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Gene expression plasticity as a mechanism of coral adaptation to a variable environment

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Local adaptation is ubiquitous, but the molecular mechanisms that give rise to this ecological phenomenon remain largely unknown. A year-long reciprocal transplant of mustard hill coral (*Porites astreoides*) between a highly environmentally variable inshore habitat and a more stable offshore habitat demonstrated that populations exhibit phenotypic signatures that are consistent with local adaptation. We characterized the genomic basis of this adaptation in both coral hosts and their intracellular symbionts (*Symbiodinium* sp.) using genome-wide gene expression profiling. Populations differed primarily in their capacity for plasticity: following transplantation to a novel environment, inshore-origin coral expression profiles became significantly more similar to the local population's profiles than those in offshore-origin corals. Furthermore, elevated plasticity of the environmental stress response expression was correlated with lower susceptibility to a natural summer bleaching event, suggesting that plasticity is adaptive in the inshore environment. Our results reveal a novel genomic mechanism of resilience to a variable environment, demonstrating that corals are capable of a more diverse molecular response to stress than previously thought.

Populations can respond to spatial environmental variation by specializing to their local environments[1–3]. When this specialization results in a higher fitness of local individuals compared with foreign transplants, populations are considered to be locally adapted[1]. Numerous examples of local adaptation across taxa have been described[2] but the molecular mechanisms that underpin these patterns are not well understood[3].

Gene expression is the proximate mechanism that links genotype to phenotype[4] and plays a central role in cellular adaptation to environmental change[5]. Expression regulation can be highly heritable[6–8] and recent work suggests that expression patterns can evolve in response to differential selection between environments[9]. Once local adaptation has been inferred through higher-order phenotypic traits, it becomes possible to correlate transcriptomic responses with quantitative trait variation across populations[10] and thus to identify the molecular phenotypes putatively facilitating adaptation to spatially varying environments.

This insight will be particularly important for non-model organisms in which traditional genetics approaches aimed at identifying the molecular basis of adaptation are still unfeasible. A reciprocal transplant experiment (Supplementary Fig. 1) demonstrated that inshore and offshore populations of *P. astreoides* corals in the Lower Florida Keys, USA, exhibit elevated growth as well as protein and lipid content in their home reef environment, consistent with local adaptation[11]. Inshore corals from these same populations also exhibit greater thermal tolerance than corals from the offshore reef[12,13]. Here we employ genome-wide gene-expression profiling using TagSeq[14,15] in conjunction with gene coexpression network analysis[16] to explore the relationship between gene expression patterns of the host and symbiont and phenotypic trait variation in the same corals used in the reciprocal transplant experiment[11].

Results and discussion

To quantitatively characterize the variation in gene expression among our coral samples we conducted a discriminant analysis of principal components (DAPC)[17] using all genes that were represented by at least 10 unique transcript counts in more than 90% of samples[18] (host $n=7{,}008$; symbiont $n=1{,}174$). The purpose of DAPC is to find the axis in the multivariate space along which the difference between pre-specified sample groups is maximized[17]. Multidimensional gene expression vectors for additional samples can then be projected onto this axis to quantify genome-wide differences in gene expression in the context of the pre-specified contrast.

First, we used DAPC to differentiate between the genome-wide gene expression profiles of inshore and offshore corals in their respective native environments (Fig. 1). Gene expression in the coral host was clearly differentiated among native inshore and offshore populations (solid distributions, Fig. 1a), and for *Symbiodinium* the difference was also present but less pronounced (Fig. 1b). We then used the native population axis as a measuring scale to score the fragments transplanted across habitats to quantify how far corals were able to shift their expression upon transplantation. The magnitude of this shift represents a quantitative measure of genome-wide gene expression plasticity. We inferred the sizes of these shifts in inshore and offshore corals and their symbionts (the lengths of the orange and blue arrows in Fig. 1, respectively) using Markov chain Monte Carlo (MCMC) linear mixed models incorporating a random effect of coral genotype and derived the *P* value for the population-specific difference by analysing 2,800 MCMC samples of parameter estimates. Inshore corals transplanted to offshore reefs exhibited significantly larger gene expression shifts than offshore corals transplanted to inshore reefs ($P_{MCMC}<0.001$), which resulted in a much closer (although still imperfect) recapitulation of 'native-like' gene expression profiles (Fig. 1a). A similar trend was observed in *Symbiodinium* gene expression profiles ($P_{MCMC}=0.07$, Fig. 1b).

