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Genome-wide SNP analysis reveals an increase in adaptive genetic variation through selective breeding of coral

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1 **Genome-wide SNP analysis reveals an increase in adaptive genetic variation through**
2 **selective breeding of coral**

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4 Running Head:

5 Molecular basis of selective breeding

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17 **Keywords:** coral, bleaching, heat tolerance, selective breeding, SNPs, heritability

18

19 **Abstract**

20 Marine heat waves are increasing in magnitude, duration, and frequency as a result of climate
21 change and are the principal global driver of mortality in reef-building corals. Resilience-based
22 genetic management may increase coral heat tolerance, but it is unclear how temperature
23 responses are regulated at the genome level and thus how corals may adapt to warming
24 naturally or through selective breeding. Here we combine phenotypic, pedigree, and genomic
25 marker data from colonies sourced from a warm reef on the Great Barrier Reef reproductively

26 crossed with conspecific colonies from a cooler reef to produce combinations of warm
27 purebreds and warm-cool hybrid larvae and juveniles. Inter-population breeding created
28 significantly greater genetic diversity across the coral genome compared to breeding between
29 populations and maintained diversity in key regions associated with heat tolerance and fitness.
30 High-density genome-wide scans of single nucleotide polymorphisms (SNPs) identified alleles
31 significantly associated with larval families reared at 27.5°C (87 – 2,224 loci), including loci
32 putatively associated with proteins involved in responses to heat stress (cell membrane
33 formation, metabolism, and immune responses). Underlying genetics of these families
34 explained 43% of PCoA multilocus variation in survival, growth, and bleaching responses at
35 27.5°C and 31°C at the juvenile stage. Genetic marker contribution to total variation in fitness
36 traits (narrow-sense heritability) was high for survival but not for growth and bleaching in
37 juveniles, with heritability of these traits being higher at 31°C relative to 27.5°C. While based
38 on only a limited number of crosses, the mechanistic understanding presented here
39 demonstrates that allele frequencies are affected by one generation of selective breeding, key
40 information for the assessments of genetic intervention feasibility and modelling of reef
41 futures.

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44

45 **Introduction**

46 Coral reefs globally are undergoing significant degradation due to mass coral mortality driven
47 by high sea surface temperatures (Heron et al., 2017; Hughes et al., 2018). Increasingly warm
48 seawater temperatures and losses in coral cover are also leading to changes in fundamental
49 reproductive processes (Baums et al., 2019; Hughes et al., 2019; Shlesinger & Loya, 2019),
50 with major implications for ecosystem resilience and recovery in the aftermath of severe, acute

51 events such as mass coral bleaching. To safeguard the persistence and resilience of coral reefs,
52 assisted evolution interventions to increase the heat tolerance of corals at the early life-history
53 stages as well as in adults are being tested in the laboratory and the field (National Academies
54 of Sciences and Medicine, 2019; Reef Restoration and Adaptation Program: Intervention
55 Technical 2019). These interventions may also protect populations of high importance.
56 Assisted evolution interventions include selective breeding within species, hybridisation
57 between species, and microbial manipulations (Chakravarti, Beltran, & Oppen, 2017; Chan,
58 Peplow, Menéndez, Hoffmann, & van Oppen, 2019; Damjanovic, Blackall, Webster, & Oppen,
59 2017; Morgans, Hung, Bourne, & Quigley, 2019; Quigley, Bay, & van Oppen, 2019; van
60 Oppen, Oliver, Putnam, & Gates, 2015). The knowledge gained from experimentation of
61 genetic interventions will facilitate optimisation of breeding designs, reduce the incidences and
62 impacts of trait trade-offs and the help to determine the probabilities of success and timeframes
63 associated with genetic gains.

64
65 Improved forecasting of future coral reef health based on the scope and rates of recovery is
66 urgently needed. This will rely on estimating rates of acclimatisation and adaptation to predict
67 how populations will respond to a suite of selective pressures. The incorporation of the effects
68 of selection and adaptation to understand population evolutionary trajectories has been applied
69 to the conservation of species like Eltham's butterfly and bighorn sheep (Creech et al., 2017;
70 Roitman et al., 2017). Corals exist close to their thermal limits (reviewed in Drury, 2019) but
71 exhibit a wide range of phenotypes in response to heat stress (Ainsworth et al., 2015; Kenkel
72 et al., 2013), suggestive of a high adaptive capacity predicated on genetic diversity across
73 populations and species (Matz, Treml, Aglyamova, & Bay, 2018). There is evidence that corals
74 have increased their heat tolerance by $\sim 0.5^{\circ}\text{C}$ in the last decade, which suggests substantial
75 capacity to adapt to increasing temperatures (Sully, Burkepile, Donovan, Hodgson, & van

76 Woesik, 2019). Part of this heat tolerance can be attributed to the diversity and relative
77 abundance of dinoflagellate symbionts (family Symbiodiniaceae) that inhabit coral tissues
78 (Baird, Bhagooli, Ralph, & Takahashi, 2009; reviewed in Quigley, Baker, Coffroth, Willis, &
79 van Oppen, 2018). In particular, changes in symbiont community composition can attribute
80 1.0-1.5°C of increased tolerance to adults (Berkelmans & van Oppen, 2006) and 26x increased
81 tolerance in juvenile corals relative to warm-warm and cool-warm crosses (Quigley, Randall,
82 van Oppen, & Bay, 2020) and has been found to explain up to 24% of bleaching variability
83 (Mizerek, Baird, & Madin, 2018). The genomic architecture of heat tolerance is often polygenic
84 (Bay & Palumbi, 2015; Jin et al., 2016). Multiple candidate genes of varying effect sizes have
85 been examined, including heat shock proteins and genes involved in immunity (Louis,
86 Bhagooli, Kenkel, Baker, & Dyal, 2017), although some target genes may more broadly be
87 associated with stress tolerance generally and not heat tolerance specifically. Therefore, to
88 understand corals adaptive capacity, comprehensive knowledge is needed concerning the
89 variation in alleles at particular loci and the topology of polymorphic genomic regions. This
90 knowledge can then be used to understand their influence in driving the emergence of different
91 phenotypes that ultimately enable adaptation in populations over ecological and evolutionary
92 time frames.

