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SYMPOSIUM

Unique Genomic and Phenotypic Responses to Extreme and Variable pH Conditions in Purple Urchin Larvae

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Synopsis Environmental variation experienced by a species across space and time can promote the maintenance of genetic diversity that may be adaptive in future global change conditions. Selection experiments have shown that purple sea urchin, *Strongylocentrotus purpuratus*, populations have adaptive genetic variation for surviving pH conditions at the "edge" (pH 7.5) of conditions experienced in nature. However, little is known about whether populations have genetic variation for surviving low-pH events beyond those currently experienced in nature or how variation in pH conditions affects organismal and genetic responses. Here, we quantified survival, growth, and allele frequency shifts in experimentally selected developing purple sea urchin larvae in static and variable conditions at three pH levels: pH 8.1 (control), pH 7.5 (edge-of-range), and pH 7.0 (extreme). Variable treatments recovered body size relative to static treatments, but resulted in higher mortality, suggesting a potential tradeoff between survival and growth under pH stress. However, within each pH level, allele frequency changes were overlapping between static and variable conditions, suggesting a shared genetic basis underlying survival to mean pH regardless of variability. In contrast, genetic responses to pH 7.5 (edge) versus pH 7.0 (extreme) conditions were distinct, indicating a unique genetic basis of survival. In addition, loci under selection were more likely to be in exonic regions than regulatory, indicating that selection targeted protein-coding variation. Loci under selection in variable pH 7.5 conditions, more similar to conditions periodically experienced in nature, performed functions related to lipid biosynthesis and metabolism, while loci under selection in static pH 7.0 conditions performed functions related to transmembrane and mitochondrial processes. While these results are promising in that purple sea urchin populations possess genetic variation for surviving extreme pH conditions not currently experienced in nature, they caution that increased acidification does not result in a linear response but elicits unique physiological stresses and survival mechanisms.

Introduction

The annual average global atmospheric carbon dioxide concentration recently reached 417 ppm, likely the highest level in the past 20 million years (Rackley 2017; IPCC Forthcoming 2020). Consequently, global climate is changing, with alterations not only in mean conditions, but also in the variability and frequency of extreme events (Rahmstorf and Coumou 2011; McNeil and Sasse 2016; Kwiatkowski and Orr 2018). This increase in variability is driving novel conditions that exceed previous extremes (Easterling et al. 2000). For organisms to persist under these drastic changes, physiological and genetic responses will be required (Somero 2010; Hoffmann and Sgrò 2011; Vázquez et al. 2017). However, whether populations have the adaptive potential to survive conditions beyond those currently experienced in nature remains a critical area of investigation for understanding species resilience (Lande and Shannon 1996; Flanagan

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et al. 2018). Further, it is unclear if environmental variation attenuates or exacerbates physiological stress and if adaptation to fluctuating versus static conditions uses the same adaptive genetic variation.

While much of our understanding of fitness in variable environments draws from studies on temperature variation (Beardmore and Levine 1963; Long 1970; Bozinovic et al. 2011; Estay et al. 2011; Folguera et al. 2011; Shama 2017), other factors are simultaneously shifting due to human activities. Oceans, in particular, are becoming more acidic due to the dissolution of atmospheric carbon dioxide into sea surface waters, declining by an approximate range of 0.017–0.027 pH units per decade since the late 1980s (IPCC Forthcoming 2020). This process, known as ocean acidification (OA), decreases ocean pH while simultaneously depleting the seawater of natural carbonate, the building block for calcium carbonate (Doney et al. 2009), creating a physiologically challenging environment for many species. Maintaining acid–base balance is essential to maintain cellular functioning, and alterations to environmental pH require energetically costly intracellular compensation (Fabry et al. 2008; Esbaugh et al. 2012; Stumpp et al. 2012; Mangan et al. 2017). Organisms that develop calcareous skeletons or shells are faced with the additional difficulty of laying down and maintaining these structures under biochemically unfavorable conditions, which can lead to negative impacts on growth and survival (Kroeker et al. 2010; Byrne et al. 2013). However, some marine environments experience natural fluctuations in pH across space and time. In the California Current Marine Ecosystem (CCME), upwelling can drive diurnal fluctuations of up to 0.67 pH units, reaching the low open ocean pH levels predicted for the end-of-the-century (Yu et al. 2011; Evans et al. 2013; Chan et al. 2017; IPCC Forthcoming 2020). Coupled with increasing atmospheric carbon dioxide, sea surface waters within the CCME frequently experience lower pH conditions than 8.1, the open ocean average (Chan et al. 2017). Consequently, populations within this system harbor physiological mechanisms and genetic variation to tolerate low pH events (Evans et al. 2013; Pespeni et al. 2013a; Brennan et al. 2019), making them an ideal model to understand how adaptation will likely proceed as pH continues to decrease.

Our understanding of how variable pH conditions that mirror natural conditions may impact species persistence and performance is limited. Previous work has shown both negative and positive effects of pH fluctuations on organismal performance and appears to depend on factors such as the species, population origin, or trait of interest. Particularly interesting is a potential rescue effect, or mitigation, where pH variability may improve a suite of characteristics including behavior in non-calcifiers (Jarrold et al. 2017) and survivorship (Dufault et al. 2012) and growth in calcifiers (Frieder et al. 2014). Conversely, growth in some calcifying species has been shown to be negatively impacted by varying pH (Price et al. 2012; Li et al. 2016). Thus, while impacts of static low pH on marine calcifiers are generally accepted as negative, impacts of fluctuating pH appear to be less conclusive. For most calcifying species that reside specifically in pH-fluctuating environments like the CCME, questions remain as to what role naturally varying pH will play in species' responses to OA as they reach the edge of their current natural low pH range and more rapidly and frequently encounter extreme levels beyond their current range.

