distinct between

369 parental populations of 12-week-old seedling of the perennial herb *Arabidopsis lyrata*, and

370 expression in intraspecific hybrids was frequently more similar to that of the maternal than

371 paternal population. Only 15 DEGs were found between the hybrid produced in one direction and

372 its maternal population, yet > 8800 DEGs were found when compared to its paternal population

373 (Videvall et al., 2016). Interestingly, maternal effects were weaker in the hybrid cross of the other

374 direction, with 334 and 661 DEGs observed when compared to its maternal and paternal

375 population respectively (Videvall et al., 2016). Only one previous study has examined maternal

376 effects in coral hybrid gene expression and only coral larvae were studied. Consistent with our

377 findings, Dixon et al. (2015) showed that gene expression of intraspecific *A. millepora* hybrid

378 larvae was similar to that of their maternal population (i.e., up to 2,000 genes in hybrids followed

379 the expression patterns of the maternal population). In these studies (Dixon et al., 2015 and

380 Videvall et al., 2016) however, maternal effects were examined in early life stages only (i.e., 12-

381 week-old seedling and 6-day-old larvae). Our results show that maternal effects can continue to

382 influence gene expression of hybrid corals up to the age of at least seven months, indicating the

383 potential long-term nature of maternal effects.

384

385 While differences in gene expression patterns were obvious between reciprocal hybrids as well as

386 between the hybrid TL and its paternal purebred, it was unclear what pathways and mechanisms

387 were linked to these differences and underpinned observed phenotypic differences (Chan et al.,

388 2018). Gene ontology (GO) analyses revealed underrepresentation of a very broad GO category,

389 "cytosol", in both pairs of comparison. It is also possible that maternal provisioning had long-

390 lasting effects in offspring (that were seven months old) and was responsible for the phenotypic

391 and gene expression differences (i.e., poorly provisioned offspring may exhibit pervasive

392 differences in transcription). Future studies on maternal effects in corals will benefit from

393 quantifying differences in maternal provisioning between the parental species, such as

394 lipid/protein content of eggs and early larvae.

395

396 In contrast, clear pathways involved in maternal effects were observed in the intraspecific *A.*

397 *millepora* hybrid larvae (Dixon et al., 2015). Analyses of cellular component categories of

398 tolerance-associated genes (i.e., genes for which expression levels prior to stress predicted the

399 probability of larval survival under stress) showed enrichment of nuclear-encoded mitochondrial

400 membrane components in hybrid coral larvae whose parents come from a warmer latitude (Dixon

401 et al., 2015). The most upregulated GO categories were energy production and conversion, and

402 encompassed mitochondrial proteins, suggesting mitochondrial protein variation in larvae may

403 have contributed to maternal effects in thermal tolerance (Dixon et al., 2015).

404

405 The difference in GO associated patterns between these two studies may be due to 1) the parental

406 populations chosen for hybridization, 2) the symbiotic/aposymbiotic nature of the corals and 3)

407 the life stage of the corals. Parental populations of the same species from different latitudes were

408 selected in Dixon et al. (2015), whereas parental populations of two different species from the

409 same reef were chosen for this study. The differences in parental thermal regimes in Dixon et al.

410 (2015) may lead to clearer maternal effects in thermal stress-related GO categories. Moreover,

411 gene expression responses of aposymbiotic larvae in Dixon et al. (2015) were likely different

412 from coral recruits (in this study) that were associated with a high density of microalgal

413 endosymbionts. The effects of maternal provisioning on gene expression is also likely to be

414 stronger in early larvae than in seven-month-old recruits. Hence, the contrasting results of the two

415 studies are unsurprising. Further, mitochondrial genes may not show maternal patterns if

416 maternal provisioning was responsible for the phenotypic maternal patterns observed in these

417 corals.

