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

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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 restoration programs using 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 of 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 of 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 cross were incubated in one of two temperatures: ambient reef temperatures (27˚C) or an acute heat stress (35.5˚C). Within the 60L flow through FSW tanks, larvae were contained in

individual mesh bottomed 20ml wells (to allow for water circulation). We stocked 20 larvae from each cross into six replicate wells and randomly distributed each cross among floating six-well plates to control for position effects (six wells per cross per treatment in total). To reach 35.5˚C in the heat stress groups the temperature was raised from 27˚C by 1˚C per hour. Water temperatures were maintained thereafter by computer-controlled heaters heating the water incoming to the system. To develop a high-resolution time series of larval mortality we monitored larval survival with manual counts of each well every 12 hours for the next ten days. All larval survival data is available at https://github.com/hollandelder/Platygyradaedalea_HeritabiltyandMarkersofThermalTolerance.

Statistical analysis of heat tolerance

We investigated the effect of heat stress using a Kaplan Meier Survival Analysis and a Cox Proportional Hazards (CPH) model that compared individual survival at 35.5˚C in the context of cross and temperature (Kaplan and Meier 1958 and Cox 1972). This analysis allowed us to determine the strength of genetic effects between crosses on the survival of larvae at high temperatures. For this analysis, we used the Survival package in R (Therneau 2015). Our model fit individual survival, allowing slopes to vary for temperature, cross, and the interaction of temperature and cross. The code for the Kaplan Meier survival plots and CPH model can be referenced at https://github.com/hollandelder/Platygyradaedalea_HeritabiltyandMarkersofThermalTolerance. We were also able to obtain Kaplan Meier estimates for the probability of survival given hour of exposure to heat stress, providing statistics for comparing crosses.

We used these data to estimate narrow-sense heritability of variation in heat tolerance (Falconer & Mackay, 1996; Wilson et al., 2010). For this analysis, we used a model for assessing

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

216 sample using 2bRAD, a sequencing-based approach for SNP genotyping (Wang et al. 2012) that
217 has been previously applied in corals (Dixon et al., 2015; Howells et al., 2016). We labeled each
218 library with a sample-specific pair of barcodes and combined libraries in equimolar ratios based
219 on qPCR using SensiFAST SYBR Hi-ROX master mix (Bioline), then sequenced the combined
220 pools on four lanes of 50bp single end reads (Illumina HiSeq2500) at Ramaciotti Centre for
221 Genomics at the University of New South Wales (Sydney, Australia).

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

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

233 After alignment to the reference, we further filtered sequence data to remove loci with
234 weak or ambiguous matches, those with low coverage, those that occurred in too few samples,
235 and samples with too many missing loci using the following process. First, we removed all loci
236 with ambiguous matches identified by mapping to two or more regions in the reference. Then we
237 removed weak matches or those that did not span at least 32 of a 36 base sequence and matched
238 fewer than 30 of the bases within that span of sequence. We then filtered loci coverage with an

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

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

260 To investigate the functional implications of genetic associations, we mapped each
261 2bRAD tag to the *P. daedalea* transcriptome (Kirk et al., 2017). To find the gene function, we

262 searched for the UniProt match for the annotation of each matched transcript. We required a
263 match of 34 base pairs of the 36 base pair tag so that matches would be unambiguous. This was
264 necessary, because of the short length of the restriction fragments analyzed here. It is likely that
265 if these standards were relaxed, more of the differentially enriched genomic regions would map
266 to functional regions but could also introduce false positives.
267
268 **Results**
269
270 *Survival in heat stress experiments*
271 A Cox proportional hazards (CPH) model revealed significant effects of heat x cross (p-
272 value < 0.0001). Endpoint survival probabilities ranged more among crosses at 35.5˚C (0.405 to
273 0.750) than at 27˚C (0.861 to 0.975) (Fig. 3). Cross DB had the greatest survival probability at
274 35.5˚C 0.75 (+/- 0.04), while cross CA had the lowest survival probability 0.41 (+/- 0.04) (Fig.3).
275 Coral E provided sperm in both EC and EF crosses which are both in the top four survival
276 probabilities 0.68 (+/- 0.04) and 0.64 (+/- 0.04) respectively. Coral C provided eggs in crosses
277 DC and BC and provided sperm in cross CA, and this coral is associated with the bottom three
278 survival probabilities 0.48 (+/- 0.04), 0.44 (+/- 0.05), and 0.40 (+/- 0.04). These analyses
279 demonstrate substantial variation in heat tolerance among larval crosses which can be attributed
280 to genetic background.
281
282 *Quantitative genetic analysis of variation in heat tolerance*
283 Variation in survival during heat stress was highly heritable, with additive genetic
284 variation explaining 66% of variation in survival (i.e. h^2 = 0.66; Table 1). We also estimated h^2