We focus on the ecologically and economically important calcifying species resident to the CCME, the purple sea urchin, *Strongylocentrotus purpuratus*. This species inhabits a broad range across the west coast of North America where it experiences natural fluctuations in pH geographically and temporally (Evans et al. 2013; Pespeni et al. 2013b), with diurnal fluctuations as great a range as 0.8 pH units and lowest pH values measured at 7.43 pH units (Chan et al. 2017). Previous work has shown that *S. purpuratus* harbors adaptive standing genetic variation to rapidly adapt to low pH conditions that fall within the range typically experienced in nature (Pespeni et al. 2013a; Brennan et al. 2019). Further, this species can be readily spawned in the laboratory, generating hundreds of thousands of offspring harnessing the high genetic variation found in the wild. By rearing replicate pools of these larvae in a selective environment, we can identify genetic variants that shift in frequency consistently across replicates, thus identifying the standing genetic variation and related physiological functions that enable survival across a single generation (Pespeni et al. 2013a; Brennan et al. 2019). Single generation experiments are extremely useful for this long-lived species that requires 2 years before reaching reproductive maturity (Leahy 1986).

Here, we use *S. purpuratus* to conduct a single-generation selection experiment to identify the genetic variation underlying adaptation to static and fluctuating low pH conditions. Specifically, we leverage variable and static selection regimes at pH conditions that fall within and outside the range typically experienced by this species in the wild to address the following objectives: (1) test the

prediction that variability in low and extreme pH "rescues" larval phenotypes, resulting in higher larval survival and larger body size compared with their static counterparts, (2) determine if mean pH, regardless of variability, drives selective responses, and (3) determine if larvae can use the same genomic and physiological machinery to survive as pH stress extends beyond the range experienced in nature.

Methods

Sea urchin collection and experiment

In June 2018, 25 adult *S. purpuratus* (14 females and 11 males) were collected from San Diego, CA, USA, and shipped overnight to the University of Vermont. While the frequency and magnitude of low static and fluctuating pH events in the southern part of the CCME is notably less than that of more northern regions (Hofmann et al. 2011; Yu et al. 2011; Evans et al. 2013), previous work has shown that purple urchin have high gene flow of low pH-adaptive genetic variants throughout the CCME (Palumbi and Wilson 1990). Further, recent work utilizing a similar experimental system and sourcing adults from the same location has shown there are low pH-adaptive alleles for responding to levels at the edge of this species' low pH range (Brennan et al. 2019). Immediately upon arrival, adults were induced to spawn by injecting 0.5 M KCl into the peristome (Strathmann 1987; Brennan et al. 2019). Eggs were filtered over 215-micron mesh and density was determined to partition 70,000 eggs from each female into each of three static pH conditions: 8.1, 7.5, and 7.0 (210,000 eggs/female). The first two pH conditions were chosen to represent different degrees of pH in the environment frequently encountered by this species in the wild. The control pH condition (8.1) represents a benign treatment that reflects the current average open ocean pH conditions (Chan et al. 2017). Similarly, the intermediate pH condition (7.5) is at the edge of the pH range that purple sea urchin naturally experience in the CCME (Evans et al. 2013; Chan et al. 2017), but is also equal to the predicted end-of-century open ocean pH levels should carbon emissions continue "business as usual" (IPCC Forthcoming 2020). The extreme low pH condition (7.0) falls outside of the range typically experienced in the wild. However, continued decreases in ocean pH mean that fluctuations in low pH will continue to reach levels below which sea urchin have experienced previously. For example, recent pH levels have dropped below 7.5, indicating that new extreme lows are beginning to occur (Chan et al. 2017). Therefore, we chose the extreme pH of 7.0 in order to understand how this species may respond to a novel, potentially impending, pH condition. Eggs for both the static and variable culturing treatments were fertilized in their respective static pH counterparts, with evenly pooled sperm from all males in 22-micron filtered, UV-sterilized seawater at 14°C with a salinity of 31 ppt.

After verifying approximately ≥95% fertilization success (determined by the appearance of the fertilization envelope a few minutes after the addition of sperm), fertilized eggs were pooled across all females within each of the three static pH conditions (980,000 eggs per static pH condition). At 24 h post fertilization ["day 1" (D1)], hatched blastula larvae were sampled from the three static pH pools ($N_{replicates}$ = 6; 11,250 eggs per pH), and remaining blastulae were seeded into replicate culturing vessels ($N_{replicates}$ = 6; 11,250 eggs per 3.7 L vessel; and seeding density: 3 larvae/mL) for each of the six different pH regimes: one control and two treatments that remained at a static pH (pH 8.1, 7.5, and 7.0) and three variable treatments that varied by 0.6 pH units over the course of 24 h (pH 8.1–7.5, pH 7.8–7.2, and pH 7.3–6.7) (Fig. 1A), the daily change in pH that occurs during upwelling season in the CCME (Evans et al. 2013; Chan et al. 2017). It is important to note that the variable pH 8.1 treatment is meant to mimic the pH range commonly experienced in the CCME during upwelling (8.1–7.5) and has a mean pH of 7.8, whereas the edge and extreme varying pH treatments vary by the same amount but with the means matching their static edge and extreme pH treatment counterparts (pH 7.5 and pH 7.0).

Experimental system and water chemistry

Larvae were reared in a custom-designed, recirculating larval culturing system that allowed for continuous water movement set to a flow rate of 0.5 mL/s from the top of each replicate culturing vessel exiting through a mesh covered cylinder in the bottom third of the vessel. The vessel design and flow rate were experimentally determined to minimize congregation of larvae at the outflow. For optimal water chemistry, 22-micron filtered natural seawater was brought to UVM from the University of New Hampshire Coastal Marine Laboratory and UV-sterilized upon arrival. Seawater was maintained at a temperature of 14°C with a salinity of 31 ppt. pH and temperature were measured by computer-monitored Hamilton Polilyte pH probes that were calibrated with three Thermo Scientific Orion pH buffers (4.01, 7.00,

