



DIFFERENCES IN THE REGULATION OF GROWTH AND BIOMINERALIZATION GENES REVEALED THROUGH LONG-TERM COMMON-GARDEN ACCLIMATION AND EXPERIMENTAL GENOMICS IN THE PURPLE SEA URCHIN

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Across heterogeneous landscapes, populations may have adaptive differences in gene regulation that adjust their physiologies to match local environments. Such differences could have origins in acclimation or in genetically fixed variation between habitats. Here we use common-garden experiments to evaluate differences in gene expression between populations of the purple sea urchin, *Strongylocentrotus purpuratus*, spanning 1700 km and average temperature differences of 5°C to 8°C. Across expression profiles from 18,883 genes after 3 years of common conditions, we find highly correlated expression patterns (Pearson's $r = 0.992$) among most genes. However, 66 genes were differentially expressed, including many ribosomal protein and biomineratization genes, which had higher expression in urchins originally from the southern population. Gene function analyses revealed slight but pervasive expression differences in genes related to ribosomal function, metabolism, transport, "bone" development, and response to stimuli. In accord with gene expression patterns, a post-hoc spine regrowth experiment revealed that urchins of southern origin regrew spines at a faster rate than northern urchins. These results suggest that there may be genetically controlled, potentially adaptive differences in gene regulation across habitats and that gene expression differences may be under strong enough selection to overcome high, dispersal-mediated gene flow in this marine species.

KEY WORDS: Climate change, ecological genomics, gene flow, natural selection, RNA-seq, *Strongylocentrotus purpuratus*.

Differences in the regulation of gene expression are essential for species persistence across diverse habitats (King and Wilson 1975; Whitehead and Crawford 2006a). Moreover, evolution of gene regulation has been shown to be an important mode of local adaptation when there is high variability in environmental conditions within and among regions of a species' range (Gilchrist and

Huey 2004; Swindell et al. 2007; Levine et al. 2011). This may be particularly true for many marine and plant species that are widely distributed, inhabiting diverse physical and biological environmental conditions (Waples 1998; Grosberg and Cunningham 2001; Sanford and Kelly 2010; Whitehead et al. 2011). However, in these systems, there are two major challenges to understanding

the role of gene expression evolution in adaptive diversification. First, gene regulation is inherently flexible, and so distinguishing genetically determined differences between populations from environmentally induced, phenotypically plastic gene expression differences is difficult. Second, gene flow among regions poses a challenge for local adaptation; differences between populations, whether neutral or adaptive, are likely to be diminished when there is a high degree of genetic connectivity among regions (Slatkin 1973; Endler 1977; Galindo et al. 2010; Pespeni et al. 2012).

A common strategy for identifying the underlying role of phenotypic plasticity in population differences is to physiologically erase acclimatory differences by growing individuals in common-garden conditions (Clausen et al. 1948; Prosser 1986; Hochachka and Somero 2002). The objective of the “common-garden acclimation” is to reset environmental history and reveal genetically controlled differences in phenotypic traits, traits as diverse as growth morphology, life history, and behavior (Oleksyn et al. 1998; Billerbeck et al. 2001; Laugen et al. 2003; Chiba et al. 2007). This approach is beginning to be used to critically evaluate transcriptome-wide gene expression differentiation across natural environmental gradients (Larsen et al. 2007; Chevron et al. 2008; Whitehead et al. 2011). In some cases, a phenomenon termed counter-gradient evolution has been observed. For example, populations growing in colder habitats may possess higher growth potential in warmer temperatures than warm-habitat natives (Laugen et al. 2003) to compensate for the inhibitory effect of low temperatures on metabolism. These gene regulatory compensations minimize the metabolic effects of temperature in different thermal habitats (Conover and Schultz 1995).

Many marine species live across environmental gradients over 1000s of km, and these populations often show distinct growth and reproductive patterns. For example, for temperate species, reproductive timing tends to be earlier in the spring for southern populations and later in the summer for northern ones (Conover 1992). Temperature, light levels, day length, ocean acidity, diet, and many other variables vary across the latitudinal range of species as diverse as reef building corals and temperate sea urchins. A few common-garden experiments have been performed on such species to evaluate the possibility of fixed interpopulation differences in environmental interactions (Conover 1998; Hutchings et al. 2007). However, the common assumption has been that high dispersal potential in many of these species, because of long-ranging planktonic larvae, often selects for species that are adapted to average conditions or that they plastically respond to environmental cues (Palumbi 1994; Kirkpatrick and Barton 1997; Warner 1997; Case and Taper 2000; Hollander 2008; Sanford and Kelly 2010).

The purple sea urchin, *Strongylocentrotus purpuratus*, plays a central role in shaping the inter- and subtidal ecological communities in the rocky reefs along the Eastern Pacific coast (Pearse

2006; Rogers-Bennett 2007). As voracious kelp grazers that are predated on by otters, spiny lobsters and sheep head wrasse, they are at the crux of a delicate balance between a healthy kelp forest and a species poor urchin “barren” (Tegner and Dayton 1991). The removal of natural predators by humans can tip the balance to urchin barrens, whereas storms and disease can decimate urchin populations allowing kelp forests to reestablish (Ebeling et al. 1985; Lafferty 2004; Rogers-Bennett 2007). In addition to being one of the most well-studied marine ecology models, the purple sea urchin has been used as a model in developmental biology for over a century owing to its large transparent eggs and embryos and ease of culture. The purple sea urchin’s central role in developmental and molecular biology has resulted in the sequencing of its genome (Sea Urchin Genome Sequencing Consortium and others 2006), yielding an excellent resource for investigations in ecological genomics, evolution, and developmental biology.

Purple sea urchin adults, embryos, and larvae can be readily cultured in the lab (Leahy 1986). Each life-history stage serves as excellent model for different studies in biology. For example, taking advantage of the regenerative abilities of echinoderms (Carnevali 2006), tube foot tissue, spines, or coelomocytes (echinoderm immune cells) can be sampled from the same adult individual across its long life, greater than 50 years (Ebert 1967; Sea Urchin Genome Sequencing Consortium and others et al. 2006), allowing various investigations in the same genetic background. However, for breeding and evolution experiments, rearing across multiple generations is less feasible because culturing animals from metamorphosis through reproductive maturity takes considerable care and the animals are not reproductively mature until 2 to 3 years of age, although some may produce gametes as early as 11 months (Leahy 1986).

Considering their broad latitudinal distribution and diverse habitat, the purple sea urchin is an ideal system to test for genetic differences in gene regulation. Their habitat is highly variable at small and large scales, as they inhabit rocky intertidal and shallow subtidal zones from Alaska to Baja California, Mexico. They are broadcast spawners, releasing eggs and sperm into surrounding waters where fertilization occurs; resulting larvae may spend weeks to months swimming and feeding before settling in a suitable habitat (Strathmann 1978). Accordingly, previous studies have shown little to no neutral population genetic structure (Palumbi and Wilson 1990; Edmonds et al. 1996; Olivares-Banuelos et al. 2008) with no fixed allelic differences in more than 12,000 polymorphisms between Boiler Bay, Oregon and San Diego, California, spanning 1700 km of coastline (Pespeni et al. 2010). Purple sea urchins are also highly fecund (Strathmann 1978) and have large population sizes (Ebert and Russell 1988; Pearse 2006). Theoretically, these species characteristics of high fecundity, large population sizes, and high neutral gene flow maximize the effects of

natural selection and minimize the effects of random genetic drift (Palumbi 1992; Hartl and Clark 1997), making this study system robust for tests of natural selection. However, we previously identified signals of selection concentrated in the upstream, putatively regulatory regions, of certain gene classes (Pespeni et al. 2012), suggesting that there may be adaptive differences between alleles and between populations in gene regulation among populations. These results suggest the possibility that the high degree of environmental variability across the species range could promote evolution in gene regulation.

Here, we cultured *S. purpuratus* from northern (Oregon) and southern (San Diego) populations for 3 years in common-garden conditions in an intermediate locality in Monterey, California. The urchins were maintained in outdoor flow-through aquaria allowing them to experience natural daily, seasonal and interannual fluctuations in environmental conditions such as temperature and pH. Using an RNA-Seq approach (De Wit et al. 2012), we measured the expression of 18,883 genes across the purple sea urchin transcriptome. Our goals in this study were to (1) determine if there were differences in gene regulation among populations after long-term common garden acclimation, (2) test if differences in gene regulation could be attributed to natural selection through gene association tests, and (3) explore if physiological differences in gene expression were associated with morphological differences in growth between populations and could be explained by counter-gradient evolution. We tested for signals of selection by testing for the non-random distribution of gene expression differences with respect to the biological function of gene classes (Lemos et al. 2005; Haygood et al. 2007; Pespeni et al. 2012). Our results show significant differences in the regulation of genes involved in growth and biomineralization. Gene association studies identify many suites of genes that appear to be under differential regulation in the two populations, suggesting the possibility of widespread gene regulatory differences along this heterogeneous species range.

Methods

URCHIN COLLECTION AND ACCLIMATION

We collected adult urchins in 2007 from Boiler Bay, Oregon (44.8N / 124.1W) and La Jolla, California (32.8N / 117.3 W) and shipped them to the Hopkins Marine Station of Stanford University, Pacific Grove, California (36.6N / 121.9W). We maintained the urchins in outdoor flow-through aquaria with each individual urchin housed in a labeled 20 cm square plastic box with four 2.5-cm-diameter holes drilled into each side of the container to allow ample seawater flow through. Each urchin was fed kelp (*Macrocystis pyrifera*) into their container *ad libitum*.

