Non-parallel transcriptional divergence during parallel adaptation

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Abstract

How underlying mechanisms bias evolution toward predictable outcomes remains an area of active debate. In this study, we leveraged phenotypic plasticity and parallel adaptation across independent lineages of Trinidadian guppies (*Poecilia reticulata*) to assess the predictability of gene expression evolution during parallel adaptation. We observed substantial gene expression plasticity as well as the evolution of expression plasticity itself across populations. Genes exhibiting expression plasticity *within* populations were more likely to also differ in expression *between* populations, with the direction of expression divergence more likely to be opposite than in the same direction as plasticity. While we also found more overlap than expected by chance in genes differentially expressed between high- and low-predation populations from different lineages, the majority of differentially expressed genes were not shared between distinct evolutionary lineages. Our data suggest alternative transcriptional configurations associated with shared phenotypes, highlighting a role for transcriptional flexibility associated with parallel phenotypic evolution in a species known for rapid adaptation.

Key words: *Poecilia reticulata*, transcriptomics, phenotypic plasticity, adaptation, parallel evolution

Introduction

Phenotypic evolution is biased by the mechanisms that link genetic variation to phenotypic variation, i.e. the genotype-phenotype map (Alberch, 1991; Pigliucci, 2010). Mechanisms linking genotype to phenotype are characterized by both robustness and flexibility. Robust developmental processes buffer phenotypes from noise such that multiple transcriptional, biochemical, or cellular network configurations give rise to similar organismal phenotypes, while flexibility in these processes allows for phenotypic plasticity in response to environmental conditions (Marder & Goaillard, 2006; Siegal & Leu, 2014; Wagner, 2011). Though contrasting, both phenomena are ultimately bounded by phenotypic, developmental, genetic, and mechanistic constraints. The complex nature of the genotype-phenotype map and the existence of 'many-to-one' mappings across hierarchical levels of biological organization make it challenging to understand how mechanistic properties facilitate or constrain adaptive evolution. Here, we combine studies of phenotypic plasticity and parallel adaptation to assess how mechanistic biases shape evolutionary trajectories across timescales.

Studies of parallel adaptation can reveal biases in mechanisms of evolution by asking whether similar mechanism are associated with repeated, independent evolutionary transitions. Similar mechanisms underlying parallel evolutionary transitions suggest the genotype-phenotype map is constrained by limits on the possible ways to construct adaptive phenotypes (Losos, 2011). In contrast, non-shared mechanisms suggest that mechanistic flexibility may facilitate evolution by providing multiple alternative 'solutions' to a given adaptive problem and providing alternative paths by which organisms can reach adaptive peaks (Badyaev & Morrison, 2018; Drion, O'Leary, & Marder, 2015; Grashow, Brookings, & Marder, 2009). Such mechanistic flexibility may allow organisms to more easily reach the same adaptive peak from alternative starting points, and additionally buffer organisms from pleiotropic effects via compensatory mechanisms that rely on the many-to-one mappings created by these alternative paths.

58 One potential consequence of the biological robustness generated by many-to-one
59 mappings is that variation in the function of only a small number of key genes will alter organismal
60 phenotypes while variation in most genes has no phenotypic consequences (Yang, Maclean,
61 Park, Zhao, & Zhang, 2017), either because variation does not propagate to higher levels of
62 organization and/or because variation is compensatory. In this case, phenotypic evolution may
63 rely on a limited number of mechanistic paths, repeatedly targeting those mechanisms that that
64 yield the greatest phenotypic responses with the smallest pleiotropic costs. Indeed, compelling
65 examples demonstrate that similar phenotypes share underlying neural, physiological, molecular,
66 and/or genetic mechanisms, even across highly divergent taxa (e.g. Insel and Young, 2000;
67 Manceau et al., 2010; Pankey et al., 2014; Rosenblum et al., 2010). In contrast, other studies
68 demonstrate flexibility in underlying mechanisms suggesting that different mechanistic 'solutions'
69 can give rise to shared phenotypes in closely related species, among populations of the same
70 species, or even among individuals of the same population (e.g. Abouheif and Wray, 2002;
71 Crawford and Oleksiak, 2007; Drion et al., 2015; Grashow et al., 2009; Mandic et al., 2018).
72 Nevertheless, even if mechanistic flexibility is a common feature of robust biological networks,
73 shared genetic background or patterns of pleiotropy in a lineage may still direct evolutionary paths
74 toward predictable mechanistic pathways, especially at long evolutionary timescales (Gompel &
75 Prud'homme, 2009; Stern & Orgogozo, 2008). Empirical evidence for both shared and distinct
76 mechanisms underlying parallel phenotypic evolution leaves open the question of when and why
77 either pattern dominates.

