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

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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 &

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

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

131 In response to experimental upwelling, *S. purpuratus* exhibits transgenerational and
132 developmental plasticity of differential gene expression, DNA methylation, and several
133 performance traits including larval growth rate, biomineralization, and lipid content (Bogan et al.,
134 2023; Strader et al., 2019, 2020, 2022; Wong et al., 2018, 2019). Phenotypic data for larval body
135 size and biomineralization from the experiment that we describe here were reported by Strader et
136 al. 2022 who detected significant genetic variation for the plasticity of both phenotypes in response

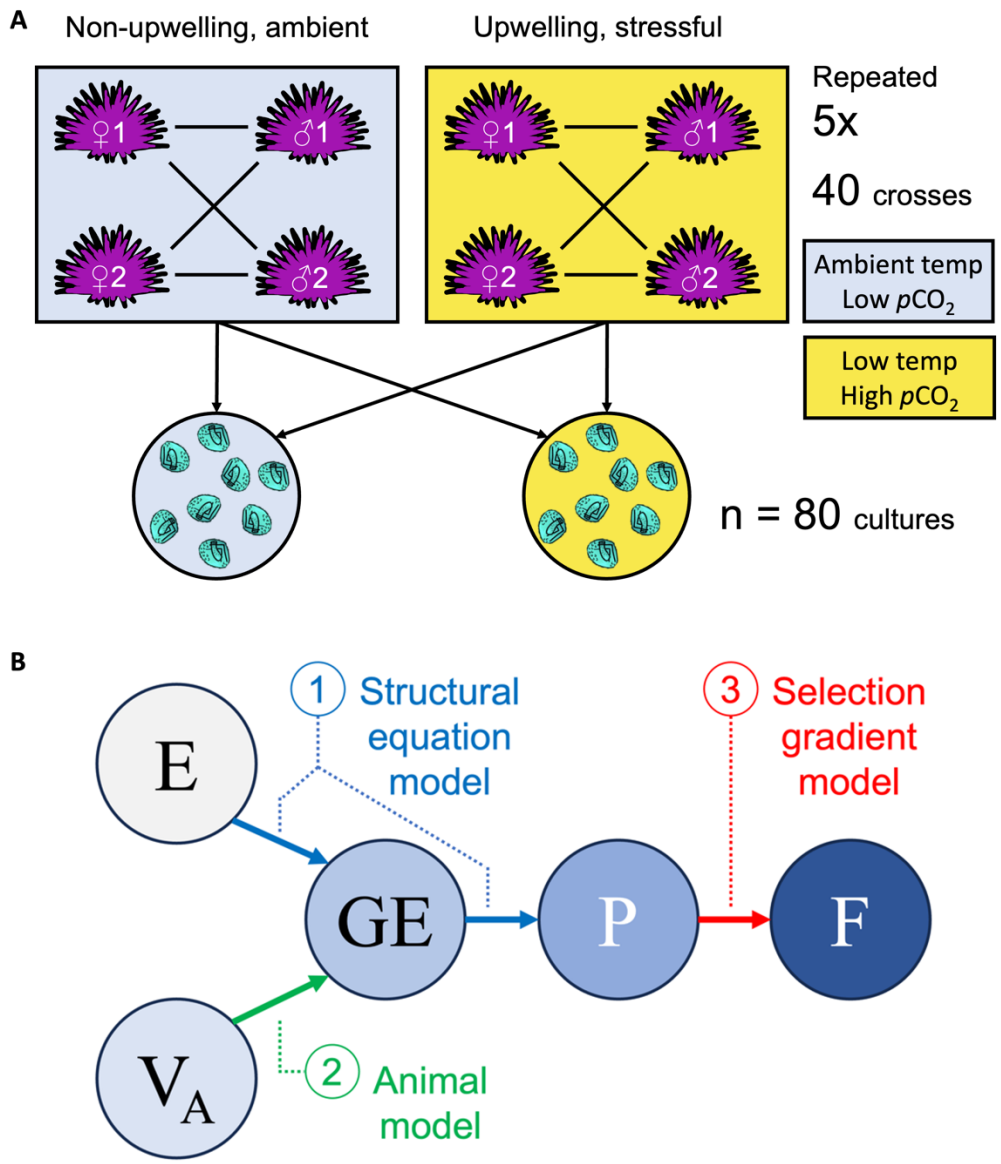

Figure 1 | *Crossing and experimental designs*. **(A)** This graphic depicts adult and larval conditioning to ambient, non-upwelling (blue) and stressful, experimental upwelling conditions (yellow). Crosses between cohorts of conditioned adults are depicted with lines. Reciprocal rearing of offspring 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

171 *2.1 Differential expression induced by parental and developmental upwelling – RNA-seq*
172 *alignment, quality checking, and filtering* – Following trimming, RNA-seq libraries achieved a
173 mean size of 37.07 ± 5.09 million reads and a mean mapping efficiency of 74.14 ± 1.80%. After
174 read-count filtering for transcripts with > 0.5 CPM in at least 75% of all samples, 12,953 transcripts
175 were retained for downstream analysis. Outlier detection was performed with filtered read count
176 data using arrayQualityMetrics v3.54.0 (Kauffmann et al., 2009), which flagged two half sibling
177 crosses from parental and developmental upwelling treatments as significant outliers (Fig. S2–S3).
178 Removal of these two samples brought the RNA-seq sample size to n = 78. Mean variation in gene
179 expression across samples equaled a BCV of 0.12. Library and alignment metrics are further
180 described in Supplemental File 1.
181 Parental conditioning to upwelling induced upregulation of 1,582 transcripts and
182 downregulation of 1,539 in larval offspring. These differentially expressed genes (DEGs) included
183 50 and 29 upregulated and downregulated transcripts with an absolute $\log_2$FC > 1.0 (Fig. 2). DEGs
184 induced by parental upwelling were enriched for 71 biological process (BP) GO terms, 49
185 molecular function (MF) terms, and 30 cellular component (CC) terms. Upregulated transcripts
186 included enrichment for the BP/MF terms involved in cellular signaling, cell adhesion,
187 transmembrane transport, and development localized to CC terms including the endoplasmic
188 reticulum, cell membrane protein complexes, and extracellular region. Downregulated transcripts
189 were enriched for BP/MF terms involved in ATP metabolism, ribosomal structure/biogenesis,
190 mitochondrial organization, and oxidoreductase activity localized to the ribosome, mitochondrial
191 matrix, and cytosol (Fig. S4–S6).
192

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

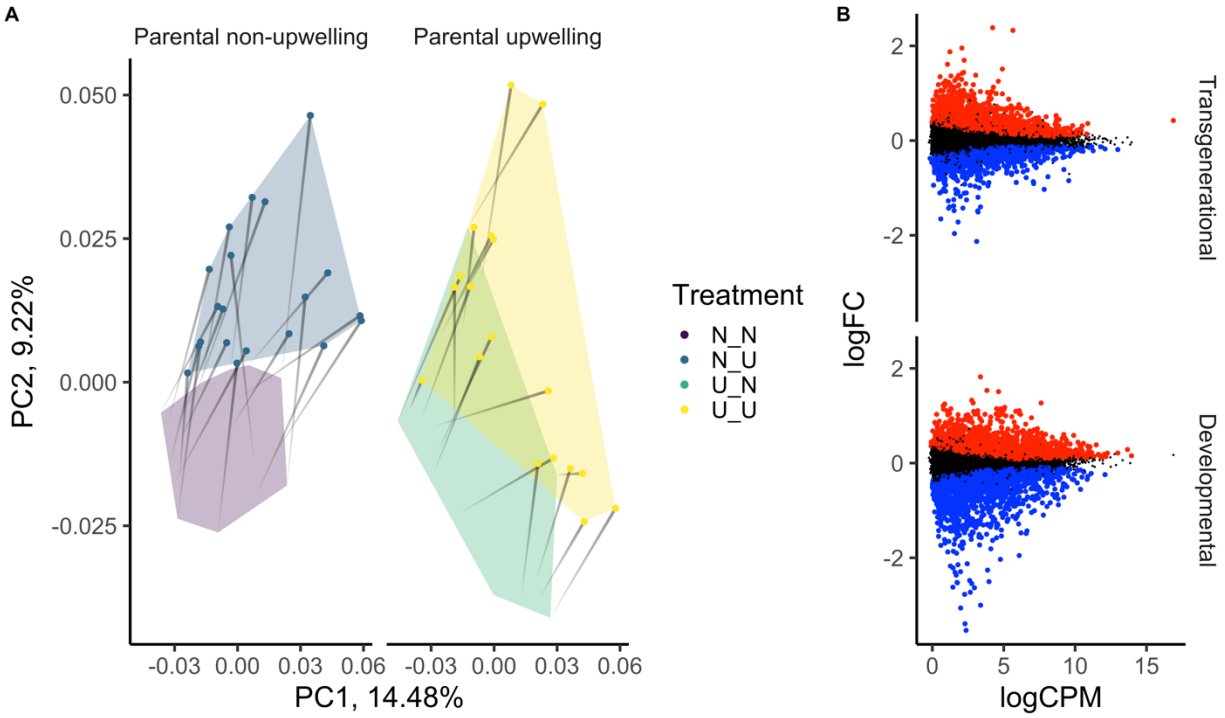

Figure 2 | *Differential expression induced by parental and developmental exposure to upwelling*. **(A)** Loading of samples to principal coordinate axes derived from filtered, normalized read counts. Parental and developmental treatments are represented by color. Paths connect single crosses and their 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.

