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Report

Multilocus Adaptation Associated with Heat Resistance in Reef-Building Corals

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Summary

The evolution of tolerance to future climate change depends on the standing stock of genetic variation for resistance to climate-related impacts [1, 2], but genes contributing to climate tolerance in wild populations are poorly described in number and effect. Physiology and gene expression patterns have shown that corals living in naturally high-temperature microclimates are more resistant to bleaching because of both acclimation and fixed effects, including adaptation [3]. To search for potential genetic correlates of these fixed effects, we genotyped 15,399 single nucleotide polymorphisms (SNPs) in 23 individual tabletop corals, *Acropora hyacinthus*, within a natural temperature mosaic in backreef lagoons on Ofu Island, American Samoa. Despite overall lack of population substructure, we identified 114 highly divergent SNPs as candidates for environmental selection, via multiple stringent outlier tests, and correlations with temperature. Corals from the warmest reef location had higher minor allele frequencies across these candidate SNPs, a pattern not seen for noncandidate loci. Furthermore, within backreef pools, colonies in the warmest microclimates had a higher number and frequency of alternative alleles at candidate loci. These data suggest mild selection for alternate alleles at many loci in these corals during high heat episodes and possible maintenance of extensive polymorphism through multilocus balancing selection in a heterogeneous environment. In this case, a natural population harbors a reservoir of alleles preadapted to high temperatures, suggesting potential for future evolutionary response to climate change.

Results

Thermal Environment

On Ofu Island in the National Park of American Samoa, a natural temperature mosaic over a small spatial scale provides an ideal natural laboratory for studying temperature adaptation. Two adjacent backreef pools differ in temperature regime: the highly variable (HV) pool experiences temperatures as high as 35°C and as low as 24.5°C [4], whereas the moderately variable (MV) pool ranges from 32°C–25°C (Figure 1A; Figure S1 available online). Corals in these pools bleach at temperatures of 33°C–35°C [5, 6] but tolerate cool temperatures of 24°C for over a week (R.A.B. and S.R.P., unpublished data). As a result, temperature variability in the HV pool appears to be stressful at the extreme upper ranges. We have previously shown that colonies of *Acropora hyacinthus* (sp. E in [7]) from the HV pool are more heat resistant in standardized tests conducted over 24 hr [3] or 72 hr [5].

We mapped temperatures experienced by 23 *A. hyacinthus* colonies over a 7 month period, February 2011 to August 2011. Colonies in the HV pool surpassed 31°C, the approximate expected bleaching threshold for American Samoa (http://www.coralreefwatch.noaa.gov), 0.47%–0.95% of the time (average = 0.67%), whereas individuals from the MV pool spent only 0%–0.20% of the time above 31°C (average = 0.09%) (Figure 1B). The amount of time spent above 31°C among individuals is also significantly more variable in the HV pool (F test p = 0.01). Similar differences occur between pools at 32°, 33°, and 34°C thresholds.

Colony SNP Differences and F_{ST} Outliers

To identify single nucleotide polymorphism (SNP) correlates of environment, we constructed and sequenced cDNA libraries from these same 23 *A. hyacinthus* colonies from the HV pool (n = 10) and MV pool (n = 13). Mapping reads to the annotated transcriptome from Barshis et al. [6] showed 107,693 high-quality SNPs, of which 15,399 could be confidently called in all individuals. F_{ST} calculations between the HV and MV pools showed higher F_{ST} values than expected across the 15,399 SNPs for all F_{ST} greater than 0.05 (95.5% randomizations had corrected Kolmogrov-Smirnov test $p \leq 0.05$, Figure S2). An F_{ST} outlier test showed 2,807 significant outliers (false discovery rate [FDR]-corrected $p \leq 0.05$), of which 1,445 had $F_{ST} > 0.05$. Because such tests likely yield many false positives [8], we also conducted a bootstrap resampling scheme. We conducted multiple runs of the simulation-based outlier analysis and discarded loci that did not appear in at least 70% of analyses, a cutoff commonly used for phylogenetic analyses [9]: a total of 251 SNPs passed this bootstrap filter.

