Title: Genetic variation in heat tolerance of the coral *Platygyra daedalea* offers the potential for adaptation to ocean warming.

Running head: Genetics of corals' heat tolerance

Holland Elder[1]*

Virginia Weis[1]

Jose Montalvo-Proano[2,3]

Veronique J.L Mocellin[2]

Andrew H. Baird[3]

Eli Meyer[1, 4]

Line K. Bay[2, 4]

1. Oregon State University, Corvallis, OR, USA. 97331
2. Australian Institute of Marine Science, 1526 Cape Cleveland Road, Cape Cleveland 4810, Queensland, Australia
   AIMS@JCU
3. ARC Centre of Excellence for Coral Reef Studies, James Cook University, 1 James Cook Dr, Douglas QLD 4814, Australia
4. These authors contributed equally

*corresponding author

760-622-9116

hollandelder@gmail.com

Keywords: coral reefs; resilience, heritability, genomic markers, allele frequency change

Paper type: Primary research article.

Global Change Biology

Abstract

Reef-building corals are foundational species in coral reef ecosystems and are threatened by many stressors including rising ocean temperatures. In 2015/16 and 2016/17, corals around the world experienced consecutive bleaching events and most coral populations are projected to experience temperatures above their current bleaching thresholds annually by 2050. Adaptation to higher temperatures is therefore necessary if corals are to persist in a warming future. While many aspects of heat stress have been well studied, few data are available for predicting the capacity for adaptive cross-generational responses in corals. To address this knowledge gap, we quantified the heritability and genetic variation associated with heat tolerance in *Platygyra daedalea* from the Great Barrier Reef (GBR). We tracked the survival of replicate quantitative genetic crosses (or families) of coral larvae from six parents in a heat stress selection experiment. We also identified allelic shifts in heat-selected survivors *versus* paired, non-selected controls of the same coral crosses. We estimated narrow sense heritability to be 0.66 and detected a total of 1,069 single nucleotide polymorphisms (SNPs) associated with heat tolerance. An overlap of 148 unique SNPs shared between experimental crosses indicates that specific genomic regions are responsible for heat tolerance of *P. daedalea* and some of these SNPs fall in coding regions. These findings suggest that this *P. daedalea* population has the genetic prerequisites for adaptation to increasing temperatures. This study also provides knowledge for the development of high throughput genomic tools to screen for variation within and across populations to harness or enhance adaptation through assisted gene flow and assisted migration.

Introduction

Increasing ocean temperatures brought about by human induced climate change have increased the frequency and severity of coral bleaching events worldwide to the point that it is recognized as a primary threat to coral reefs (Hutchings et al., 2019). Bleaching is the process of loss of dinoflagellate symbionts (Family Symbiodiniaceae) from host corals (Lajeunesse et al., 2018). Severe heat stress and mass bleaching caused by marine heat waves can result in rapid and widespread coral mortality (Glynn, 1993). Global and regional bleaching events have resulted in a substantial loss of coral cover worldwide over the last few decades (Hughes et al., 2018). Over the last five years alone, 30% of reefs in the Great Barrier Reef (GBR) showed decreases in coral cover (Hughes et al., 2019; Hughes et al., 2018; Long Term Reef Monitoring Program, 2019). For coral reefs to persist in an ocean that will continue to warm for the foreseeable future, corals will need to rapidly become more tolerant or resilient to higher temperatures. Consequently, there is now an urgent need to understand adaptive and evolutionary capacity of corals to this threat.

Mechanisms of thermal and bleaching tolerance are diverse. For example, the presence of low background densities of heat tolerant symbiont species can aid in recovery from heat stress (Bay et al., 2016; Berkelmans & van Oppen, 2006; Manzello et al., 2019; Oliver & Palumbi, 2011; Silverstein, Cunning, & Baker, 2014). In addition, plastic genomic responses including, epigenetic alterations and changes in gene expression can result in acclimatization in corals (Barshis et al., 2013; Dixon et al., 2018; Kenkel et al., 2013; Liew et al., 2020; Oliver & Palumbi, 2011; Thomas et al., 2019). Many studies have documented genetic variation in coral host heat tolerance that supports the potential for adaptation (Dixon et al., 2015; Howells et al.,

2016; Kenkel et al., 2013; Meyer et al., 2009; Woolsey et al., 2014). However, there are few datasets that predict adaptation and evolution in corals. Working knowledge of the genomic regions that influence heat tolerance, the extent to which coral host genetics contributes to heat tolerance, and its cross generational persistence in a population will be essential to predict corals' responses into the future. This knowledge will also inform conventional management approaches as well as assisted gene flow and assisted migration. to enhance the tolerance of local populations (van Oppen et al., 2015).

Adaptation requires genetic variation underpinning fitness-related phenotypes. If variation in a fitness-related phenotype has a genetic basis and is heritable and not otherwise constrained, then selection can act to further propagate it within populations (Charmantier & Garant, 2005). Narrow sense heritability describes the total phenotypic variance in a trait that can be attributed to parental or additive genetics and is used to determine the genetic contribution to traits such as heat tolerance (Falconer & Mackay, 1996; Lynch & Walsh, 1998). Estimates of narrow sense heritability are essential for calculating selection differentials and in order to use the breeder's equation to model the persistence of a trait in a population (Falconer & Mackay, 1996; Lynch & Walsh, 1998). Specifically, estimates of narrow sense heat heritability in heat tolerance in corals will allow us to calculate potential rates of adaptation under various selection scenarios.

Current estimates of heritability suggest that the genetic variation to support adaptation of increased heat tolerance is present in coral populations in many different regions. For example, a heritability value of 0.89 was found for bleaching resistance in *Orbicella faveolata* in the Caribbean (Dziedzic et al. 2019) and values between 0.48 and 0.74 for heat tolerance for *Platygyra daedalea* in the Persian Gulf (Kirk et al., 2018). Heritability was similarly high (0.87)

101 for heat tolerance in the larvae of the coral *A. millepora* from the GBR (Dixon et al., 2015).
102 However, heritability can vary substantially between species, between populations of
103 conspecifics, and is influenced by the environment in which it is measured (Falconer & Mackay,
104 1996; Lynch & Walsh, 1998), therefore a heritability estimate from the Persian Gulf should not
105 be used to estimate selection for heat tolerance or potential for adaptation in a population on the
106 GBR (Dixon et al., 2015). To understand the heritability of heat tolerance, selection for heat
107 tolerance, and the potential for adaptation in GBR populations, we need more heritability
108 estimates of heat tolerance from populations on the GBR.

109 *Platygyra daedalea* is a good candidate for estimates of heritability because it is widely
110 distributed in the Indo-Pacific and occurs in the much warmer Persian Gulf. Heritability was
111 recently estimated for a population of *P. daedalea* in the Persian Gulf, which provides an
112 opportunity to compare of heritability between populations from warmer and relatively cooler
113 environments, which could help reveal different genetic mechanisms of heat tolerance of this
114 species between the two environments (Kirk et al., 2017). The Gulf regularly experiences
115 temperatures of 33 - 34˚C in the summer and the bleaching threshold for *P. daedalea* is between
116 35 and 36˚C (Howells et al., 2016; Riegl et al., 2011). Indeed, the bleaching threshold
117 temperature for the coral assemblages is 3 - 7˚C higher in the Gulf when compared to other parts
118 of the world (29 - 32˚C), including the GBR (Berkelmans, 2002; Coles & Riegl, 2013; Hoegh-
119 Guldberg, 1999). This makes the comparison of heritability and genomic heat tolerance of these
120 two regions important for understanding how increased thermal tolerance evolves.