Without common environmental conditions across one to a few generations, differences in gene expression could be due to

differences in maternal effects or other environmental cues experienced during early development (Kinne 1962; Zamer and Mangum 1979; Kawecki and Ebert 2004; Sanford and Kelly 2010). In shorter-lived species, experimental tests for local adaptation are ideally performed after a few generations in common conditions (see Sanford and Worth 2010), however, the purple sea urchin is a long-lived species. In the wild, an individual urchin may live more than 50 years and is not reproductive until after 2 to 3 years (Ebert 1967; Leahy 1986; Sea Urchin Genome Sequencing Consortium and others 2006). In addition, it is difficult to rear urchins to reproductive maturity in the laboratory setting (Leahy 1986). For these reasons, we allowed an extensive acclimatization period of 3 years, much longer than the accepted standard time of a few weeks to 6 months (Hochachka and Somero 2002; Whitehead and Crawford 2006b; Whitehead et al. 2011). This also allowed us to synchronize reproductive cycles and cues across populations, as *S. purpuratus* is gravid once per year and the timing varies slightly due to differences in regional environmental cues (Pearse et al. 1986; Lester et al. 2007b). Synchronizing reproductive state eliminated any gene expression differences that may have existed due to differences in reproductive status.

RNA EXTRACTION AND SAMPLE PREPARATION

Methods broadly follow the protocol in De Wit et al. (2012). To capture transcript abundance at homeostasis, we sampled tube foot tissue from six individuals from each population after 3 years in common-garden conditions. All individuals were sampled over the course of 1 h. Individuals were randomized with respect to population origin and brought into the lab in sets of four individuals for tissue sampling. Individual urchins were placed in small containers of filtered seawater and extended tube feet were snipped with surgical scissors. Approximately 30 µg snipped tube foot tissue was collected with a 1000 ml pipette with the tip trimmed and put in a 1.5 ml tube. Tissue was flushed three times with 1 ml of filtered seawater each time. Tissue was homogenized using the TissueLyser for 2 min at 30 Hz in RLT buffer (Qiagen, Valencia, CA). We proceeded directly to total RNA extraction using the RNeasy kit (Qiagen) and purified poly-A containing mRNA using the TruSeq kit (Illumina, San Diego, CA). We prepared samples for sequencing using the TruSeq kit following the manufacturer's protocol. We multiplexed six samples per lane (three from each population) and sequenced samples on two lanes of Illumina's HiSeq platform (second-generation flow cell, University of Utah, Microarray and Genomics Core Facility). This yielded an average of 12.9 million 50 base pair reads per sample.

DATA PROCESSING AND ANALYSIS

We processed raw sequence data removing adapter sequences and trimming for quality and length using FASTX toolkit programs (http://hannonlab.cshl.edu/fastx_toolkit/index.html). This

resulted in an average of 12 million reads per sample. We mapped all reads of each sample to all 29,130 predicted genes of the purple sea urchin genome (downloaded from www.sbase.org) using Burrows–Wheeler Aligner (BWA; Li and Durbin 2009). From the resultant alignment files, we counted the number of reads that mapped singly to each gene using custom Python scripts (available at <http://sfg.stanford.edu/>). We counted reads that mapped singly, to one place across all gene sequences, because reads that map to multiple reference sequences may do so due to sequence similarity due to gene duplication or homologous regions across gene families, or errors in the assembly of the reference genome. As it is not possible to distinguish among these possibilities, the most conservative approach is to only consider reads that map uniquely to one position across the reference transcriptome.

For each sample, we scaled the number of reads of each gene to account for any differences in sequencing depth among samples using DESeq (Anders 2010). To reduce noise in the data, we excluded genes with an average of less than five counts per individual or with a standard deviation greater than the mean within each population set. This resulted in gene expression data for 18,883 genes. We identified genes significantly differentially expressed between Oregon and San Diego using DESeq (Anders 2010) and corrected for false discovery rate using the Benjamini–Hochberg approach (Benjamini and Hochberg 1995). We generated a colorimetric representation of the magnitude of gene expression using the heatmap.2 function in the gplots package (Warnes et al. 2006) in R (R Development Core Team 2009). We performed hierarchical clustering of the genes using the Euclidean distance matrix and the complete agglomeration method implemented with the heatmap.2 function. To be able to visualize genes with high and low expression levels in a single graph, we normalized each gene by dividing the number of counts for each sample by the average number of counts across all samples for a given gene.

To test for the concentration of genes with high differences in transcript abundance between northern and southern urchins in proteins with specific biological functions, we characterized each gene using UniProt identifiers (Bairoch et al. 2009) and Gene Ontology (GO) biological process categories (Ashburner et al. 2000). In addition to GO-defined categories, we generated an urchin-specific list of biominerization genes identified from the literature and defined it as a functional category (Livingston et al. 2006; Oliveri et al. 2002). We tested for a correlation between membership in a functional category and magnitude of difference in gene expression using gene score resampling implemented in ErmineJ (Lee et al. 2005) using log-transformed *P*-values from the test for gene expression differences as scores. We excluded functional categories with less than 10 gene members and more than 100 gene members to reduce noise and exclude excessively broad functional categories, respectively. Statistical significance was determined by 10,000 permutations and *P*-values were cor-

rected for false discovery rate using the Benjamini–Hochberg approach (Benjamini and Hochberg 1995). A significantly enriched category in this analysis does not mean that all genes in that category showed signs of differential expression; instead, in this case, using *P*-values from a test for differential expression, an enriched category means that genes in that category were nonrandomly distributed toward the tail of the distribution of *P*-values relative to all genes not in that category. We characterized functional categories into broader functional groups by collapsing terms based on common “parent” terms or higher-level functional categories. Collapsed categories were validated using the program REViGO (Supek et al. 2011). In addition, because GO categories are not optimized for sea urchins, we also developed a term-search screen for groups of genes using keyword searching of BLAST identifications, testing for groups that had significantly high or low expression using contingency tables with multiple test corrections.

SPINE REGROWTH EXPERIMENT

To test if fine-scale physiological gene expression data accurately predicted phenotypic growth differences, we performed a post-hoc spine regrowth experiment. We chose to measure spine regrowth after experimental spine ablation (even though gene expression was measured from tube feet) because spine regrowth can be readily measured, many spines can be trimmed from a single individual, it is a rapid response to damage, and it should reflect the short-term growth status of an individual. These urchins had already achieved about maximum size during our 3-year husbandry and as a result, growth rate experiments on whole animals would have been less revealing. We expected a positive relationship between ribosomal protein gene expression in tube foot tissue and spine growth because ribosomes are a fundamental structure involved in cellular growth and repair. Many studies have used rRNA levels as a proxy for growth potential (Dahlhoff 2004). Our approach uses mRNA levels of ribosomal proteins.

From each of 25 adult urchins from each population, we trimmed 10 large spines in a row along the oral–aboral axis to within 2 mm of the test. At days 5, 10, 15, 20 and 30 posttrimming, we removed a spine from each individual. We measured spine regrowth as the distance in millimeters from the cut (visible as a nub) to the growing tip of the spine using precision calipers and photomicroscopy. Differences in growth were tested using a one-tailed *t*-test. We performed this experiment twice, once in the summer of 2011 and once in the winter of 2012, after 4 years of common-garden conditions, to determine if predicted differences were persistent.

SEA SURFACE TEMPERATURES

Average monthly sea surface temperatures were calculated from NOAA Pathfinder data (<http://data.nodc.noaa.gov/pathfinder/>

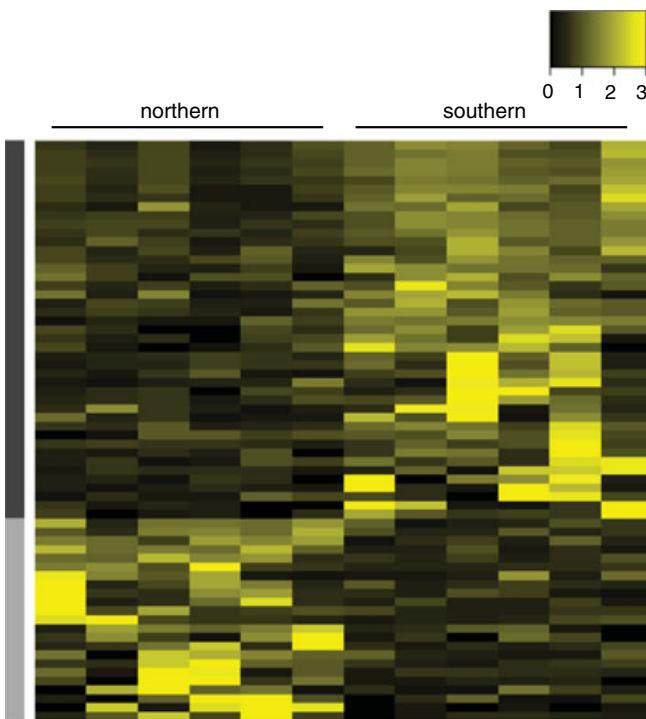


Figure 1. Heat map of the 66 differentially expressed genes between common-garden acclimated northern and southern urchins (FDR, $P < 0.05$). Each column represents data from an individual; each row represents a gene. Each gene is normalized to the mean number counts for that gene, so the color scale indicates relative fold differences in expression. The dark grey bar highlights the 43 genes with higher expression in northern urchins. The light grey bar highlights the 23 genes with higher expression in southern urchins.

