Title: Transgenerational effects alter the fitness consequences and genetic architecture of phenotypic plasticity and its regulatory pathways

Authors and affiliations: Samuel N. Bogan[1], Marie E. Strader[1,2], and Gretchen E. Hofmann[1]

[1]Department of Ecology, Evolution and Marine Biology, University of California Santa Barbara, Santa Barbara, CA, USA

[2]Department of Biology, Texas A&M University, College Station, TX, USA

Corresponding author:

Samuel N. Bogan

samuel.n.bogan@gmail.com

Keywords: Gene expression, plasticity, genetic variation, adaptation, acclimation, global change

Abstract

Parental exposure to environmental stress can influence phenotypic plasticity by offspring developing under that stressor. Transgenerational effects may also reshape natural selection on developmental plasticity by influencing its fitness consequences and expression of its genetic variation. We tested these hypotheses in the purple sea urchin *Strongylocentrotus purpuratus*, an invertebrate exposed to coastal upwelling (periods of low temperature and pH impacting biomineralization and performance). We conditioned parents and larvae to experimental upwelling and integrated RNA-seq, phenotyping of body size and biomineralization, and measured fitness-correlated traits in a quantitative genetic experiment. Larvae developing under upwelling induced widespread differential expression (DE), decreased biomineralization, and reduced body size. We detected fitness benefits for increased biomineralization and reduced size under upwelling indicative of adaptive plasticity, but only when larvae were spawned from parents exposed to upwelling. Larval DE was largely associated with adaptive phenotypic plasticity. Negative genetic correlation in DE was abundant between genes associated with adaptive plasticity. However, genetic correlations in DE associated with body size plasticity were significantly more positive in larvae from upwelling-exposed parents. These results show that transgenerational effects modify the fitness landscape and genetic architecture of phenotypic plasticity and its regulatory pathways.

Introduction

The phenotypic plasticity of physiology and performance has received attention for its ability to facilitate adaptive organismal responses to environmental change on ecological timescales (Bozinovic & Pörtner, 2015; Ghalambor et al., 2007; Hofmann & Todgham, 2010; Million et al., 2022) and, increasingly, its potential to evolve and promote persistence under future climates (Corl et al., 2018; Kelly, 2019; Vinton et al., 2022). We define phenotypic plasticity as an environmental effect on a phenotype's expression independent of genetic variation in that trait (Scheiner & Lyman, 1991). Plasticity can be adaptive, neutral, or maladaptive (Donelan et al., 2020) and has become a focal point for conservation and management strategies (Donelson et al., 2023). The evolution of plasticity relies on the strength and direction of natural selection acting upon it (Hendry, 2016) and heritable genetic variance underpinning it (Kovuri et al., 2023; Scheiner & Lyman, 1991). The plasticity of complex traits is often driven by multiple regulatory pathways that can possess positive or negative genetic covariance with one another, facilitating or constraining integrated responses to natural selection (de Miguel et al., 2022; S.-Y. Kim et al., 2016; Petino Zappala et al., 2024). Molecular pathways responsible for organismal responses to environmental change are often studied using RNA-seq, but in the absence of data on the heritability of differential gene expression or its fitness effects (Rivera et al., 2021). Using a quantitative genetic breeding design (Fig. 1; Fig. S1) integrating RNA-seq with multiple-trait phenotyping in the purple sea urchin *Strongylocentrotus purpuratus*, we examined the genetic variation and fitness effects of phenotypic and gene expression plasticity. Deriving genetic and fitness parameters from a transgenerational experiment, we determined how parental environment affected the genetic architecture and fitness consequences of plasticity and its regulatory pathways.

A small number of empirical studies on single or multiple traits have uncovered patterns of genetic variation for (mal)adaptive plasticity that help explain its evolution. For example, heat tolerant populations of Anolis lizards exhibit canalization of gene expression whose differential expression (DE) is maladaptive under acute thermal stress. There is greater interpopulation divergence at *cis*-regulatory sites of these maladaptive DE genes, suggesting that genetic variation in maladaptive plasticity fueled adaptive canalization of pathways related to thermal stress (Campbell-Staton et al., 2021). Similarly, species adapted to high altitudes exhibit the loss of ancestral plasticity in several pathways that, when induced under chronic low oxygen, result in maladaptive pathologies (Durmowicz et al., 1993; Velotta et al., 2018). In each example, it remains unclear whether canalization was driven by greater standing genetic variation and evolvability underpinning maladaptive plasticity prior to selection or the shear strength of negative selection against maladaptive responses. We address this challenge by integrating fitness associated measures of plasticity across many gene expression traits with estimates of their genetic variation.

A larger body of theory has predicted the joint influence of plasticity's genetic variance and fitness effects on adaptation. Adaptive plasticity possessing low genetic variation may enable populations to persist under novel environments at the cost of limiting adaptation. Alternatively, maladaptive plasticity may drive adaptation if it possesses a high degree of genetic variance and can express otherwise cryptic phenotypes that are subsequently canalized (Brennan et al., 2022; Ghalambor et al., 2007). Adaptive plasticity may experience evolutionary increases and improve populations' abilities to cope with predictable environmental variation when plasticity is sufficiently heritable (Bitter et al., 2021). Non-heritable, maladaptive plasticity may result in the evolution of genetic compensation to counteract its effects (Crispo, 2007; Kelly, 2019; Morris & Rogers, 2013). Essential to these predictions is whether and how genetic variation in plasticity

92 covaries with its fitness consequences. Theoreticians have urged the need for empirical research
93 in this area (Chevin & Hoffmann, 2017).

94 Performance traits and their phenotypic plasticity can be highly polygenic, regulated by
95 multiple physiological pathways as identified by cross-environment genome-wide association
96 studies (X. Li et al., 2018; Liu et al., 2021; Nguyen Ba et al., 2022). The multivariate nature of
97 genetic variants and molecular pathways that shape plasticity adds complexity to how its fitness
98 effects and genetic variation shape the evolution of plasticity by natural selection. The most
99 common method used to characterize molecular pathways associated with plastic responses to
100 environmental change is RNA-seq (Evans & Hofmann, 2012; Martyniuk, 2020; Rivera et al.,
101 2021). However, changes in gene expression across environments can be a consequence of stress
102 and reduced performance or adaptive, plastic responses. Without fitness data, results from RNA-
103 seq studies are black boxes with respect to whether DE is attributed to the maladaptive effects of
104 stress or adaptive plasticity.

105 The multitude of molecular pathways that underpin plasticity are not likely to exhibit
106 unidirectional, integrated responses to selection. Distinct pathways that jointly influence a single
107 trait can share positive or negative genetic correlations due to pleiotropic alleles and linkage
108 disequilibrium (Hekerman et al., 2005; Mauro & Ghalambor, 2020). RNA-seq experiments can be
109 integrated with quantitative genetic breeding designs to (i) measure the additive genetic variation
110 and heritability of DE and (ii) estimate genetic variance-covariance (*G*) matrices of DE traits and,
111 consequently, their genetic correlation (Blows et al., 2015). In environmental studies, it is possible
112 to model changes in *G* and genetic correlation between environments as well as environment-
113 dependent fitness effects. Progress has been made in determining how developmental
114 environments influence genetic correlation between traits (Sgrò & Hoffmann, 2004; Wood &

Brodie, 2015) and their fitness effects (Kingsolver & Gomulkiewicz, 2003). Whether parental environments reshape natural selection on traits through these mechanisms is poorly understood, however (Donelson et al., 2018).

Using the purple sea urchin *Strongylocentrotus purpuratus* as an ecologically important model invertebrate (Pearse, 2006), we integrated RNA-seq with assays of performance and fitness-correlated traits in a quantitative genetic crossing design of larval families exposed to ecologically relevant, abiotic stress (Figures 1A & S1). We input these data to structural equation models of environmental change in gene expression and performance (Kline, 2015), a quantitative genetic animal model of additive genetic variation (Wilson et al., 2010), and selection gradient models of natural selection (Lande & Arnold, 1983) as shown in Figure 1B. We quantified the heritability of gene expression plasticity and its effect on adaptive phenotypic plasticity before modeling covariance in heritability and fitness effects. Adults and larvae were conditioned to ecologically relevant abiotic stress applied in these experiments that mimicked variation in temperature and pCO_2 under coastal upwelling frequently experienced by *S. purpuratus*. Upwelling occurs when wind-driven, upward movement of deep seawater lowers the temperature and increases the pCO_2 of surface oceans (Gruber et al., 2012).

In response to experimental upwelling, *S. purpuratus* exhibits transgenerational and developmental plasticity of differential gene expression, DNA methylation, and several performance traits including larval growth rate, biomineralization, and lipid content (Bogan et al., 2023; Strader et al., 2019, 2020, 2022; Wong et al., 2018, 2019). Phenotypic data for larval body size and biomineralization from the experiment that we describe here were reported by Strader et al. 2022 who detected significant genetic variation for the plasticity of both phenotypes in response

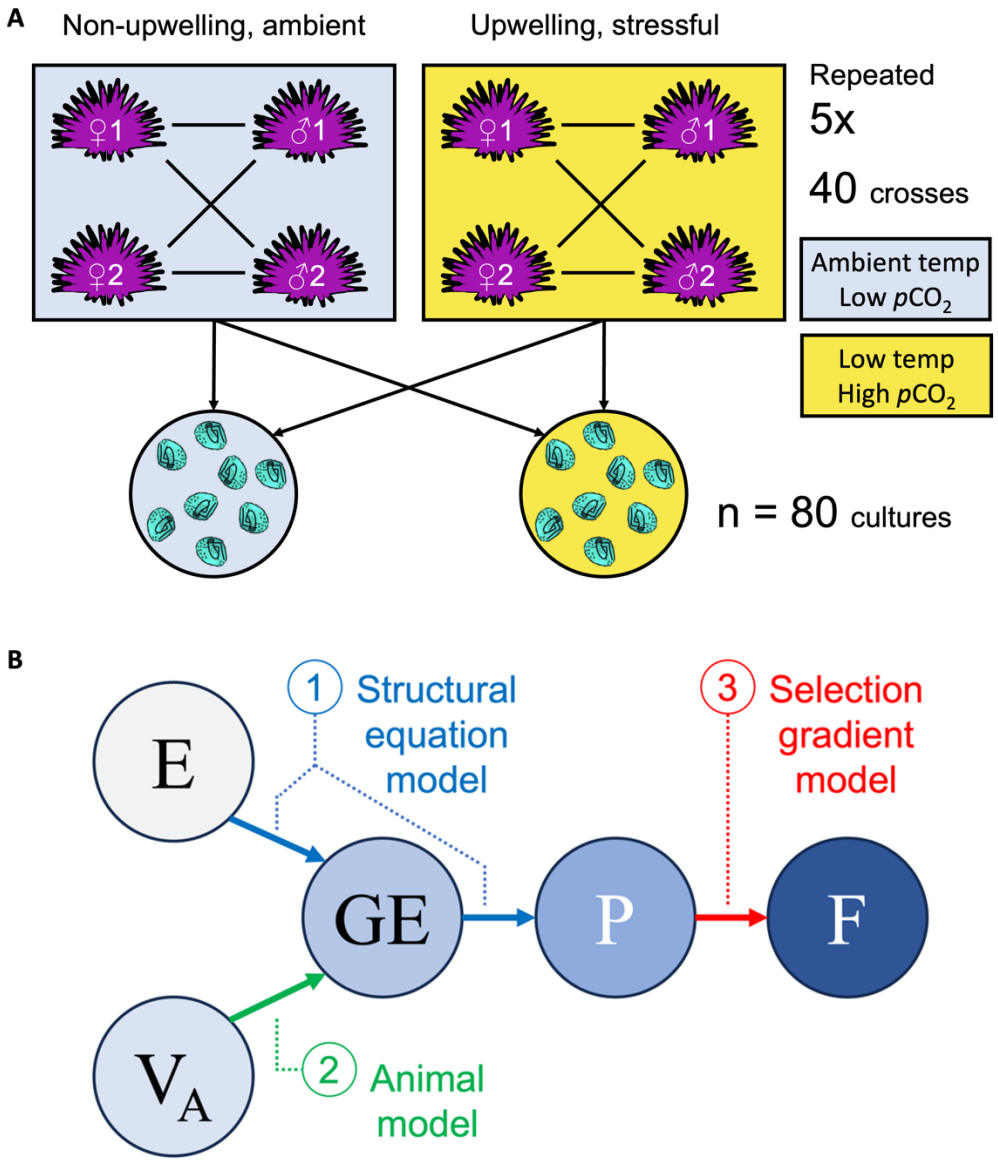


Figure 1 | *Crossing and experimental designs*. **(A)** This graphic depicts adult and larval conditioning to ambient, non-upwelling (blue) and stressful, experimental upwelling conditions (yellow). Crosses between cohorts of conditioned adults are depicted with lines. Reciprocal rearing of offspring resulting from each cross is depicted with arrows directed toward larval non-upwelling and upwelling conditions. **(B)** A visual representation of modeling approaches used to integrate environmental treatments ("E"), additive genetic variance ("V_A"), gene expression ("GE"), phenotypes ("P"), and fitness correlating traits ("F"). Structural equation modeling is visualized in blue which estimated the effect of differential expression on phenotypic plasticity induced by environmental treatments. Animal models predicting additive genetic variance in gene expression are green. Selection gradient models predicting the fitness effects of differential expression are red. Environmental treatments are grey. Parameters predicted by modeling approaches are visualized in shades of blue.

149 to upwelling (Strader et al., 2022). Here we examine whether these reported phenotypic and
150 molecular responses to upwelling are indeed adaptative and, if so, whether parental environments
151 alter the fitness effects and genetic variation of environmental responses by offspring.
152
153 **2. Results**
154 Coastal upwelling causes stressful conditions for *S. purpuratus* larvae (Strader et al., 2020,
155 2022; Wong et al., 2018, 2019). This was similarly observed here, as evidenced by an increased
156 percent abnormality in larvae in cultures conditioned to the treatment transgenerationally and
157 developmentally (Strader et al., 2022). Developmental and parental conditioning to experimental
158 upwelling induced widespread differential expression (DE) as shown in Figure 2. DE induced by
159 developmental upwelling was primarily associated with decreases in larval body size and, to a
160 lesser extent, increases in biomineralization (Fig. 3). The plasticity of body size and
161 biomineralization both yielded significant fitness effects measured as variance in the proportion of
162 normal development among larval families. However, fitness effects were contingent upon the
163 parental environment from which larvae were spawned. Following parental upwelling, reductions
164 in larval body size and plastic increases in biomineralization were both adaptive. Using a
165 heritability threshold of $h^2 \geq 0.2$ (Orton, 2020), 56.80 – 59.93% of adaptive gene expression
166 plasticity exhibited significant heritability (Fig. 4). The heritability of DE did not vary between
167 transcripts associated with adaptive versus maladaptive changes in biomineralization. However,
168 DE associated with adaptive reductions in body size was significantly less heritable than DE
169 associated with maladaptive increases in size (Fig. 4). We further describe these results below.
170

2.1 Differential expression induced by parental and developmental upwelling – RNA-seq alignment, quality checking, and filtering – Following trimming, RNA-seq libraries achieved a mean size of 37.07 ± 5.09 million reads and a mean mapping efficiency of 74.14 ± 1.80%. After read-count filtering for transcripts with > 0.5 CPM in at least 75% of all samples, 12,953 transcripts were retained for downstream analysis. Outlier detection was performed with filtered read count data using arrayQualityMetrics v3.54.0 (Kauffmann et al., 2009), which flagged two half sibling crosses from parental and developmental upwelling treatments as significant outliers (Fig. S2–S3). Removal of these two samples brought the RNA-seq sample size to n = 78. Mean variation in gene expression across samples equaled a BCV of 0.12. Library and alignment metrics are further described in Supplemental File 1.

Parental conditioning to upwelling induced upregulation of 1,582 transcripts and downregulation of 1,539 in larval offspring. These differentially expressed genes (DEGs) included 50 and 29 upregulated and downregulated transcripts with an absolute $\log_2$FC > 1.0 (Fig. 2). DEGs induced by parental upwelling were enriched for 71 biological process (BP) GO terms, 49 molecular function (MF) terms, and 30 cellular component (CC) terms. Upregulated transcripts included enrichment for the BP/MF terms involved in cellular signaling, cell adhesion, transmembrane transport, and development localized to CC terms including the endoplasmic reticulum, cell membrane protein complexes, and extracellular region. Downregulated transcripts were enriched for BP/MF terms involved in ATP metabolism, ribosomal structure/biogenesis, mitochondrial organization, and oxidoreductase activity localized to the ribosome, mitochondrial matrix, and cytosol (Fig. S4–S6).