121 The genetic architecture of heat tolerance can also influence the response to selection and
122 the ability to develop markers of tolerance for conservation management as well as new
123 restoration approaches like assisted gene flow and assisted migration. Understanding the genetic

124 architecture of heat tolerance involves identifying coding and non-coding regions in the genome
125 that influence thermal tolerance. For example, in plants, allele frequency analysis has identified
126 markers associated with traits such as yield, drought tolerance and heat tolerance (Singh et al.,
127 2017; Venuprasad et al., 2009; Vikram et al., 2012; M. Wang et al., 2019). These markers can be
128 used to identify individuals in wild populations with increased heat tolerance. Numerous studies
129 have found differential gene expression associated with thermal tolerance in corals e.g. (Barshis
130 et al., 2013; Kenkel et al., 2013; Ruiz-jones & Palumbi, 2017; Traylor-knowles et al., 2017).
131 Fewer have identified genomic markers in coding and non-coding genes that are associated with
132 a thermally tolerant phenotype (Bay & Palumbi, 2014; Dixon et al., 2015; Dziedzic et al., 2019;
133 Jin et al., 2016). While these studies represent progress in identifying markers which connect the
134 genotype to the phenotype of heat tolerance, they also represent only two species from the Indo-
135 Pacific and one from the Caribbean.
136 To understand the potential for the GBR coral *Platygyra daedalea* to persist in a warming
137 environment through selection and genetic adaptation, we estimated heritability and identified
138 genomic regions associated with heat tolerance. Estimating heritability of heat tolerance allowed
139 us to quantify the host genetic contribution to this trait. We identified markers associated with
140 heat tolerant phenotypes by conducting a heat selection experiment and then assessing changes
141 in allele frequency in the survivors. Sequencing our heat-selected pools of bi-parental crosses
142 allowed us to identify markers associated with heat tolerance and examine how widespread these
143 markers were across families in both coding and non-coding genomic regions. This study
144 addresses fundamental questions regarding the adaptive potential of corals to heat stress and
145 provides evidence of their ability to survive and persist in a changing environment.
146

Methods

Experimental crosses and larval culture

To study the genetic basis and heritability in heat tolerance, we produced a series of crosses which are larval pools produced with sperm and eggs from two different parental colonies. For these experiments, we used 6 colony fragments of *Platygyra daedalea* collected from Esk Reef, an inshore reef in the central region of the Great Barrier Reef (18˚ 46'21.95" S, 146˚ 31' 19.33" E) with Australian Institute of Marine Science (AIMS) permit G12/35236.1. The corals were maintained in the National Sea Simulator at AIMS at 27˚C in flow-through sea water aquaria with 25% reduced ambient sunlight. In November 2016 (the 5th night after the full moon, at 18:30) the six parental colonies were isolated in ~60L bins with 0.04-µm filtered sea water (FSW). Between 18:30-19:00, the colonies spawned and gamete bundles were collected. After collection, gamete bundles were washed with FSW. Sperm and eggs were then separated using 60 µm mesh sieves. Eggs were thrice rinsed to eliminate residual sperm. We combined gametes from pairs of colonies according to a partial diallele design in Figure 1 to produce 10 families (hereafter termed crosses). Each cross was maintained at a density of 0.5 to 1.0 planula larvae per ml in separate 12L culture vessels with flow-through FSW at 27˚C and gentle aeration.

Heat stress experiments

To quantify the variation in coral heat tolerance, we measured larval survival during controlled heat stress exposure for each cross following Dixon et al. (2015). Larvae from each

170 cross were incubated in one of two temperatures: ambient reef temperatures (27˚C) or an acute
171 heat stress (35.5˚C). Within the 60L flow through FSW tanks, larvae were contained in
172 individual mesh bottomed 20ml wells (to allow for water circulation). We stocked 20 larvae
173 from each cross into six replicate wells and randomly distributed each cross among floating six-
174 well plates to control for position effects (six wells per cross per treatment in total). To reach
175 35.5˚C in the heat stress groups the temperature was raised from 27˚C by 1˚C per hour. Water
176 temperatures were maintained thereafter by computer-controlled heaters heating the water
177 incoming to the system. To develop a high-resolution time series of larval mortality we
178 monitored larval survival with manual counts of each well every 12 hours for the next ten days.
179 All larval survival data is available at https://github.com/hollandelder/Platygyradaedalea
180 _HeritabiltyandMarkersofThermalTolerance.
181
182 *Statistical analysis of heat tolerance*
183 We investigated the effect of heat stress using a Kaplan Meier Survival Analysis and a
184 Cox Proportional Hazards (CPH) model that compared individual survival at 35.5˚C in the
185 context of cross and temperature (Kaplan and Meier 1958 and Cox 1972). This analysis allowed
186 us to determine the strength of genetic effects between crosses on the survival of larvae at high
187 temperatures. For this analysis, we used the Survival package in R (Therneau 2015). Our model
188 fit individual survival, allowing slopes to vary for temperature, cross, and the interaction of
189 temperature and cross. The code for the Kaplan Meier survival plots and CPH model can be
190 referenced at https://github.com/hollandelder/Platygyradaedalea_HeritabiltyandMarkersof
191 ThermalTolerance. We were also able to obtain Kaplan Meier estimates for the probability of
192 survival given hour of exposure to heat stress, providing statistics for comparing crosses.

193 We used these data to estimate narrow-sense heritability of variation in heat tolerance
194 (Falconer & Mackay, 1996; Wilson et al., 2010). For this analysis, we used a model for assessing
195 heritability using restricted maximum likelihood (REML) implemented in the software package
196 Wombat (Meyer 2007). We followed the same procedure we have previously described for
197 analysis of genetic variation in coral larvae (Dixon et al., 2015; Meyer et al., 2009). This analysis
198 uses known half-sibling relationships among larval biparental crosses to partition variance into
199 genetic and non-genetic components from which narrow-sense heritability (h^2) can be calculated
200 (Lynch & Walsh, 1998). We estimated heritability of survival from the time-course survival
201 described above, at the experiment endpoint, and from the cumulative probability of survival.
202
203 *Genetic analysis of selection for heat stress tolerance*
204 To identify genetic markers under selection during acute heat exposure, we conducted a
205 controlled stress experiment to eliminate the most heat sensitive genotypes in each pool. To
206 provide sufficient larvae for these experiments, we stocked larvae from each cross into replicated
207 flow-through culture containers in each of two treatments (500 larvae per container). We
208 maintained four replicate containers per cross at ambient reef temperatures (27˚C) throughout the
209 experiment and exposed another four containers to a heat stress treatment. For this treatment, we
210 gradually increased temperatures by 1˚C per hour to a maximum temperature of 35.5˚C then
211 maintained that temperature thereafter. Temperatures were maintained as described above by
212 incubating the flow-through culture containers in larger 60L flow-through tanks. We sampled
213 100 - 150 larvae from each replicate after 53 h, and preserved larvae in 100% ethanol for
214 subsequent genetic analysis.

We prepared genotyping libraries from genomic DNA from pooled larval samples. We extracted genomic DNA using the Omega Bio-tek E.Z.N.A. Tissue Kit (Omega Bio-tek, Norcross, GA). We genotyped thousands of single nucleotide polymorphisms (SNPs) in each sample using 2bRAD, a sequencing-based approach for SNP genotyping (Wang et al. 2012) that has been previously applied in corals (Dixon et al., 2015; Howells et al., 2016). We labeled each library with a sample-specific pair of barcodes and combined libraries in equimolar ratios based on qPCR using SensiFAST SYBR Hi-ROX master mix (Bioline), then sequenced the combined pools on four lanes of 50bp single end reads (Illumina HiSeq2500) at Ramaciotti Centre for Genomics at the University of New South Wales (Sydney, Australia).

Prior to statistical analysis, we processed the raw data to exclude low-quality reads. We removed any reads that had ten or more bases that we considered low quality (Q-score > 30). We removed any reads having twelve bases matching Illumina adaptors. This quality filtering process produced a set of high-quality reads for genotyping analysis. All filtering scripts used are available at (https://github.com/Eli-Meyer/sequence_processing).

To enable comparisons between this genomic data set from a GBR *P. daedalea* population with the unusually heat-tolerant *P. daedalea* population from the Persian Gulf (Howells et al., 2016), we mapped our reads against a reference we previously developed from larvae of Persian Gulf *P. daedalea* (SRA accession: SRP066627). We aligned high quality reads against this reference and filtered alignments to determine allele frequencies at each position as described previously (Howells et al., 2016; Wang et al., 2012).

After alignment to the reference, we further filtered sequence data to remove loci with weak or ambiguous matches, those with low coverage, those that occurred in too few samples, and samples with too many missing loci using the following process. First, we removed all loci

238 with ambiguous matches identified by mapping to two or more regions in the reference. Then we
239 removed weak matches or those that did not span at least 32 of a 36 base sequence and matched
240 fewer than 30 of the bases within that span of sequence. We then filtered loci coverage with an
241 initial coverage filter requiring that there needed to be at least 10x coverage of a tag. We also
242 removed monomorphic loci as well as those not found in 25% of samples. We removed samples
243 with < 30,000 loci. We required that each locus be genotyped in at least seven out of eight
244 replicates for each cross in order to compare alleles at that locus across replicates in each
245 treatment. We also removed tags with more than two SNPs, because they are likely to come from
246 repetitive regions. Finally, we filtered so there would be one SNP per tag to control for non-
247 independence and linkage. All 2bRAD sequence filtering was conducted using scripts available
248 at (https://github.com/Eli-Meyer/2brad_utilities).