Version5.2/) extracted using the xtracto_3D script (http://coastwatch.pfle.noaa.gov/xtracto/R/code/xtracto_3D_bdap.R) in the R programming environment (R Development Core Team 2009). Monthly average temperatures were collected across a 20-year period from January 1, 1990 to December 30, 2010 for Boiler Bay, Oregon; Hopkins Marine Station, Pacific Grove, California; and La Jolla, California. Sea surface temperatures were collected for regions approximately 5 to 10 km offshore from each site because satellite imaging does not consistently produce data for the very nearshore environment potentially because of cloud cover. Additional temperature data were collected from the common-garden aquaria at Hopkins Marine Station and from the intertidal zone at Boiler Bay, Oregon.

Results

To test if urchins from different populations regulate gene transcript abundance differently even after 3 years in common-garden conditions, we sequenced RNA from 12 individuals multiplexed across two Illumina HiSeq lanes. This resulted in an average of

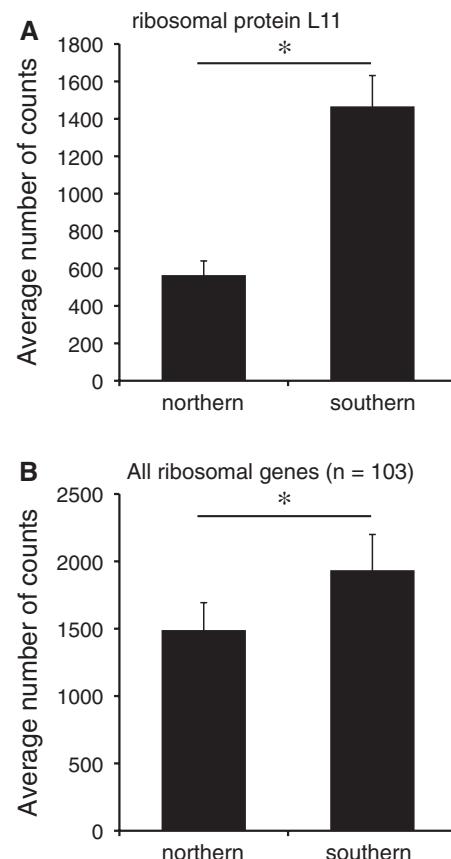


Figure 2. Average gene expression for (A) ribosomal protein L11, a differentially expressed gene (corrected $P < 0.0001$), and (B) all 103 ribosomal related genes assayed in this study (paired t-test, $P < 0.0001$). Error bars indicate standard error.

12 million, 50 base pair reads per individual after clipping adapter sequences and trimming for quality and length. We mapped reads to the *S. purpuratus* transcript sequence library and counted singly mapped reads for each gene for each individual. After screening for quality and noise reduction, this resulted in gene expression data for 18,883 genes.

The two populations showed highly similar transcriptional profiles: the correlation coefficient in expression among our 18,883 genes was 0.992 (Pearson's correlation, $P < 0.0001$). Nevertheless, there were 66 differentially expressed genes, 0.35% of all genes assayed (Fig. 1, $P < 0.05$ after FDR correction, see Table S1 for complete list). There was a high degree of interindividual variability as can be seen by the colorimetric representation of gene expression magnitude in Figure 1. Among these 66 differentially expressed genes, 43 had average higher expression in southern urchins than northern urchins.

Of particular note among these differentially expressed genes was the ribosomal protein L11 with 2.6-fold higher expression in southern urchins than northern urchins and low variance within each population (Fig. 2A). Among the 103 genes related to

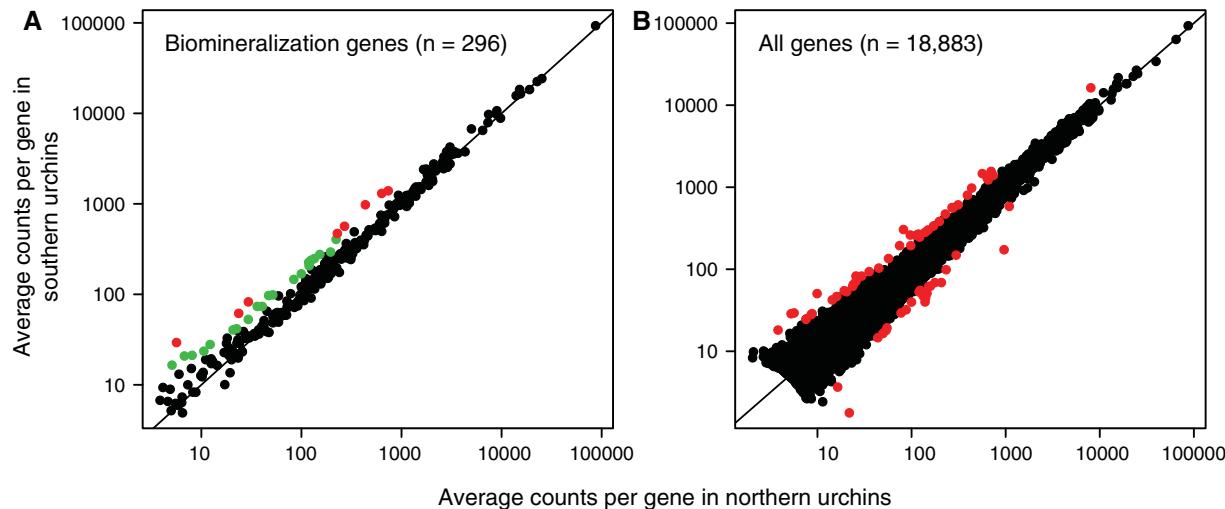


Figure 3. Scatter plot of average gene expression for northern urchins versus southern urchins for (A) all genes related to biomineralization assayed in this study ($n = 296$) and (B) all genes assayed in this study ($n = 18,883$). Red dots highlight genes differentially expressed (FDR, $P < 0.05$). Green dots highlight genes differentially expressed among the more limited dataset of biomineralization genes (q -value < 0.05). The diagonal line represents the 1 : 1 line.

ribosomal function in our dataset, 85% had higher expression in southern urchins than northern urchins with an average 29.8% higher expression in southern urchins (Fig. 2B, paired t -test, $P < 0.0001$).

We tested for differential expression among 296 biomineralization genes known for sea urchins (Livingston et al. 2006; Oliveri et al. 2002). Among these genes, 29 were significantly differentially expressed (using q -values to define the false discovery rate among this more limited dataset). This proportion (9.8%) is higher than the proportion of biomineralization genes in the entire dataset (1.6%, Fisher's exact test, $P < 0.0001$). All 29 genes were more highly expressed in southern urchins than northern urchins with an average 2.0 fold higher expression in southern urchins (Fig. 3A, green dots). Among these 29 genes, eight were also our list of globally differentially expressed genes (the difference was due to more stringent multiple test correction in the complete dataset; Fig. 3, red dots).

We extended this type of analysis to a variety of other gene functional categories, and found a large number of categories in which most genes had higher expression in San Diego (Fig. 4, black columns) or Oregon (Fig. 4, grey columns, χ^2 -test, $P < 0.0001$ in each case). In addition to the ribosomal protein genes described earlier, San Diego urchins had higher numbers of genes with higher expression in categories for electron transport and protein translation termination. By contrast, Oregon urchins had larger numbers of genes with high expression in cell size regulation, axolemma proteins and genes involved in secretion by the smooth endoplasmic reticulum. An additional interesting category with high expression in northern urchins is vitellogenesis. This

category includes dozens of proteins with the ankyrin 2,3/unc44 domain and is joined by higher expression in Oregon urchins for the sperm bindin gene that is expressed on egg surfaces. Of course, we isolated our RNA from tube feet alone, and it is surprising to find ovary genes expressed in this tissue. Either this mis-expression in northern urchins is accentuated, or these results reflect a shift in spawning season in urchins despite their 3-year common-garden conditions. Differences in the endoplasmic reticulum category can trace to proteins of the saccin family, a group of DNAj chaperonins that help fold proteins correctly—either at creation or perhaps during cellular stress. Seventeen of 18 Saccin's are expressed more highly in Oregon urchins, one significantly so (Table S1), and overall expression levels are 50% higher in Oregon animals.

Overall, we found that 907 of the 2621 functional categories tested (34.6%) were enriched for differential expression among the populations (FDR; $P < 0.05$, see Table S2 for complete list). There were 8,994 unique genes represented in these 907 categories, representing 47.6% of all the genes in this study.

Although we found many categories of genes with highly significant differences in the numbers of genes upregulated in northern versus southern populations (Fig. 4B), the absolute expression difference in the genes in these categories was usually slight. In general, percent change in expression was between -30 to $+50\%$ (comparing BB to SD expression; Fig. 4A), and few of the individual genes in these categories were significantly different after multiple test corrections. As a result, the gene expression signature we find between common-garden urchins is one of slight changes in a large number of genes.

