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Genetic markers for antioxidant capacity in a reef-building coral

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The current lack of understanding of the genetic basis underlying environmental stress tolerance in reef-building corals impairs the development of new management approaches to confronting the global demise of coral reefs. On the Great Barrier Reef (GBR), an approximately 51% decline in coral cover occurred over the period 1985–2012. We conducted a gene-by-environment association analysis across 12° latitude on the GBR, as well as both in situ and laboratory genotype-by-phenotype association analyses. These analyses allowed us to identify alleles at two genetic loci that account for differences in environmental stress tolerance and antioxidant capacity in the common coral *Acropora millepora*. The effect size for antioxidant capacity was considerable and biologically relevant (32.5 and 14.6% for the two loci). Antioxidant capacity is a critical component of stress tolerance because a multitude of environmental stressors cause increased cellular levels of reactive oxygen species. Our findings provide the first step toward the development of novel coral reef management approaches, such as spatial mapping of stress tolerance for use in marine protected area design, identification of stress-tolerant colonies for assisted migration, and marker-assisted selective breeding to create more tolerant genotypes for restoration of denuded reefs.

INTRODUCTION

The impacts of global climate change, ocean acidification, and other anthropogenic disturbances are growing concerns for coral reef ecosystems (*1*–*3*). Coral bleaching [that is, the loss of obligate dinoflagellate photosymbionts, *Symbiodinium* spp., and/or their photopigments from coral tissues (*4*)] is a common stress response in corals; it results from a variety of factors, including high and low temperatures (*5*), high irradiance levels, low salinity, sedimentation, pollution, and herbicides (*6*). Extended periods of higher-than-usual summer temperatures have caused mass coral bleaching events and have led to considerable coral mortality worldwide. The capacity of corals to resist thermal bleaching is reduced when they are also exposed to high nutrient levels associated with terrestrial runoff (*7*). Furthermore, terrestrial runoff on its own is also known to have a negative impact on coral health and can cause coral bleaching (*8*). Similar to coral reefs in other regions, the Great Barrier Reef (GBR) is experiencing environmental challenges due to land modification in adjacent coastal areas, which leads to discharges of sediments, chemicals, and nutrients into nearshore waters (*9*).

Intraspecific variation in bleaching tolerance thresholds is common both among and within coral populations (*10*). This can be attributed to acclimatization and adaptation to local environments by the coral host, as well as endosymbiotic dinoflagellates and other microbial symbiont communities (*11*, *12*). Unveiling factors underpinning intraspecific variation in coral stress tolerance will not only enhance our understanding of biological functioning but also provide information that is relevant to coral reef management and restoration approaches. For instance, the identification of coral stress tolerance genes will allow relatively tolerant wild colonies to be selected for fragmentation, reseeding, and restoration (*13*) based on a simple genotyping assay. Alternatively, such colonies could be used for selective breeding to rear offspring with enhanced environmental stress tolerance (*14*) or for assisted translocation (*15*–*17*).

Divergent selection pressures exerted by contemporary environmental gradients provide researchers an excellent opportunity to examine the genetic basis of intraspecific phenotypic variation in adaptive traits. The GBR extends over 14° latitude, providing an extensive north-to-south temperature gradient, and also spans a cross-shelf (west to east) water quality gradient associated with proximity to agriculture on adjacent coastlines and terrestrial runoff during the wet season through several large river systems (*9*). Here, we use gene-by-environment and genotype-by-phenotype association analyses to identify quantitative trait loci (QTLs) for antioxidant capacity or environmental stress tolerance in the genome of the coral animal *Acropora millepora*.

RESULTS

Target single nucleotide polymorphisms

Initially, 19 single nucleotide polymorphism (SNP) markers were targeted. Nine of these were obtained from a genetic linkage study of *A. millepora* (*18*) and were selected on the basis of a study that revealed frequency variations in SNP alleles in populations of *A. millepora* originating from two environmentally distinct reefs on the GBR (*19*). In addition, another nine SNPs were selected from a gene-by-environment study (*20*) that showed significant correlations in five of the loci examined, and one SNP was included on the basis of its putative function in ribosomal protein synthesis (table S1). Gene annotations were obtained from previously published studies (*18*, *19*).