Developmental exposure to upwelling induced upregulation of 2,246 transcript and downregulation of 2,205. With a >1.0 log_2FC cutoff, these included 38 upregulated and 184 downregulated transcripts (Fig. 2). 39.73% of DEGs induced by transgenerational effects were also differentially expressed in response to developmental conditioning. Developmental upwelling DEGs were enriched for 89 BP GO terms, 53 MF terms, and 37 CC terms. Upregulated transcripts were enriched for BP/MF terms related to chromatin remodeling, mitochondrial organization, ATP metabolism, cellular responses to stress and DNA damage, ubiquitination, and ribosomal structure/biogenesis. These upregulated terms were localized to catalytic complexes, nuclear and organelle lumen, ribosomes, and the nucleolus. Downregulated transcripts were enriched for

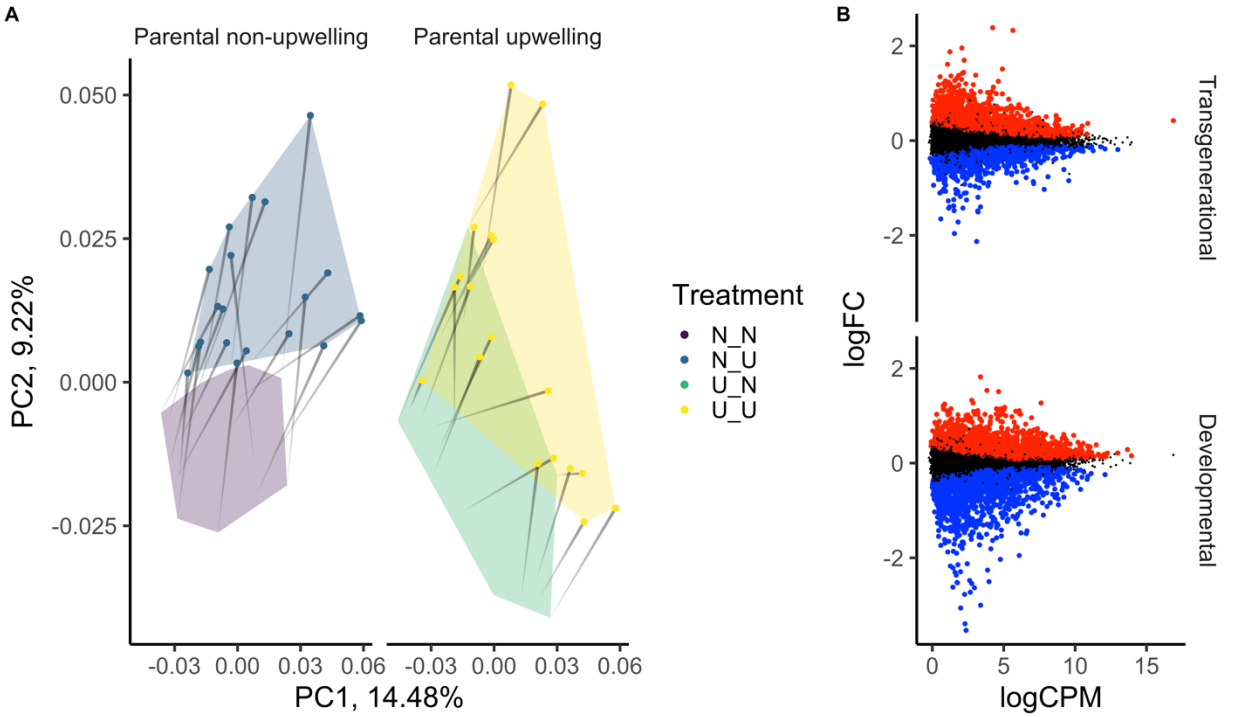


Figure 2 | *Differential expression induced by parental and developmental exposure to upwelling*. **(A)** Loading of samples to principal coordinate axes derived from filtered, normalized read counts. Parental and developmental treatments are represented by color. Paths connect single crosses and their change in loading between non-upwelling ("N"; no point) and upwelling ("U"; point) treatments. **(B)** Mean difference plots of transcript logFC across average CPM per transcript, grouped by parental and developmental effects of upwelling. Significant downregulation is depicted in blue and upregulation in red.

BP/MF terms related to cytoskeletal organization, cell adhesion, oxidoreductase activity, and metabolism of lipids and carbohydrates localized to the cytoskeleton, cell junctions, cell membranes, and endosomes (Fig. S7 – S9).

Upregulated transcripts related to ribosomal function included several ribosomal subunits and 16 DEAD-box proteins involved in the initiation of translation. Included in cellular responses to stress was the significant upregulation of 8 heat shock proteins including 3 Hsp70 and 5 Hsp40 chaperones. Interestingly, 8 heat shock proteins were significantly downregulated in response to upwelling, indicating that they were more highly expressed under warmer conditions. These included all 3 Hsp90 isoforms present in the *S. purpuratus* genome as well as 2 Hsp70 and 3 Hsp40 chaperones. These functional enrichment results demonstrate a suite of complex molecular responses to multivariate, abiotic environmental change brought on by experimental upwelling, a third of which were commonly induced by parental and larval conditioning.

2.2. Associations between differential expression and adaptive versus maladaptive plasticity – Structural equation models (SEMs) predicting the effect of differential expression (DE) on plastic changes in body size and biomineralization in response to developmental upwelling identified 231 transcripts associated with increased body size, 564 with reduced body size, 125 associated with increased biomineralization, and 113 with decreased biomineralization. Selection gradient models predicting the proportion of normal development per replicate culture (a fitness correlated trait) as a function of body size or biomineralization and their plasticity estimated fitness benefits of (i) plastic reductions in size under upwelling and (ii) maintenance of or plastic increases in biomineralization under upwelling. When larvae were spawned from parents exposed to upwelling, plastic increases in biomineralization incurred a selection gradient of 0.40 ± 0.09 (Fig. 3A) and plastic reductions in body size incurred a weaker selection gradient of 0.13 ± 0.04 (Fig.

3B). Interestingly, the plasticity of both traits did not exhibit detectable fitness costs or benefits when larvae were spawned from non-upwelling parents. These selection gradient coefficients represent the slopes of logistic regressions between larval survival (abnormality) and phenotypic plasticity visualized in Figures 3A and 3B. Thus, DE driving reductions in body size or increases in biomineralization were associated with adaptive or weakly adaptive phenotypic outcomes.

As the absolute fold change of a transcript's DE increased, the adaptive effect of DE on reduced body size and/or increased biomineralization significantly increased. From here forward, 'adaptive' or 'maladaptive' effects refer to selection on larvae from upwelling-exposed parents unless otherwise specified. Associations between DE and effects on phenotypic plasticity toward maladaptive directions were weaker, demonstrating that DE induced by developmental upwelling was predominantly associated with adaptive plasticity (Fig. 3C). Transcripts associated with reduced body size under upwelling conditions were enriched for BP/MF terms involved in cellular signaling, transmembrane transport, and ribosomal biogenesis localized to cell junctions and the nucleolus. In stark contrast to transcripts associated with plasticity of larval body size, DE driving increases or decreases in biomineralization exhibited no functional enrichment across all GO term categories.

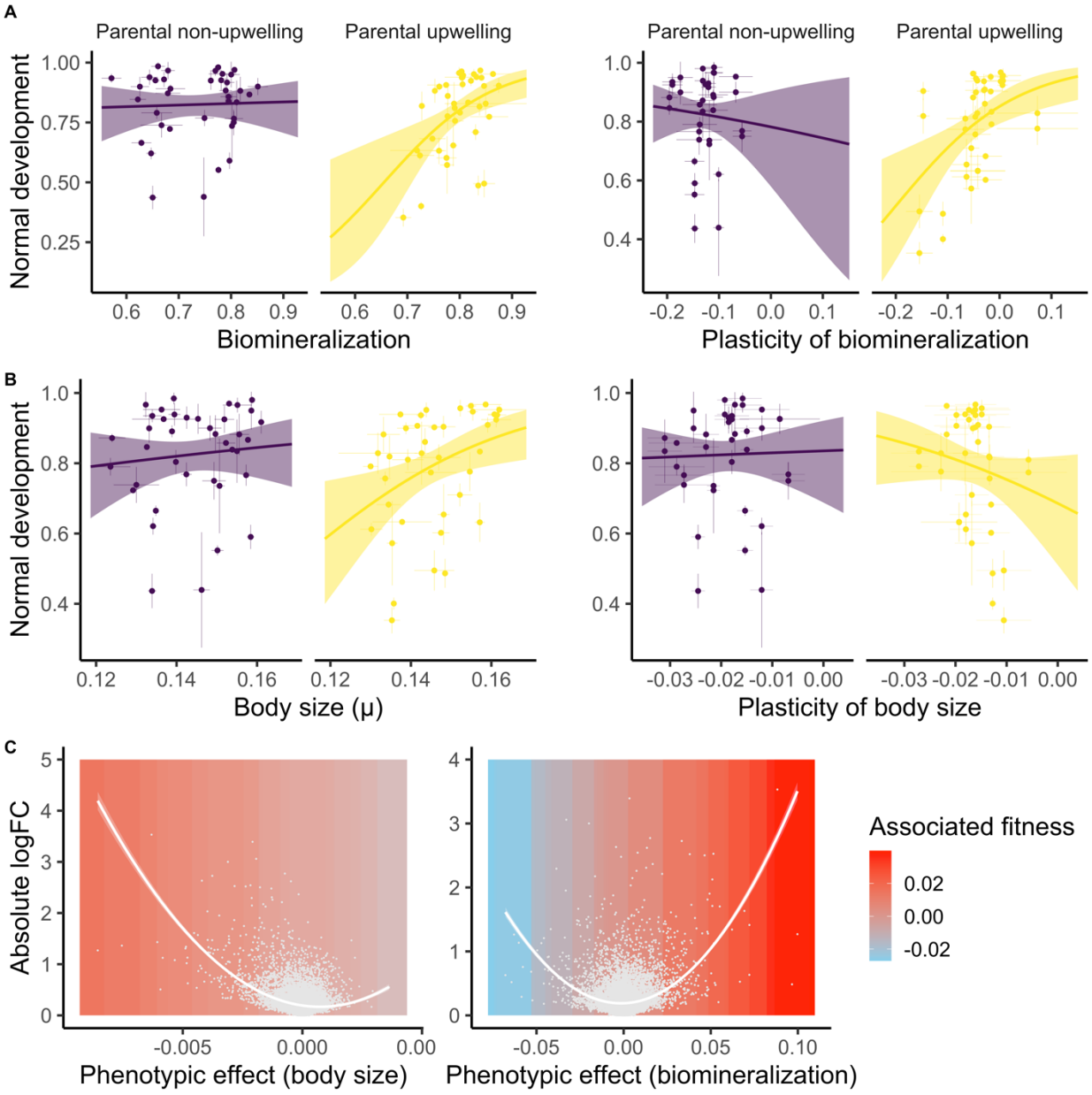


Figure 3 | *Effects of differential expression on adaptive plasticity*. **(A – B)** The effects of phenotypic values and plasticity in biomineralization (spicule length normalized by body length) and body size (maximum body length) on proportion of normal development grouped by parental environment are plotted in A and B, respectively. Points represent phenotypic means (left) or mean reaction norms (right) of crosses reared in each environment. Environments are depicted by color such that non-upwelling is blue and upwelling is yellow. Error bars depict standard deviation in each trait among replicates of each cross. **(C)** Absolute logFC of differential expression induced by developmental upwelling is plotted across differential expression's association with upwelling effects on body size and biomineralization in A and B, respectively. Points represent transcripts. Solid lines depict fitted quadratic curves ± 95% CI. Plot background color corresponds to the product of differential expression's phenotypic effect on the plasticity of body size or biomineralization and the selection gradient for plasticity of each trait under parental upwelling, resulting in an inferred fitness induced by differential expression.

2.3. The transcriptome-wide heritability of adaptive and maladaptive differential expression – DE was highly heritable across most genes, but levels of heritability varied according to associations between DE and adaptive plasticity. DE was more heritable than baseline gene expression (CPM) by 19.95% h^2 ($p < 2.2e^{-16}$) and the two variables were uncorrelated (Fig. 4A). 60.46% of significant DE induced by developmental conditioning exhibited h^2 greater than or equal to 0.20 (i.e., at least 20% of in DE variance was heritable). The average h^2 of DE was 0.2674 ± 0.1592. 7.59% of DEGs were both heritable and associated with adaptive plasticity in body size, but 59.93% of adaptive DE related to body size was heritable. These transcripts exhibited similar h^2 relative to all DE: 0.2613 ± 0.1516. Substantially less DE was heritable and associated with maladaptive plasticity in body size, equaling only 3.59% of all DEGs. However, maladaptive DE related to body size exhibited greater heritability with a mean h^2 of 0.2984 ± 0.1600. 56.80 % of DEGs associated with adaptive increases in biomineralization were heritable, with a mean h^2 of 0.3709 ± 0.1224. 1.55% of DEG's were heritable and associated with adaptive increases in biomineralization. 0% of DEGs that were significantly associated with maladaptive decreases in biomineralization were heritable.

DE associated with adaptive reductions in body size under upwelling was significantly less heritable than DE associated with maladaptive increases in size. As the effect of DE on adaptive decreases in body size grew, h^2 significantly decreased (β = -0.96; $p = 5.77e^{-8}$) as well as the probability of $h^2 \geq 0.2$ (β = -5.47; $p = 2.16e^{-8}$). Conversely, this means h^2 and the probability of heritability increased as maladaptive effects of DE on increased body size grew (Fig. 4B). The phenotypic effect of DE on adaptive biomineralization plasticity had a negative but insignificant effect on its heritability (Fig. 4B).

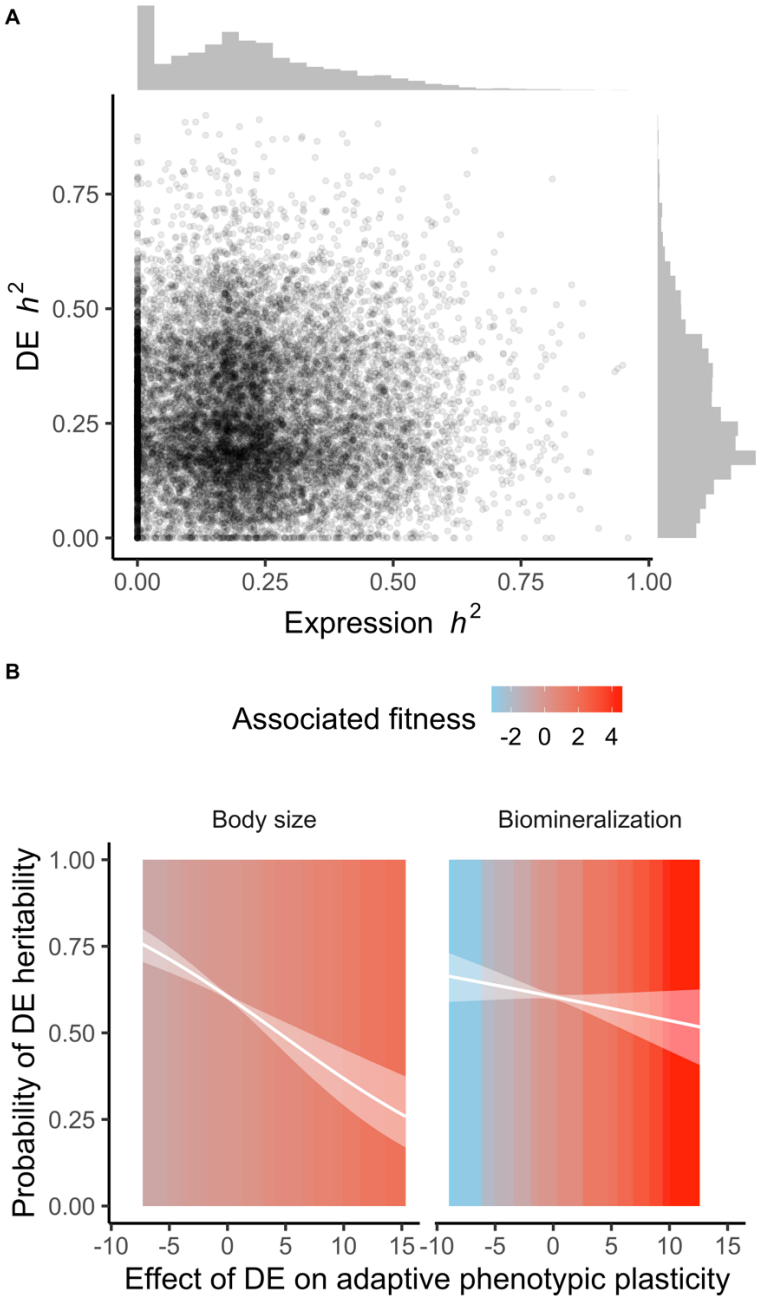


Figure 4 | *Heritability of differential expression and its effect on adaptive phenotypic plasticity*. **(A)** h^2 of differential expression (DE) induced by developmental upwelling is plotted across h^2 of baseline gene expression transcriptome wide. The distributions of h^2 for baseline and differential expression are plotted adjacent to the x and y axes, respectively. **(B)** The probability of differential expression $h^2 \geq 0.20$ is plotted as a logistic curve ± 95% CI across differential expression's effect on biomineralization and body size signed for the effect's direction toward adaptive plasticity. Background color represents the coefficient for DE's effect on phenotypic plasticity multiplied by the selection gradient acting on the plasticity of each phenotype under parental upwelling (e.g., the associated fitness outcome of DE's effect on plasticity).

Adaptive DE remained significantly less heritable when considering the cumulative fitness effects of plasticity-associated DE. The cumulative fitness effect of DE was measured by summing DE's phenotypic effects on both traits and multiplying these effects by the selection gradients acting on each trait's plasticity. This cumulative fitness effect was negatively correlated with the probability of heritability (β = -0.05; p = 0.00376). For illustrative purposes, variation in DE's logFC, h^2, and total effect on adaptive plasticity are plotted as a fitness landscape in Figure 5. Negative fitness effects were enriched among transcripts with DE $h^2 > 0.2$ and logFC > 1.0 while positive fitness effects clustered at a lower h^2 among downregulated transcripts (Fig. 5). Minimal changes in gene expression did not result in strong effects on fitness, as evidence by a valley of neutral costs associated with low DE (Fig. 4 – 5).