To investigate these differences in more detail and to explore their relationship with other quantitative traits, we conducted weighted gene coexpression network analysis (WGCNA[16]) on the host and symbiont datasets. The 7,008 coral host genes were assigned to 14 coexpression modules, 3 of which showed significant correlations with the site of transplantation and the density of symbiont cells (Fig. 2a,b; $n=2{,}814$ genes total). Genes in the Turquoise ($n=1{,}781$) and Lightcyan ($n=31$) modules were upregulated, while genes in the Yellow module ($n=814$) were downregulated at the inshore

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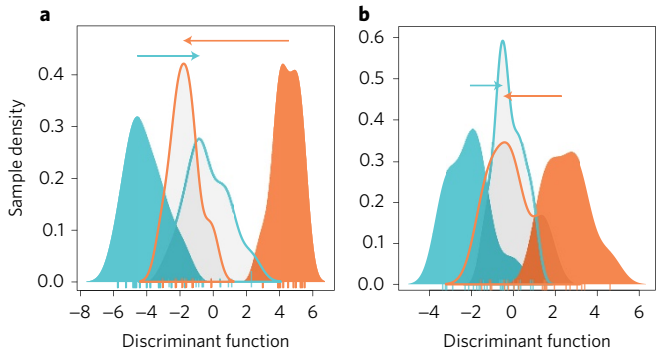

Figure 1 | Population-level variation in genome-wide gene expression plasticity. a, Coral host genes ($n=7{,}008$). **b**, Algal symbiont genes ($n=1{,}174$). The *x* axis is the direction in multivariate gene expression space along which the difference between native inshore and offshore corals is maximized (inshore–offshore discriminant function). The curves are density plots of the four sample groups. Orange (turquoise) indicates corals originating from inshore (offshore) reefs; solid fills indicate corals in their native habitat, whereas transparent fills represent corals transplanted to alternative habitats. Projections of individual samples onto the discriminant function axis are indicated by tick marks. The arrows above the density plots indicate the mean changes in the gene expression profiles as a result of transplantation. Inshore corals are capable of significantly higher genome-wide gene expression plasticity in response to transplantation ($P_{MCMC}<0.001$) and their *Symbiodinium* symbionts exhibit the same trend ($P_{MCMC}=0.07$).

site (Fig. 2b). Closer examination of module eigengene (the first principal component of the expression matrix) expression patterns revealed that the Turquoise module responds to transplantation in both inshore- and offshore-origin corals; however, the magnitude of this response was notably lower in offshore-origin corals (Fig. 3a). Changes in the Yellow module were even more origin-specific, again with minimal regulation observed in offshore-origin corals (Fig. 3b). All of the remaining modules that correlate significantly with transplantation (Pink, Lightcyan and Blue, Fig. 2b) seem to be equally plastic in both populations (Supplementary Fig. 2). Overall, these observations confirm that offshore corals are less capable of gene expression plasticity.

Functional enrichment analysis of the plastic host modules that show the strongest positive (Turquoise module) and negative (Yellow module) correlations with symbiont density indicated differential regulation of the environmental stress response (ESR) among populations. The top 'biological process' gene ontology (GO) enrichment for the Turquoise module was GO:0033554 (cellular response to stress; false discovery rate (FDR) = 0.05) and the top term for the Yellow module was GO:0042254 (ribosome biogenesis; FDR < 0.001, Supplementary Table 1). Taken together, these results demonstrate that corals transplanted to inshore reefs upregulated cellular stress response genes, including molecular chaperones such as heat shock proteins, whereas inshore-origin corals also downregulated ribosome biogenesis, a hallmark of the ESR[5].