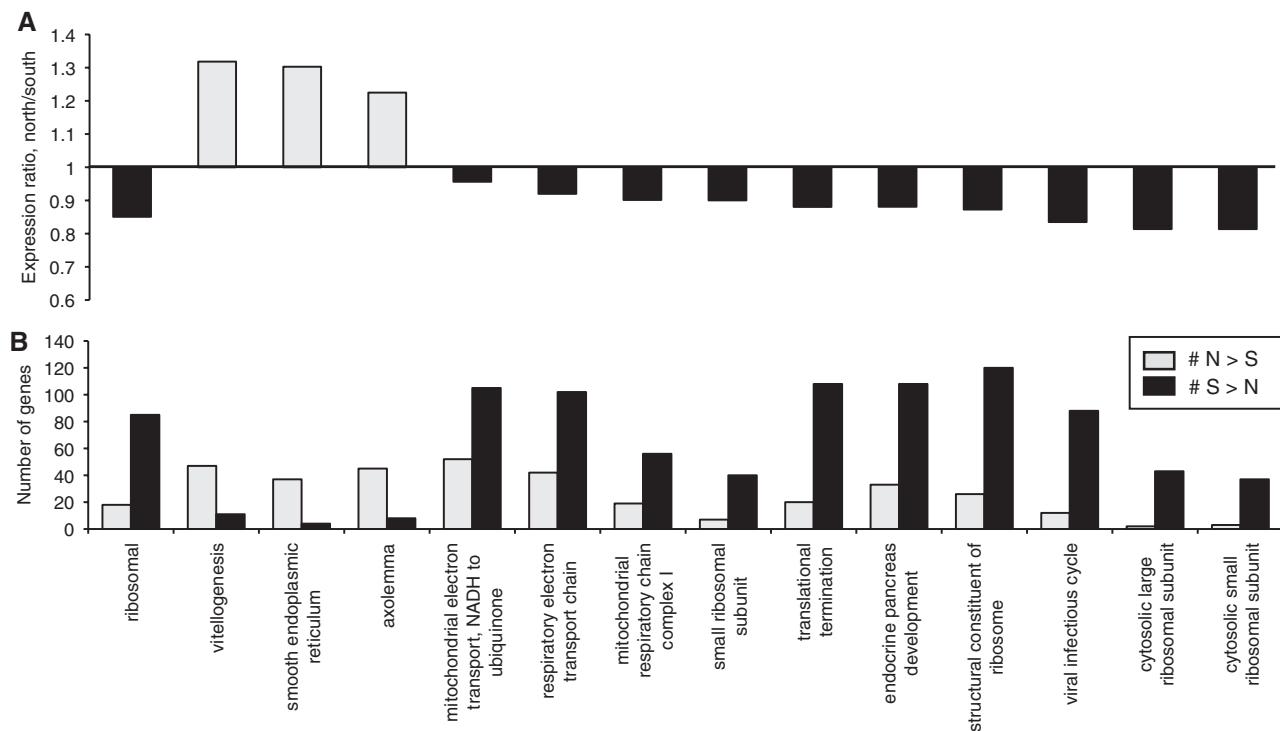


Figure 4. Differential expression among gene ontology (GO) categories for northern versus southern urchins. (A) Average shift in gene expression amount among genes in fourteen significant GO categories, calculated as the ratio of expression in urchins from Boiler Bay, Oregon to San Diego, California. (B) The number of genes among in each category that have higher expression in Boiler Bay, Oregon (light grey columns) and San Diego (black columns). χ^2 tests show these values differ significantly from 1:1 expectations in each of these comparisons ($P < 0.0001$).

Patterns of higher ribosomal and metabolic gene expression in southern urchins could result in faster growth. To provide a simple, initial test of whether southern urchins possessed higher growth potential after common-garden exposure, we performed a spine regrowth experiment. We reasoned that spine regrowth would parallel normal metabolic processes and perhaps would provide a repeatable index of individual growth potential. We found that indeed, after 10 days of spine regrowth, southern urchins had 10% longer regrown spines than northern urchins (Fig. 5A, Day 10, one-tailed t -test, $P < 0.05$). This 10% advantage continued through the 30-day duration of the experiment (Fig. 5B, C, Day 30, one-tailed t -test, $P < 0.001$). We repeated the experiment after 4 years of common gardening to find the differences in growth were maintained (Fig. 5D).

ENVIRONMENTAL DIFFERENCES

Urchin tanks in Monterey experienced temperatures ranging from 12.5°C to 15°C during 2010 and 2011 when animals were sampled for mRNA (Fig. 6A). Summer temperatures were approximately 1.5°C warmer than winter temperatures in 2011 but were about equal in 2010 (Fig. 6A). The Monterey tanks did not experience water above 17°C or below 11°C during 2010 or 2011: water temperature was between 12°C and 15°C 94% to 97% of the

time in those 2 years. These values are similar to values recorded offshore (Hopkins Marine Station: $13.1 \pm 1.5^\circ\text{C}$; Fig. 6B).

We do not have similar data from the low intertidal habitats of our urchin populations. However, offshore water in La Jolla, California, is substantially warmer ($17.9 \pm 2.5^\circ\text{C}$) than in Monterey due to its position in the Southern California Bight (Huyer 1983). Temperature records for intertidal mussel beds in Boiler Bay, Oregon, are available from parts of 2002 and 2004 (Fig. 6A): if we remove the temperature records at low tide, we see summer temperatures ranging from 9°C to 11.5°C. Strong upwelling in Boiler Bay (Menge et al. 2004; Hauri et al. 2009) drops the summertime temperature there during the months of June, July, and August.

In addition to temperature data from Monterey, we also collected pH data from the same tanks. Due to local upwelling, the pH of coastal water in Monterey Bay falls during the summer (Menge et al. 2004; Hauri et al. 2009): during 2010 and 2011, the monthly mean pH was never above 7.95, and reached 7.65 for 3 months in 2011 (Fig. S1). We have no comparable pH data from Boiler Bay and San Diego, but upwelling is low in San Diego and ocean pH hovers near 7.9 to 8.0 (Hauri et al. 2009; Hofmann et al. 2011). Coastal Oregon is washed by periodic upwelling and so we expect pH to be highly variable at Boiler Bay.

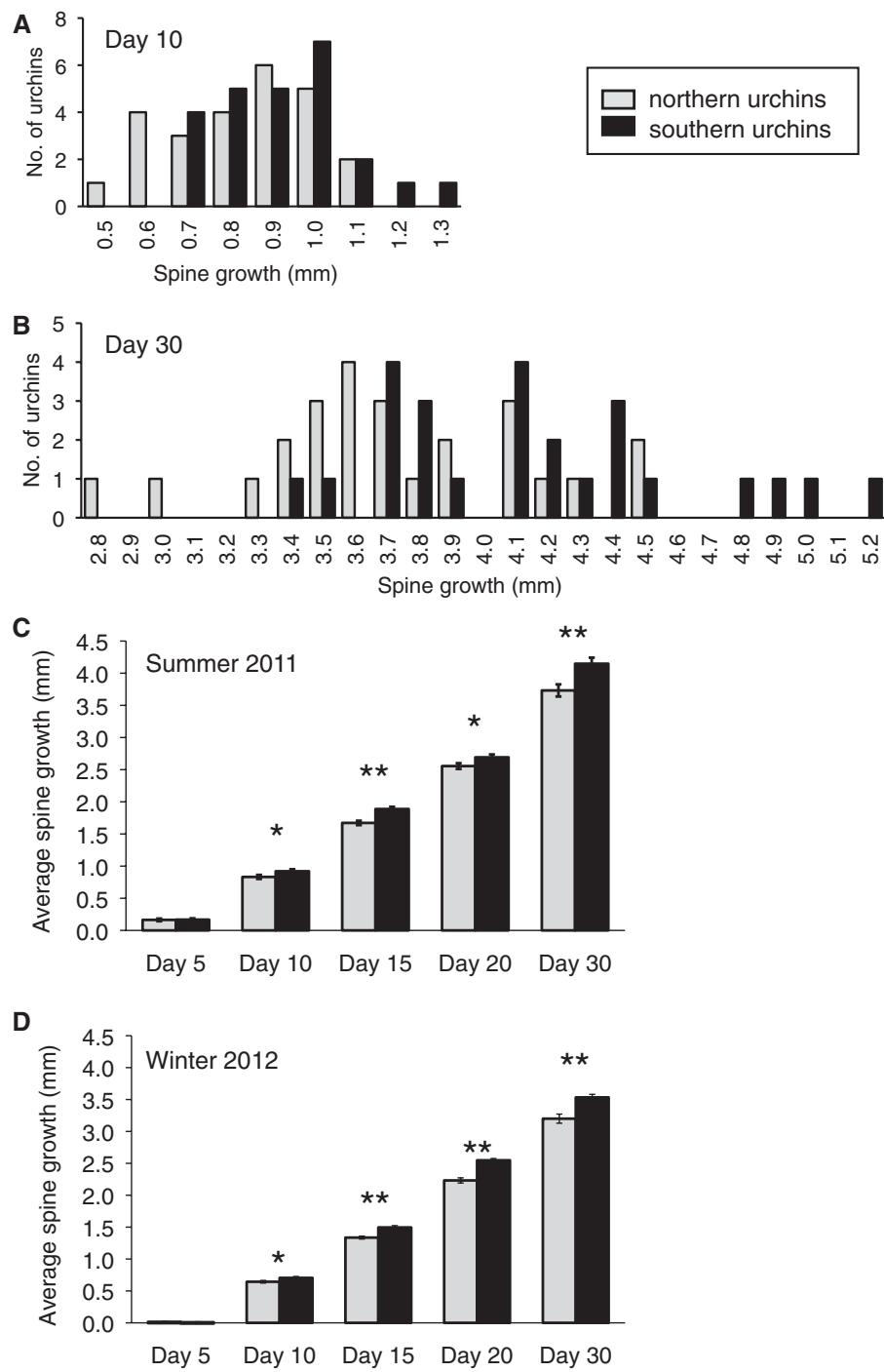


Figure 5. Histograms of spine growth after (A) 10 days and (B) 30 days. (C) Average spine growth at the five sampled time points across the duration of the experiment. (D) A replicate of the experiment after 4 years in common-garden conditions shows maintained differences in growth. Data from northern urchins are in light grey and southern urchins in black. One asterisk indicates $P < 0.05$ and two asterisks indicate $P < 0.001$. Error bars indicate standard error.