Genetic variance-covariance matrices (*G* matrices) were estimated for sibships' logFC values for differentially expressed genes associated with adaptive plasticity of body size and biomineralization. *G* matrices enable identification of genetically correlated modules of traits and axes of genetic variation (Steppan et al., 2002). *G* matrices are thus integral to the effects of natural selection on multivariate phenotypic space (S. J. Arnold et al., 2001). Genetically-correlated modules of DE genes were identified by screening for genes with logFC values that were less than a genetic distance of 0.5 from one another, where genetic distance equals 1 – genetic correlation. DE associated with adaptive plasticity in body size was composed of 28 genetically correlated modules of 564 genes. DE associated with adaptive plasticity in biomineralization was composed of 32 genetically correlated modules of 125 genes. Genetic correlations between logFC values were primarily positive and exhibited larger absolute correlation among logFCs sharing positive genetic correlation (Fig. 6).

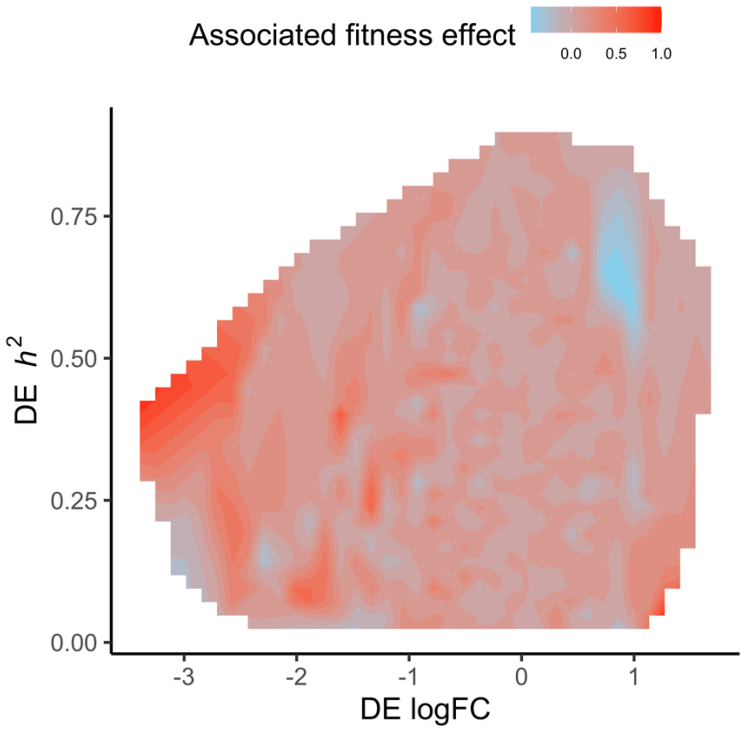


Figure 5 | *Transcriptome wide distribution of differential expression, its heritability, and associated fitness effects.* The distribution of heritability for differential expression (DE) in response to upwelling is plotted as h^2 across the distribution of DE's fold change (logFC). Color represents the fitness outcome associated with DE's effect on phenotype: the summed products of DE's phenotypic effects on body size and biomineralization multiplied by the selection gradients acting on the plasticity of each trait.

G matrices for larvae from upwelling versus non-upwelling parents exhibited differences in genetic correlation for DE associated with adaptive plasticity in body size. Mantel tests were used to evaluate variation between parental treatments in genetic correlation matrices of DE associated with adaptive plasticity in body size ($Z = 40477.59$; $p < 1e10^{-4}$) and biomineralization ($Z = 1438.176$; $p < 1e10^{-4}$). For both traits, some correlations shifted from negative-to-positive and positive-to-negative under parental upwelling (Fig. 6). Even in the absence of environmental effects, random chance in changes to the sign of genetic correlation are expected. Thus, a chi-squared test was used to determine if there were greater or fewer positive-to-negative shifts than expected by chance alone. Genetic correlations in body size DE exhibited significant shifts from negative-to-positive under parental upwelling ($\chi^2 = 1767.7$; $p < 2e10^{-16}$).

Parental treatment groups contained different genotypes, presenting a potential confound of transgenerational effects on genetic correlation. Permutations of the experimental pedigree were performed, dividing it into two random halves with equal representation of parental treatment groups. Random permutation revealed that 7.25% of enrichment for positive, directional changes to genetic correlations in body size DE were attributed to pedigree structure rather than environment. It was also significantly more likely that directional shifts in genetic correlation under parental upwelling were attributed to environment rather than pedigree structure (χ^2 = 1639.6; $p < 2e10^{-16}$). 94.53% of upwelling-induced directional shifts to genetic correlations in biomineralization DE were attributable to pedigree structure. Thus, there were detectable effects of parental environment on the genetic architecture of pathways associated with adaptive body size plasticity, but not biomineralization.

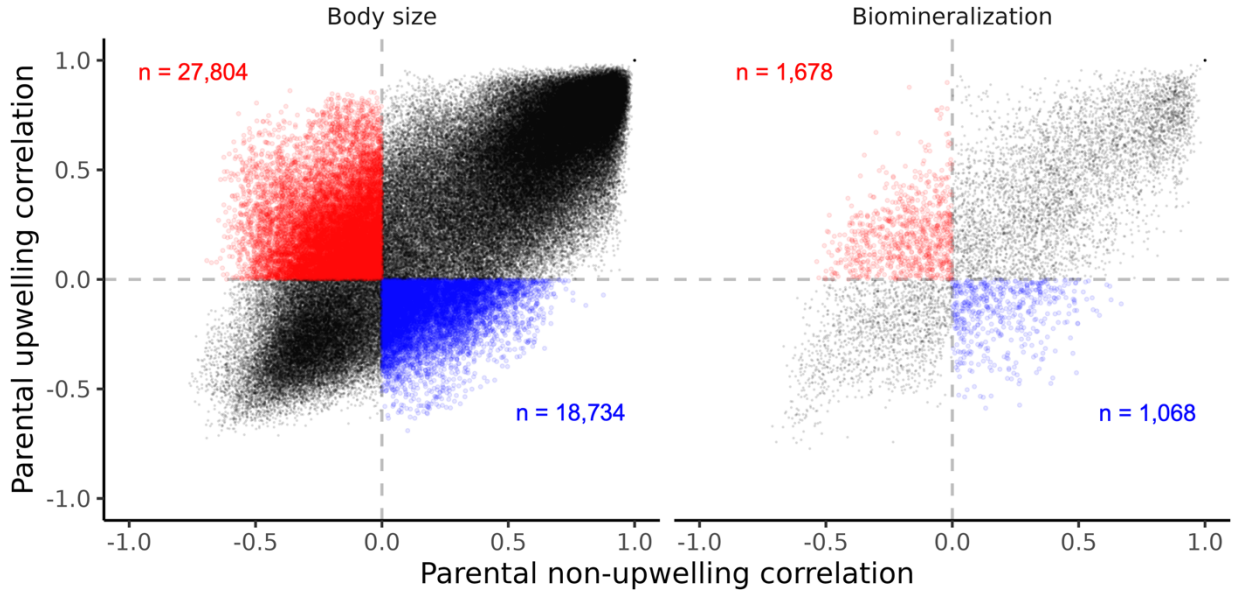


Figure 6 | *Changes in genetic correlation between parental upwelling treatments for log foldchanges (logFC) of differential expression (DE) associated with adaptive plasticity in larval body size and biomineralization*. X and Y axes represent genetic correlations extracted from *G* matrices of logFC values estimated from larvae of non-upwelling and upwelling conditioned parents. Correlations that shifted from negative to positive under parental upwelling are enlarged and colored in red. Correlations that shifted from positive to negative under parental upwelling are enlarged and colored in blue.

Heritable DE associated with plasticity in body size was enriched for functions associated with ribosomal biogenesis and maintenance (Fig. 7). Transcripts with high DE heritability and adaptive reductions in body size under upwelling were enriched for the BP/MF GO terms related to ribosomal biogenesis, RNA processing, and transmembrane transport localized to the nucleolus. Heritable DE associated with maladaptive increases body size was enriched for BP/MF terms related to amide formation (a component of peptide synthesis during translation) and ribosome structure localized to the cytosolic ribosome and large ribosomal subunit (Fig. 7). Heritable DE with adaptive effects on the plasticity of biomineralization was not enriched for GO terms.

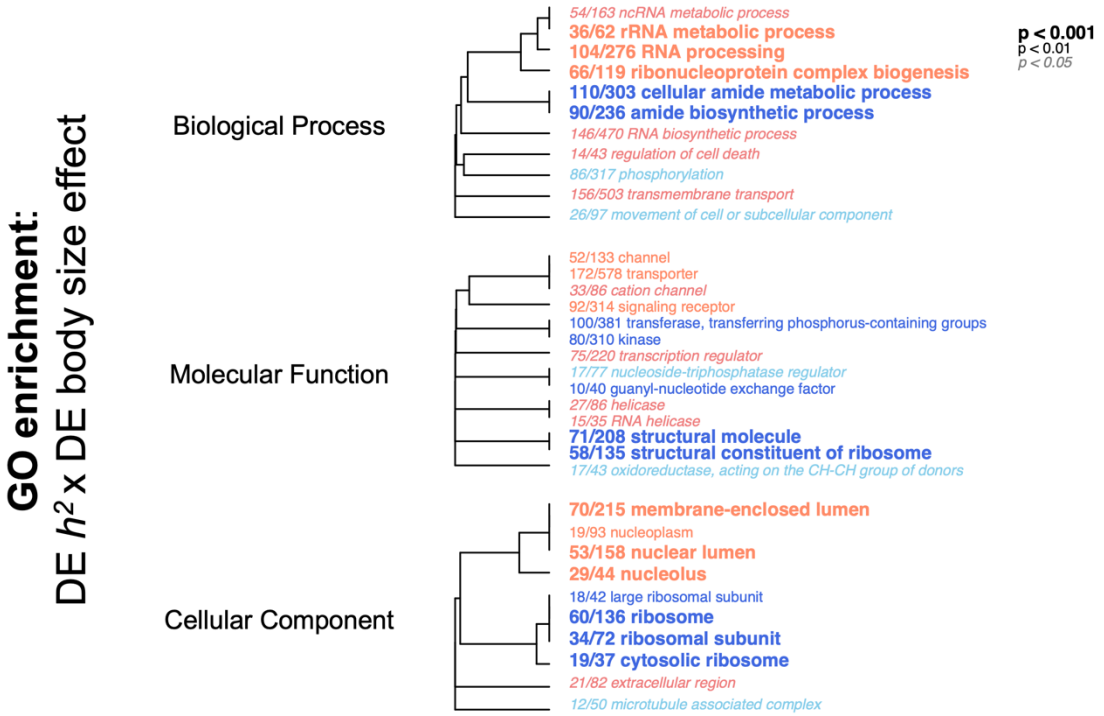


Figure 7 | *Functional enrichment of differentially expressed genes with high heritability and strong absolute effects on the plasticity of body size*. Enriched gene ontology (GO) terms are derived from a Mann Whitney U test of variation in the product of differential expression's heritability (h^2) and DE's effect on adaptive plasticity of body size in response to upwelling. Trees depict clustering of GO terms based on shared transcripts. Red GO terms are enriched within heritable differential expression associated with adaptive reductions in body size in response to developmental upwelling exposure. Blue terms are enriched within heritable differential expression associated with maladaptive increases in body size under upwelling.

4. Discussion

3.1. Reduced heritability of transcriptional responses associated with adaptive plasticity – We observed that the transcriptome wide heritability of DE significantly decreased when DE was associated with adaptive, phenotypic responses to developmental conditioning under upwelling (Figures 4 – 5). This extent of genetic variation for DE, and its decline in DE associated with adaptive plasticity, confirms observations in other studies of plants (He et al., 2021) and animals (Campbell-Staton et al., 2021; Oostra et al., 2018). However, past research has assigned putatively adaptive and maladaptive roles to molecular responses based on prior findings. Here, we directly integrated gene expression plasticity with fitness measures. Quantifying cross-environment genetic correlations (a measure of genetic variance in plasticity) for gene expression in a tropical butterfly, Oostra et al. detected poor genetic variation for DE between two seasonal morphological phenotypes (Oostra et al., 2018). The authors proposed that minimal genetic variation for transcriptional plasticity resulted from a canalized response to a highly reliable seasonal cue (Oostra et al., 2018). Numerous studies have reported moderate-to-high genetic variation in DE measured as genotype x environment (GxE) interactions shaping expression in animals (Grishkevich & Yanai, 2013; Huang et al., 2020; McCairns et al., 2016). Oostra et al. provide one of the only measures of additive genetic variance in the reaction norm of gene expression rather than variance attributed to GxE. Thus, it is notable that V_A of DE that they detected is strikingly lower than what we observed here.

Multiple factors may contribute to high genetic variance and heritability for transcriptional plasticity under upwelling in *S. purpuratus*. *S. purpuratus* larvae are widely dispersed during their planktonic phase resulting in high connectivity across spatial scales and high genetic diversity within populations (Edmands et al., 1996; Palumbi & Wilson, 1990; Pespeni & Palumbi, 2013).

Its dispersal distances can be wide enough that larvae are frequently transported across areas of major and minor upwelling in the California Current and Southern California Bight (Chan et al., 2017; Pespeni & Palumbi, 2013; Zaytsev et al., 2003). *S. purpuratus* populations exhibit evidence of local adaptation to regional differences in pCO_2 despite high rates of gene flow (Evans et al., 2013; Pespeni et al., 2013). These findings suggest that an individual population should possess high genetic variation in trait means and/or plastic responses associated with coastal upwelling, and that reductions in genetic variation of adaptive plasticity may occur due to selection. Lastly, genetic variation is often more highly expressed under stressful conditions (Hoffmann et al., 1999). We observed moderate-to-high levels of developmental abnormality in many larval crosses indicative of a baseline level of stress across replicates brought on by parental conditioning or stress incurred *in situ* prior to adult collection.

How V_A of DE covaries with its fitness effects is more poorly understood than genetic variation in DE itself. Studying Anolis lizards, Campbell-Staton et al. found increased frequencies of SNPs within *cis*-regulatory regions of genes associated with putatively maladaptive decreases in CTmax under thermal stress. *Cis*-regulatory mutations proximal to genes with putatively adaptive DE did not vary relative to genomic background. This study's design differed from our own in that plasticity was measured at a population level – distinct genotypes were reared under each treatment rather than single genotypes or families being split across treatments. Plasticity was thus summarized as a mean change in phenotype in each population (Campbell-Staton et al., 2021). Our study also expanded on Campbell-Staton's approach of putatively identifying adaptive DE by directly measuring fitness in the same individuals that gene expression was characterized in.

Our findings are among the first to directly associate transcriptome-wide changes in gene expression with phenotypic plasticity, genetic variation, and fitness effects. h^2 was high for DE induced by upwelling, but it decreased for DE associated with adaptive phenotypic plasticity (Figures 4 – 5). Our study cannot pinpoint specific genetic variants underpinning adaptive plasticity, but two hypotheses are worth highlighting. Firstly, plastic responses that were adaptive may incur strong positive or stabilizing selection in nature resulting in decreased genetic variance (Price & Langen, 1992). This argument is similar to that made by Oostra et al. regarding fixed levels of morphological plasticity within populations (Oostra et al., 2018). Theory also predicts decreased genetic variance for plasticity caused by the fixation of plastic alleles in populations inhabiting more variable environments that select for plasticity (Jong & Gavrilets, 2000). Alternative hypotheses may explain reduced genetic variation in DE associated with adaptive versus maladaptive plasticity, however

One hypothesis proposes that maladaptive plasticity stems from the expression of deleterious cryptic genetic variants under novel environments that have were not subject to prior negative selection (Acasuso-Rivero et al., 2019; R. F. Schneider & Meyer, 2017). Despite aiming to experimentally simulate upwelling conditions, laboratory conditioning almost certainly introduced *S. purpuratus* to novel environments. Secondly, populations subject to stabilizing selection on a trait incur net negative fitness even when the phenotypic equals the fitness optimum because deviation from that optimum imposes fitness costs (Brady et al., 2019; Burt, 1995; Hansen et al., 2006). Therefore, reactions norms that possess high genetic variation and heritability may be intrinsically likely to exhibit apparent negative directional selection on reaction norms when selection is truly stabilizing.

448 Despite reductions in genetic variation for adaptive DE, substantial genetic variation and
449 heritability remained. If we assume responses to upwelling are under positive or stabilizing
450 selection for high plasticity in some populations, genetic variation for adaptive plasticity could be
451 maintained by fluctuating selection caused by (i) variation in the severity and frequency of coastal
452 upwelling across the wide range and dispersal distance of *S. purpuratus* (Chan et al., 2017; Kelly
453 et al., 2013) or (ii) the seasonal nature of upwelling intensity (Lynn & Simpson, 1987; Quilfen et
454 al., 2021). For example, Hallson et al. observed that lines of seed beetles reared under fluctuating
455 selection acting on thermal performance exhibited significantly greater genetic variance for
456 thermal plasticity of developmental rate after 18 generations of selection (Hallsson & Björklund,
457 2012). In summary, positive selection on DE may have caused its reduction in genetic variation,
458 but plastic alleles may not have fixed due to the variable and fluctuating landscape of upwelling
459 and its selective pressures across the range of *S. purpuratus*.

