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SYMPOSIUM

Signs of Adaptation to Local pH Conditions across an Environmental Mosaic in the California Current Ecosystem

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Synopsis Little is known about the potential for rapid evolution in natural populations in response to the high rate of contemporary climatic change. Organisms that have evolved in environments that experience high variability across space and time are of particular interest as they may harbor genetic variation that can facilitate evolutionary response to changing conditions. Here we review what is known about genetic capacity for adaptation in the purple sea urchin, *Strongylocentrotus purpuratus*, a species that has evolved in the upwelling ecosystem of the Northeast Pacific Ocean. We also present new results testing for adaptation to local pH conditions in six populations from Oregon to southern California. We integrate data on 19,493 genetic polymorphisms with data on local pH conditions. We find correlations between allele frequency and rank average time spent at pH <7.8 in 318 single-nucleotide polymorphisms in 275 genes. Two of the genes most correlated with local pH are a protein associated with the cytoskeleton and a proton pump, with functional roles in maintenance of cell volume and with internal regulation of pH, respectively. Across all loci tested, high correlations with local pH were concentrated in genes related to transport of ions, biomineralization, lipid metabolism, and cell–cell adhesion, functional pathways important for maintaining homeostasis at low pH. We identify a set of seven genes as top candidates for rapid evolutionary response to acidification of the ocean. In these genes, the putative low-pH-adapted allele, based on allele frequencies in natural populations, rapidly increases in frequency in purple sea urchin larvae raised at low pH. We also found that populations from localities with high pH show a greater change in allele frequency toward putative low-pH-adapted alleles under experimental acidification, compared with low-pH populations, suggesting that both natural and artificial selection favor the same alleles for response to low pH. These results illustrate that purple sea urchins may be adapted to local pH and suggest that this species may possess the genetic capacity for rapid evolution in response to acidification. This adaptive capacity likely comes from standing genetic variation maintained in nature by balancing selection across the spatial and temporal environmental mosaic that characterizes the California Current Ecosystem.

Introduction

Species with broad geographic distributions generally persist across a wide range of environmental conditions (Endler 1977). This persistence may be achieved by physiological plasticity, evolutionary adaptation to local environmental conditions, or a combination of both physiological and genetic mechanisms (Hochachka and Somero 2002; Sanford and Kelly 2011). Dispersal between environmentally distinct regions, however, may hinder the evolutionary process of adaptation because locally adapted alleles are homogenized by gene flow among regions (Slatkin 1987). Such gene flow leads to the wide distribution of most alleles (Pespeni et al. 2012; Pespeni and Palumbi 2013) and the potential for offspring from most populations to possess adaptive alleles for a wide variety of environments. The natural combination of spatial and temporal heterogeneity yields a rich environmental mosaic in which to understand

how organisms persist under variable conditions. Studies in such systems will be critical for predicting the capacity for evolutionary responses to future, rapid environmental changes.

Here we focus on the purple sea urchin, *Strongylocentrotus purpuratus*, as a model organism for understanding the adaptive genetic landscape for a high-dispersal species across an environmental mosaic. The purple sea urchin is a broadcast spawning marine invertebrate that can be found in most inter-tidal and sub-tidal rocky reefs from the cold waters of Alaska to the warmer waters of Baja California, Mexico (Rogers-Bennett 2007). The genomic tools, ecological understanding, and increasing environmental monitoring associated with purple sea urchins is yielding important insights into how environmental variability and the evolutionary force of balancing selection may maintain adaptive genetic variation in populations of purple sea urchins (Sea Urchin Genome Sequencing Consortium et al. 2006; Pespeni et al. 2012; Evans et al. 2013; Kelly et al. 2013; Pespeni and Palumbi 2013). This reservoir of adaptive alleles may be critical for evolutionary responses by purple urchins and other marine species to future climatic change. As a result, insights into the capacity and limits of standing genetic variation to fuel future adaptive shifts for this species may be illustrative of other species in the Eastern Pacific and elsewhere.

This article includes three main sections. First, we review recent evolutionary insights into the population biology of the purple sea urchin. Second, we review evidence for the evolution of gene regulation and the role of spatial and temporal balancing selection in allowing this species to persist across an environmental mosaic. Third, we present new results from previously published data that demonstrate a link between local pH and genetic variation. Our previous work showed how low pH was an agent of selection at scores of genes across the genomes of larval purple sea urchins (Pespeni et al. 2013b). The current work adds to the environmental context of these results and provides key candidate genes and pathways that may enable resilience in this, and other, species. We explore natural variation among populations in their degree of adaptation to local pH and responsiveness to experimental acidification.

Evolutionary history and population genomics of the purple sea urchin

Purple sea urchins and their congeners radiated in the North Pacific Ocean from the arctic southward approximately 10 million years ago during the Late Miocene (Biermann et al. 2003; Lee 2003). They evolved after the beginning of strong upwelling that began 15–12 million years ago (Vermeij 1989; Jacobs et al. 2004), along with many other ecologically and economically important groups of diverse taxa on the West Coast, such as giant kelp, Pacific salmon, Sebastes rockfish, and abalone (Jacobs et al. 2004). The burst of species-formation in the late Miocene established the rich, upwelling-dependent ecosystem that has characterized the West Coast of North America for millions of years (Estes and Steinberg 1988). The current purple sea urchins' range spans this ecosystem from Alaska to Baja California (Rogers-Bennett 2007). The environmental conditions under which they evolved and in which they exist today are a mosaic of seasonally and spatially variable temperature and pH due to the interaction of upwelling with coastal topography and bathymetry (Estes and Steinberg 1988; Menge 2000; Feely et al. 2008).