418

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

420 ***conditions***

421 Elevated temperature and pCO_2 conditions had a– negative impact on survival and size of the

422 corals used in this study (Table 1), yet gene expression within an offspring group did not differ

423 between ambient and elevated conditions (Figure 1). Nevertheless, gene expression changes

424 under short-term acute stress are commonly found in coral. This often involves the regulation of

425 genes encoding heat shock proteins, ion transport, apoptosis, immune responses and/or oxidative

426 stress (Barshis et al., 2013; Desalvo et al., 2008; Meyer et al., 2011; Ruiz-Jones & Palumbi,

427 2017). The absence of DEGs in corals under ambient versus elevated conditions was unexpected

428 and may be due to the relatively mild and long-term nature of the treatments. The elevated

429 conditions of this study (ambient +1 °C, 685 ppm $p\mathrm{CO_2}$) were relatively mild compared to many

430 other longer-term studies (e.g., ambient +7 and + 12 ºC, Maor-Landaw et al., 2017; 856-3880

431 ppm $p\mathrm{CO_2}$, Vidal-Dupiol et al., 2013). In addition, gene expression responses of corals under

432 long-term stress have been shown to differ from those under short-term stress. Despite significant

433 differences in $\mathrm{CO_2}$ concentration under control and natural $\mathrm{CO_2}$ seep sites (i.e., ~355 versus 998

434 ppm), only 61 DEGs were found in *A. millepora* from the two sites (Kenkel et al., 2017).

435 Similarly, the expression of calcification-related genes changed significantly in *A. millepora*

436 subjected to short-term (i.e., 3 days) high $p\mathrm{CO_2}$ exposure (Moya et al., 2012, 2015), but far fewer

437 DEGs were found as exposure time increased (Moya et al., 2015; Rocker et al., 2015). Since

438 cellular stress gene expression responses can be transient (Kültz, 2003), certain expression

439 changes may only be detectable during the initial exposure and therefore fewer differentially

440 expressed genes are generally found in long-term studies.

441

442 **Conclusions and future studies**

443 This study showed that maternal effects manifested as gene expression differences in

444 interspecific hybrids of the coral *A. tenuis* and *A. loripes*. We also showed that maternal effects

445 can persist to at least seven months of age in coral and were likely responsible for the phenotypes

446 of F1 hybrids. However, the pathways and mechanisms responsible for the phenotypic

447 differences were unknown and exposure to elevated temperature and pCO_2 conditions did not

448 result in differential coral gene expression. Although the composition of bacterial and microalgal

449 endosymbiont communities of these corals was similar under ambient and elevated conditions

450 and between hybrids and purebreds, these microbes may have expressed different genes and

451 contributed to holobiont phenotypic differences. Future studies will benefit from examining the

452 gene expression of these microbial communities alongside the host. Other less studied members

453 of the coral holobiont, such as viruses and fungi (that were not examined), may also have

454 contributed to coral survival and size differences between offspring groups and treatment

455 conditions. Further, post-transcriptional and epigenetic regulation (e.g., DNA methylation) may

456 have varied between treatments and hybrid and purebreds and may have resulted in phenotypic

457 differences (Dimond et al., 2017). Future studies should consider adopting a multi-omics

458 approach and assessing other members of the coral-associated microbiome to explore other

459 mechanisms that underpin the phenotype of the coral holobiont.

466
467 **Author's contribution.** W.Y.C., M.J.H.O., L.P. and A.H. designed the study. W.Y.C. and
468 L.P. conducted the experiment. L.P. carried out the laboratory work. J.C., W.Y.C. and A.H.
469 undertook bioinformatic and statistical analyses. W.Y.C. and M.J.H.O. wrote much of the
470 manuscript and all authors contributed to the final edited version of the manuscript.

471
472 **Data Availability Statement**
473 Raw sequences are available in GenBank (SRR12695232 to SRR12695253, project accession
474 no.: PRJNA665083) and the R scripts for statistical analyses are available as Appendix S1.

475
476 **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.

481 **References**

482 Alleman, M., & Doctor, J. (2000). Genomic imprinting in plants: Observations and evolutionary
483   implications. *Plant Molecular Biology*, *43*(2), 147–161.
484   https://doi.org/10.1023/A:1006419025155

485 Barfield, S. J., Aglyamova, G. V., Bay, L. K., & Matz, M. V. (2018). Contrasting effects of
486   *Symbiodinium* identity on coral host transcriptional profiles across latitudes. *Molecular*
487   *Ecology*, *27*(15), 3103–3115. https://doi.org/10.1111/mec.14774

488 Barshis, D. J., Ladner, J. T., Oliver, T. A., Seneca, F. O., Traylor-Knowles, N., & Palumbi, S. R.
489   (2013). Genomic basis for coral resilience to climate change. *Proceedings of the National*
490   *Academy of Sciences*, *110*(4), 1387–1392. https://doi.org/10.1073/pnas.1210224110

491 Beemelmanns, A., & Roth, O. (2016). Biparental immune priming in the pipefish *Syngnathus*
492   *typhle*. *Zoology*, *119*(4), 262–272. https://doi.org/10.1016/j.zool.2016.06.002