460 *3.2. Functional enrichment within heritable, adaptive plasticity* – Fitness benefits of
461 plasticity during developmental conditioning to upwelling can be contingent on whether affected
462 pathways are developmentally canalized – e.g., whether DE can be induced by any process other
463 than ontogeny (Siegal & Bergman, 2002). Under this framework, DE that compensates for
464 pathological deviations from developmentally canalized processes are expected to be more
465 associated with adaptive plasticity. Variation in ribosomal function during development bears
466 harmful effects on organismal function (Freed et al., 2010; Ordas et al., 2008), and multiple GO
467 terms enriched among transcripts differentially expressed in response to developmental upwelling
468 exposure were indicative of cellular responses to ribotoxic stress.
469

Heritable and adaptive changes in gene expression were enriched for GO terms associated with ribosomal biogenesis while maladaptive, heritable DEGs retained enriched functions indicative of ribotoxic stress such as downregulated ribosomal subunits (Fig. 7). Induced ribotoxic stress can result in the downregulation of ribosomal subunits in tandem with HSF1's induction of the cytosolic, 70 kda heat shock protein Hsc70 and Hsp40 chaperones (Albert et al., 2019). These are the two classes of heat shock proteins that were upregulated in response to developmental upwelling. However, hsp's were collectively split between being upregulated or downregulated in response to upwelling, potentially due to positive correlations between the expression of some chaperones and temperature, and thus require further scrutiny (Feder & Hofmann, 1999). Abiotic stress can perturbate ribosomal function via denaturation of ribosomal proteins/RNAs or misfolding of nascent proteins that disrupt proximal ribosomes (De & Mühlemann, 2022; Iordanov et al., 1998). The extent to which ribosomal biogenesis and structure was overrepresented among transcripts with heritable, (mal)adaptive plasticity suggests that maintenance of translation and ribotoxic stress responses may be critical for acclimation and adaptation to upwelling.

3.3.Transgenerational effects reshape fitness consequences and genetic architecture of adaptive transcriptional and phenotypic plasticity – The heritability of phenotypic plasticity, and how this heritability covaries with its fitness effects, can impact the evolution of plasticity and the traits it acts on (Fox et al., 2019; West-Eberhard, 2003). However, evolutionary biology has a limited ability to predict when plasticity and its genetic variation is subject to selection (P. A. Arnold et al., 2019; H. M. Schneider, 2022; Van Buskirk & Steiner, 2009). Under global change, these ecological and evolutionary effects are important for predicting whether future climates will drive canalization of tolerance or increase acclimatory potential via directional selection on heritable plasticity (Svensson et al., 2020; Van ASCH et al., 2007). In this study, we observed that

493 transgenerational effects of parental conditioning to an ecologically relevant stressor modified the
494 fitness effects of phenotypic plasticity and the genetic architecture of associated regulatory
495 pathways. By reshaping the genetic (co)variation and fitness effects of phenotypic plasticity,
496 transgenerational effects may influence its evolution by natural selection.

497 Genetic variance-covariance matrices (G matrices) can constrain responses to selection in
498 multivariate trait space when genetic covariance or correlation between traits is strongly negative
499 (Agrawal & Stinchcombe, 2008). G matrices of absolute logFC values for differential expression
500 associated with plasticity demonstrated a high degree of positive genetic correlation. This suggests
501 that adaptive responses by some of the genetically correlated modules associated with adaptive
502 plasticity should positively correlate in response natural selection. Among DE associated with
503 adaptive plasticity in body size, genetic correlations between logFC values of thousands of genes
504 significantly shifted from negative to positive in offspring of upwelling-conditioned parents (Fig.
505 6). Environments can influence genetic correlations between traits by inducing or silencing the
506 expression of genetic variation underpinning those traits (Bogan & Yi, 2024; Wood & Brodie,
507 2015). For polygenic traits, this can lead to the expression of variants that share more positive or
508 more negative genetic correlation in a given environment. Effects of developmental environments
509 on genetic correlation have been reported in pairs of traits (Fischer et al., 2016; Sgrò & Hoffmann,
510 2004; Wood & Brodie, 2015). Our results reveal that not only developmental, but parental
511 environment, influences the expression of genetic variation and genetic correlations across
512 thousands of gene expression traits associated with phenotypic plasticity. Increases in positive
513 genetic correlation among DE associated with adaptive body size plasticity reduced genetic
514 tradeoffs that may constrain adaptive evolution (Fig. 6).

Upwelling-induced increases in positive genetic correlation (Fig. 6) coincided with an increase in phenotypic plasticity's fitness effects (Fig. 3). Natural selection on phenotypic plasticity requires that it imposes fitness costs or benefits that have been historically difficult to detect. Recently, plasticity's costs have been shown to be contingent on ecological and evolutionary contexts such as fluctuating environments (Schaum et al., 2022), differences between sexes and morphotypes (Hangartner et al., 2022; Svensson et al., 2020), and fitness trade-offs with between plasticity other traits (Bogan et al., 2024; van Heerwaarden & Kellermann, 2020). Our findings that the fitness costs of plasticity in larval biomineralization and body size depended on parental environment expands our knowledge of eco-evolutionary processes influencing plasticity's costs. To our knowledge, this is the first demonstration that parental environments influence the costs of phenotypic plasticity in offspring. Plasticity's fitness costs may have been exacerbated in larvae reared from upwelling-exposed parents due to resource limitation imposed by parental stress during conditioning. However, past work in *S. purpuratus* has shown that maternal conditioning to upwelling during gametogenesis does not alter egg size or protein content and improves lipid provisioning (Wong et al., 2018, 2019). This lipid provisioning may be insufficient to overcome resource limitation and the cost of allocation to plasticity in larvae that are developmentally and transgenerationally exposed to upwelling.

4. Conclusion

Understanding plasticity's fitness costs and genetic variation will improve predictions of adaptive responses by biodiversity to climate change. By leveraging RNA-seq integrated with measures of performance and fitness correlating traits, we resolved a transcriptome scale picture of heritability in the regulatory and physiological pathways that underpin adaptive and maladaptive

538 responses to an ecologically relevant stressor. In our case study of a pervasive coastal herbivore,
539 parental exposure to simulated upwelling increased the fitness effects of plastic responses to
540 upwelling by offspring and reduced negative genetic correlations between differentially expressed
541 genes associated with adaptive plasticity. Ecological and physiological research on *S. purpuratus*
542 has demonstrated a high level of resilience to environmental perturbations such as marine
543 heatwaves relative to other coastal fauna (Chamorro et al., 2023; Rogers-Bennett & Catton, 2019;
544 Smale, 2020) and potential local adaptation to upwelling (Kelly et al., 2013; Pespeni et al., 2013;
545 Pespeni & Palumbi, 2013). Our findings further support the potential for *S. purpuratus* to maintain
546 this resilience via evolution of phenotypic plasticity in multiple physiological pathways and
547 underscore the influence of transgenerational effects on plasticity's evolution. More broadly, they
548 reveal evolutionary roles of transgenerational effects as modulators of fitness landscapes and
549 genetic (co)variation driving evolution by natural selection.
550
551 **5. Methods**
552 *5.1 Adult conditioning, crossing, and larval culture* – Adult urchins were collected from 2
553 coastal sites in the Santa Barbara Channel in August and September of 2018: Naples Reef
554 (34.4221216, -119.95154) on August 23, 2018, and from Arroyo Quemado Reef (34.46774988, -
555 120.11905). Adults were distributed across 4 90 L tanks per parental treatment at a density of 10
556 urchins per tank. Adults acclimated to parental treatments for approximately 4 months: non-
557 upwelling = 17°C and 596 µatm pCO_2; upwelling = 12.8°C and 1117 µatm pCO_2. Flow rates to
558 adult tanks equaled 20 l h^{-1}. Adults were fed fresh *Macrocystis pyrifera ad libitum* with food
559 changes and tank cleanings conducted once per week. Seawater temperature was controlled using

560 heat pumps regulated by Nema 4X digital temperature controllers. pCO_2 was controlled using a
561 flow-through CO_2 mixing system adapted from Fangue *et al*. (Fangue et al., 2010).

562 Fertilizations were conducted using a staggered cross-classified North Carolina II breeding
563 design. During each phase of the staggered cross, 2 males and 2 females from a common adult
564 condition were reciprocally crossed and their resulting offspring were cultured under non-
565 upwelling and upwelling conditions until the prism stage of larval development. Each cross x larval
566 treatment group was reared using 2 technical replicate culture buckets, resulting in 16 larval
567 cultures per staggered cross. This process was repeated 5x for non-upwelling and upwelling
568 crosses, alternating in order between parental treatments, resulting in 40 crosses reared across 160
569 technical replicates and 80 biological replicates (Fig. 1A).

570 Larvae were cultured in replicate flow-through 6 L nested buckets (i.e., an inner bucket
571 with 30 µM mesh openings nested in an exterior bucket) at a flow rate of 3 L h^{-1} and a density of
572 10 larvae ml^{-1} until the early prism stage of larval development signified by the onset of tripartite
573 gut differentiation. Temperature and pH were regulated in larval culture buckets as described
574 above for adult conditioning. Point measures of temperature, salinity, total alkalinity, and pH for
575 adult and larval tanks are described by Strader et al. 2022.

576 *5.2. Phenotyping of performance and fitness-correlated traits* – Three phenotypes were
577 measured in larval cultures: (i) percent developmental abnormality (a corollary of survival), (ii)
578 larval body size, and (iii) spicule length per unit body size (a corollary of biomineralization).
579 Morphometric measurements of body size and spicule length were performed on $n \geq 30$ larvae per
580 technical replicated stored in 2% formalin buffered with 100 mM $NaBO_3$ (pH 8.7) in filtered sea
581 water. Body size was defined as the maximum linear distance of a prism body and spicule length
582 defined as length from the tip of the body rod to the branching point of the post-oral rod.

583 Abnormality was scored on $n \geq 30$ larvae during sampling and was measured as the percentage of
584 larvae exhibiting unsuccessful gastrulation. Because RNA-seq was performed using pooled RNA
585 samples per culture, performance and fitness-correlating phenotypes were integrated with gene
586 expression data using culture means rather than per-animal values.

587 *5.3. RNA extraction, sequencing, and bioinformatic processing* – Total RNA was extracted
588 with Trizol from pooled samples of 6,000 larvae per culture replicate. Extractions were performed
589 on 1 technical replicate per cross x developmental treatment resulting in 80 RNA extractions. Total
590 RNA quantity and quality was evaluated via Nanodrop, gel electrophoresis, and Qubit
591 quantification before library preparation. RNA-seq libraries were prepared using polyA
592 enrichment and were quality checked via LabChip GX. Strand specific PE 150 bp reads were
593 sequenced on an Illumina HiSeq 4000 platform.

594 Illumina Universal Adapters were removed from paired end reads using CutAdapt v4.4
595 (Martin, 2011) and reads were trimmed and quality filtered using Trimmomatic v0.39 set to paired
596 end mode, trailing and leading clip = 3, sliding window = 4:15, minimum length = 36, and
597 headcrop = 10 (Bolger et al., 2014). All trimmed reads passed quality check via FastQC v0.12.1
598 (Andrews, n.d.). Forward strands of paired reads were aligned to the 'Spur_5.1' reference genome
599 assembly (Sodergren et al., 2006) using hisat2 v2.2.1 (D. Kim et al., 2019). Forward rather than
600 paired reads were aligned to increase the number of genes included in the dataset after filtering for
601 read count. Resulting SAM alignments were sorted and converted to BAM using SAMtools v1.6
602 (H. Li et al., 2009). Reads were counted per transcript from sorted BAM files using featureCounts
603 v1.6.3 input with the 'Spur_5.1' gtf annotation set to a MAPQ alignment quality cutoff of 10 (Liao
604 et al., 2014). Read counts were performed with transcript annotations rather than genes to ensure
605 accurate estimation of additive genetic variation for transcriptional traits, which may vary between

transcript isoforms. Transcripts of the same parent gene made up 9.14% of the final filtered data set used for differential expression analysis and quantitative genetic models. DE transcripts associated with adaptive plasticity in larval body size and biomineralization included 0 pairs of transcript isoforms of the same parent gene, avoiding any confounds of genetic correlation.

5.4 Tests of differential expression – Transcript read counts were normalized in edgeR v3.40.2 as counts per million (CPM). Read counts were filtered to keep all transcripts exhibiting CPM > 0.5 in at least 75% of the 80 replicates. Differential expression (DE) was modeled using a negative binomial generalized linear model (glm) fitted with edgeR's robust, tagwise dispersion parameter using the robust iteration of the model fitting function 'GLMQLFit' and the DE test function 'GLMQLFTest' (Robinson et al., 2010). Expression was predicted as a function of two non-interacting, categorical variables for parental and developmental environment. Models were fit with non-interacting environmental predictors because the study's design only enabled the measurement of V_A for developmental rather than transgenerational plasticity. Fitting an interaction between both effects would confound interpretation of V_A for developmental plasticity. Significant DE was evaluated using FDR adjusted p-values (alpha < 0.05). Functional enrichment was tested using a rank-based Mann Whitney U test of Gene Ontology terms input with logFC coefficients for DE. This test determines whether a given GO term's logFC distribution is significantly skewed from the mean of the background, filtered transcriptome (Wright et al., 2015).

5.5 Measuring the adaptive differential expression – The effect of DE on the plasticity of body size and biomineralization (body size-normalized spicule length) was measured using structural equation models (SEMs). SEMs were derived from two linear models: (i) phenotype predicted as a function of transcript abundance, developmental environment, and parental environment and (ii) scaled, signed transcript abundance predicted as a function of developmental

and parental environments. Scaled transcript abundance was signed such that samples with low expression resulting in a negative scaled value were multiplied by the direction of the transcript's DE under upwelling. The effect of developmental environment on phenotype mediated by DE was estimated for each transcript using mediation analysis performed with the 'mediate' function of the R package mediation v4.5.0 set to 1000 simulations (Tingley et al., 2014). Positive mediation effects indicated that changes in gene expression in the direction of DE were associated with higher levels of body size or biomineralization under upwelling. Negative mediation effects indicated that DE was associated with reduced phenotypic values under upwelling. To understand how the strength of DE impacted phenotypic outcomes, linear regressions were performed between transcriptome wide, absolute logFC and a second-order, quadratic polynomial for the phenotypic effects output by SEM.

The fitness costs and benefits of plasticity in body size and biomineralization were measured using a Lande & Arnold selection gradient model (Lande & Arnold, 1983) as described in Chapter Two, whereby a fitness correlated trait (proportion of normal development, a larval corollary of survival) was modeled as a function of developmental and parental environment, body size or biomineralization per cross in each environment, and the plasticity of body size or biomineralization of a cross between developmental environments. Selection gradient models included a random effect identifying each cross and controlling for genetic covariance using a relatedness matrix generated from custom code (see https://github.com/snbogan/QG_Purp_RNA) such that offspring-parent relatedness equals 0.5, full-sibling relatedness equals 0.5, half sibling relatedness equals 0.25, and unrelated cohorts share a relatedness of 0.

651 Selection gradient models were fitted in brms v2.19.6 (Bürkner, 2017), an R interface to
652 the Bayesian programming language Stan (Carpenter et al., 2017). Models assumed uniform
653 priors, employed 40,000 MCMC iterations with a 5,000 iteration warm up, and a beta-distributed
654 generalized linear regression model family. Beta distribution was selected because the proportion
655 of normal development is constrained between 0 and 1. A Bayesian approach was selected for
656 fitting because of the flexibility of packages such as brms for accommodating relatedness matrices
657 within a beta distributed model family. These selection gradient models predicted whether positive
658 versus negative plasticity of larval body size and biomineralization promoted greater fitness under
659 upwelling stress. The significance of fitness effects were tested using probability of direction,
660 which determines whether the ≥ 95% of posterior distribution falls above or below 0 (Makowski
661 et al., 2019). Selection gradient coefficients were then multiplied with the phenotypic effects of
662 DE on plasticity for body size and biomineralization to calculate the associated fitness effect of
663 transcriptional plasticity.

664 *5.6 Estimating the heritability of gene expression and its plasticity* – V_A for gene expression
665 and DE were measured across all transcripts using animal models fit with the 'relmatLmer'
666 function of the R package lme4qtl v0.2.2 (Ziyatdinov et al., 2018). Within animal models, DE was
667 measured as the foldchange of gene expression across developmental environment for a given
668 cross. Mean-standardized CPM (gene expression) was predicted as a function of fixed effects for
669 developmental and parental environment, random effects for dam and sire, and a random effect for
670 cross identity. Genetic covariance between crosses was estimated using the relatedness matrix
671 described above. Mean-standardized DE fold changes were predicted using an identical animal
672 model lacking a fixed effect for developmental environment. h^2 was derived from each model as
673 the heritable proportion of total variance in gene expression or DE. Differences in h^2 of baseline

gene expression and gene expression were modeled transcriptome wide using a beta distributed glm fitted using the R package betareg v3.1-4 (Grün et al., 2012).

Covariation between DE's heritability and adaptive, phenotypic effects were modelled using three different approaches addressing the questions (i) 'Does the probability of heritability ($h^2 \geq 0.2$) vary as a function of DE's effect on adaptive plasticity in body size or biomineralization?', (ii) 'Does total heritability (h^2 as a continuous variable) vary as a function of DE's phenotypic effects?', and (iii) 'Does total heritability vary according to the fitness costs of DE's combined effect on plasticity in body size and biomineralization?'. Tests of questions i and ii were performed by modelling the probability of $h^2 \geq 0.2$ using binomial glm's. Continuous h^2 was modeled as a beta-distributed glm. Each model type fitted two continuous predictor variables for the phenotypic effect of DE on body size and biomineralization signed toward the adaptive direction of that effect under parental upwelling, which induced yielded fitness effects of plasticity as opposed to neutral effects under parental non-upwelling. Models of continuous h^2 set parameters for DE's phenotypic effects as a second order polynomial to accommodate non-linear variation in h^2 across the parameter space. For question iii, DE's total effect on adaptive plasticity was calculated as the summation of the SEM-predicted coefficient for DE of transcript *i*'s effect (E) on body size (S) and biomineralization (B) multiplied by the selection gradient (β) acting on each plastic trait under upwelling, such that adaptive plasticity associated with transcript $i = (E_{S,i} \times \beta_{S,i}) + (E_{B,i} \times \beta_{B,i})$. Covariance between DE h^2 and its total adaptive effect on plasticity was modeled using a beta-distributed glm in the R package betareg v3.1-4 (Grün et al., 2012).

