25. P. Loubeyre *et al.*, *High Press. Res.* **24**, 25–31 (2004).
26. P. Loubeyre *et al.*, *Phys. Rev. B* **86**, 144115 (2012).
27. A. F. Goncharov *et al.*, *Phys. Rev. B* **87**, 024101 (2013).
28. V. Dzyabura, M. Zaghoo, I. F. Silvera, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 8040–8044 (2013).
29. Z. M. Geballe, R. Jeanloz, *J. Appl. Phys.* **111**, 123518 (2012).
30. M. Matzen *et al.*, *Phys. Plasmas* **12**, 055503 (2005).
31. L. M. Barker, R. E. Hollenbach, *J. Appl. Phys.* **43**, 4669 (1972).
32. G. I. Kerley, Equations of state for composite materials, *Technical Report No. SAND2003-3613*, Sandia National Laboratories (2003).
33. N. C. Holmes, M. Ross, W. J. Nellis, *Phys. Rev. B* **52**, 15835–15845 (1995).
34. J. E. Bailey *et al.*, *Phys. Rev. B* **78**, 144107 (2008).
35. R. C. Clay III *et al.*, *Phys. Rev. B* **89**, 184106 (2014).

CORAL REEFS

Genomic determinants of coral heat tolerance across latitudes

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As global warming continues, reef-building corals could avoid local population declines through "genetic rescue" involving exchange of heat-tolerant genotypes across latitudes, but only if latitudinal variation in thermal tolerance is heritable. Here, we show an up–to–10-fold increase in odds of survival of coral larvae under heat stress when their parents come from a warmer lower-latitude location. Elevated thermal tolerance was associated with heritable differences in expression of oxidative, extracellular, transport, and mitochondrial functions that indicated a lack of prior stress. Moreover, two genomic regions strongly responded to selection for thermal tolerance in interlatitudinal crosses. These results demonstrate that variation in coral thermal tolerance across latitudes has a strong genetic basis and could serve as raw material for natural selection.

Worldwide, coral reefs are threatened by increasing temperatures associated with climate change (*1, 2*). Models predict that even a modest increase in the thermal tolerance of reef-building corals over 40 to 80 years would lower their extinction risk dramatically (*3*). Corals are capable of physiological acclimatization to elevated temperature, and it has been argued that in such long-lived organisms acclimatization rather than genetic adaptation will play the leading role in their response to climate change (*4*). Here, we present data for the heritable basis of temperature tolerance that supports the potential for rapid adaptation at the genetic level based on standing genetic variation.

Many coral species maintain high genetic connectivity across thousands of kilometers and inhabit latitudinal ranges that span considerable temperature gradients (*5, 6*). However, it remains unclear to what extent latitudinal variation in coral thermal physiology is heritable and could fuel genetic rescue via exchange of temperature-tolerant immigrants across latitudes (*7*). We used quantitative genetic, functional genomic, and quantitative trait loci analyses to address this question in *Acropora millepora* corals from thermally divergent locations separated by 5° of latitude: Princess Charlotte Bay (PCB) and Orpheus Island (OI, Fig. 1A).

Ten crosses were established according to a diallel scheme by cross-fertilizing gametes from four adult colonies from the two locations (Fig. 1B). Larval families were cultured in triplicate for 5 days until embryonic development was complete and sampled for tag-based RNA-sequencing analysis (*8*). Separately, larval crosses were scored for heat tolerance, measured as odds of survival after 27 and 31 hours at 35.5°C. The target temperature was reached by ramping over 12 hours at the rate of 0.63°C per hour, less than half of the warming rate on a reef flat during a tidal cycle (*4*).

Survival rates varied substantially among families (Fig. 1D). A mixed-effects generalized linear model with random effects of sire, dam, and their interaction as predictors indicated that the combined parental effects (i.e., broad-sense heritability) accounted for 87% of total deviance in odds of larval survival (Fig. 1E, 95% credible interval of the posterior: 72 to 99%). Proportions of deviance resulting from sire, dam, and their interaction were estimated at 11%, 66%, and 12%, respectively, although the credible intervals were wide because of the limited scope of our crossing design (Fig. 1E). Parents from the warmer location (PCB) conferred significantly higher thermo-tolerance to their offspring relative to parents from the cooler location (OI), with a PCB dam conferring a five-fold increase ($P_{MCMC} < 0.001$; MCMC, Markov chain Monte Carlo) and a PCB sire conferring an additional twofold increase ($P_{MCMC} = 0.048$) in survival odds (Fig. 1F).