The maintenance of homeostasis in the face of environmental variability is a physiological challenge faced by all organisms, but sessile animals, such as reef-building corals, are particularly susceptible as they cannot change habitats to escape stressors[5]. Inshore reefs in the Florida Keys are more variable than offshore reefs for multiple environmental parameters[19], including temperature, and inshore corals exhibit elevated thermal tolerance[12,13]. In the present study, thermal stress was probably responsible for upregulation of the ESR at inshore reefs, as samples were collected at the end of the 2012 summer[11]. *In situ* recordings for the 6 months preceding sample collection show greater variance in the daily temperature range at inshore reefs, as well as multiple time-periods in which temperatures were 1–2 °C higher than at offshore reefs (Supplementary Fig. 3). In addition, coral bleaching—the stress-induced functional loss of the endosymbionts, a common result of elevated temperature[20]—was observed at the time of sample collection, but only in offshore-origin corals that were transplanted to inshore reefs (Supplementary Fig. 4).

To see whether higher ESR expression could have contributed to improved coral fitness under elevated summer temperatures, we examined whether it was associated with the maintenance of symbiont densities at the inshore reef (Fig. 3c–e). Indeed, there was a highly significant positive relationship for 40 genes from the host Turquoise module that were annotated as cellular response to stress genes ($R=0.39$, $P=0.01$, Fig. 3c), while 17 genes from the host Yellow module that represent the GO term 'small ribosomal subunit' (and were expected to be down-regulated during ESR) exhibited a negative trend ($R=-0.29$, $P=0.09$, Fig. 3d). We also examined the relationship between symbiont density and ESR expression plasticity by regressing symbiont density in the inshore-transplanted fragment against the change in ESR expression in that fragment compared with the fragment of the same genotype at the offshore site. For this analysis, a combined ESR eigengene was constructed based on the expression of the 40 cellular response to stress genes from the Turquoise module and 55 ribosome biogenesis genes from the Yellow module. We observed a positive correlation, the strongest that we examined (Fig. 3e, $R=0.49$), although it was only marginally significant due to the reduction in sample size (5 inshore-origin fragment pairs and 10 offshore-origin pairs, $P=0.06$). Furthermore, there was no relationship between the expression of ESR genes in the absence of stress at the offshore site and the maintenance of symbiont densities at the inshore site ($P=0.29$, Fig. 3f). This indicates that differential thermal tolerance was due to the varying capacity for gene expression plasticity rather than elevated pre-emptive expression ('frontloading') of stress-response genes[21].

Furthermore, these results suggest that variation in the bleaching susceptibility of the holobiont (the combination of host and symbiont) could be explained by the ability of the coral host to mitigate intracellular damage resulting from environmental stress. This is in contrast to many other coral species where variation in the bleaching susceptibility is largely attributable to differences in symbiont genotype[22,23], although there are other explanations for the correlation between ESR expression and symbiont density. One possibility is the exact opposite of our hypothesis: that the higher ESR is caused by the lack of bleaching, perhaps because the coral has to tolerate reactive oxygen species produced by heat-stressed symbionts[24]. However, this explanation seems unlikely as we observed no molecular signs of stress in the symbionts (Supplementary Table 1). Another explanation could be that a higher ESR promotes not resistance to bleaching, but more rapid recovery. As our corals were sampled more than three weeks after the last prolonged heating episode at the inshore site (Supplementary Fig. 3), it is possible that some fragments had bleached and recovered before our sampling time-point. Further experiments are required to investigate the physiological underpinnings of the correlation between ESR and symbiont density.