5.7. Estimating G matrices – Genetic variance-covariance matrices of absolute logFC values for DE associated with adaptive plasticity in larval body size and biomineralization were estimated by fitting multivariate animal models representing all possible pairs of DEGs in each

group. Additive genetic variance in each absolute logFC trait and covariances between pairs were extracted from the multivariate models and assembled into *n*-by-*n* variance-covariance matrices, where *n* equals the number of DEGs associated with adaptive plasticity in body size or biomineralization. Genetic variance-covariance matrices were then converted to correlation matrices using the cov2corr() function of R 'stats' v4.2.2. The number of genetically-correlated modules of adaptive plasticity-associated DEGs was estimated from *G* matrices using hierarchical clustering of genetic distances (1 – genetic correlation). A distance threshold of < 0.5 was used to assign DEGs to their shared modules. Hierarchical clustering was performed using the hclust() function of R stats v4.2.2.

Variation in logFC *G* matrices between larvae of upwelling and non-upwelling parents was quantified. First, two multivariate animal models fitted with pedigrees of parental non-upwelling or parental upwelling families were fitted for all pairs of genes with DE associated with adaptive plasticity in larval body size or biomineralization. As described above, genetic (co)variances from each model were used to populate parental upwelling and non-upwelling *G* matrices which were converted to correlation matrices. Significant differences in correlation matrix structure between parental treatments were evaluated using a three-step approach. Two-sided Mantel tests were used to compare parental environment matrices, applying separate tests to logFC associated with adaptive plasticity in body size and adaptive biomineralization plasticity. Mantel tests were run using the mantel.test() function of the R package 'ape' v5.8 using 10,000 iterations (Paradis et al., 2004; Paradis & Schliep, 2019). Next, a chi-squared test was used to determine if the number of negative-to-positive changes in genetic correlation under upwelling relative to positive-to-negative changes was greater than expected by chance.

719 To determine whether significant enrichment in negative-to-positive changes in genetic
720 correlation was attributed to parental environment or the pedigree structure of populations in
721 parental treatment groups (i.e., differences in genetic (co)variance between animals used in each
722 treatment), random permutations of the experimental pedigree were constructed. These
723 permutations split the pedigree into two halves which evenly represented families of parental
724 treatment groups. Random permutations of split pedigrees were input into animal models and
725 resulting *G* matrices. Chi-squared tests of enrichment for directional changes in genetic correlation
726 between permuted matrices were performed, which determined the effect of pedigree structure on
727 apparent effects of parental environment on genetic correlations. In Results, enrichment of
728 directional changes in genetic correlation between parental environment are reported alongside the
729 percentage of these effects that were attributed to pedigree structure rather than treatment.
730
731 **Acknowledgements**
732 We thank Juliet Wong, Logan Kozal, Terence Leach, Jannine Chamorro, Maddie Housh,
733 and Olivia Simon for their assistance in adult conditioning and experimental culturing. We thank
734 Matthew Wolak, Katie Lotterhos, Steven Roberts, Yaamini Venkataraman, and Alan Downey-Wall
735 for his insights on quantitative genetic and transcriptomic analyses. This research was funded by
736 United States National Science Foundation awards IOS-1656262 and OPP-2053726 to GEH.
737 Diving and boating resources were provided by Santa Barbara Coastal Long Term Ecological
738 Research program (NSF award OCE-1831937; Director: Dr. Robert Miller).
739
740
741

Data availability

Raw RNA-seq data are available on the Short Read Archive under Bioproject PRJNA1169019. All phenotypic data, pedigree data, and code are hosted on the Github repository https://github.com/snbogan/QG_Purp_RNA. An archived copy of this Github repository is available on Zenodo under the DOI [will be produced upon acceptance].

Author Contributions

SNB, MS, and GEH conceived of the aims and scope of the study. MS led experimental design and phenotyping. GEH assisted SB assisted with experimental culturing. SB and MS assisted with seawater chemistry and phenotyping. SB performed RNA extractions, oversaw sequencing, bioinformatics, and all statistical analyses. SB wrote the manuscript. All authors edited and revised the manuscript.

Declarations

The authors declare no competing interests.

References

Acasuso-Rivero, C., Murren, C. J., Schlichting, C. D., & Steiner, U. K. (2019). Adaptive phenotypic plasticity for life-history and less fitness-related traits. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1904), 20190653. https://doi.org/10.1098/rspb.2019.0653

Agrawal, A. F., & Stinchcombe, J. R. (2008). How much do genetic covariances alter the rate of adaptation? *Proceedings of the Royal Society B: Biological Sciences*, *276*(1659), 1183–1191. https://doi.org/10.1098/rspb.2008.1671

Albert, B., Kos-Braun, I. C., Henras, A. K., Dez, C., Rueda, M. P., Zhang, X., Gadal, O., Kos, M., & Shore, D. (2019). A ribosome assembly stress response regulates transcription to maintain proteome homeostasis. *eLife*, *8*, e45002. https://doi.org/10.7554/eLife.45002

Andrews, S. R. (n.d.). *Babraham Bioinformatics—FastQC A Quality Control tool for High Throughput Sequence Data*. Retrieved July 7, 2023, from https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Arnold, P. A., Nicotra, A. B., & Kruuk, L. E. B. (2019). Sparse evidence for selection on phenotypic plasticity in response to temperature. *Philosophical Transactions of the Royal Society B*, *374*(1768), 20180185. https://doi.org/10.1098/rstb.2018.0185

Arnold, S. J., Pfrender, M. E., & Jones, A. G. (2001). The adaptive landscape as a conceptual bridge between micro- and macroevolution. *Genetica*, *112*(1), 9–32. https://doi.org/10.1023/A:1013373907708

Bitter, M. C., Wong, J. M., Dam, H. G., Donelan, S. C., Kenkel, C. D., Komoroske, L. M., Nickols, K. J., Rivest, E. B., Salinas, S., Burgess, S. C., & Lotterhos, K. E. (2021). Fluctuating selection and global change: A synthesis and review on disentangling the

roles of climate amplitude, predictability and novelty. *Proceedings of the Royal Society B*, *288*(1957), 20210727. https://doi.org/10.1098/rspb.2021.0727

Blows, M. W., Allen, S. L., Collet, J. M., Chenoweth, S. F., & McGuigan, K. (2015). The Phenome-Wide Distribution of Genetic Variance. *The American Naturalist*, *186*(1), 15–30. https://doi.org/10.1086/681645

Bogan, S. N., Porat, O. I., Meneses, M. J., & Hofmann, G. E. (2024). Thermal plasticity has higher fitness costs among thermally tolerant genotypes of Tigriopus californicus. *Functional Ecology*, *38*(7), 1562–1577. https://doi.org/10.1111/1365-2435.14568

Bogan, S. N., Strader, M. E., & Hofmann, G. E. (2023). Associations between DNA methylation and gene regulation depend on chromatin accessibility during transgenerational plasticity. *BMC Biol*. https://doi.org/10.1186/s12915-023-01645-8

Bogan, S. N., & Yi, S. V. (2024). Potential Role of DNA Methylation as a Driver of Plastic Responses to the Environment Across Cells, Organisms, and Populations. *Genome Biology and Evolution*, *16*(2), evae022. https://doi.org/10.1093/gbe/evae022

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bonamour, S., Chevin, L.-M., Charmantier, A., & Teplitsky, C. (2019). Phenotypic plasticity in response to climate change: The importance of cue variation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1768), 20180178. https://doi.org/10.1098/rstb.2018.0178

Bozinovic, F., & Pörtner, H.-O. (2015). Physiological ecology meets climate change. *Ecology and Evolution*, *5*(5), 1025–1030. https://doi.org/10.1002/ece3.1403

812 Brady, S. P., Bolnick, D. I., Angert, A. L., Gonzalez, A., Barrett, R. D. H., Crispo, E., Derry, A.
813 M., Eckert, C. G., Fraser, D. J., Fussmann, G. F., Guichard, F., Lamy, T., McAdam, A. G.,
814 Newman, A. E. M., Paccard, A., Rolshausen, G., Simons, A. M., & Hendry, A. P. (2019).
815 Causes of maladaptation. *Evolutionary Applications*, *12*(7), 1229–1242.
816 https://doi.org/10.1111/eva.12844
817 Brennan, R. S., deMayo, J. A., Dam, H. G., Finiguerra, M. B., Baumann, H., & Pespeni, M. H.
818 (2022). Loss of transcriptional plasticity but sustained adaptive capacity after adaptation
819 to global change conditions in a marine copepod. *Nature Communications*, *13*(1), 1147.
820 https://doi.org/10.1038/s41467-022-28742-6
821 Bürkner, P.-C. (2017). brms: An R package for Bayesian multilevel models using Stan. *Journal*
822 *of Statistical Software*, *80*, 1–28. https://doi.org/10.18637/jss.v080.i01
823 Burt, A. (1995). The Evolution of Fitness. *Evolution*, *49*(1), 1–8. https://doi.org/10.1111/j.1558-
824 5646.1995.tb05954.x
825 Campbell-Staton, S. C., Velotta, J. P., & Winchell, K. M. (2021). Selection on adaptive and
826 maladaptive gene expression plasticity during thermal adaptation to urban heat islands.
827 *Nature Communications*, *12*(1), Article 1. https://doi.org/10.1038/s41467-021-26334-4
828 Carpenter, B., Gelman, A., Hoffman, M. D., Lee, D., Goodrich, B., Betancourt, M., Brubaker,
829 M., Guo, J., Li, P., & Riddell, A. (2017). Stan: A Probabilistic Programming Language.
830 *Journal of Statistical Software*, *76*, 1–32. https://doi.org/10.18637/jss.v076.i01
831 Chamorro, J. D., Macdonald, A. M., & Hofmann, G. E. (2023). Transgenerational plasticity as a
832 mechanism of response to marine heatwaves in the purple sea urchin, *Strongylocentrotus*
833 *purpuratus*. *Frontiers in Marine Science*, *10*. https://doi.org/10.3389/fmars.2023.1212781

834 Chan, F., Barth, J. A., Blanchette, C. A., Byrne, R. H., Chavez, F., Cheriton, O., Feely, R. A.,
835 Friederich, G., Gaylord, B., & Gouhier, T. (2017). Persistent spatial structuring of coastal
836 ocean acidification in the California Current System. *Scientific Reports*, *7*(1), 2526.
837 Chevin, L.-M., & Hoffmann, A. A. (2017). Evolution of phenotypic plasticity in extreme
838 environments. *Philosophical Transactions of the Royal Society B: Biological Sciences*,
839 *372*(1723), 20160138. https://doi.org/10.1098/rstb.2016.0138
840 Corl, A., Bi, K., Luke, C., Challa, A. S., Stern, A. J., Sinervo, B., & Nielsen, R. (2018). The
841 Genetic Basis of Adaptation following Plastic Changes in Coloration in a Novel
842 Environment. *Current Biology*, *28*(18), 2970-2977.e7.
843 https://doi.org/10.1016/j.cub.2018.06.075
844 Crispo, E. (2007). THE BALDWIN EFFECT AND GENETIC ASSIMILATION: REVISITING
845 TWO MECHANISMS OF EVOLUTIONARY CHANGE MEDIATED BY
846 PHENOTYPIC PLASTICITY. *Evolution*, *61*(11), 2469–2479.
847 https://doi.org/10.1111/j.1558-5646.2007.00203.x
848 de Miguel, M., Rodríguez-Quilón, I., Heuertz, M., Hurel, A., Grivet, D., Jaramillo-Correa, J. P.,
849 Vendramin, G. G., Plomion, C., Majada, J., Alía, R., Eckert, A. J., & González-Martínez,
850 S. C. (2022). Polygenic adaptation and negative selection across traits, years and
851 environments in a long-lived plant species (Pinus pinaster Ait., Pinaceae). *Molecular*
852 *Ecology*, *31*(7), 2089–2105. https://doi.org/10.1111/mec.16367
853 De, S., & Mühlemann, O. (2022). A comprehensive coverage insurance for cells: Revealing links
854 between ribosome collisions, stress responses and mRNA surveillance. *RNA Biology*,
855 *19*(1), 609–621. https://doi.org/10.1080/15476286.2022.2065116

856 Donelan, S. C., Hellmann, J. K., Bell, A. M., Luttbeg, B., Orrock, J. L., Sheriff, M. J., & Sih, A.
857 (2020). Transgenerational Plasticity in Human-Altered Environments. *Trends in Ecology*
858 *& Evolution*, *35*(2), 115–124. https://doi.org/10.1016/j.tree.2019.09.003
859 Donelson, J. M., Gaitan-Espitia, J. D., Hobday, A. J., Mokany, K., Andrew, S. C., Boulter, S.,
860 Cook, C. N., Dickson, F., Macgregor, N. A., Mitchell, N. J., Pickup, M., & Fox, R. J.
861 (2023). Putting plasticity into practice for effective conservation actions under climate
862 change. *Nature Climate Change*, *13*(7), Article 7. https://doi.org/10.1038/s41558-023-
863 01706-4
864 Donelson, J. M., Salinas, S., Munday, P. L., & Shama, L. N. S. (2018). Transgenerational
865 plasticity and climate change experiments: Where do we go from here? *Glob. Chang.*
866 *Biol.*, *24*(1), 13–34.
867 Dore, A. A., McDowall, L., Rouse, J., Bretman, A., Gage, M. J. G., & Chapman, T. (2018). The
868 role of complex cues in social and reproductive plasticity. *Behavioral Ecology and*
869 *Sociobiology*, *72*(8), 124. https://doi.org/10.1007/s00265-018-2539-x
870 Durmowicz, A. G., Hofmeister, S., Kadyraliev, T. K., Aldashev, A. A., & Stenmark, K. R. (1993).
871 Functional and structural adaptation of the yak pulmonary circulation to residence at high
872 altitude. *Journal of Applied Physiology*, *74*(5), 2276–2285.
873 https://doi.org/10.1152/jappl.1993.74.5.2276
874 Edmands, S., Moberg, P. E., & Burton, R. S. (1996). Allozyme and mitochondrial DNA evidence
875 of population subdivision in the purple sea urchin Strongylocentrotus purpuratus. *Marine*
876 *Biology*, *126*(3), 443–450. https://doi.org/10.1007/BF00354626

877 Evans, T. G., Chan, F., Menge, B. A., & Hofmann, G. E. (2013). Transcriptomic responses to
878 ocean acidification in larval sea urchins from a naturally variable pH environment. *Mol.*
879 *Ecol.*, *22*(6), 1609–1625.
880 Evans, T. G., & Hofmann, G. E. (2012). Defining the limits of physiological plasticity: How
881 gene expression can assess and predict the consequences of ocean change. *Philosophical*
882 *Transactions of the Royal Society B: Biological Sciences*, *367*(1596), 1733–1745.
883 https://doi.org/10.1098/rstb.2012.0019
884 Fangue, N. A., O'Donnell, M. J., Sewell, M. A., Matson, P. G., MacPherson, A. C., & Hofmann,
885 G. E. (2010). A laboratory-based, experimental system for the study of ocean
886 acidification effects on marine invertebrate larvae. *Limnol. Oceanogr. Methods*, *8*(8),
887 441–452.
888 Feder, M. E., & Hofmann, G. E. (1999). HEAT-SHOCK PROTEINS, MOLECULAR
889 CHAPERONES, AND THE STRESS RESPONSE: Evolutionary and Ecological
890 Physiology. *Annual Review of Physiology*, *61*(1), 243–282.
891 https://doi.org/10.1146/annurev.physiol.61.1.243
892 Fischer, E. K., Ghalambor, C. K., & Hoke, K. L. (2016). Plasticity and evolution in correlated
893 suites of traits. *Journal of Evolutionary Biology*, *29*(5), 991–1002.
894 https://doi.org/10.1111/jeb.12839
895 Fox, R. J., Donelson, J. M., Schunter, C., Ravasi, T., & Gaitán-Espitia, J. D. (2019). Beyond
896 buying time: The role of plasticity in phenotypic adaptation to rapid environmental
897 change. *Philosophical Transactions of the Royal Society B: Biological Sciences*,
898 *374*(1768), 20180174. https://doi.org/10.1098/rstb.2018.0174

899 Freed, E. F., Bleichert, F., Dutca, L. M., & Baserga, S. J. (2010). When ribosomes go bad:
900 Diseases of ribosome biogenesis. *Molecular BioSystems*, *6*(3), 481–493.
901 https://doi.org/10.1039/B919670F
902 Ghalambor, C. K., McKAY, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-
903 adaptive phenotypic plasticity and the potential for contemporary adaptation in new
904 environments. *Functional Ecology*, *21*(3), 394–407. https://doi.org/10.1111/j.1365-
905 2435.2007.01283.x
906 Grishkevich, V., & Yanai, I. (2013). The genomic determinants of genotype × environment
907 interactions in gene expression. *Trends in Genetics*, *29*(8), 479–487.
908 https://doi.org/10.1016/j.tig.2013.05.006
909 Gruber, N., Hauri, C., Lachkar, Z., Loher, D., Frölicher, T. L., & Plattner, G.-K. (2012). Rapid
910 Progression of Ocean Acidification in the California Current System. *Science*, *337*(6091),
911 220–223. https://doi.org/10.1126/science.1216773
912 Grün, B., Kosmidis, I., & Zeileis, A. (2012). Extended Beta Regression in R: Shaken, Stirred,
913 Mixed, and Partitioned. *Journal of Statistical Software*, *48*, 1–25.
914 https://doi.org/10.18637/jss.v048.i11
915 Hallsson, L. R., & Björklund, M. (2012). Selection in a fluctuating environment leads to
916 decreased genetic variation and facilitates the evolution of phenotypic plasticity. *Journal*
917 *of Evolutionary Biology*, *25*(7), 1275–1290. https://doi.org/10.1111/j.1420-
918 9101.2012.02512.x
919 Hangartner, S., Sgrò, C. M., Connallon, T., & Booksmythe, I. (2022). Sexual dimorphism in
920 phenotypic plasticity and persistence under environmental change: An extension of

theory and meta-analysis of current data. *Ecology Letters*, *25*(6), 1550–1565. https://doi.org/10.1111/ele.14005