Discussion

Sequencing RNA from long-term common-garden acclimated sea urchins of distant, environmentally distinct populations, we found highly correlated patterns of individual gene expression with 66 significantly differentially expressed genes. There were

however heightened differences in expression for genes involved in metabolism and biominerization, all with higher expression in southern urchins. In addition, subtle differences are observed in closer inspection of functional groups of genes: 907 categories of genes showed shifts in gene expression, comprising 47.6% of the

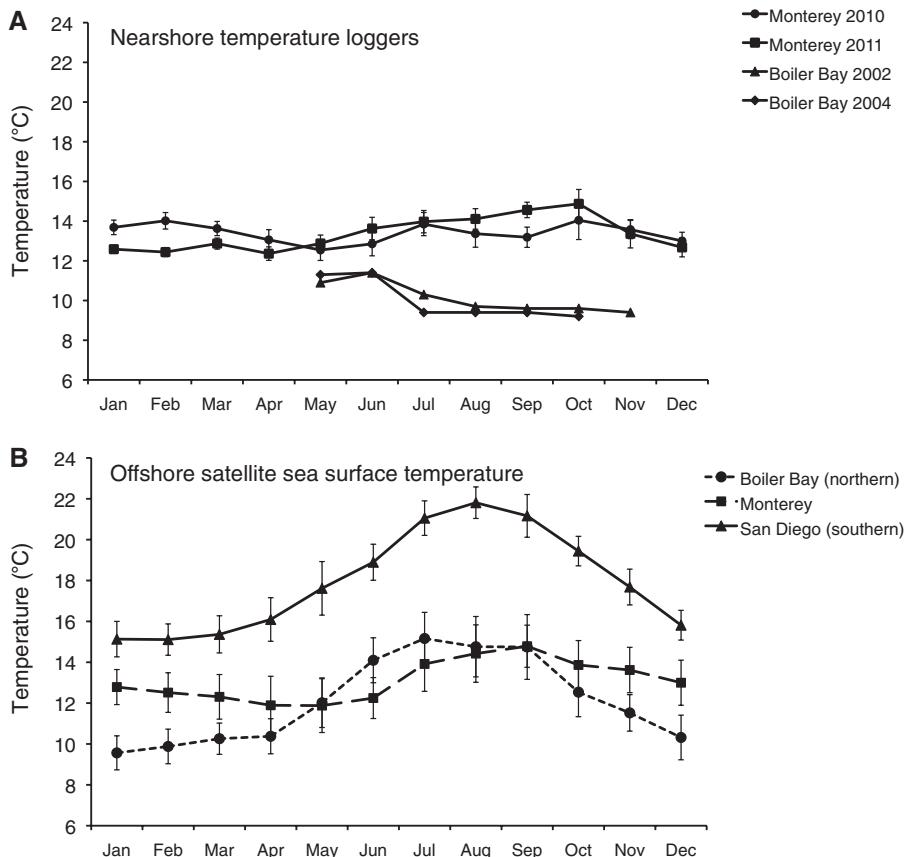


Figure 6. Temperature data for the (A) nearshore environment from intertidal and aquaria temperature loggers, and (B) offshore environment from satellite sea surface temperature data.

genes measured in this study. However, expression differences across these categories seldom exceeded 50%. Not surprisingly, these gene classes acted on many different cellular functions including metabolism, growth, biomineratization, RNA processing, and stress response. Overall, expression differences were slight among a large number of genes.

DIFFERENCES IN GROWTH AMONG POPULATIONS

Our results suggest southern urchins have higher scope for growth than northern urchins in Monterey Bay common-garden conditions. Scope for growth is the difference between energy input to an organism as food and output as respiratory metabolism, yielding the energy available for growth or reproduction. Scope for growth can be negative or positive, and can be a good measure of physiological stress (Bayne et al. 1979; Widdows and Johnson 1988; Naylor et al. 1989). Evidence for higher scope for growth in southern urchins includes (1) higher expression of ribosomal and metabolic genes in southern urchins, (2) higher expression of biomineratization genes in southern urchins, and (3) transcriptome-wide regulatory differences in genes related to metabolism and transport of nutrients. The higher scope for growth in the southern urchins would suggest that they were less

stressed in the relatively colder common garden than the northern urchins in the relatively warmer conditions with respect to their native habitat. Elevated expression of stress response genes in northern urchins corroborate this suggestion.

In particular, the higher expression of ribosomal proteins, approximately 30% higher in southern urchins, suggests a higher growth potential in these urchins. The ratio of RNA to DNA has been widely used in marine invertebrates and fishes as a biochemical indicator of growth (Dahlhoff 2004). In this study, we take the elevated expression of ribosomal proteins as an indication of a higher RNA : DNA ratio in southern urchins because RNA and ribosomal protein levels are tightly correlated (Kennell and Magasanik 1962; Matchett 1968). A previous study in another ecologically important intertidal invertebrate, the mussel *Mytilus californianus*, showed that field-acclimated mussels from Boiler Bay (the source site for the northern urchins in this study) had lower growth and metabolic activity than mussels in a neighboring site in Oregon that had higher food availability (Dahlhoff and Menge 1996). The authors demonstrated that these differences were physiologically plastic: the RNA : DNA ratios of reciprocally transplanted mussels converged on those of the site to which they were transplanted (Dahlhoff and Menge 1996). In contrast, in

this study with sea urchins, given the same food availability and environmental conditions in flow through aquaria in Monterey for 3 years, our results revealed persistent differences in growth potential among populations; these differences may be genetically controlled.

A second, correlated, class of genes is related to energy metabolism. Among 157 genes related to mitochondrial electron transport, 105 (67%) are more highly expressed in southern urchins ($P < 0.0001$). Likewise, two thirds of genes involved in protein translation are more highly expressed in San Diego animals ($P < 0.0001$). Both classes of genes might result in higher metabolic activity and higher growth potential.

TESTING GRADIENT ADAPTATION

To determine if gene expression patterns suggesting differences in metabolism resulted in differences in growth, we measured the regrowth rate of trimmed spines. Previous studies have shown spine growth in urchins is related to food availability and positive scope for growth (Ebert 1968; Minor and Scheibling 1997). We found that spines of southern urchins grew about 10% faster, suggesting that gene regulatory differences permit a slight increase in growth potential in southern populations. By contrast, Ebert (2010) found no correlation of latitude with growth or survival in *S. purpuratus*, although these field studies were unable to control for food supply. The most significant latitudinal shifts seen between San Diego and Oregon for this species were a reduction in size of adults and a reduction of recruits in the south (Ebert 2010). Overall, our simple spine experiments show a persistent difference in San Diego versus Oregon animals in growth rate despite a 3-year acclimation to the same conditions.

We observe signs of elevated metabolism and faster spine growth in southern population urchins under common-garden conditions with northern urchins. These results are counter to predictions based on counter-gradient evolution to compensate for temperature differences among latitudes. Counter-gradient variation, also known as temperature compensation in the physiology literature, predicts higher metabolic rates and faster growth for higher latitude or higher altitude organisms when brought into common-garden conditions with their lower latitude / altitude conspecifics (Levins 1969; Conover and Schultz 1995). This variation is due to natural selection improving metabolism to counter poorer growing conditions in higher latitudes or altitudes that have colder temperatures and/or shorter growing seasons. Such environments require higher metabolism to maintain normal body size, growth, or swimming abilities for the species across the environmental gradient along the species range (Berven 1982; Crawford and Powers 1992; Laugen et al. 2003). Counter-gradient variation, where genetic differences oppose environmental effects to maintain a phenotype, has been detected in over 60 species, primarily amphibians and fishes (Conover et al. 2009).