249 To compare allele frequencies between control larvae and the survivors of heat stress, we
250 compared replicate samples from each treatment within each cross. Since these samples
251 represented pools of large numbers of individuals (>100) rather than single diploid individuals,
252 we interpreted nucleotide frequencies in each sample directly as allele frequencies in the pool,
253 following the same general approach previously described as Pool-Seq (Kofler et al., 2011). We
254 applied a stringent coverage threshold of 80x to minimize sampling error that is likely to distort
255 estimates of allele frequencies at lower coverage. To test for differences in allele frequencies
256 between control larvae and the those surviving heat stress, we used logistic regression to analyze
257 the number of observations of each allele in each sample, conducting generalized linear models
258 in R (glm with argument "cross=binomial(logit)"). This allowed us to determine the effects of
259 treatment on allele frequencies at each locus, identifying loci associated with heat tolerance in

260 each cross. We examined the presence and absence of SNPs identified as associated with heat
261 tolerance from the allele frequency analysis between all combinations of two and three crosses.
262 To investigate the functional implications of genetic associations, we mapped each
263 2bRAD tag to the *P. daedalea* transcriptome (Kirk et al., 2017). To find the gene function, we
264 searched for the UniProt match for the annotation of each matched transcript. We required a
265 match of 34 base pairs of the 36 base pair tag so that matches would be unambiguous. This was
266 necessary, because of the short length of the restriction fragments analyzed here. It is likely that
267 if these standards were relaxed, more of the differentially enriched genomic regions would map
268 to functional regions but could also introduce false positives.
269
270 **Results**
271
272 *Survival in heat stress experiments*
273 A Cox proportional hazards (CPH) model revealed significant effects of heat x cross (p-
274 value < 0.0001). Endpoint survival probabilities ranged more among crosses at 35.5˚C (0.405 to
275 0.750) than at 27˚C (0.861 to 0.975) (Fig. 3). Cross DB had the greatest survival probability at
276 35.5˚C 0.75 (+/- 0.04), while cross CA had the lowest survival probability 0.41 (+/- 0.04) (Fig.3).
277 Coral E provided sperm in both EC and EF crosses which are both in the top four survival
278 probabilities 0.68 (+/- 0.04) and 0.64 (+/- 0.04) respectively. Coral C provided eggs in crosses
279 DC and BC and provided sperm in cross CA, and this coral is associated with the bottom three
280 survival probabilities 0.48 (+/- 0.04), 0.44 (+/- 0.05), and 0.40 (+/- 0.04). These analyses
281 demonstrate substantial variation in heat tolerance among larval crosses which can be attributed
282 to genetic background.

283

284 *Quantitative genetic analysis of variation in heat tolerance*

285 Variation in survival during heat stress was highly heritable, with additive genetic
286 variation explaining 66% of variation in survival (i.e. $h^2 = 0.66$; Table 1). We also estimated h^2
287 using the survival probabilities from the Kaplan Meier analysis, and recovered a similar estimate
288 ($h^2 = 0.64$). Both metrics reflect the total mortality over the entire time course.

289

290 *Genomic analysis of markers under selection during heat stress*

291 Genomic analysis of heat stressed versus control larval pools found that experimental
292 treatment altered allele frequencies at hundreds of loci. Logistic regression of multilocus SNP
293 genotypes from surviving heat stress versus control larval pools (Fig. 4) uncovered tens-to-
294 hundreds of markers in each cross that were significantly affected by heat stress (Table 2). The
295 number of alleles significantly associated with survival under heat stress differed widely among
296 crosses. This variation was not correlated with the number of markers genotyped in each sample
297 ($P = 0.47$, $R^2 = 0.067$), genetic distance between the parents ($P = 0.46$, $R^2 = 0.071$), or heat
298 tolerance of each cross ($P = 0.49$, $R^2 = 0.063$) (Table 2). The variation was also not associated
299 with a particular parent. For example, parents C and D each produced crosses with few markers
300 under selection (BC and DB), but the combination of these parents in cross DC had the most:
301 366 SNPs associated with survival under heat stress (Table 2). This comparison suggests the
302 large number of alleles changing in frequency in cross DC results from a combination of parental
303 alleles at many loci, producing larvae with multiple deleterious alleles.

304 To evaluate the generalizability of genetic associations we compared these significant
305 loci among crosses. A proportion of markers were consistently associated with heat stress across

306 multiple crosses; 2 - 17 markers identified in each cross were shared with one or more crosses,
307 and 2 - 5 of these were identified in all three (Figure 5). Altogether, 148 unique markers were
308 present in at least 2 crosses (Table 3). The same two markers were found in seven crosses, a
309 single marker overlapped in six crosses, and the same four markers overlapped in five crosses
310 (Table 3). However, most markers associated with heat tolerance in one cross were not
311 associated with heat tolerance in unrelated crosses. For example, cross DC shared no parents
312 with crosses EF or FA and 82-92% of the markers identified in each cross were not associated
313 with heat stress in the other two.

314 Altogether, 22 markers could be unambiguously assigned to a position in the
315 transcriptome and five SNPs were located in annotated transcripts (Table 4). Reproducible
316 associations across different genetic backgrounds were also apparent for four transcripts without
317 matches in UniProt. Furthermore, five loci were associated with heat tolerance in more than one
318 family (Table 4).

319

320 **Discussion**

321

322 *Genetic background affects heat tolerance*

323 Our survival model shows that heat tolerance is significantly affected by genetic
324 background. Survival probabilities varied significantly between crosses indicating that genetics
325 contributed to variation in heat tolerance. Coral parent E was associated with crosses that had
326 high survival probabilities and Coral parent C was associated with crosses that had lower
327 survival probabilities. We conclude that parental genetics contribute to heat tolerance of

328 offspring and consideration of parental genetics will be important in coral and conservation as
329 well as assisted gene flow and assisted migration.
330
331 *Heritability and modeling heat tolerance*
332 Quantifying the amount of genetic variance that contributes to a phenotype is important
333 for evaluating adaptive potential of a population (Falconer & Mackay, 1996). Our results add to
334 only three published narrow sense heritability estimates for heat tolerance in corals, all of which
335 find this trait is highly heritable (Dixon et al., 2015; Dziedzic et al., 2019; Kirk et al., 2018). Our
336 study is the only estimate of heritability for a sub-massive and relatively heat tolerant coral
337 species from the GBR. Our result for *P. daedalea* likewise indicates that heritability in larval
338 survival after heat stress was substantial ($h^2 = 0.66$) and suggests that the *P. daedalea*
339 populations have substantial standing genetic variation which contributes to heat tolerance and
340 that this population has an innate capacity to adapt increased to some level of ocean warming.
341 Heritability estimates from different populations can give us valuable information about
342 selection in a given geographic region. Heritability estimates are dependent on the environment
343 and the standing genetic variation in the population for which the estimate is made (Falconer &
344 Mackay, 1996). If there are low levels of genetic variation contributing to a trait and all parents
345 express the same phenotype, the heritability estimate will be lower (Falconer & Mackay, 1996).
346 A novel comparison is the contrast of the heritability estimate (0.48) found by Kirk and
347 colleagues for *P. daedalea* in the Persian Gulf (Kirk et al., 2018). The Persian Gulf *P. daedalea*
348 has lived in a very shallow and warm sea for thousands of years (Purkis & Riegl, 2012), which
349 may mean that a degree of selection for heat tolerance has already taken place resulting in less
350 genetic diversity in the population (Falconer & Mackay, 1996). The lower heritability value is

351 evidence to support this and the higher degree of heritability for heat tolerance in the central
352 GBR *P. daedalea* population suggests that until very recently, heat stress has not been the
353 predominant selective pressure on this population.