Hansen, T. F., Carter, A. J. R., & Pélabon, C. (2006). On Adaptive Accuracy and Precision in Natural Populations. *The American Naturalist*, *168*(2), 168–181. https://doi.org/10.1086/505768

He, F., Steige, K. A., Kovacova, V., Göbel, U., Bouzid, M., Keightley, P. D., Beyer, A., & de Meaux, J. (2021). Cis-regulatory evolution spotlights species differences in the adaptive potential of gene expression plasticity. *Nature Communications*, *12*(1), 3376. https://doi.org/10.1038/s41467-021-23558-2

Hekerman, P., Zeidler, J., Bamberg-Lemper, S., Knobelspies, H., Lavens, D., Tavernier, J., Joost, H.-G., & Becker, W. (2005). Pleiotropy of leptin receptor signalling is defined by distinct roles of the intracellular tyrosines. *The FEBS Journal*, *272*(1), 109–119. https://doi.org/10.1111/j.1432-1033.2004.04391.x

Hendry, A. P. (2016). Key questions on the role of phenotypic plasticity in eco-evolutionary dynamics. *Journal of Heredity*, *107*(1), 25–41. https://doi.org/10.1093/jhered/esv060

Hoffmann, A. A., Merilä, J., Hoffmann, A. A., & Merilä, J. (1999). Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology & Evolution*, *14*(3), 96–101. https://doi.org/10.1016/S0169-5347(99)01595-5

Hofmann, G. E., & Todgham, A. E. (2010). Living in the Now: Physiological Mechanisms to Tolerate a Rapidly Changing Environment. *Annual Review of Physiology*, *72*(1), 127–145. https://doi.org/10.1146/annurev-physiol-021909-135900

Huang, W., Carbone, M. A., Lyman, R. F., Anholt, R. R. H., & Mackay, T. F. C. (2020). Genotype by environment interaction for gene expression in Drosophila melanogaster. *Nature Communications*, *11*(1), 5451. https://doi.org/10.1038/s41467-020-19131-y

Huyer, A. (1983). Coastal upwelling in the California current system. *Progress in Oceanography*, *12*(3), 259–284. https://doi.org/10.1016/0079-6611(83)90010-1

Iordanov, M. S., Pribnow, D., Magun, J. L., Dinh, T.-H., Pearson, J. A., & Magun, B. E. (1998). Ultraviolet Radiation Triggers the Ribotoxic Stress Response in Mammalian Cells *. *Journal of Biological Chemistry*, *273*(25), 15794–15803. https://doi.org/10.1074/jbc.273.25.15794

Jong, G. de, & Gavrilets, S. (2000). Maintenance of genetic variation in phenotypic plasticity: The role of environmental variation. *Genetics Research*, *76*(3), 295–304. https://doi.org/10.1017/S0016672300004729

Kauffmann, A., Gentleman, R., & Huber, W. (2009). arrayQualityMetrics—A bioconductor package for quality assessment of microarray data. *Bioinformatics*, *25*(3), 415–416. https://doi.org/10.1093/bioinformatics/btn647

Kelly, M. W. (2019). Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1768), 20180176. https://doi.org/10.1098/rstb.2018.0176

Kelly, M. W., Padilla-Gamiño, J. L., & Hofmann, G. E. (2013). Natural variation and the capacity to adapt to ocean acidification in the keystone sea urchin *Strongylocentrotus purpuratus*. *Glob. Chang. Biol.*, *19*(8), 2536–2546.

963 Kim, D., Paggi, J. M., Park, C., Bennett, C., & Salzberg, S. L. (2019). Graph-based genome
964 alignment and genotyping with HISAT2 and HISAT-genotype. *Nature Biotechnology*,
965 *37*(8), Article 8. https://doi.org/10.1038/s41587-019-0201-4
966 Kim, S.-Y., Metcalfe, N. B., & Velando, A. (2016). A benign juvenile environment reduces the
967 strength of antagonistic pleiotropy and genetic variation in the rate of senescence.
968 *Journal of Animal Ecology*, *85*(3), 705–714. https://doi.org/10.1111/1365-2656.12468
969 Kingsolver, J. G., & Gomulkiewicz, R. (2003). Environmental Variation and Selection on
970 Performance Curves1. *Integrative and Comparative Biology*, *43*(3), 470–477.
971 https://doi.org/10.1093/icb/43.3.470
972 Kline, R. B. (2015). *Principles and practice of structural equation modeling, fourth edition*.
973 Guilford Publications.
974 Kovuri, P., Yadav, A., & Sinha, H. (2023). Role of genetic architecture in phenotypic plasticity.
975 *Trends in Genetics*, *0*(0). https://doi.org/10.1016/j.tig.2023.04.002
976 Lande, R., & Arnold, S. J. (1983). The measurement of selection on correlated characters.
977 *Evolution*, *37*(6), 1210–1226. https://doi.org/10.2307/2408842
978 Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G.,
979 Durbin, R., & 1000 Genome Project Data Processing Subgroup. (2009). The Sequence
980 Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079.
981 https://doi.org/10.1093/bioinformatics/btp352
982 Li, X., Guo, T., Mu, Q., Li, X., & Yu, J. (2018). Genomic and environmental determinants and
983 their interplay underlying phenotypic plasticity. *Proceedings of the National Academy of*
984 *Sciences*, *115*(26), 6679–6684. https://doi.org/10.1073/pnas.1718326115

985 Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: An efficient general purpose program
986 for assigning sequence reads to genomic features. *Bioinformatics*, *30*(7), 923–930.
987 https://doi.org/10.1093/bioinformatics/btt656
988 Liu, N., Du, Y., Warburton, M. L., Xiao, Y., & Yan, J. (2021). Phenotypic Plasticity Contributes
989 to Maize Adaptation and Heterosis. *Molecular Biology and Evolution*, *38*(4), 1262–1275.
990 https://doi.org/10.1093/molbev/msaa283
991 Lynn, R., & Simpson, J. (1987). The California Current System—The Seasonal Variability of Its
992 Physical Characteristics. *JOURNAL OF GEOPHYSICAL RESEARCH-OCEANS*,
993 *92*(C12), 12947-. https://doi.org/10.1029/JC092iC12p12947
994 Makowski, D., Ben-Shachar, M. S., Chen, S. H. A., & Lüdecke, D. (2019). Indices of Effect
995 Existence and Significance in the Bayesian Framework. *Frontiers in Psychology*, *10*.
996 https://www.frontiersin.org/articles/10.3389/fpsyg.2019.02767
997 Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads.
998 *EMBnet.Journal*, *17*(1), Article 1. https://doi.org/10.14806/ej.17.1.200
999 Martyniuk, C. J. (2020). Perspectives on transcriptomics in animal physiology studies.
1000 *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*,
1001 *250*, 110490. https://doi.org/10.1016/j.cbpb.2020.110490
1002 Mauro, A. A., & Ghalambor, C. K. (2020). Trade-offs, Pleiotropy, and Shared Molecular
1003 Pathways: A Unified View of Constraints on Adaptation. *Integrative and Comparative*
1004 *Biology*, *60*(2), 332–347. https://doi.org/10.1093/icb/icaa056
1005 McCairns, R. J. S., Smith, S., Sasaki, M., Bernatchez, L., & Beheregaray, L. B. (2016). The
1006 adaptive potential of subtropical rainbowfish in the face of climate change: Heritability

and heritable plasticity for the expression of candidate genes. *Evolutionary Applications*, *9*(4), 531–545. https://doi.org/10.1111/eva.12363

Million, W. C., Ruggeri, M., O'Donnell, S., Bartels, E., Conn, T., Krediet, C. J., & Kenkel, C. D. (2022). Evidence for adaptive morphological plasticity in the Caribbean coral, *Acropora cervicornis*. *Proceedings of the National Academy of Sciences*, *119*(49), e2203925119. https://doi.org/10.1073/pnas.2203925119

Morris, M. R. J., & Rogers, S. M. (2013). Overcoming maladaptive plasticity through plastic compensation. *Current Zoology*, *59*(4), 526–536. https://doi.org/10.1093/czoolo/59.4.526

Nguyen Ba, A. N., Lawrence, K. R., Rego-Costa, A., Gopalakrishnan, S., Temko, D., Michor, F., & Desai, M. M. (2022). Barcoded bulk QTL mapping reveals highly polygenic and epistatic architecture of complex traits in yeast. *eLife*, *11*, e73983. https://doi.org/10.7554/eLife.73983

Oostra, V., Saastamoinen, M., Zwaan, B. J., & Wheat, C. W. (2018). Strong phenotypic plasticity limits potential for evolutionary responses to climate change. *Nature Communications*, *9*(1), Article 1. https://doi.org/10.1038/s41467-018-03384-9

Ordas, B., Malvar, R. A., & Hill, W. G. (2008). Genetic variation and quantitative trait loci associated with developmental stability and the environmental correlation between traits in maize. *Genetics Research*, *90*(5), 385–395. https://doi.org/10.1017/S0016672308009762

Orton, T. J. (2020). Chapter 3—Review of Genetics (From The Perspective of A Plant Breeder). In T. J. Orton (Ed.), *Horticultural Plant Breeding* (pp. 39–71). Academic Press. https://doi.org/10.1016/B978-0-12-815396-3.00003-2

1029 Palumbi, S. R., & Wilson, A. C. (1990). Mitochondrial Dna Diversity in the Sea Urchins
1030 Strongylocentrotus Purpuratus and S. Droebachiensis. *Evolution*, *44*(2), 403–415.
1031 https://doi.org/10.1111/j.1558-5646.1990.tb05208.x
1032 Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of Phylogenetics and Evolution in
1033 R language. *Bioinformatics*, *20*(2), 289–290.
1034 https://doi.org/10.1093/bioinformatics/btg412
1035 Paradis, E., & Schliep, K. (2019). ape 5.0: An environment for modern phylogenetics and
1036 evolutionary analyses in R. *Bioinformatics*, *35*(3), 526–528.
1037 https://doi.org/10.1093/bioinformatics/bty633
1038 Pearse, J. S. (2006). Ecological role of purple sea urchins. *Science*, *314*(5801), 940–941.
1039 https://doi.org/10.1126/science.1131888
1040 Pespeni, M. H., Chan, F., Menge, B. A., & Palumbi, S. R. (2013). Signs of adaptation to local pH
1041 conditions across an environmental mosaic in the California Current Ecosystem. *Integr.*
1042 *Comp. Biol.*, *53*(5), 857–870.
1043 Pespeni, M. H., & Palumbi, S. R. (2013). Signals of selection in outlier loci in a widely
1044 dispersing species across an environmental mosaic. *Mol. Ecol.*, *22*(13), 3580–3597.
1045 Petino Zappala, M. A., Mensch, J., Carreira, V., Soto, I., & Fanara, J. J. (2024). Genome Wide
1046 Association Studies of Early Fitness Traits in Drosophila melanogaster Unveil Plasticity
1047 and Decoupling of Different Aspects of Phenotype. *Evolutionary Biology*, *51*(1), 69–81.
1048 https://doi.org/10.1007/s11692-023-09619-y
1049 Price, T., & Langen, T. (1992). Evolution of correlated characters. *Trends in Ecology &*
1050 *Evolution*, *7*(9), 307–310. https://doi.org/10.1016/0169-5347(92)90229-5

1051 Quilfen, Y., Shutler, J., Piolle, J.-F., & Autret, E. (2021). Recent trends in the wind-driven
1052 California current upwelling system. *Remote Sensing of Environment*, *261*, 112486.
1053 https://doi.org/10.1016/j.rse.2021.112486
1054 Renaud, L.-A., Festa-Bianchet, M., & Pelletier, F. (2022). Testing the match–mismatch
1055 hypothesis in bighorn sheep in the context of climate change. *Global Change Biology*,
1056 *28*(1), 21–32. https://doi.org/10.1111/gcb.15923
1057 Rivera, H. E., Aichelman, H. E., Fifer, J. E., Kriefall, N. G., Wuitchik, D. M., Smith, S. J., &
1058 Davies, S. W. (2021). A framework for understanding gene expression plasticity and its
1059 influence on stress tolerance. *Molecular Ecology*, *30*(6), 1381–1397.
1060 https://doi.org/10.1111/mec.15820
1061 Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: a Bioconductor package for
1062 differential expression analysis of digital gene expression data. *Bioinformatics*, *26*(1),
1063 139–140.
1064 Rogers-Bennett, L., & Catton, C. A. (2019). Marine heat wave and multiple stressors tip bull
1065 kelp forest to sea urchin barrens. *Scientific Reports*, *9*(1), Article 1.
1066 https://doi.org/10.1038/s41598-019-51114-y
1067 Schaum, C.-E., Buckling, A., Smirnoff, N., & Yvon-Durocher, G. (2022). Evolution of thermal
1068 tolerance and phenotypic plasticity under rapid and slow temperature fluctuations. *Proc.*
1069 *Biol. Sci.*, *289*(1980), 20220834.
1070 Scheiner, S. M., & Lyman, R. F. (1991). The genetics of phenotypic plasticity. II. Response to
1071 selection. *Journal of Evolutionary Biology*, *4*(1), 23–50. https://doi.org/10.1046/j.1420-
1072 9101.1991.4010023.x

1073 Schneider, H. M. (2022). Characterization, costs, cues and future perspectives of phenotypic
1074 plasticity. *Annals of Botany*, *130*(2), 131–148. https://doi.org/10.1093/aob/mcac087
1075 Schneider, R. F., & Meyer, A. (2017). How plasticity, genetic assimilation and cryptic genetic
1076 variation may contribute to adaptive radiations. *Molecular Ecology*, *26*(1), 330–350.
1077 https://doi.org/10.1111/mec.13880
1078 Sgrò, C. M., & Hoffmann, A. A. (2004). Genetic correlations, tradeoffs and environmental
1079 variation. *Heredity*, *93*(3), 241–248. https://doi.org/10.1038/sj.hdy.6800532
1080 Siegal, M. L., & Bergman, A. (2002). Waddington's canalization revisited: Developmental
1081 stability and evolution. *Proceedings of the National Academy of Sciences*, *99*(16), 10528–
1082 10532. https://doi.org/10.1073/pnas.102303999
1083 Smale, D. A. (2020). Impacts of ocean warming on kelp forest ecosystems. *New Phytologist*,
1084 *225*(4), 1447–1454. https://doi.org/10.1111/nph.16107
1085 Sodergren, E., Weinstock, G. M., Davidson, E. H., Cameron, R. A., Gibbs, R. A., Angerer, R. C.,
1086 Angerer, L. M., Arnone, M. I., Burgess, D. R., Burke, R. D., Coffman, J. A., Dean, M.,
1087 Elphick, M. R., Ettensohn, C. A., Foltz, K. R., Hamdoun, A., Hynes, R. O., Klein, W. H.,
1088 Marzluff, W., … Wright, R. (2006). The Genome of the Sea Urchin Strongylocentrotus
1089 purpuratus. *Science*, *314*(5801), 941–952. https://doi.org/10.1126/science.1133609
1090 Steppan, S. J., Phillips, P. C., & Houle, D. (2002). Comparative quantitative genetics: Evolution
1091 of the G matrix. *Trends in Ecology & Evolution*, *17*(7), 320–327.
1092 https://doi.org/10.1016/S0169-5347(02)02505-3
1093 Strader, M. E., Kozal, L. C., Leach, T. S., Wong, J. M., Chamorro, J. D., Housh, M. J., &
1094 Hofmann, G. E. (2020). Examining the role of DNA methylation in transcriptomic
1095 plasticity of early stage sea urchins: Developmental and maternal effects in a kelp forest

herbivore. In *Frontiers in Marine Science* (Vol. 7, p. 205). https://doi.org/10.3389/fmars.2020.00205

Strader, M. E., Wolak, M. E., Simon, O. M., & Hofmann, G. E. (2022). Genetic variation underlies plastic responses to global change drivers in the purple sea urchin, *Strongylocentrotus purpuratus*. *Proceedings. Biological Sciences*, *289*(1981), 20221249. https://doi.org/10.1098/rspb.2022.1249

Strader, M. E., Wong, J. M., Kozal, L. C., Leach, T. S., & Hofmann, G. E. (2019). Parental environments alter DNA methylation in offspring of the purple sea urchin, *Strongylocentrotus purpuratus*. *J. Exp. Mar. Bio. Ecol.*, *517*, 54–64.