93

94 Evolutionary genetics describes gene frequency changes across populations and over time. The
95 analysis of genetic diversity in wild and artificially-bred populations is central to understanding
96 the rates and potential of adaptation. Shallow and deep whole genome sequencing is now
97 widely available and relatively cost effective, including sequencing whole genome single-
98 nucleotide polymorphisms (SNPs) (Davey et al., 2011; Helyar et al., 2011) and are increasingly
99 illuminating the ecological and evolutionary mechanisms coral use to respond to their
100 environment (Dixon et al., 2015; Fuller et al., 2019; Palumbi, Barshis, Traylor-Knowles, &

101 Bay, 2014). SNPs are particularly valuable for identifying common variants underlying trait
102 variation and can be applied to genetic conservation practices through breeding programs that
103 utilize quantitative genetic principles, breeding values, and narrow and broad-sense heritability
104 (h^2 , H^2) (Visscher, Hill, & Wray, 2008). SNPs are intrinsically linked to the h^2 given the
105 proportionality to the product of SNP quantity and effect size (Holland et al., 2019). This
106 marker-assisted selection approach identifies markers ultimately underlying the heritability
107 estimates, although single alleles may explain only a small proportion of the measured heritable
108 variation (Manolio et al., 2009). Specifically, SNPs provide the marker data used as inputs for
109 relatedness models, that when combined with phenotypic data, are used to calculate additive
110 genetic effects and variances and finally, heritability and the potential for selection (O'hara,
111 Cano, Ovaskainen, Teplitsky, & Alho, 2008).

112

113 Here we apply high-density genome-wide marker sequencing to samples collected from
114 selectively bred reproductive crosses to elucidate how artificially produced corals may evolve
115 under heat stress. Selective breeding of corals from historically warmer reefs with others
116 sourced from cooler reefs and then exposing juveniles to temperature tolerant symbionts like
117 *Durusdinium trenchii* significantly improved coral fitness when exposed to 31°C (e.g.
118 increased survival, growth and bleaching tolerance (Quigley, Randall, van Oppen, & Bay,
119 2020)), even upon inspection of a limited number of crosses produced from only five parents.
120 Here we link those traits measured in the five reproductive crosses to their underlying allele
121 frequency changes and genomic contribution (h^2) and identify the putative causative variants
122 separating the five families to explain and potentially link these heritability estimates at both
123 ambient and elevated temperatures to explain the genetic variation in these three quantitative
124 traits.

125

126 **Materials and Methods**

127 **Spawning, larval rearing, settlement and symbiont infection**

128 Gravid adult corals of the species *Acropora spathulata* were collected from one reef in the far
129 north of the Great Barrier Reef (Tijou, far northern GBR; 13°10'44.0"S, 143°56'54.6"E, permit
130 G16/38488.1) and one reef in the central GBR (Backnumbers, central GBR; 18°30'49.8"S,
131 147°09'10.7"E, permit G12/35236.1) and brought back to the National Sea Simulator (SeaSim)
132 for coral spawning in November 2017 as outlined in (Quigley et al., 2020). Briefly, on the night
133 of spawning the separated eggs and sperm from three far northern colonies were mixed with
134 those of three central colonies, resulting in larvae from 30 distinct familial crosses that were
135 raised in 27.5°C ultrafiltered, flow-through seawater (see Table 1 for pedigree and parental
136 colony designations). Only five families were available at the time of larval settlement and
137 used to produce recruits (hereafter referred to as juveniles given measurements were taken after
138 70 days of development). Here we focus on those five familial crosses sequenced at both the
139 larval stage (27.5°C) and the juvenile stage (27.5°C and 31°C). These crosses include three
140 produced from two parents sourced from the warm far northern reef (WW1, WW2, WW3), one
141 cross with a warm dam and cool sire (WC) and one with a cool dam and warm sire (CW).
142 Aposymbiotic juveniles were infected with *Symbiodinium tridacnidorum*, *Cladocopium*
143 *goreaui*, and *Durusdinium trenchii* with cultured material obtained from the AIMS Symbiont
144 Culture Facility (further details in Quigley et al., 2020). Full details of the collection,
145 environmental parameters of each location, permits, spawning, larval and juvenile rearing, and
146 symbiosis establishment can be found in Quigley et al., 2020. Briefly, symbiosis establishment
147 in coral juveniles was performed by adding 1×10^5 ml⁻¹ of one of the three cultured symbiont
148 strains separately to each juvenile tank (n = 3 replicate tanks per culture), suspending the flow
149 to allow for contact between corals and symbionts, and repeating one further time until
150 infection was confirmed with microscopy.

151

152 **Larval sampling and trait measurements in juveniles**

153 *Acropora spathulata* larvae were sampled into 100% ethanol after rearing at 27.5°C ~2.5 days
154 after fertilization. DNA from tissue samples were extracted using a modified SDS protocol
155 previously used for sequencing of single coral eggs (Quigley, Willis, & Bay, 2017). Juvenile
156 growth (percent change in area), bleaching (percent change in coloration), and survival (alive
157 or dead) were quantified after 70 days of experimental exposure to 27.5°C and 31°C, and a
158 detailed assessment of this physiological data can be found in (Quigley et al., 2020).

159

160 **Sampling, DNA extraction, library preparation, sequencing**

161 Individual larvae were genotyped (total n = 68) across five families representing three
162 population crosses (n = WW: 34, WC: 15, CW: 19). All samples were sequenced on the
163 Illumina HiSeq2500 and genotyped using proprietary DArT-seqTM technology at Diversity
164 Arrays Technology (Canberra, Australia). Digestion and ligation reactions were carried out
165 with the PstI and HpaII restriction enzymes following (Jaccoud, Peng, Feinstein, & Kilian,
166 2001; Kilian et al., 2012). DNA purification and library preparation followed DArT proprietary
167 methods (Kilian et al., 2012). As part of the DArT pipeline, raw sequence data was filtered
168 based on Q-scores and filtered against viral and bacterial databases to remove contamination.
169 The restriction site fragments generated were used to call single nucleotide polymorphisms
170 (SNPs) with the KDCompute pipeline and DArTsoft14 algorithm (Diversity Arrays
171 Technology, <http://www.kddart.org/kdcompute.html>). SNPs were referenced against the
172 *Acropora digitifera* genome (Shinzato et al., 2011) and further quality controlled and filtered
173 to produce a reduced dataset of only high quality loci using both proprietary DART software
174 and the following criteria, which included filtering loci for average repeatability of alleles at
175 the locus ('filter.repavg', >99%), collapsing duplicated sequences ('keep monomorphs'),

176 individuals with missing data ('filter.callrate', >25%), filtering by Minor Allele Frequencies (<
177 0.02), coverage (< 8 read depth), and filtering loci out of Hardy-Weinberg Equilibrium.