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Of the 19 loci examined, five loci (C20479S292, C29226S281, C70S236, C60613S230, and C16774S791) had clear calling of two alleles and showed good DNA amplifications, and were therefore used in further analyses of allele frequencies. These five SNP loci belong to five different genetic linkage groups (*18*). The remaining 14 loci showed more than two alleles, poor genotyping results, or little variation among populations, and were therefore excluded from further analyses.

Detection of outlier populations

Using the five target SNPs, seven of the 25 populations were identified as outliers on the basis of Mahalanobis distance measures. Five populations among the divergent populations detected in this study were from offshore reefs in the southern GBR region (Ross Reef, Darley Reef, Boulton Reef, Goble Reef, and 21–121 Reef), and two populations were from the southernmost central reefs (High Peak Reef and North Keppel Island Reef) (fig. S1). These populations have been previously found to be genetically distinct on the basis of analysis of microsatellite loci (*21*) and were excluded from the gene-by-environment association analysis.

Gene-by-environment association analysis

Spearman's rank correlation tests on the 18 populations showed that multiple environmental variables were significantly correlated ($P < 0.05$) with two of the five SNP markers (C29226S281 and C70S236), which showed high frequencies of G and T alleles in environments characterized by poor water quality, low mean sea surface temperature (SST), and high annual SST range (Fig. 1). F1

Spatial clustering of water quality and temperature variables

Principal components analysis (PCA) revealed that most reefs were grouped into two clusters on the basis of two water quality variables (chlorophyll and NO_3 concentration) and one temperature variable (range in SST).The pattern of clustering indicates that environmental conditions at Halftide Rocks Reef, Humpy Island Reef, Halfway Island Reef, and Magnetic Island Reef are different from those at other locations (fig. S2). Principal component 1 explained 76.2% of the variation of the three variables.

Combined effects of NO_3 concentration and temperature range

A multiple regression model, accounting for both the NO_3 and the SST range variables, explained 48 and 58% of the variability in allele frequency patterns for the C29226S281 and C70S236 loci, respectively, with a confidence level of more than 99% (table S2).

Natural bleaching experiment

To evaluate the efficacy of these two markers as QTLs, we examined the in situ bleaching responses of different genotypes of *A. millepora* to high temperature and water quality (fig. S3). Surveys of 150 colonies of *A. millepora* across five sites in the Palm Islands (central GBR), sampled during temperature-induced bleaching in the summer of 2006, revealed an approximately 12% higher frequency of allele T at C70S236 (corrected $P = 0.0469$) in nonbleached colonies compared to bleached colonies (Fig. 2A). In the summer of 2009, an extended F2 period of extensive rainfall caused increased turbidity and decreased salinity, whereas the temperature did not increase above the long-term average summer maxima (*22*) (fig. S3). Of the 165 colonies genotyped from four sites in the Palm Islands, nonbleached colonies showed an approximately 28% higher frequency of allele G at C29226S281 (corrected $P = 0.0092$) compared to bleached colonies (Fig. 2A).