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

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

223 *2.2. Associations between differential expression and adaptive versus maladaptive*
224 *plasticity* – Structural equation models (SEMs) predicting the effect of differential expression (DE)
225 on plastic changes in body size and biomineralization in response to developmental upwelling
226 identified 231 transcripts associated with increased body size, 564 with reduced body size, 125
227 associated with increased biomineralization, and 113 with decreased biomineralization. Selection
228 gradient models predicting the proportion of normal development per replicate culture (a fitness
229 correlated trait) as a function of body size or biomineralization and their plasticity estimated fitness
230 benefits of (i) plastic reductions in size under upwelling and (ii) maintenance of or plastic increases
231 in biomineralization under upwelling. When larvae were spawned from parents exposed to
232 upwelling, plastic increases in biomineralization incurred a selection gradient of 0.40 ± 0.09 (Fig.
233 3A) and plastic reductions in body size incurred a weaker selection gradient of 0.13 ± 0.04 (Fig.

234 3B). Interestingly, the plasticity of both traits did not exhibit detectable fitness costs or benefits
235 when larvae were spawned from non-upwelling parents. These selection gradient coefficients
236 represent the slopes of logistic regressions between larval survival (abnormality) and phenotypic
237 plasticity visualized in Figures 3A and 3B. Thus, DE driving reductions in body size or increases
238 in biomineralization were associated with adaptive or weakly adaptive phenotypic outcomes.

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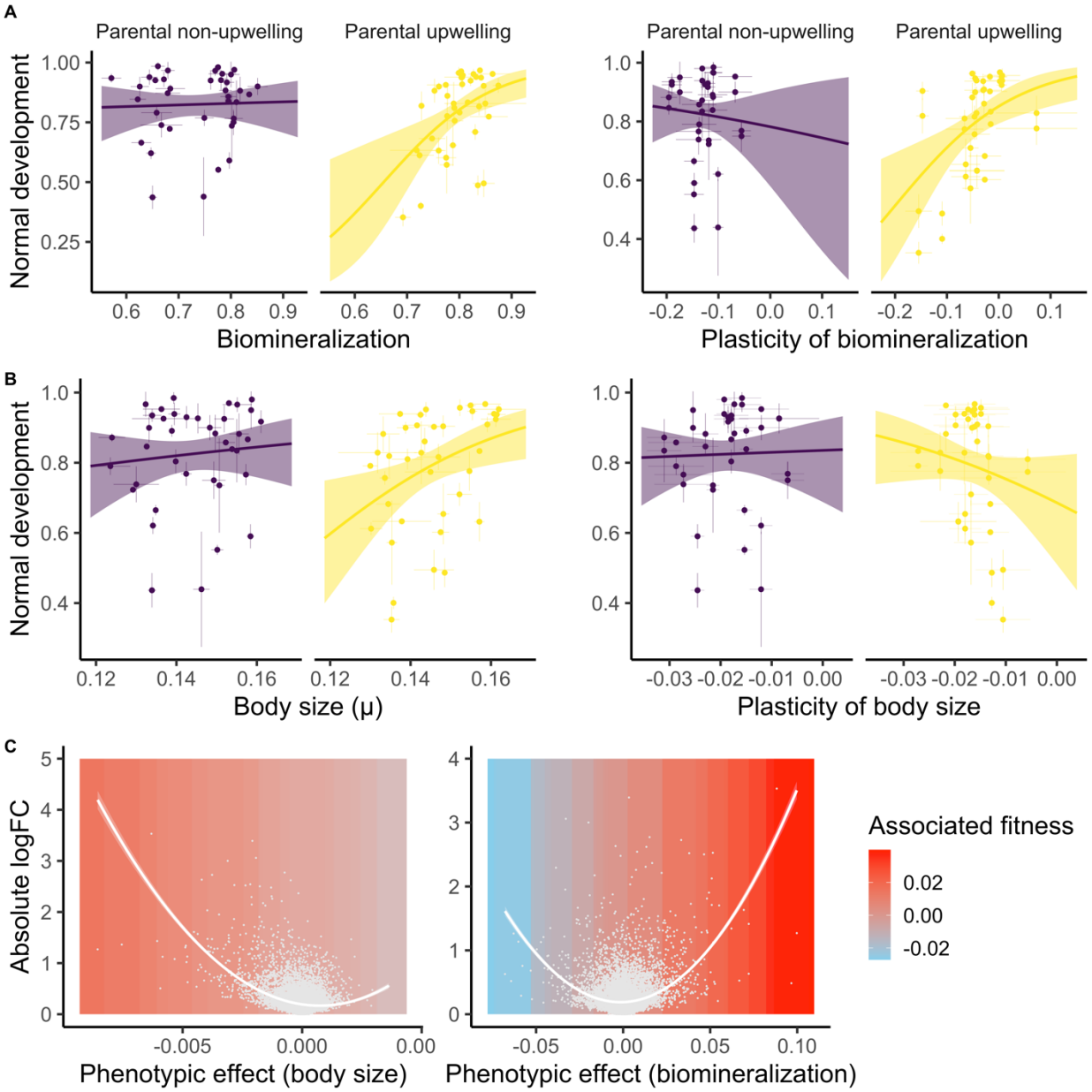

Figure 3 | *Effects of differential expression on adaptive plasticity*. **(A – B)** The effects of phenotypic values and plasticity in biomineralization (spicule length normalized by body length) and body size (maximum body length) on proportion of normal development grouped by parental environment are 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.

