Title: Rare genetic variation is important for survival in extreme low pH conditions

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Abstract

Population persistence through increasingly frequent extreme environmental fluctuations will require selection on standing genetic variation. While some species have shown the capacity to adapt to mean future conditions, the ability to survive and potentially adapt to extreme events is unknown. Here we used pooled capture sequencing to test for adaptive capacity and identify genetic variation responsive to moderate (pH 8.0) and extreme (pH 7.5) low pH conditions using single generation selection experiments on hundreds of thousands of *Strongylocentrotus purpuratus* sea urchin larvae generated from wild-caught adults. The single generation of selection showed consistent shifts in allele frequencies across replicate cultures and increased linkage disequilibrium around selected loci, revealing selective sweeps from standing variation. We found extreme pH selection targeted alleles at low frequency in the population while variants that responded to both moderate and extreme pH selection started at higher allele frequencies, suggesting maintenance by balancing selection. Variants with the greatest changes in allele frequencies performed functions related to lipid metabolism, pH tolerance, membrane trafficking, and regulation of actin/cytoskeleton dynamics. These results highlight that survival in extreme conditions relies on low frequency standing genetic variation that must be maintained by large population sizes.

Introduction

As temperatures increase and oceans become more acidic, many marine species are at high risk of decline and extinction [1]. In addition to changing global averages, the frequency and intensity of extreme events are also increasing [2]. With small or gradual changes in conditions, organisms may acclimate through physiological plasticity or migrate to suitable habitats where possible, but some amount of genetic adaptation will be necessary for continued population persistence, particularly in the context of extreme events [3]. Indeed, extreme events rather than average conditions set the physiological and biogeographic limits of individuals and populations [4-6]. Though limits set by rare extreme events will be critical for population persistence, the genetic mechanisms that could allow such rapid adaptation have rarely been explored [7].

Evolve or select & resequence, a type of experimental evolution, is particularly powerful and promising for understanding capacity to adapt to future conditions and extreme events. In this approach, one artificially induces the selective treatment and directly measures genetic response during adaptation [8-11]. While evolve and resequence studies typically leverage 15-20 generations of selection [10,12], we have empirically found that adaptive genetic variation can be identified with a single generation of selection by using 1000s of small offspring generated from highly heterozygous, outbred parents collected from the wild [13].

The purple sea urchin, *Strongylocentrotus purpuratus*, is an ideal model for understanding the process of adaptation from adaptive standing genetic variation. This species is found on inter- and subtidal rocky reefs and kelp forests across a broad latitudinal range from Alaska to Baja California, Mexico in the California Current Marine Ecosystem (CCME). They experience high heterogeneity in environmental conditions in both time and space [14-16], increasing the likelihood of the presence of adaptive standing genetic variation [17-19]. Further, this species is a good system to understand the genetics of rapid adaptation due to large census and effective population sizes that contribute to high standing genetic variation [20,21]. While gene flow is high due to pelagically dispersing larvae, populations nevertheless show increased frequencies of variants putatively adaptive to local temperature and pH conditions [22]. Previous studies have shown that *S. purpuratus* larvae have the physiological and genetic capacity to adaptively respond to an acidifying ocean [13,15,23], but these studies have focused on relatively

64 mild conditions and have not investigated the capacity to respond to extreme pH conditions that
65 are predicted to increase in frequency in the near future [2,24].
66 Here, we perform single generation selection experiments in moderate (pH 8.0) and
67 extreme low (pH 7.5) pH conditions using purple sea urchin larvae generated from 25 wild-
68 caught adults. We test the hypotheses that (1) there will be unique genetic variation responsive
69 to extreme low pH conditions, and (2) that genomic patterns of variation, such as linkage
70 disequilibrium and starting allele frequencies, will provide insight into the evolutionary
71 mechanisms that maintain adaptive standing genetic variation for survival in moderate and
72 extreme conditions, and (3) pooled sequencing of genomic DNA from larvae before and after
73 treatments will show consistent changes in allele frequency across replicate cultures due to
74 selective mortality through development.
75
76 **Methods**
77 *Sample collection & experiment:*
78 Purple sea urchin adults were collected in September 2016 from San Diego, CA, shipped
79 overnight to the University of Vermont, and the experiment began immediately upon their arrival
80 (n=25 total: 14 females and 11 males). Spawning was induced with 0.5M KCl in Instant Ocean
81 artificial seawater (ASW) (Instant Ocean, Blacksburg, VA) at 14°C and salinity of 35ppt. For
82 each female, 200,000 eggs were put into each treatment, moderate (pH 8.0) and extreme (pH 7.5)
83 low pH, and fertilized by evenly pooled sperm from all males. We chose these pH conditions
84 based on empirical data collected in the CCME. The current average open ocean and intertidal
85 pH is 8.1, though conditions in the CCME frequently drop to pH 8.0 (daily), and only rarely drop
86 as low as pH 7.5 (once in three month upwelling period; [14,16,24,25]). Fertilized eggs were
87 pooled across all females by pH and seeded into four replicate culturing vessels per treatment
88 (37,000 eggs per 3.7 L vessel). Developing embryos were sampled at day 1 and day 7 post
89 fertilization for morphometric and genomic analyses. See the supplemental methods for
90 expanded details.
91
92 *Morphometrics*:
93 7-day old 4-armed pluteus larvae were photographed for morphometric analysis using a
94 Photometrics Scientific CoolSNAP EZ camera (Tuscon, AZ) connected to a Zeiss Axioscop 2