78 One feature of biological networks that may channel divergence into particular paths is
79 environmentally induced plasticity. First, both plastic and evolutionary processes may rely on
80 those mechanistic paths that yield the greatest phenotypic responses. In this case, we expect
81 plastic and evolutionary responses to share underlying mechanisms, although they may not act
82 in the same direction. Theory predicts that plastic and evolutionary changes will be in the same
83 direction when plasticity in a novel environment is adaptative, increasing immediate survival and

84 allowing time for evolutionary divergence via co-option of mechanisms involved in environmentally
85 induced responses (Baldwin, 1896; Ghalambor, McKay, Carroll, & Reznick, 2007; Lande, 2009;
86 West-Eberhard, 2003). In contrast, plastic and evolutionary changes will be in opposite directions
87 when plasticity in a novel environment is non-adaptive thereby increasing the strength of selection
88 or when plastic responses 'overshoot' adaptive optima and are compensated by selection
89 (Conover, Duffy, & Hice, 2009; Ghalambor et al., 2007; Grether, 2005; Velotta & Cheviron, 2018).
90 Importantly, plasticity may facilitate adaptation under either scenario and empirical studies
91 document both patterns. In addition, plastic and evolutionary processes may rely on shared
92 mechanistic paths because the mechanisms mediating phenotypic plasticity promote the
93 accumulation of cryptic genetic variation that is released under new environmental conditions,
94 thereby fostering associations between plasticity and divergence (Draghi & Whitlock, 2012;
95 Espinosa-Soto, Martin, & Wagner, 2011). Finally, while associations between phenotypic
96 plasticity and evolution have primarily been considered in the context of phenotypic change,
97 associations between developmental plasticity and genetic divergence at the level of underlying
98 mechanisms could also arise if initially plastic compensatory or homeostatic responses to
99 maintain, rather than alter, organism level phenotypes become genetically fixed as adaptation
100 proceeds (Velotta & Cheviron, 2018), in which case we may also expect the evolution of plasticity
101 itself.

102 In the present study, we take advantage of parallel phenotypic evolution in independent
103 lineages of Trinidadian guppies (*Poecilia reticulata*) to explore patterns of flexibility and constraint
104 in transcriptional mechanisms mediating repeated adaptation. We compare whole-brain gene
105 expression patterns based on developmental experience and genetic background to test four
106 hypotheses: (1) that genes with significant expression plasticity are more likely to show genetic
107 divergence in expression, (2) that the direction of plastic responses predicts the direction of
108 genetic divergence, (3) that gene expression plasticity itself evolves, and (4) that parallel
109 phenotypic adaptation across independent lineages relies on shared gene expression changes.

110

111 *Guppies as a model system*

112 Guppies have become a model system in ecology and evolutionary biology due to
113 repeated, independent adaptation of natural populations to distinct predation environments
114 (Endler, 1995; Haskins, Haskins, McLaughlin, & Hewitt, 1961; D. Reznick, Butler IV, & Rodd,
115 2001). In Trinidad, high- and low-predation population pairs from different river drainages
116 represent independent evolutionary lineages (Barson, Cable, & Van Oosterhout, 2009; Fraser,
117 Künstner, Reznick, Dreyer, & Weigel, 2015; Gilliam, Fraser, & Alkins-Koo, 1993; Willing et al.,
118 2010) in which colonization of low-predation environments has led to parallel, adaptive changes
119 in life history traits, morphology, and behavior (Endler, 1995; Magurran, 2005; D. A. Reznick,
120 Bryga, & Endler, 1990; D. Reznick et al., 2001; D. N. Reznick, 1997). For example, low-predation
121 guppies shoal less tightly (Huizinga, Ghalambor, & Reznick, 2009; Magurran & Seghers, 1990b,
122 1991), escape more slowly (Ghalambor, Reznick, & Walker, 2004), are slower to re-commence
123 movement following a predator encounter (Elvidge, Ramnarine, & Brown, 2014; Harris,
124 Ramnarine, Smith, & Pettersson, 2010), and perform fewer predator inspections (Magurran &
125 Seghers, 1990a, 1994) than their high-predation counterparts.