Laboratory heat stress experiment

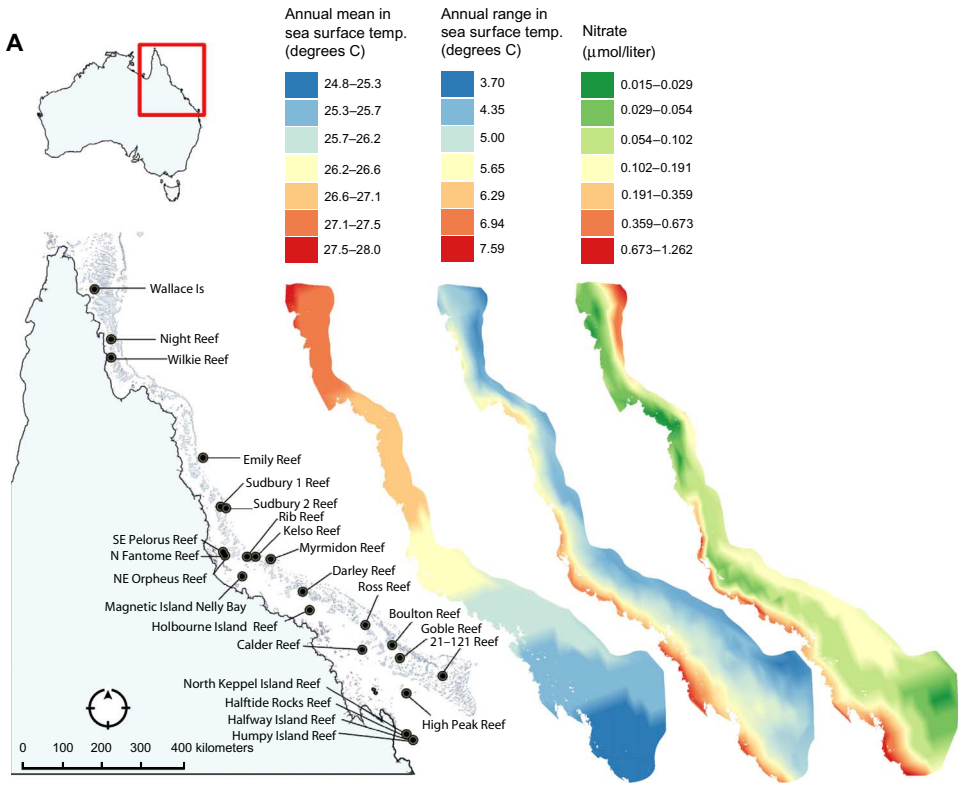
The two loci were validated further in a laboratory heat stress experiment (*23*, *24*). We analyzed the maximum quantum yield of photosystem II (F_v/F_m), a measure of the photochemical efficiency of *Symbiodinium*, and the coenzyme Q (CoQ) pool redox state (%$CoQH_2$; the proportion of reduced CoQ to oxidized CoQ), a measure of the antioxidant capacity of the coral animal (*23*), for different genotypes at the two SNP loci. It has been demonstrated that thermal stress, both short term and prolonged, causes a 10 to 15% oxidation of the CoQ pool in corals (*23*, *25*).

Coral bleaching is understood to occur when the coral host's capacity to detoxify reactive oxygen species (ROS) is overwhelmed (*2*). ROS leak from compromised algal symbionts into the coral host cytosol and, additionally, are produced by host mitochondria (*2*). As one of the defense systems against ROS, the mitochondria contain high levels of the antioxidant ubiquinol (reduced CoQ) (*26*). Colonies of the GG and TT genotypes for C29226S281 and C70S236, respectively, maintained about 3 to 6% higher $CoQH_2$ levels in both 27° and 32°C treatments compared to other genotypes [$P = 0.0096$ (27°C) and $P = 0.0013$ (32°C) for C29226S281; $P = 0.0014$ (27°C) and $P < 0.0001$ (32°C) for C70S236] (Fig. 2C). Consistent with higher levels of %$CoQH_2$, we found about 2 and 20% higher F_v/F_m in the C70S236 TT compared to the CC genotype in both 27° and 32°C treatments, respectively, over the course of the experiment [$P = 0.0001$ (27°C) and $P = 0.0007$ (32°C)] (fig. S4). A considerable amount of variation in $CoQH_2$ level was explained by the QTL genotypes, representing 14.6 and 32.5% for C29226S281 and C70S236, respectively. The effect size for F_v/F_m was smaller, with 10.5% of the variance attributable to the genotype for C70S236, and there was no significant genotype effect for C29226S281.