Despite broad environmental heterogeneity along the species' range, previous studies using mitochondrial and allozyme markers found little to no population structure along the species' range (Palumbi and Wilson 1990; Edmands et al. 1996; Pespeni and Palumbi 2013). A genome-wide scan of 12,431 single-nucleotide polymorphisms (SNPs) shows that all alleles at these sites are present in two populations separated by over 1700 km (Boiler Bay, Oregon, and San Diego, California). That is, there are no fixed differences in alleles between these populations at these 12,431 sites across the genome (Pespeni et al. 2010). The lack of strong genetic structure and the sharing of alleles in space are consistent with the high potential of sea urchins for dispersal, resulting from external fertilization and a life-history stage of swimming and feeding larvae (Strathmann 1978).

While there is high potential for dispersal and general genetic homogeneity among purple sea urchin populations, there are several signals of limited gene flow at specific genes across the genome suggestive of local adaptation. Although there are no fixed allelic differences between urchins from the coasts of Oregon and California, genome-wide patterns of allele frequencies are different enough to allow populations to be distinguished using principal component analysis, partitioning genetic variance in space (Fig. 1A) (Pespeni et al. 2010). Analyses integrating data on gene function identify specific classes of genes that may be important for local adaptation; specifically genes in the Ubiquitin protein-degradation pathway show high levels of genetic differentiation relative to the genome-wide average (Pespeni et al. 2012). The differentiated Ubiquitin genes are E3 ligases, target-specific regulators of several cellular

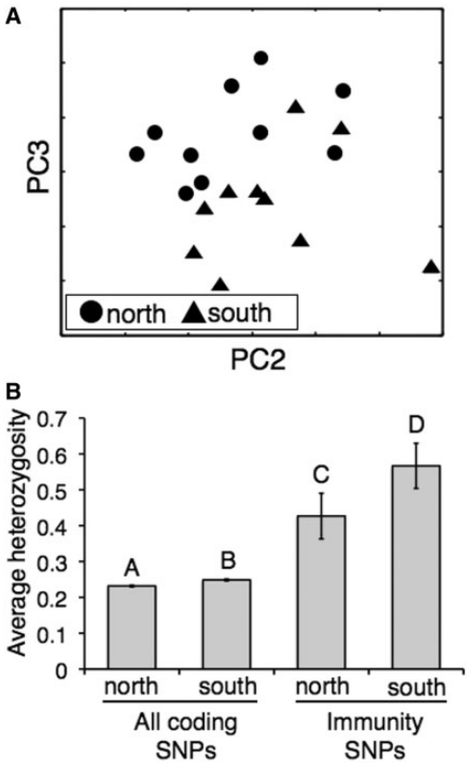

Fig. 1 Population differentiation and signs of local adaptation in the highly dispersing purple sea urchin. Individuals were sampled from Boiler Bay, Oregon (north) and San Diego, California (south). (**A**) Separation of populations based on the partitioning of variance in the genetic variation of 12,431 genome-wide polymorphisms using principal component analysis. Adapted from Pespeni et al. (2010). (**B**) Signs of balancing selection in the elevated heterozygosity in the coding regions of immune-related genes. While levels of heterozygosity across all loci are higher in southern urchins, heterozygosity in immunity genes is disproportionately higher in southern populations where disease pressure is higher, and thus suggestive of local adaptation. Adapted from Pespeni et al. (2012).

processes from metabolism to development to programmed cell death (Pespeni et al. 2012). Additionally, there is an excess of allelic diversity in immunity-related genes in the southern population where incidence of disease is high, suggesting that there is a selective pressure for higher diversity in immune genes, similar to what has been observed in major histocompatibility complex (MHC) loci in vertebrate adaptive immune systems (Fig. 1B) (Pespeni et al. 2012). Taken together, these results suggest that natural selection has acted on specific genes across the genome leaving signatures of reduced gene flow in some gene classes and elevated genetic diversity in others. These complex patterns of diversity across the genome may be made possible by the high levels of genetic diversity and the high rates of recombination in this species, and evolutionary processes such as balancing selection and soft sweeps (Hermisson and Pennings 2005; Pespeni and Palumbi 2013).

A follow-up study on putative adaptive loci suggests that a combination of natural selection and local retention of alleles in the area of the Southern California Bight may allow purple sea urchin populations to adapt to the distinct environmental conditions that characterize that region (Pespeni and Palumbi 2013). Seven genes were identified as putatively adaptive or putatively neutral based on the genome-scan study (Pespeni et al. 2010). These genes were studied in greater detail by the direct sequencing of many more individuals sampled from six populations spanning most of the species' range from Bamfield, Canada, to Punta San Carlos, Mexico (Pespeni and Palumbi 2013). The putative adaptive loci showed signatures of selection while the putative neutral loci generally did not (discussed in more detail in the following section). Patterns across all five putative adaptive loci showed reduced gene flow into and out of the San Diego population situated in the Southern California Bight; a pattern not observed in the putative neutral genes (Pespeni and Palumbi 2013). The Southern California Bight harbors warmer waters as the coastal reefs are situated inshore of a broad continental shelf protected from upwelling, and distinct from the colder, upwelling coastlines to the north and south (Bakun and Parrish 1982; Smith and Eppley 1982; Zaytsev et al. 2003; Mann and Lazier 2006). The signature of differentiation in San Diego suggests that adaptation to local temperature, pH, or disease might be operating. In this region, the scale of environmental variation might be larger than the scale of larval dispersal, allowing a multi-generational build-up of different alleles.

Evolution of gene regulation and balancing selection

Adaptive differences between populations or species can often be attributed to differences in the regulation of gene expression (King and Wilson 1975; Whitehead and Crawford 2006). This is particularly true when there is a high degree of spatial and temporal environmental variability in the species' range (Gilchrist and Huey 2004; Swindell et al. 2007; Levine et al. 2011). In this section, we discuss several recent studies that suggest important roles for regulatory differentiation and balancing selection in allowing populations of purple sea urchins to persist in environments that are highly variable in space and time. In the population genomics study

mentioned in the previous section, differentiation in Ubiquitin-related genes occurs in the upstream putative regulatory regions of these genes (Pespeni et al. 2012). Detailed sequence-analyses of these upstream regions identified known regulatory motifs, similar to those observed in the regulatory regions of well-studied genes of purple sea urchins, suggesting that genetic variation in these regions may have functional consequences for gene regulation (Pespeni et al. 2012).