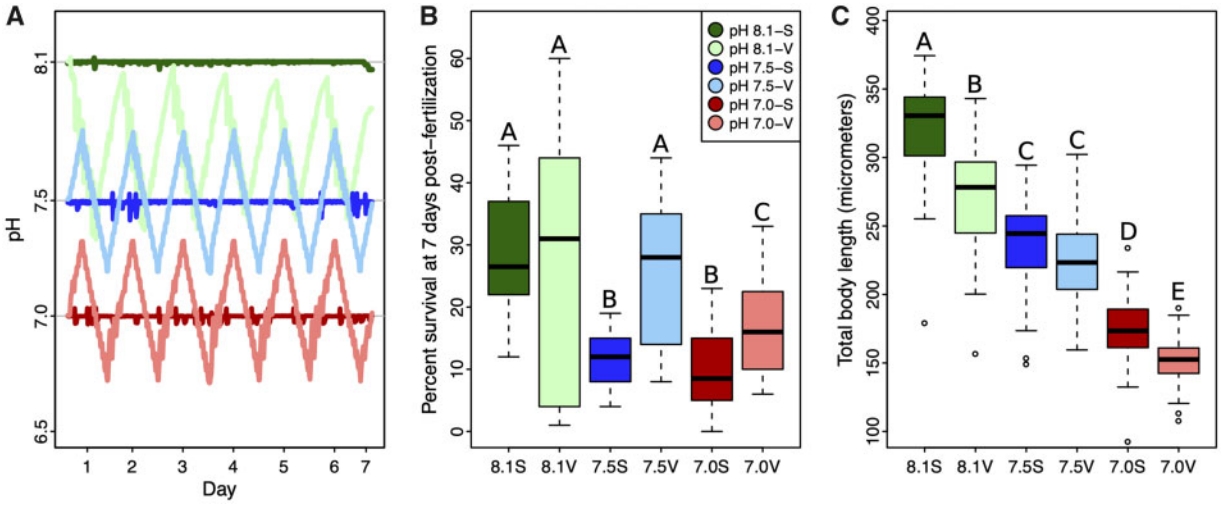

Fig. 1 (**A**) pH measurements from the larval culturing system day 1 (D1) post-fertilization to end of the experiment (day 7 [D7] post-fertilization), recorded every 15 min from each of the six header tanks. The dark shades of color show the three static treatments (pH 8.1, 7.5, and 7.0) and the light shades of color show their varying counterparts. See legend in B. (**B**) Tukey's boxplot of survival (percentage) after 7 days of development in each treatment ($n = 12–18$ survival estimates per pH condition). Letters above each boxplot indicate results from *post hoc* tests where different letters indicate significantly different groups. (**C**) Total body length (micrometers) of sampled surviving larvae ($n = 48–55$ measurements per pH condition) after 7 days of development.

and 10.01). pH levels were controlled through communication between the pH probe, a computer system (RCK systems, San Diego, CA) and a solenoid valve that would release pure CO_2 gas through airstone bubblers measured and dosed every 10 s to maintain static or follow programed variable pH conditions. Temperature was maintained by heat exchangers attached to the header tanks. CO_2 scrubbers were attached to the protein skimmers in the sump tanks for the pH 8.1 static (control) and variable conditions in order to help maintain control pH conditions for the former and to help pH increase when scheduled for the latter. Additional water chemistry measurements were taken on days 1, 4, and 7 post-fertilization (Supplementary Table S1), with temperature, salinity, and pH measured around the same time of day and water collected and sealed for follow-up titration to determine total alkalinity (TA). TA was measured with a Mettler Toledo G10S Titrator, standardized to Andrew Dicksons' seawater standards (Dickson 2010). All of these water chemistry measurements were entered into the CO2Sys_v.2.1 program to calculate pCO_2 (Pierrot et al. 2006). On days 3 and 5 post-fertilization, recirculation was paused and larvae were fed 1000 cells/mL each of *Dunaliella* spp. and *Rhodomonas* spp. (Pespeni et al. 2013a; Brennan et al. 2019) and allowed to feed for 1 h before recirculation resumed. Larvae were reared in the culture vessels until 7 days old, the pluteus larval stage, at which point they were sampled for morphometric, survival, and genomic analyses (below).

Morphometrics and survival estimates

Seven-day old plutei were preserved in calcium carbonate-buffered formalin in seawater, and then photographed for morphometric analysis as in Brennan et al. 2019 ($n = 48–55$ per pH condition). Total body length, represented as the midline body length extended to the top of the arms, was measured using *ImageJ* software (Schneider et al. 2012), calibrated with a stage micrometer. Data analysis was conducted in R (R Core Team 2015) via a generalized linear model, with the family parameter set to "gaussian" and pH as the main effect.

To estimate survival to day 7 (D7), culturing vessels were gently stirred, then three samples of 33 mL were collected from each vessel and preserved in calcium carbonate-buffered formalin in seawater. Based on the starting density of 3 larvae/mL, 100% survival would yield ~100 larvae in 33 mL. Estimated percent survival was thus calculated based on the number of larvae counted over the expected 100 larvae for each of the three replicate samples per vessel. Larvae were considered to be alive if they had the pluteus larval developmental form, regardless of the number of arms, as low pH has been shown to cause developmental delay in urchins (Kurihara and Shirayama 2004). Abnormal larvae were minimal and not included in the alive count. Data analysis was conducted in R via a logistic regression using the *glm* function, with the response variable (survival) represented as two counts: "alive" or "dead" and pH treatment as the main effect. *Post hoc* comparisons

were conducted using Tukey's multiple comparison of means in the *multcomp* package (Hothorn et al. 2008).

DNA sequencing, processing, mapping, and SNP-calling

Four replicate pools of starting larval populations (D1, $n = 11{,}250$ larvae) per initial static pH condition (pH 8.1, pH 7.5, and pH 7.0) as well as the pools of surviving larvae (D7, $n = \sim$1000–3000 larvae) from each of the six culture vessels per pH condition, were collected and spun down to remove excess seawater, flash frozen in liquid nitrogen, and stored at −80°C until extractions for genomic analyses. DNA was extracted with a Zymo ZR-Duet DNA/RNA MiniPrep Plus Kit (Zymo, Irvine, CA). DNA was shipped over dry ice to Rapid Genomics (Gainesville, FL) for library preparation and capture-sequencing with 46,316 custom 120 bp probes, with two probes designed per gene based on the *S. purpuratus* genome v. 3.1: one in an exonic region and the other in a putative regulatory region falling within 1000 bp upstream of the transcription start site. Samples were barcoded, pooled, and then sequenced as 150 bp paired-end reads on a single Illumina HiSeqX lane.

Raw DNA reads had Illumina adapters removed and were quality filtered with *trimmomatic-0.36* (Bolger et al. 2014) accepting final reads ≥35 bp in length. Filtered reads were mapped to the *S. purpuratus* genome v. 3.1 (build 7, echinobase.org) with BWA-MEM (Li 2013). SNPs were called using *Varscan* (Koboldt et al. 2012), resulting in 19,529,443 SNPs that were then further filtered in *R* (R Core Team 2015) by depth (≥30, Ferretti et al. 2013), minor allele frequency (0.025), and a high coverage filter of three times the median coverage. This resulted in 54,427 high-quality SNPs for downstream analyses.