To elucidate molecular processes underlying this variation, we identified genes whose expression before stress predicted the odds of larval survival under stress (Fig. 2A), which we term tolerance-associated genes (TAGs). At the 5% false discovery rate (FDR), 1973 TAGs were identified (Fig. 2B). In heat-tolerant larvae, gene ontology (GO) categories related to oxidoreductase activity and extracellular matrix were significantly enriched in the up-regulated gene set, whereas categories related to transmembrane transporter and motor activity were significantly enriched in the down-regulated gene set (Fig. 2C). An analysis of cellular component categories additionally revealed enrichment of nuclear-encoded mitochondrial membrane components (Fig. 2D and fig. S3), potentially a manifestation of mitochondrial variation that could contribute to the high maternal effect on heat tolerance (Fig. 1E).

Higher coral heat tolerance has been attributed to "frontloading," where elevated baseline expression of stress response genes primes the organism for stress (*9*). Alternatively, higher tolerance could be due to the lack of prior stress, in which case the expression of TAGs should be unrelated or opposite to the heat stress response. We compared the TAGs to gene expression in adult parental colonies after 3 days of heat stress (31.5°C, figs. S3 and S4) and to published data on larvae after 4 hours or 5 days of heat stress (*8*), based on patterns of up- and down-regulation within eukaryotic orthologous group (KOG) gene classes (*10*) (Fig. 3A). The adult heat stress response was quite similar to the 5-day larval heat stress response (Fig. 3B). The TAGs expression was significantly negatively correlated with long-term heat stress response in larvae (Fig. 3C) and in adults (albeit marginally significant: $P_{cor.test} = 0.06$). This indicates that the larval heat tolerance we detected most likely arose from the absence of preexisting stress, not from prior up-regulation of heat stress genes through frontloading.

The KOG class most enriched in up-regulated TAGs was energy production and conversion and encompassed mitochondrial proteins (Fig. 3A and fig. S3), further supporting the possible contribution of mitochondrial variation to the maternal effect on heat tolerance (Fig. 1E). Alternatively, maternal effect could be due to epigenetic modification

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of the nuclear genome, in which case variation in TAGs expression would likely also be under maternal control. We undertook a weighted gene correlation network analysis (*11*) to investigate this possibility and found a predominance of genetic rather than maternal effects: Expression variation of over 2700 genes was transmitted from adult corals to their larval offspring irrespective of whether the adults were used as sires or as dams (fig. S6 and accompanying text). The number of genes affected by maternal effects ranged from none to nearly 2000 among dams, which would be surprising if maternal effects were due to ubiquitously present genome-wide epigenetic modifications. The best correlation with heat tolerance was observed for biparentally rather than maternally controlled genes (fig. S6, B to E), which corresponds well with the observation that higher heat tolerance was contributed by both PCB parents (Fig. 1F). However, because the strongest maternal effects both on gene expression and on larval heat tolerance were observed for the same parent (dam C), the role of epigenetic modifications cannot be ruled out.

To further demonstrate that larval heat tolerance has a genetic basis and can respond to selection, we quantified genomic effects of artificial selection by heat in two interlatitudinal reciprocal crosses (AC and CA). Selected samples consisted of the last 30 to 50 heat stress–surviving larvae out of the initial ~1000, whereas control samples consisted of 50 larvae from unstressed cultures. This experiment was performed with two culture replicates from each cross, resulting in eight compared groups. Larvae were individually genotyped (*n* = 326) by using 2bRAD methodology (*12*) to construct a genetic linkage map and to identify genomic regions displaying reproducible allele frequency shifts in response to heat selection.