95 compound microscope (Jena, Germany). Larval body size data were analyzed in R [26] with a
96 generalized linear mixed model in the package lme4 [27], with pH as a fixed effect and culturing
97 vessel as a random effect. See the supplemental methods for expanded details.
98
99 *DNA sequencing, Mapping, & SNP-calling:*
100 DNA was extracted from pools of larvae for each day and replicate vessel using a Zymo
101 ZR-Duet DNA/RNA MiniPrep Plus Kit (Zymo, Irvine, CA). High quality DNA was shipped to
102 Rapid Genomics (Gainesville, FL) for library prep, capture, and sequencing. Following library
103 generation, DNA libraries were captured with 46,316 custom probes. Probes were designed to
104 capture two 120 base pair regions per gene: one within exon boundaries and one in putative
105 regulatory regions. Barcoded samples were then pooled and sequenced using 100 bp paired-end
106 sequencing on one lane of an Illumina HiSeq 3000.
107 Raw paired-end reads were quality trimmed and mapped to the *S. purpuratus* genome 3.1
108 (build 7) with bwa mem [28]. Variants were identified using *mpileup* in Samtools and filtered for
109 minor allele frequency (maf) of 0.01, quality greater than 20, bi-allelic SNPs only, and no
110 missing data. Sequencing depth can influence the accuracy of allele frequency estimation, and
111 we used only variants where each pool was sequenced to a depth of > 40x and with an average
112 minimum depth across all pools > 50x, as recommended by Schlotterer et al. [29]. Mean
113 maximum coverage cutoff was 372. Finally, we removed off target variants, which were defined
114 as any variant greater than 2kb from a probe. This filtering process resulted in 77,449 variant
115 sites. See supplemental methods for more details. Code for data processing and analyses can be
116 found on our GitHub page: https://github.com/PespeniLab/urchin_sel_ms_2018.
117
118 *Detecting changes in allele frequency:*
119 Cochran-Mantel-Haenszel (CMH) tests were conducted in R (*mantelhaen.test*) to identify
120 significant shifts in allele frequency in response to pH treatment. CMH tests are a standard
121 method of identifying changes in allele frequency in experimental evolution studies [10]. To
122 identify consistent changes in allele frequency through developmental time, we compared allele
123 frequency estimates for the four replicate samples from day 1 at pH 8.0 (T_0) to the four replicate
124 samples from day 7 at pH 8.0 and to the four replicate samples from day 7 at pH 7.5. P-values
125 were corrected using the *qvalue* package in R [30]. These data could be affected by long range

126 linkage disequilibrium [31]; therefore, we take a conservative approach and consider q-values <
127 0.001 significant.
128 A principal components analysis (PCA) was used to visualize relationships between
129 allele frequencies of treatment replicates using the R package *pcadapt* [32]. We calculate
130 nucleotide diversity for each treatment using a sliding window approach in Popoolation with the
131 *variance-sliding* command [33]. Window size was set to 400 bp with step size of 200 bp.
132 Distributions of nucleotide diversity were compared with a Kolmogorov-Smirnov test using the
133 *ks.test* function in R.
134 Following a selective sweep, sites under selection should show a pattern of higher linkage
135 disequilibrium (LD) than variants responding to drift alone. To test for this pattern, we compared
136 LD estimates among pairs of SNPs across the genome for selected (CMH $q < 0.001$) and neutral
137 (non-selected, CMH $q \geq 0.001$) variants using LDx [34] (supplemental methods). LDx uses a
138 maximum likelihood approach and leverages haplotype information of SNPs present on single
139 reads to estimate LD between pairs of variants. An exponential decay model was fit to each
140 group of LD estimates using the R package *nls* [35]. To assess whether the levels of LD present
141 in selected sites could be due to the lower number of variants than is present in neutral sites, we
142 randomly subsampled all variants 500 times to match the number of selected loci and compare
143 these decay curves to the observed decay in LD using a Kolmogorov-Smirnov test.
144 To assess the distribution of starting allele frequencies of adaptive loci, selected loci were
145 polarized by the frequency of the allele increasing in frequency in response to pH selection (i.e.,
146 the putative adaptive allele). Neutral loci ($q \geq 0.001$) were polarized in the same way, though this
147 is a random assignment given the lack of a significant shift in frequency. To ensure the patterns
148 observed were not a byproduct of the CMH statistic having the greatest power to identify
149 variants at low starting allele frequencies, the observed allele frequency distributions were
150 compared to permuted distributions with no true biological signal as described in the
151 supplemental methods.
152 Gene ontology (GO) enrichment was performed using the weight algorithm in topGO
153 version 2.22.0 [36]. GO terms for each gene were retrieved from EchinoBase
154 (http://www.echinobase.org/). Enrichment tests were conducted for each set of significant
155 variants and genes that had any SNPs in genic or intergenic regions were considered the target
156 set. Any gene with multiple significant variants was only considered once.