Principal components analysis (PCA) was used to visualize variation across all identified SNPs among treatments and days. Significance among groups was tested using PERMANOVA implemented using the adonis function in the vegan package (v. 2.4-2). Cochran–Mantel–Haenszel (CMH) tests were used to identify specific loci with consistent changes in allele frequencies across replicates between pairs of groups with replicates randomly downsampled to four to have matched numbers of replicates for all contrasts. The CMH statistical test is an accurate method for identification of loci with changes in allele frequency in response to experimental selection (Vlachos et al. 2019). The test relies on sufficient replication; simulation studies show that the four replicates per condition should result in sufficient power to identify selected loci (Kofler and Schlötterer 2014). We tested for differences in allele frequency relative to both the D1, pH 8.1 samples and the D7, pH 8.1 static samples. Results from all contrasts are presented in Supplementary Table S2. We focus downstream analyses using the contrasts to D7, pH 8.1 static samples to control for potential changes in allele frequency due to laboratory adaptation. Linkage disequilibrium decays quickly in *S. purpuratus*, over a few hundred base pairs (Brennan et al. 2019). As such, we considered loci associated with each gene region (combining coding and regulatory regions for a given gene) to be independent, resulting in 4548 independent regions. Given the low linkage disequilibrium, the number of independent regions represents the number of independent tests. We thus used a stringent Bonferroni correction to adjust for multiple testing, where 0.05/4548 resulted in a *P*-value threshold of $1.1 \times 1\ 0^{-5}$. To test for correlations of changes in allele frequency within and between the low and extreme static and variable pH conditions, we used Pearson's correlation. Finally, loci were categorized as exonic (synonymous, non-synonymous), intronic, promoter, or intergenic and linked to genes using SnpEff (Cingolani et al. 2012). χ^2 tests were used to test if any group contained significantly more loci responding to selection than expected by chance. Loci significantly changing in allele frequency for each treatment were tested for functional enrichment of gene ontology (GO) categories. GO terms for annotated genes were downloaded from EchinoBase (www.echinobase.org). TOPGO v. 2.36.0 was used to test for significant enrichment of function categories using the weight algorithm and limited to terms that had at least five annotated genes (Alexa and Rahnenfuhrer 2006). Similarity among significant GO terms ($P < 0.05$) was calculated with GOSemSim in R using Wang's method, which calculates similarity based on the topology of the GO graph structure (Yu et al. 2010). This similarity measure was converted to a dissimilarity matrix, hierarchically clustered, and plotted using ggdendro in R (de Vries and Ripley 2016).

Code and details to reproduce all analyses are available at https://github.com/PespeniLab/spoa_static_vs_variable.

Results

Survival and morphometrics

pH condition had a significant effect on both larval survival and body size where lower pH, generally, resulted in higher mortality and smaller body size. Larval survival ($n = 12$–18 samples per pH

treatment) decreased by 20% after 7 days in the static pH 7.5 (edge) and pH 7.0 (extreme) conditions, compared with the static control, pH 8.1 (Fig. 1B, $P < 0.001$). No difference was found in survival between the edge and extreme static conditions ($P = 0.44$). Under the pH 7.5 selection regime, variability improved survival relative to static conditions such that survival was comparable to control static conditions (pH 8.1). A similar mitigation was observed for pH 7.0 under variable conditions, which was increased compared with its static counterpart (Fig. 1B, $P < 0.001$), though survival did not improve to match control conditions. In agreement with survival results, total body length of 7-day plutei showed a similar reduction as pH decreased, from a mean size of 319 µm to 230 and 175 µm for the static pH 7.5 and 7.0 conditions, respectively (Fig. 1C, $P < 0.001$). However, variable conditions did not result in the same pattern of recovery. In contrast, variable conditions had either similar sized (pH 7.5, mean = 225 µm) or smaller individuals (pH 8.1 and 7.0, mean = 268 and 151 µm, respectively) than their static counterparts (Fig. 1C, $P < 0.001$).

Genomic analyses

Genome-wide allele frequency estimates across all 54,427 high quality SNPs identified from pooled, capture-sequenced genomic DNA showed low variation among replicates within each treatment group using PCA (Fig. 2A). Samples clustered separately according to mean pH and day of sampling (PERMANOVA, $P < 0.05$), where samples moved sequentially further in PC space from the D1, pH 8.1 control as the low pH condition intensified. Accordingly, the largest differences observed were between D1 control static condition and D7, pH 7.0 (Fig. 2A). Static and variable treatments within a pH overlapped in their distributions except for pH 7.0 (PERMANOVA, $P = 0.04$), suggesting stronger selection in response to mean pH rather than variability. Despite broad differences in allele frequencies and high levels of mortality, no differences in estimates of nucleotide diversity were observed among day or treatment groups (Supplementary Table S3).

CMH tests identified loci consistently responding to pH selection after 7 days of development compared with the D7, static pH 8.1. Across the treatments, decreasing pH resulted in a greater number of loci with significant changes in allele frequencies relative to the static pH 8.1 control, a pattern that agrees with the increasing distance in principle component space as pH decreases. We observed limited changes in allele frequency between the static pH 8.1 and variable (mean pH 7.8) conditions, with only six divergent loci ($P < 1.1 \times 10^{-5}$). More divergence was observed in pH 7.5 with 24 and 34 pH-selected loci for static and variable treatments, respectively (Fig. 2B). However, of these loci, only four were overlapping (7.4% of pH 7.5 selected loci). We observed the largest degree of allelic divergence from the control at pH 7.0 with 602 and 253 significant loci in the respective static and variable treatments and 106 overlapping variants between them (14.2% of the pH 7.0 selected loci; Fig. 2B). Between the pH