493 Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and
494   powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B*
495   *(Methodological)*, *57*(1), 289–300. https://doi.org/10.2307/2346101

496 Bindoff, N., Cheung, W., Kairo, J., Arístegui, J., Guinder, V., Hallberg, R., Hilmi, N., Jiao, N.,
497   Karim, M., Levin, L., O'Donoghue, S., Cuicapusa Purca, S., Rinkevich, B., Suga, T.,
498   Tagliabue, A., & Williamson, P. (2019). Changing ocean, marine ecosystems, and
499   dependent communities. In *IPCC special report on the ocean and cryosphere in a*
500   *changing climate* (pp. 477–587). [H-O Pörtner, DC Roberts, V Masson-Delmotte, P Zhai,
501   M Tignor, E Poloczanska, K Mintenbeck, A Alegría, M Nicolai, A Okem, J Petzold, B
502   Rama, NM Weyer (eds.)]. Cambridge: University Press.

503 Bischoff, A., & Müller–Schärer, H. (2010). Testing population differentiation in plant species –
504   how important are environmental maternal effects. *Oikos*, *119*(3), 445–454.
505   https://doi.org/10.1111/j.1600-0706.2009.17776.x

506 Blackall, L. L., Wilson, B., & van Oppen, M. J. H. (2015). Coral-the world's most diverse
507   symbiotic ecosystem. *Molecular Ecology*, *24*(21), 5330–5347.
508   https://doi.org/10.1111/mec.13400

509 Blighe, K., Rana, S., & Lewis, M. (2018). *EnhancedVolcano: Publication-ready volcano plots*
510   *with enhanced colouring and labeling*.
511   https://bioconductor.org/packages/devel/bioc/vignettes/EnhancedVolcano/inst/doc/
512   EnhancedVolcano.html

513 Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina
514   sequence data. *Bioinformatics*, *30*(15), 2114–2120.
515   https://doi.org/10.1093/bioinformatics/btu170

516 Buerger, B., Alvarez-Roa, C., Coppin, C., Pearce, S., Chakravarti, J., Oakeshott, J., Edwards, O.,
517   & Van Oppen, M. J. H. (2020). *Long-term heat exposure of algal symbionts increases*
518   *coral bleaching tolerance*.

519 Bushnell, B. (2020). *BBMap*. https://sourceforge.net/projects/bbmap/

520 Chan, W. Y., Peplow, L. M., Menéndez, P., Hoffmann, A. A., & Oppen, M. J. H. van. (2019).
521   The roles of age, parentage and environment on bacterial and algal endosymbiont
522   communities in *Acropora* corals. *Molecular Ecology*, *28*(16), 3830–3843. https://doi.org/
523   10.1111/mec.15187

524 Chan, W. Y., Peplow, L. M., Menéndez, P., Hoffmann, A. A., & van Oppen, M. J. H. (2018).
525   Interspecific hybridization may provide novel opportunities for coral reef restoration.
526   *Frontiers in Marine Science*, *5*. https://doi.org/10.3389/fmars.2018.00160

527 Chen, Z. J. (2013). Genomic and epigenetic insights into the molecular bases of heterosis. *Nature*
528   *Reviews Genetics*, *14*(7), 471–482. https://doi.org/10.1038/nrg3503

529 Davies, S. W., Marchetti, A., Ries, J. B., & Castillo, K. D. (2016). Thermal and pCO$_2$ stress elicit
530   divergent transcriptomic responses in a resilient coral. *Frontiers in Marine Science*, *3*.
531   https://doi.org/10.3389/fmars.2016.00112

532 Desalvo, M. K., Voolstra, C. R., Sunagawa, S., Schwarz, J. A., Stillman, J. H., Coffroth, M. A.,
533   Szmant, A. M., & Medina, M. (2008). Differential gene expression during thermal stress
534   and bleaching in the Caribbean coral *Montastraea faveolata*. *Molecular Ecology*, *17*(17),
535   3952–3971. https://doi.org/10.1111/j.1365-294X.2008.03879.x

536 Dimond, J. L., Gamblewood, S. K., & Roberts, S. B. (2017). Genetic and epigenetic insight into
537   morphospecies in a reef coral. *Molecular Ecology*, *26*(19), 5031–5042.
538   https://doi.org/10.1111/mec.14252