While these studies reveal putative functional diversity in the regulatory regions of genes, it remained to be determined whether there are differences in gene regulation between populations. Pespeni et al. (2013a) conducted the first long-term, common-garden acclimation study of adult purple sea urchins from distant populations (Pespeni et al. 2013a). The goal of common-garden acclimation is to erase differences in environmental history to reveal genetically controlled differences, in this case differences in gene regulation (Prosser 1986; Hochachka and Somero 2002). Pespeni et al. grew urchins from San Diego, California, and Boiler Bay, Oregon, together at the Hopkins Marine Station in Monterey, California, for 3 years and then sampled tissue from these co-cultured individuals to assess transcriptome-wide patterns of gene expression. The authors measured transcript abundance levels of 18,883 genes using RNA-sequencing. While most of the transcriptome showed equivalent levels of gene expression between the populations sampled, the study revealed persistent differences in the regulation of genes related to biomineralization and growth (Pespeni et al. 2013a).

Expression in genes related to biomineralization and metabolism was consistently higher in southern urchins, compared with northern urchins (Fig. 2). The differences predict that southern urchins would have higher scope for growth than would northern urchins under the common-garden conditions. A post hoc experiment confirmed that southern urchins indeed regrew spines at a faster rate than did northern urchins (Pespeni et al. 2013a). These results demonstrated that the first-order transcriptome-wide gene expression phenotype has morphological consequences. Higher scope for growth in southern urchins could suggest that urchins native to the region of the Southern California Bight thrive in the cooler, resource-abundant conditions of the Monterey Bay common-garden (urchins were fed *ad libitum*) relative to the urchins native to the Oregon coast that experienced relatively warmer temperatures and higher pH levels in the Monterey Bay common-garden. These gene-regulatory

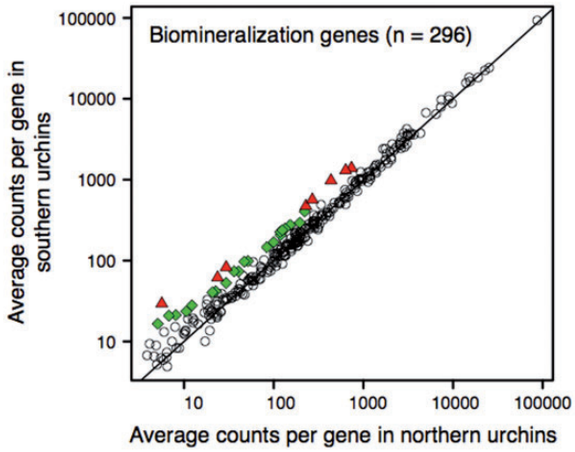

Fig. 2 Persistent differences in the regulation of 10% of biomineralization genes between long-term, common-garden-acclimated purple sea urchins from Boiler Bay, Oregon, and San Diego, California. Each point in the scatter plot represents the average gene expression in northern urchins versus southern urchins for the 296 genes related to biomineralization in the purple sea urchin; triangles show genes differentially expressed across all 18,883 genes tested for differences in expression (FDR $P < 0.05$), diamonds add genes differentially expressed out of the biomineralization subset (q-value < 0.05), while open circles show the genes related to biomineralization which were not differentially expressed between populations (FDR $P > 0.05$). The diagonal line represents the 1:1 line. Among the 10% differentially expressed genes, gene expression is higher in southern urchins than in northern ones in all cases. Adapted from Pespeni et al. (2013a).

differences appear to be genetically controlled and may have consequences for locally-adapted populations of urchins as they experience changing climatic regimes. Changes in environmental conditions that affect growth or biomineralization, such as changes in temperature or pH, may have uneven effects across populations.

The evolution of gene regulation and balancing selection are likely important forces in maintaining functional diversity in an environmentally heterogeneous seascape. As described in the previous section, Pespeni and Palumbi tested five loci that had high genetic differentiation in the previous genome scan (Pespeni et al. 2010) for signs of natural selection at the sequence level using direct sequencing in purple sea urchins from populations spanning much of the species' range from Canada to Baja California, Mexico (Pespeni and Palumbi 2013). The putative adaptive loci showed signatures of selection including correlations with temperature rather than with latitude or an excess of changes in amino acids, in contrast to the putative neutral loci tested. Two of the five putative adaptive loci tested were transcription factors. Both of these genes show high levels of amino-acid changing

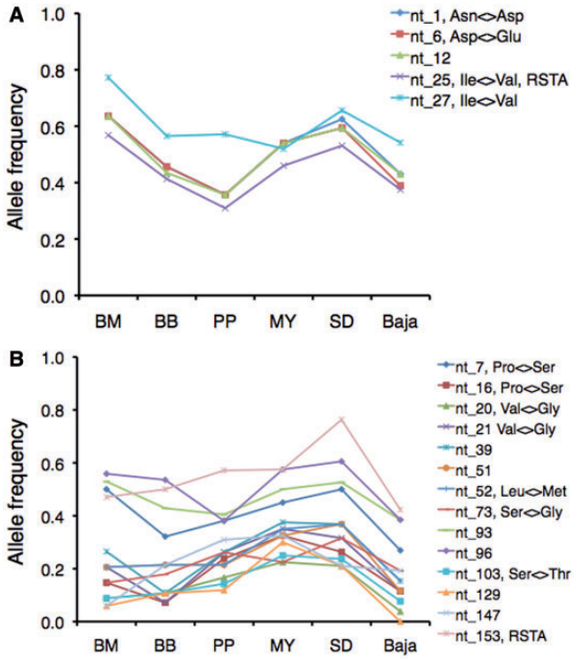

Fig. 3 Excess variation in amino acids and signals of spatial or temporal balancing selection in two transcription-factor proteins: transcription factor 25 (**A**, SPU_015723) and transcription factor (**B**, SPU_002852). Lines show allele frequencies of each SNP in populations from north to south, Bamfield, Canada (BM), Boiler Bay, Oregon (BB), Patrick's Point, California (PP), Monterey, California (MY), San Diego, California (SD), and three sites sampled in Baja California, Mexico (Baja). RSTA (Restriction-Site Tiling Analysis) denotes the polymorphisms originally identified as differentiated between populations. Adapted from Pespeni and Palumbi (2013).

polymorphisms maintained in the six populations studied (dN/dS >> 1), as well as signals of spatial or temporal balancing selection (see Fig. 3A and B for allele frequencies across populations) (Pespeni and Palumbi 2013). Many alleles in these transcription factor genes differ in their sequences of amino acids. The function of these transcription factors is either particularly immune to variation in amino acids or there is a great deal of functional variation among individuals in the genes regulated by these transcription factors.