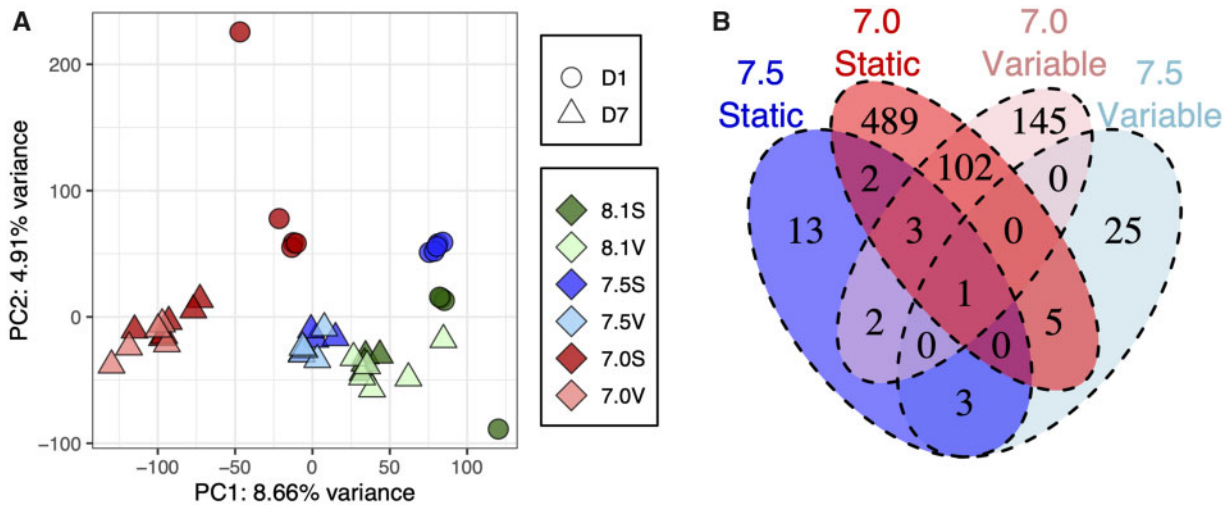

Fig. 2 (**A**) Principal component analysis (PCA) of the 54,427 high quality SNPs for the six pH conditions and two sampled time periods. Color indicates pH condition in the bottom portion of the legend key, where dark shades of color are static and light shades of color are variable. Shapes (circle or triangle) denote which day post-fertilization; circles are samples from D1 of development and triangles from samples after 7 days of development. (**B**) *Venn diagram* of loci with significant changes in allele frequency in response to selection at pH 7.5 and 7.0 for static and variable conditions (compared to D7, static pH 8.1).

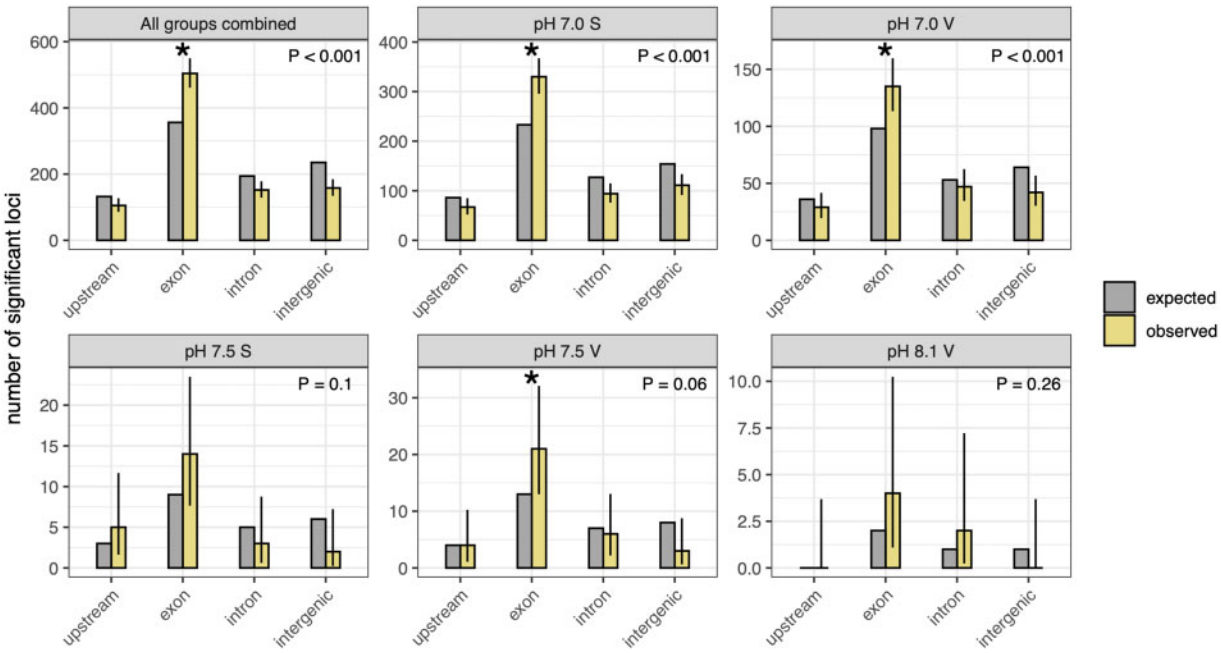

Fig. 3 Number of loci significantly changing in frequency in different regions of the genome. *P*-values in the top right corners indicate corrected significance from χ^2 tests, where values are FDR corrected to account for multiple testing. Within each panel, expected values are from χ^2 tests and observed values are the actual counts of significant loci. Error bars for observed values represent the 95% confidence interval calculated with the Clopper–Pearson method (Clopper and Pearson 1934). Asterisks indicate groups with a significantly greater number of loci than expected by chance (corresponding grey bar), as determined by binomial *post hoc* tests. Note that we have chosen to collapse synonymous and non-synonymous loci in exonic regions as linkage disequilibrium extends at least 100 bp (Brennan et al. 2019) inhibiting identification of specific loci targeted by selection. See Supplementary Fig. S1 for these loci separated.

treatments, we observed little overlap in responsive loci (1% of static selected loci; 0.3% of variable selected loci; Fig. 2B), indicating the genetic responses to different degrees of pH stress were largely unique. Finally, we found that loci in exonic regions were more likely to be targets of selection than loci in promoter, intronic, or intergenic regions ($\chi^{2df\ =\ 3}$ = 100, $P < 0.001$; Fig. 3). This pattern was driven by pH 7.0 variable and static ($P < 0.001$) and pH 7.5 variable ($P = 0.055$), though both pH 7.5 static and pH 8.1 variable follow a similar trend (Fig. 3).