DISCUSSION

The three independent data sets obtained in our study (summarized in Table 1) provide compelling evidence that C29226S281 and C70S236 T1 are true QTLs; the presence of G and T alleles at these loci, respectively, is indicative of a relatively higher antioxidant capacity and tolerance to temperature stress and/or low water quality, especially in the homozygous state. Whereas the 3 to 6% higher $CoQH_2$ levels in colonies of the GG and TT genotypes for C29226S281 and C70S236, respectively, might seem insubstantial as compared to other genotypes in both 27° and 32°C experimental treatments (Fig. 2B), this difference is considerable and biologically relevant given that severe coral bleaching causes an approximately 10% drop in CoQH2% (*23*). Both genetic markers are located in genes that play a role in the ubiquitination process, which is known to be involved in the coral thermal stress response (*20*, *27*). Frontloading of stress tolerance genes (such as heat shock proteins) and antioxidant enzymes, as demonstrated in our laboratory experiment, has also been observed in a transcriptomic study of experimentally heat-stressed colonies of the congener *Acropora hyacinthus* in American Samoa (*27*).

A number of invertebrates, including oysters (*28*) and corals (*29*, *30*), are known to recruit the same pathways in response to a range of environmental stressors as well as pathogen infection. This response involves a defense against ROS (*2*) (for example, antioxidant and chaperone proteins), apoptosis, cytoskeleton reorganization, and the innate immune



B

Category	Environmental variable	C20479S292-T(G)	C70S236-(T)C	C60613S230-T(G)	C16774S791-A(C)	C29226S281-A(G)
Temperature	Mean SST	−0.164	−0.483**	−0.229	0.063	−0.549**
	Minimum SST	−0.016	−0.525**	−0.056	0.086	−0.385
	Maximum SST	0.061	−0.122	−0.192	−0.005	−0.356
	Range SST	0.055	0.529**	−0.145	0.038	0.315
Water quality	Secchi depth	−0.276	−0.115	0.248	−0.151	0.114
	Chlorophyll	0.133	0.463*	−0.079	0.098	0.448*
	Total dissolved nitrogen	0.370	0.018	0.080	0.293	−0.271
	Total dissolved phosphorus	0.340	0.153	0.077	0.274	0.256
	Suspended solids	0.237	0.453*	−0.002	0.148	0.180
	Dissolved inorganic phosphorus	0.234	0.517**	0.106	0.463*	0.523**
	Particulate phosphorus	0.251	0.397	−0.154	0.094	0.031
	Nitrate	0.259	0.634**	0.401*	0.045	0.664**
	Nitrite	0.238	0.450*	0.137	−0.142	0.048
	Ammonia	0.101	−0.080	−0.203	0.194	−0.422*
	Particulate nitrogen	0.217	0.443*	−0.069	0.027	0.305
	Silicate	0.279	0.048	−0.177	0.220	−0.218

Fig. 1. Gene-by-environment associations. (**A**) Map showing mean SST and range in SST, nitrate gradients, and locations of 25 populations of *A. millepora* sampled on the GBR. (**B**) Correlation coefficients (ρ) between environmental gradients and allele frequencies. SNP alleles in brackets were used for the analysis. *P = 0.05 to 0.1; **P < 0.05.

response (*27*, *31*). High temperature and poor water quality commonly lead to oxidative stress and subsequent bleaching due to a reduction in *Symbiodinium* photosynthetic efficiency caused by increased *Symbiodinium* density (*32*, *33*) and by photoinhibition of photosystem II (*4*). High levels of ROS may trigger the coral host innate immune response, leading to high levels of the reactive nitrogen species, nitric oxide, a common immune pathway in animals (*34*). The two markers identified in our study are QTLs for antioxidant capacity, and their role in the coral stress response to both high and low temperatures as well as poor water quality is in line with our current understanding of common invertebrate responses to environment- and pathogen-induced stress.

Nevertheless, our results suggest that some level of response specificity of the markers may exist. The laboratory heat stress experiment demonstrated that the effect size of C70S236 on $CoQH_2$ and F_v/F_m (antioxidant capacity and photochemical efficiency) was larger than that of C29226S281. Therefore, C70S236 may have a greater influence on