178

179 **Statistical Analyses**

180 **Multivariate statistics**

181 SNP metrics were recalculated in R for post-filtered data using the 'DArTR package' (Gruber,
182 Unmack, Berry, & Georges, 2018). PCoAs were constructed from individual larval SNP
183 genotypes with Gower PCoA ordination and Euclidean distances using the packages
184 'Adegenet' and 'DArTR' (Gruber et al., 2018; Jombart, 2008). Clusters of genetically related
185 individuals were inferred using Discriminant Analysis of Principal Components (Jombart,
186 Devillard, & Balloux, 2010) constructed with and without the between- and within- group
187 variance components from PCoA outputs. Multiple loading thresholds were explored,
188 including the default value of the third quartile (75%) of x-values (Grünwald, Kamvar, &
189 Everhart, Jombart & Collins, 2015). Diversity metrics were calculated using 'DArTR'
190 following (Sherwin, Chao, Jost, & Smouse, 2017). These analyses are similar to classic
191 population genetic analyses that measure differentiation via F_{st} , in which high allele frequency
192 differentiation suggests population differentiation although they can be fraught with false
193 positives (Rajora, 2019).

194 Given the influence of close relationships in STRUCTURE analyses (Anderson & Dunham,
195 2008), DAPC was used as an alternative method to infer admixture (Grünwald et al., 2010).
196 Hence, "true k " was calculated based on Bayesian Information Criteria (BIC, Jombart et al.,
197 2010), in which the largest drop in BIC was at $k = 5$.

198

199 **Single Nucleotide Polymorphism annotation**

200 SNPs significantly contributing to the PCoA separation between each family cross were
201 identified, translated to nucleotide sequences by searching and aligning sequences to the
202 *Acropora digitifera* genome (v. OIST_v1.1) using custom scripts. All nucleotide matches were
203 downloaded and re-formatted into a searchable BLAST database using the “blastdbcmd”
204 command. Nucleotide matches were blasted to annotated proteins (OIST v.0.9, see reference
205 for full instructions on annotations). Predicted proteins were filtered for only protein matches
206 with E-values $<1e-4$ were retained ([https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastx&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome)
207 [blastx&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastx&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome)).

208

209 **Modelling SNP frequency distributions**

210 The distributions of expected Hardy-Weinberg (HW) equilibrium genotype frequencies were
211 simulated using a custom R script (Chivers, 2011) for three population sizes of the SNP profiles
212 from single larval samples (number of larvae in each population, $n = 34, 19, 15$). A null
213 distribution of allele frequencies in a population that is not evolving follows HW proportions.
214 SNP profiles were constructed from single larval samples for all families as well as
215 bioinformatically pooled by geographic source of parental corals (WW, WC, CW). Parameters
216 included were number of parents, total population sizes, number of genotypes, and number of
217 generations to simulate in randomly mated populations. Expected HW genotype frequencies
218 were statistically compared to the experimentally observed genotype frequencies from the
219 families and a multidimensional, distribution-free Kolmogorov-Smirnov Two-Sample test was
220 used to assess statistical significance of differences between paired continuous two-
221 dimensional distributions using the package ‘Peacock.test’ (Fasano & Franceschini, 1987).

222

223 **Narrow-sense heritability**

224 Physiological data on survival, bleaching, and growth (Quigley et al., 2020) were combined
225 with pedigree information (Supplementary Table 1) to calculate narrow-sense heritability
226 estimates for each trait at 27.5°C and 31°C. Relatedness between individual juveniles (sibships
227 and family groups) are calculated from the pedigree input as a random effect. Narrow-sense
228 heritability estimates (h^2) were derived from additive genetic variance calculated from the
229 ‘animal model’ (Kruuk, 2004) using the package ‘MCMCglmm’ (Hadfield, 2010), in which
230 the coefficient of relatedness between individual juveniles is parameterized as a random effect.
231 The ‘animal model’ is a type of specialized mixed-effect model that calculates narrow-sense
232 heritability by parameterizing relatedness between measured individuals using random effects
233 (Kruuk, 2004). Hence, this analysis calculates the relative contribution of genetic and
234 environmental influence on phenotypic variance of each trait (Wilson et al., 2010). This
235 analysis builds upon that performed in Quigley et al., 2020 by: 1) using the underlying
236 physiological data and 2) extending the mixed effects models used in that study that
237 parameterized the influence of population of origin (WW, WC, CW) and parental cross (WW1,
238 WW2, WW3, WC, CW) on survival, growth, and bleaching. Three Symbiodiniaceae strains
239 were used for inoculation of juveniles. For clarity, heritability estimates for only *Cladocopium*,
240 the punitively principal symbiont of *Acropora spathulata* on the GBR (*sensu* van Oppen,
241 Palstra, Piquet, & Miller, 2001), is presented in the main results and the additional strains in
242 the Supplementary Information.

243 Models of h^2 of juvenile survival at both temperatures were run with time as a fixed factor,
244 pedigree and individual as random factors, using binary logistic regression (categorical
245 distribution), 150×10^6 iterations, 0.1% burn-in of total iterations and at multiple thinning
246 levels of 7500 (27.5°C: *Symbiodinium tridacnidorum*, *Cladocopium goreau*, and *Durusdinium*
247 *trenchii*, 31°C: *C. goreau* and *D. trenchii*) or 37,500 (31°C: *S. tridacnidorum*). Models of h^2
248 of juvenile bleaching and growth at both temperatures were run using 1.5×10^6 iterations, a

249 thinning level of 1,500 and a burn-in of 10% of the total iterations. Fixed and random factors
250 were parameterized in the survival models with a Gaussian distribution.

251 A non-informative flat prior specification was used, following an inverse gamma distribution
252 (Wilson et al., 2010). Assumptions of chain mixing, normality of posterior distributions and
253 autocorrelation were met. The posterior heritability was calculated by dividing the model
254 variance attributed to relatedness by the sum of additive and residual variance. Given
255 differences in iterations and thinning levels across models, a scaling factor ($x' = x - x_{\min}/x_{\max}$
256 $- x_{\min}$) was applied to the posterior distributions of each trait using the “rescale” function in the
257 ‘plotrix’ package (Lemon, 2006) to visualize each heritability estimate equally to ease
258 interpretation. Specifically, each posterior was re-scaled to the minimum ($y = 0.00598$) and
259 maximum ($y = 57.42421$) y-density values for the *S. tridacnidorum* treatment at 31°C.

260 **Results**

261

262 **Interpopulation differentiation**

263 After filtering, 9,031 high quality SNPs remained from the larval samples collected at ambient
264 temperature across 68 individually genotyped larvae from the five families ($n = 7 - 20$ for
265 each). The five families separated in multidimensional space, in which genetic variation
266 explained 43% of the variation between multilocus genotypes from the five families (PC1-PC3:
267 25.8; 13.4; 4.1%) (Figure 1A). Families that shared the same sire (Tijou 2A) roughly clustered
268 together in Principle Components Analysis (PCoA) space (WW1 and CW with WW2), whereas
269 the other two families were more divergent.