Temperature and Colony Genetics

As a second way to sort loci for environmental selection, we filtered outlier loci for those at which individual genotypes correlated highly with individual temperature measurements. We used permutational ANOVA to compare genotype with fraction of time spent above 31°C for each individual, a metric that is consistent across multiple seasons ($p < 0.01$; Figures 3A and S1). Among all SNPs, this analysis yielded 1,006 loci associated with temperature (uncorrected $p \leq 0.05$). Of these, 114 were also F_{ST} outliers with $\geq$70% bootstrap score and $F_{ST} > 0.05$. Because these loci passed tests for outlier status, high F_{ST}, and genotype correlation with colony temperature, we used these 114 SNPs as candidates for environmental selection (Table S1).

For each SNP, we denoted the most frequent allele as the "reference" allele and the other allele as the "alternate" or "minor" allele. One unexpected pattern was that minor allele frequencies for candidate SNPs were generally higher in the HV pool, instead of having been evenly distributed among populations as were the noncandidate SNPs (Figure 2). For example, a homolog of the deformed epidermal autoregulatory factor (*DEAF1*) contains eight candidate SNPs. For seven of these SNPs, all individuals in the HV pool possessed at least one copy of the alternate allele (Figure 3B). Yet in the MV pool, the reference homozygote was the most common genotype. Across all candidate SNPs, each HV pool coral had alternate alleles at, on average, 57 of the 114 candidate loci, compared

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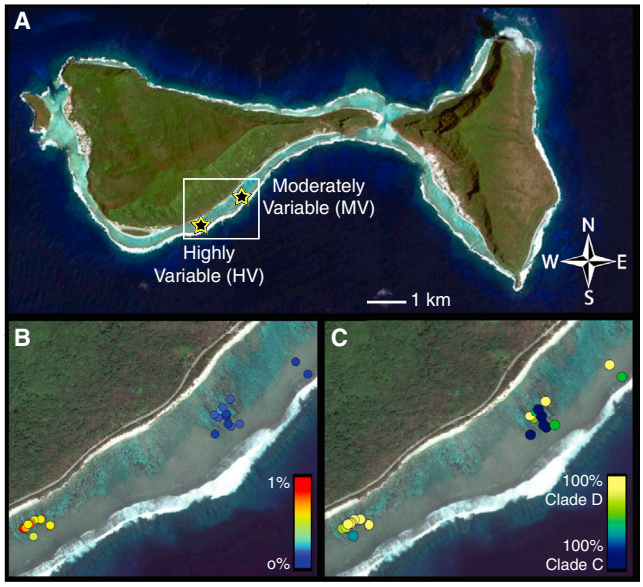

Figure 1. Sampling Sites, Thermal Environment, and Symbiont Type

(A) Map of sampled sites on Ofu Island, American Samoa. The white box highlights the region that is expanded in (B) and (C).

(B) Proportion of time spent above 31°C for each individual sampled from the highly variable (HV) and moderately variable (MV) pools.

(C) Results from symbiont typing analysis.

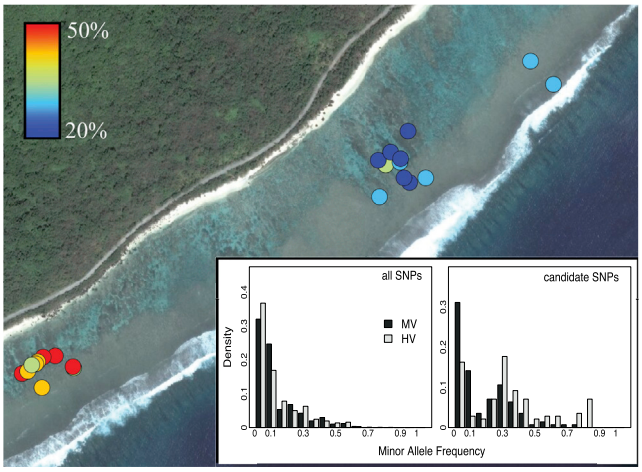

Figure 2. Polymorphism at 114 Candidate SNPs Is Maintained across a Heterogeneous Environment

Top: frequency of alternate (minor) alleles in candidate genes in the HV and MV pools. Colors indicate the percent of candidate loci with minor alleles for each individual. Inset shows histograms of minor allele frequency distributions. Left panel of inset shows distributions for all 15,399 SNPs, whereas the right panel shows distributions for 110 candidate SNPs for environmental selection.

to 32 loci in MV individuals ($p < 0.001$; Figure 2; Table S2). We also found a strong correlation between the fraction of time spent above 31°C for each colony and the fraction of loci with alternative alleles (Figure 3C: $R^2 = 0.74$, $p < 0.001$).