269 *2.3. The transcriptome-wide heritability of adaptive and maladaptive differential*
270 *expression* – DE was highly heritable across most genes, but levels of heritability varied according
271 to associations between DE and adaptive plasticity. DE was more heritable than baseline gene
272 expression (CPM) by 19.95% h^2 ($p < 2.2e^{-16}$) and the two variables were uncorrelated (Fig. 4A).
273 60.46% of significant DE induced by developmental conditioning exhibited h^2 greater than or
274 equal to 0.20 (i.e., at least 20% of in DE variance was heritable). The average h^2 of DE was 0.2674
275 ± 0.1592. 7.59% of DEGs were both heritable and associated with adaptive plasticity in body size,
276 but 59.93% of adaptive DE related to body size was heritable. These transcripts exhibited similar
277 h^2 relative to all DE: 0.2613 ± 0.1516. Substantially less DE was heritable and associated with
278 maladaptive plasticity in body size, equaling only 3.59% of all DEGs. However, maladaptive DE
279 related to body size exhibited greater heritability with a mean h^2 of 0.2984 ± 0.1600. 56.80 % of
280 DEGs associated with adaptive increases in biomineralization were heritable, with a mean h^2 of
281 0.3709 ± 0.1224. 1.55% of DEG's were heritable and associated with adaptive increases in
282 biomineralization. 0% of DEGs that were significantly associated with maladaptive decreases in
283 biomineralization were heritable.
284 DE associated with adaptive reductions in body size under upwelling was significantly less
285 heritable than DE associated with maladaptive increases in size. As the effect of DE on adaptive
286 decreases in body size grew, h^2 significantly decreased (β = -0.96; $p = 5.77e^{-8}$) as well as the
287 probability of $h^2 \geq 0.2$ (β = -5.47; $p = 2.16e^{-8}$). Conversely, this means h^2 and the probability of
288 heritability increased as maladaptive effects of DE on increased body size grew (Fig. 4B). The
289 phenotypic effect of DE on adaptive biomineralization plasticity had a negative but insignificant
290 effect on its heritability (Fig. 4B).
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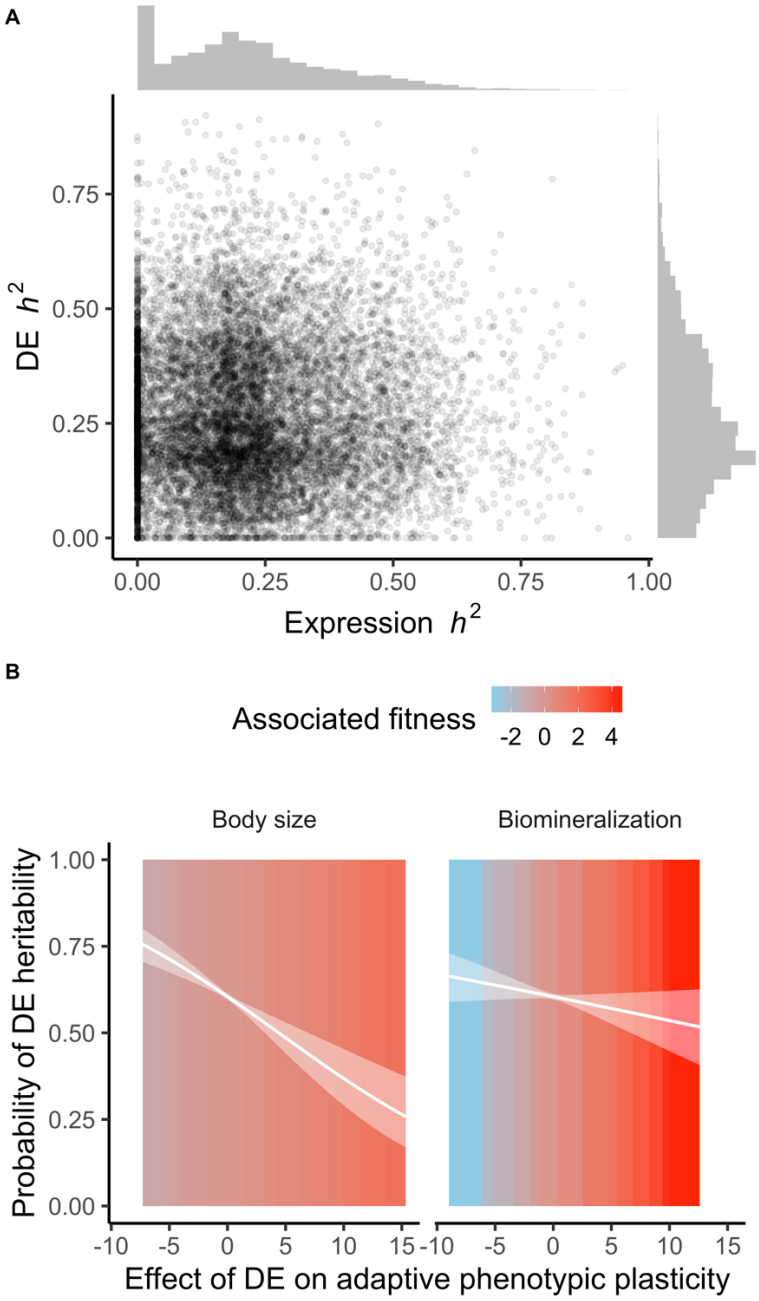

Figure 4 | *Heritability of differential expression and its effect on adaptive phenotypic plasticity*. **(A)** h^2 of differential expression (DE) induced by developmental upwelling is plotted across h^2 of baseline gene expression transcriptome wide. The distributions of h^2 for baseline and differential 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).

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

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

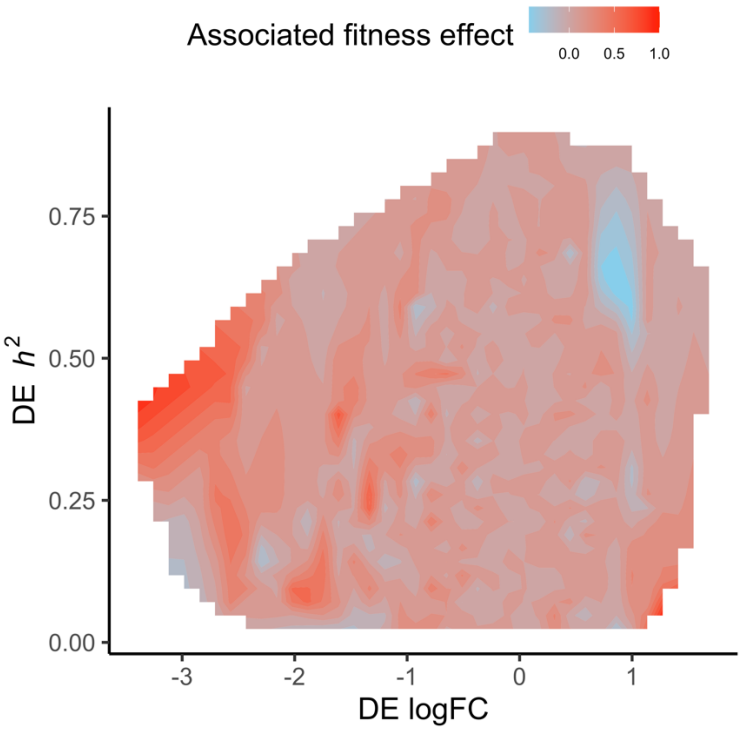

Figure 5 | *Transcriptome wide distribution of differential expression, its heritability, and associated fitness effects.* The distribution of heritability for differential expression (DE) in response to upwelling is plotted as h^2 across the distribution of DE's fold change (logFC). Color represents 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 ($\chi^2 = 1767.7$; $p < 2e10^{-16}$).

342 Parental treatment groups contained different genotypes, presenting a potential confound
343 of transgenerational effects on genetic correlation. Permutations of the experimental pedigree were
344 performed, dividing it into two random halves with equal representation of parental treatment
345 groups. Random permutation revealed that 7.25% of enrichment for positive, directional changes
346 to genetic correlations in body size DE were attributed to pedigree structure rather than
347 environment. It was also significantly more likely that directional shifts in genetic correlation
348 under parental upwelling were attributed to environment rather than pedigree structure (χ^2 =
349 1639.6; $p < 2e10^{-16}$). 94.53% of upwelling-induced directional shifts to genetic correlations in
350 biomineralization DE were attributable to pedigree structure. Thus, there were detectable effects
351 of parental environment on the genetic architecture of pathways associated with adaptive body size
352 plasticity, but not biomineralization.
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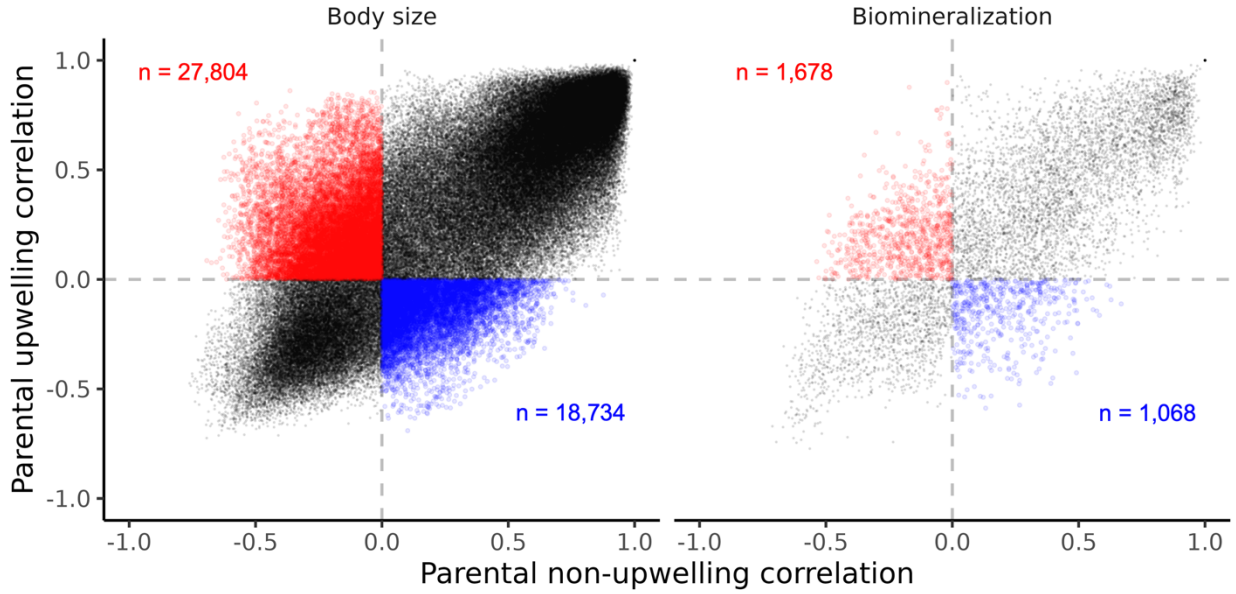