270

271 Analysis of population structure using Discriminant Analysis of Principle Components
272 (DAPC) further confirmed this pattern of clustering between WW1 and CW along the first two

273 discriminant functions. The 95% confidence ellipses demonstrate that the dispersion of
274 multilocus genotypes also varied across the families, where WW2 shows the greatest and WW1
275 shows the least variance. Cluster membership probabilities calculated from DAPC analysis also
276 showed flat distributions in between-group and within-group allele variances, demonstrating
277 that admixture has occurred, especially between WW3, WC, WW1, and to a lesser extent with
278 WW2, and particularly CW.

279

280 **Allele frequency profiles**

281 The majority of loci were fixed at either 0 or 1. The average distribution of loci in population
282 purebred crosses was significantly different compared to interpopulation crosses, with a greater
283 abundance of loci at intermediate frequencies in the interpopulational families (compared to
284 null distributions; Kolmogorov-Smirnov Two-Sample test, $p = 2.1e-26$ and $1.3e-21$, Figure 2).
285 This suggests that these interpopulation hybrids have significantly greater genetic diversity
286 than within population purebreds. When individual purebred families were analysed, the
287 majority of the on average reduced diversity (e.g. lower abundance of loci at intermediate
288 frequencies) was attributed to crosses WW1 and WW2, whereas WW3 demonstrated a
289 similarly greater abundance of loci at intermediate frequencies as the interpopulational crosses
290 (Supplementary Figure 1). WW1 and WW2 exhibited significantly different distributions
291 compared to the distribution of WC ($p = 5.5e-15$ and $2.4e-4$, respectively) and CW ($p = 1.2e-$
292 11 and $6.7e-4$, respectively) (Figure 2). In contrast, the distribution of loci in the WW3 cross
293 was not significantly different compared to WC ($p = 8.7e-2$) or CW ($p = 0.77$). All families
294 differed significantly from expected, modelled HW frequencies when modelled again
295 populations of the same size ($p = WW$ and WC: $1.03e-45$; CW: $2.2e-46$). Observed
296 heterozygosity (H_o), and alpha Diversity (${}^0D_\alpha$ and ${}^2D_\alpha$ at $q = 0$ and 2) was highest in the
297 interpopulation crosses (WC, CW) compared to the purebreds, with the exception of WW2,

298 which was also high (Table 1). Alpha Entropy at $q = 1$ was the highest in WC, WW2 and lowest
299 in WW3.

300

301 **QTL analysis**

302 SNPs were correlated with the five families reared under ambient temperature. The number of
303 SNPs associated with these five families varied depending on threshold cut-offs ranging from
304 2,224 SNPs (75% quartile) to 87 SNPs at the stricter threshold value (99% quartile). Twenty-
305 six of the 87 SNPs (30%) could not be assigned annotations within the *A. digitifera* genome, a
306 common issue in coral and symbiont 'omics (Figure 3 inset, *sensu* (Barshis et al., 2013; Peng
307 et al., 2010; Shinzato et al., 2011; Sogin, Putnam, Anderson, & Gates, 2016)).

308

309 In the 87 SNPs that contributed to the separation of crosses at ambient temperatures, we
310 identified proteins involved in a range of biological processes, mainly immunity and stress, cell
311 functioning and metabolism, and calcification (Figure 3 inset). Stress related proteins included
312 lysosomal-trafficking regulator-like proteins, CEPU-1-like protein, E3 ubiquitin-protein ligase
313 RNF213-like, spondin, NFX1-type zinc finger-containing protein, NACHT,
314 MAP/microtubule affinity and other processes, NLRC3-like proteins. Proteins involved with
315 collagen production varied across families, as well as 2 dTDP-glucose 4,6-dehydratase-like
316 and two sodium bicarbonate transporter-like proteins. A host of kinase related proteins,
317 including those involved in cell functioning and transcription were detected.

318

319 PCoA loadings defined cut-offs for significant SNPs and hence assigned proteins.
320 Homeodomain-interacting proteins, NFX1-type zinc finger-containing proteins and src
321 proteins (Figure 3A), which are broadly associated with transcription, immunity and stress, and
322 cell functioning were broadly positively related to the separation of families WW2/WW1/CW

323 and WW3 and WC along PCoA axis 1 (Figure 1A). Alternatively, differences in cell
324 functioning proteins were more negatively associated with their separation. Proteins
325 contributing to the separation of WW2/WW3, WW1 and CW, and WC along PCoA2 were
326 associated with responses to UV radiation, and MAP proteins (associated with cell functioning
327 and immunity/stress, respectively) whereas multiple proteins associated with immunity and
328 transcription were negatively associated with their separation.

329

330 **Narrow-sense heritability**

331 Population of origin (WW, WC, CW), family cross identity (WW1, WW2, WW3, WC, CW)
332 and symbiont type significantly influenced juvenile responses to growth, bleaching and
333 survivorship at 27.5°C and 31°C (full summary in Quigley et al., 2020). Specifically, juveniles
334 with at least one parental coral sourced from the northern GBR survived significantly better,
335 grew more, and bleached less at 31°C compared to other crosses, especially if infected with the
336 Symbiodiniaceae symbiont, *D. trenchii*.

337

338 Physiological data for juvenile survival, growth, and bleaching were previously presented in
339 Quigley et al., 2020. These raw data were used to calculate Bayesian narrow-sense heritability
340 (h^2) estimates for survival, bleaching, and growth demonstrate that only survival of corals
341 infected with *C. goreau* was strongly affected by temperature (Figure 4, Supplementary Table
342 1). Bleaching responses were generally associated with low heritability estimates at both
343 temperatures, although juveniles associated with *C. goreau* exhibited more moderate
344 heritability estimates. Heritable genotypic variation across families contributed little to
345 differences in growth rates at 27.5 and 31°C (Table 2). With the other three Symbiodiniaceae
346 taxa, h^2 estimates across the three traits varied in juveniles from the five families infected at
347 27.5 and 31°C (Supplementary Figure 1, Supplementary Table 1).

348

349 **Discussion**

350 **Selective breeding produces increased occurrence of alleles with intermediate frequencies**
351 **on which natural selection can act**

352

353 Selective breeding is one genetic intervention strategy that may quickly increase adaptive
354 genetic variation in corals, as such facilitating adaptation to increasing sea surface temperatures
355 (Chan, Hoffmann, & van Oppen, 2019; van Oppen et al., 2015). This approach has been used
356 across a range of aquaculture and mariculture species to improve a number of commercially
357 important traits. Commercially important species like oysters and mussels share similar life-
358 history attributes with corals, including the production of numerous, potentially low-quality
359 gametes, and high levels of genetic diversity (e.g. R-strategist) (Ellegren & Galtier, 2016). In
360 those systems, only a few generations of selective breeding has resulted in 30% higher growth
361 under elevated pCO₂ conditions compared to wild populations (Parker et al., 2012) and 50%
362 higher growth in redclaw crayfish (Stevenson, Jerry, & Owens, 2013) without significantly
363 eroding genetic diversity compared to wild populations after pooling breeding combinations
364 (O'Connor, Dove, & Knibb, 2016). Selective breeding over only one generation in corals has
365 shown that significant increases in heat tolerance of larvae (Dixon et al., 2015) and juveniles
366 (Quigley et al., 2020) are possible using these methods. Lessons learnt here may therefore
367 provide insights into the mechanisms underpinning the success of these techniques in a wild
368 marine context.