539 Dixon, G. B., Davies, S. W., Aglyamova, G. V., Meyer, E., Bay, L. K., & Matz, M. V. (2015).
540   Genomic determinants of coral heat tolerance across latitudes. *Science*, *348*(6242), 1460–
541   1462. https://doi.org/10.1126/science.1261224

542 Donohue, K. (2009). Completing the cycle: Maternal effects as the missing link in plant life
543   histories. *Philosophical Transactions of the Royal Society B: Biological Sciences*,
544   *364*(1520), 1059–1074. https://doi.org/10.1098/rstb.2008.0291

545 Galloway, L. F., & Etterson, J. R. (2007). Transgenerational plasticity is adaptive in the wild.
546   *Science*, *318*(5853), 1134–1136. https://doi.org/10.1126/science.1148766

547 Gandolfo, L. C., & Speed, T. P. (2018). Rle plots: Visualizing unwanted variation in high
548   dimensional data. *PLOS ONE*, *13*(2), e0191629.
549   https://doi.org/10.1371/journal.pone.0191629

550 Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., Adiconis, X.,
551   Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A.,
552   Rhind, N., di Palma, F., Birren, B. W., Nusbaum, C., Lindblad-Toh, K., … Regev, A.
553   (2011). Trinity: Reconstructing a full-length transcriptome without a genome from RNA-
554   Seq data. *Nature Biotechnology*, *29*(7), 644–652. https://doi.org/10.1038/nbt.1883

555 Harii, S., Yamamoto, M., & Hoegh-Guldberg, O. (2010). The relative contribution of
556   dinoflagellate photosynthesis and stored lipids to the survivorship of symbiotic larvae of
557   the reef-building corals. *Marine Biology*, *157*(6), 1215–1224.
558   https://doi.org/10.1007/s00227-010-1401-0

559 Helmkampf, M., Bellinger, M. R., Frazier, M., & Takabayashi, M. (2019). Symbiont type and
560   environmental factors affect transcriptome-wide gene expression in the coral *Montipora*
561   *capitata*. *Ecology and Evolution*, *9*(1), 378–392. https://doi.org/10.1002/ece3.4756

562 Isomura, N., Iwao, K., & Fukami, H. (2013). Possible natural hybridization of two
563   morphologically distinct species of *Acropora* (Cnidaria, Scleractinia) in the Pacific:
564   Fertilization and larval survival rates. *PLOS ONE*, *8*(2), e56701.
565   https://doi.org/10.1371/journal.pone.0056701

566 Kendziorski, C. M., Zhang, Y., Lan, H., & Attie, A. D. (2003). The efficiency of pooling mRNA
567   in microarray experiments. *Biostatistics*, *4*(3), 465–477.
568   https://doi.org/10.1093/biostatistics/4.3.465

Kenkel, C. D., & Bay, L. K. (2017). Novel transcriptome resources for three scleractinian coral species from the Indo-Pacific. *GigaScience*, *6*(9). https://doi.org/10.1093/gigascience/gix074

Kenkel, C. D., Moya, A., Strahl, J., Humphrey, C., & Bay, L. K. (2017). Functional genomic analysis of corals from natural CO_2-seeps reveals core molecular responses involved in acclimatization to ocean acidification. *Global Change Biology*, *24*(1), 158–171. https://doi.org/10.1111/gcb.13833

Killian, J. K., Nolan, C. M., Stewart, N., Munday, B. L., Andersen, N. A., Nicol, S., & Jirtle, R. L. (2001). Monotreme IGF2 expression and ancestral origin of genomic imprinting. *Journal of Experimental Zoology*, *291*(2), 205–212. https://doi.org/10.1002/jez.1070

Kopylova, E., Noé, L., & Touzet, H. (2012). SortMeRNA: Fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. *Bioinformatics*, *28*(24), 3211–3217. https://doi.org/10.1093/bioinformatics/bts611

Kültz, D. (2003). Evolution of the cellular stress proteome: From monophyletic origin to ubiquitous function. *Journal of Experimental Biology*, *206*(18), 3119. https://doi.org/10.1242/jeb.00549

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, *9*(4), 357–359. https://doi.org/10.1038/nmeth.1923

Li, B., & Dewey, C. N. (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics*, *12*(1), 323. https://doi.org/10.1186/1471-2105-12-323

Li, L., Lu, K., Chen, Z., Mu, T., Hu, Z., & Li, X. (2008). Dominance, overdominance and epistasis condition the heterosis in two heterotic rice hybrids. *Genetics*, *180*(3), 1725–1742. https://doi.org/10.1534/genetics.108.091942