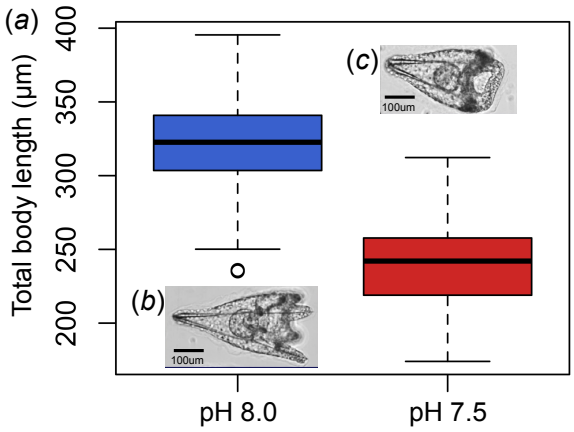

Figure 1: Morphometric analysis of total larval body length. (a) Boxplot of body size in each selection regime. Inset pictures show representative individuals found in pH 8.0 (b) and 7.5 (c).

Results

Morphometrics

Mean total larval body length was significantly lower in pH 7.5 (mean ± standard error: 239.1 ± 3.1 micrometers) as compared to pH 8.0 (320.9 ± 2.9 micrometers) (Fig. 1a, $P < 0.001$). Overall, larvae in the extreme low pH treatment were more stunted, with smaller bodies and spines compared to moderately low pH conditions (Fig. 1b-c). These results suggest that physiological and selective impact was stronger in pH 7.5 than pH 8.0. While we were unable to measure mortality with these data, estimates from other experiments in our lab show 30% survival in ambient (pH 8.1) conditions and 10% survival in extreme low (pH 7.5) conditions after 7 days.

Consistent allele frequency changes among replicate selection lines

We identified 75,368 variable sites present in or near 9,828 genes. 1,078 variants (in 816 genes) showed consistent changes in allele frequency (AF) in response to selection at pH 7.5 and 724 variants (in 579 genes) in response to pH 8.0 (CMH, $q < 0.001$; see Table S1 for a summary of results). 177 (11%) of these significant variants overlapped (in 144 genes, which is 11% of unique genes). However, assessing the overlap of genes that had loci targeted by selection, rather than overlap of specific variants, 205 genes overlapped (17% of unique genes). Overall, 2% of

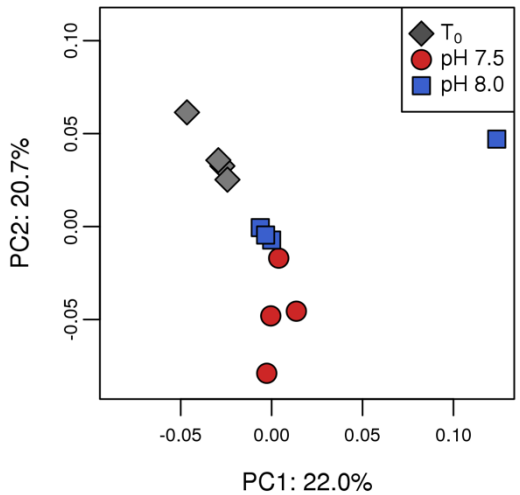

Figure 2: Principal component analysis of allele frequencies for all 75,368 SNPs at time point zero (T_0) and after seven days in the two the pH treatments.

177 variants surveyed (1,625/77,449) and 12% of genes surveyed (1,200/9,828) were identified as
178 responsive to one or both pH treatments at *q*-value < 0.001.
179 Of the total assayed variants, 50,686 (67%) were in genic regions while 24,677 (33%)
180 were in intergenic regions. Matching expectations of chance sampling, for pH 7.5 selected loci,
181 725 (67%) were genic and 353 (33%) were intergenic (chi square, $P > 0.05$). Similarly, pH 8.0
182 selected loci consist of 506 (70%) genic and 218 (30%) intergenic loci (chi square, $P > 0.05$).
183 Given the rapid decay in linkage disequilibrium (see below), these results suggest that there are
184 both important coding and putative regulatory pH-responsive loci segregating in populations.
185 Principal component analysis (PCA) showed that the variance in allele frequencies
186 among larvae sampled from replicate culture vessels clustered by day and treatment (Fig. 2). pH
187 7.5 samples show the largest shift from the starting allele frequencies as expected with increased
188 selective mortality due to treatment. Note that one of the D7 pH 8.0 samples was an outlier. To
189 ensure that this sample did not artificially reduce power, we removed this sample and down
190 sampled all replicates to n=3, and reran the CMH test. We found the same relative numbers of
191 significant variants for both pH treatments.
192
193 *Signals of shared and pH-specific selection*
194 Patterns of LD among loci showed the highest LD between SNP pairs involving pH 7.5
195 loci, followed by pH 8.0 selected loci. Further, both sets of selected loci had higher LD than the

196 neutral expectation and putatively neutral sites matched genome wide expectations when
197 controlling for the number of variants sampled (Fig. 3 and Fig. S1). We observed rapid decay of
198 LD within 200 base pairs, which is expected given the high levels of genetic diversity, large
199 effective population size, high fecundity, and high gene flow of this species.