126 Building on decades of work comparing high- and low-predation populations from the wild,
127 more recent laboratory breeding designs have disentangled genetic and environmental influences
128 of predation on phenotypic differences. These studies demonstrate that a combination of genetic
129 and environmental influences shape guppy life history (Torres Dowdall et al., 2012), morphology
130 (Fischer, Soares, Archer, Ghalambor, & Hoke, 2013; C. A. Handelsman, Ruell, Torres-Dowdall,
131 & Ghalambor, 2014; Ruell et al., 2013; Torres-Dowdal, Handelsman, Reznick, & Ghalambor,
132 2012), physiology (Fischer, Harris, Hofmann, & Hoke, 2014; Corey A. Handelsman et al., 2013),
133 and behavior (Fischer, Ghalambor, & Hoke, 2016b; Huizinga et al., 2009; Torres-Dowdal et al.,
134 2012). Yet despite many years of work at the level of organism level phenotypes, the mechanisms
135 underlying adaptive phenotypic differences in guppies remain largely unexplored. A single study

136 characterized brain gene expression in multiple populations from the same lineage during the
137 earliest stages of adaptation (~3 generations after colonization of low-predation environments)
138 and found a negative relationship between phenotypic plasticity and adaptive divergence
139 (Ghalambor et al., 2015).

140 In the current study, we compare the effects of genetic background (high- versus low-
141 predation populations) and developmental environment (rearing with and without predator cues)
142 on brain gene expression patterns in two parallel, independent evolutionary lineages of guppies.
143 These lineages diverged at least 600,000 years ago, with subsequent, more recent colonization
144 of low-predation environments by high-predation ancestors within each river drainage (Fajen &
145 Breden, 1992; Willing et al., 2010). We here compare whole-brain gene expression patterns *within*
146 and *between* lineages based on evolutionary history with and rearing with predators. We focus
147 on gene expression specifically in the brain because the previous gene expression study in
148 guppies used brain tissue (Ghalambor et al., 2015) and because of previous evidence for
149 behavioral plasticity in our focal populations (Fischer et al., 2016b).

150 To test our hypotheses we (1) quantify the extent of overlap in genes exhibiting significant
151 expression plasticity as well as genetic expression divergence within each drainage, (2) examine
152 associations in the direction of plastic and genetic expression differences, (3) ask whether
153 expression plasticity itself evolves, and (4) assess whether parallel phenotypic adaptation across
154 lineages relies on shared gene expression mechanisms. First, if developmental plasticity does
155 indeed predict genetic divergence, we expect a within-lineage association in gene identity and/or
156 expression direction among those genes showing plastic and genetic expression differences.
157 Second, if gene expression plasticity itself evolves, we expect the extent and/or direction of plastic
158 responses to differ between genetic backgrounds. Finally, if only a few gene expression
159 configurations (i.e. mechanistic paths) can give rise to shared adaptive behavioral phenotypes,
160 we expect parallel adaptation to low-predation habitats to be characterized by parallel evolution
161 in a set of genes that are largely shared between lineages. In contrast, if transcriptional

162 mechanisms are flexible, we expect gene expression divergence in largely non-overlapping gene
163 sets. Taken together, our results allow us to assess the flexibility of transcriptional mechanisms
164 of adaptation across timescales – from developmental plasticity, to genetic divergence, to parallel
165 adaptation across lineages.
166

167 **Material and Methods**

168 *Fish collection and rearing*

169 We established lab populations of guppies from high- and low-predation populations pairs
170 collected from the Aripo and Quare river drainages in 2012 and 2014, respectively. To maintain
171 the genetic variation of the original wild-caught fish, we established 20 - 30 unique family lines for
172 each population (i.e., for each generation a single female from each family was crossed to a single
173 male from another family) (D. N. Reznick & Bryga, 1987). We used second-generation lab born
174 fish from these unique family lines in this study to minimize environmental and maternal effects.
175 At birth, we split second-generation siblings into rearing environments with (pred+) or without
176 (pred-) predator chemical cues, and they remained in these environments until the completion of
177 the experiment (as in Fischer et al., 2016). We used only mature males in this study. To maximize
178 the range of genetic variation captured among focal fish, all males in a given experimental group
179 (i.e. population and rearing environment) were from distinct families; however, our split-brood
180 design allowed us to simultaneously leverage genetic similarity among siblings and control for the
181 effects of genetic background on developmental responses. Figure 1 provides an overview of our
182 experimental design and interpretation of comparisons.