We observed correlated changes in allele frequencies within and between pH conditions (Fig. 4). Within pH 7.0, changes in allele frequency in response to static and variable conditions were strongly correlated (Pearson's $r = 0.54$; Fig. 4A). Conversely, static and variable responses within pH 7.5 showed a weaker correlation ($r = 0.21$; Fig. 4B). Comparing static and variable responses between pH 7.0 and 7.5 revealed different patterns. Changes in frequency in response to static pH were strongly correlated between pH 7.5 and 7.0 ($r = 0.47$; Fig. 4C) but the correlation between the variable treatments was much weaker ($r = 0.25$; Fig. 4D).

Functional enrichment analyses revealed unique and shared biological processes subject to selection among the experimental groups (Fig. 5). See Supplementary Figure S1 and Supplementary Tables S4–S8 for enrichment results for cellular components, molecular function, and for D1 versus D7 comparisons. The variable pH 8.1 treatment (mean pH 7.8) was enriched for one category related to hormone-mediated signaling. In response to pH 7.5 conditions, the majority of categories enriched for changes in allele frequency were involved in the biosynthesis, metabolism, transportation, or modification of lipids. Three categories were shared between the pH 7.5 static and variable conditions, all related to phosphatidylinositol metabolism, signaling, and phosphorylation. In response to extreme pH 7.0 conditions, allele frequency changes were also enriched for lipid and phospholipid processes. However, there were several processes unique to pH 7.0, including transmembrane and mitochondrial processes, and RNA processing. Although the pH 7.0 static and variable treatments had overlapping and correlated genetic responses (Figs. 2B and 4A), only one GO term overlapped between the two treatments: cellular protein complex disassembly. In static and variable pH 7.0 conditions, selection disproportionately targeted exonic regions. Consistent with overall enrichment in these conditions, selected exonic loci in static

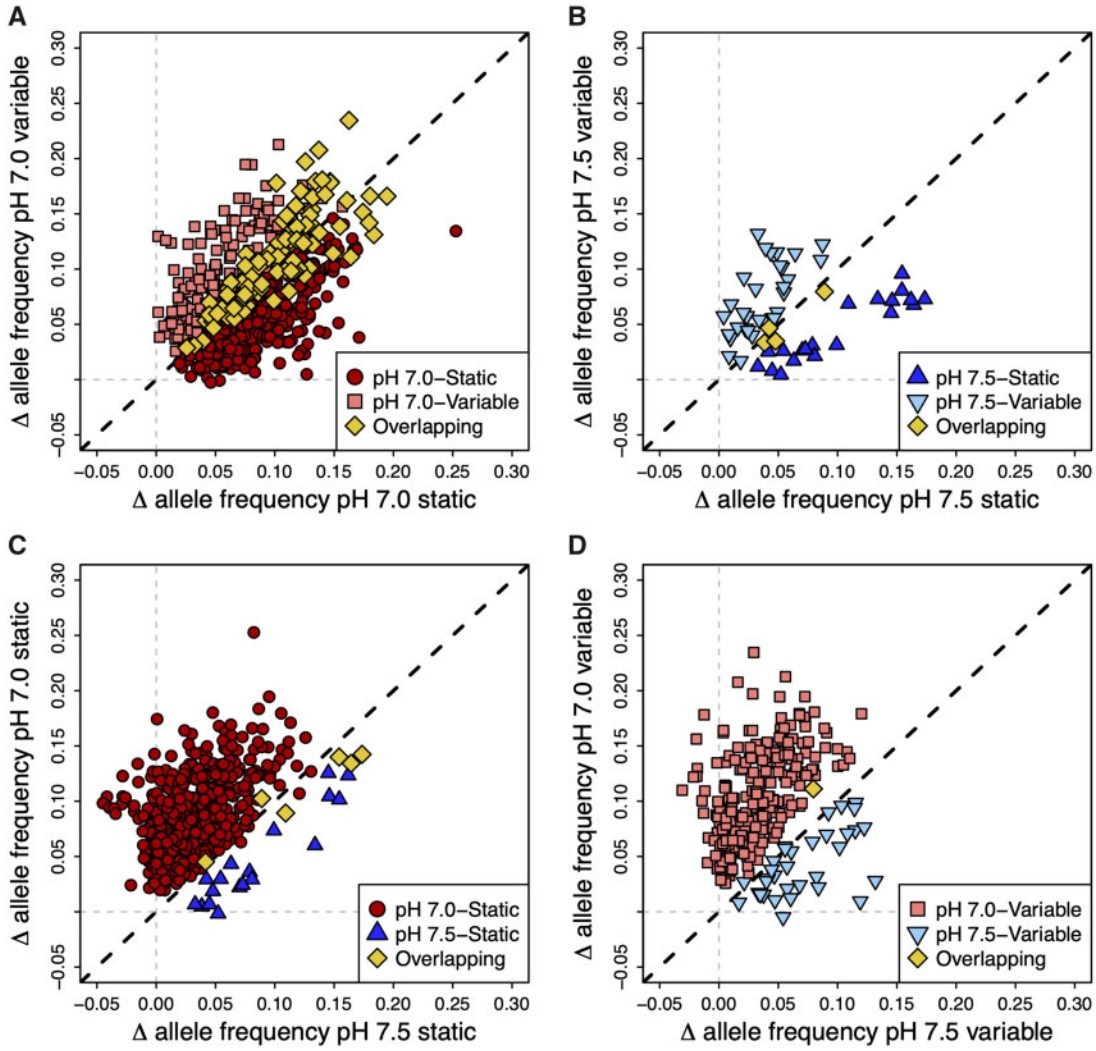

Fig. 4 Correlation between loci showing significant changes in allele frequency ($P < 1.1 \times 10^{-5}$) in response to selection in different treatments. Change in allele frequency for all plots is relative to frequencies of D7, static pH 8.1 at D7. Color and shape indicate each treatment while loci significant for both treatments (overlapping) in each panel are represented by yellow diamonds (**A**) within pH 7.0, static versus variable ($r = 0.54$), (**B**) within pH 7.5, static versus variable ($r = 0.21$), (**C**) within static, pH 7.0 versus pH 7.5 ($r = 0.47$), and (**D**) within variable, pH 7.0 versus pH 7.5 ($r = 0.25$).

pH 7.0 included immune responses (Supplementary Table S9) while exonic loci in variable pH 7.0 were enriched for vesicle transport and metabolic processes (Supplementary Table S10). Static conditions at different pH shared enrichment for immune responses, while the variable conditions shared no common functional categories. Interestingly, there were similar numbers of enriched categories for surviving pH 7.0 and pH 7.5, despite the 10-fold greater number of loci changing in allele frequency in response to the more extreme pH environment.