285 using the survival probabilities from the Kaplan Meier analysis, and recovered a similar estimate
286 (h^2 = 0.64). Both metrics reflect the total mortality over the entire time course.
287
288 *Genomic analysis of markers under selection during heat stress*
289 Genomic analysis of heat stressed versus control larval pools found that experimental
290 treatment altered allele frequencies at hundreds of loci. Logistic regression of multilocus SNP
291 genotypes from surviving heat stress versus control larval pools (Fig. 4) uncovered tens-to-
292 hundreds of markers in each cross that were significantly affected by heat stress (Table 2). The
293 number of alleles significantly associated with survival under heat stress differed widely among
294 crosses. This variation was not correlated with the number of markers genotyped in each sample
295 (P = 0.47, R^2 = 0.067), genetic distance between the parents (P = 0.46, R^2 = 0.071), or heat
296 tolerance of each cross (P = 0.49, R^2 = 0.063) (Table 2). The variation was also not associated
297 with a particular parent. For example, parents C and D each produced crosses with few markers
298 under selection (BC and DB), but the combination of these parents in cross DC had the most:
299 366 SNPs associated with survival under heat stress (Table 2). This comparison suggests the
300 large number of alleles changing in frequency in cross DC results from a combination of parental
301 alleles at many loci, producing larvae with multiple deleterious alleles.
302 To evaluate the generalizability of genetic associations we compared these significant
303 loci among crosses. A proportion of markers were consistently associated with heat stress across
304 multiple crosses; 2 - 17 markers identified in each cross were shared with one or more crosses,
305 and 2 - 5 of these were identified in all three (Figure 5). Altogether, 148 unique markers were
306 present in at least 2 crosses (Table 3). The same two markers were found in seven crosses, a
307 single marker overlapped in six crosses, and the same four markers overlapped in five crosses

308 (Table 3). However, most markers associated with heat tolerance in one cross were not
309 associated with heat tolerance in unrelated crosses. For example, cross DC shared no parents
310 with crosses EF or FA and 82-92% of the markers identified in each cross were not associated
311 with heat stress in the other two.

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

317

318 **Discussion**

319

320 *Genetic background affects heat tolerance*

321 Our survival model shows that heat tolerance is significantly affected by genetic
322 background. Survival probabilities varied significantly between crosses indicating that genetics
323 contributed to variation in heat tolerance. Coral parent E was associated with crosses that had
324 high survival probabilities and Coral parent C was associated with crosses that had lower
325 survival probabilities. We conclude that parental genetics contribute to heat tolerance of
326 offspring and consideration of parental genetics will be important in coral and conservation as
327 well as assisted gene flow and assisted migration.