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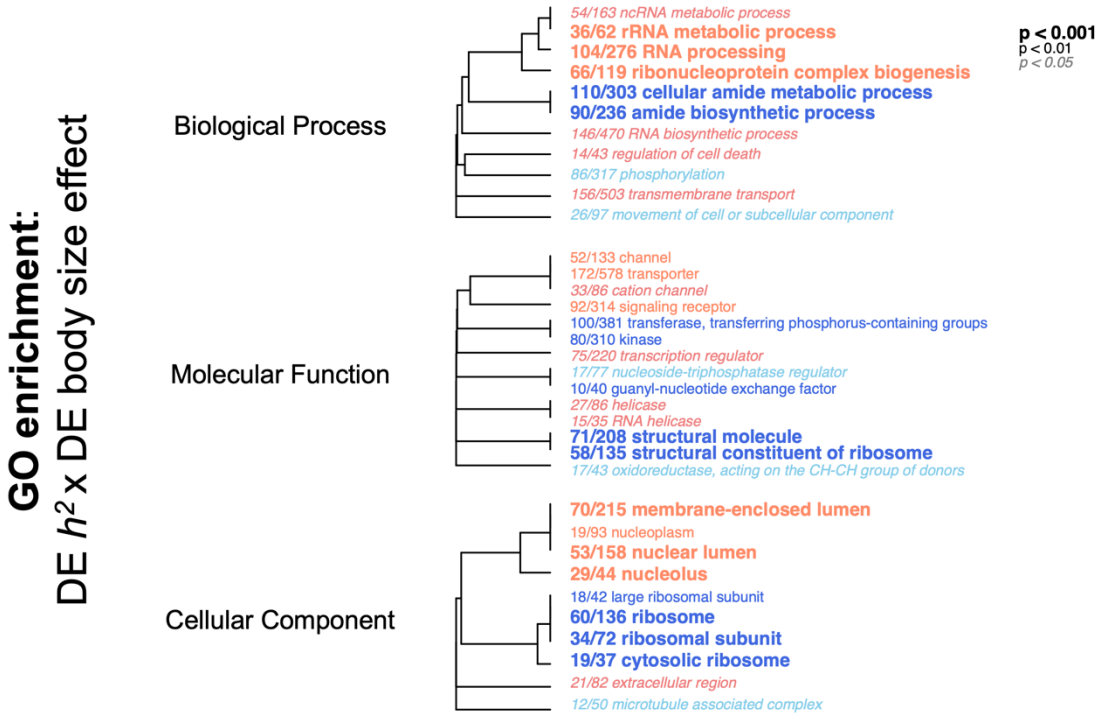
355 **Figure 6** | *Changes in genetic correlation between parental upwelling treatments for log foldchanges (logFC) of*
356 *differential expression (DE) associated with adaptive plasticity in larval body size and biomineralization*. X and Y
357 axes represent genetic correlations extracted from *G*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

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



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371 **Figure 7** | *Functional enrichment of differentially expressed genes with high heritability and strong absolute effects*
372 *on the plasticity of body size*. Enriched gene ontology (GO) terms are derived from a Mann Whitney U test of variation
373 in the product of differential expression's heritability (h^2) and DE's effect on adaptive plasticity of body size in
374 response to upwelling. Trees depict clustering of GO terms based on shared transcripts. Red GO terms are enriched
375 within heritable differential expression associated with adaptive reductions in body size in response to developmental
376 upwelling exposure. Blue terms are enriched within heritable differential expression associated with maladaptive
377 increases in body size under upwelling.
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380

381 **4. Discussion**

382 *3.1. Reduced heritability of transcriptional responses associated with adaptive plasticity –*
383 We observed that the transcriptome wide heritability of DE significantly decreased when DE was
384 associated with adaptive, phenotypic responses to developmental conditioning under upwelling
385 (Figures 4 – 5). This extent of genetic variation for DE, and its decline in DE associated with
386 adaptive plasticity, confirms observations in other studies of plants (He et al., 2021) and animals
387 (Campbell-Staton et al., 2021; Oostra et al., 2018). However, past research has assigned putatively
388 adaptive and maladaptive roles to molecular responses based on prior findings. Here, we directly
389 integrated gene expression plasticity with fitness measures. Quantifying cross-environment
390 genetic correlations (a measure of genetic variance in plasticity) for gene expression in a tropical
391 butterfly, Oostra et al. detected poor genetic variation for DE between two seasonal morphological
392 phenotypes (Oostra et al., 2018). The authors proposed that minimal genetic variation for
393 transcriptional plasticity resulted from a canalized response to a highly reliable seasonal cue
394 (Oostra et al., 2018). Numerous studies have reported moderate-to-high genetic variation in DE
395 measured as genotype x environment (GxE) interactions shaping expression in animals
396 (Grishkevich & Yanai, 2013; Huang et al., 2020; McCairns et al., 2016). Oostra et al. provide one
397 of the only measures of additive genetic variance in the reaction norm of gene expression rather
398 than variance attributed to GxE. Thus, it is notable that V_A of DE that they detected is strikingly
399 lower than what we observed here.

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

404 Its dispersal distances can be wide enough that larvae are frequently transported across areas of
405 major and minor upwelling in the California Current and Southern California Bight (Chan et al.,
406 2017; Pespeni & Palumbi, 2013; Zaytsev et al., 2003). *S. purpuratus* populations exhibit evidence
407 of local adaptation to regional differences in pCO_2 despite high rates of gene flow (Evans et al.,
408 2013; Pespeni et al., 2013). These findings suggest that an individual population should possess
409 high genetic variation in trait means and/or plastic responses associated with coastal upwelling,
410 and that reductions in genetic variation of adaptive plasticity may occur due to selection. Lastly,
411 genetic variation is often more highly expressed under stressful conditions (Hoffmann et al., 1999).
412 We observed moderate-to-high levels of developmental abnormality in many larval crosses
413 indicative of a baseline level of stress across replicates brought on by parental conditioning or
414 stress incurred *in situ* prior to adult collection.

415 How V_A of DE covaries with its fitness effects is more poorly understood than genetic
416 variation in DE itself. Studying Anolis lizards, Campbell-Staton et al. found increased frequencies
417 of SNPs within *cis*-regulatory regions of genes associated with putatively maladaptive decreases
418 in CTmax under thermal stress. *Cis*-regulatory mutations proximal to genes with putatively
419 adaptive DE did not vary relative to genomic background. This study's design differed from our
420 own in that plasticity was measured at a population level – distinct genotypes were reared under
421 each treatment rather than single genotypes or families being split across treatments. Plasticity was
422 thus summarized as a mean change in phenotype in each population (Campbell-Staton et al., 2021).
423 Our study also expanded on Campbell-Staton's approach of putatively identifying adaptive DE by
424 directly measuring fitness in the same individuals that gene expression was characterized in.
425

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

438 One hypothesis proposes that maladaptive plasticity stems from the expression of
439 deleterious cryptic genetic variants under novel environments that have were not subject to prior
440 negative selection (Acasuso-Rivero et al., 2019; R. F. Schneider & Meyer, 2017). Despite aiming
441 to experimentally simulate upwelling conditions, laboratory conditioning almost certainly
442 introduced *S. purpuratus* to novel environments. Secondly, populations subject to stabilizing
443 selection on a trait incur net negative fitness even when the phenotypic equals the fitness optimum
444 because deviation from that optimum imposes fitness costs (Brady et al., 2019; Burt, 1995; Hansen
445 et al., 2006). Therefore, reactions norms that possess high genetic variation and heritability may
446 be intrinsically likely to exhibit apparent negative directional selection on reaction norms when
447 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

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

484 *3.3.Transgenerational effects reshape fitness consequences and genetic architecture of*
485 *adaptive transcriptional and phenotypic plasticity* – The heritability of phenotypic plasticity, and
486 how this heritability covaries with its fitness effects, can impact the evolution of plasticity and the
487 traits it acts on (Fox et al., 2019; West-Eberhard, 2003). However, evolutionary biology has a
488 limited ability to predict when plasticity and its genetic variation is subject to selection (P. A.
489 Arnold et al., 2019; H. M. Schneider, 2022; Van Buskirk & Steiner, 2009). Under global change,
490 these ecological and evolutionary effects are important for predicting whether future climates will
491 drive canalization of tolerance or increase acclimatory potential via directional selection on
492 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).