Discussion

We find that as seawater acidification increases in intensity, both the physiological (Fig. 1) and genomic (Figs. 2–4) consequences become more extreme. In particular, when pH conditions extend beyond what is currently experienced in nature, the adverse effects on the survival and growth of developing sea urchin larvae are most pronounced. We demonstrate that while fluctuating pH conditions can reduce mortality relative to static conditions, the same effect is not seen for body size. Yet, we simultaneously show that adaptive genetic variation for extreme and fluctuating pH is present in wild populations. Loci targeted by selection were disproportionately found in genes related to lipid metabolism (pH 7.5) and membrane function (pH 7.0) and in exonic regions, indicating that changes to protein function enabled survival particularly in extreme pH 7.0 conditions. Importantly, the genetic responses to pH 7.5 (edge) and pH 7.0 (extreme) conditions were

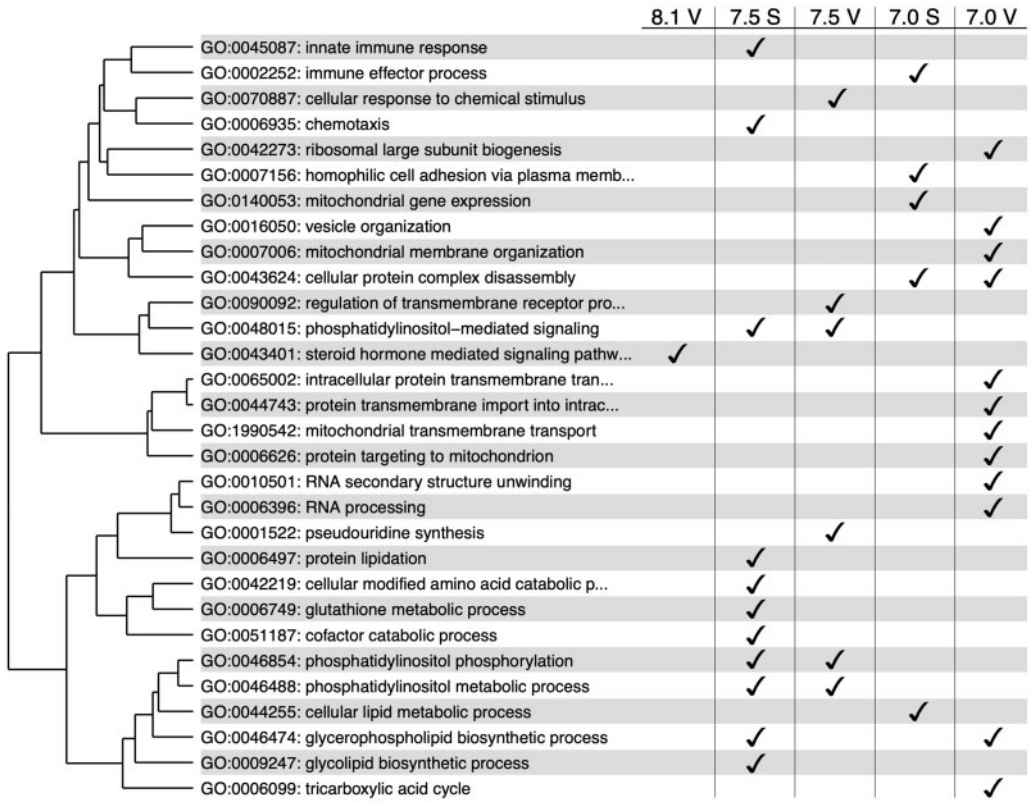

Fig. 5 GO enrichment results for biological processes. Checks indicate enrichment ($P_{adj} < 0.05$) for significant changes in allele frequency for loci in the category (row) in response to the treatment indicated (column). GO terms are clustered by similarity according to their topology in the GO graph structure. See the "Methods" section for details.

unique, indicating that the physiological processes enabling persistence are similarly unique. This suggests that changes along the logarithmic pH scale do not elicit a linear physiological response. Further, the distinct responses suggest that adaptation to gradual decreases in average pH may not facilitate persistence to extreme pH conditions, a hypothesis that warrants further investigation.

Tradeoff between survival and growth

Strongylocentrotus purpuratus relies on amorphous calcium carbonate to lay down its skeleton during development (Addadi et al. 2003; Politi et al. 2008; Vidavsky et al. 2014) and, as pH decreases, the increase in energetic demands to develop skeletal structure leads to developmental abnormalities and higher mortality rates (Stumpp et al. 2012; Pan et al. 2015). Accordingly, the increased mortality and decreased size with increasing pH stress observed here (Fig. 1A) match expectations from previous work that has also shown reductions in size and survival under decreased pH conditions (Kurihara 2008; Ries et al. 2009; Stumpp et al. 2012). However, we find that fluctuating pH drives higher survival, but not body size (Fig. 1B, C), partially matching our predictions. Previous work has identified both positive (Dufault et al. 2012) and negative (Mangan et al. 2017; Onitsuka et al. 2018; Chan and Tong 2020) impacts of pH fluctuations on survival and growth. For purple sea urchin, fluctuations in pH may serve as a buffer, where periodic exposure to less stressful pH conditions enable more typical development that increases survival. Alternatively, a fluctuating environment is regularly experienced during the spawning season (Miller and Emlet 1997) and may represent the conditions to which individuals are adapted and thus best able to survive.