The linkage map contained 1448 markers in 14 linkage groups (LGs) and had a total length of 1358 centimorgans (cM). In both crosses, the selection was predominantly against paternally derived haplotypes (fig. S7), resulting in markedly different genome-wide patterns of selection between reciprocal crosses (Fig. 4). The strength of negative selection, measured as a decrease in survival of larvae bearing the less-preferred haplotype, reached unity in LG 10 in the CA cross (i.e., the less-preferred haplotype was completely eliminated from the larval pool) and 0.91 in LG 5 in the AC cross. No statistically significant signatures of selection were observed when comparing pairs of unselected
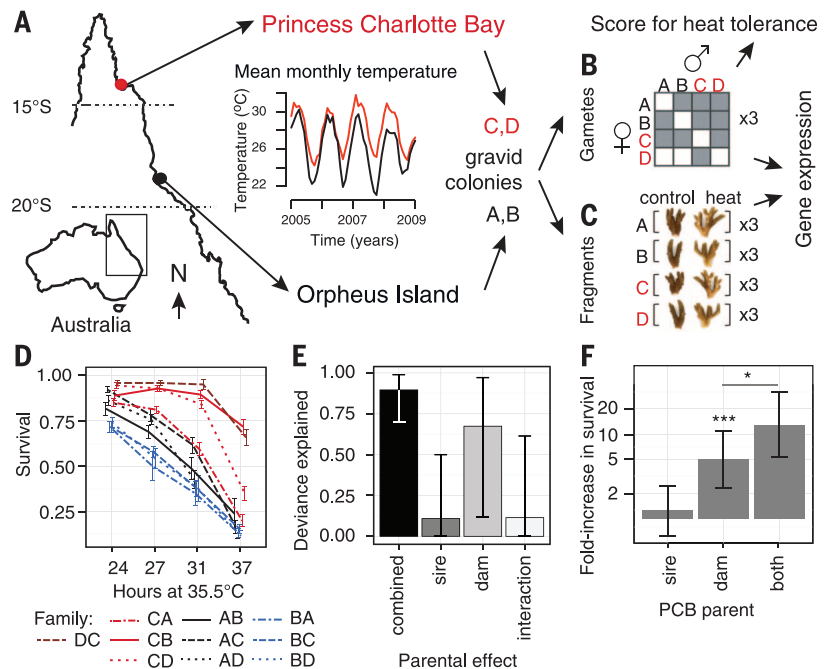

Fig. 1. Experimental design and quantitative genetics of larval heat tolerance. (**A**) Sampling locations and their annual temperature regimes on the Great Barrier Reef, Australia. (**B**) Crossing design matrix where solid squares represent established crosses. (**C**) Experimental design to quantify gene expression differences between parental colonies under heat stress (31.5°C for 3 days). (**D**) Mortality curves ± SE for each larval family. In the family identifier, the first letter is dam (mother); the second letter is sire (father). (**E**) Proportion of total deviance explained by parental effects. (**F**) Increase in odds of larval survival with parents from the warmer location (PCB) relative to the larvae with both parents from the cooler location (OI). ***$P <$ 0.001, *$P <$ 0.05. Whiskers on (E) and (F) denote 95% credible interval of the posterior.
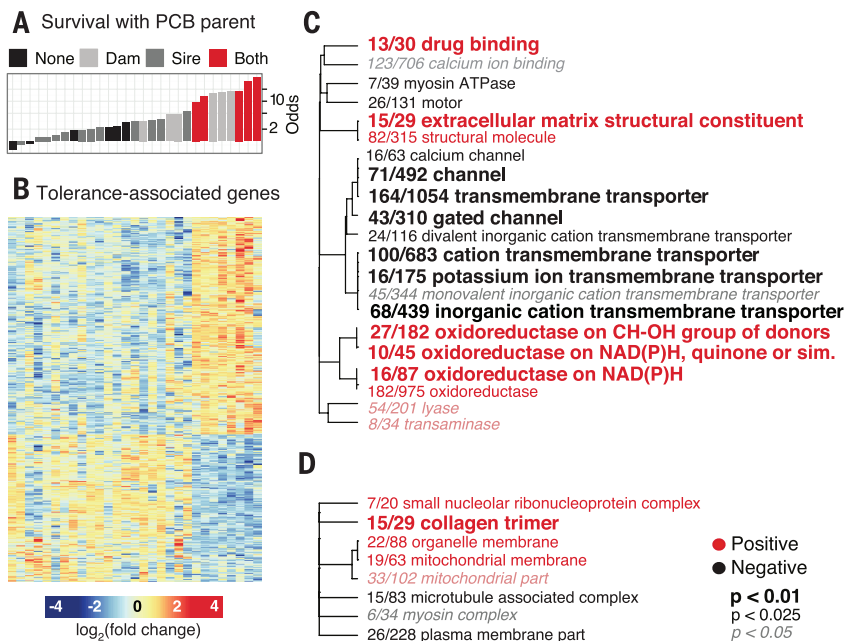