In *Symbiodinium*, 1,174 genes were assigned to seven coexpression modules, none of which showed correlations with symbiont density, although the Red module was correlated with chlorophyll *a* content (Fig. 2c,d). Expression in two modules was correlated with the site of transplantation, and expression plasticity in the Red module tended to differ among populations, with inshore symbionts showing a greater change than offshore symbionts (Supplementary Fig. 5). The Red module comprised few genes ($n=98$) with the most significantly enriched (although not formally significant following FDR-correction) GO term being GO:0009521 (photosystem; Supplementary Table 1). The genes in the module annotated with this

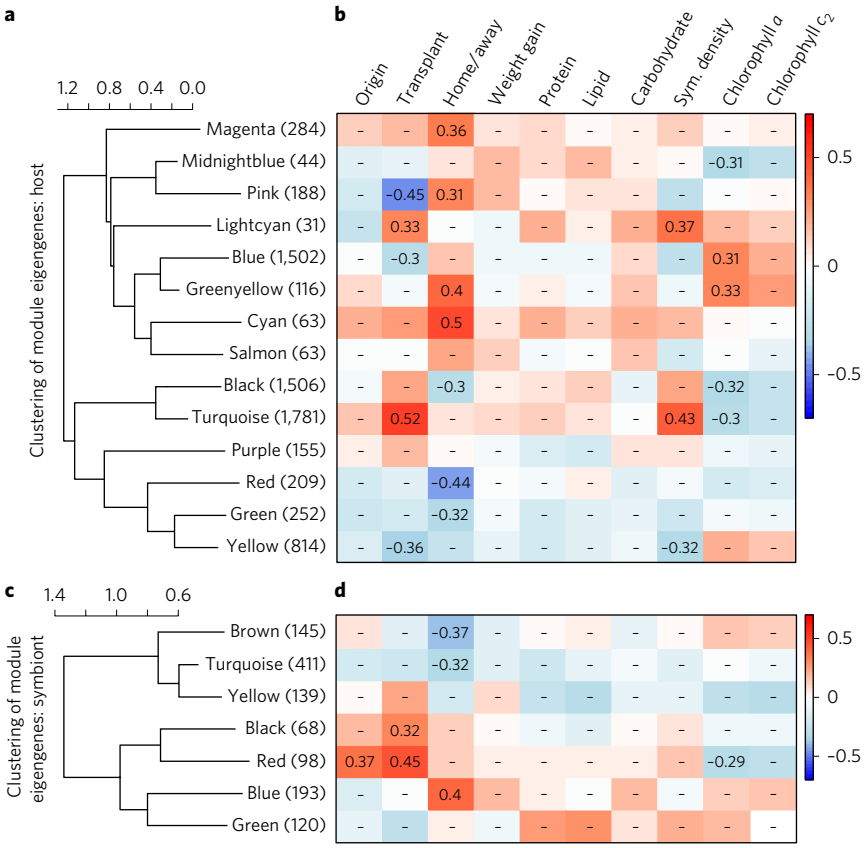

Figure 2 | Hierarchical clustering dendrogram of module eigengenes (the first principal component of a module, representative of the overall expression profile for genes within that module) and heat maps of module-trait correlations. a,**b**, Coral host. **c**,**d**, *Symbiodinium*. In **a** and **c**, the number of genes in each module is indicated in parentheses, and the scale corresponds to the average distance between all inter-cluster pairs. In **b** and **d**, red indicates a positive correlation, blue a negative correlation, and Pearson's *R* for significant correlations ($P < 0.05$) are reported. 'Origin', 'Transplant' and 'Home/away' are categorical traits, coded in binary, indicating site of origin (inshore = 1, offshore = 0), site of transplant (inshore = 1, offshore = 0) or whether corals were transplanted to the native or foreign reef site (native = 1, non-native = 0), respectively. Sym., symbiont.

GO term included components of the peripheral light-harvesting complex, such as fucoxanthin-chlorophyll *a*/*c* binding proteins. A decrease in peripheral light-harvesting complexes limits the risk of photodamage to D1 reaction centre proteins and has been proposed as a photoprotection mechanism of *in hospite Symbiodinium* in response to stress[25]. If *Symbiodinium* self-protection was the reason for the elevated thermal tolerance of inshore-origin corals, inshore-origin symbionts should exhibit downregulation of these photosystem genes at inshore reefs where bleaching was observed (Supplementary Fig. 4). However, the opposite pattern was evident: native inshore-origin *Symbiodinium* exhibited elevated expression in comparison with offshore-origin transplants (Supplementary Fig. 5). Moreover, none of the identified symbiont modules was functionally associated with stress response (Supplementary Table 1). This suggests that symbiont performance under thermal stress is maintained not by the symbionts' own stress protection mechanisms, but through plasticity of the host ESR genes.