Another study explored the evolution of gene regulation in purple sea urchins to find high levels of genetic variation and signals of positive selection in the regulatory regions of six out of eight genes tested (Garfield et al. 2012). These genes, involved in early development of sea urchins, were chosen for investigation because their regulatory regions had been well characterized in previous studies, not because they had been identified as putative targets of selection. This locus selection process makes the signals of natural selection all the more striking and suggests that gene regulatory pathways central in early development may be more responsive to selective pressures and less canonical than generally considered. It is also noteworthy that the high levels of genetic diversity and signals of balancing selection are harbored in a single population sampled off Santa Barbara, California (Garfield et al. 2012). In sum, these studies highlight the role of balancing selection in maintaining polymorphisms that vary gene regulation within and among populations of a species in a heterogeneous land- or seascape as a ripe area for further investigation, particularly in the context of rapidly changing climatic conditions.

Environmental variability and adaptive genetic variation in a changing climate

The maintenance of adaptive genetic variation in the spatially and temporally heterogeneous landscape illustrated in the above studies could allow populations or species to adaptively evolve in response to climatic change. This adaptive genetic variation may be more likely to be present in natural populations if populations have previously experienced selective pressures in response to spatial and temporal environmental variability in their ecological and evolutionary history (Lande and Shannon 1996). Little is known, however, about the ability of populations or species to genetically evolve in response to climatic change (Hoffmann and Sgrò 2011). Even less is known about how adaptive genetic variation may be partitioned in space among populations (but see Langer et al. 2009; Byrne et al. 2011; Parker et al. 2011; Kelly et al. 2013).

The acidification of the oceans presents a major threat to organisms that secrete calcium carbonate skeletons and shells, such as corals, mussels, urchins, and plankton (Harley et al. 2006; Hoegh-Guldberg et al. 2007; Doney et al. 2009). The California Current Ecosystem, however, is an excellent natural laboratory for studying the potential for evolutionary adaptation to low pH. Regional differences in the seasonal upwelling of cold, low-pH, nutrient-rich waters along the coasts of Oregon and California create a spatially and temporally variable environmental mosaic of pH conditions (Feely et al. 2008). Organisms living in this habitat seasonally experience pH conditions similar to those predicted for the year 2100 (Hofmann et al. 2011).

Several studies to date have demonstrated a capacity for evolution in response to high-CO_2 in a range of marine species including three urchin species, a bryozoan species, a mussel species, and multiple strains of a coccolithophore species (Langer et al.

2009; Ridgwell et al. 2009; Findlay et al. 2011; Parker et al. 2011; Pistevos et al. 2011; Sunday et al. 2011; Foo et al. 2012; Kelly et al. 2013; Pespeni et al. 2013b). In these studies, organisms from one or more natural populations were brought into the laboratory and cultured under conditions of high CO_2. These experiments revealed that the organisms have standing genetic variation for heritable responses to climatic change.

In the present study, we address which natural populations of purple sea urchins harbor genetic variation for evolutionary response to low pH by testing for correlations between local pH conditions experienced in nature and local frequencies of alleles. We also test if putative low-pH adapted alleles identified in natural populations are the alleles that show the greatest responses to experimental acidification. If populations are adapted to local pH conditions, we expect to validate two simple predictions: (1) that populations experiencing chronically high CO_2 harbor alleles that improve survival under conditions of high CO_2 and (2) that these alleles should show the greatest response to experimental acidification in populations that do not chronically experience high CO_2. To validate the first prediction, we expect to find more alleles correlated with local pH than expected by chance and that these correlated alleles would be in genes predicted to play important roles in maintaining homeostasis under high-CO_2 conditions. To validate the second prediction, we expect that larvae from populations that experience low CO_2 in nature would show greater changes in allele frequency in response to experimental acidification in the putative high-CO_2 adapted genes.

Correlations with local pH conditions

In a previously published study, we collected adult purple sea urchins from six populations along the species' range from Oregon to Southern California (Pespeni et al. 2013b). In the laboratory, we spawned urchins, fertilized eggs, and raised resulting embryos and larvae at ambient (400 µatm) and elevated (900 µatm) CO_2 to match present-day, global, mean atmospheric CO_2 and a fossil-fuel intensive projection, respectively (Moss et al. 2010). We measured allele frequencies at 19,493 SNPs in pools of ~1000 individuals at two stages in development, Day-1 blastula and Day-7 four-arm plutei, for each condition and each population. In the present analysis, we used estimates of allele frequencies from Day-1 blastula in ambient CO_2 to represent allele frequencies in the wild (40 alleles sampled; 20 parents: 10 females and 10 males contributed gametes to each population pool). See Appendix 1 for more details on the methods of larval culture and genome analysis. Adjacent to each collection site, we took high-frequency measurements of local pH using an autonomous pH sensor deployed from April to September, 2011 at each study site (F. Chan et al., in preparation). We calculated the amount of time spent below pH 7.8 for each site to capture the severity of low pH conditions experienced there. Sites were ranked 1 through 6 based on time spent below pH 7.8, with the site ranked number 1 having spent the most time under low-pH conditions (Table 1).