183 All guppies were individually housed in 1.5 liter tanks on a 12:12 hour light cycle at
184 Colorado State University. Fish were fed a measured food diet once daily, receiving Tetramin™
185 tropical fish flake paste and hatched *Artemia* cysts on an alternating basis. Prior to tissue
186 collection for this study, behavioral and hormone data were collected in an identical manner for
187 all fish and these results are reported elsewhere (Fischer et al., 2016b). All experimental methods

188 were approved by the Colorado State University Animal Care and Use Committee (Approval #12-
189 3818A).
190
191 *Tissue collection and processing*
192 We collected brain tissue from the Aripo and Quare lineage males described above in
193 2013 and 2015, respectively. To standardize any effects of recent experience, behavior, and
194 circadian rhythm on gene expression, we collected whole brains within 10 minutes of lights-on.
195 We interpret our transcriptional data as baseline, in the sense that fish were minimally stimulated
196 prior to tissue collection, and expression levels should therefore reflect genetic background and
197 developmental experience more strongly than responses to immediate environmental context.
198 Fish were anesthetized by immersion in ice water followed by rapid decapitation. Whole brains
199 were removed, flash frozen in liquid nitrogen, and stored at -80°C until further processing. Tissue
200 collection took <2 minutes, rapid enough to minimize changes in gene expression responses to
201 handling and dissection.
202 We extracted total RNA from brain tissue using the Qiagen RNeasy Lipid Tissue Mini Kit
203 (Qiagen, Germany) following manufacturer guidelines. We prepared separate sequencing
204 libraries for each individual using the NEBNext Ultra RNA Library Prep Kit for Illumina (New
205 England Biolabs, Massachusetts, USA) following manufacturer instructions. Libraries were
206 sequenced on an Illumina HiSeq 2000 at the Florida State University College of Medicine
207 Translational Science Laboratory (Tallahassee, Florida) in May 2014 (Aripo dataset) and January
208 2016 (Quare dataset). For the Aripo dataset, 40 samples (N=10 per group) were combined with
209 unique barcodes into eight samples per pool and each pool was sequenced on a single lane. For
210 the Quare dataset, 60 samples (N=12-14 per group) were combined into three pools with 20
211 samples per pool and each pool was sequenced in two separate lanes. Blocks of individuals run
212 in the same week were processed and sequenced together, and experimental groups were
213 balanced across sequencing lanes.

214

215 *Transcript abundance estimation*

216 We received 1.45 billion 100-bp paired-end reads that passed the HiSeq quality filter,
217 averaging 14.5 million reads per sample. We used Rcorrector to amend Illumina sequencing
218 errors (Song & Florea, 2015), and removed adapter sequences and trimmed reads for high-quality
219 sequence using Trim Galore! (Babraham Bioinformatics, Babraham Institute). Following
220 developer recommendations, we used a quality score of 33, a stringency of 5, and a minimum
221 read length of 36bp. We aligned corrected, trimmed reads from both datasets to the guppy
222 genome (http://uswest.ensembl.org/Poecilia_reticulata; downloaded November 2019) and
223 estimated transcript abundance using STAR (Dobin et al., 2013) with default parameters. On
224 average, 91% (range: 88.8% – 94.2%) of sequences per individual mapped to the guppy genome
225 assembly.