515 Upwelling-induced increases in positive genetic correlation (Fig. 6) coincided with an
516 increase in phenotypic plasticity's fitness effects (Fig. 3). Natural selection on phenotypic
517 plasticity requires that it imposes fitness costs or benefits that have been historically difficult to
518 detect. Recently, plasticity's costs have been shown to be contingent on ecological and
519 evolutionary contexts such as fluctuating environments (Schaum et al., 2022), differences between
520 sexes and morphotypes (Hangartner et al., 2022; Svensson et al., 2020), and fitness trade-offs with
521 between plasticity other traits (Bogan et al., 2024; van Heerwaarden & Kellermann, 2020). Our
522 findings that the fitness costs of plasticity in larval biomineralization and body size depended on
523 parental environment expands our knowledge of eco-evolutionary processes influencing
524 plasticity's costs. To our knowledge, this is the first demonstration that parental environments
525 influence the costs of phenotypic plasticity in offspring. Plasticity's fitness costs may have been
526 exacerbated in larvae reared from upwelling-exposed parents due to resource limitation imposed
527 by parental stress during conditioning. However, past work in *S. purpuratus* has shown that
528 maternal conditioning to upwelling during gametogenesis does not alter egg size or protein content
529 and improves lipid provisioning (Wong et al., 2018, 2019). This lipid provisioning may be
530 insufficient to overcome resource limitation and the cost of allocation to plasticity in larvae that
531 are developmentally and transgenerationally exposed to upwelling.
532
533 **4. Conclusion**
534 Understanding plasticity's fitness costs and genetic variation will improve predictions of
535 adaptive responses by biodiversity to climate change. By leveraging RNA-seq integrated with
536 measures of performance and fitness correlating traits, we resolved a transcriptome scale picture
537 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

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

610 *5.4 Tests of differential expression* – Transcript read counts were normalized in edgeR
611 v3.40.2 as counts per million (CPM). Read counts were filtered to keep all transcripts exhibiting
612 CPM > 0.5 in at least 75% of the 80 replicates. Differential expression (DE) was modeled using a
613 negative binomial generalized linear model (glm) fitted with edgeR's robust, tagwise dispersion
614 parameter using the robust iteration of the model fitting function 'GLMQLFit' and the DE test
615 function 'GLMQLFTest' (Robinson et al., 2010). Expression was predicted as a function of two
616 non-interacting, categorical variables for parental and developmental environment. Models were
617 fit with non-interacting environmental predictors because the study's design only enabled the
618 measurement of V_A for developmental rather than transgenerational plasticity. Fitting an
619 interaction between both effects would confound interpretation of V_A for developmental plasticity.
620 Significant DE was evaluated using FDR adjusted p-values (alpha < 0.05). Functional enrichment
621 was tested using a rank-based Mann Whitney U test of Gene Ontology terms input with logFC
622 coefficients for DE. This test determines whether a given GO term's logFC distribution is
623 significantly skewed from the mean of the background, filtered transcriptome (Wright et al., 2015).

624 *5.5 Measuring the adaptive differential expression* – The effect of DE on the plasticity of
625 body size and biomineralization (body size-normalized spicule length) was measured using
626 structural equation models (SEMs). SEMs were derived from two linear models: (i) phenotype
627 predicted as a function of transcript abundance, developmental environment, and parental
628 environment and (ii) scaled, signed transcript abundance predicted as a function of developmental

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

640 The fitness costs and benefits of plasticity in body size and biomineralization were
641 measured using a Lande & Arnold selection gradient model (Lande & Arnold, 1983) as described
642 in Chapter Two, whereby a fitness correlated trait (proportion of normal development, a larval
643 corollary of survival) was modeled as a function of developmental and parental environment, body
644 size or biomineralization per cross in each environment, and the plasticity of body size or
645 biomineralization of a cross between developmental environments. Selection gradient models
646 included a random effect identifying each cross and controlling for genetic covariance using a
647 relatedness matrix generated from custom code (see https://github.com/snbogan/QG_Purp_RNA)
648 such that offspring-parent relatedness equals 0.5, full-sibling relatedness equals 0.5, half sibling
649 relatedness equals 0.25, and unrelated cohorts share a relatedness of 0.
650

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

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

676 Covariation between DE's heritability and adaptive, phenotypic effects were modelled
677 using three different approaches addressing the questions (i) 'Does the probability of heritability
678 ($h^2 \geq 0.2$) vary as a function of DE's effect on adaptive plasticity in body size or
679 biomineralization?', (ii) 'Does total heritability (h^2 as a continuous variable) vary as a function of
680 DE's phenotypic effects?', and (iii) 'Does total heritability vary according to the fitness costs of
681 DE's combined effect on plasticity in body size and biomineralization?'. Tests of questions i and
682 ii were performed by modelling the probability of $h^2 \geq 0.2$ using binomial glm's. Continuous h^2
683 was modeled as a beta-distributed glm. Each model type fitted two continuous predictor variables
684 for the phenotypic effect of DE on body size and biomineralization signed toward the adaptive
685 direction of that effect under parental upwelling, which induced yielded fitness effects of plasticity
686 as opposed to neutral effects under parental non-upwelling. Models of continuous h^2 set parameters
687 for DE's phenotypic effects as a second order polynomial to accommodate non-linear variation in
688 h^2 across the parameter space. For question iii, DE's total effect on adaptive plasticity was
689 calculated as the summation of the SEM-predicted coefficient for DE of transcript *i*'s effect (*E*) on
690 body size (*S*) and biomineralization (*B*) multiplied by the selection gradient (β) acting on each
691 plastic trait under upwelling, such that adaptive plasticity associated with transcript $i = (E_{S,i} \text{ x } \beta_{S,i})$
692 $+ (E_{B,i} \text{ x } \beta_{B,i})$. Covariance between DE h^2 and its total adaptive effect on plasticity was modeled
693 using a beta-distributed glm in the R package betareg v3.1-4 (Grün et al., 2012).

694 *5.7. Estimating G matrices* – Genetic variance-covariance matrices of absolute logFC
695 values for DE associated with adaptive plasticity in larval body size and biomineralization were
696 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.

To determine whether significant enrichment in negative-to-positive changes in genetic correlation was attributed to parental environment or the pedigree structure of populations in parental treatment groups (i.e., differences in genetic (co)variance between animals used in each treatment), random permutations of the experimental pedigree were constructed. These permutations split the pedigree into two halves which evenly represented families of parental treatment groups. Random permutations of split pedigrees were input into animal models and resulting *G* matrices. Chi-squared tests of enrichment for directional changes in genetic correlation between permuted matrices were performed, which determined the effect of pedigree structure on apparent effects of parental environment on genetic correlations. In Results, enrichment of directional changes in genetic correlation between parental environment are reported alongside the percentage of these effects that were attributed to pedigree structure rather than treatment.