Increased plasticity is predicted to evolve in a population if reaction norms vary across genotypes and the slope of the reaction norm is positively correlated with fitness[26,27]. Assuming that the expression of stress response genes is energetically costly[28], a trade-off is expected where enhanced plasticity would be beneficial in a variable environment, such as the inshore reef, but detrimental in a stable environment, such as the offshore reef. We estimated the costs of stress response expression plasticity using the selection gradient method[29,30] by regressing the relative fitness (weight gain) of corals in their home environment against mean expression at home and the magnitude of the expression change on transplantation (plasticity). The partial regression coefficient for the plasticity term provides an estimate of cost while controlling for the trait's mean. A positive value indicates selection for plasticity, while a negative value indicates selection against plasticity[31]. The estimated plasticity coefficient for expression of cellular stress response genes ($n = 40$ genes) was positive for inshore-origin corals (0.11) and negative for offshore-origin corals (−0.02), suggesting that divergent selection acted among populations, consistent with environmental variation among reef sites. However, as neither coefficient was significantly different from zero, additional work is needed to confirm this hypothesis.

Given the difference in thermal regimes between inshore and offshore reefs in the Lower Florida Keys it is expectable that inshore corals have adapted and/or acclimated to their native reef environments[11] and exhibit elevated thermotolerance[12,13]. The surprising result of our study is that these higher-order phenotypic responses may be explained by a differential capacity for gene expression plasticity. The population-level difference in gene expression plasticity described here is also in contrast to another recently reported mechanism for coral adaptation to temperature variation. *Acropora hyacinthus* corals from more thermally variable pools exhibited constitutive upregulation of ESR transcripts, or frontloading[21]. However, while ESR plasticity is associated with higher bleaching resistance (that is, the maintenance of *Symbiodinium* densities, Fig. 3e), we find no relationship between bleaching resistance and the baseline ESR expression levels as observed in the same genotypes at the stable offshore reef environment; the trend is in fact negative (Fig. 3f). The difference between bleaching-resistance strategies (plasticity versus frontloading) may be due to the frequency at which coral