Although temperatures can differ an average of 5°C to 8°C between Boiler Bay and San Diego, there may be other environmental differences among these localities that could exact stronger selection. The metabolic differences observed here could be due to counter-gradient evolution in response to food quality and food availability differences. Southern urchins may actually be in the “poorer” quality habitat because of lower coastal upwelling and lower productivity. In addition, habitat quality and food availability has declined dramatically over the last 50 years along the southern California coast (Foster and Schiel 1985). This decline has largely been due to kelp forest decline in response to higher incidents of warm water stress, human induced habitat destruction, coastal development, increasing turbidity and siltation. In addition, removal of urchin predators such as the sheepshead wrasse and spiny lobster, has led to increased urchin numbers, more urchin barrens, and potentially a higher degree of intraspecific competition for food (Tegner and Dayton 1991; Lafferty 2004). The provision of high-quality *M. pyrifera* kelp *ad libitum* in our common-garden conditions may have been an environmental boon for a southern urchin adapted to low-quality or low-abundance food. However, further studies are needed to determine if metabolic differences among populations are due to counter-gradient variation in response to food quality and availability differences among latitudes.

Previous population genetic data found evidence that there may be local adaptation and particularly high genetic differentiation in urchins in the Southern California Bight. Pespeni et al. (2012) measured excess heterozygosity in immunity-related genes in San Diego versus Boiler Bay urchins, which correlates with the higher incidence of disease along the southern California coast (Lester et al. 2007a). Follow-on surveys (Pespeni et al. 2012) showed that genetic differences at many loci with high coast-wide genetic differentiation were concentrated around San Diego. Although this is not the southern-most population of sea urchins, its unique position far from the cold-water, nutrient rich California Current may set it apart environmentally from other populations to the north or to the south.

NATURAL ACIDIFICATION AND GENE REGULATION

The largest shift in expression we see in any gene class is for biominerization genes where expression is about twofold higher in southern urchins (Fig. 3). In particular, three spicule matrix proteins are among the 66 differentially expressed genes (Table S1). These proteins are found within skeletons of larvae and adults (Livingston et al. 2006). It is possible that biominerization gene expression is increased for the same reasons that ribosomal protein, metabolic enzymes, and translational machinery are increased: for example, these genes may all be related to increased growth. However, the higher overexpression of biominerization genes (2 \times) compared to ribosomal, metabolic, or translational

genes (5%–15%) suggests that there may be an additional factor—pH stress. San Diego urchins typically live in pH conditions that largely resemble open ocean levels, varying between 7.9 and 8.2 (Hofmann et al. 2011, see Fig. 2) but in coastal Monterey, urchins experience periodic acidification due to local upwelling (Fig. S1). Under such conditions, physiological predictions are that calcification is more energetically costly (Kroeker et al. 2010), and might demand higher expression of calcification genes. By contrast, Boiler Bay urchins live in an upwelling environment that periodically lowers pH (Menge et al. 2004 and references therein). As a result, Boiler Bay urchins may already show adaptations to low pH not seen in La Jolla animals, and not have been under pH stress during our experiments. Our experiment was not designed to reveal the impact of adaptation to low pH, but provides a set of hypotheses to test in the future.

GENE EXPRESSION EVOLUTION ACROSS POPULATIONS

Our results suggest three evolutionary signals across populations acting on gene regulation. First, gene expression patterns for most genes are highly correlated between localities: the correlation coefficient between expression values for San Diego and Oregon urchins across 18,883 genes was 0.992 ($P < 0.0001$). This probably reflects stabilizing selection for regulation of gene expression across environments (Lemos et al. 2005). Second, there are small but widespread shifts in gene expression across many genes. These are not large enough to affect the correlation discussed earlier because they are usually shifts of only 10%–20% (Fig. 4). However, they occur across a wide range of gene classes and among many genes. These slight but widespread regulatory differences could be due to the slow erosion of phenotypically plastic differences in gene regulation. In other words, after 3 years in common conditions, the urchins may maintain regulatory differences attributable to their different environmental histories rather than genetic differences. Experiments in both fish and anemone clones demonstrated that differences in early developmental conditions can have persistent and irreversible physiological effects through an organism's life (Kinne 1962; Zamer and Mangum 1979).

Alternatively, these results could suggest that there may be genetic differences between populations in a smaller number of higher-level gene expression regulators, resulting in the small but pervasive gene expression differences seen here. This kind of shift has been described in yeast, where a broad “environmental stress response” alters the transcription of many genes (Gasch et al. 2000) after a change in environment. Such changes mirror many of the ones seen here, with shifts in metabolic enzymes, ribosomal protein genes and other genes involved in cell growth and RNA processing. A key insight from these results is that many genes might be affected by a smaller number of regulatory elements held

in common. As a result, evolution of slight differences at 1000s of genes would not demand independent evolution at 1000s of regulatory regions but perhaps involve a far fewer number of changes.

Third, there are a small number of genes with quite large gene regulation differences despite common environments. These expression patterns at 66 genes may be under directional selection in different environments. This form of spatial or temporal balancing selection is particularly relevant for a species distributed across diverse habitats; alternative alleles (in our case for gene expression variants) might be maintained in the population as a whole despite the homogenizing effects of gene flow by recurring selection every generation or as conditions change in time (Levene 1953; Felsenstein 1976; Gillespie 1976; Hedrick 2006).

Both stabilizing and balancing selection have been observed in gene regulatory studies of natural populations of *Drosophila* (Lemos et al. 2005; Levine et al. 2011), *Fundulus* (Oleksiak et al. 2002; Whitehead and Crawford 2006b) and stickleback (Jones et al. 2012) fish, suggesting that these modes of regulatory evolution may be the norm for ecological adaptation along environmental gradients without obvious barriers to dispersal. In addition, in the context of a high gene flow species distributed across a heterogeneous landscape, adaptive differences may be more likely to occur in gene regulation as opposed to protein function because there are more genetic targets for mutation, for example *cis*- and *trans*- regulatory regions, transcription factors, enhancers, etc. versus mutations that would need to affect specific functional regions of a three-dimensional protein (Wray 2007).

These signals of selection also match predictions based on our previous genome-wide studies of genetic diversity in these two purple sea urchin populations (Pespeni et al. 2010, 2012). A survey of 12,431 polymorphisms showed a high degree of genetic similarity between these populations (Pespeni et al. 2010). However, there was a concentration of high F_{ST} polymorphisms in the upstream putatively regulatory regions of genes, particularly genes related to proteolysis (Pespeni et al. 2012), suggesting adaptive differences in gene regulation between these populations. The present study confirms regulatory differences in these genes between populations: of the 12 E3 ligase genes present in this dataset and identified in the previous study (Pespeni et al. 2012), 10 had higher expression in northern urchins than southern urchins.

IMPLICATIONS FOR CLIMATE CHANGE

Taken together these results suggest that the gene regulatory machinery of purple sea urchin populations may be partially adapted to local conditions, and that climate shifts will require regulatory evolution. Urchins from the northern population, when exposed to warmer water conditions, have lower scope for growth than

urchins from the southern population. These data do not translate easily into predictions about climate change because we raised urchins under benign conditions with little or no acute heat or pH stress. Osovitz and Hofmann (2005) showed that Oregon urchins expressed heat shock proteins more quickly after acute heat shock than southern populations, and other work on the physiology of west coast marine invertebrates suggests that transient temperature extremes are more important than mean water temperatures (Helmuth et al. 2010). Future investigations that test for genetically controlled physiological differences are needed to help us better understand and predict future population dynamics in changing climate conditions (Helmuth et al. 2010; Hoffmann and Sgrò 2011).

Conclusions

This study provides an experimental demonstration of how gene regulation has likely evolved in populations distributed along a latitudinal environmental gradient. We find a pattern of broad but shallow gene regulatory differences: many genes are expressed differently but the level of difference is slight. There are two classes of exceptions—a suite of 66 genes with individually significant gene expression differences, and a class of genes involved in biominerilization that has a strong average difference between common-garden populations. These differences paired with differences in spine regrowth suggest that there may be genetically controlled differences in physiological performance between populations along the latitudinal gradient of the west coast of North America.

In all, these data begin to close gaps in our understanding of the mechanisms of adaptive regulatory evolution in natural populations distributed across a heterogeneous landscape. Nevertheless, further work testing the regulatory machinery of identified genes and more studies identifying the genetic underpinnings of physiological tolerance and adaptive capacity will improve our ability to predict how populations will respond to changing climate conditions and our understanding of the mechanisms of adaptive evolution in the face of gene flow.

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LITERATURE CITED

- Anders, S., and W. Huber. 2010. Differential expression analysis for sequence count data. *Genome Biology* 11:R106.
- Ashburner, M., C. Ball, J. Blake, D. Botstein, H. Butler, J. Cherry, A. Davis, K. Dolinski, S. Dwight, and J. Eppig. 2000. Gene ontology: tool for the unification of biology. *Nat. Genet.* 25:25–29.