We identified genes potentially adapted to local pH conditions by testing for a correlation between the allele frequency in the population and the rank local pH. We identified 318 SNPs present in 275 genes correlated with local pH ($P<0.01$), 65% more than expected by chance out of the 19,493 SNPs tested. There were 1439 SNPs correlated with local pH at the $P<0.05$ level, 47% more than expected by chance, although neither set survived correction for false discovery rate (FDR $P>0.05$). The top 10 annotated genes with correlated SNPs are listed in Table 2. They include a cytoskeletal protein, a proton-pump protein, and a sterol-carrier protein, proteins that based on previous studies may play roles in internal regulation of pH and in cellular stability at low pH (Putnam 1998; Szászi et al. 2001; Pörtner 2008). See Supplementary Table S1

Table 1 Sampling locations, abbreviations, coordinates, and local pH conditions for six adult urchin collection sites

Location	Site abbrev.	Latitude	Longitude	Rank acidity	Mean pH	SD pH
Fogarty Creek, OR	FC	44.81	124.1	1	7.97	0.18
Strawberry Hill, OR	ST	44.25	124.1	2	8.02	0.17
Van Damme State Park, CA	VD	39.28	123.8	4	7.96	0.09
Bodega Marine Reserve, CA	BD	38.31	123.1	3	7.99	0.14
Terrace Point, CA	TP	36.95	122.1	6	8.10	0.11
Alegria, CA	AL	34.25	119.6	5	8.08	0.13

Rank acidity was determined by the amount of time spent at pH < 7.8 at each site from April to September, 2011, a period of time that spanned the upwelling season for all sites.

Table 2 Top 10 genes with allele frequencies in natural populations correlated to local pH conditions (out of 318 SNPs with $P<0.01$)

Gene annotation	Gene ID	SNP position	Avg. no. reads per sample	Correlation (r)	P-value
Cytoskeleton associated protein	SPU_004863	261	1254	0.96	0.001
Glutamic-oxaloacetic transaminase 2	SPU_014067	372	1201	0.82	0.002
Vacuolar proton pump delta polypeptide	SPU_012628	192	817	0.81	0.002
Lysyl-tRNA synthetase	SPU_008981	522	1057	0.81	0.002
Immunomodulatory protein	SPU_004320	239	572	0.81	0.001
Endoplasmic reticulum-golgi intermediate	SPU_017463	270	1043	0.80	0.002
Sterol carrier protein 2 isoform 1 proprotein	SPU_006383	432	830	0.78	0.002
Hydroxyacyl-coenzyme A	SPU_008554	768	1614	0.76	0.002
Frizzled homolog 8a	SPU_022916	945	935	0.76	0.001
Eukaryotic translation initiation factor 3	SPU_026863	1281	1505	0.74	0.002

For additional candidates, see Supplementary Table S1 for the complete list of 318 SNPs correlated with local pH and site allele frequency data.

Table 3 Functional classes of proteins with an overrepresentation of SNPs correlated with local pH conditions ($P<0.01$)

Functional category	GO ID	No. SNPs	No. Genes	P-value
Energy coupled proton transport, against electrochemical gradient	GO:0015988	285	32	0.000
Biomineralization	custom list	884	97	0.001
Cell–cell adhesion	GO:0016337	695	63	0.001
Proton transport	GO:0015992	364	45	0.002
Lipid oxidation	GO:0034440	425	37	0.003
Anion transport	GO:0006820	499	60	0.003
Fatty acid catabolic process	GO:0009062	404	35	0.008
Homophilic cell adhesion	GO:0007156	266	30	0.008
Response to amine stimulus	GO:0014075	182	19	0.009
Total no. unique genes			299	

for a complete list of 318 SNPs with population allele frequencies. These results suggest the action of natural selection in response to local pH conditions, as there are more SNPs than expected by chance that were correlated with pH. Moreover, the identified SNPs are in genes predicted to be important for physiological response to low pH.

Functional enrichment

Another signal of natural selection is the non-random concentration of SNPs correlated with local pH in specific functional classes of genes. We characterized the protein function of all genes represented by the 19,493 SNPs using the Gene Ontology database (Ashburner et al. 2000) and generated our own list of biomineralization genes based on the purple sea urchin literature (Pespeni et al. 2013a) (see Methods in Appendix 1 for more details). We then performed functional enrichment analyses; for each functional class, we tested for a correlation between membership in that class and the strength of correlations to local pH of the SNPs in that class relative to permuted data (see Methods in Appendix 1 for more details). Significant results in this test would indicate that correlations to local pH are not random with respect to protein function, suggesting that natural selection broadly affects genes with specific functions that improve survival under conditions of local pH. Out of these 1307 categories tested, we found nine functional classes of genes whose members had SNPs that were more highly correlated with local pH conditions ($P<0.01$, Table 3). These categories did not survive FDR correction (Benjamini and Hochberg 1995). However, categories related to proton transport, cell adhesion, and biomineralization were overrepresented in the top nine categories based on their representation across the complete list of 1307 categories tested (Fisher's exact test, $P<0.001$, $P<0.01$, and $P<0.05$, respectively). We predicted the involvement of genes related to the processing of lipids and to the

homeostasis of ions, based on the classes of genes that responded to experimental acidification (Pespeni et al. 2013b). Indeed, oxidation of lipids, catabolism of fatty acids, and anionic transport were among the top nine categories ($P < 0.01$). In sum, eight of the nine categories enriched for high correlations to local pH play physiological roles expected, on the basis of previous work, to be involved in maintaining homeostasis at low pH (Pörtner et al. 2005; Melzner et al. 2009; Todgham and Hofmann 2009; O'Donnell et al. 2010; Spicer et al. 2011; Stumpp et al. 2011; Pespeni et al. 2013b).

SNP correlations with local pH and functional enrichment may not survive multiple test correction because of high levels of gene flow and high degrees of spatial and temporal environmental heterogeneity, weakening the strength of these population-level signatures of natural selection. However, patterns are stronger than expected by chance and it is striking that genes and functional classes of proteins most correlated with local pH are those predicted to play roles in growing and surviving at low pH.