226

227 *Data filtering and screening*

228 Preliminary cluster analyses revealed retinal contamination in a subset of our Aripo
229 dataset brain samples. While opsins are expressed at low levels in the brain, the very high
230 expression levels (>10,000 copies) in three samples pointed to retinal contamination. To deal with
231 this issue, we devised a sample filtering and screening procedure to remove genes in which
232 expression differences between samples were likely dominated by retinal contamination. Briefly,
233 we first filtered genes with low expression, then we used contigs annotated as known retinal genes
234 (Rhodopsin, red/green-sensitive opsins, blue-sensitive opsins) as seed contigs to identify other
235 contamination-related transcripts based on high positive correlations of expression levels with
236 seed genes. We calculated the gene-wise sum of correlations between candidate genes and seed
237 genes and performed multiple hypothesis testing using a false discovery rate (FDR) controlling
238 procedure. The nominal level of FDR was set to α=0.2 to remove presumptive contaminant
239 contigs. Using this approach, we identified 1,559 contigs as presumptive retina-enriched genes

240 (~ 3% of all contigs in our final assembly) which we removed from both datasets in all subsequent
241 analyses (Table S1). More detailed descriptions of statistical procedures are in the Supplemental
242 Methods.
243
244 *Differential expression analysis*
245 Due to differences in timing of fish rearing, sample processing, and sequencing, we did
246 not combine Aripo and Quare datasets for statistical analysis. We instead performed analysis in
247 an identical fashion for both drainages and subsequently conducted separate analyses to test
248 hypotheses concerning mechanistic parallelism across drainages (see below). Standard
249 differential expression analysis packages could not accommodate the random effects in our
250 experimental design (i.e. family and week; see below) but including these effects improved model
251 fit and reflected our split-brood experimental design. Upon fitting a generalized linear mixed model
252 with negative binomial link to each data set (detailed in the Supplemental Methods), a portion of
253 genes had statistically significant random effects due to either family or week or both. Model
254 comparisons between the generalized linear model without random effects and the generalized
255 linear mixed model including week and family effects supported incorporating random effects, as
256 likelihood ratio tests indicated better fit of the mixed models for 391 out of 19,004 genes in the
257 Aripo dataset and 1,194 out of 19,902 genes in the Quare dataset. For consistency, we performed
258 differential expression analysis using generalized linear mixed models with random effects for all
259 genes as further detailed in the Supplemental Materials.
260 We normalized read counts using DESeq2 in R (Love, Huber, & Anders, 2014) and
261 performed differential expression analysis using the lme4 package in R (github.com/lme4).
262 Normalized count data were modeled using a generalized linear mixed model with negative
263 binomial distribution. We included population of origin, rearing environment, and their interaction
264 as fixed effects. In addition, we included family (siblings split across rearing environments) and
265 week (tissue was collected from animals in balanced blocks across multiple weeks) as random

266 effects. A Wald's test provided p-values for main effects and interaction effects for each gene
267 (Lehmann & Romano, 2005). We adjusted p-values for multiple hypothesis testing using a direct
268 approach for FDR correction (Storey, 2002) as implemented in the fdrtool package in R (Strimmer,
269 2008). We considered transcripts differentially expressed (DE) if the adjusted p-value for an effect
270 was below 0.05. To examine whether differential expression calls were influenced by transcript
271 abundance, we compared mean and median counts of DE and non-DE genes using two sample
272 t-tests and Wilcoxon rank sum tests. We also compared overall patterns of gene-wise variance
273 between DE and non-DE groups with respect to either population or rearing effect using Siegel-
274 Tukey tests and Kolmogorov-Smirnov tests. We performed GO term enrichment analysis for all
275 sets of DE transcripts using annotation information for 'Biological Processes' in the topGO
276 package in R (Alexa & Rahnenfuhrer, 2016).
277
278 *Overlap in identity and expression direction of DE gene sets within lineages*
279 To determine if transcripts exhibiting expression plasticity in response to rearing with
280 predators were more likely to also exhibit evolutionary divergence between high- and low-
281 predation populations, we evaluated overlap in transcripts differentially expressed between
282 populations and between rearing conditions as well as their directions of expression change. To
283 test the hypothesis that initial plastic responses following colonization of low-predation
284 environments might drive subsequent divergence, we specifically compared pattern of ancestral
285 plasticity (i.e. high-predation fish reared with and without predators) to genetic divergence (high-
286 versus low-predation fish reared in the novel predator-free environment) within each drainage.
287 We used post-hoc tests of simple effects to identify DE genes in these comparisons and estimate
288 log-fold gene expression changes. We then used log-fold expression changes to determine
289 whether gene expression differences were in the same/concordant direction (i.e. upregulated in
290 high-predation populations and in response to rearing with predator cues, or down-regulated in
291 high-predation populations and in response to rearing with predator cues) or opposite/non-

292 concordant direction (upregulated in high-predation populations but down-regulated in response
293 to rearing with predators, or vice versa).