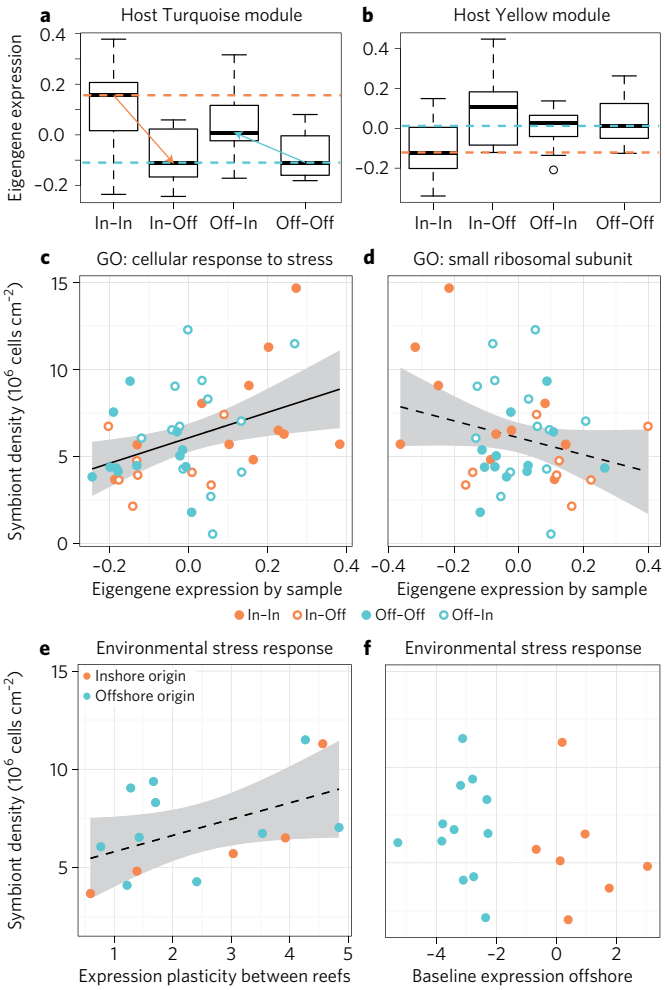

Figure 3 | Inshore coral hosts show greater gene expression plasticity of major symbiont-correlated modules than offshore corals. a,**b**, The boxplots show the median and interquartile range of module eigengenes (the first principal component of the expression matrix) with respect to the site of origin and transplant environment for the host Turquoise (**a**) and Yellow (**b**) modules. Whiskers extend 1.5 times beyond the interquartile range and open circles indicate outliers. Coloured dashed lines indicate the origin-specific medians. **c**,**d**, Corals with the strongest ESR maintained the highest symbiont densities. The regression of the symbiont densities on the eigengenes for the significantly enriched (FDR ≤ 0.05) gene ontology terms cellular response to stress ($n = 40$ genes, host Turquoise module, R: 0.39, 95% CI (0.10, 0.61), $P = 0.01$) (**c**) and small ribosomal subunit ($n = 17$ genes, host Yellow module, R: −0.26, 95% CI (−0.51, 0.04), $P = 0.09$) (**d**) are shown. **e**, Coral genotypes with a greater plasticity of combined ESR expression (eigengene of cellular response to stress and ribosome biogenesis GO categories from the Turquoise and Yellow modules) maintained higher symbiont densities when transplanted to inshore reefs (R: 0.49, 95% CI (−0.03, 0.80), $P = 0.06$). **f**, Combined ESR expression in the absence of stress at the offshore site was not associated with a better ability to maintain symbiont densities at the inshore site (R: −0.25, 95% CI (−0.64, 0.23), $P = 0.29$). Solid lines indicate significant associations ($P < 0.05$), dashed lines indicate marginally significant associations ($P < 0.1$) and shading around regression lines indicates the 95% confidence interval.

populations are exposed to thermal stress events. The dominant cycle of temperature fluctuations in the Florida Keys occurs on an annual scale[11], while the corals studied by Barshis *et al.*[21] experience dominant fluctuations on a daily basis, during tidal cycles. Theory predicts that constitutive expression of an adaptive phenotype will be favoured over plasticity if the environment fluctuates more rapidly than the typical response time[26]. The constitutive upregulation of ESR genes by corals in tidal pools[21] suggests that these populations integrate over the periodicity of stress events, analogous to a constant stress environment. In contrast, the variable expression of ESR genes in the inshore coral populations observed here suggests that these corals have adopted an alternate solution, employing adaptive plasticity to cope with annual cycles of temperature variation in the Florida Keys. However, it is important to keep in mind that *Acropora* spp. and *Porites* spp. are divergent lineages and macroevolutionary changes in physiology could also be responsible for their divergent gene expression responses.

Taken together, our results show that inshore corals from a more variable thermal environment developed an ability to more dynamically regulate expression of environmental stress response genes, which is associated with maintenance of *Symbiodinium* densities following thermal stress. Understanding the capacity of coral populations to adapt or acclimatize to local thermal stress is paramount for predicting the responses of corals to future climate change. Plasticity may accelerate evolution by facilitating genetic variance[32], as well as by allowing coral populations to exploit novel emerging conditions[33]. Future work should aim to investigate how different strategies of constitutive frontloading and expression plasticity affect the capacity of coral populations and species to adapt to changing climates in the long term.