328

329 *Heritability and modeling heat tolerance*

330 Quantifying the amount of genetic variance that contributes to a phenotype is important
331 for evaluating adaptive potential of a population (Falconer & Mackay, 1996). Our results add to
332 only three published narrow sense heritability estimates for heat tolerance in corals, all of which
333 find this trait is highly heritable (Dixon et al., 2015; Dziedzic et al., 2019; Kirk et al., 2018). Our
334 study is the only estimate of heritability for a sub-massive and relatively heat tolerant coral
335 species from the GBR. Our result for *P. daedalea* likewise indicates that heritability in larval
336 survival after heat stress was substantial ($h^2 = 0.66$) and suggests that the *P. daedalea*
337 populations have substantial standing genetic variation which contributes to heat tolerance and
338 that this population has an innate capacity to adapt to some level of ocean warming.
339 Heritability estimates from different populations can give us valuable information about
340 selection in a given geographic region. Heritability estimates are dependent on the environment
341 and the standing genetic variation in the population for which the estimate is made (Falconer &
342 Mackay, 1996). If there are low levels of genetic variation contributing to a trait and all parents
343 express the same phenotype, the heritability estimate will be lower (Falconer & Mackay, 1996).
344 A novel comparison is the contrast of the heritability estimate (0.48) found by Kirk and
345 colleagues for *P. daedalea* in the Persian Gulf (Kirk et al., 2018). The Persian Gulf *P. daedalea*
346 has lived in a very shallow and warm sea for thousands of years (Purkis & Riegl, 2012), which
347 may mean that a degree of selection for heat tolerance has already taken place resulting in less
348 genetic diversity in the population (Falconer & Mackay, 1996). The lower heritability value is
349 evidence to support this and the higher degree of heritability for heat tolerance in the central
350 GBR *P. daedalea* population suggests that until very recently, heat stress has not been the
351 predominant selective pressure on this population.

352 Responses to selection depend on genetic variation in the trait under selection (Falconer
353 & Mackay, 1996; Lynch & Walsh, 1998) and our study contributes to building the necessary
354 quantitative genetic data to model heat tolerance under various selection regimes. In its simplest
355 form, this can be expressed in the breeder's equation, which describes the response to selection
356 as the product of the selection differential (S) and heritability of the trait under selection. To our
357 knowledge, selection differentials have not been empirically estimated in corals, but considering
358 the widespread reductions in coral populations following coral bleaching (Bellwood et al., 2004;
359 Bruno & Selig, 2007; De'ath et al., 2012; Hoegh-Guldberg et al., 2007; Hughes et al., 2017,
360 2018) they are likely high for sensitive species. For truncation selection, where all individuals
361 with trait values above a threshold contribute offspring to the next generation, selection
362 differentials are equal to the difference in mean trait values between the selected population and
363 the original population (Falconer & Mackay, 1996; Lynch & Walsh, 1998) expressed as standard
364 deviations of the population mean. If the selection differential equals one standard deviation, all
365 parents with traits one standard deviation above the population mean contribute to the next
366 generation. Based on the distribution of cumulative survival probabilities in heat stress from our
367 experiment (0.57 ± 0.18) and the heritability of variation in heat tolerance we estimated from
368 these data (0.66), the Breeder's Equation predicts a response to selection of 0.12. We would
369 expect that if larvae of the next generation after selection were subjected to the same heat stress
370 treatment, the average mortality would be 0.48 (0.59 - 0.12), a 20% reduction in overall
371 mortality. If instead of the high h^2 estimated in our study (0.66), we modeled the responses to
372 selection using a low h^2 value of 0.1, we would predict only a 3% reduction in mortality in the
373 following generation. While the estimates above are underpinned by too many assumptions to
374 predict responses in natural populations of corals, the comparison highlights how empirical

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

398 small effect alleles can affect heat tolerance (Bay & Palumbi, 2014; Kirk et al., 2018). These
399 differences may also result in part from epistatic interactions where the effects of variation at
400 these loci depend on the genetic background. Few data on either frequencies of heat tolerance
401 alleles or epistatic effects are available for any coral species. However, the repeated observation
402 of some of these associations across different genetic backgrounds provides strong evidence that
403 these markers are linked to genetic factors contributing to variation in heat tolerance.

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

412

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

414 In our study, we found a subset of regions in coding genomic regions associated with heat
415 tolerance, five of which were observed in multiple crosses (Tables 3 and 4). These SNPS were
416 found in genes associated with the extra-cellular matrix and metabolism. There were also SNPs
417 in four unannotated transcripts. These SNP-containing genes found in this study are candidates
418 for further study of their role in this trait and could serve as heat tolerance markers.