354 Responses to selection depend on genetic variation in the trait under selection (Falconer
355 & Mackay, 1996; Lynch & Walsh, 1998) and our study contributes to building the necessary
356 quantitative genetic data to model heat tolerance under various selection regimes. In its simplest
357 form, this can be expressed in the breeder's equation, which describes the response to selection
358 as the product of the selection differential (S) and heritability of the trait under selection. To our
359 knowledge, selection differentials have not been empirically estimated in corals, but considering
360 the widespread reductions in coral populations following coral bleaching (Bellwood et al., 2004;
361 Bruno & Selig, 2007; De'ath et al., 2012; Hoegh-Guldberg et al., 2007; Hughes et al., 2017,
362 2018) they are likely high for sensitive species. For truncation selection, where all individuals
363 with trait values above a threshold contribute offspring to the next generation, selection
364 differentials are equal to the difference in mean trait values between the selected population and
365 the original population (Falconer & Mackay, 1996; Lynch & Walsh, 1998) expressed as standard
366 deviations of the population mean. If the selection differential equals one standard deviation, all
367 parents with traits one standard deviation above the population mean contribute to the next
368 generation. Based on the distribution of cumulative survival probabilities in heat stress from our
369 experiment (0.57 ± 0.18) and the heritability of variation in heat tolerance we estimated from
370 these data (0.66), the Breeder's Equation predicts a response to selection of 0.12. We would
371 expect that if larvae of the next generation after selection were subjected to the same heat stress
372 treatment, the average mortality would be 0.48 (0.59 - 0.12), a 20% reduction in overall
373 mortality. If instead of the high h^2 estimated in our study (0.66), we modeled the responses to

374 selection using a low h^2 value of 0.1, we would predict only a 3% reduction in mortality in the
375 following generation. While the estimates above are underpinned by too many assumptions to
376 predict responses in natural populations of corals, the comparison highlights how empirical
377 estimations of narrow-sense heritability can be used to predict adaptive responses. The relevance
378 of these estimates for population responses depends on the extent to which larval survival during
379 heat stress is directly under selection in natural populations, or alternatively the effectiveness of
380 larval heat tolerance as a proxy for heat tolerance in adult colonies.
381
382 *Allele frequency analysis*
383 Physiological and fitness traits can be determined by single or few loci of large effects,
384 but commonly they are underpinned by many loci of small effect. Our genomic analysis
385 identified tens-to-hundreds of genetic markers associated with heat tolerance in each cross, based
386 on changes in allele frequencies between heat selected and control pools. Comparing between
387 crosses allowed us to investigate the generality of these patterns among different genetic
388 backgrounds. This comparison revealed a number of markers with consistent changes in allele
389 frequencies in multiple crosses (Table 3, 4, and Supplemental Table 1). However, many of the
390 associations detected in each cross were not shared in comparisons of multiple crosses. The lack
391 of overlap for many markers likely results from the underlying distribution of those alleles in our
392 parental population. In isolated population the loci contributing to a trait are predicted to be
393 fewer and they are of larger effect size (Savolainen et al., 2013). However, in populations
394 experiencing immigration, the locus effect size and the number of loci that contribute to a trait
395 become more variable (Savolainen et al., 2013). Corals can migrate in their larval phase before
396 metamorphosis into an adult, so it is entirely possible that individuals originating from disparate

397 reefs were among those chosen for our parental population. The observation that many markers
398 associated with heat stress were specific to individual crosses suggests that many alleles of small
399 effect contribute to variation in heat tolerance and that many different combinations of these
400 small effect alleles can affect heat tolerance (Bay & Palumbi, 2014; Kirk et al., 2018). These
401 differences may also result in part from epistatic interactions where the effects of variation at
402 these loci depend on the genetic background. Few data on either frequencies of heat tolerance
403 alleles or epistatic effects are available for any coral species. However, the repeated observation
404 of some of these associations across different genetic backgrounds provides strong evidence that
405 these markers are linked to genetic factors contributing to variation in heat tolerance.

406 Future studies can use markers identified in this and other studies. Starting with genetic
407 markers associated with habitat variation in previous studies (Capper et al., 2015), we can test
408 these associations through a combination of laboratory experiments and extensive population
409 surveys of allele frequencies (Jin et al., 2016). These studies revealed that the frequencies of
410 alleles associated with enhanced tolerance for environmental stress range from 0.48 in some
411 populations to 0.92 in others (Jin et al., 2016). Further studies are needed to extend these surveys
412 across species, markers, and geographic regions to develop an ecosystem-scale perspective on
413 the potential for coral adaptive responses to ocean warming.

414

415 *SNPs associated with heat tolerance occurring in coding genes*

416 In our study, we found a subset of regions in coding genomic regions associated with heat
417 tolerance, five of which were observed in multiple crosses (Tables 3 and 4). These SNPS were
418 found in genes associated with the extra-cellular matrix and metabolism. There were also SNPs

419 in four unannotated transcripts. These SNP-containing genes found in this study are candidates
420 for further study of their role in this trait and could serve as heat tolerance markers.
421 Three SNPs were associated with coding genes that play roles in the extracellular matrix:
422 (1) GPI_Mannosyltransferase_2, which functions in the assembly of
423 glycosylphosphatidylinositol (GPI) anchors that attach proteins to cell membranes (Kang et al.
424 2005), (2) cad96ca, an E type cadherin, that initiates cell-cell adhesion (Lodish et al. 2013), and
425 (3) metalloendopeptidase, a metal-dependent protease that restructures the extracellular matrix
426 (Lodish et al. 2013) (Table 4). A SNP was also found in Serine/threonine-protein kinase (PAK3)
427 a member of cell regulatory pathways influencing regulation of cytoskeletal formation, cell
428 migration, and the cell cycle (Deleris et al. 2011). Three coral crosses had a SNP in the Krebs
429 cycle enzyme isocitrate dehydrogenase (IDH) (Garret & Grisham, 2013). This enzyme has been
430 identified in gene expression studies as important to the coral thermal stress response (Kenkel,
431 Meyer, et al., 2013; Polato et al., 2010). IDH participates in handling reactive oxygen species
432 and is one of the 44 proteins identified as the minimal stress proteome (Kultz, 2005). The SNP in
433 IDH associated with heat tolerance further strengthens its proposed role in the thermal stress
434 response in corals.
435 Many studies have pointed out the abundance of unannotated genes in the genomes of
436 early-diverging animals and the absence of any pathways to understand their function. The four
437 SNPs that we found which were unannotated in the transcriptome show overlap between crosses
438 highlighting the potential importance of understanding the role these unknown genes in thermal
439 tolerance. These four unannotated transcripts warrant further functional investigations, such as
440 full sequencing, domain analysis, and expression in functional assays. Knowing the function of

441 these genes will be important for having a complete picture of the alleles controlling thermal
442 tolerance.

443 Comparisons of populations of *P. daedalea* in more typical reef temperature conditions
444 (this study) and unusually warm conditions (Kirk et al., 2018) will be important to understanding
445 the genomic basis of heat tolerance. Two SNPs from the present study were also significantly
446 associated with heat tolerance in the Persian Gulf population and thus represent excellent
447 candidates for further study. This could include screens for natural abundance among thermal
448 habitats and potential validation for gene function through knock-down from CRISPR-Cas9
449 induced mutagenesis (Cleves et al., 2018). This unexpected lack of shared markers is further
450 evidence that many alleles of small effect contribute to heat tolerance and that they vary in their
451 distribution across populations.

452

453 *Conclusions*

454 Our findings demonstrate that heat tolerance is highly heritable (i.e., > 60%) in the coral
455 *Platygyra daedalea* from the GBR demonstrating high potential for adaptation. The heat stress
456 time course (Table 1) supports the conclusion that genetic variation explains a substantial
457 fraction of the variation in heat tolerance among our crosses. Our analysis identified hundreds of
458 markers associated with heat tolerance, including markers that were repeatedly associated with
459 heat tolerance across different genetic backgrounds. This supports the conclusion that heat
460 tolerance is a complex polygenic trait determined by many alleles of small affect that are likely
461 to be species and population specific. Despite this complexity, we found markers in multiple
462 crosses that may be important for heat tolerance in *P. daedalea*. These markers associated with
463 heat tolerance in our study of *P. daedalea* from typical coral reef conditions in the GBR will

464 provide a valuable comparison with future genomic studies of heat tolerant populations from the
465 Persian Gulf. The four unannotated transcripts found in this study warrant further functional
466 investigations to find their role in the animal and examine how they might function in heat
467 tolerance. Our findings build on the growing body of evidence that natural populations of corals
468 harbor substantial genetic variation in heat tolerance that can support adaptive responses to
469 selection and identify genetic markers for heat tolerance in a globally distributed coral species.
470 These heat tolerance markers are essential to any type of reseeding or assisted gene flow and
471 assisted migration programs, which may be necessary to maintaining reefs in the future as we
472 work to reduce CO_2 emissions and mitigate the effects of climate change.
473

474 **Acknowledgements**

475 We thank the staff of the National Sea Simulator at the Australian Institute of Marine Science for
476 their assistance in the collection and husbandry of the corals used in these experiments. We also
477 thank Ida Bjornsbo for her tireless assistance in counting coral larvae at all hours of the day and
478 night. This research was conducted under Great Barrier Reef Marine Park Authority permit
479 (G11/34671.1) and supported with funding from the National Science Foundation Graduate
480 Research Fellowship to Holland Elder, National Science Foundation Graduate Research
481 Opportunities Worldwide to Holland Elder and internal grants from AIMS to Line K. Bay.
482

483 **Authors' contributions**

484 E.M., H.E., and L.B. conceived the experiments. H.E., V.M., J.M., and L.B. conducted
485 experiments, L.B. and A.B. provided advice and logistical support through the project, H.E.

conducted genetic and statistical analysis and wrote the manuscript. L.B. and E.M. helped with genetic and statistical analysis and L.B., V.W. and A. B. helped write the manuscript.