Fig. 2. Gene expression associated with larval heat tolerance. (**A**) Bar chart for survival odds under heat stress for each larval culture, ranked in increasing order. (**B**) Heat map of 1973 genes (rows) for which the expression before heat stress predicts the survival odds under stress. Columns are larval cultures ordered as in the bar chart above (A). (**C** and **D**) GO categories significantly enriched with genes either positively (red) or negatively (black) associated with heat tolerance. The dendrograms depict the sharing of genes between categories; the fractions correspond to genes with an unadjusted $P <$ 0.05 relative to the total number of genes within the category. (C) Molecular function. ATPase, adenosine triphosphatase; NAD(P)H, reduced form of nicotinamide adeninine dinucleotide. (D) Cellular component.
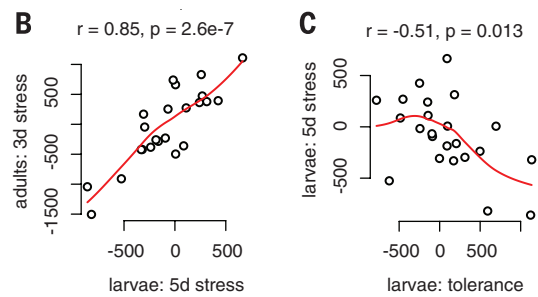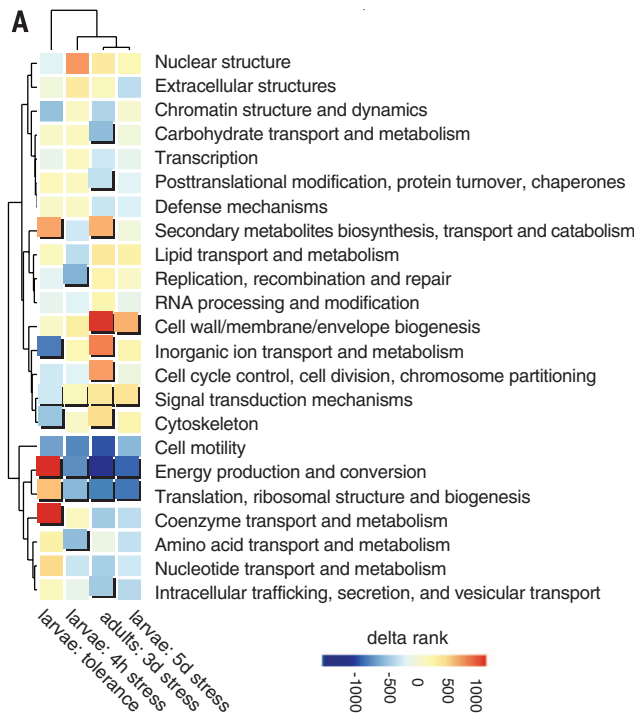
Fig. 3. Coral gene expression responses compared among data sets. (**A**) Heat map of enrichment of KOG classes (rows) by differentially expressed genes in different data sets (columns). The KOG classes significantly enriched (FDR = 0.05) with up- or down-regulated genes are identified by raised tiles. (**B**) Correlation of KOG delta ranks between larval response to 5-day heat stress and adult response to 3-day heat stress. (**C**) Correlation of KOG delta ranks between larval heat tolerance and larval heat stress response. The red lines on (B) and (C) are loess regression.