419 Three SNPs were associated with coding genes that play roles in the extracellular matrix:
420 (1) GPI_Mannosyltransferase_2, which functions in the assembly of

421 glycosylphosphatidylinositol (GPI) anchors that attach proteins to cell membranes (Kang et al.
422 2005), (2) cad96ca, an E type cadherin, that initiates cell-cell adhesion (Lodish et al. 2013), and
423 (3) metalloendopeptidase, a metal-dependent protease that restructures the extracellular matrix
424 (Lodish et al. 2013) (Table 4). A SNP was also found in Serine/threonine-protein kinase (PAK3)
425 a member of cell regulatory pathways influencing regulation of cytoskeletal formation, cell
426 migration, and the cell cycle (Deleris et al. 2011). Three coral crosses had a SNP in the Krebs
427 cycle enzyme isocitrate dehydrogenase (IDH) (Garret & Grisham, 2013). This enzyme has been
428 identified in gene expression studies as important to the coral thermal stress response (Kenkel,
429 Meyer, et al., 2013; Polato et al., 2010). IDH participates in handling reactive oxygen species
430 and is one of the 44 proteins identified as the minimal stress proteome (Kultz, 2005). The SNP in
431 IDH associated with heat tolerance further strengthens its proposed role in the thermal stress
432 response in corals.

433 Many studies have pointed out the abundance of unannotated genes in the genomes of
434 early-diverging animals and the absence of any pathways to understand their function. The four
435 SNPs that we found which were unannotated in the transcriptome show overlap between crosses
436 highlighting the potential importance of understanding the role these unknown genes in thermal
437 tolerance. These four unannotated transcripts warrant further functional investigations, such as
438 full sequencing, domain analysis, and expression in functional assays. Knowing the function of
439 these genes will be important for having a complete picture of the alleles controlling thermal
440 tolerance.

441 Comparisons of populations of *P. daedalea* in more typical reef temperature conditions
442 (this study) and unusually warm conditions (Kirk et al., 2018) will be important to understanding
443 the genomic basis of heat tolerance. Two SNPs from the present study were also significantly

444 associated with heat tolerance in the Persian Gulf population and thus represent excellent
445 candidates for further study. This could include screens for natural abundance among thermal
446 habitats and potential validation for gene function through knock-down from CRISPR-Cas9
447 induced mutagenesis (Cleves et al., 2018). This unexpected lack of shared markers is further
448 evidence that many alleles of small effect contribute to heat tolerance and that they vary in their
449 distribution across populations.
450
451 *Conclusions*
452 Our findings demonstrate that heat tolerance is highly heritable (i.e., > 60%) in the coral
453 *Platygyra daedalea* from the GBR demonstrating high potential for adaptation. The heat stress
454 time course (Table 1) supports the conclusion that genetic variation explains a substantial
455 fraction of the variation in heat tolerance among our crosses. Our analysis identified hundreds of
456 markers associated with heat tolerance, including markers that were repeatedly associated with
457 heat tolerance across different genetic backgrounds. This supports the conclusion that heat
458 tolerance is a complex polygenic trait determined by many alleles of small affect that are likely
459 to be species and population specific. Despite this complexity, we found markers in multiple
460 crosses that may be important for heat tolerance in *P. daedalea*. These markers associated with
461 heat tolerance in our study of *P. daedalea* from typical coral reef conditions in the GBR will
462 provide a valuable comparison with future genomic studies of heat tolerant populations from the
463 Persian Gulf. The four unannotated transcripts found in this study warrant further functional
464 investigations to find their role in the animal and examine how they might function in heat
465 tolerance. Our findings build on the growing body of evidence that natural populations of corals
466 harbor substantial genetic variation in heat tolerance that can support adaptive responses to

selection and identify genetic markers for heat tolerance in a globally distributed coral species. These heat tolerance markers are essential to any type of reseeding or assisted gene flow and assisted migration programs, which may be necessary to maintaining reefs in the future as we work to reduce CO_2 emissions and mitigate the effects of climate change.