Our results suggest that, for early-in-development larvae, smaller body size may enable increased survival under low pH conditions; fluctuating conditions increase survival but decrease body size relative to static treatments (Fig. 1B, C). Decreasing pH consistently results in smaller larvae through development for *S. purpuratus*, among other sea urchin species as well (Yu et al. 2011; Pespeni et al. 2013a; Suwa et al. 2013; Brennan et al. 2019), but the adaptive significance of this change has been unclear. Larvae under low pH dedicate much of their energy to acid–base balance at the cost of growth (Stumpp

et al. 2012; Pan et al. 2015). We hypothesize that smaller size and lower growth under low pH conditions decreases the total energy budget and allows for increased survival. Further, the success of metamorphosis is dependent on lipid energy reserves, which may be similarly increased by reducing size during development (Sewell 2005; Byrne et al. 2008). This is further corroborated by our GO enrichment results, which show increasing enrichment for genes related to lipid metabolism as pH decreases (Fig. 5). While small size during development may increase early survival or success of metamorphosis, it could also reduce overall population fitness. For example, small larvae experience higher predation rates (Allen 2008) and may develop into smaller, less fecund adults (Dupont et al. 2013). Future work should address the consequences of reduced body size on energetic demands, survival, metamorphosis, and adult fitness in *S. purpuratus*.

Surviving in pH 7.5 (edge) versus pH 7.0 (extreme) conditions

Previous work has shown that populations of *S. purpuratus* harbor sufficient standing genetic variation to respond to static pH levels within (pH 7.8) and at the edge (pH 7.5) of their current range (Pespeni et al. 2013a; Brennan et al. 2019). Here, we demonstrate how adaptation may proceed as pH begins to extend beyond what is currently experienced in the wild. We find that responses to pH conditions beyond the natural range (pH 7.0) are not merely more extreme changes in allele frequency at the loci underlying adaptation to pH conditions within the natural range of pH variability (pH 7.5). Rather, selection targets a unique set of loci (Fig. 4B). Under extreme static pH conditions, we find selected loci in gene functions related to immune response, cell adhesion, mitochondrial gene expression, and lipid metabolism (Fig. 5). Immune response under extremely stressful conditions is not unexpected (Bibby et al. 2008; Brothers et al. 2016), especially in such an energetically-costly environment as low pH for calcifying larvae that need to maintain acid–base physiology (Stumpp et al. 2012; Pan et al. 2015). Indeed, perhaps the enrichment for mitochondrial gene expression is being used by larvae under extreme conditions for energy allocation in order to pay the cost of surviving and maintaining cellular processes in novel pH conditions.

Loci under selection at the edge of the natural pH range (pH 7.5) are unique from those responding to pH 7.0 but overlap in a number of gene functions including immune response and lipid metabolism. Lipids are important for cellular membrane structure and serve as critical energy stores for calcifying marine invertebrates (Sewell 2005; Schoepf et al. 2013). Indeed, gene functions related to lipid metabolic processes were the only ones enriched to varying degrees across all of the edge and extreme pH treatments (Fig. 5). Lipid energy allocation in sea urchins is essential for successful metamorphosis, as metamorphosing larvae spend weeks nutritionally reliant on the lipid stores from the pluteus larval stage (Sewell 2005). Thus, high enrichment for lipid metabolism may confer a selective advantage for larvae subjected to low pH levels at the edge of their range. While we see no difference in survival between pH 7.5 and pH 7.0 static conditions (Fig. 1B), the noticeably lower enrichment of lipid metabolism in static pH 7.0 conditions compared with static pH 7.5 suggests that genetic variation in lipid metabolism genes was critical for survival in pH 7.5. Without selection for these alleles, larvae in pH 7.0 may accrue a greater metabolic cost of pH stress resulting in negative carryover effects to later life history stages, such as metamorphosis and juvenile survival. Finally, for static pH 7.5, we observe unique enrichment for gene functions related to chemotaxis. Chemotaxis is especially important for phagocytes in sea urchin, which make up the majority of the coelomocytes, the first line of cellular defense in the sea urchin innate immune system (Smith et al. 2006), and have been shown to be negatively impacted by acidification (Brothers et al. 2016).

Surviving static versus fluctuating pH conditions

Our findings suggest that genetic responses to variability are similar to static conditions (Fig. 4); response to selection is correlated between the regimes, and related genetic mechanisms are used to respond to static and variable conditions. Considering the high amount of environmental variation in the CCME, along with the high levels of standing genetic variation found in this species (Sodergren et al. 2006; Pespeni and Palumbi 2013c), one explanation is that previous selection in fluctuating pH has led to genetic variation that is adaptive in pH-variable environments and similar mechanisms are used to survive static conditions with the same mean pH. We find enrichment in pH 7.0 (extreme) variable conditions for loci in genes related to lipid metabolism, ribosomal and RNA structure, and mitochondria structure and transport (Fig. 5). Previous OA research in copepods

has shown multigenerational selection on ribosomal structure and oxidative phosphorylation, a key metabolic process that produces ATP and occurs within the mitochondria (De Wit et al. 2016).

The extreme variable pH 7.0 treatment exhibited a trade-off between survival and growth, with higher survival in the pH 7.0 variable conditions compared with the pH 7.0 static, but at the cost of reduced size in surviving larvae. Integrating organismal and gene function results, purple urchin larvae may focus on energy production and allocation in order to survive extreme fluctuating pH conditions, putting more energy into survival over development. Differential energy allocation has been found previously in *S. purpuratus* (Pan et al. 2015) and multiple calcifying marine gastropod species, which use "dwarfing" as an adaptive strategy to tolerate low pH (Garilli et al. 2015). Metabolomic work in another marine calcifier, *Pocillopora damicornis* coral, conveys the importance of cellular structure and maintenance for survival under pH stress, and suggests the energetic expense of this could come at a cost on growth (Sogin et al. 2016). Overall, while there is limited overlap in the specific functional categories enriched for static and variable pH conditions, enriched functions serve a shared purpose: maintenance of structural integrity and regulation of metabolism to simultaneously manage development, growth, and survival as pH decreases and/or fluctuates.

Conclusion

Understanding the genetic and phenotypic responses of organisms to their environment is pertinent, especially in a rapidly changing climate characterized by increases in extreme and variable conditions. Here, we show that purple sea urchins have greater adaptive potential than previously thought, with genetic variation available to respond to extreme static and variable pH conditions. pH variability, while leading to survivors with smaller bodies, did increase larval survival. High levels of standing genetic variation, coupled with natural variation in pH conditions across space and time, appear to promote the maintenance of adaptive potential for purple sea urchin populations. Future studies, however, should explore carryover effects to later life history stages and across generations and test the hypothesis that these factors promote adaptive potential to global change conditions in a broader phylogenetic framework. Ultimately, environmental change may be buffered against for species that have sufficient genetic variation for responding to such change.

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Supplementary data

Supplementary data are available at *ICB* online.

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