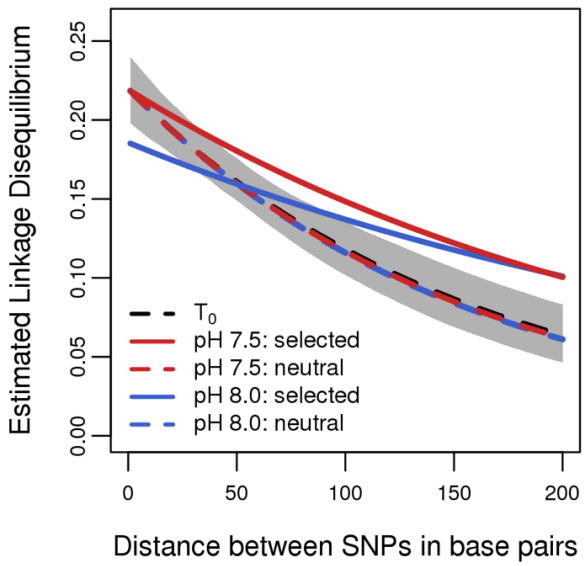

Figure 3: Decay in linkage disequilibrium with physical distance between SNP pairs. Dashed lines are neutral loci and solid red and blue lines represent decay in pH 7.5 and 8.0 selected variants, respectively. Solid grey shading is the 95% distribution of 500 permutations of down sampled random variants across the genome (see text for details).

200 Shifts in allele frequency showed unique and shared signals between pH selection
201 regimes (Fig. 4a, S2). Significant loci specific to pH 7.5 show a correlation in average allele
202 frequency change of 0.68 with their non-significant pH 8.0 counterparts ($P < 0.001$) suggesting
203 that the same loci were responding to treatment though to a lesser degree in pH 8.0; pH 8.0
204 significant loci reveal the same pattern with a correlation of 0.69 ($P < 0.001$). As expected, loci
205 that were identified as significant in both treatments showed the strongest correlation in allele
206 frequency change ($r^2 = 0.87$, $P < 0.001$). Interestingly, the loci with the most extreme shifts in
207 allele frequency were significant in both selection regimes (upper right quadrant of Fig. 4a, Fig.
208 S3).

209 To understand how variants are segregating and maintained in populations, we explored
210 the starting allele frequencies (T_0) for loci identified as responsive to pH 7.5, pH 8.0, and in both
211 treatments (Fig. 4b). All three sets of selected loci had significantly lower starting allele
212 frequencies than neutral loci (KS test, $P < 0.001$). Loci responsive to the most extreme selection

213 regime, pH 7.5, had significantly lower starting allele frequency than loci responsive pH 8.0 (KS
214 test, $P < 0.05$) and than loci responsive to both pH treatments (KS test, $P < 0.1$). Loci responsive
215 to just pH 8.0 and to both treatments had similar distributions of starting allele frequency (KS
216 test, $P = 0.38$). We also compared observed allele frequency distributions of selected loci to the
217 allele frequency distributions of the same number of randomly sampled loci across the genome
218 (Fig. 4B, S4). The allele frequency distribution for all three groups of selected variants were
219 significantly different from all 1000 randomly sampled distributions (KS tests, $P < 0.001$).
220 Lastly, we randomly shuffled sample IDs and compared starting allele frequencies of observed
221 and permuted "selected" loci. These permutations showed that the CMH statistic was not biased
222 towards loci that started at low frequencies (Fig. S5). These results suggest that loci underlying
223 low pH adaptation are rare in the starting population and more severe selection targets less
224 common variants. However, loci responsive to both pH treatments are more common than pH-
225 specific loci.
226 This overall pattern an excess of low allele frequency variants among selected loci is
227 consistent across functional classes of variants (non-synonymous, synonymous, intronic,