Lippman, Z. B., & Zamir, D. (2007). Heterosis: Revisiting the magic. *Trends in Genetics*, *23*(2), 60–66. https://doi.org/10.1016/j.tig.2006.12.006

Maor-Landaw, K., Ben-Asher, H. W., Karako-Lampert, S., Salmon-Divon, M., Prada, F., Caroselli, E., Goffredo, S., Falini, G., Dubinsky, Z., & Levy, O. (2017). Mediterranean versus Red sea corals facing climate change, a transcriptome analysis. *Scientific Reports*, *7*, 42405. https://doi.org/10.1038/srep42405

Matsuura, K. (2020). Genomic imprinting and evolution of insect societies. *Population Ecology*, *62*(1), 38–52. https://doi.org/10.1002/1438-390X.12026

McCormick, M. I. (1999). Experimental test of the effect of maternal hormones on larval quality of a coral reef fish. *Oecologia*, *118*(4), 412–422. https://doi.org/10.1007/s004420050743

Metzger, D. C. H., & Schulte, P. M. (2016). Maternal stress has divergent effects on gene expression patterns in the brains of male and female threespine stickleback. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1839), 20161734. https://doi.org/10.1098/rspb.2016.1734

Meyer, E., Aglyamova, G. V., & Matz, M. V. (2011). Profiling gene expression responses of coral larvae (*Acropora millepora*) to elevated temperature and settlement inducers using a novel RNA-Seq procedure. *Molecular Ecology*, *20*(17), 3599–3616. https://doi.org/10.1111/j.1365-294X.2011.05205.x

Miller, M., Zhang, C., & Chen, Z. J. (2012). Ploidy and hybridity effects on growth vigor and gene expression in *Arabidopsis thaliana* hybrids and their parents. *G3: Genes, Genomes, Genetics*, *2*(4), 505–513. https://doi.org/10.1534/g3.112.002162

614 Mommer, B. C., & Bell, A. M. (2014). Maternal experience with predation risk influences
615   genome-wide embryonic gene expression in threespined sticklebacks (*Gasterosteus*
616   *aculeatus*). *PLOS ONE*, *9*(6), e98564. https://doi.org/10.1371/journal.pone.0098564
617 Mousseau, T. A., & Dingle, H. (1991). Maternal effects in insect life histories. *Annual Review of*
618   *Entomology*, *36*(1), 511–534. https://doi.org/10.1146/annurev.en.36.010191.002455
619 Moya, A., Huisman, L., Ball, E. E., Hayward, D. C., Grasso, L. C., Chua, C. M., Woo, H. N.,
620   Gattuso, J.-P., ForêT, S., & Miller, D. J. (2012). Whole transcriptome analysis of the coral
621   *Acropora millepora* reveals complex responses to CO_2-driven acidification during the
622   initiation of calcification. *Molecular Ecology*, *21*(10), 2440–2454.
623   https://doi.org/10.1111/j.1365-294X.2012.05554.x
624 Moya, A., Huisman, L., Forêt, S., Gattuso, J.-P., Hayward, D. C., Ball, E. E., & Miller, D. J.
625   (2015). Rapid acclimation of juvenile corals to CO_2-mediated acidification by
626   upregulation of heat shock protein and bcl-2 genes. *Molecular Ecology*, *24*(2), 438–452.
627   https://doi.org/10.1111/mec.13021
628 Parekh, S., Ziegenhain, C., Vieth, B., Enard, W., & Hellmann, I. (2016). The impact of
629   amplification on differential expression analyses by RNA-seq. *Scientific Reports*, *6*(1),
630   25533. https://doi.org/10.1038/srep25533
631 Renfree, M. B., Suzuki, S., & Kaneko-Ishino, T. (2013). The origin and evolution of genomic
632   imprinting and viviparity in mammals. *Philosophical Transactions of the Royal Society B:*
633   *Biological Sciences*, *368*(1609), 20120151. https://doi.org/10.1098/rstb.2012.0151
634 Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015).
635   Limma powers differential expression analyses for RNA-sequencing and microarray
636   studies. *Nucleic Acids Research*, *43*(7), e47–e47. https://doi.org/10.1093/nar/gkv007
637 Roach, D. A., & Wulff, R. D. (1987). Maternal effects in plants. *Annual Review of Ecology and*
638   *Systematics*, *18*(1), 209–235. https://doi.org/10.1146/annurev.es.18.110187.001233
639 Rocker, M. M., Noonan, S., Humphrey, C., Moya, A., Willis, B. L., & Bay, L. K. (2015).
640   Expression of calcification and metabolism-related genes in response to elevated pCO_2
641   and temperature in the reef-building coral *Acropora millepora*. *Marine Genomics*, *24 Pt*
642   *3*, 313–318. https://doi.org/10.1016/j.margen.2015.08.001
643 Rosenberg, E., Koren, O., Reshef, L., Efrony, R., & Zilber-Rosenberg, I. (2007). The role of
644   microorganisms in coral health, disease and evolution. *Nature Reviews Microbiology*,
645   *5*(5), 355–362. https://doi.org/10.1038/nrmicro1635
646 Ruiz-Jones, L. J., & Palumbi, S. R. (2017). Tidal heat pulses on a reef trigger a fine-tuned
647   transcriptional response in corals to maintain homeostasis. *Science Advances*, *3*(3),
648   e1601298. https://doi.org/10.1126/sciadv.1601298
649 Shama, L. N. S., Mark, F. C., Strobel, A., Lokmer, A., John, U., & Wegner, K. M. (2016).
650   Transgenerational effects persist down the maternal line in marine sticklebacks: Gene
651   expression matches physiology in a warming ocean. *Evolutionary Applications*, *9*(9),
652   1096–1111. https://doi.org/10.1111/eva.12370
653 Shinzato, C., Shoguchi, E., Kawashima, T., Hamada, M., Hisata, K., Tanaka, M., Fujie, M.,
654   Fujiwara, M., Koyanagi, R., Ikuta, T., Fujiyama, A., Miller, D. J., & Satoh, N. (2011).
655   Using the *Acropora digitifera* genome to understand coral responses to environmental
656   change. *Nature*, *476*(7360), 320–323. https://doi.org/10.1038/nature10249
657 Shoguchi, E., Beedessee, G., Tada, I., Hisata, K., Kawashima, T., Takeuchi, T., Arakaki, N.,
658   Fujie, M., Koyanagi, R., Roy, M. C., Kawachi, M., Hidaka, M., Satoh, N., & Shinzato, C.