Methods

Sample collection and processing. The transplantation experiment has been fully described previously[11]. Briefly, 15 genotypes (individual colonies) of *P. astreoides* from both an inshore and an offshore reef ($n = 30$ total) in the Lower Florida Keys, USA, were fragmented, and pieces of each genotype were outplanted at both native and foreign sites (Supplementary Fig. 1) under Florida Keys National Marine Sanctuary permit no. 2011–115. Following one year of transplantation, coral growth rates, energetic stores (total protein, lipid and carbohydrate content), symbiont densities and chlorophyll content were measured for each genotype. Tissue samples of 1 cm^2 were immediately taken from each coral fragment on field collection and preserved in RNALater (Ambion, Life Technologies) on ice. Samples were stored at −80 °C until processing. Total RNA was extracted using an RNAqueous kit (Ambion, Life Technologies), with minor modifications. Samples homogenized in a lysis buffer were kept on ice for 1 h with occasional vortexing to increase RNA yields, which was followed by centrifugation for 2 min at 16,100*g* to precipitate skeleton fragments and other insoluble debris; 700 µl of the supernatant was used for RNA purification. At the final elution step, the same 25 µl of elution buffer was passed twice through the spin column to maximize the concentration of eluted RNA. Samples were DNAse treated as described previously[34]. One microgram of total RNA per sample was used for tag-based RNA-seq, or TagSeq[14], with modifications for sequencing on the Illumina platform. TagSeq has recently been demonstrated to generate more accurate estimates of protein-coding transcript abundances than standard RNA-seq, at a fraction of the cost[15].

Bioinformatic analysis. A total of 45 libraries prepared from each biological sample were sequenced on the Illumina HiSeq 2500 at the Genome Sequencing and Analysis Facility at UT Austin. Although 60 samples were originally outplanted (30 paired genotype replicates, 15 from inshore and 15 from offshore), 9 were lost due to hurricane damage[11] and 6 more were discarded during sample preparation for poor RNA or cDNA quality. Overall, 527.9 million raw reads were generated, with individual counts ranging from 3.2 to 26.3 million per sample (median = 11.1 million reads, NCBI SRA accession code: PRJNA327141). A custom perl script was used to discard reads sharing the same sequence as the read and degenerate adaptor (PCR duplicates) and to trim the leader sequence from remaining reads. The fastx_toolkit (http://hannonlab.cshl.edu/fastx_toolkit) was then used to trim reads after a homopolymer run of 'A' ≥ 8 bases was encountered, to retain reads with a minimum sequence length of 20 bases, and to quality filter, requiring PHRED of at least 20 over 90% of the read. A total of 0.3–2.1 million reads per sample (median = 0.9 million reads) remained after quality filtering. The *P. astreoides* transcriptome[35] was concatenated to a *Symbiodinium* Clade A reference[36], as *P. astreoides* host A4/A4a-type symbionts in the Florida Keys[12,37]. Filtered reads were mapped to this combined reference transcriptome with Bowtie2[38], using the -sensitive-local flag. Read counts were assembled by isogroup (groups of sequences putatively originating from the same gene, or with sufficiently high sequence similarity to justify the assumption that they serve the same function) using a custom perl script. Reads mapping to multiple isogroups were discarded. This count file was split into host-specific and symbiont-specific isogroup

files for subsequent analyses. In total, 137,542–769,503 unique reads per sample (median = 354,184 reads) mapped to 26,000 host isogroups and 7,393–66,623 unique reads per sample (median = 26,760 reads) mapped to 21,257 symbiont isogroups.

Differential expression, coexpression network and functional enrichment analyses. Analyses were carried out in the R statistical environment[39]. Low-expression genes (those with less than 10 counts in more than 90% of samples)[18] were removed from the dataset, leaving 7,008 and 1,174 highly expressed genes in the host and symbiont datasets, respectively. Gene counts in both the host and symbiont datasets were normalized and log-transformed using a regularized log transform with the command rlog() in DESeq2[40] for subsequent analyses.