Because these differences might include a pool effect, not just a temperature difference, we also compared corals living in different temperature microclimates within pools. Although we had little power to detect significant trends within pools, we saw a positive trend within the MV when the number of alternate alleles at candidate loci was compared to thermal environment (Figure 3C). Although outliers in the HV pool obscured a similar trend, grouping colonies based on microclimate showed a similar pattern—individuals in warmer microclimates within the HV pool harbored more alternate alleles at candidate loci (Figure S3).

Overall colony genetic relationships also show higher variance in the HV pool. Our initial principal-component analysis (PCA) (Figure 4A) had four obvious outliers, three from the HV pool and one from the MV pool. The outlier from the MV pool (AH28) has the highest fraction of candidate loci with alternate alleles and lives in the third warmest microclimate. With these individuals removed, the PCA yielded six outliers, all from the HV pool. Individuals did not strictly cluster by pool; rather, warm microclimate colonies were scattered across the PCA, and HV individuals from the coolest microclimate group were grouped within the cooler MV cluster (Figure S4). Other principal-component (PC) axes were related more directly to temperature. PC4 was highly correlated with time above 31°C and was strongly influenced by variation in candidate SNPs: 34 of these loci (29.8%) were weighted in the top 5% on PC4.

Location, Temperature, and Symbionts

Every individual we sampled hosts a mixture of the thermally tolerant clade D of *Symbiodinium* and the less tolerant clade C [10–13]. However, most colonies in the HV pool were dominated (>85%) by clade D, whereas colonies from the MV pool more often hosted either clade C or D (Figure 1C). Because of this location effect, symbiont clade covaried with temperature across pools. However, within pools, the relationship was weak ($R^2 = 0.09$, $p = 0.16$), and there were no strong genetic correlations of any of our loci with symbiont type within the MV or HV pools. These data suggest that symbiont type does not play a major role at the loci we characterize here.

Gene Families Associated with Selection Candidates

Of 92 contigs that contain our 114 candidate SNPs, 68 had annotations from protein databases (Table S1) [6]. Candidate loci included proteins involved in heat stress response, including heat shock genes (3 contigs), protein recycling (3 contigs), and apoptosis. Although these gene families are known to be involved in heat stress reaction in corals [6, 14–16], formal gene ontology (GO) analysis showed no gene categories with significant enrichment (FDR $p > 0.05$) among our candidate genes.

Discussion

SNP variation at 114 loci is correlated with thermal environment in the coral *A. hyacinthus*. Many loci appear to be involved, rather than a few key genes. Similar to other studies of the evolution of complex environmental traits in natural populations, this result is consistent with a model of multigenic adaptation that is the cumulative result of a number of small-effect variants across many different cellular pathways [17, 18]. This pattern is similar to that observed in several forest tree species where adaptive variation is not the result of one allelic variant but rather the product of a number of small-effect variants [18]. In effect, thermal tolerance may be a quantitative trait conferred by alleles at many interacting loci. Such adaptive genetic variation over small spatial scales may provide a source of beneficial variation as climate change increases global ocean temperatures.