(2018). Two divergent *Symbiodinium* genomes reveal conservation of a gene cluster for sunscreen biosynthesis and recently lost genes. *BMC Genomics*, *19*(1), 458. https://doi.org/10.1186/s12864-018-4857-9

Slot, M., Palow, D. T., & Kitajima, K. (2013). Seed reserve dependency of *Leucaena leucocephala* seedling growth for nitrogen and phosphorus. *Functional Plant Biology*, *40*(3), 244–250. https://doi.org/10.1071/FP12255

Strader, M. E., Kozal, L. C., Leach, T. S., Wong, J. M., Chamorro, J. D., Housh, M. J., & Hofmann, G. E. (2020). Examining the role of DNA methylation in transcriptomic plasticity of early stage sea urchins: Developmental and maternal effects in a kelp forest herbivore. *Frontiers in Marine Science*, *7*. https://doi.org/10.3389/fmars.2020.00205

Thamban, T., Agarwaal, V., & Khosla, S. (2020). Role of genomic imprinting in mammalian development. *Journal of Biosciences*, *45*(1), 20. https://doi.org/10.1007/s12038-019-9984-1

The UniProt Consortium. (2015). UniProt: A hub for protein information. *Nucleic Acids Research*, *43*(D1), D204–D212. https://doi.org/10.1093/nar/gku989

van Oppen, M. J. H., Catmull, J., McDonald, B. J., Hislop, N. R., Hagerman, P. J., & Miller, D. J. (2002). The mitochondrial genome of *Acropora tenuis* (cnidaria; scleractinia) contains a large group i intron and a candidate control region. *Journal of Molecular Evolution*, *55*(1), 1–13. https://doi.org/10.1007/s00239-001-0075-0

van Oppen, M. J. H., Puill-Stephan, E., Lundgren, P., De'ath, G., & Bay, L. K. (2014). First-generation fitness consequences of interpopulational hybridisation in a Great Barrier Reef coral and its implications for assisted migration management. *Coral Reefs*, *33*(3), 607–611. https://doi.org/10.1007/s00338-014-1145-2