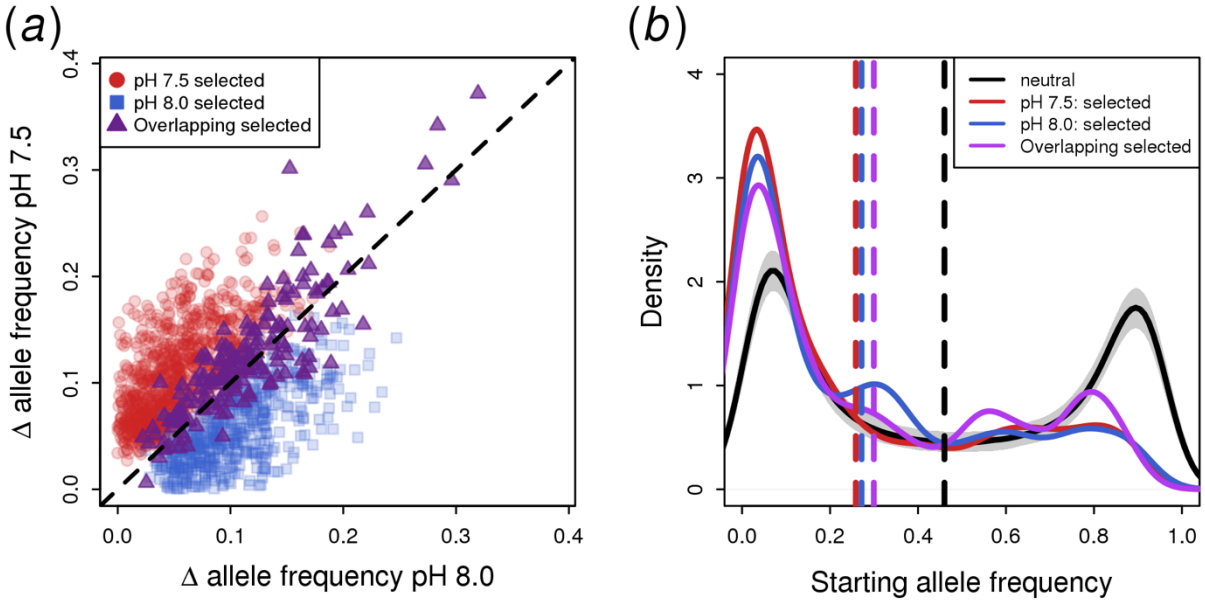

Figure 4: (a) Mean change in allele frequency for variants showing significant shifts under pH 7.5 and 8.0. Dashed black line represents the 1:1 expectation. (b) Allele frequency at T_0 polarized by the allele increasing in frequency during selection. Colored solid lines are the density plot distributions for each set of selected loci. The solid black line represents the median expectation of neutral loci from 1000 permutations of down sampling to the same number of variants as selected loci; grey shading is the 95% distribution of the permutation. Dashed vertical lines are the mean allele frequencies of each set of variants.

228 intergenic; Fig. S6). Low starting allele frequencies of responsive variants could suggest that
229 surviving offspring were from a single parent. However, this is unlikely to be the case due to the
230 rapid decay of linkage disequilibrium for selected loci relative to neutral loci. In addition, the
231 large number of responsive loci and genes suggests that survival in low pH conditions is a highly
232 polygenic trait, thus it is unlikely that surviving offspring had all the adaptive alleles from a
233 single parent.

234 Genetic diversity at T_0 was 0.0158 and 0.0129 and 0.0128 after seven days at pH 8.0 and
235 7.5, respectively. Both pH selection regimes resulted in a decrease in genetic diversity compared
236 to T_0 (KS test, $P < 0.001$). The decrease in diversity between the pH treatments was not different
237 (KS test, $P = 0.27$), demonstrating that, while the selection regimes were of different magnitude,
238 the relative loss of genetic diversity through time was similar.

239 Functional enrichment analysis revealed that genes under selection were enriched for
240 specific biological functions (Table S2). We observed enrichment for 19, 12, and 10 biological
241 processes GO terms for pH 7.5, pH 8.0, and overlapping variants, respectively. Of these, only
242 hexose metabolic process (GO:0019318) was significant across all three variant sets. Other
243 related terms shared across sets included those involved in mitotic damage control (GO:0044818;
244 GO:0007095; GO:0044774), cell growth (GO:0007099; GO:0030307; GO:0032467),
245 metabolism and energy production (GO:0001678; GO:0009267; GO:0019318; GO:0006096).
246

247 **Discussion**

248 We show that genetic response to moderate and extreme low pH relies on both shared
249 and unique mechanisms and that rare variants are important for survival in extreme low pH
250 conditions while common (intermediate frequency) variants are important for survival in both
251 moderate and extreme low pH regimes. Patterns of linkage disequilibrium and starting allele
252 frequencies suggest that both neutral and selective processes maintain adaptive variants in
253 natural populations. We further demonstrate the utility of single generation selection experiments
254 to identify the genetic basis of adaptation, which is particularly useful for testing capacity for
255 response to conditions that will be chronic in the future but are not so in nature today. Using
256 sequence capture of genomic DNA, we quantified shifts in allele frequency that represent
257 differential survival of genotypes during low pH selection in sea urchins. This approach has wide
258 potential application for identifying responsive genetic variants from wild populations. Our