- Bairoch, A., L. Bougueret, S. Altairac, V. Amendolia, A. Auchincloss, G. Argoud-Puy, K. Axelsen, D. Baratin, M. Blatter, and B. Boeckmann. 2009. The universal protein resource (UniProt) 2009. *Nucleic Acids Res.* 37:D169–D174.
- Bayne, B., M. Moore, J. Widdows, D. Livingstone, P. Salkeld, D. Crisp, R. Morris, J. Gray, A. Holden, and R. Newell. 1979. Measurement of the responses of individuals to environmental stress and pollution: studies with Bivalve Molluscs [and Discussion]. *Philos. Trans. R. Soc. Lond. B, Biol. Sci.* 286:563–581.
- Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Series B (Methodological)* 51:289–300.
- Berven, K. A. 1982. The genetic basis of altitudinal variation in the wood frog *rana sylvatica*. I. An experimental analysis of life history traits. *Evolution* 36:962–983.
- Billerbeck, J. M., T. E. Lankford, and D. O. Conover. 2001. Evolution of intrinsic growth and energy acquisition rates. I. Tradeoffs with swimming performance in *Menidia menidia*. *Evolution* 55:1863–1872.
- Carnevali, M. 2006. Regeneration in Echinoderms: repair, regrowth, cloning. *Invertebr. Surv. J.* 3:64–76.
- Case, T. J., and M. L. Taper. 2000. Interspecific competition, environmental gradients, gene flow, and the coevolution of species' borders. *Amer. Nat.* 155:583–605.
- Cheviron, Z. A., A. Whitehead, and R. T. Brumfield. 2008. Transcriptomic variation and plasticity in rufous collared sparrows (*Zonotrichia capensis*) along an altitudinal gradient. *Mol. Ecol.* 17:4556–4569.
- Chiba, S., S. A. Arnott, and D. O. Conover. 2007. Coevolution of foraging behavior with intrinsic growth rate: risk-taking in naturally and artificially selected growth genotypes of *Menidia menidia*. *Oecologia* 154:237–246.
- Clausen, J., D. Keck, and W. Hiesey. 1948. Experimental studies on the nature of species III. Environmental responses of climatic races of Achillea. Carnegie Institute of Washington Publication 581. Carnegie Institute, Washington, DC.
- Conover, D. 1992. Seasonality and the scheduling of life history at different latitudes. *J. Fish Biol.* 41:161–178.
- Conover, D. O. 1998. Local adaptation in marine fishes: evidence and implications for stock enhancement. *Bull. Mar. Sci.* 62:477–493.
- Conover, D. O., and E. T. Schultz. 1995. Phenotypic similarity and the evolutionary significance of counter-gradient variation. *Trends Ecol. Evol.* 10:248–252.
- Crawford, D. L., and D. A. Powers. 1992. Evolutionary adaptation to different thermal environments via transcriptional regulation. *Mol. Biol. Evol.* 9:806–813.
- Conover, D. O., T. A. Duffy, and L. A. Hice. 2009. The covariance between genetic and environmental influences across ecological gradients. *Ann. N. Y. Acad. Sci.* 1168:100–129.
- Dahlhoff, E. P. 2004. Biochemical indicators of stress and metabolism: applications for marine ecological studies. *Annu. Rev. Physiol.* 66:183–207.
- Dahlhoff, E. P., and B. A. Menge. 1996. Influence of phytoplankton concentration and wave exposure on the ecophysiology of *Mytilus californianus*. *Mar. Ecol. Prog. Ser.* 144:97–107.
- De Wit, P., M. H. Pespeni, J. T. Ladner, D. J. Barshis, F. Seneca, H. Jaris, N. Overgaard Therkildsen, M. Morikawa, and S. R. Palumbi. 2012. The Simple Fool's Guide to population genomics via RNA-Seq: an introduction to high-throughput sequencing data analysis. *Molec. Ecol. Res.* 12:1058–1067.
- Ebeling, A., D. Laur, and R. Rowley. 1985. Severe storm disturbances and reversal of community structure in a southern California kelp forest. *Mar. Biol.* 84:287–294.

- Ebert, T. 2010. Demographic patterns of the purple sea urchin *Strongylocentrotus purpuratus* along a latitudinal gradient, 1985–1987. *Mar. Ecol. Prog. Ser.* 406:105–120.
- Ebert, T. A. 1967. Negative growth and longevity in the purple sea urchin *Strongylocentrotus purpuratus* (Stimpson). *Science* 157:557–558.
- . 1968. Growth rates of the sea urchin *Strongylocentrotus purpuratus* related to food availability and spine abrasion. *Ecology* 49:1075–1091.
- Ebert, T. A., and M. P. Russell. 1988. Latitudinal variation in size structure of the West Coast Purple Sea Urchin: a correlation with headlands. *Limnol. Oceanogr.* 33:286–294.
- Edmands, S., P. E. Moberg, and R. S. Burton. 1996. Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin *Strongylocentrotus purpuratus*. *Mar. Biol.* 126:443–450.
- Endler, J. A. 1977. Geographic variation, speciation and clines. Princeton University Press, Princeton, New Jersey.
- Felsenstein, J. 1976. The theoretical population genetics of variable selection and migration. *Annu. Rev. Genet.* 10:253–280.
- Foster, M. S., and D. R. Schiel. 1985. Ecology of giant kelp forests in California: a community profile. San Jose State Univ., Moss Landing, CA.
- Galindo, H. M., A. S. Pfeiffer Herbert, M. A. McManus, Y. Chao, F. Chai, and S. R. Palumbi. 2010. Seascapes genetics along a steep cline: using genetic patterns to test predictions of marine larval dispersal. *Mol. Ecol.* 19:3692–3707.
- Gasch, A. P., P. T. Spellman, C. M. Kao, O. Carmel-Harel, M. B. Eisen, G. Storz, D. Botstein, and P. O. Brown. 2000. Genomic expression programs in the response of yeast cells to environmental changes. *Mol. Biol. Cell* 11:4241–4257.
- Gilchrist, G. W., and R. B. Huey. 2004. Plastic and genetic variation in wing loading as a function of temperature within and among parallel clines in *Drosophila subobscura*. *Integr. Comp. Biol.* 44:461–470.
- Gillespie, J. H. 1976. The role of migration in the genetic structure of populations in temporally and spatially varying environments II. Island Models. *Theor. Popul. Biol.* 10:227–238.
- Grosberg, R., and C. Cunningham. 2001. Genetic structure in the sea. Pp. 61–84 in M. D. Bertness, S. Gaines, and M. E. Hay, eds. *Marine Community Ecology*. Sinauer Associates, Sunderland, MA.
- Hartl, D., and A. Clark. 1997. *Principles of population genetics*. Sinauer Associates, Sunderland, MA.
- Hauri, C., N. Gruber, G. K. Plattner, S. Alin, R. A. Feely, B. Hales, and P. A. Wheeler. 2009. Ocean acidification in the California current system. *Oceanography* 22:60–71.
- Haygood, R., O. Fedrigo, B. Hanson, K. D. Yokoyama, and G. A. Wray. 2007. Promoter regions of many neural-and nutrition-related genes have experienced positive selection during human evolution. *Nat. Genet.* 39:1140–1144.
- Hedrick, P. 2006. Genetic polymorphism in heterogeneous environments: the age of genomics. *Ann. Rev. Ecol., Evol. Syst.* 37:67–93.
- Helmhuth, B., B. R. Broitman, L. Yamane, S. E. Gilman, K. Mach, K. Mislan, and M. W. Denny. 2010. Organismal climatology: analyzing environmental variability at scales relevant to physiological stress. *J. Exp. Biol.* 213:995–1003.
- Hochachka, P. W., and G. N. Somero. 2002. *Biochemical adaptation: mechanism and process in physiological evolution*. Oxford Univ. Press, New York, NY.
- Hoffmann, A. A., and C. M. Sgrò. 2011. Climate change and evolutionary adaptation. *Nature* 470:479–485.
- Hofmann, G. E., J. E. Smith, K. S. Johnson, U. Send, L. A. Levin, F. Micheli, A. Paytan, N. N. Price, B. Peterson, and Y. Takeshita. 2011. High-Frequency dynamics of ocean pH: A multi-ecosystem comparison. *PLoS One* 6:e28983.
- Hollander, J. 2008. Testing the grain size model for the evolution of phenotypic plasticity. *Evolution* 62:1381–1389.
- Hutchings, J. A., D. P. Swain, S. Rowe, J. D. Eddington, V. Puvanendran, and J. A. Brown. 2007. Genetic variation in life-history reaction norms in a marine fish. *Proc. R. Soc. B: Biol. Sci.* 274:1693–1699.
- Huyer, A. 1983. Coastal upwelling in the California Current system. *Prog. Oceanogr.* 12:259–284.
- Jones, F. C., M. G. Grabherr, Y. F. Chan, P. Russell, E. Mauceli, J. Johnson, R. Swofford, M. Pirun, M. C. Zody, and S. White. 2012. The genomic basis of adaptive evolution in three spine sticklebacks. *Nature* 484:55–61.
- Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. *Ecol. Lett.* 7:1225–1241.
- Kennell, D., and B. Magasanik. 1962. The relation of ribosome content to the rate of enzyme synthesis in *Aerobacter aerogenes*. *Biochim. Biophys. Acta (BBA)-Specialized Section on Nucleic Acids and Related Subjects* 55:139–151.
- King, M., and A. Wilson. 1975. Evolution at two levels in humans and chimpanze. *Science* 188:107–116.
- Kinne, O. 1962. Irreversible nongenetic adaptation. *Comp. Biochem. Physiol.* 5:265–282.
- Kirkpatrick, M., and N. Barton. 1997. Evolution of a species' range. *Am. Nat.* 150:1–23.
- Kroeker, K. J., R. L. Kordas, R. N. Crim, and G. G. Singh. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol. Lett.* 13:1419–1434.
- Lafferty, K. 2004. Fishing for lobsters indirectly increases epidemics in sea urchins. *Ecol. Appl.* 14:1566–1573.
- Larsen, P. F., E. E. Nielsen, T. D. Williams, J. Hemmer-Hansen, J. K. Chipman, M. Kruhøffer, P. Groenkjaer, S. G. George, L. Dyrskjot, and V. Loeschke. 2007. Adaptive differences in gene expression in European flounder (*Platichthys flesus*). *Mol. Ecol.* 16:4674–4683.
- Laugen, A., A. Laurila, K. Räsänen, and J. Merilä. 2003. Latitudinal counter-gradient variation in the common frog (*Rana temporaria*) development rates—evidence for local adaptation. *J. Evol. Biol.* 16:996–1005.
- Leahy, P. S. 1986. Laboratory culture of *Strongylocentrotus purpuratus* adults, embryos, and larvae. *Methods Cell Biol.* 27:1–13.
- Lee, H., W. Braynen, K. Keshav, and P. Pavlidis. 2005. ErmineJ: tool for functional analysis of gene expression datasets. *BMC Bioinform.* 6:269.
- Lemos, B., C. D. Meiklejohn, M. Cáceres, and D. L. Hartl. 2005. Rates of divergence in gene expression profiles of primates, mice, and flies: stabilizing selection and variability among functional categories. *Evolution* 59:126–137.
- Lester, S., E. Tobin, and M. Behrens. 2007a. Disease dynamics and the potential role of thermal stress in the sea urchin, *Strongylocentrotus purpuratus*. *Can. J. Fish. Aquat. Sci.* 64:314–323.
- Lester, S. E., S. D. Gaines, and B. P. Kinlan. 2007b. Reproduction on the edge: large-scale patterns of individual performance in a marine invertebrate. *Ecology* 88:2229–2239.
- Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. *Am. Nat.* 87:331–333.
- Levine, M. T., M. L. Eckert, and D. J. Begun. 2011. Whole-genome expression plasticity across tropical and temperate *Drosophila melanogaster* populations from Eastern Australia. *Mol. Biol. Evol.* 28:249–256.
- Levins, R. 1969. Thermal acclimation and heat resistance in *Drosophila* species. *Am. Nat.* 103:483–499.
- Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25:1754–1760.
- Livingston, B., C. Killian, F. Wilt, A. Cameron, M. Landrum, O. Ermolaeva, V. Sapojnikov, D. Maglott, A. Buchanan, and C. Ettensohn. 2006. A