The resulting sample group sizes for gene expression analysis were: inshore to inshore ($n=11$), inshore to offshore ($n=9$), offshore to inshore ($n=13$) and offshore to offshore ($n=12$). A DAPC was used to compare expression of all 7,008 highly expressed genes for host corals, and 1,174 genes for symbionts using the adegenet package[17,41]. A discriminant function was built by defining native transplants as groups (inshore corals transplanted to inshore reefs, and offshore corals transplanted to offshore reefs). To compare gene expression plasticity in response to transplantation among populations, we used the MCMCglmm package[42] in R to model DAPC score as a function of origin plus the origin-specific effect of being transplanted away from home (these parameters correspond to the means of solid-fill curves and sizes and directions of arrows in Fig. 1). With this model we generated 2,800 sets of parameter estimates representing samples from the posterior. To derive MCMC-based *P* values we calculated the difference between the absolute values of origin-specific effects of being transplanted away from home (the length of the orange arrow minus the length of blue arrow in Fig. 1). For all sampled parameter sets, the *P* value is the number of negative differences divided by the total number of sampled parameter sets.

WGCNA analysis was carried out following tutorials for undirected WGCNA on RNAseq data[16,43,18]. The analysis was blind to experimental design and involved five steps: (1) variance stabilized counts were filtered for outlier samples (one host and one symbiont sample were discarded in this step, leaving $n=8$ inshore to offshore samples for the host and $n=10$ inshore to inshore samples for the symbiont); (2) Pearson correlations for all gene pairs across all samples were computed to construct a similarity matrix of gene expression, retaining the sign of the expression change (signed networks); (3) expression correlations were transformed into connection strengths (connectivities) through a power adjacency function, using a soft thresholding power of 6 for the host and 10 for the symbiont, based on the scale-free topology fit index (Supplementary Fig. 6); (4) hierarchical clustering of genes based on topological overlap (sharing of network neighbourhood) was performed to identify groups of genes whose expression covaried across samples (network modules), retaining modules with at least 30 genes and merging highly similar modules (with module eigengenes correlated at $R>0.85$) (Supplementary Figs 7, 8); and (5) external trait data was related to the expression of inferred modules (Fig. 2).

Functional enrichment analyses were conducted using the GO_MWU package to identify over-representation of particular functional groups within modules of the host and symbiont datasets, based on GO classification[44]. For each GO term, the number of annotations assigned to genes within a module was compared to the number of annotations assigned to the rest of the dataset, to evaluate whether any ontologies were more highly represented within the module than was expected by chance (Fisher's exact test).

Plasticity versus baseline expression and selection gradient estimation. The ESR eigengene was calculated by sample for functionally annotated genes within the Turquoise ($n=40$, 'cellular response to stress') and Yellow modules ($n=55$, 'ribosome biogenesis'). The difference between native and non-native expression of the ESR eigengene was then calculated for individual genotypes ($n=5$ paired inshore-origin corals, $n=10$ paired offshore-origin corals) as a metric of expression plasticity and this metric was regressed against *Symbiodinium* cell density at the inshore reef site. Baseline expression of the ESR eigengene in the absence of stress at the offshore reef site was also regressed against the symbiont cell density quantified for those same genotypes at inshore reefs to quantify the contribution of frontloading[21] to the maintenance of *Symbiodinium* densities following environmental stress.

We quantified the costs of stress response expression plasticity for inshore and offshore populations by relating fitness to the mean expression of the 40 genes that comprise the cellular response to stress functional enrichment (Supplementary Table 1) at native reefs and the difference in expression between environments following[29,30]. Relative fitness was defined as the growth of an individual genotype at its native reef relative to the maximum possible weight gained by a coral within each environment[11]. The mean expression level and the difference in expression in response to transplantation (expression plasticity) were calculated for individual genotypes using standardized DAPC scores for this gene subset.

Code availability. The current protocol and bioinformatics scripts for TagSeq are maintained at https://github.com/z0on/tag-based_RNAseq. R scripts and input files for conducting the gene expression and statistical analyses described here can be found on the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.2bv13. R scripts and example input files for the GO_MWU package are maintained at https://github.com/z0on/GO_MWU.

Data availability. Raw Illumina sequence data that support the findings of this study can be obtained from NCBI's SRA under the accession code PRJNA327141.

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Author contributions

C.D.K. and M.V.M. conceived and designed experiments. C.D.K. performed experiments, analysed data and wrote the first draft of the manuscript. Both authors contributed to revisions.

Additional information

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Competing interests

The authors declare no competing financial interests.