Vidal-Dupiol, J., Zoccola, D., Tambutté, E., Grunau, C., Cosseau, C., Smith, K. M., Freitag, M., Dheilly, N. M., Allemand, D., & Tambutté, S. (2013). Genes related to ion-transport and energy production are upregulated in response to CO_2-driven pH decrease in corals: New insights from transcriptome analysis. *PLOS ONE*, *8*(3), e58652. https://doi.org/10.1371/journal.pone.0058652

Videvall, E., Sletvold, N., Hagenblad, J., Ågren, J., & Hansson, B. (2016). Strong maternal effects on gene expression in *Arabidopsis lyrata* hybrids. *Molecular Biology and Evolution*, *33*(4), 984–994. https://doi.org/10.1093/molbev/msv342

Vollmer, S. V., & Palumbi, S. R. (2002). Hybridization and the evolution of reef coral diversity. *Science*, *296*(5575), 2023–2025. https://doi.org/10.1126/science.1069524

Warne, R. W., Kardon, A., & Crespi, E. J. (2013). Physiological, behavioral and maternal factors that contribute to size variation in larval amphibian populations. *PLOS ONE*, *8*(10), e76364. https://doi.org/10.1371/journal.pone.0076364

Willis, B. L., van Oppen, M. J. H., Miller, D. J., Vollmer, S. V., & Ayre, D. J. (2006). The role of hybridization in the evolution of reef corals. *Annual Review of Ecology, Evolution, and Systematics*, *37*(1), 489–517. https://doi.org/10.1146/annurev.ecolsys.37.091305.110136

Young, M. D., Wakefield, M. J., Smyth, G. K., & Oshlack, A. (2010). Gene ontology analysis for RNA-seq: Accounting for selection bias. *Genome Biology*, *11*(2), R14. https://doi.org/10.1186/gb-2010-11-2-r14
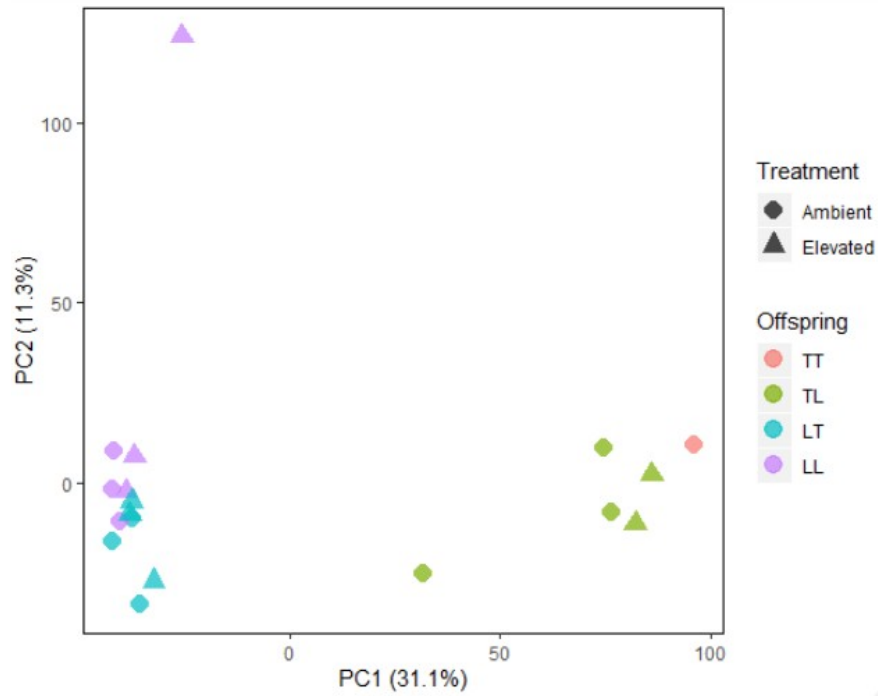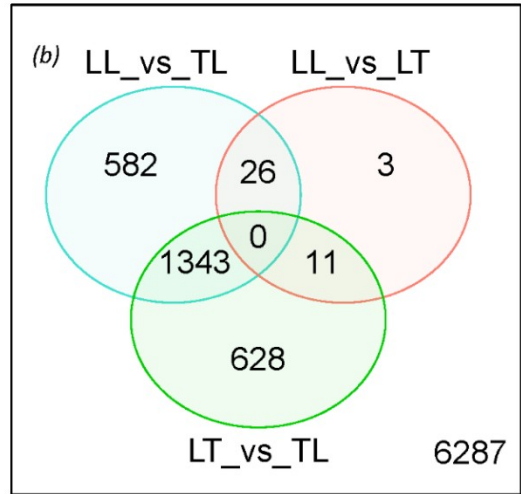
702