- genome-wide analysis of biomineralization-related proteins in the sea urchin *Strongylocentrotus purpuratus*. *Dev. Biol.* 300:335–348.
- Matchett, W. H. 1968. Ribosomal precursors and ribonucleic acid synthesis in *Escherichia coli*. *J. Bacteriol.* 96:997–1005.
- Menge, B. A., C. Blanchette, P. Raimondi, T. Freidenburg, S. Gaines, J. Lubchenco, D. Lohse, G. Hudson, M. Foley, and J. Pamplin. 2004. Species interaction strength: testing model predictions along an upwelling gradient. *Ecol. Monogr.* 74:663–684.
- Minor, M., and R. Scheibling. 1997. Effects of food ration and feeding regime on growth and reproduction of the sea urchin *Strongylocentrotus droebachiensis*. *Mar. Biol.* 129:159–167.
- Naylor, C., L. Maltby, and P. Calow. 1989. Scope for growth in *Gammarus pulex*, a freshwater benthic detritivore. *Hydrobiologia* 188:517–523.
- Oleksiak, M. F., G. A. Churchill, and D. L. Crawford. 2002. Variation in gene expression within and among natural populations. *Nat. Genet.* 32:261–266.
- Oleksyn, J., J. Modrzynski, M. Tjoelker, P. Reich, and P. Karolewski. 1998. Growth and physiology of *Picea abies* populations from elevational transects: common garden evidence for altitudinal ecotypes and cold adaptation. *Funct. Ecol.* 12:573–590.
- Olivares-Banuelos, N. C., L. M. Enriquez-Paredes, L. B. Ladah, and J. De La Rosa-Velez. 2008. Population structure of purple sea urchin *Strongylocentrotus purpuratus* along the Baja California peninsula. *Fish. Sci.* 74:804–812.
- Oliveri, P., D. M. Carrick, and E. H. Davidson. 2002. A regulatory gene network that directs micromere specification in the sea urchin embryo. *Dev. Biol.* 246:209–228.
- Osovitz, C. J., and G. E. Hofmann. 2005. Thermal history-dependent expression of the hsp 70 gene in purple sea urchins: biogeographic patterns and the effect of temperature acclimation. *J. Exp. Mar. Biol. Ecol.* 327:134–143.
- Palumbi, S. 1992. Marine speciation on a small planet. *Trends Ecol. Evol.* 7:114–118.
- . 1994. Genetic divergence, reproductive isolation, and marine speciation. *Annu. Rev. Ecol. Syst.* 25:547–572.
- Palumbi, S. R., and A. C. Wilson. 1990. Mitochondrial DNA diversity in the sea urchins *Strongylocentrotus purpuratus* and *S. Droebackiensis*. *Evolution* 44:403–415.
- Pearse, J. S. 2006. Ecological role of purple sea urchins. *Science* 314:940–941.
- Pearse, J. S., V. B. Pearse, and K. K. Davis. 1986. Photoperiodic regulation of gametogenesis and growth in the sea urchin *Strongylocentrotus purpuratus*. *J. Exp. Zool.* 237:107–118.
- Pespeni, M., T. Oliver, M. Manier, and S. Palumbi. 2010. Restriction Site Tiling Analysis: accurate discovery and quantitative genotyping of genome-wide polymorphisms using nucleotide arrays. *Genome Biol.* 11:R44.
- Pespeni, M. H., D. A. Garfield, M. K. Manier, and S. R. Palumbi. 2012. Genome-wide polymorphisms show unexpected targets of natural selection. *Proc. R. Soc. B: Biol. Sci.* 279:1412–1420.
- Prosser, C. L. 1986. *Adaptational biology: molecules to organisms*. Wiley, New York, NY.
- R Development Core Team. 2009. *R: A language and environment for statistical computing*. Foundation for Statistical Computing, Vienna, Austria.
- Rogers-Bennett, L. 2007. The ecology of *Strongylocentrotus franciscanus* and *Strongylocentrotus purpuratus*. Pp. 393–425 in J. M. Lawrence, ed. *Edible sea urchins: biology and ecology*. Elsevier, Amsterdam, The Netherlands.
- Sanford, E., and M. Kelly. 2010. Local adaptation in marine invertebrates. *Ann. Rev. Mar. Sci.* 3:509–535.
- Sanford, E., and D. J. Worth. 2010. Local adaptation along a continuous coastline: prey recruitment drives differentiation in a predatory snail. *Ecology* 91:891–901.
- Sea Urchin Genome Sequencing Consortium, E. Sodergren, G. M. Weinstock, E. H. Davidson, R. A. Cameron, R. A. Gibbs, R. C. Angerer, L. M. Angerer, M. I. Arnone, D. R. Burgess, et al. 2006. The genome of the sea urchin *Strongylocentrotus purpuratus*. *Science* 314:941–952.
- Slatkin, M. 1973. Gene flow and selection in a cline. *Genetics* 75:733–756.
- Strathmann, R. 1978. Length of pelagic period in echinoderms with feeding larvae from the Northeast Pacific. *J. Exp. Mar. Biol. Ecol.* 34:23–28.
- Supek, F., M. Bošnjak, N. Škunca, and T. Šmuc. 2011. REVIGO summarizes and visualizes long lists of Gene Ontology terms. *PLoS One* 6:e21800.
- Swindell, W., M. Huebner, and A. Weber. 2007. Plastic and adaptive gene expression patterns associated with temperature stress in *Arabidopsis thaliana*. *Heredity* 99:143–150.
- Tegner, M., and P. Dayton. 1991. Sea urchins, El Niños, and the long term stability of Southern California kelp forest communities. *Mar. Ecol. Prog. Ser.* 77:49–63.
- Waples, R. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *J. Hered.* 89:438–450.
- Warner, R. 1997. Evolutionary ecology: how to reconcile pelagic dispersal with local adaptation. *Coral Reefs* 16:115–120.
- Warnes, G., B. Bolker, and T. Lumley. 2006. gplots: various R programming tools for plotting data. R package version 2.3. 1.
- Whitehead, A., and D. L. Crawford. 2006a. Variation within and among species in gene expression: raw material for evolution. *Mol. Ecol.* 15:1197–1211.
- . 2006b. Neutral and adaptive variation in gene expression. *Proc. Natl. Acad. Sci. U.S.A.* 103:5425–5430.
- Whitehead, A., J. L. Roach, S. Zhang, and F. Galvez. 2011. Genomic mechanisms of evolved physiological plasticity in killifish distributed along an environmental salinity gradient. *Proc. Natl. Acad. Sci. U.S.A.* 108:6193–6198.
- Widdows, J., and D. Johnson. 1988. Physiological energetics of *Mytilus edulis*: scope for growth. *Mar. Ecol. Prog. Ser.* 46:113–121.
- Wray, G. A. 2007. The evolutionary significance of *cis*-regulatory mutations. *Nat. Rev. Genet.* 8:206–216.
- Zamer, W. E., and C. P. Mangum. 1979. Irreversible nongenetic temperature adaptation of oxygen uptake in clones of the sea anemone *Haliplanella luciae* (Verrill). *Biol. Bull.* 157:536–547.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Table S1.

Table S2.

Figure S1.