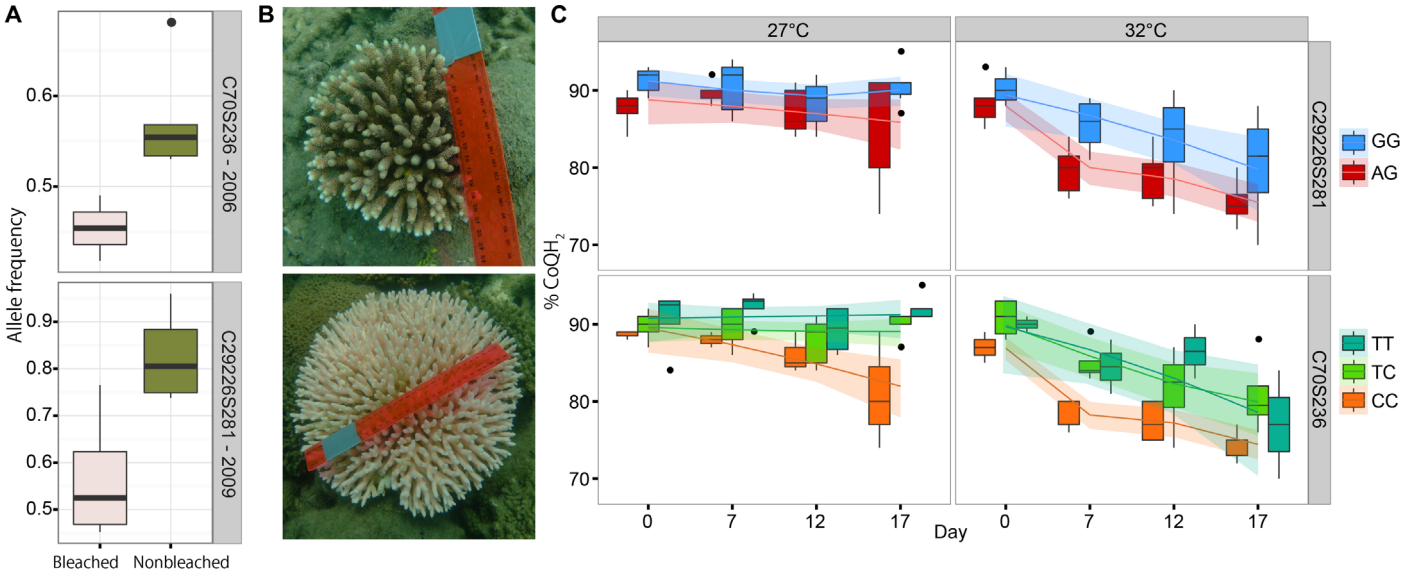


Fig. 2. Genotype-by-phenotype associations. (**A**) Relationships between bleaching responses observed in 2006 (N = 150 coral colonies) and 2009 (N = 165 coral colonies) and allele frequencies at C70S236 (corrected P = 0.0469) and C29226S281 (corrected P = 0.0092) markers. Frequencies of G and T are shown for C29226S281 and C70S236 markers, respectively. (**B**) Photos showing nonbleached (top) and bleached (bottom) corals. (**C**) The relationships between $CoQH_2$ and genotypes (N = 8 coral colonies). P = 0.0096 (27°C) and P = 0.0013 (32°C) for C29226S281; P = 0.0014 (27°C) and $P < 0.0001$ (32°C) for C70S236.

Table 1. Summary of results. Main results from the three independent data sets obtained in this study.

Data set	Locus C70S236	Locus C29226S281
Gene-by-environment correlation (poor water quality, high SST range, and low mean SST)	Higher frequency of T allele	Higher frequency of G allele
2006 nonbleached versus bleached corals (temperature stress)	12% higher frequency of T allele in nonbleached colonies	No difference
2009 nonbleached versus bleached corals (salinity and turbidity stress)	No difference	28% higher frequency of G allele in nonbleached colonies
Experimental heat stress	Higher antioxidant capacity ($CoQH_2$) in TT genotypes: 35.2% explained by genotype	Higher antioxidant capacity ($CoQH_2$) in GG genotypes: 14.6% explained by genotype
	Resistance to photosynthetic damage (F_v/F_m) in TT genotypes: 10.5% explained by genotype	

temperature-related stress tolerance. This is consistent with the gene-by-environment association analysis that showed a significant correlation between allele frequencies at C70S236 and range in SST (Fig. 1). Furthermore, the natural bleaching data demonstrated that the bleaching tolerance of colonies collected in the summer that was characterized by high temperature (2006) was significantly associated with allele frequencies at the C70S236 locus, whereas C29226S281 showed allelic associations with bleaching conditions caused by poor water quality (Fig. 2A and fig. S3). This also explains the smaller (but still significant) genotypic effects on tolerance to heat stress at the C29226S281 locus in the laboratory experiment. We predict that these two SNP loci will represent markers for tolerance to a range of other environmental stressors that are known to lead to cellular oxidative stress.