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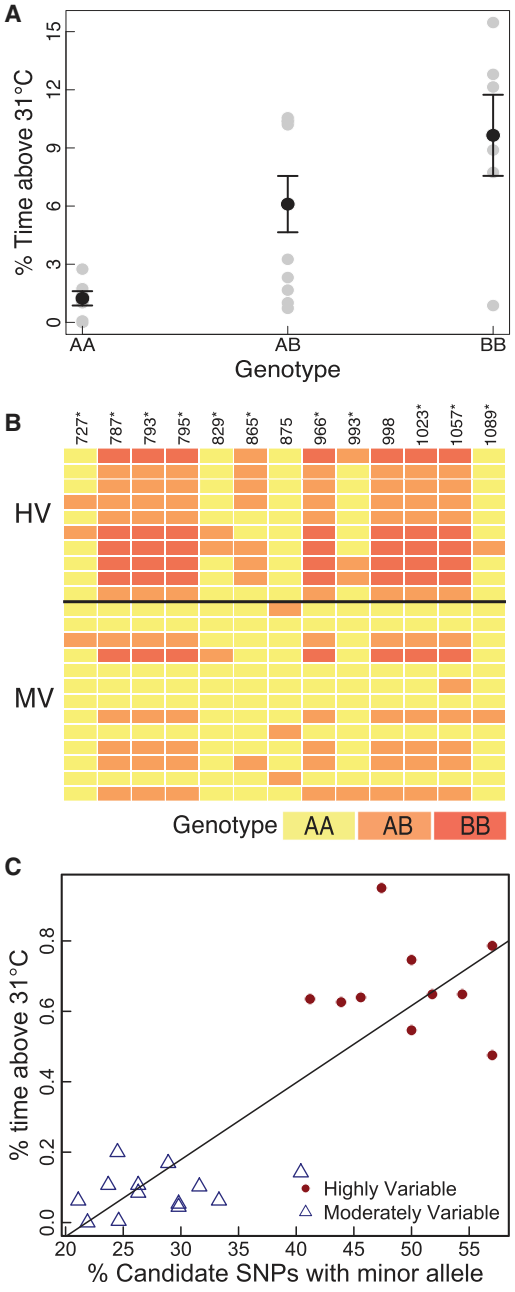

Figure 3. Relationship between Genotype and Thermal Environment

(A) Relationship between genotype and fraction of time spent above 31°C for the *DEAF1* homolog. Error bars indicate SEM for each genotype category.
(B) Genotypes at 13 SNPs of the *DEAF1* homolog, which could be genotyped in all individuals. Here, each row represents an individual coral colony, and each column represents an SNP. Column names indicate the position of the SNP within the contig from the *A. hyacinthus* transcriptome assembly [10]. Positions with amino acid changing SNPs are marked with an asterisk (*).
(C) Significant relationship ($R^2 = 0.74$, $p < 0.001$) between the number of loci at which a coral has alternative alleles for candidate loci and the temperature at which that coral lives.

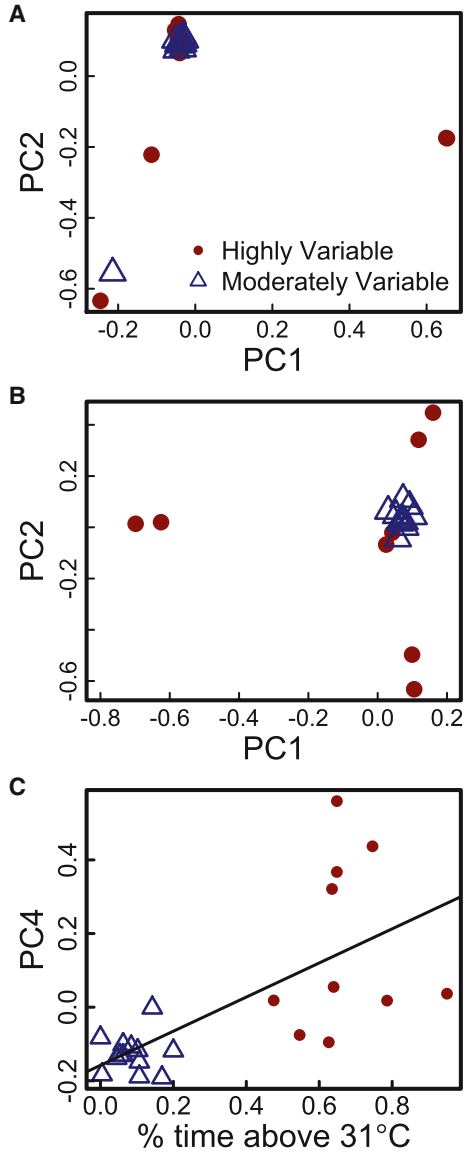

Figure 4. Principal-Component Analysis of 15,399 SNPs for *A. hyacinthus* Colonies on the Backreef of Ofu Island

Principal-component analysis (PCA) of 15,399 SNPs genotyped in 23 *A. hyacinthus* individuals.
(A) PCA with all individuals.
(B) PCA with four outlier individuals removed.
(C) Correlation ($R^2 = 0.42$, $p = 0.0004$) between the fourth principal component axis (PC4) and the proportion of time an individual spends above 31°C.