References

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

Bay, L. K., Doyle, J., Logan, M., & Berkelmans, R. (2016). Recovery from bleaching is mediated by threshold densities of background thermo-tolerant symbiont types in a reef-building coral. *Royal Society Open Science*, *3*(160322).

Bay, R. a, & Palumbi, S. R. (2014). Report Multilocus Adaptation Associated with Heat Resistance in Reef-Building Corals. *Current Biology*, 1–5. https://doi.org/10.1016/j.cub.2014.10.044

Bellwood, D. R., Hughes, T. P., Folke, C., & Nystro, M. (2004). Confronting the coral reef crisis. *Nature*, *429*(June), 827–833.

Berkelmans, R. (2002). Time-integrated thermal bleaching thresholds of reefs and their variation on the Great Barrier Reef. *Marine Ecology Progress Series*, *229*, 73–82.

Berkelmans, R., & van Oppen, M. J. H. (2006). The role of zooxanthellae in the thermal tolerance of corals: a “nugget of hope” for coral reefs in an era of climate change. *Proceedings. Biological Sciences / The Royal Society*, *273*(1599), 2305–2312. https://doi.org/10.1098/rspb.2006.3567

Bruno, J. F., & Selig, E. R. (2007). Regional Decline of Coral Cover in the Indo-Pacific : Timing , Extent , and Subregional Comparisons. *PloS One*, *8*. https://doi.org/10.1371/journal.pone.0000711

Capper, R. L., Jin, Y. K., Lundgren, P. B., Peplow, L. M., Matz, M. V, & Oppen, M. J. H. Van. (2015). Quantitative high resolution melting : two methods to determine SNP allele frequencies from pooled samples. *BMC Genetics*, *16*(62), 1–13. https://doi.org/10.1186/s12863-015-0222-z

Charmantier, A., & Garant, D. (2005). Environmental quality and evolutionary potential : lessons from wild populations. *Proceedings of the Royal Society*, *272*, 1415–1425. https://doi.org/10.1098/rspb.2005.3117

Cleves, P. A., Strader, M. E., Bay, L. K., Pringle, J. R., & Matz, M. V. (2018). CRISPR/Cas9-mediated genome editing in a reef building coral. *PNAS*, *115*(20), 5235–5240. https://doi.org/10.1073/pnas.1722151115

Coles, S. L., & Riegl, B. M. (2013). Thermal tolerances of reef corals in the Gulf : A review of the potential for increasing coral survival and adaptation to climate change through assisted translocation. *Marine Pollution Bulletin*, *72*(2), 323–332. https://doi.org/10.1016/j.marpolbul.2012.09.006

Cunning, R., Vaughan, N., Gillette, P., Capo, T. R., Mate, J. L., & Baker, A. C. (2015). Dynamic regulation of partner abundance mediates response of reef coral symbioses to environmental change. *Ecology*, *96*(5), 1411–1420.

Cunning, R, Silverstein, R. N., & Baker, A. C. (2015). Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. *Proc. R. Soc. B*, *282*(20141725).

Cunning, Ross, Silverstein, R. N., & Baker, A. C. (2018). Symbiont shuffling linked to differential photochemical dynamics of Symbiodinium in three Caribbean reef corals. *Coral Reefs*, *37*(1), 145–152. https://doi.org/10.1007/s00338-017-1640-3

De'ath, G., Fabricius, K. E., Sweatman, H., & Puotinen, M. (2012). The 27 – year decline of coral cover on the Great Barrier Reef and its causes. *PNAS*, *109*(44), 17995–17999. https://doi.org/10.1073/pnas.1208909109

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

Dixon, G., Davies, S., Aglyamova, G., Meyer, E., Bay, L. K., & Matz, M. V. (2015). Genomic Determinants of coral heat tolerance across latitudes. *Science*, *348*(6242), 1460–1462.

Dixon, G., Liao, Y., Bay, L. K., & Matz, M. V. (2018). Role of gene body methylation in acclimatization and adaptation in a basal metazoan. *PNAS*, *115*(52), 13342–13346. https://doi.org/10.1073/pnas.1813749115

Dziedzic, K. E., Elder, H., & Meyer, E. (2019). Heritable variation in bleaching responses and its functional genomic basis in reef-building corals (Orbicella faveolata). *Molecular Ecology*, 1–16.

Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics*.

Garret, R. H., & Grisham, C. M. (2013). *Biochemistry*.

Glynn, P. W. (1993). Coral reef bleaching: ecological perspectives. *Coral Reefs*, *12*, 1–17. https://doi.org/10.1007/BF00303779

Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research*, *50*(8), 839–866. https://doi.org/10.1071/MF99078

Hoegh-Guldberg, O., Harvell, C. D., Sale, P. F., Edwards, A. J., Caldeira, K., Knowlton, N., Eakin, C. M., Muthiga, N., Bradbury, R. H., Dubi, A., Hatziolos, M. E., Mumby, P. J., Hooten, A., Steneck, R. S., Greenfield, P., Gomez, E., & Iglesias-Prieto, R. (2007). Coral

Reefs Under Rapid Climate Change and Ocean Acidification. *Science*, *318*(5857), 1737–1742.

Howells, E. J., Abrego, D., Meyer, E., Kirk, N., & Burt, J. A. (2016). Host adaptation and unexpected symbiont partners enable reef-building corals to tolerate extreme temperatures. *Global Change Biology*, 1–13. https://doi.org/10.1111/gcb.13250

Hughes, T., Kerry, J., Baird, A., Connolly, S., Chase, T., Dietzel, A., Hill, T., Hoey, A., Hoogenboom, M., Jacobson, M., Kerswell, A., Madin, J., Mieog, A., Paley, A., Pratchett, M., Torda, G., & Woods, R. (2019). Global warming impairs stock - recruitment dynamics of corals. *Nature*, *568*, 387–390. https://doi.org/10.1038/s41586-019-1081-y

Hughes, T. P., Kerry, J. T., Álvarez-noriega, M., Álvarez-romero, J. G., Anderson, K. D., Baird, A. H., Babcock, R. C., Beger, M., Bellwood, D. R., Berkelmans, R., Bridge, T. C., Butler, I. R., Byrne, M., Cantin, N. E., Comeau, S., Connolly, S. R., Cumming, G. S., Dalton, S. J., Diaz-Pulido, G., … Wilson, S. K. (2017). Global warming and recurrent mass bleaching of corals. *Nature*, *543*, 373–377. https://doi.org/10.1038/nature21707

Hughes, T. P., Kerry, J. T., Baird, A. H., Connolly, S. R., Dietzel, A., Eakin, C. M., Heron, S. F., Hoey, A. S., Hoogenboom, M. O., Liu, G., Mcwilliam, M. J., Pears, R. J., Pratchett, M. S., Skirving, W. J., Stella, J. S., & Torda, G. (2018). Global warming transforms coral reef assemblages. *Nature*, *556*, 492–496.

Hutchings, P., Kingsford, M., & Hoegh-Guldberg, O. (2019). *The Great Barrier Reef: Biology, Environment, and Management*. Csiro Publishing.

Jin, Y. K., Lundgren, P., Lutz, A., Raina, J., Howells, E. J., Paley, A. S., Willis, B. L., & Oppen, M. J. H. Van. (2016). *Genetic markers for antioxidant capacity in a reef-building coral*. *May*.