Environmental variables related to temperature and water quality have cumulative effects on coral fitness (*3*, *35*, *36*). However, mean SST and NO_3 concentration at the sampling sites in this study have an inverse relationship, suggesting that the populations investigated are exposed to either high mean SSTs or high NO_3, rather than to both simultaneously. In contrast, range in SST is positively correlated with NO_3 concentration across sampling locations, indicating that locations with poor water quality (that is, high nutrient concentrations) are characterized by wider temperature fluctuations (Fig. 1). Four populations (Halftide Rocks, Magnetic Island, Humpy Island, and Halfway Island Reefs) that showed allelic differentiation from the remainder of the populations at the two loci are characterized by both high temperature fluctuation and poor water quality (as represented by high chlorophyll and NO_3 concentrations)

(fig. S2). Furthermore, a multiple regression model that included NO_3 concentration and range in SST showed a significant fit to the allele frequency data for both loci (C29226S281 and C70S236). This increase in the goodness of fit of the model, despite a nonsignificant and small effect of range in SST when analyzed independently (table S2), suggests that there may be synergistic effects between selection driven by NO_3 concentration and range in SST. Finding different allele frequencies in thermally variable environments is consistent with previous experimental evidence demonstrating that corals from fluctuating habitats are more tolerant to temperature stress (*37*).

Whereas phenotypic traits are not necessarily under the control of the QTL itself [that is, they can instead be regulated by genes in the same linkage group (*38*)], the two SNP markers identified in our study are representative of the hard-wired genetic components underpinning phenotypic variation and can be used in a wide range of applications relevant to coral reef management and restoration. Extrinsic factors, such as temperature and water quality, are critical drivers of the degradation of coral populations. Spatial mapping of the C29226S281 and C70S236 genotypes can provide high-resolution data, which are used to predict the environmental stress susceptibility of corals and allow the identification of resilient and susceptible populations and individuals. Additional QTLs can be developed to provide more confidence in such initiatives. In combination with the information on connectivity among populations, this will enable the identification of key targets for conservation, such as highly resilient populations that have the ability to seed surrounding reefs. Furthermore, active human interference through assisted migration and selective breeding may be necessary to facilitate the survival of coral reefs in the future (*14*). Individuals with double homozygous genotypes at the two genetic markers, such as those identified in our study, can be targeted to select stress-tolerant brood stock for translocation and selective breeding to restore highly damaged reefs. A great advantage of the use of genetic markers for identifying resilient corals, rather than relying on historical environmental averages, is the greater resolution associated with it (that is, to the colony rather than reef level). Detection of genetically determined phenotypic variants within the population minimizes issues often encountered in transplant studies, such as acclimatization-associated trade-offs and environmentally regulated heritable factors (*11*). In conclusion, our study has revealed two QTLs for antioxidant capacity and environmental stress tolerance in reef-building corals. The identification of stress-tolerant genotypes will facilitate exploration of new management and restoration options for the world's rapidly degrading coral reefs.

MATERIALS AND METHODS

Study sites

Samples of the coral *A. millepora*, collected for a separate study of large-scale gene flow (*21*), were pooled and genotyped for each population as described by Capper *et al.* (*19*). A total of 25 populations were sampled, spanning ~1550 km (~12° latitude) of the GBR (Fig. 1 and table S4). At each location, one branch was collected from each of the 35 to 56 colonies. Coral branches were collected in an area of approximately 300 × 300 m, at a depth of between 1 and 11 m at each site, and then fixed in absolute ethanol for DNA analysis. Of these 25 populations, 20 populations formed a part of the large-scale population genetic study (*21*).