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742 **Data availability**

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

747

748 **Author Contributions**

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

754

755 **Declarations**

756 The authors declare no competing interests.

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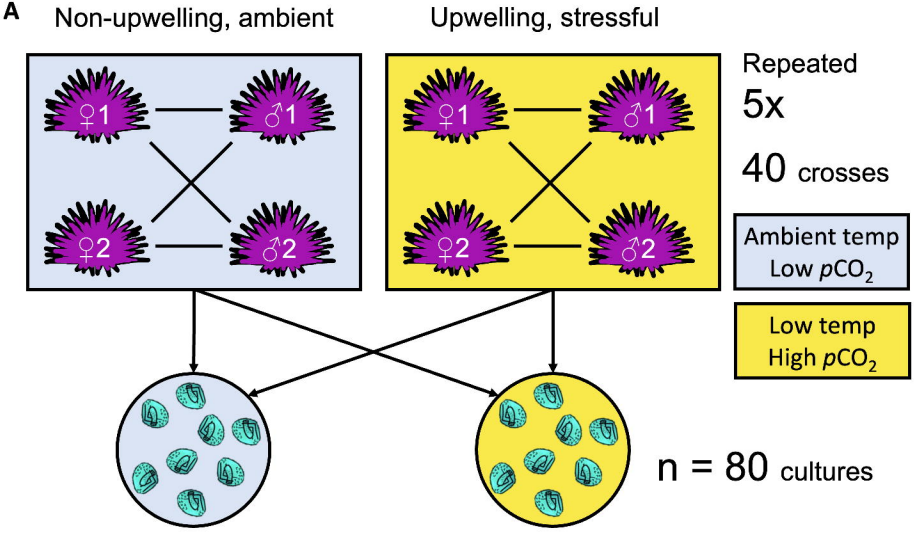
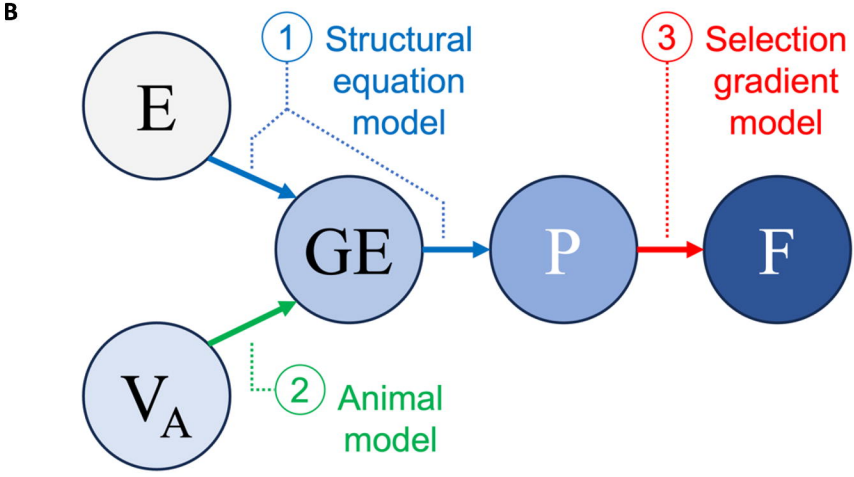
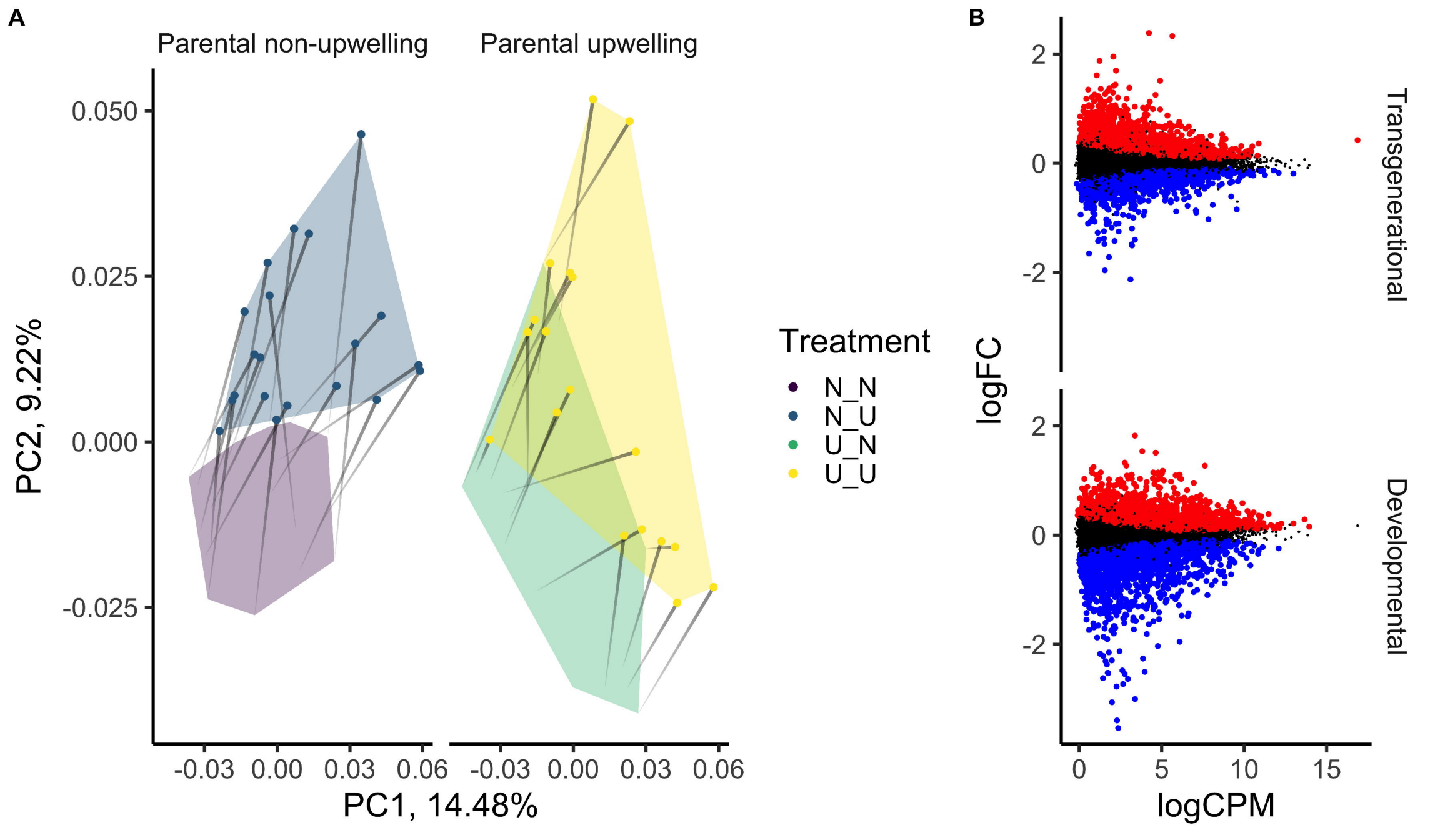
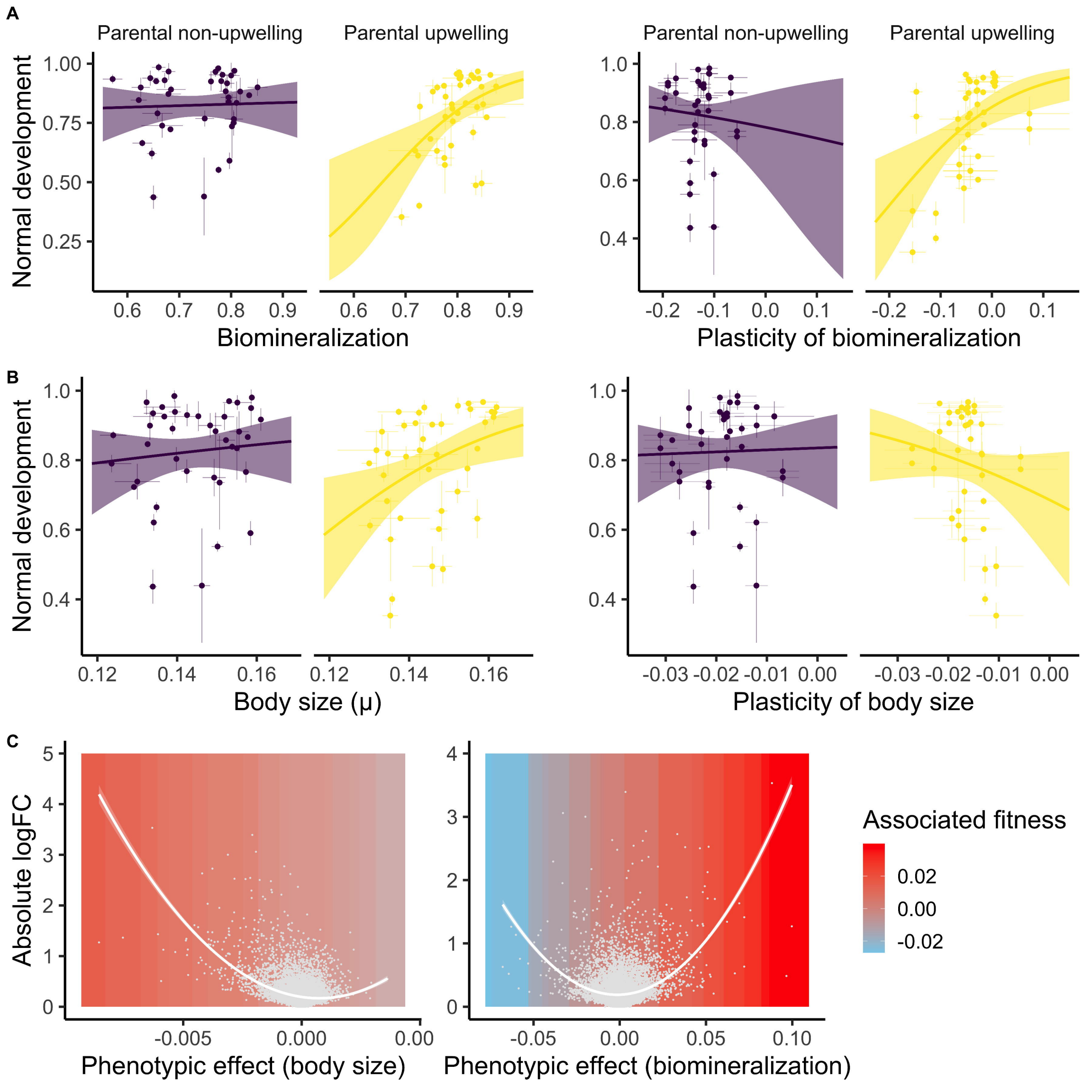
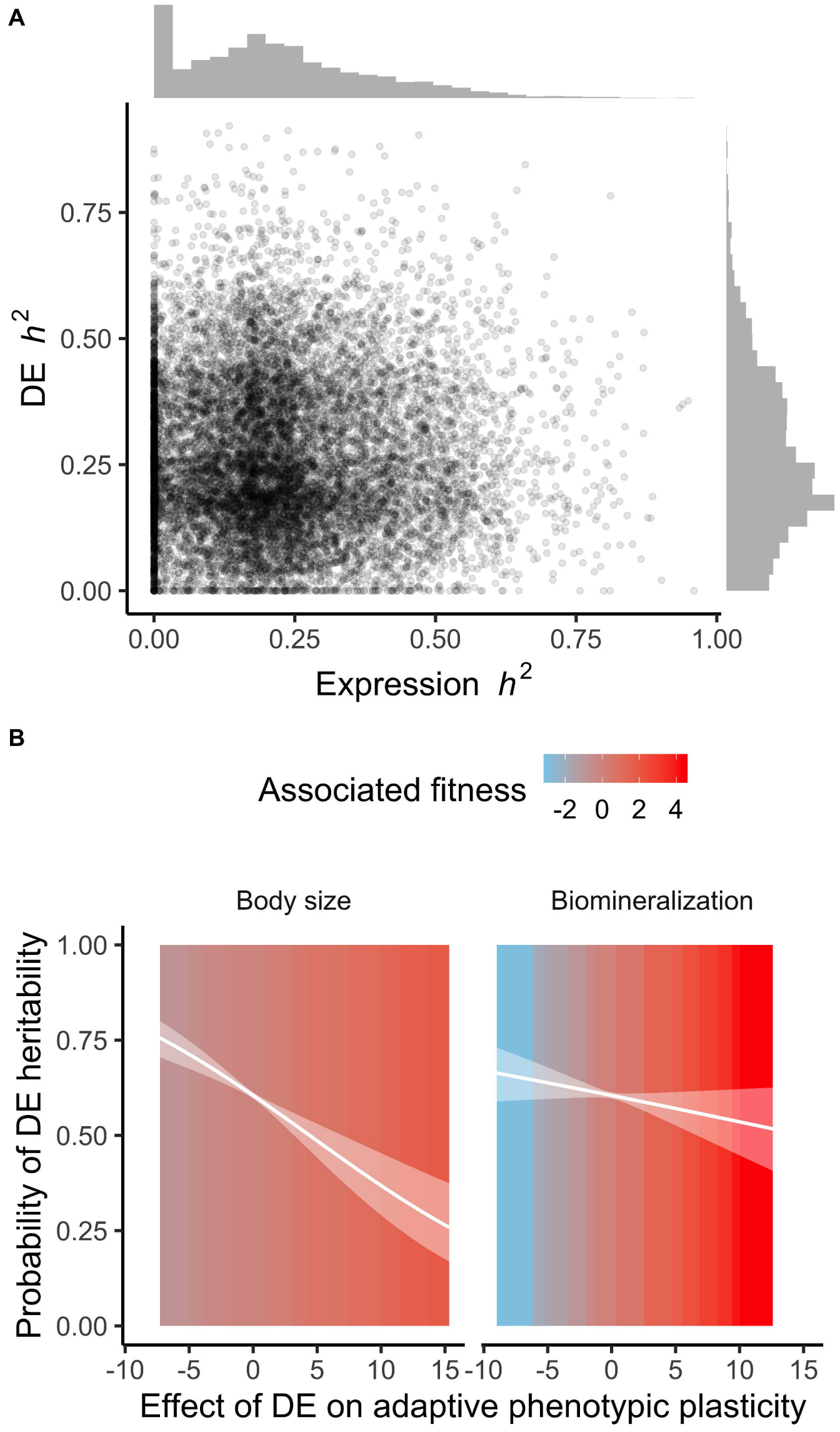
1166