580 Kenkel, C. D., Goodbody-Gringley, G., Caillaud, D., Davies, S. W., Bartels, E., & Matz, M. V.
581 (2013). Evidence for a host role in thermotolerance divergence between populations of the
582 mustard hill coral (Porites astreoides) from different reef environments. *Molecular Ecology*,
583 *22*(16), 4335–4348. https://doi.org/10.1111/mec.12391
584 Kenkel, C. D., Meyer, E., & Matz, M. V. (2013). Gene expression under chronic heat stress in
585 populations of the mustard hill coral (Porites astreoides) from different thermal
586 environments. *Molecular Ecology*, *22*(16), 4322–4334. https://doi.org/10.1111/mec.12390
587 Kirk, N., Howells, E., Abrego, D., Burt, J., & Meyer, E. (2018). Genomic and transcriptomic
588 signals of thermal tolerance in heat-tolerant corals (Platygyra daedalea) of the
589 Arabian/Persian Gulf. *Molecular Ecology*, *27*, 5180–5194.
590 Kofler, R., Orozco-terWengel, P., de Maio, N., Pandey, R. V., Nolte, V., Futschik, A., Kosiol,
591 C., & Schlötterer, C. (2011). Popoolation: A toolbox for population genetic analysis of next
592 generation sequencing data from pooled individuals. *PLoS ONE*, *6*(1).
593 https://doi.org/10.1371/journal.pone.0015925
594 Kultz, D. (2005). Molecular And Evolutionary Basis of the Cellular Stress Response. *Annual*
595 *Review Physiology*, *67*, 225–257.
596 https://doi.org/10.1146/annurev.physiol.67.040403.103635
597 Lajeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C.
598 R., Santos, S. R., Lajeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., &
599 Reimer, J. D. (2018). Systematic Revision of Symbiodiniaceae Highlights the Antiquity and
600 Diversity of Coral Endosymbionts Article Systematic Revision of Symbiodiniaceae
601 Highlights the Antiquity and Diversity of Coral Endosymbionts. *Current Biology*, *28*(16),
602 2570-2580.e6. https://doi.org/10.1016/j.cub.2018.07.008

Liew, Y. J., Howells, E. J., Wang, X., Michell, C. T., Burt, J. A., Idaghdour, Y., & Aranda, M. (2020). Intergenerational epigenetic inheritance in reef-building corals. *Nature Climate Change*, *10*, 254–259. https://doi.org/10.1038/s41558-019-0687-2

Long Term Reef Monitoring Program. (2019). *Annual Report on Reef Condition: Mixed bill of health for the Great Barrier Reef*. https://www.aims.gov.au/reef-monitoring/gbr-condition-summary-2018-2019

Lynch, M., & Walsh, B. (1998). *Genetics and Analysis of Quantitative Traits*.

Manzello, D. P., Jankulak, M., Matz, M. V, Enochs, I. C., Valentino, L., Carlton, R. D., Kolodziej, G., Serrano, X., & Towle, E. K. (2019). Role of host genetics and heat - tolerant algal symbionts in sustaining populations of the endangered coral Orbicella faveolata in the Florida Keys with ocean warming. *Global Change Biology*, *25*, 1016–1031. https://doi.org/10.1111/gcb.14545

Marshall, P. A., & Baird, A. H. (2000). Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. *Coral Reefs*, *19*(2), 155–163. https://doi.org/10.1007/s003380000086

Meyer, E., Davies, S., Wang, S., Willis, B., Abrego, D., Juenger, T., & Matz, M. (2009). Genetic variation in responses to a settlement cue and elevated temperature in the reef-building coral Acropora millepora. *Marine Ecology Progress Series*, *392*, 81–92. https://doi.org/10.3354/meps08208

Mieog, J. C., Van Oppen, J. H., Berkelmans, R., Stam, W. T., & Olsen, J. L. (2009). Quantification of algal endosymbionts (Symbiodinium) in coral tissue using real-time PCR. *Molecular Ecology Resources*, *9*, 74–82. https://doi.org/10.1111/j.1755-0998.2008.02222.x

Núnez-Pons, L., Bertocci, I., & Baghdasarian, G. (2017). Symbiont dynamics during thermal acclimation using cnidarian-dinoflagellate model holobionts. *Marine Environmental Research*, *130*, 303–314. https://doi.org/10.1016/j.marenvres.2017.08.005

Oliver, T. a., & Palumbi, S. R. (2011). Do fluctuating temperature environments elevate coral thermal tolerance? *Coral Reefs*, *30*(2), 429–440. https://doi.org/10.1007/s00338-011-0721-y

Oliver, T. A., & Palumbi, S. R. (2011). Many corals host thermally resistant symbionts in high-temperature habitat. *Coral Reefs*, *30*, 241–250. https://doi.org/10.1007/s00338-010-0696-0

Polato, N. R., Voolstra, C. R., Schnetzer, J., DeSalvo, M. K., Randall, C. J., Szmant, A. M., Medina, M., & Baums, I. B. (2010). Location-specific responses to thermal stress in larvae of the reef-building coral Montastraea faveolata. *PloS One*, *5*(6), e11221. https://doi.org/10.1371/journal.pone.0011221

Purkis, S. J., & Riegl, B. M. (2012). Geomorphology and Reef Building in the SE Gulf. In *Coral Reefs of the Gulf: Adaptation to Climatic Extremes in the World's Hottest Sea* (pp. 33–50). https://doi.org/10.1007/978-94-007-3008-3

Riegl, B., Purkis, S., Al-cibahy, A., Abdel-moati, M., & Hoegh-Guldberg, O. (2011). Present Limits to Heat-Adaptability in Corals and Population-Level Responses to Climate Extremes. *PloS One*, *6*(9). https://doi.org/10.1371/journal.pone.0024802

Ruiz-jones, L. J., & Palumbi, S. R. (2017). Tidal heat pulses on a reef trigger a fine-tuned transcriptional response in corals to maintain homeostasis. *Science Advances*, *3*(e1601298), 1–11.

Savolainen, O., Lascoux, M., & Merilä, J. (2013). Ecological genomics of local adaptation. *Nature Reviews Genetics*, *14*(November), 807–820. https://doi.org/10.1038/nrg3522

Silverstein, R. N., Cunning, R., & Baker, A. C. (2014). Change in algal symbiont communities

after bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Global Change Biology*, 236–249. https://doi.org/10.1111/gcb.12706

Singh, D., Singh, C. K., Sewak, R., Tomar, S., & Pal, M. (2017). Genetics and Molecular Mapping of Heat Tolerance for Seedling Survival and Pod Set in Lentil. *Crop Science*, *57*, 3059–3067. https://doi.org/10.2135/cropsci2017.05.0284

Thomas, L., López, E. H., Morikawa, M. K., & Palumbi, S. R. (2019). Transcriptomic resilience , symbiont shuffling , and vulnerability to recurrent bleaching in reef - building corals. *Molecular Ecology*, *28*, 3371–3382. https://doi.org/10.1111/mec.15143

Traylor-knowles, N., Rose, N. H., & Palumbi, S. R. (2017). The cell specificity of gene expression in the response to heat stress in corals. *Journal of Experimental Biology*, *220*, 1837–1845. https://doi.org/10.1242/jeb.155275

Traylor-knowles, N., Rose, N. H., Sheets, E. A., & Palumbi, S. R. (2017). Early Transcriptional Responses during Heat Stress in the Coral Acropora hyacinthus. *Biological Bulletin*, *232*, 91–100.

van Oppen, M. J. H., Oliver, J. K., Putnam, H. M., & Gates, R. D. (2015). Building coral reef resilience through assisted evolution. *Proceedings of the National Academy of Sciences*, *112*(8), 2307–2313. https://doi.org/10.1073/pnas.1422301112

Venuprasad, R., Dalid, C. O., Del Valle, M., Zhoa, D., Espiritu, M., Sta Cruz, M. T., Amante, M., Kumar, A., & Atlin, G. N. (2009). Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theoretical Applied Genetics*, *120*, 177–190. https://doi.org/10.1007/s00122-009-1168-1

Vikram, P., Swamy, B. P. M., Dixit, S., Ahmed, H., Cruz, M. T. S., Singh, A. K., Ye, G., &