Patterns and Process of Natural Selection

Correlations between individual genetics and climate variables have been discovered in a number of species, including forest trees [19, 20], herbaceous plants [21, 22], and humans [23]. In our system, 114 SNPs, about 0.7% of the total, have signals of selection, a fraction similar to other marine studies that used comparable F_{ST} outlier approaches filtered by multiple criteria. For example, DeWit and Palumbi [24] found 3% of 21,579 SNPs to be F_{ST} outliers in populations of red abalone, and Hess et al. [25] found the same fraction in Pacific lamprey. However, those studies were done over much larger spatial scales (600–2,000 km). Corals such as *A. hyacinthus* have a 7–10 day period of free-swimming larval development [26]. Because pools are so close together compared to dispersal scale, selective differentiation at over 100 loci probably demands high selection pressure on settling coral larvae. The genetic load implied by this selection may require many settling coral larvae to die as a result of mismatches between genotype and environment [27].

Our data show that candidate SNPs for environmental selection have higher minor allele frequencies in the HV pool compared to the MV pool and that corals with a larger number of alternative alleles live at higher local temperatures (Figure 3A). These patterns are consistent with spatial balancing selection, when habitats with different selection pressures select for different alleles from a common gene pool [28]. Because the HV pool is small relative to the remainder of the reef, the overall frequencies of beneficial alleles are expected to be small. Colonies may be succeeding in several different ways depending on their genetic makeup, and it is possible that the number of loci at which an individual has the HV alternate allele relates to the individual's fitness (Figures 2 and 3A).

The observed pattern also suggests a fitness tradeoff: if an alternative allele is beneficial in the HV pool, but not detrimental elsewhere, then that allele should rise to fixation. The maintenance of polymorphism therefore suggests that either the two environments favor different genotypes or sufficient time has not passed for them to rise to fixation. Further studies on fitness effects of putatively adaptive variants will help differentiate between these possible scenarios.

Heat Tolerance and Local Microclimate

Corals in the HV and MV pools, including some of the same corals used here, show different levels of heat tolerance and also show marked differences in gene expression during experimental heat stress [6]. Palumbi et al. [3] performed reciprocal transplants between the HV and MV pools and showed that about half the increase of heat tolerance in the HV pool was due to acclimation of *A. hyacinthus*, and approximately half was due to fixed effects, including adaptation and epigenetic effects. These studies also examined differential gene expression during heat stress, and many of the dominant pathways are the same as those that we found to have candidate SNPs.

For example, studies of stress response in coral have found regulation of heat shock proteins and related transcription factors [6, 14, 15]. In our data, we see a signal for selection in proteins that interact with heat shock proteins, such as *HSC70*. The ubiquitin pathway is also known to be involved in heat stress and thermal tolerance [29, 30]. In addition, regulation of antioxidant defense and apoptosis genes, many of which were identified as selection candidates, is known to change expression in response to heat stress in corals [6, 15, 16]. Perhaps such physiological responses to stress have become incorporated into the genome through coding-region changes that alter gene function in an environment where individuals regularly experience stressful conditions. It is also possible that our focus on coding-region SNPs has missed numerous SNPs in gene regulatory regions, which appear to be common in genetic systems research (see [31] for review). Lastly, pool effects besides temperature may play a role in selection among our candidate loci.

Standing Variation and Climate Change Response

Our results pinpoint SNPs that are potentially adapted to different levels of thermal variability between pools. The data suggest standing variation in thermal tolerance within populations of *A. hyacinthus* and that selection in the HV pool produces genetic change at a number of loci. As ocean temperatures increase, beneficial genetic variants may become important for the maintenance of populations and species. Especially in highly dispersing species, these adapted populations might be able to seed more susceptible ones. Many current models that predict species response to climate change, however, assume that thermal tolerance is static not only within a population but also over time. For example, the model used by the National Oceanic and Atmospheric Administration to predict coral reef bleaching as well as long-term response assumes a fixed bleaching threshold that varies over latitude, but not temporally [32]. Studies have shown that incorporating adaptation into these models could greatly impact predictions for the future of coral reefs [33, 34].

Our results uncover a wealth of possible beneficial genetic variation and suggest a capacity for future adaptation of coral populations. Given that there is a role for genetic adaptation in thermal tolerance, future studies could focus on the rates at which this adaptation occurs. The relationship between the adaptive rate and the rate of temperature increase will determine whether natural selection can potentially mitigate negative impacts of anthropogenic climate change.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, four figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.10.044.

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