703 **Figures**

704
705 Figure 1. Principal component analyses of the offspring groups using normalized counts (i.e.,
706 log-CPM) of the 8,880 genes retained post filtering. The maternal parent is listed prior to the
707 paternal parent for the abbreviation of the offspring groups, where "T" is *A. tenuis* and "L" is *A.*
708 *loripes*.
709

	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

711

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*.
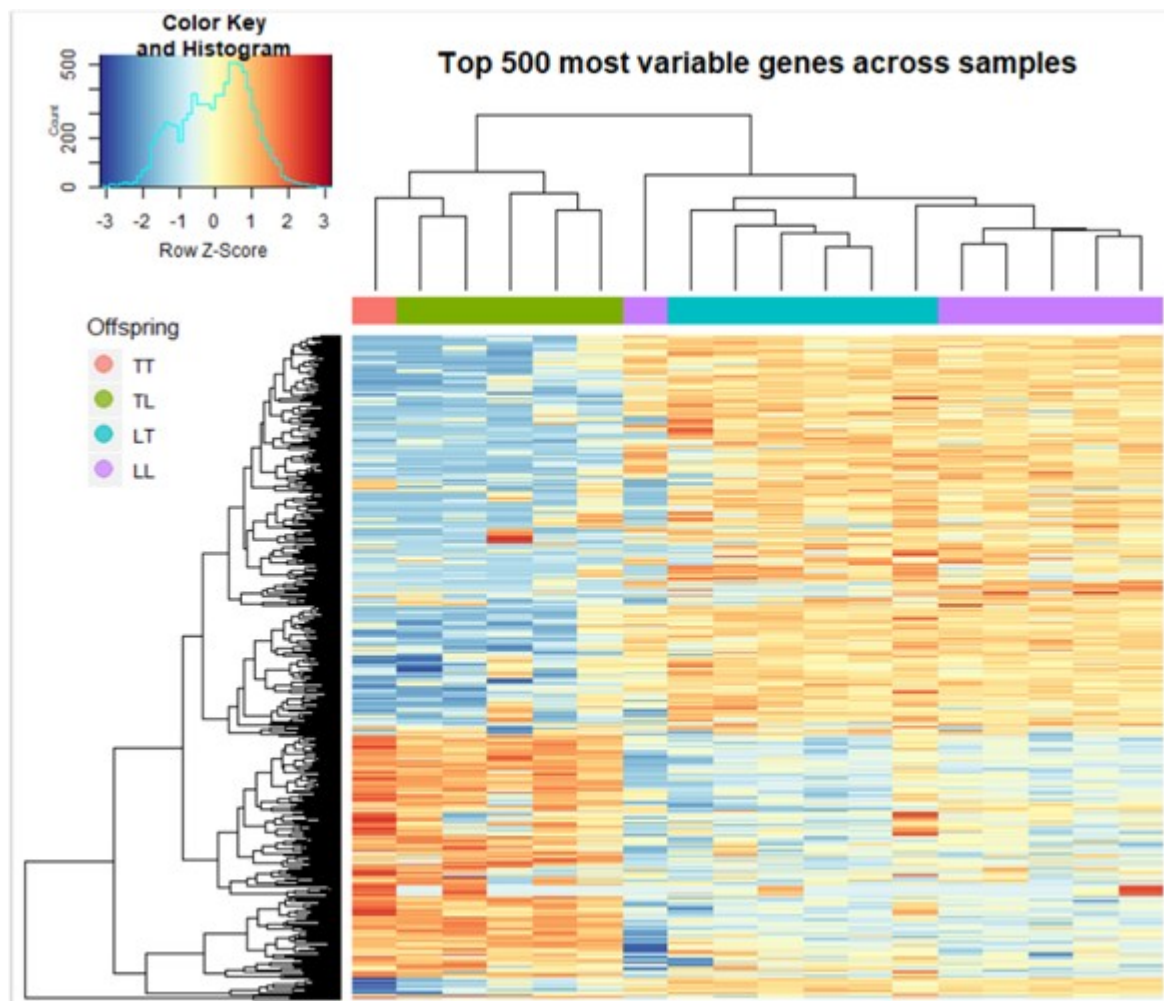
718

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.

724 Table 1. A summary of the key conclusions from previous works on the phenotypes and
725 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
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

726