672 Kumar, A. (2012). Field Crops Research Bulk segregant analysis : " An effective approach
673 for mapping consistent-effect drought grain yield QTLs in rice ." *Field Crops Research*,
674 *134*, 185–192. https://doi.org/10.1016/j.fcr.2012.05.012
675 Wang, M., Liu, W., Jiang, B., & Peng, Q. (2019). Genetic Analysis and Related Gene Primary
676 Mapping of Heat Stress Tolerance in Cucumber Using Bulked Segregant Analysis.
677 *Horticulture Science*, *54*(3), 423–428. https://doi.org/10.21273/HORTSCI13734-18
678 Wang, S., Meyer, E., McKay, J., & Matz, M. (2012). 2b-RAD: a simple and flexible method for
679 genome-wide genotyping. *Nature Methods*, *9*(August), 808–810.
680 http://www.nature.com/nmeth/journal/v9/n8/abs/nmeth.2023.html
681 Wilson, A. J., Clements, M. N., Morrissey, M. M., Re, D., Postma, E., Walling, C. A., Kruuk, L.
682 E. B., & Nussey, D. H. (2010). An ecologist ' s guide to the animal model. *Journal of*
683 *Animal Ecology*, *79*, 13–26. https://doi.org/10.1111/j.1365-2656.2009.01639.x
684 Woolsey, E. S., Keith, S. a., Byrne, M., Schmidt-Roach, S., & Baird, a. H. (2014). Latitudinal
685 variation in thermal tolerance thresholds of early life stages of corals. *Coral Reefs*, *34*, 471–
686 478. https://doi.org/10.1007/s00338-014-1253-z
687

688 **Tables**

Table 1. Estimates of narrow-sense heritability of thermal tolerance obtained from different survival metrics. Censored and uncensored KM estimates refer to cumulative probability of survival estimated with or without censoring individuals surviving at the experimental endpoint.

Data	*Survival*	h^2
Survival at 240hr	59%	0.66
KM Estimate	59%	0.64

689

Table 2. The number of loci at which allele frequencies were significantly altered in survivors of thermal stress in each cross.

Cross	*Significant markers*	*Markers genotyped*	*Parental distance*	*Survival (10 d)*
AB	76	15,217	0.247	0.637
AF	34	19,399	0.245	0.551
BA	95	11,616	0.247	0.742
BC	69	22,820	0.257	0.442
CA	75	23,173	0.246	0.406
DB	44	23,999	0.256	0.758
DC	336	17,548	0.246	0.483
EC	77	16,734	0.262	0.683
EF	124	13,904	0.257	0.654
FA	139	16,691	0.245	0.639

690

691

692

693

694 **Table 3**. Markers identified in four or more crosses.

Marker	SNP Location	Marker Found in Crosses	Number Crosses
denovoLocus34739	1, 21	AB, AF, BC, CA, DC, EF, FA	7
denovoLocus44191	16, 17	AB, AF, BA, CA, DB, EF, FA	7
denovoLocus9359	1	AB, BC, CA, DC, EF, FA	6
denovoLocus121909	11	AB, BA, CA, EC, FA	5
denovoLocus1291	4, 6	AB, BA, BC, DB, EF	5
denovoLocus22543	1, 25	BA, CA, EC, EF, FA	5
denovoLocus93322	3, 25	AB, AF, DC, EC, EF	5
denovoLocus13062	26, 36	CA, EC, EF, FA	4
denovoLocus18723	1	BA, DB, DC, EC	4
denovoLocus29413	1	BC, DC, EF, FA	4
denovoLocus36225	1, 4	AB, AF, EC, EF	4
denovoLocus51123	4	AB, AF, DC, EF	4

695

Table 4. SNP markers affected by heat stress that map to transcripts. Listed in order of the number of crosses the SNP marker was found.

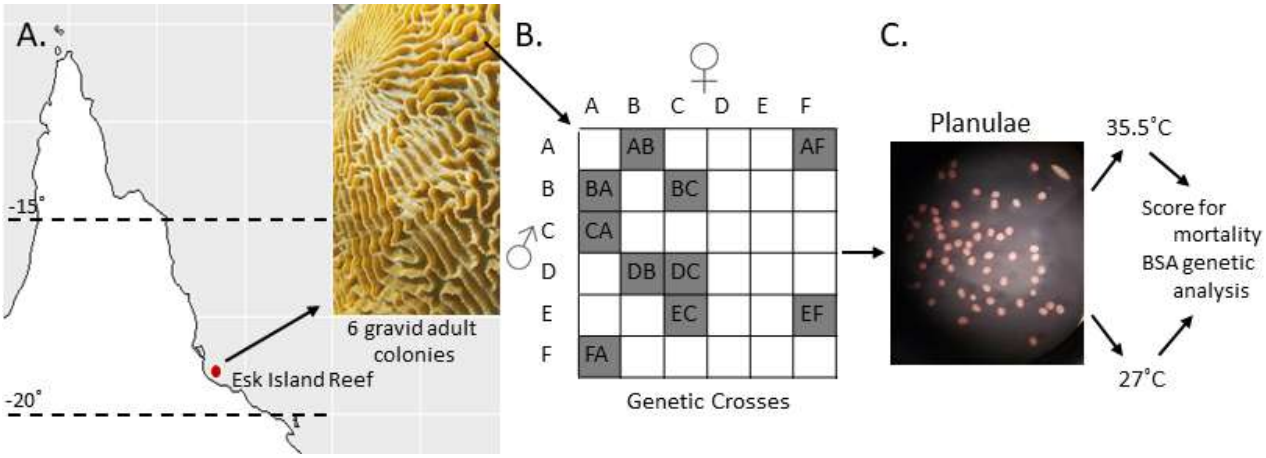
Markers	*Crosses*	*Transcripts*	*Descriptions (UniProt match)*
denovoLocus32940	AF, DC, EF	c31022_g1_i1	Not annotated (no match)
denovoLocus14433	BA, DC	c40970_g1_i1	Not annotated (no match)
denovoLocus24976	DC, EF	c17319_g1_i1	Not annotated (no match)
denovoLocus55443	DB, DC	c19185_g1_i1	Not annotated (no match)
denovoLocus31374	FA	c12147_g2_i1	GPI mannosyltransferase 2 (U3DAR7)
denovoLocus2995	EC	c8644_g1_i2	Putative cad96ca fragment (L7MF99)
denovoLocus4290	AB	c36043_g1_i1	Metalloendopeptidase (A7RVF7)
denovoLocus7677	DC	c16415_g1_i3	S/T-protein kinase PAK 3 (T2MHJ0)
denovoLocus22915	AF, BA, CA	c15457_g1_i1	Isocitrate dehydrogenase 1 (E9C6G6)

696

697

698 **Figures**

699



700

701 **Fig. 1. Crossing design and heat stress experiments.** (**A**) Six *Platygyra daedalea* colonies

702 were sampled from Esk Reef on the GBR. (**B**) Crossing design used to produce ten crosses

703 among those parental colonies. (**C**) Representative micrograph (10X) of planula larvae used for

704 these experiments (53 hours post-fertilization).

705

706

707

708

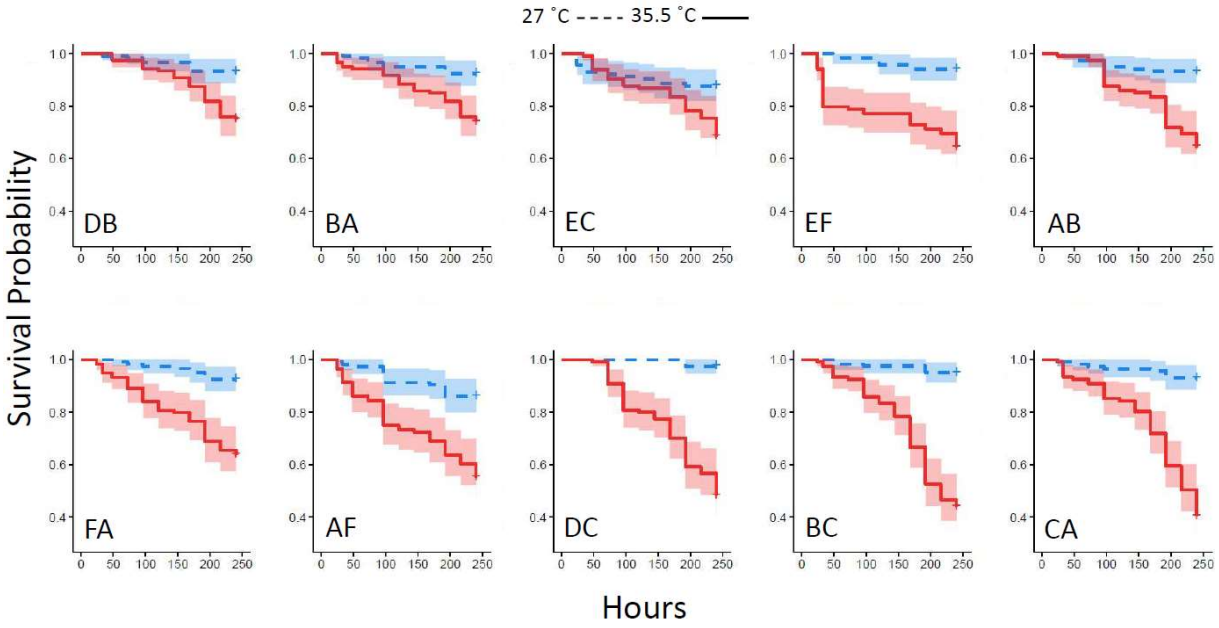


Fig. 3. Heat tolerance differs among crosses. Kaplan Meier plots of cumulative probability of survival for each family in control 27.5˚C (blue) and elevated 35.5˚C (red) temperature. Panels are arranged in order of heat tolerance, based on survival probability at 240 hr. Shaded regions represent 95% confidence intervals.