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Authors' contributions

E.M., H.E., and L.B. conceived the experiments. H.E., V.M., J.M., and L.B. conducted 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.

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686 **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

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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

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692 **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

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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)

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Figures

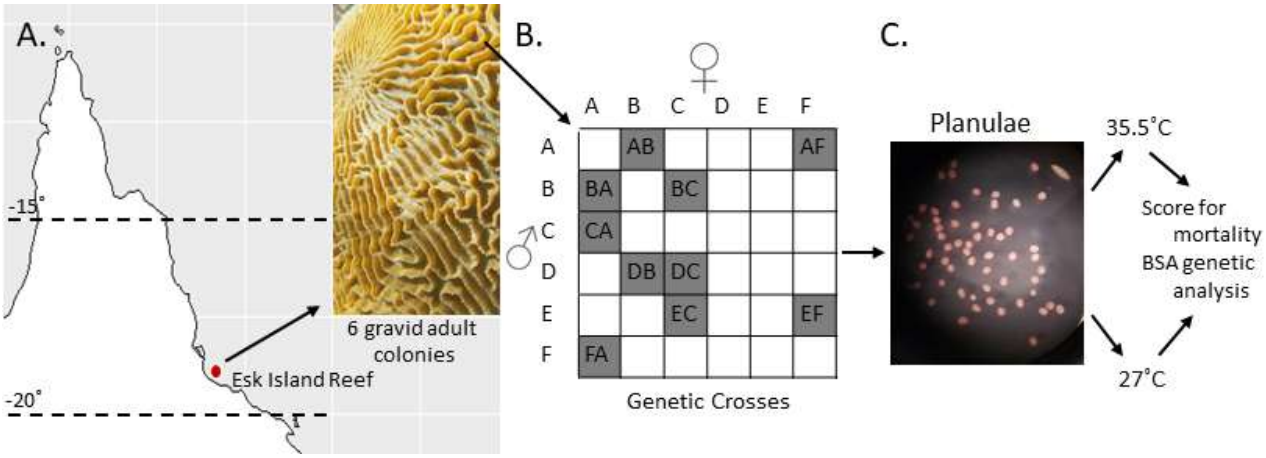

Fig. 1. Crossing design and heat stress experiments. (**A**) Six *Platygyra daedalea* colonies were sampled from Esk Reef on the GBR. (**B**) Crossing design used to produce ten crosses among those parental colonies. (**C**) Representative micrograph (10X) of planula larvae used for these experiments (53 hours post-fertilization).