Fig. 4. Manhattan plot of allele frequency difference after selection by heat. (**A**) Selection effects in CA family. (**B**) Selection effects in AC family. (**C**) Differences in allele frequencies among control samples. Red points show markers at 5% FDR according to the Fisher's combined probability test; blue bars identify regions with significant clustering of such markers (according to 100,000 bootstrapped replicates).

samples (Fig. 4C). Selection against paternal haplotypes aligns well with the putative involvement of mitochondria in heat tolerance determination: Such selection could be due to poor compatibility of certain paternal nuclear alleles with maternal

mitochondria under stress (*13*). There were two genes encoding mitochondrial transporters within 100 kilobases of the top marker under selection (CA cross, LG 10), one of which was significantly associated with larval heat tolerance at the gene expression level (fig. S8 and table S1).

Thermal tolerance measured in our experiments represents just one of many aspects of coral physiology potentially relevant for adaptation to climate change. For example, although molecular responses of larvae and adults to thermal stress are similar (Fig. 3B), larval thermal tolerance may not necessarily translate into adult thermal tolerance. Nonetheless, our study demonstrates heritability of coral stress-related phenotypic and molecular traits and thus highlights the adaptive potential stemming from standing genetic variation in coral metapopulations. Several lines of evidence point toward the importance of mitochondria and mitochondrial-nuclear interactions in determining heat tolerance, including its predominantly maternal inheritance (Fig. 1E), altered expression of mitochondrial proteins in heat-tolerant larvae (Fig. 2D and fig. S3), persistent selection against paternal haplotypes in reciprocal crosses under heat stress (Fig. 4 and fig. S7), and two genes encoding mitochondrial proteins in the vicinity of the major heat-selected marker (fig. S8). High maternal effect on larval thermal tolerance could also be partially due to epigenetic modification, which remains poorly understood in corals. Most importantly, the strong response of two genomic regions to heat selection (Fig. 4) directly confirms that natural variation in heat tolerance is both heritable and evolvable. The genetic rescue scenario, therefore, emerges as a plausible mechanism of rapid coral adaptation to climate change, especially if the natural connectivity of corals across latitudes is enhanced by assisted colonization efforts (*14*).

REFERENCES AND NOTES

1. T. P. Hughes *et al.*, *Science* **301**, 929–933 (2003).
2. O. Hoegh-Guldberg *et al.*, *Science* **318**, 1737–1742 (2007).
3. C. A. Logan, J. P. Dunne, C. M. Eakin, S. D. Donner, *Glob. Change Biol.* **20**, 125–139 (2014).
4. S. R. Palumbi, D. J. Barshis, N. Traylor-Knowles, R. A. Bay, *Science* **344**, 895–898 (2014).
5. D. J. Ayre, T. P. Hughes, *Evolution* **54**, 1590–1605 (2000).
6. M. J. H. Van Oppen, L. M. Peplow, S. Kininmonth, R. Berkelmans, *Mol. Ecol.* **20**, 4899–4914 (2011).
7. P. K. Ingvarsson, *Trends Ecol. Evol.* **16**, 62–63 (2001).
8. E. Meyer, G. V. Aglyamova, M. V. Matz, *Mol. Ecol.* **20**, 3599–3616 (2011).
9. D. J. Barshis *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 1387–1392 (2013).
10. R. L. Tatusov *et al.*, *BMC Bioinformatics* **4**, 41 (2003).
11. P. Langfelder, S. Horvath, *BMC Bioinformatics* **9**, 559 (2008).
12. S. Wang, E. Meyer, J. K. McKay, M. V. Matz, *Nat. Methods* **9**, 808–810 (2012).
13. L. A. Hoekstra, M. A. Siddiq, K. L. Montooth, *Genetics* **195**, 1129–1139 (2013).
14. O. Hoegh-Guldberg *et al.*, *Science* **321**, 345–346 (2008).

Editor's Summary

Some like it hot

Coral reefs are threatened by increasing temperatures. Acute temperature increases stress and
damage corals. However, more gradual temperature changes can result in adaptation and subsequent
tolerance for higher temperatures. Dixon *et al.* show that the heat tolerance that currently exists across
coral populations from different latitudes can be inherited. Thus, natural variation in temperature
tolerance may facilitate rapid adaptation among corals as our climate warms.

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