Svensson, E. I., Gomez-Llano, M., & Waller, J. T. (2020). Selection on phenotypic plasticity favors thermal canalization. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(47), 29767–29774. https://doi.org/10.1073/pnas.2012454117

Tingley, D., Yamamoto, T., Hirose, K., Keele, L., & Imai, K. (2014). mediation: R package for causal mediation analysis. *UCLA Statistics/American Statistical Association*. https://dspace.mit.edu/handle/1721.1/91154

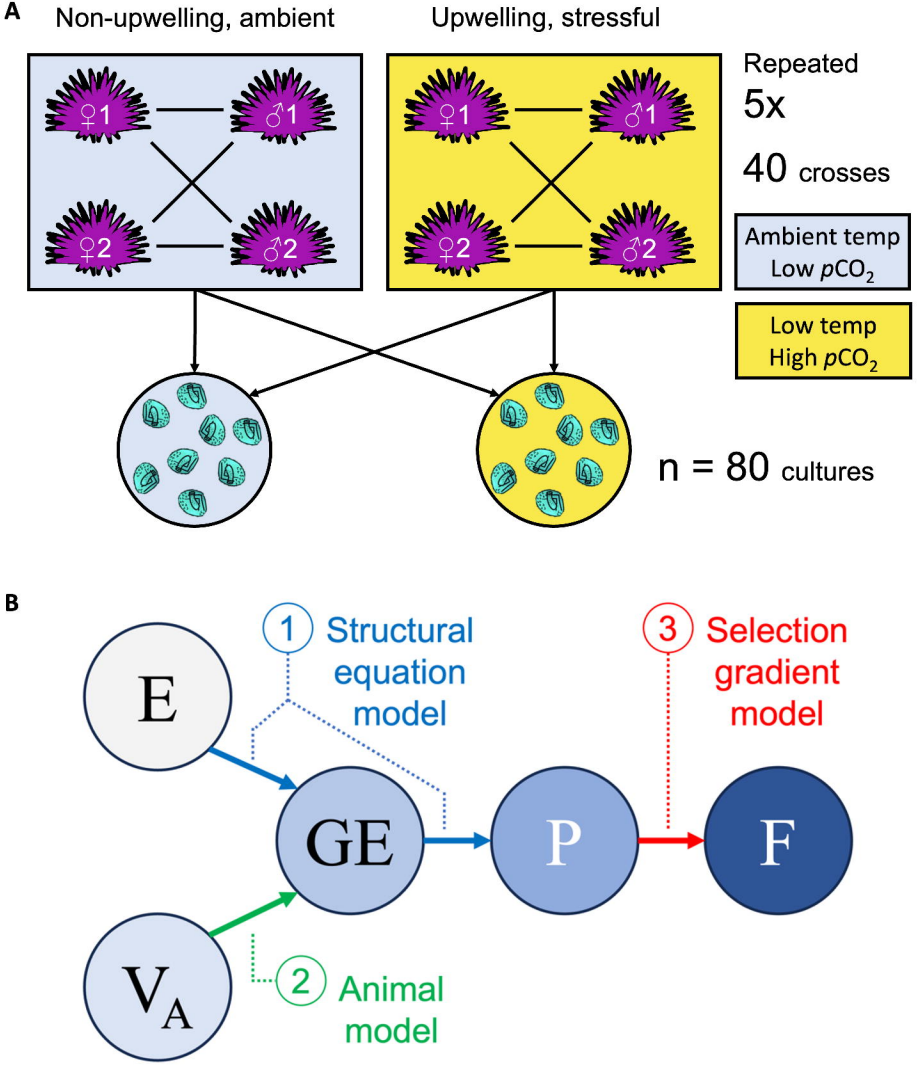
Valladares, F., Gianoli, E., & Gómez, J. M. (2007). Ecological limits to plant phenotypic plasticity. *New Phytologist*, *176*(4), 749–763. https://doi.org/10.1111/j.1469-8137.2007.02275.x

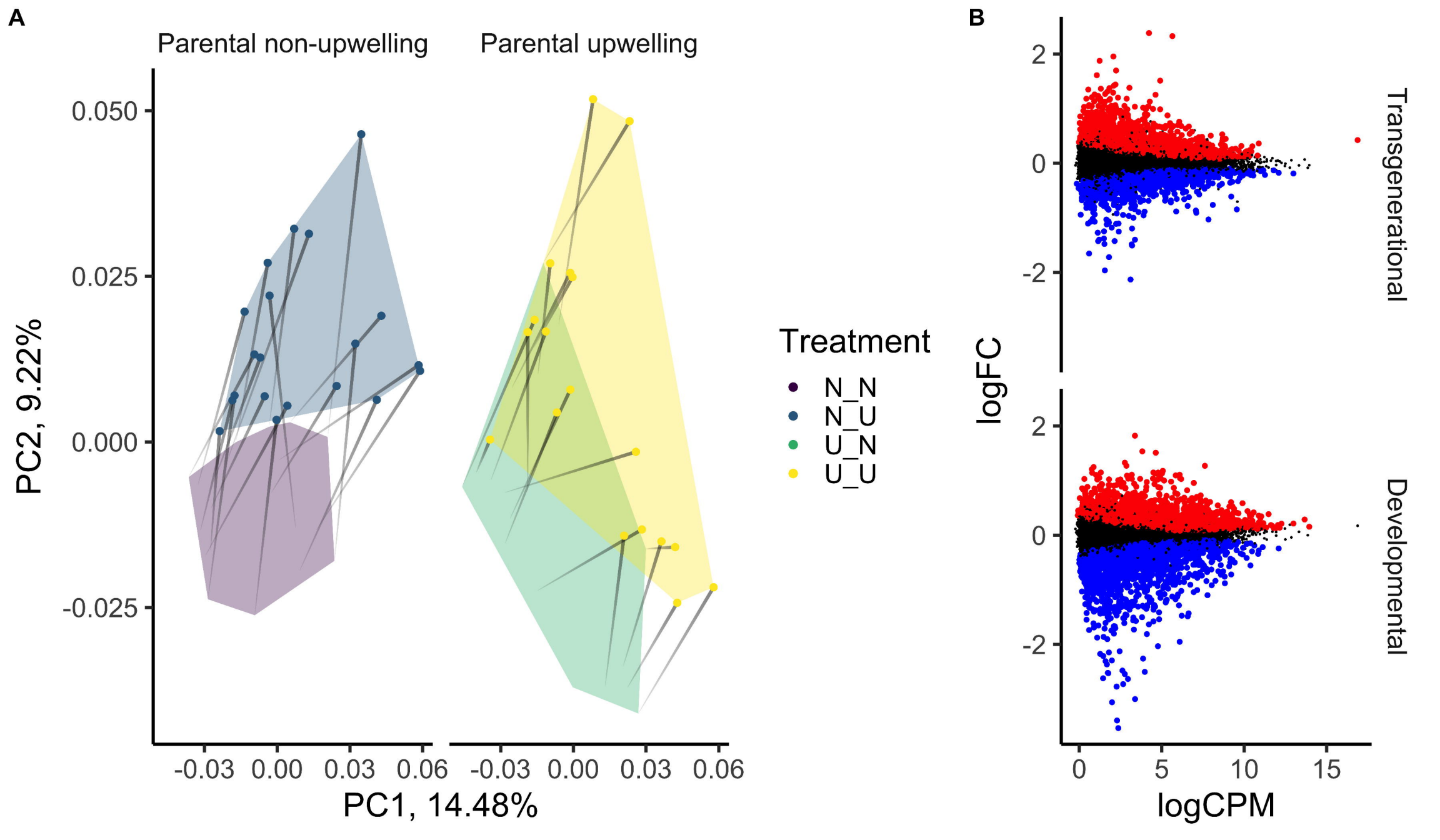
Van ASCH, M., Van TIENDEREN, P. H., Holleman, L. J. M., & Visser, M. E. (2007). Predicting adaptation of phenology in response to climate change, an insect herbivore example. *Global Change Biology*, *13*(8), 1596–1604. https://doi.org/10.1111/j.1365-2486.2007.01400.x

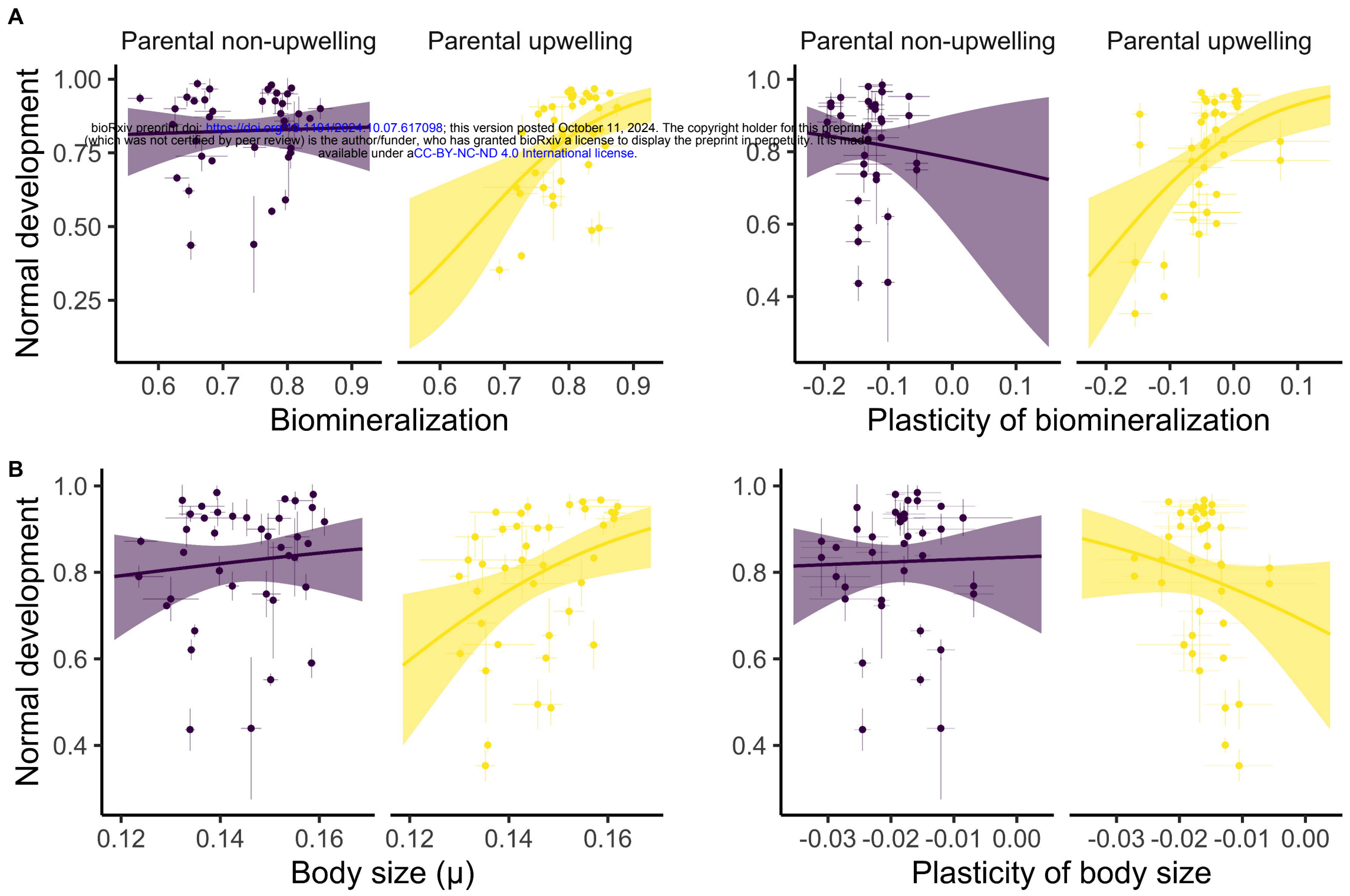
1119 Van Buskirk, J., & Steiner, U. K. (2009). The fitness costs of developmental canalization and
1120 plasticity. *Journal of Evolutionary Biology*, *22*(4), 852–860.
1121 https://doi.org/10.1111/j.1420-9101.2009.01685.x
1122 van Heerwaarden, B., & Kellermann, V. (2020). Does plasticity trade off with basal heat
1123 tolerance? *Trends in Ecology & Evolution*, *35*(10), 874–885.
1124 https://doi.org/10.1016/j.tree.2020.05.006
1125 Velotta, J. P., & Cheviron, Z. A. (2018). Remodeling Ancestral Phenotypic Plasticity in Local
1126 Adaptation: A New Framework to Explore the Role of Genetic Compensation in the
1127 Evolution of Homeostasis. *Integrative and Comparative Biology*, *58*(6), 1098–1110.
1128 https://doi.org/10.1093/icb/icy117
1129 Velotta, J. P., Ivy, C. M., Wolf, C. J., Scott, G. R., & Cheviron, Z. A. (2018). Maladaptive
1130 phenotypic plasticity in cardiac muscle growth is suppressed in high-altitude deer mice.
1131 *Evolution; International Journal of Organic Evolution*, *72*(12), 2712–2727.
1132 https://doi.org/10.1111/evo.13626
1133 Vinton, A. C., Gascoigne, S. J. L., Sepil, I., & Salguero-Gómez, R. (2022). Plasticity's role in
1134 adaptive evolution depends on environmental change components. *Trends in Ecology &*
1135 *Evolution*, *37*(12), 1067–1078. https://doi.org/10.1016/j.tree.2022.08.008
1136 Weinig, C. (2000). Plasticity Versus Canalization: Population Differences in the Timing of
1137 Shade-Avoidance Responses. *Evolution*, *54*(2), 441–451. https://doi.org/10.1111/j.0014-
1138 3820.2000.tb00047.x
1139 West-Eberhard, M. J. (2003). *Developmental Plasticity and Evolution*. Oxford University Press.

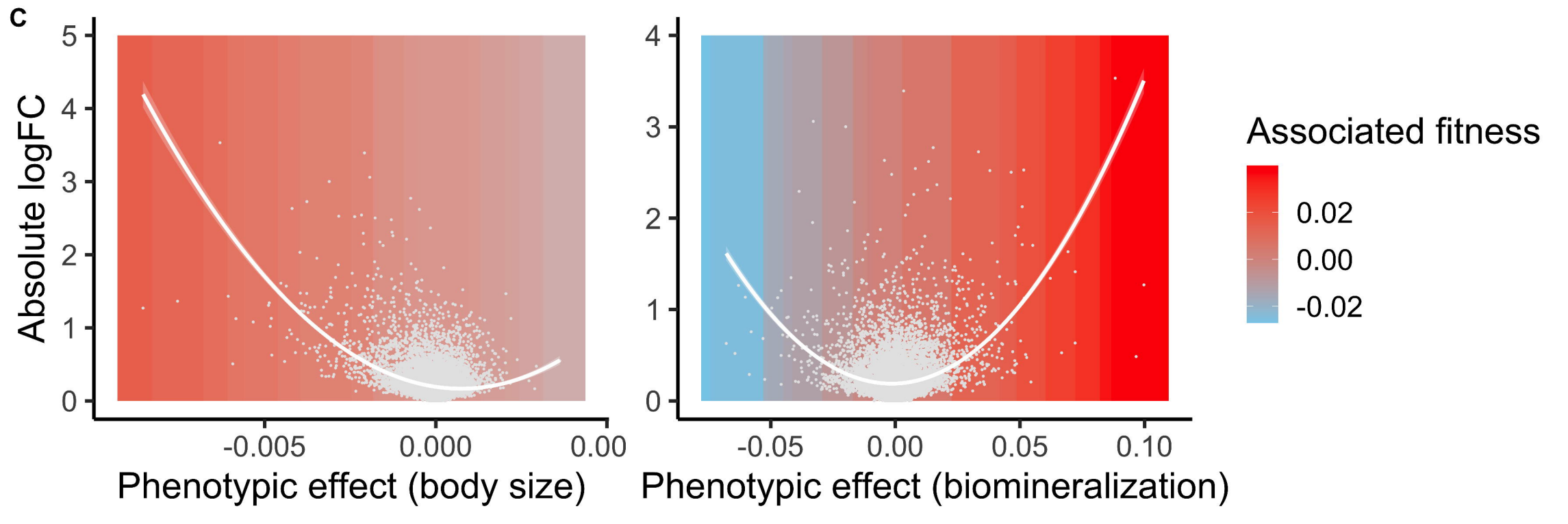
1140 Wilson, A. J., Réale, D., Clements, M. N., Morrissey, M. M., Postma, E., Walling, C. A., Kruuk,
1141 L. E. B., & Nussey, D. H. (2010). An ecologist's guide to the animal model. *Journal of*
1142 *Animal Ecology*, *79*(1), 13–26. https://doi.org/10.1111/j.1365-2656.2009.01639.x
1143 Wong, J. M., Johnson, K. M., Kelly, M. W., & Hofmann, G. E. (2018). Transcriptomics reveal
1144 transgenerational effects in purple sea urchin embryos: Adult acclimation to upwelling
1145 conditions alters the response of their progeny to differential pCO2 levels. *Mol. Ecol.*,
1146 *27*(5), 1120–1137.
1147 Wong, J. M., Kozal, L. C., Leach, T. S., Hoshijima, U., & Hofmann, G. E. (2019).
1148 Transgenerational effects in an ecological context: Conditioning of adult sea urchins to
1149 upwelling conditions alters maternal provisioning and progeny phenotype. *J. Exp. Mar.*
1150 *Bio. Ecol.*, *517*, 65–77.
1151 Wood, C. W., & Brodie, E. D., III. (2015). Environmental effects on the structure of the G-
1152 matrix. *Evolution*, *69*(11), 2927–2940. https://doi.org/10.1111/evo.12795
1153 Wright, R. M., Aglyamova, G. V., Meyer, E., & Matz, M. V. (2015). Gene expression associated
1154 with white syndromes in a reef building coral, Acropora hyacinthus. *BMC Genomics*, *16*,
1155 371.
1156 Xiu, P., Chai, F., Curchitser, E. N., & Castruccio, F. S. (2018). Future changes in coastal
1157 upwelling ecosystems with global warming: The case of the California Current System.
1158 *Scientific Reports*, *8*(1), Article 1. https://doi.org/10.1038/s41598-018-21247-7
1159 Zaytsev, O., Cervantes-Duarte, R., Montante, O., & Gallegos-Garcia, A. (2003). Coastal
1160 Upwelling Activity on the Pacific Shelf of the Baja California Peninsula. *Journal of*
1161 *Oceanography*, *59*(4), 489–502. https://doi.org/10.1023/A:1025544700632