369

370 We found that alleles at intermediate frequencies were at greater abundances than expected
371 under HWE in WC and CW crosses relative to the on average distribution of loci in WW
372 crosses, suggesting an overall increase in genetic diversity resulting from selective breeding of

373 corals from different regions of the GBR. Distributions of allele frequencies typically follow
374 that most loci nearly reach fixation at either 0 or 1, where alleles at intermediate frequencies
375 are less common but are important given an increased abundance of intermediate alleles are
376 the raw material for selection (Jombart & Collins, 2015). Hence, genetic material on which
377 selective adaptive processes can operate likely exists at greater abundances in the WC and CW
378 relative to the WW crosses. Hybridisation may lead to increased performance/fitness relative
379 to the parental generation, with this increased performance having been linked to dominant,
380 regulatory, single quantitative trait nucleotides (Jakobson & Jarosz, 2019). Increases in genetic
381 diversity, hybrid vigour and genetic rescue are well-known but inconsistent features of intra-
382 and inter-specific hybridization (Chan, Hoffmann & van Oppen, 2019; Flowers et al., 2019;
383 Hazzouri et al., 2019; Weeks et al., 2011) but has not yet been demonstrated in the selective
384 breeding of corals. For example, it was unknown whether the breeding of divergent populations
385 would cause a decrease in genetic diversity due to processes like genome incompatibility
386 (Hogenboom, 1975). These results support that even in a small number of crosses and
387 contributing parents, genetic diversity improves. Observed heterozygosity and alpha Diversity
388 (${}^0D_\alpha$ and ${}^2D_\alpha$ at $q = 0$ and 2) was highest in the interpopulation crosses (WC, CW). The ${}^0D_\alpha$
389 metric is sensitive to the presence of rare alleles, which is important given breeding across
390 populations may initially introduce novel mutants (Sherwin et al., 2017), suggesting that WC,
391 CW, and WW2 had an increased occurrence of rare variants. ${}^1H_\alpha$, also known as the Shannon
392 Information criteria, is informative as a natural measure of evolvability, and was highest in WC
393 and WW2. Finally, preliminary analysis of adult colonies shows Tijou corals had on average
394 higher observed heterozygosity compared to Backnumbers corals ($H_o = 0.065$ vs. 0.0217 ,
395 unpublished data), suggesting that there could have been a risk of reduced diversity in
396 offspring. Importantly, we did not observe a loss of genetic diversity by crossing these two
397 divergent populations.

398

399 Finally, we found that interpopulation selective breeding significantly changed the resulting
400 allele frequencies of offspring compared to modelled HWE distributions. This is perhaps
401 unsurprising given assumptions of random mating, no gene flow, and infinite population sizes
402 were not met. However, this suggests the influence of breeding on the genetic architecture of
403 the resulting F1 generation. Therefore, the interbreeding of even a small number of corals from
404 different reefs across the GBR may result in extensive introgression and therefore accelerate
405 the potential for adaptation to warming, although the production of F2 generations would be
406 needed to confirm this.

407

408 We also demonstrate that variants associated with immune responses, growth and cellular
409 operating may be re-arranged during breeding but are maintained within the next generation.
410 This suggests that the important functional diversity (i.e., at stress tolerance genes) associated
411 with focal populations can be maintained in the breeding process. In response to heat stress,
412 corals alter their gene and protein expression patterns, as reflected in changes in their structural
413 lipids, metabolism, and immune responses (Barshis et al., 2013; Sogin et al., 2016). Differences
414 in proteins associated with collagen production and sodium bicarbonate transport were
415 important in differentiating the five families produced in our study. Collagen is important for
416 the production of the extracellular matrix, required for multicellularity and the spatial
417 organization of functional units of cells (Helman et al., 2008) whereas bicarbonate transporters
418 are pivotal for coral calcification and hence growth (Zoccola et al., 2015). Basic cellular
419 functioning also potentially varied through the differences in dTDP-glucose 4,6-dehydratase-
420 like proteins detected and their involvement in the non-oxidative pentose phosphate pathway
421 (Buerger, Wood-Charlson, Weynberg, Willis, & van Oppen, 2016; Yuyama, Watanabe, &
422 Takei, 2011), critical for glucose utilization. Differences between crosses in these foundational

423 processes like cellular organization and biomineralization therefore suggests that even breeding
424 across relatively few individuals has the potential to substantially create distinct genetic
425 combinations.

426

427 Protein NLRC3-like, which has been previously implicated in acroporid immune suppression
428 in response to heat stress by acting in Toll-like receptor modification (Zhou et al., 2017), also
429 varied significantly across families. Other proteins involved in immunity and stress were also
430 detected (lysosomal-trafficking regulator-like proteins), and these have also been linked to the
431 mounting of innate immune responses through Toll-like receptor activity in mice (Westphal et
432 al., 2017). Additional immunity related proteins were detected, including CEPU-1-like protein
433 (Spaltmann & Brummendorf, 1996), E3 ubiquitin-protein ligase RNF213-like (Iguchi et al.,
434 2019), NACHT (Hamada et al., 2013), and spondin (Palmer & Traylor-Knowles, 2012).
435 Interestingly, NFX1-type zinc finger-containing protein was found here and downregulated in
436 resistant corals exposed to disease (Polato et al., 2010). Expression of nascent polypeptide-
437 associated complex subunit alpha protein (Bellantuono, Hoegh-Guldberg, & Rodriguez-
438 Lanetty, 2012) was similarly downregulated in resistant corals, suggesting that these proteins
439 provide an important role in protective immune responses.

440 Genotyping individual aposymbiotic larva from the five families reared under ambient
441 conditions (27.5°C) provides foundational knowledge as to how selective breeding influences
442 underlying genetic architecture. It also sheds light on the underlying molecular origins and
443 mechanisms of heritability, a long-standing goal of quantitative genetics (Jakobson & Jarosz,
444 2019). Broad and narrow-sense heritability has been quantified for a range of traits, but the
445 underlying mechanisms have rarely been described (Dixon et al., 2015; Dzedzic, Elder, &
446 Meyer, 2017). The majority of alleles fixed at the extremes of allele frequency distributions
447 (“U-shaped” *sensu* Hill, Goddard, & Visscher, 2008) are likely driven by the small number of

448 parents from which the larvae in each cross were derived (5 unique parental colonies used
449 across the families, Supplementary Table 1), but may also suggest selection against
450 heterozygotes during the aquarium rearing period. Interpopulation hybrids displayed greater
451 genetic diversity relative to within population purebreds, which may be the result of the varying
452 effects of selection on purebreds versus hybrids in the aquarium environment. U-shaped
453 distributions may arise under strong cases of artificial selection (e.g. aquariums) combined with
454 rare mutations (Hill et al., 2008).