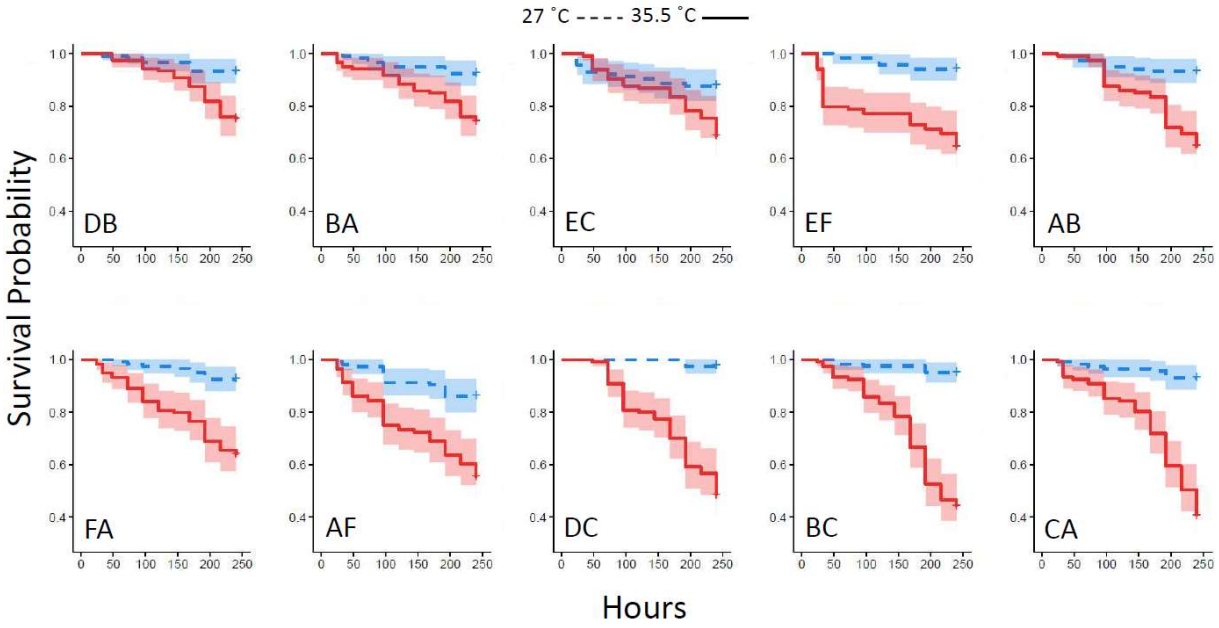

Fig. 3. Heat tolerance differs among crosses. Kaplan Meier plots of cumulative probability of survival for each family in control 27.5˚C (blue) and elevated 35.5˚C (red) temperature. Panels are arranged in order of heat tolerance, based on survival probability at 240 hr. Shaded regions represent 95%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