Top candidates for response to acidification of the ocean

We identified seven genes as top candidates that showed (1) signs of adaptation to local pH conditions and (2) responsiveness to experimental acidification (Fig. 4). These genes include phosphoribosyltransferase, a catalytic and regulatory protein involved in

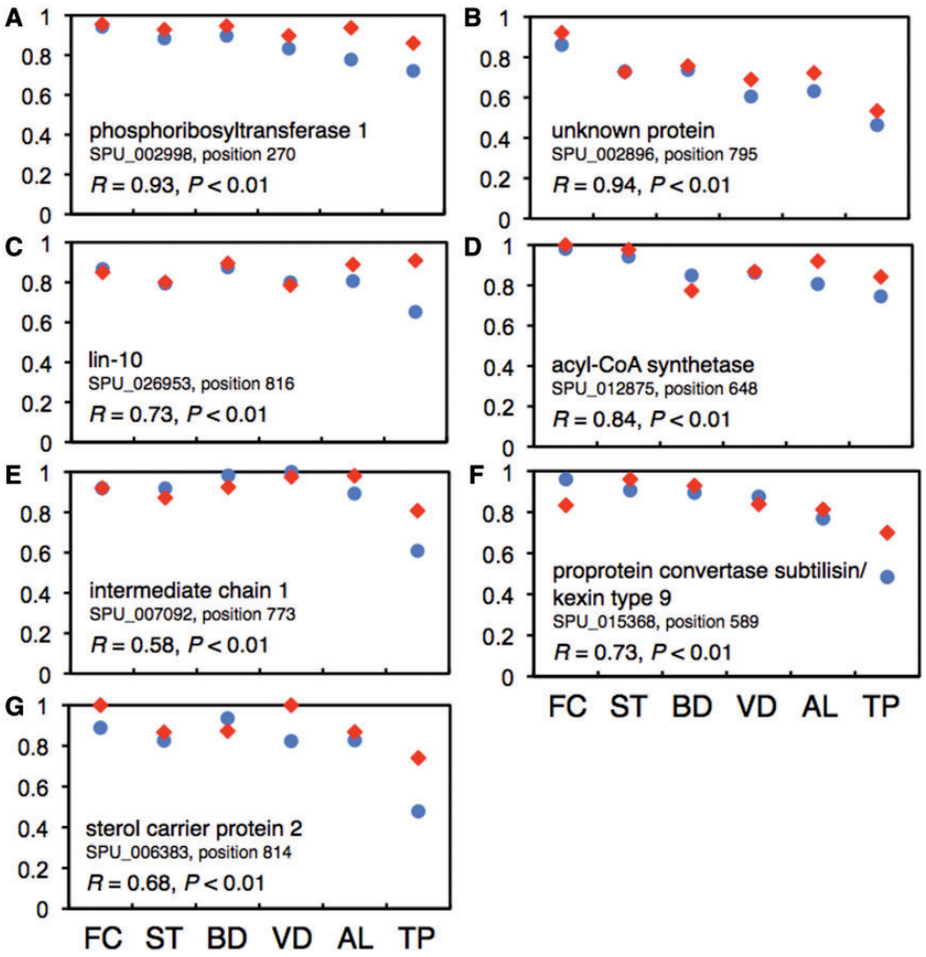

Fig. 4 Top-candidate genes for response to acidification are correlated with local pH and show strong responses to experimental acidification in high-pH-adapted populations. Allele frequency plotted by population ordered by rank time spent at pH < 7.8, generally north to south, but see Table 1 for latitude and longitude. The sites were Fogarty Creek, Oregon (FC), Strawberry Hill, Oregon (ST), and in California at Bodega Marine Reserve (BD), Van Damme State Park (VD), Alegria, Santa Barbara (AL), and Terrace Point, Santa Cruz (TP). Circles represent allele frequencies in nature estimated from larvae sampled at Day 1 under ambient CO_2; diamonds represent allele frequencies after experimental acidification, Day 7 at elevated CO_2. Values of *R* and *P* are from the correlations between allele frequencies in the wild (Day 1, ambient CO_2, blue dots) and rank time spent at pH < 7.8.

nucleotide synthesis and salvage; lin-10, a gene that functions in development; a sterol-carrier protein; a non-specific lipid-transfer protein; acyl-CoA synthetase, a metabolic enzyme involved in fatty-acid synthesis; proprotein convertase subtilisin/kexin type 9, a protein that plays a regulatory role in cholesterol homeostasis; and a protein of unknown function. Three of these six annotated genes play roles in lipid-processing, similar to the classes of genes previously shown to respond to experimental acidification (Pespeni et al. 2013b). These seven candidates show clinal patterns with respect to time spent at pH < 7.8 and show increases in frequency of the putatively low-pH-adapted allele in response to experimental acidification, particularly in populations from high-pH regions. These genes are candidates worthy of further investigation in acidification experiments on natural populations in this and other species. These genes might also be evaluated as biomarkers to predict the sensitivity or capacity for resilience of particular populations.

Signals of adaptive genetic variation

If populations were adapted to local pH conditions via natural selection for alleles that perform best at low pH, we would expect a greater change in allele frequency during experimental acidification to occur in populations that experience high pH, compared with populations that experience low pH. In the absence of a selective force, there is an equal probability that low-pH populations would show the same magnitude of allelic change as high-pH populations. We found that indeed there was a difference in the magnitude of change in allele frequency in response to experimental acidification between populations from environments with low versus high pH (Kolmogorov–Smirnov test, $P < 0.0001$, Fig. 5). As predicted, if the difference were due to selection for low-pH adapted alleles, the magnitude of change was greater in the high-pH populations as allele frequencies shifted toward the low-pH-adapted allele ($t = 4.03$, $P < 0.0001$, Fig. 5). These results suggest that our experimental acidification mimicked low-pH conditions experienced in the wild and that the same genetic machinery is used for response both to natural and to experimental low pH.