455

456 **Functional variation associated with selectively bred families**

457 How does genomic variation lead to phenotypic differences between corals? The location and
458 effect size of the SNP difference are important to determining its eventual phenotypic effect,
459 and simplistically, differences in coding vs. non-coding regions are predicted to cause
460 phenotypic effects through a variety of mechanisms (Cavallo & Martin, 2005). Assigning
461 function to SNP differences is challenging given the majority of SNPs detected by association
462 studies are non-coding (Nishizaki & Boyle, 2017), whereas the majority of key changes are
463 coding (Cavallo & Martin, 2005), setting up a situation of difficult detection and classification.
464 Analysis of population structure using DAPC links the genomic patterns seen in the multilocus
465 genotypes with the underlying biological processes quantified in the heritability models. Using
466 this approach, we identified alleles contributing to the separation of these selectively bred
467 families, revealing that breeding of the selected populations targets changes to the immunity
468 and stress responses and growth, likely important processes in survival generally. Assigning
469 differences in SNPs to phenotypic differences between individuals will be key to understanding
470 and increasing thermal tolerance for intervention methods.

471

472 **Selective breeding influences the level of admixture and population discontinuity**

473

474 Admixture events affect members of species, populations and individuals differently (Lawson,
475 Van Dorp, & Falush, 2018). We saw this in the extent of admixture and its associated variance
476 across the five families, in which some families exhibited very little admixture relative to others
477 (especially CW). Although this can be somewhat dependent on the k structure of the model
478 used, both patterns were explored independently using two techniques (DAPC and PCoA) and
479 in conjunction with AIC, confirmed statistically the likelihood of population differentiation.
480 Therefore, this may suggest that the shared ancestry of the colony sourced from Backnumbers2
481 may be limited between the other Backnumbers and Tijou corals or whose origins were from
482 few or divergent founders (Lawson et al., 2018). This would suggest that adult colony
483 Backnumbers2 is not highly related to other Backnumbers or Tijou colonies. Furthermore,
484 PCoA and DAPC both demonstrated that the five families separate out in multidimensional
485 space given the magnitude of allelic covariance between individuals. Irrespective of population
486 labels, DAPC analysis also recapitulated the number of selectively bred families produced,
487 although interestingly, the purebred families were not assigned to single population clusters
488 but instead retained hybrid clustering structure in which the proportion of ancestry was shared
489 between multiple two to three clusters simultaneously. The discontinuity between populations
490 was also surprising, as demonstrated by the reduced spread of individuals between clusters,
491 especially in WW1, suggestive that selective breeding produces discrete differences in the
492 underlying genomic architecture of F1 individuals, even in populations likely undergoing some
493 underlying level of gene flow (Lukoschek, Riginos, & van Oppen, 2016).

494

495 **Heritability**

496 Heritability estimates provide information as to the extent to which phenotypic trait variation
497 has a genetic basis and the potential for adaptive change in these traits (Visscher et al., 2008).

498 SNPs are intrinsically linked to narrow-sense heritability given h^2 is proportional to the product
499 of the number of SNPs and their effect size (Holland et al., 2019). We found a high level of
500 variation in survival, bleaching, and growth attributed to narrow-sense heritability (h^2) when
501 pooled at the level of population cross, which varied in juveniles infected with *C. goreau*
502 across the two temperatures tested here (see Supplementary Information for further details on
503 *S. tridacnidorum* and *D. trenchii*).

504

505 Interestingly, additive genetic variance was only important in influencing survival at 31°C
506 when infected with *C. goreau* but not at 27.5°C. Hence, it is likely that there is significant
507 selection on survival at high temperatures for *C. goreau*, consistent with the generally overall
508 poorer performance of these symbionts at both temperatures (*S. tridacnidorum*) or at higher
509 temperatures in coral juveniles (*C. goreau*) (see Table 6.1 in Quigley, Baker, Coffroth, Willis,
510 & van Oppen, 2018). The shapes of the posterior heritability distributions are informative for
511 generating hypotheses concerning the underlying genomic architecture associated with
512 selective breeding. Bimodal heritability estimates can be driven by single large effect eQTLs
513 (as seen generally for traits with high heritability), whereas multiple loci of small effect size
514 generally result in unimodal distributions (Rudra et al., 2017). The influence of *cis* and *trans*
515 co-regulation of the traits may also influence the underlying distribution (Yang et al., 2014).
516 Bimodal heritability (often with wide credibility intervals) estimates can also be driven by high
517 underlying within-population variability even with large sample sizes (mirrored in the high
518 phenotypic variability between genotypes, Quigley et al., 2020), and have been recorded across
519 a range of disease-related expression of genes (Yang et al., 2014). High heritability at ambient
520 temperatures may also be common (Kronenberg et al., 2019), and potentially indicative of core
521 traits under continual selective pressures. Finally, it is likely that not all the SNPs associated
522 with these three phenotypes have been captured although narrow-sense heritability should

523 incorporate additively all the common SNPs associated (Holland et al., 2019) and it will be
524 important to use these methods under a variety of experimental settings to fully develop models
525 of adaptive selection.

526

527 **Conclusion**

528 The identification of potential allele frequency differences among familial crosses can link
529 specific genotypes to important fitness traits. Although only a small number of crosses are
530 presented in this pilot study produced from the mixing of five adult colonies, our findings
531 suggest that selective breeding has measurable effects on the genomic diversity of the resulting
532 offspring. This was apparent in the significant differences in genetic variants and phenotypes
533 associated with stress tolerance when exposed to varying temperatures. These results are in line
534 with studies demonstrating the influence of recent immigrants having significant influence on
535 allele frequency shifts.

536

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548

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550

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808 **Data Accessibility**

809 DART SNP sequencing data (larval) and physiology (juvenile) data is available through
 810 [https://github.com/LaserKate/Genome-wide-SNP-analysis-reveals-an-increase-in-adaptive-](https://github.com/LaserKate/Genome-wide-SNP-analysis-reveals-an-increase-in-adaptive-genetic-variation-through-selective-breedin.git)
 811 [genetic-variation-through-selective-breedin.git](https://github.com/LaserKate/Genome-wide-SNP-analysis-reveals-an-increase-in-adaptive-genetic-variation-through-selective-breedin.git)

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813 **Author contributions**

814 KMQ, LB, MvO designed the research; KMQ performed the lab work, analysed the data,
 815 wrote the manuscript; All authors contributed reagents and critically reviewed the
 816 manuscript.