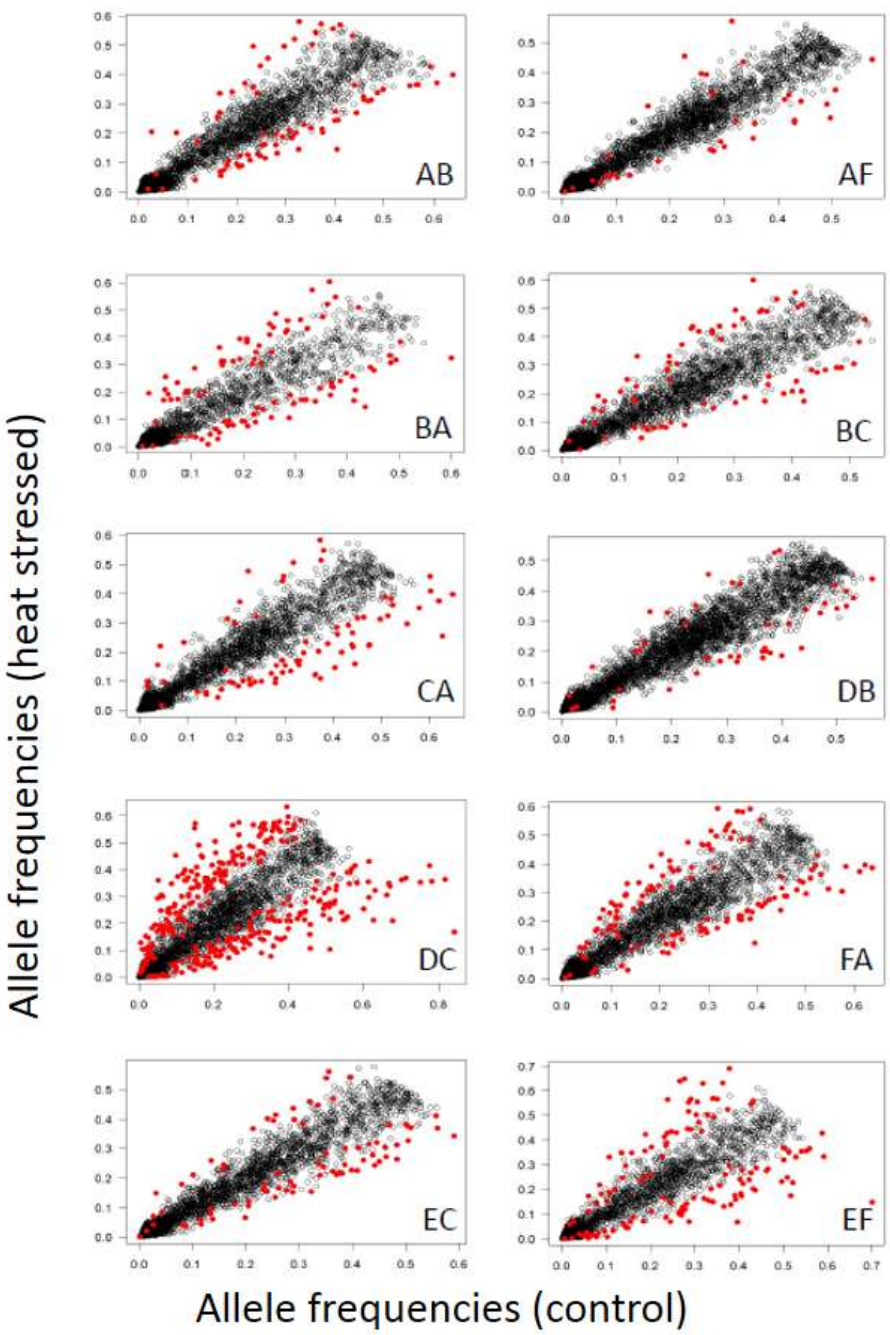


Fig. 4. Heat stress alters allele frequencies in surviving larvae. Panels show correlations between the average allele frequencies in survivors of replicate heat stress treatments relative to the corresponding controls. Markers with significantly altered allele frequencies (logistic regression, FDR < 0.05) are highlighted in red.

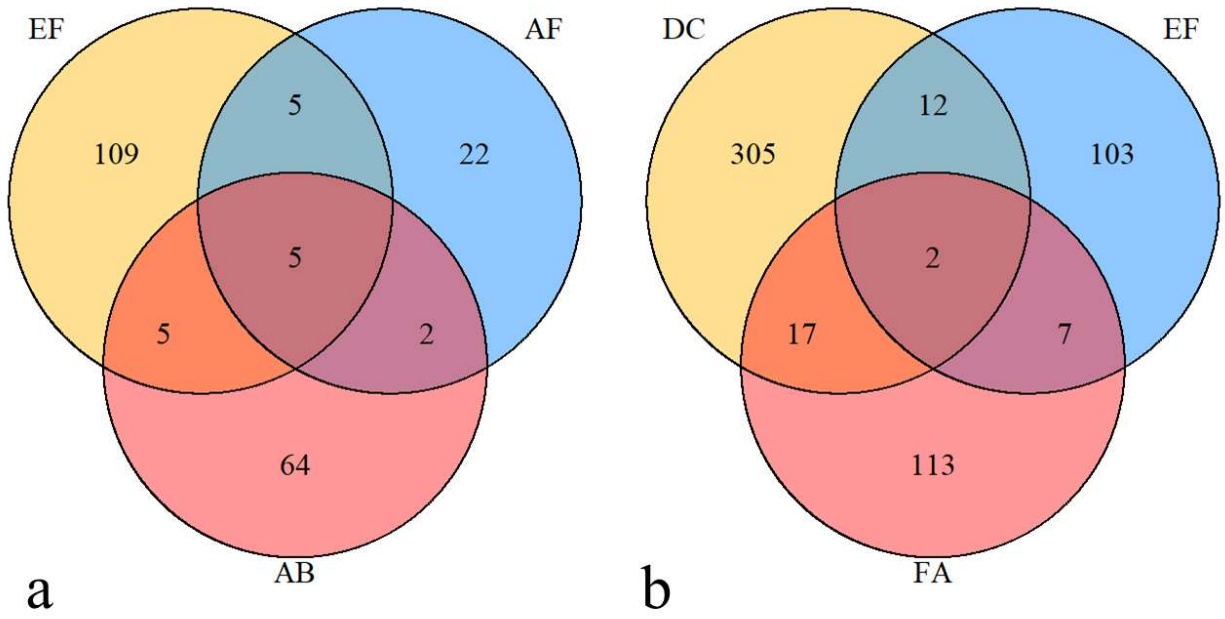


Fig. 5. Venn diagrams depicting the overlap of genetic markers in sets of three crosses

Many markers affected by heat stress were specific to each cross, but a subset of markers showed consistent changes in allele frequency during heat stress across multiple crosses. Examples shown include (**a**) crosses EF, AF, and AB; and (**b**) crosses DC, EF, and FA. The Venn diagram (**a**) shows shared markers between the three crosses that had the most shared markers and (**b**) shows shared markers between three crosses with only one shared parent. These comparisons demonstrate independent associations between heat stress and genotype at these loci across different genetic backgrounds.