Detection of outlier populations

Outlier populations were excluded from the gene-by-environment association analysis to minimize the likelihood of falsely identifying SNPs that correlate with environmental gradients due to historical factors or limits to gene flow. The R package rrcovHD was used to calculate Mahalanobis distance (*39*), which is a multivariate outlier detection analysis that can be used for identifying populations that deviate in allele frequency distribution. The calculation was iterated 1000 times using standardized allele frequencies of the five selected loci, and the number of times populations were identified as outliers was counted. Cutoff values for determining outliers were set to 500 (that is, >50% chance of being identified as an outlier). Populations identified as outliers (fig. S1) were excluded from the gene-by-environment association analysis.

Gene-by-environment association analysis

As a first step in identifying genetic markers for environmental stress tolerance in corals, we searched for correlations between allele frequencies at 5 SNP loci and 16 environmental parameters (12 water quality parameters and 4 water temperature parameters), using samples from 856 colonies collected from 18 populations after exclusion of outlier populations (Fig. 1A, fig. S1, and table S4). Satellite temperature data for the three temperature variables (that is, minimum, maximum, and range SST) were obtained from the National Oceanic and Atmospheric Administration (*40*). The three temperature variables were calculated based on a decadal average of minimum, maximum, and range summertime temperatures between 1990 and 2000. Other environmental data were obtained from spatial prediction maps on the basis of long-term monitoring data gathered by the Australian Institute of Marine Science (AIMS) (http://eatlas.org.au/data/uuid/df7012eb-a23f-4cf2-b92d-6fff77718987). Allele frequency data for the 18 populations were used to test correlations with environmental data that were obtained for the corresponding locations. Spearman's rank correlations between environmental variables and allele frequencies were calculated using the R package stats version 3.0.2. Categorical values were used for the environmental data.

Spatial clustering of water quality and temperature factors

A PCA plot was constructed based on NO_3 concentration, chlorophyll, and range in SST data to explore clustering patterns for both water quality and temperature variables in the 18 highly connected reefs. The three environmental variables were selected based on the following criteria: chlorophyll is a commonly used indicator of the ecosystem status of the GBR (*41*); NO_3 has adverse effects on coral physiology (*35*, *36*, *42*); and thermally unstable environments have a high abundance of stress-tolerant alleles in this study, with study sites showing high NO_3 concentrations being characterized by high temperature fluctuations. The R packages ggplot2 and ggfortify were used to plot the data. Categorical values were used.

Combined effects of NO_3 concentration and temperature range

The combined effects of these two environmental factors were investigated using multiple linear regression analysis. Continuous values were used for all the variables. The R package stats was used to measure the correlations.

Mendelian inheritance

Two colonies of *A. millepora* from Orpheus Island were crossed in vitro as described by van Oppen *et al.* (*17*). Larvae were reared and fixed in absolute EtOH. DNA extraction was done following previously published methods for larval (*43*) and adult (*44*) coral DNA. Both C29226S281 and C70S236 markers showed strict Mendelian segregation patterns in an analysis involving two parent colonies of *A. millepora* (heterozygote

female × homozygote male for both loci) and 50 of their offspring [AG:GG, 0.48/0.5 (C29226S281); TC:CC, 0.54/0.44 (C70S236)] (table S5).

Natural bleaching experiment

We sampled coral colonies from Cattle Bay ($N = 30$), northeast Orpheus ($N = 30$), southwest Pelorus ($N = 30$), southeast Pelorus ($N = 30$), and Pioneer Bay ($N = 30$) on 5 and 6 March 2006 and from central Pioneer Bay ($N = 48$), southern Pioneer Bay ($N = 29$), southwest Pelorus ($N = 41$), and Cattle Bay ($N = 47$) on 27, 28, and 29 March and 12 April 2009 (table S6 and fig. S5). Bleaching states were visually determined in the field (that is, healthy, pale, and bleached) (Fig. 2B). Healthy and pale conditions were treated as nonbleached. Coral nubbins were snap-frozen and stored in liquid nitrogen or directly fixed in 100% ethanol for DNA extractions. In genotyping assays, DNA samples were pooled according to bleaching condition for each population to estimate allele frequencies. All populations, regardless of the presence or absence of bleached colonies, were included in a subsequent statistical analysis. We tested whether there was a higher frequency of the T (locus 70S236) and G (locus C29226S281) alleles in bleaching-resistant colonies on the basis of the association of these genotypes with the experimental data (enhanced antioxidant capacity) and environmental correlations (high SST range and nutrient loading). We performed one-tailed t tests and adjusted P values ($\alpha = 0.05$) for multiple comparisons using the Holm-Bonferroni sequential method.