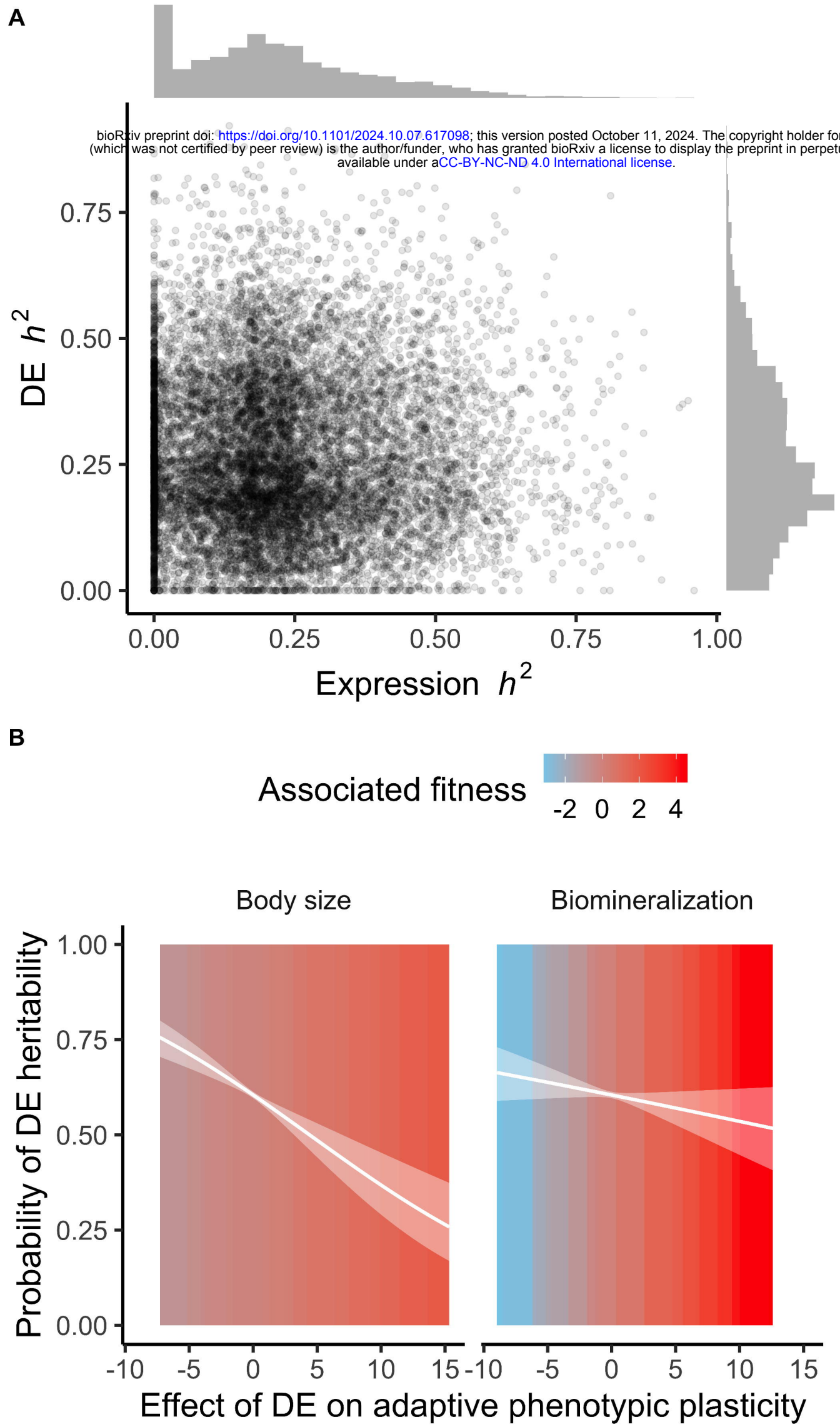
Ziyatdinov, A., Vázquez-Santiago, M., Brunel, H., Martinez-Perez, A., Aschard, H., & Soria, J. M. (2018). lme4qtl: Linear mixed models with flexible covariance structure for genetic studies of related individuals. *BMC Bioinformatics*, *19*(1), 68. https://doi.org/10.1186/s12859-018-2057-x

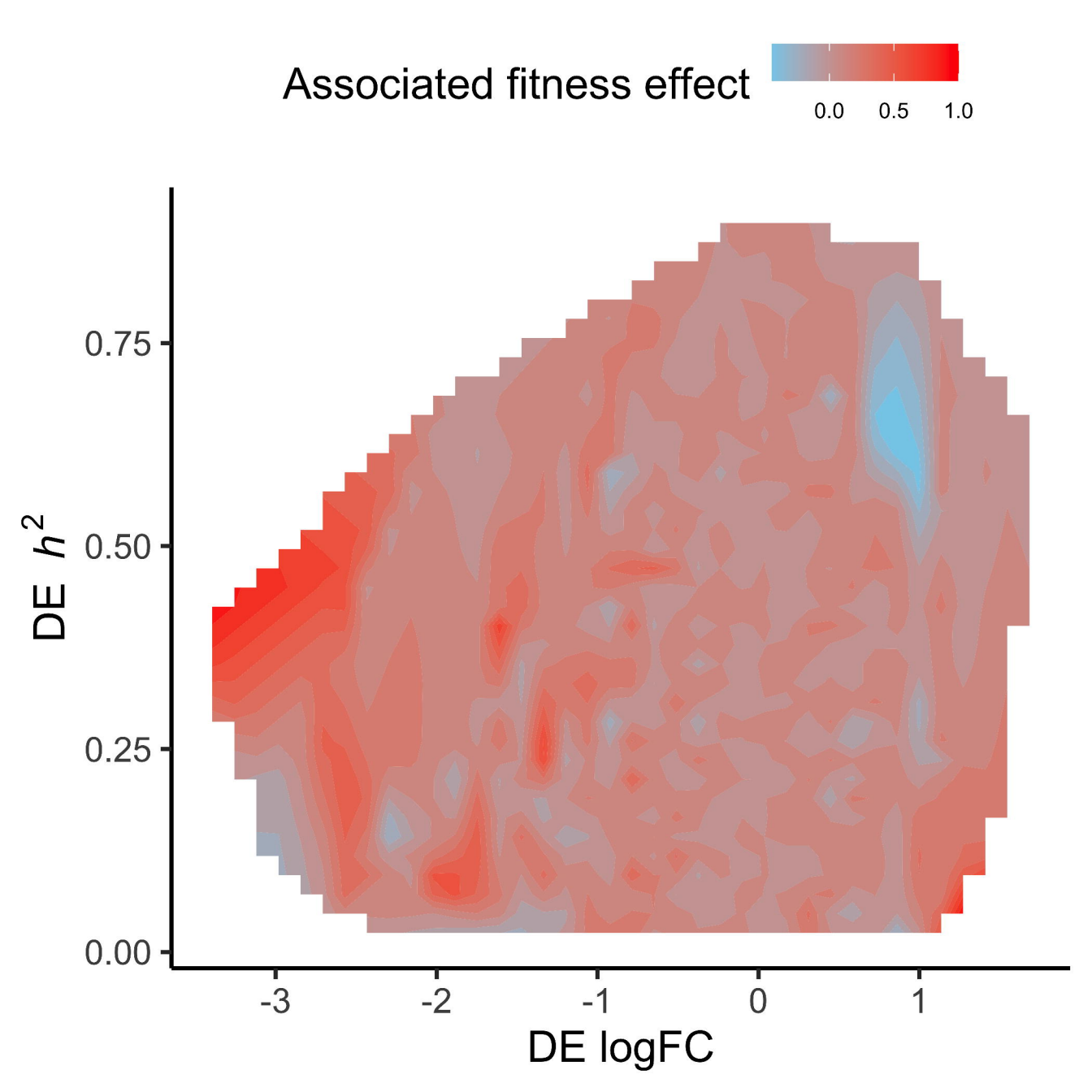

A
Non-upwelling, ambient
Upwelling, stressful
♀1
♂1
♀2
♂2
Repeated
5x
40 crosses
Ambient temp
Low pCO_2
Low temp
High pCO_2
n = 80 cultures
B
E
1 Structural equation model
3 Selection gradient model
GE
P
F
V_A
2 Animal model

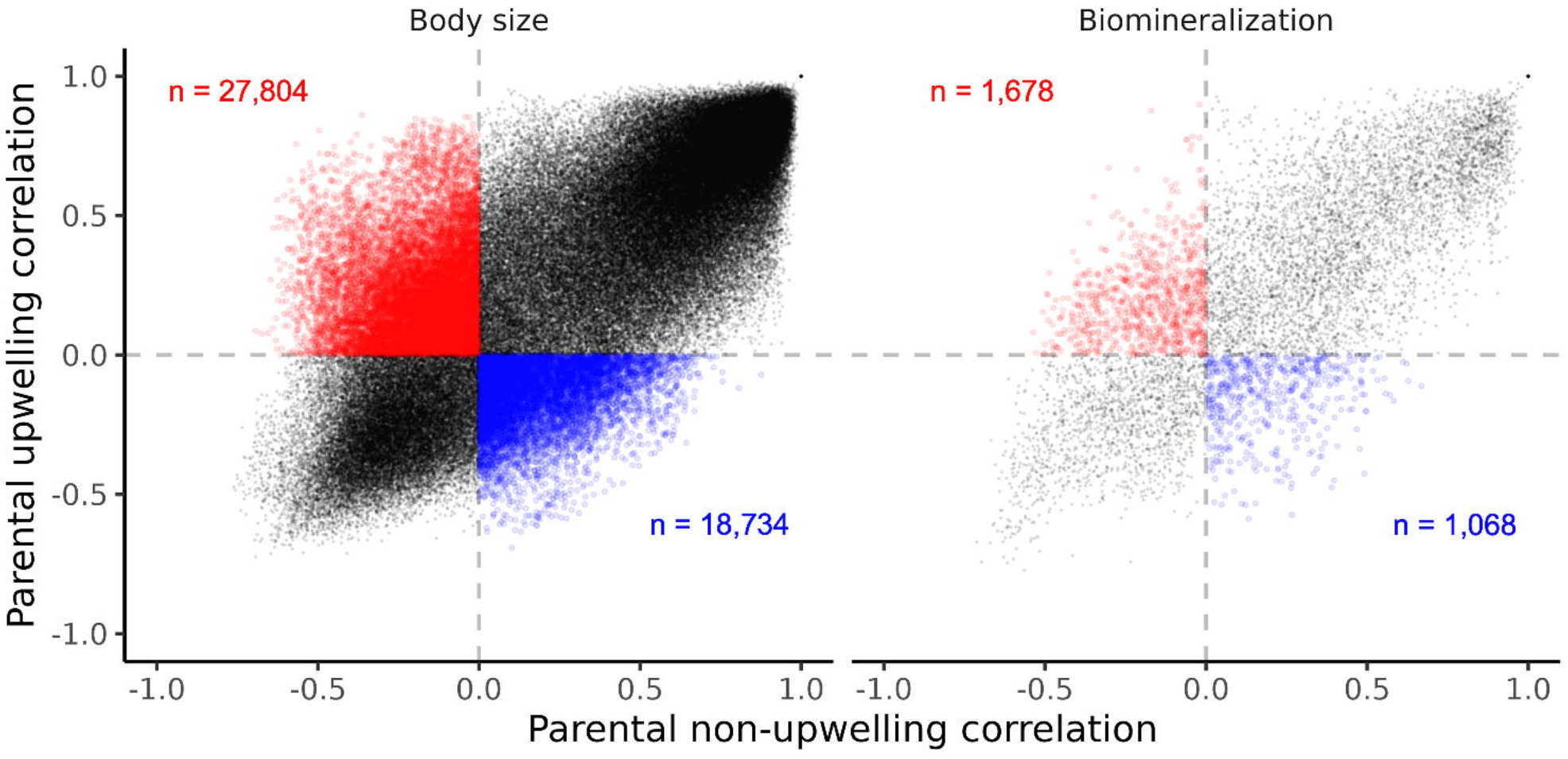

A
Parental non-upwelling
Parental upwelling
0.050
0.025
0.000
-0.025
PC2, 9.22%
-0.03
0.00
0.03
0.06
PC1, 14.48%
Treatment
N_N
N_U
U_N
U_U
B
2
0
-2
logFC
Transgenerational
Developmental
0
5
10
15
logCPM

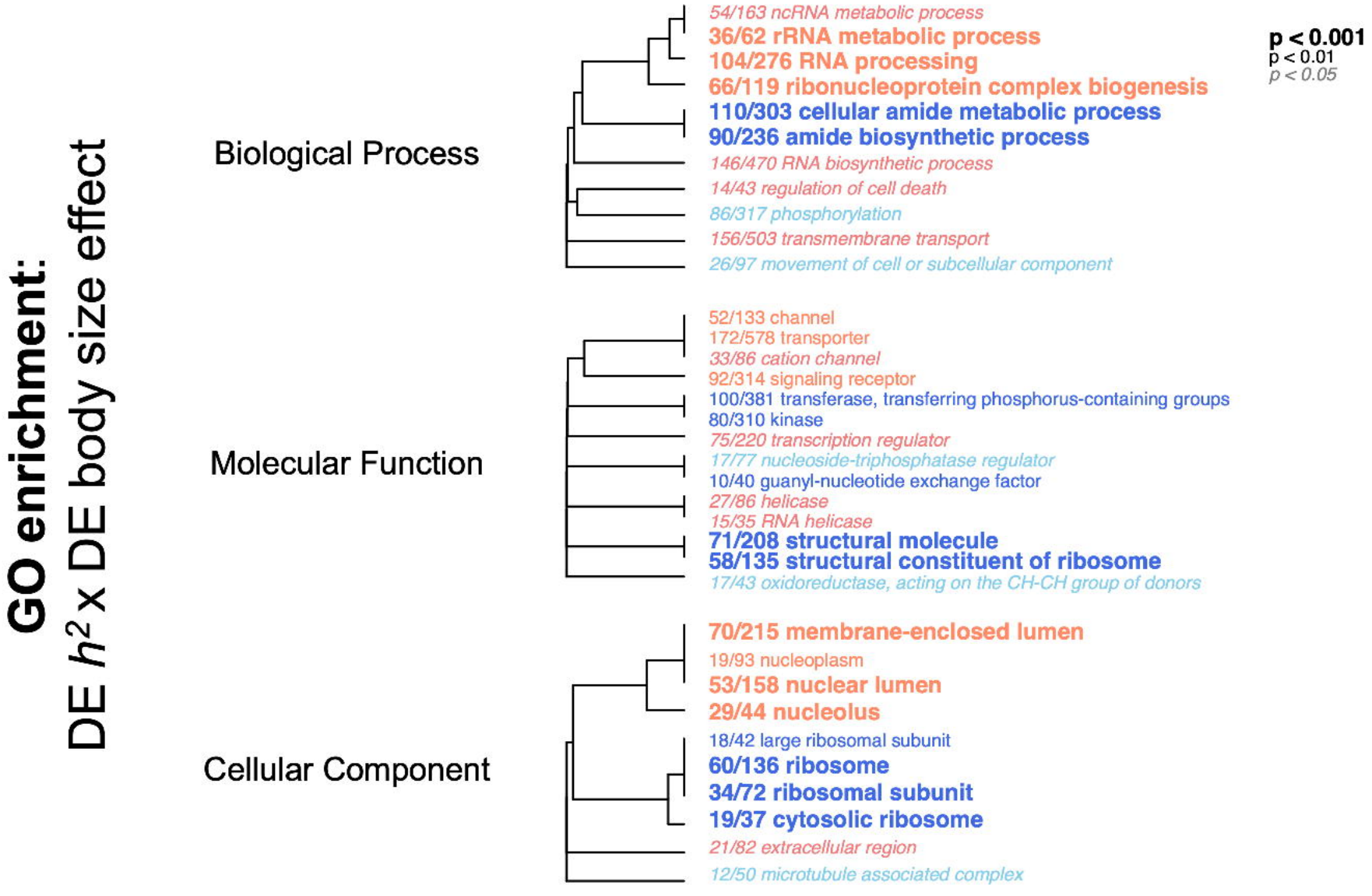







Associated fitness effect
0.0
0.5
1.0
0.75
0.50
0.25
0.00
DE h^2
-3
-2
-1
0
1
DE logFC


Body size
Biomineralization
n = 27,804
n = 18,734
n = 1,678
n = 1,068
Parental upwelling correlation
Parental non-upwelling correlation
1.0
0.5
0.0
-0.5
-1.0


GO enrichment:
DE h^2 x DE body size effect
Biological Process
Molecular Function
Cellular Component
54/163 ncRNA metabolic process
36/62 rRNA metabolic process
104/276 RNA processing
66/119 ribonucleoprotein complex biogenesis
110/303 cellular amide metabolic process
90/236 amide biosynthetic process
146/470 RNA biosynthetic process
14/43 regulation of cell death
86/317 phosphorylation
156/503 transmembrane transport
26/97 movement of cell or subcellular component
52/133 channel
172/578 transporter
33/86 cation channel
92/314 signaling receptor
100/381 transferase, transferring phosphorus-containing groups
80/310 kinase
75/220 transcription regulator
17/77 nucleoside-triphosphatase regulator
10/40 guanyl-nucleotide exchange factor
27/86 helicase
15/35 RNA helicase
71/208 structural molecule
58/135 structural constituent of ribosome
17/43 oxidoreductase, acting on the CH-CH group of donors
70/215 membrane-enclosed lumen
19/93 nucleoplasm
53/158 nuclear lumen
29/44 nucleolus
18/42 large ribosomal subunit
60/136 ribosome
34/72 ribosomal subunit
19/37 cytosolic ribosome
21/82 extracellular region
12/50 microtubule associated complex
p < 0.001
p < 0.01
p < 0.05