259 results highlight that neutral standing genetic variation maintained with large population sizes
260 will be critical for survival in extreme environmental conditions.
261
262 *Detecting adaptive loci from standing genetic variation*
263 In the short term, a selective sweep results in decreased variation and increased LD, both
264 of which can be leveraged to identify adaptive genomic regions [37,38]. Our results reveal an
265 increase in LD in regions surrounding putatively adaptive loci, and the permutation shows that
266 this increase in LD is not a byproduct of our test statistic or sampling noise (Fig. 3, S1). This
267 provides confidence that our approach identifies genomic regions that are true targets of
268 selection. In addition, because the experimental design relies on selection on standing genetic
269 variation where loci will be present on multiple haplotypes, selective sweeps will retain ancestral
270 variation, soft sweeps, and signals are expected to be weaker than a hard sweep [18,39,40].
271 Typical evolve and resequence studies rely on multiple generations of selection to
272 identify adaptive shifts in allele frequency. However, a single generation selection can be
273 leveraged for organisms with high fecundity and small offspring, which is particularly useful for
274 long-lived organisms. This approach is unique and has benefits over standard experimental
275 evolution. First, one starts with outbred wild-caught individuals, which will increase starting
276 genetic diversity relative to inbred lab strains or isofemale lines [12,41,42]. Inference is limited
277 to the genetic diversity present in the starting individuals and may miss variation present at
278 different points in space or time and will miss the potential of new beneficial mutations. For
279 organisms with large effective population sizes and high mutation rates, new adaptive mutations
280 may be an important mode for adaptation [18]. However, starting with outbred parents
281 maximizes the amount of recombined genetic variation, avoids large linkage blocks, which can
282 plague experimental evolution studies from lab lines, and thereby improves the chances of
283 identifying selected loci. Moreover, generating a large amount of offspring from all crosses of
284 many parents creates a vast number of uniquely recombined genotypes upon which selection can
285 act. Finally, because offspring are small, it is possible to subject replicates of thousands of
286 individuals to selection, which is not possible with less fecund species with larger offspring.
287 The lack of genomic resources can limit the utility of a sequence capture approach for
288 non-model organisms. One solution is to utilize resources from closely related species.
289 Alternatively, it is possible to use a transcriptome rather than whole genome for probe design or

290 to utilize expressed exome capture [43]. While this does not allow for the design of probes in
291 promoter regions, it is typically available at a lower cost than whole genome sequencing.
292 Previous work in the purple sea urchin has used pooled RNA-sequencing data to identify
293 potentially adaptive genes and shifts in allele frequencies consistent among replicate pools within
294 pH treatments [13]. We find in the present study that allele frequencies estimated based on RNA
295 and genomic DNA from the same pooled larval samples are highly correlated ($r^2 = 0.93$, $P <$
296 0.001; Fig. S7), demonstrating that RNA-seq is a viable option for generating allele frequency
297 estimates.
298
299 *Maintenance of adaptive standing genetic variation*
300 Variants that respond to extreme low pH treatment are present in the starting population
301 (T_0) at low allele frequencies relative to neutral alleles (Fig. 4B). This pattern suggests that these
302 adaptive variants are not maintained by balancing selection or overdominance, but rather by one
303 of three processes. First, these alleles may be beneficial under extreme low pH conditions but
304 neutral or slightly deleterious at ambient conditions, known as conditional neutrality [44].
305 Alternatively, antagonistic pleiotropy can alter the rank fitness of alleles across different
306 environments, thus maintaining genetic variation across space or time [45]. Finally, purple sea
307 urchins are distributed along the west coast of North America from Alaska to Baja California,
308 Mexico [21,46] where pH conditions vary due to natural processes such as upwelling, and
309 populations have putative adaptive genetic variation associated with local pH conditions [22].
310 High gene flow results in extensive transfer of genetic variation across the geographic range and
311 migration-selection balance can result in the maintenance of low frequency alleles that are not
312 adaptive in a local environment [47]. Similar results have been observed in *D. melanogaster*
313 during experimental evolution to high temperature where allele frequencies of adaptive loci were
314 shifted towards low starting values [31]. Interestingly, this low shift was only observed for
315 adaptation to high but not low temperature, providing additional evidence that the CMH statistic
316 is not inherently biased towards low starting minor allele frequencies.
317 Interestingly, loci responsive to both moderate and extreme pH treatments had higher
318 starting allele frequencies than those responsive to extreme low pH alone (Fig. 4B). These "low-
319 pH-essential" alleles may be maintained at higher allele frequency due to balancing selection in
320 the wild. That is, the spatial and temporal heterogeneity of pH conditions experienced by purple