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818 **Conflict of Interest Statement**

819 The authors declare no competing or financial interests.

820

821 **Table 1.** Genomic diversity metrics

| Cross | H_o | ${}^0D_a \pm SD$ | ${}^2D_a \pm SD$ | ${}^1H_a \pm SD$ |
|-------|-------|------------------|------------------|------------------|
| WW1 | 0.158 | 1.29±0.45 | 1.20±0.34 | 0.17±0.27 |
| WW2 | 0.219 | 1.30±0.46 | 1.22±0.36 | 0.18±0.28 |
| WW3 | 0.189 | 1.28±0.45 | 1.20±0.36 | 0.16±0.27 |
| WC | 0.223 | 1.31±0.46 | 1.23±0.37 | 0.18±0.28 |

| | | | | |
|----|-------|-----------|-----------|-----------|
| CW | 0.244 | 1.30±0.46 | 1.22±0.37 | 0.17±0.28 |
|----|-------|-----------|-----------|-----------|

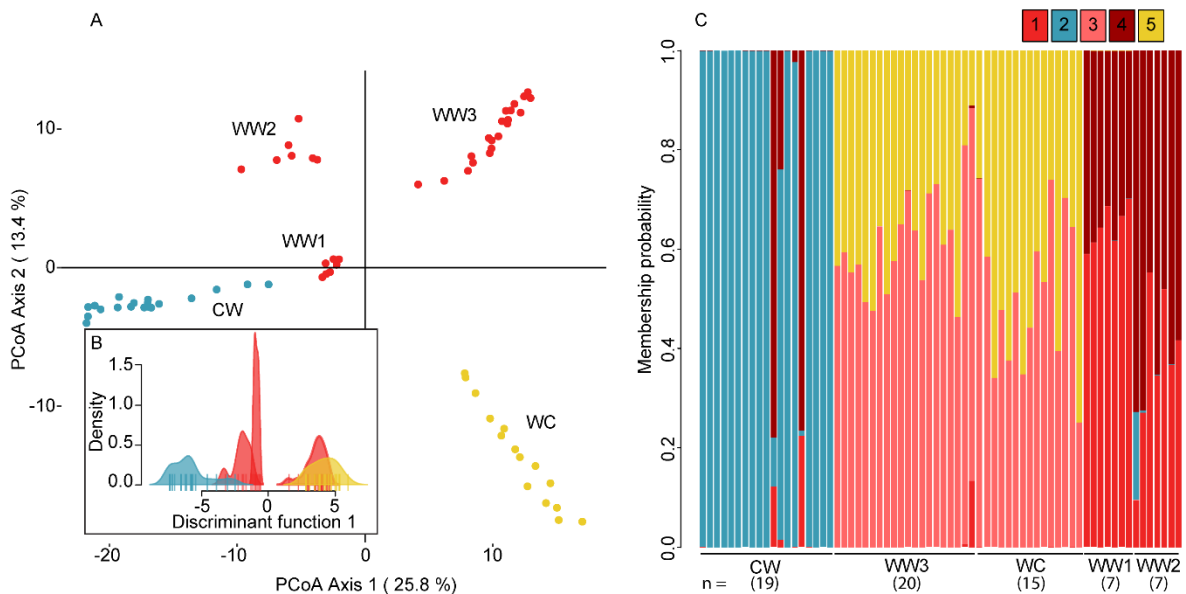
822 H_o = Average observed heterozygosity
823 ${}^0D_\alpha \pm SD$ = Alpha within-population Diversity at $q = 0$
824 ${}^2D_\alpha \pm SD$ = Alpha within-population Diversity at $q = 2$
825 ${}^1H_\alpha \pm SD$ = Alpha within-population Entropy at $q = 1$
826

827 **Table 2.** Mean narrow-sense heritability (h^2) measurements for coral juveniles infected with
828 *C. goreau*.

| | h^2 (mean \pm 95% Bayesian Credibility Intervals) | |
|-----------|---|--------------------|
| | 27.5°C | 31°C |
| Survival | 0.0007 (0.00 - 0.88) | 0.93 (0.73 - 0.98) |
| Bleaching | 0.13 (0.02 - 0.34) | 0.15 (0.03 - 0.34) |
| Growth | 0.01 (0.00- 0.76) | 0.01 (0.00 - 0.67) |

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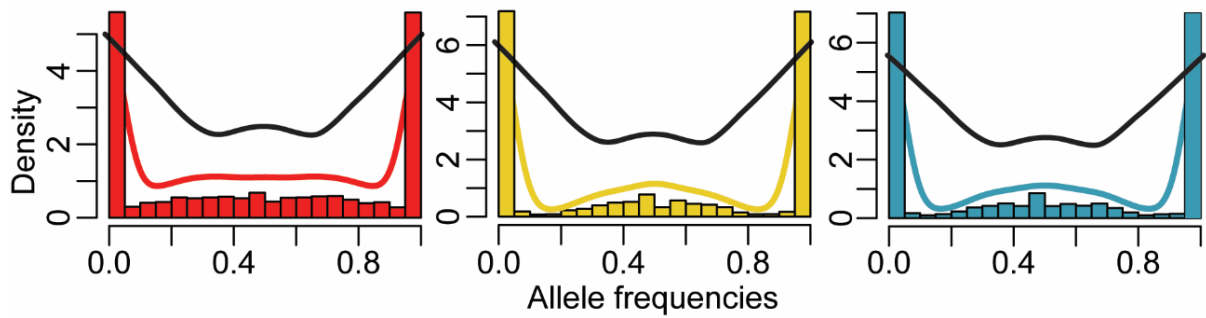
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833 **Figure 1. Genetic differential among population crosses.** Single Nucleotide Polymorphism
834 (SNP) cluster analysis for 68 individually genotyped *Acropora spathulata* larvae produced
835 from five reproductive crosses (red: WW1, WW2, WW3), warm-cool (yellow: WC), cool-
836 warm (blue: CW). (A) Principal Coordinates Analysis and (B) Discriminant Analysis of
837 Principal Components (DAPC) of genetic structure across the SNP dataset. Point colors
838 correspond to five genetic crosses. (C) Admixture inference using DAPC at $k = 5$. Each vertical
839 bar represents an individually genotyped larva, with colors indicative of the membership
840 probability for that population into each of the assigned genetic clusters.