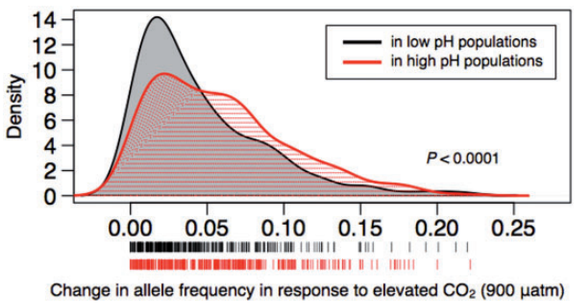

Fig. 5 Genetic change in response to experimental acidification is greatest in populations that experience higher pH in the wild ($t = 4.03$, $P < 0.0001$) in putatively pH-adapted SNPs ($n = 318$, $P < 0.01$). The solid distribution shows changes in allele frequency in response to experimental acidification in populations that experience low pH in the wild (FC and ST) while the hashed distribution shows changes in allele frequency in response to experimental acidification in populations that experience high pH in the wild (AL and TP). The area under the curve of a density function represents the probability of observing a value of x given a range of x values (calculated using the "density" function in R). The rug plots under the graph show the actual data points as tick marks. Genetic changes between wild (Day 1, ambient CO_2) and acidification treatment (Day 7, elevated CO_2) increase the frequency of alleles putatively adapted to low pH.

Summary and conclusions

We have suggested that purple sea urchin populations may harbor genetic variants that allow populations to thrive in variable environments as conditions change in space or time. We first reviewed evidence for (1) population differentiation, particularly in the warmest, most environmentally distinct region of the species' range, the Southern California Bight, (2) putative adaptive regional differences in gene regulation, particularly in genes related to growth and biomineralization, and (3) spatial or temporal balancing selection, particularly in transcription-factor proteins. We then presented new results that suggest that populations may be adapted to local pH conditions and that putative low-pH-adapted alleles are the same genetic variants selected by experimental acidification. Taken together these studies suggest that northern and southern urchin populations may have different strong suites in the rapidly changing climatic conditions: southern populations may have higher fitness in variable temperature and disease conditions while northern populations may have higher fitness in high-CO_2 conditions. The sharing of alleles across space through larval dispersal, high rates of recombination, and overall high levels of genetic diversity may allow populations of this species to evolve at the same tempo as climatic conditions change. However, the consequences of such rapid evolution, such as loss of genetic diversity, are unknown.

Our results highlight several open questions regarding the potential resilience of species in changing climatic conditions. These include: (1) What are the costs of such rapid evolution and consequent loss of genetic diversity? (2) As adaptation requires genetic variability, do organisms have the capacity to respond to concurrent environmental pressures such as changes in pH,

temperature, predators, disease, and availability of food? (3) What species-level characteristics, such as population size, levels of genetic variation, gene flow, and degree of spatial and temporal environmental heterogeneity experienced during a species' evolutionary history, predict their resilience or vulnerability to changing climatic conditions?

Our data are promising but the picture remains incomplete for purple sea urchins. In purple sea urchins and other species, more needs to be known about the capacity for multiple natural populations to respond to concurrent environmental stressors. Future environmental changes may be synergistic, rather than additive, and extremes at both ends of the thermal and pH spectra may be more common. For example, the intensity of upwelling has been increasing over the past 50 years, periodically resulting in more extreme low pHs and low temperatures while average annual temperatures have been steadily increasing (Bakun 1990; Snyder et al. 2003; Iles et al. 2012). Future interactions of climate and ecosystem will be complex and uncertain, and the effects of these interactions may be dramatic (Bakun 1990; Helmuth et al. 2010). Finally, we do not know how the patterns we observe in this species will translate to other species. Evolution in a temporally and spatially variable environment may generate the genetic diversity needed for evolutionary response to changing climatic conditions, although a deeper understanding of population-level and species-level capacity for rapid evolutionary responses is necessary for predicting and managing the consequences of climatic change.

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Supplementary data

Supplementary Data available at ICB online.

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Appendix 1

Methods

Collection and larval culture of urchins

Thirty adult purple sea urchins were collected from each study site (Table 1) and shipped to the Bodega Marine Laboratory. For each population, urchins were spawned with 0.5 M KCl in sea water and gametes of 10 females and 10 males were mixed for fertilization. Fertilized embryos from each population were divided into two groups, and the groups placed in two separate jars, one maintained at ambient pCO_2 levels (400 µatm, present-day global-mean atmospheric CO_2) and the other at elevated pCO_2 levels (900 µatm, a moderate "fossil-fuel intensive" projection for the year 2100) (Moss et al. 2010). The cultures were stocked at 0.66 larvae per milliliter, an ecologically relevant density (Strathmann 1987). The culture jars were held in seawater tables maintained at 14°C. Seawater in the jars was stirred constantly using oscillating paddles and every 2 days 90% of the seawater was exchanged, removing water with reverse filtration through mesh and replacing with filtered seawater pre-equilibrated with the desired pCO_2 level. Beginning 3 days after fertilization after each exchange of water, larvae were fed a mixture of algae (*Rhodomonas* sp. and *Dunaliella* sp.) *ad libitum*. See Pespeni et al. (2013b) for complete details of culture methods and water chemistry.

Population genomics

To summarize from the previous study, for each population, we sampled approximately 1000 live larvae at

Day 1 and Day 7 post-fertilization for each pCO_2 treatment. We extracted total RNA from each larval pool using TRIzol and prepared sequencing cDNA libraries using the TruSeq kit following the manufacturer's protocol (Illumina, San Diego, CA). Each sample was sequenced on a single Illumina HiSeq lane yielding ~80 million 50 bp reads per sample (Microarray and Genomics Core Facility, University of Utah, Salt Lake City, UT). Raw sequence data were trimmed for quality and then length using the FASTX toolkit programs (Gordon 2011) with a minimum Phred-scale quality score of 30 and a minimum length of 30 bp. We mapped the reads of each sample to all predicted purple sea urchin genes (downloaded from www.spbase.org) using the Burrows-Wheeler Aligner (BWA) program (Li and Durbin 2009) and using default parameters except $n = 0.001$, to allow for the high genetic variability in purple sea urchins (Sea Urchin Genome Sequencing Consortium et al. 2006; Pespeni and Palumbi 2013). SNPs across all samples were detected using Genome Analysis Toolkit (GATK) programs (McKenna et al. 2010) and the developer's most stringent criteria (http://www.broadinstitute.org/gatk/guide/). Estimates of frequencies of alleles for each sample of the larval pool were extracted from the resulting .vcf files by dividing the number of quality reads that mapped to the reference allele by the total number of quality mapped reads at that position, using a custom python script (available upon request). A pipeline of data-processing steps that can be customized can be found at http://sfg.stanford.edu (De Wit et al. 2012).