321 sea urchins across the species range and across their life history stages likely maintains these
322 alleles at intermediate frequencies through fluctuating selection or spatially balancing selection
323 [48]. We should note that these shared loci may also include variants involved in selection for
324 the general lab culture conditions. While the experimental design of this study precludes the
325 isolation of lab-adaptation variants, the concordance of functional classes of genes previously
326 identified as responsive to moderate low pH conditions (pH 7.8) in purple sea urchin larvae
327 generated from multiple populations [13,22] suggests that lab selection is unlikely to affect
328 general results, patterns, or conclusions in the present study.
329
330 *Mechanisms for response to low pH conditions*
331 Numerous biological processes related to maintaining homeostasis in low pH conditions
332 are overrepresented among selected loci. Across all sets of selected variants, we observe
333 enrichment in processes related to metabolism and energy production (GO:0001678,
334 GO:0009267, GO:0009061, GO:0019318, GO:0006096). Alterations to metabolic processes,
335 energy demands, and allocation are a primary response to low pH environments [49,50] and
336 previous *S. purpuratus* transcriptomic work has shown that this class of genes is differentially
337 regulated in response to low pH stress [15,51]. The production of calcium carbonate ($CaCO_3$)
338 generates excess protons that must be removed to maintain acid-base balance, and under acidic
339 conditions and under low pH conditions the energy required for acid-base regulation is increased
340 [50]. We also see shared enrichment for genes involved in cell proliferation and damage control,
341 including those implicated in cell growth and replication (GO:0030307, GO:0032467,
342 GO:0007099, GO:0006272), replication checkpoints (GO:0044818, GO:0044774, GO:0000076,
343 GO:0007095, and protein transport (GO:0046825, GO:0006606). These results match our
344 morphometric results of stunted larval growth and suggest that fine control of cellular growth
345 may be important for survival in low pH conditions. Two genes of interest that showed changes
346 in allele frequency greater than 20% in both pH treatments included ELMOD2 (SP-ELMOD2,
347 SPU_007564), the locus with the highest changes in allele frequency in both treatments, and
348 Focadhesin (KIAA1797, SPU_015184), a gene with three SNPs, two of which change amino
349 acids (upper right quadrant of Fig. 4a). ELMOD2 plays an important role in regulating
350 membrane traffic and secretion, phospholipid metabolism, and actin/cytoskeleton dynamics [52-
351 54] while Focadhesin is an important protein in subcellular structures as it integrates and receives

352 biomechanical and biochemical signals between the cytoskeleton and the extracellular matrix
353 [55,56].
354 In addition to shared responses, pH 7.5 selection, the edge of what these organisms
355 experience in nature, revealed unique adaptive targets to extreme pH selection. We observe
356 enrichment for 'actin polymerization or depolymerization' (GO:0008154). It has been
357 hypothesized that changes in actin abundance are related to cytoskeleton remodeling due to
358 intracellular stress during acclimation of oysters to climate change conditions [57,58]. Evans et
359 al., (2017) find enrichment for expression of genes involved in actin folding in *S. purpuratus*,
360 suggesting that the cytoskeleton is a target of pH stress. There is also enrichment for vacuolar
361 acidification (GO:0007035) which includes a H^+ transporting V-type ATPase (SPU_016993). V-
362 type ATPase regulate the pH of cellular compartments and can help maintain pH homeostasis
363 when extracellular pH is altered [59].
364 Finally, we identify TASK2 (SPU_003613) as under selection at pH 7.5 (Fig. 5). TASK2
365 is a pH sensitive K^+ transporter [60] and is an important component of bicarbonate (HCO_3^-)
366 uptake in mouse kidney [61]. In this mammalian system, HCO_3^- uptake occurs using a Na^+ co-
367 transporter where Na^+ gradients are maintained by Na^+ excretion through Na^+/K^+-ATPases,
368 resulting in the buildup of intracellular K^+. Alkalinzation from HCO_3^- activates TASK2 which
369 exports K^+ and re-establishes membrane polarization. HCO_3^- transport in urchin larvae is
370 similarly driven by a Na^+ co-transporter and Na^+ gradients are maintained by Na^+ excretion using
371 Na^+/K^+-ATPases [50]; it is possible that TASK2 is also involved in the urchin uptake process.
372 Under extreme pH conditions, the pH sensitivity of TASK2 may be under selection to ensure
373 continued HCO_3^- uptake, however, empirical work is needed to validate this hypothesized
374 mechanism.
375 The results of this analysis are sensitive to false negatives as the stringent cutoff of $q <$
376 0.001 may not identify loci that are truly under selection. For example, carbonic anhydrase
377 (SPU_012518) appears to be responding to pH 7.5 where 3/12 variants have *P*-values < 0.01,
378 two of which change amino acid sequence and are in linkage disequilibrium. This gene catalyzes
379 the hydration of CO_2 to bicarbonate [62] and is responsive to experimental acidification in many
380 organisms including purple sea urchin [25,51], corals [63], anemones [64], mussels [65], oysters
381 [66], and giant kelp [67], making it an ideal candidate for adaptation despite not meeting the
382 significance threshold. This protein, and potentially many others that are not statistically

383 significant, likely plays an important role in low pH adaptation and is worthy of future functional
384 investigations.