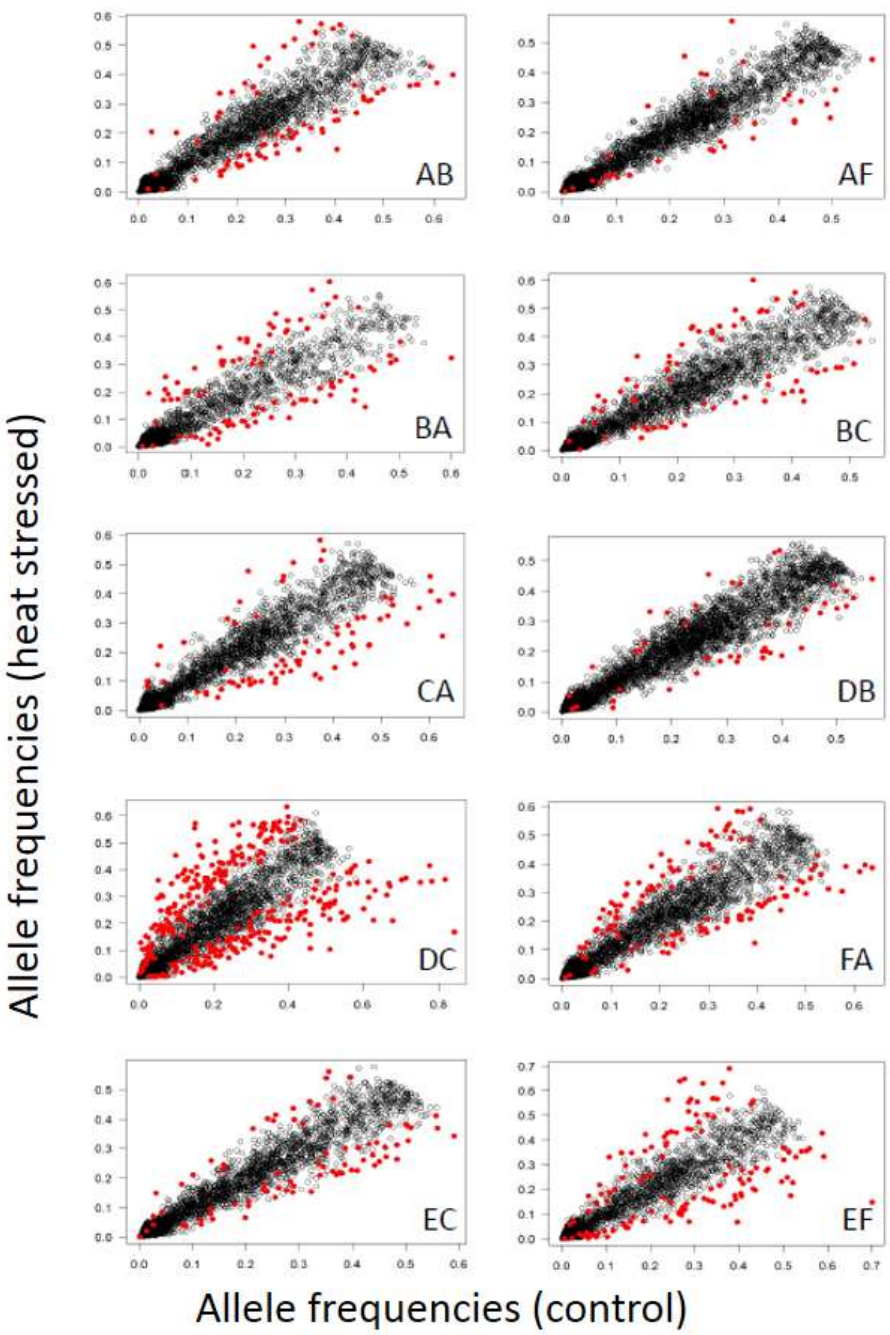

Fig. 4. Heat stress alters allele frequencies in surviving larvae. Panels show correlations between the average allele frequencies in survivors of replicate heat stress treatments relative to the corresponding controls. Markers with significantly altered allele frequencies (logistic regression, FDR 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

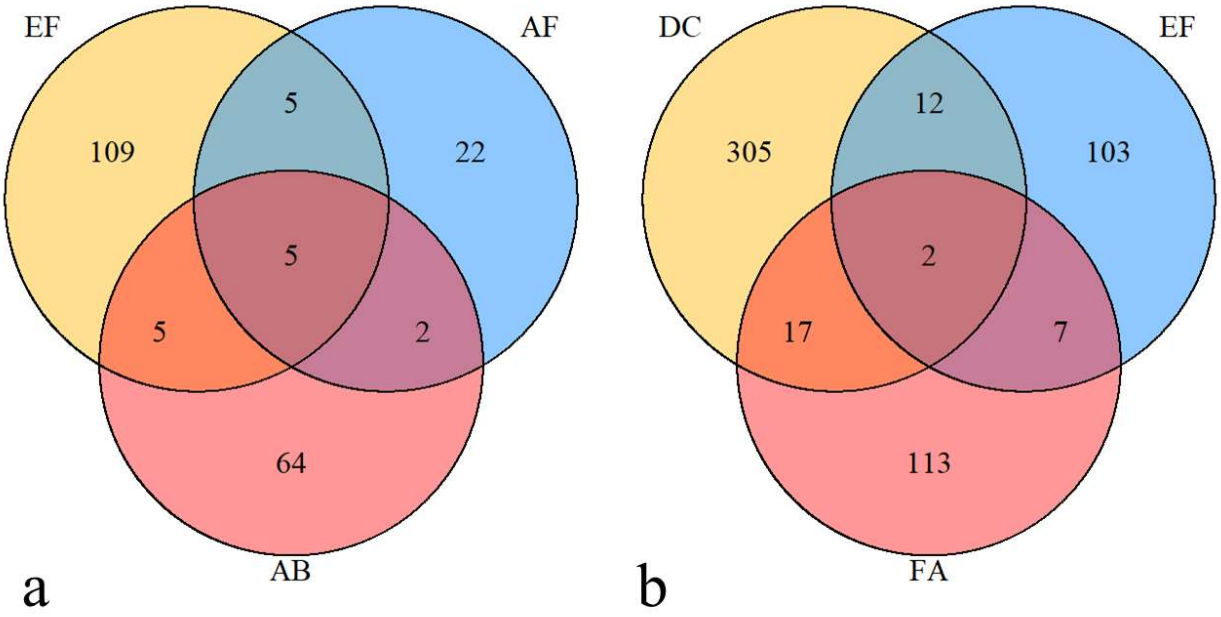

Fig. 5. Venn diagrams depicting the overlap of genetic markers in sets of three crosses

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.