Estimates of allele frequencies from larvae sampled at Day 1 from ambient CO_2 conditions were taken to represent allele frequencies present in wild populations. Estimates from larvae sampled at Day 7 after culture at elevated CO_2 were taken to represent allele frequencies after laboratory culture at high CO_2. Differences in allele frequency between these two Day × Treatment samples were taken to represent changes in allele frequency due to mortality of larvae. Alternative explanations for differences in allele frequency could be (1) differences in size of larvae and therefore in abundances of transcripts among samples (however, we measured minimal size-differences between treatments so larval size is unlikely to affect the estimates), or (2) differences in allele-specific expression, but we found no evidence for this in the data (see Pespeni et al. (2013b) for analyses and discussion).

Collection of data on environmental pH

We took high-frequency measurements of local pH using an autonomous pH sensor deployed at each study site (F. Chan et al., in preparation). The pH sensor was mounted at intertidal sites where adult sea urchins were collected so as to most accurately record the pH experienced by urchins in the wild (except for Alegria, CA, 34.47°N, where records from the nearest available sensor at Lompoc, CA, 34.72°N, were used). The pH sensor was a modified version of the SeaFET sensor (Martz et al. 2010) which utilizes an ion-sensitive field-effect transistor Honeywell DuraFET pH probe and packaged with an internal data logger and power supply (Evans et al. 2013). Measurements were taken every 10 min across the core April to September upwelling season in 2011. The amount of time spent below pH 7.8 was calculated for each site to capture the severity of low pH experienced at each site. Sites were ranked 1 through 6 based on the time spent below pH 7.8, with 1 spending the most time at low pH (Table 1).

Test for correlations between allele frequency and local pH

For each SNP, we tested for a correlation between allele frequency and rank local pH at the six study sites. We used partial Mantel tests implemented in the R programming environment (R Development Core Team 2009) using the function "mantel.partial" in the vegan library (Oksanen et al. 2008) controlling for geographic distance. *P*-values were determined by random permutation of the data and recalculation of the correlation 1000 times. Prior to testing for a correlation, we standardized the variance for each variable by dividing by the mean and subtracting one standard deviation, according to the procedure of Selkoe et al. (2010).

Test for a relationship between gene-function and correlation to local pH

We tested for the non-random concentration of SNPs with high correlations to local pH conditions in specific functional classes of genes, an approach called functional enrichment analysis. We characterized each gene using UniProt identifiers (Bairoch et al. 2009), then Gene Ontology (GO) biological process categories (Ashburner et al. 2000). We also included a list of biomineralization genes, genes important for skeletal growth in sea urchins (as in Pespeni et al. 2013a, 2013b). To reduce noise and focus on more interesting patterns across the genome, we limited analyses to functional classes that had between 10 and 100 gene members, excluding classes that were too narrow or broad, respectively. There were 1307 functional categories that had between 10 and 100 gene members. For each

functional category, we tested for a correlation between membership in that category and gene score using gene-score resampling implemented in ErmineJ (Lee et al. 2005). For gene scores, we used log-transformed *P*-value for each SNP calculated in the partial Mantel test for a correlation between allele frequency and rank pH described above. When there were multiple SNPs per gene, we used the mean score. Statistical significance was determined by 10,000 permutations, and corrected *P*-values were calculated using the Benjamini-Hochberg approach (Benjamini and Hochberg 1995). To determine whether specific functional categories were overrepresented among those with $P < 0.01$, we compared the observed frequencies versus expected frequencies above and below this cut-off of *P*-value using Fisher's exact test.

Identification of top-candidate genes and signals of selection

We identified top-candidate genes for response to acidification by exploring the 318 SNPs with correlations to local pH ($P < 0.01$). These SNPs showed general clinal patterns with respect to rank time spent at pH < 7.8 for each site. We compared allele frequencies estimated from the wild (Day 1, 400 µatm) clinal alleles with allele frequencies after 7 days at low pH (Day 7; 900 µatm). Presuming these alleles to be adapted to low pH, we looked for minimal changes in allele frequency under experimental acidification in the populations putatively adapted to low pH (FC and ST) and for changes of greater magnitude in the high-pH populations (AL and TP). If all populations had responded to the experimental acidification by Day 7, we would expect low variability in allele frequencies among populations at Day 7, for the larvae at 900 µatm. To identify the top candidates for response to acidification, we made these calculations as described and ranked loci accordingly. We also visually inspected patterns at all 318 loci by writing a script to loop through and plot all 318 loci. The top seven candidates were selected, based on these criteria, although all 318 loci with allele frequencies for each population can be found in Supplementary Table S1.

If experimental selection targeted the same alleles that were adapted to local pH conditions in the wild, we would expect the magnitude of genetic change in response to experimental acidification (low pH, high CO_2) to be greatest in populations that normally experience high pH in nature and lower in populations that typically experience low pH in nature. To test for this, we calculated the average difference in allele frequency between larvae at Day 1 (400 µatm) and larvae at Day 7 (900 µatm) in the sets of two populations with the rank-highest and rank-lowest pH conditions (FC and ST, and AL and TP, respectively). We compared the two response distributions using a Kolmogorov–Smirnov (K-S) test (ks.test function in R) and compared these average responses with low pH conditions using a *t*-test predicting the high-ranking populations to show the greatest response (t.test function in R).