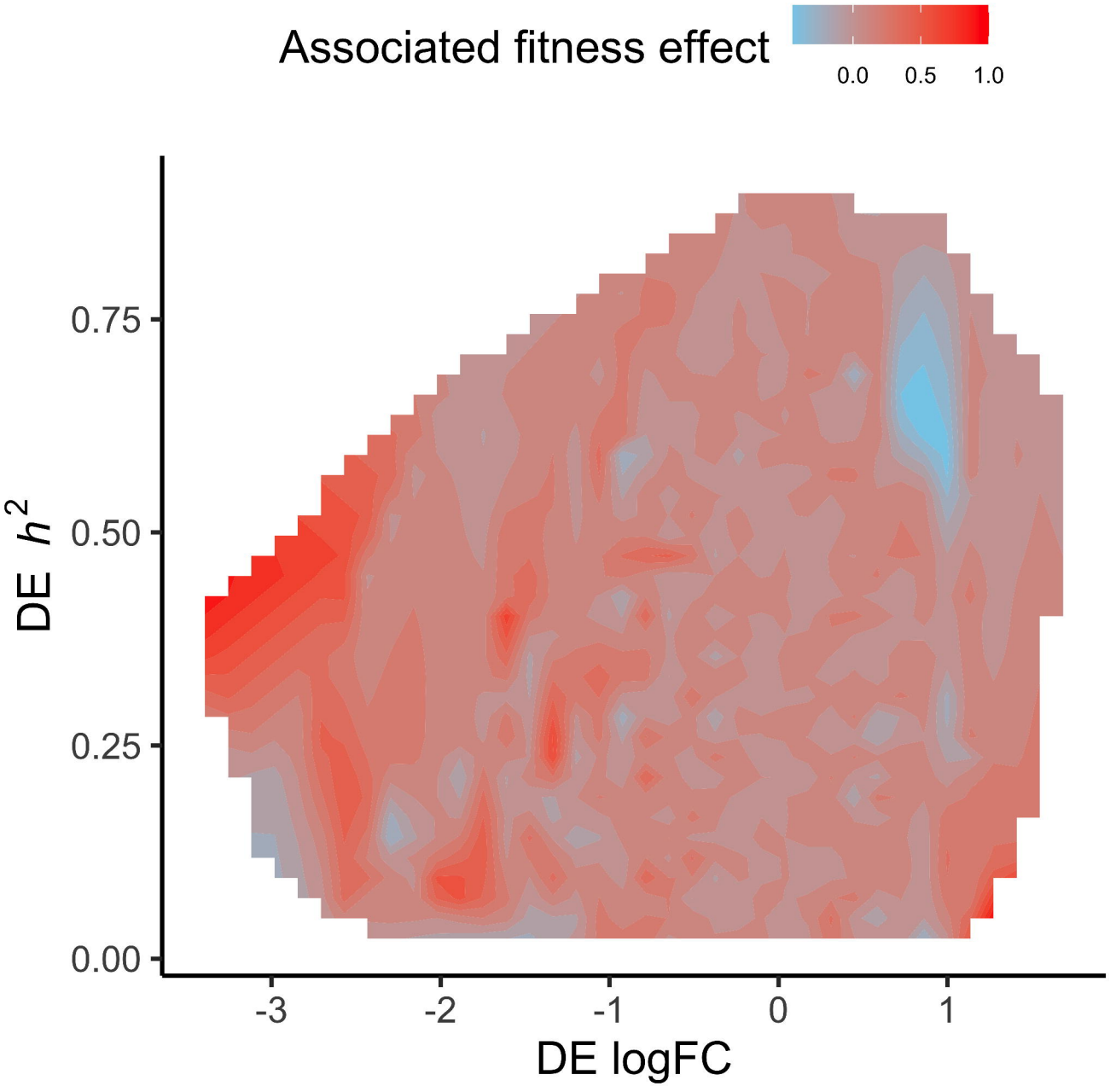

A
Non-upwelling, ambient
Upwelling, stressful
♀1
♂1
♀2
♂2
♀1
♂1
♀2
♂2
Repeated
5x
40 crosses
Ambient temp
Low pCO_2
Low temp
High pCO_2
n = 80 cultures