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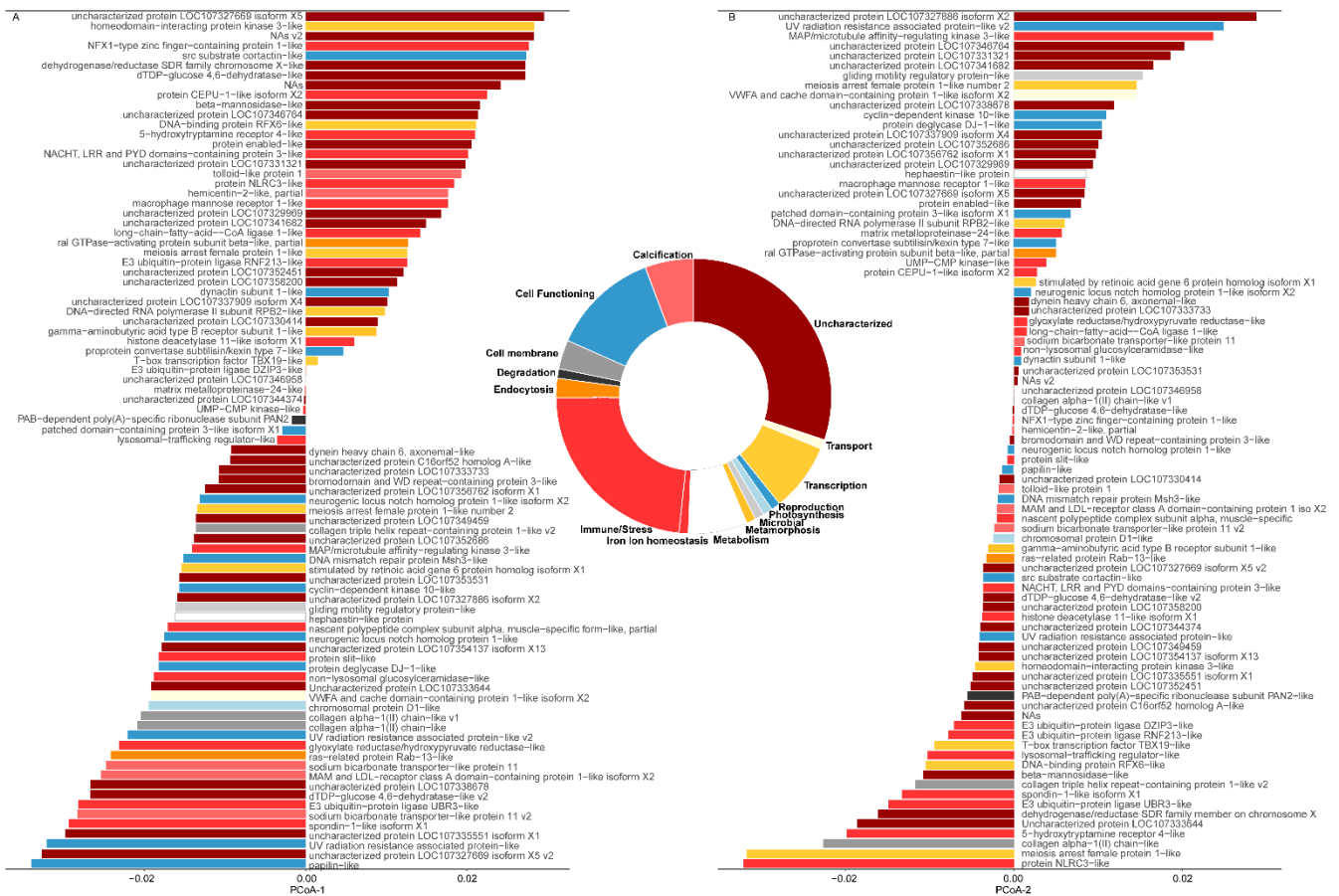
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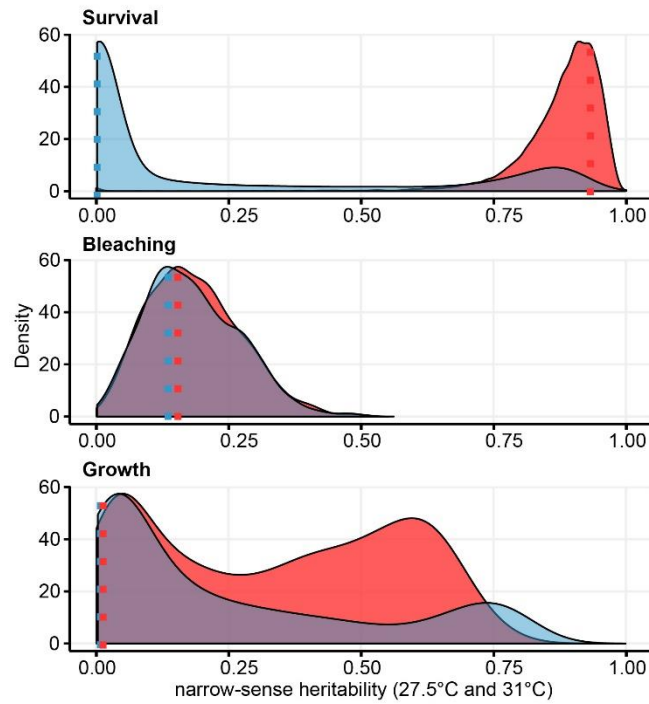
Figure 2. Selective breeding across populations creates significantly greater genetic variation across the coral genome. Distribution of allele frequencies for purebred and hybrid offspring groups consisting of warm-warm crosses (red: WW), warm-cool (yellow: WC), cool-warm (blue: CW) for individually genotyped larvae reared at 27.5°C. Black lines correspond to modelled theoretically expected Hardy-Weinberg distributions of allele frequencies simulated for the three population sizes ($n = \text{WW}: 34, \text{WC}: 15, \text{CW}: 19$). Theoretical versus expected allele frequencies differed significantly within population crosses (Kolmogorov-Smirnov Two-Sample test, WW: $p = 1.0e-45$, WC: $2.2e-46$ and CW: $1.0e-45$) and between purebred and hybrid crosses (WW versus WC: $2.1e-26$, WW versus CW: $1.3e-21$) but not between hybrids (WC versus CW: 0.34).



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Figure 3. Variation in functional proteins as a result of selective breeding of corals. (A) Relative contribution of each protein to PCoA loadings (PC1 variation). Significantly different annotated proteins across the five reproductive crosses in PCoA-1 (A) and PCoA-2 (B).

862 Proportion of proteins per functional category of proteins (inset). Colors correspond to the
863 functional categories of proteins.
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870 **Figure 4.** Narrow-sense heritability (h^2) of survival, bleaching and growth under ambient (blue:
871 27.5°C) and heat stress (red: 31°C) temperature conditions in juvenile corals infected with
872 *Cladocopium goreaui*. Dashed lines correspond to mean h^2 of the distribution.
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