Heat stress experiment

To further test the relationship between genotype and thermal bleaching at the two SNP loci, we analyzed samples of *A. millepora* from a heat stress experiment (*23*, *24*). Ten colonies of *A. millepora*, containing *Symbiodinium* C2 from a single site at Pelorus Island were used for this study. Colonies were transfered to the aquaria at AIMS and split into a total of 36 fragments, each comprising approximately 25 branches (nubbins). Fragments were placed in twelve indoor tanks in a randomized arrangement. After 2 weeks of acclimatization, the temperatures in six tanks were ramped up to 32°C over a period of 7 days, whereas the other six control tanks were maintained at 27°C throughout the experiment. Corals were sampled at four time points: after the acclimatization period ($t = 0$); after reaching 32°C in the thermal stress treatment ($t = 7$); and after 5 days ($t = 12$) and 10 days ($t = 17$) at the target temperature. Whole fragments were snap-frozen in liquid nitrogen immediately upon collection. More detailed information on the experimental design is found in the study of Raina *et al.* (*24*). For our genotype-by-phenotype association study, we used coral physiological data indicating the oxidative stress state of the coral host, including CoQ pool redox states, which was quantified by Lutz *et al.* (*23*) using liquid chromatography–mass spectrometry. We also examined genotypic associations with the maximum quantum yield of photosystem II (F_v/F_m), a measure of the photochemical efficiency of *Symbiodinium*, which was measured by Lutz *et al.* (*23*) using a pulse amplitude–modulated fluorometer.

Genotypic associations of $CoQH_2$ and F_v/F_m for each locus were tested by a generalized estimating equation model. The dependent (response) variables were $CoQH_2$ and F_v/F_m. The predictor variables were day number, treatment (temperature), and genotypes. Colony was a random factor. $CoQH_2$ and F_v/F_m data from the same colonies were partitioned into the same clusters in the model. An exchangeable correlation structure was used because multiple fragments from the same colonies were used for the same treatments (that is, intracolony correlations) (*45*). This procedure takes into account the dependence between measurements on the same colonies. Wald tests were used to examine the effect of each term added sequentially to the model. The model was fitted using the R package geepack (*46*).

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/2/5/e1500842/DC1

fig. S1. Outlier populations detected by 1000 iterations of Mahalanobis distance calculations.
fig. S2. PCA reveals two clusters of reefs on the basis of NO_3, chlorophyll, and SST range data for 18 reefs.
fig. S3. Environmental conditions at the Palm Islands.
fig. S4. Genotype-by-phenotype association.
fig. S5. Allele frequencies at C70S236 and C29226S281 in bleached and nonbleached colonies collected from the Palm Islands in 2006 and 2009.
table S1. SNP markers used in this study.
table S2. A multiple linear regression model for testing correlations between allele frequencies and two environmental variables, SST range and NO_3.
table S3. Allele frequencies at the five SNP loci in the 18 *A. millepora* populations sampled along the length of the GBR.
table S4. List of sampling locations for the gene-by-environment analysis.
table S5. Mendelian inheritance of C29226S281 and C70S236 markers.
table S6. Allele frequencies at the two QTLs in bleached and nonbleached colonies from the Palm Islands.
table S7. Genotypes at the two QTLs and $CoQH_2$ concentration of *A. millepora* colonies during the laboratory bleaching experiment.
table S8. Genotypes at the two QTLs and maximum quantum yields (F_v/F_m) of photosystem II of *A. millepora* colonies during the laboratory bleaching experiment.

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