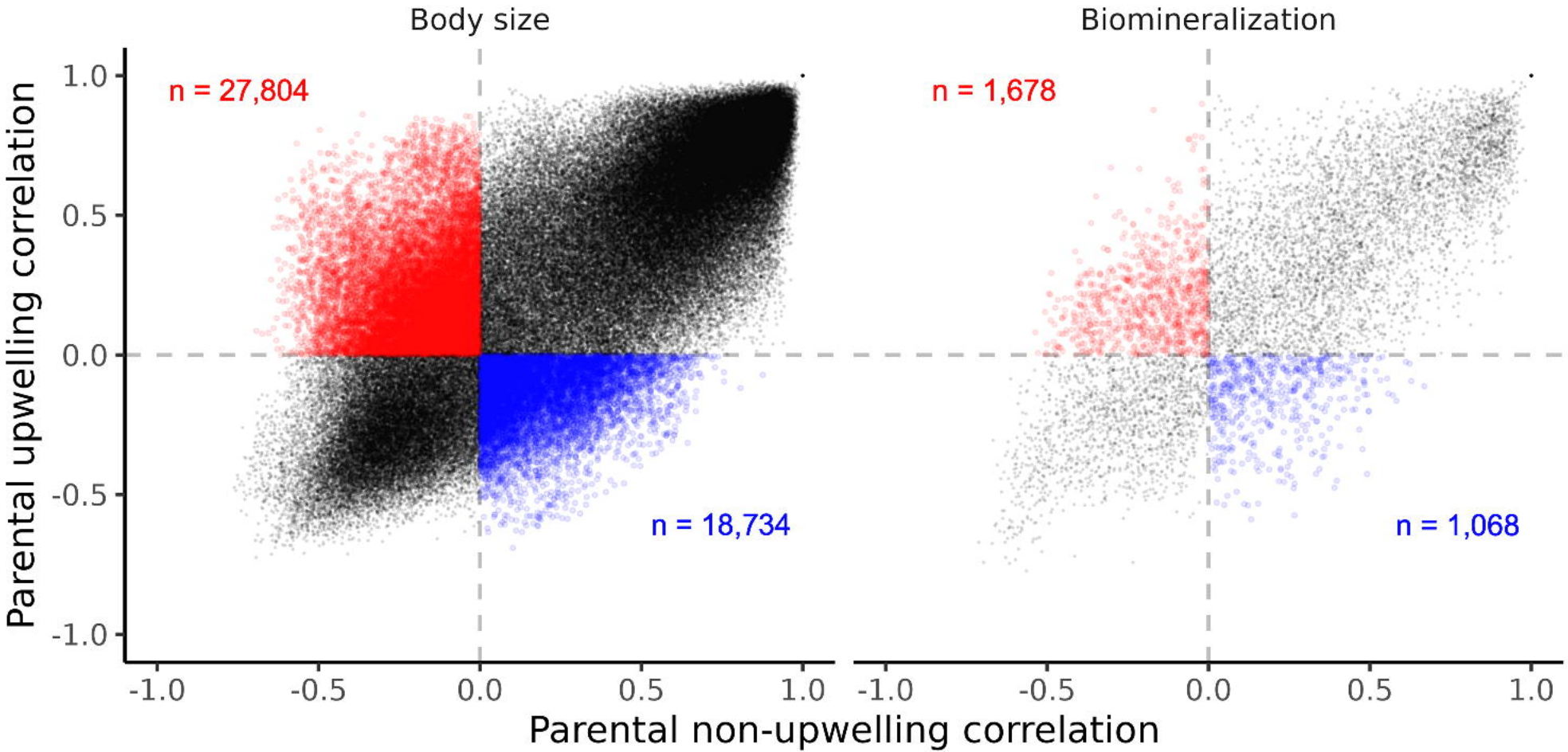

B
E
GE
P
F
V_A
1 Structural equation model
2 Animal model
3 Selection gradient model

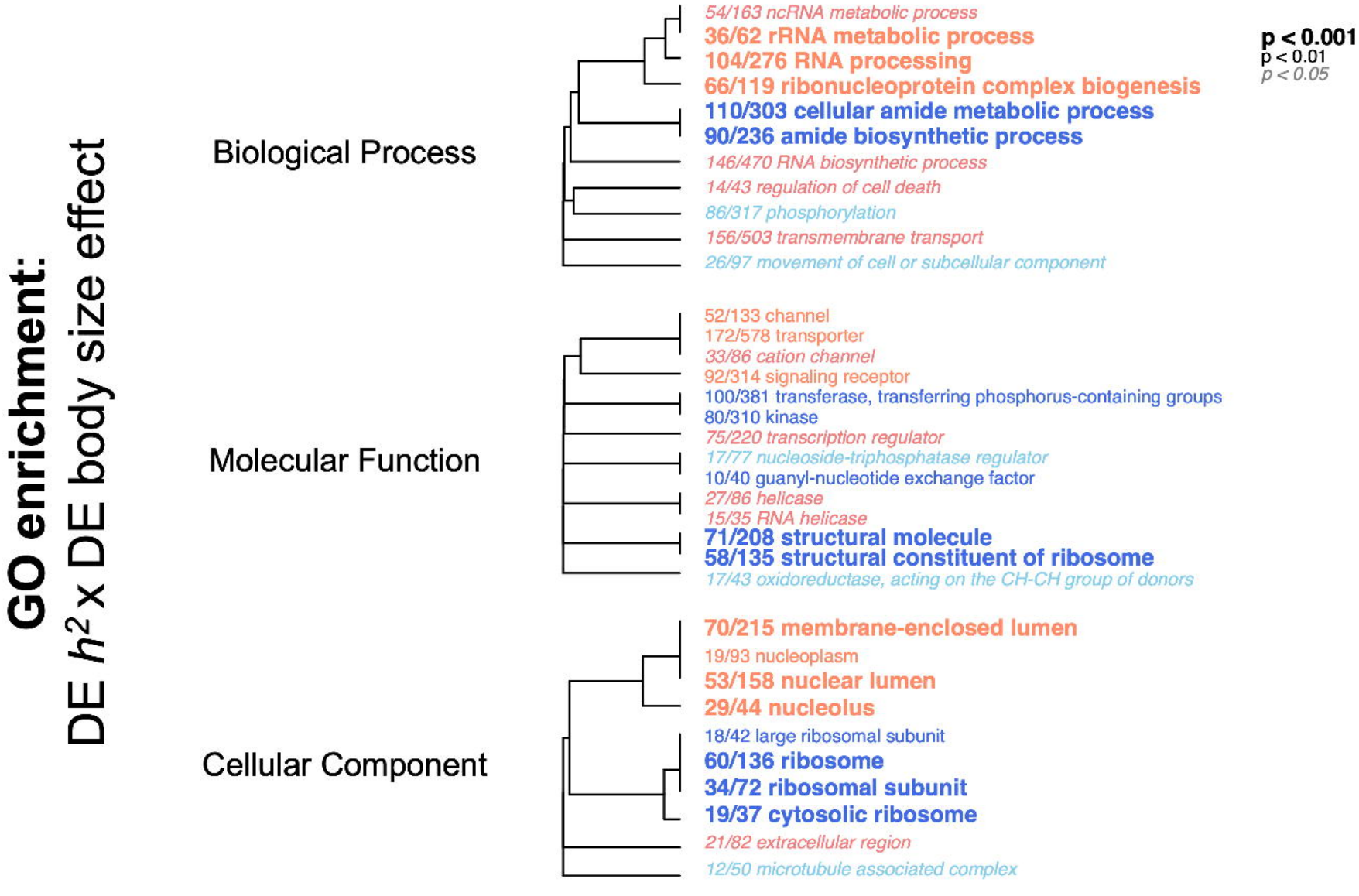

A
Parental non-upwelling
Parental upwelling
0.050
0.025
0.000
-0.025
PC2, 9.22%
-0.03
0.00
0.03
0.06
PC1, 14.48%
Treatment
N_N
N_U
U_N
U_U
B
Transgenerational
Developmental
logFC
2
0
-2
0
5
10
15
logCPM


A
Parental non-upwelling
Parental upwelling
Parental non-upwelling
Parental upwelling
Normal development
1.00
0.75
0.50
0.25
0.6
0.7
0.8
0.9
Biomineralization
1.0
0.8
0.6
0.4
-0.2
-0.1
0.0
0.1
Plasticity of biomineralization
B
Normal development
1.0
0.8
0.6
0.4
0.12
0.14
0.16
Body size (μ)
-0.03
-0.02
-0.01
0.00
Plasticity of body size
C
Absolute logFC
5
4
3
2
1
0
-0.005
0.000
0.00
Phenotypic effect (body size)
-0.05
0.00
0.05
0.10
Phenotypic effect (biomineralization)
Associated fitness
0.02
0.00
-0.02


A
DE h^2
0.00
0.25
0.50
0.75
0.00
0.25
0.50
0.75
1.00
Expression h^2
B
Associated fitness
-2
0
2
4
Body size
Biomineralization
Probability of DE heritability
0.00
0.25
0.50
0.75
1.00
-10
-5
0
5
10
15
Effect of DE on adaptive phenotypic plasticity


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
-1.0
-0.5
0.0
0.5
1.0


GO enrichment:
DE h^2 x DE body size effect
Biological Process
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
Molecular Function
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
Cellular Component
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