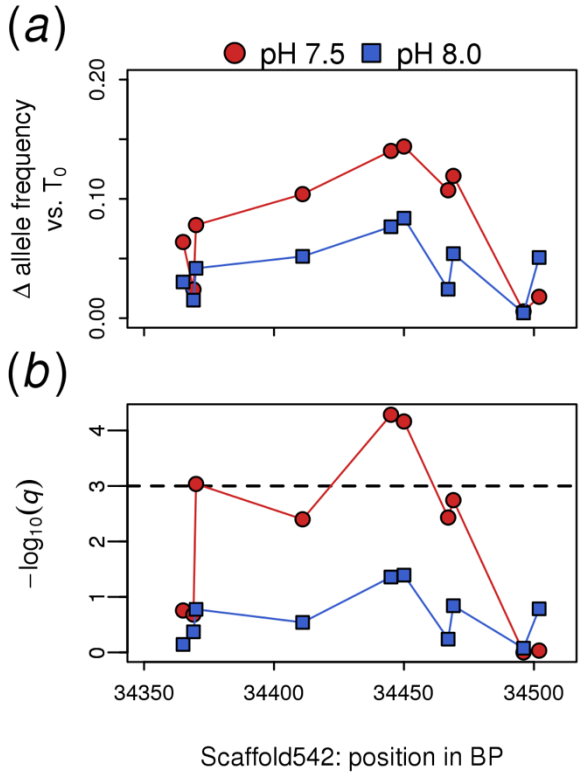

Figure 5: (a) Mean change in allele frequency of pH treatments relative to T_0 across a ~150 bp region of scaffold 542 that is ~8kb upstream of the K^+ transporter TASK2. (b) *q*-values corresponding to the allele frequency changes in (a). The dashed horizontal line indicates the significance threshold of 0.001.

385

386 **Conclusion**

387 We utilize a single generation selection experiment using an outbred, genetically diverse,
388 highly fecund species to reveal loci responding to extreme and moderate low pH selection. The
389 low variation among replicates, decreased decay of LD among selected loci, and enrichment for
390 biological functions related to pH adaptation suggest that this approach accurately identifies
391 adaptive loci. Further, we demonstrate how patterns of starting allele frequency can be used to
392 infer the mechanisms underlying maintenance of adaptive standing genetic variation. This work
393 provides a framework for future studies assessing the genetic basis of adaptive responses to
394 climate change. Increasing the temporal sampling through development would reveal how
395 different life stages differentially respond to selection, while functional studies on top candidates

396 will help to validate their mechanistic and evolutionary significance. Additionally, inter-
397 population studies will provide insight into how the adaptive potential may differ geographically
398 and be used to inform models to predict species level responses to climate change. Together, the
399 results presented here show that *S. purpuratus* possesses genetic variation that is responsive to
400 extreme low pH conditions and argues that rare genetic variation will be disproportionately
401 important for surviving future extreme conditions. Taken together, these results suggest an
402 important warning, given the low starting allele frequency and strength of selection, as evidenced
403 by the sweep patterns of linkage disequilibrium, selection by extreme events could result in a
404 major loss of standing genetic variation that could be important for response to other biotic or
405 abiotic stressors.
406
407 **Data and code availability**
408 Sequence data is available at the National Center for Biotechnology Information (NCBI SRA
409 BioProject: PRJNA479817). Phenotype data is available as part of the supplementary material.
410 All code for analysis is available at https://github.com/PespeniLab/urchin_sel_ms_2018.
411
412 **Competing Interests**
413 We have no competing interests.
414
415 **Author Contributions**
416 RB analyzed data and wrote the manuscript. AG designed and conducted the experiment and
417 assisted with data analysis and drafting the manuscript. KH helped perform the experiment and
418 analyzed the morphometric data. HH helped design aquaculture facilities and pilot the
419 experiment. MP designed the experiment and wrote the manuscript.
420
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633

634 **Figure legends:**

635 **Figure 1:** Morphometric analysis of total larval body length. (a) Boxplot of body size in each
636 selection regime. Inset pictures show representative individuals found in pH 8.0 (b) and 7.5 (c).

637

638 **Figure 2:** Principal component analysis of allele frequencies for all 75,368 SNPs at time point
639 zero (T_0) and after seven days in the two the pH treatments.

640

641 **Figure 3:** Decay in linkage disequilibrium with physical distance between SNP pairs. Dashed
642 lines are neutral loci and solid red and blue lines represent decay in pH 7.5 and 8.0 selected
643 variants, respectively. Solid grey shading is the 95% distribution of 500 permutations of down
644 sampled random variants across the genome (see text for details).

645

646 **Figure 4:** (a) Mean change in allele frequency for variants showing significant shifts under pH
647 7.5 and 8.0. Dashed black line represents the 1:1 expectation. (b) Allele frequency at T_0
648 polarized by the allele increasing in frequency during selection. Colored solid lines are the
649 density plot distributions for each set of selected loci. The solid black line represents the median
650 expectation of neutral loci from 1000 permutations of down sampling to the same number of
651 variants as selected loci; grey shading is the 95% distribution of the permutation. Dashed vertical
652 lines are the mean allele frequencies of each set of variants.

653

654 **Figure 5:** (a) Mean change in allele frequency of pH treatments relative to T_0 across a ~150 bp
655 region of scaffold 542 that is ~8kb upstream of the K^+ transporter TASK2. (b) *q*-values
656 corresponding to the allele frequency changes in (a). The dashed horizontal line indicates the
657 significance threshold of 0.001.