294 Within each drainage, we compared the overlap in genes with both statistically significant
295 genetic and plastic expression changes. We compared the association between directions of
296 plasticity and divergence (concordant vs. non-concordant) in (1) those genes with both significant
297 ancestral plasticity and significant population differences, and (2) all transcripts with significant
298 population differences in expression, including those without significant expression plasticity. The
299 second comparison addressed the possibility that even subthreshold expression plasticity in
300 response to rearing environment could influence patterns of expression divergence, especially
301 given the high false negative rate in our conservative DE analyses. Because these comparisons
302 rely on two comparisons to a single group (high predation fish reared without exposure to predator
303 cues), the estimates of plasticity and divergence are dependent. We adapted a parametric
304 bootstrap method (Efron & Tibshirani, 1993) similar to the approach implemented by (W. C. Ho &
305 Zhang, 2019) but accounting for conditional probabilities. We tested whether the conditional
306 probabilities of gene expression divergence differed for genes that exhibited ancestral plasticity
307 and those that did not. We further tested whether conditional probabilities for upregulated or
308 downregulated genes in the low predation population compared to the high predation population
309 differed depending on the direction of plasticity. We calculated parametric bootstrap confidence
310 intervals for these differences in conditional probabilities as detailed in the Supplemental
311 Methods, with confidence intervals that do not include zero indicating association between
312 divergence and plasticity.

313

314 *Analysis of evolution of expression plasticity*

315 To test for the evolution of plasticity in gene expression responses, we examined the
316 subset of transcripts with significant interaction effects in our differential expression analysis (see
317 above). We grouped genes with significant interaction effects into categories for the evolution of

318 plasticity outlined by Renn and Schumer (2013) based on *post hoc* differences between rearing
319 environments within a population ($p<0.05$): (1) Assimilated: plasticity in the ancestral high-
320 predation population but a loss of plasticity in the derived low-predation population; (2)
321 Accommodated: a change in the degree, but not the direction, of plasticity in the derived as
322 compared to the ancestral population; (3) Reversed: opposing directions of plasticity in high-
323 versus low-predation populations; (4) Evolved plastic: no plasticity in the ancestral high-predation
324 population but an emergence of plasticity in the derived low-predation population; and (5)
325 Unclassified: genes that had a significant main interaction effect but no significant *post hoc* rearing
326 differences and could therefore not be unambiguously classified into one of the other categories.
327 All statistical tests and data visualization were performed in R (version 3.5.1; The R Foundation
328 for Statistical Computing).
329
330 *Parallelism in DE gene identity and expression direction between lineages*
331 To ask whether parallel phenotypic changes across independent lineages rely on shared
332 gene expression changes, we evaluated overlap in DE transcript identity and expression direction
333 between the Aripo and Quare lineage datasets. We used chi-square tests of independence to test
334 for greater than chance overlap *between* drainages in those gene sets differentially expressed
335 based on rearing environment or population of origin *within* drainages, as well as to test for biases
336 in concordant versus non-concordant expression between drainages. We performed two
337 additional comparisons to address potential bias in our conclusions due to high false negative
338 rates in gene expression data (Rice, Schork, & Rao, 2008). First, we performed the same analysis
339 as before at two less stringent p-value cut-offs ($p=0.1$ and 0.2). Second, for comparisons of
340 expression direction, we made an additional, more conservative comparison by including not only
341 those genes differentially expressed in *both* drainages (i.e. the intersection of DE gene lists), but
342 also those differentially expressed in *either* drainage (i.e. the union of DE gene lists) in the

343 analysis. We again used log-fold expression differences to call gene expression as being in the
344 same/concordant or opposite/non-concordant direction between drainages.

345 We also performed one addition comparison to assess parallelism in a subset of the
346 population DE genes likely to arise from selection rather than other evolutionary processes, such
347 as drift. We identified to those genes mostly likely to have diverged by selection, rather than by
348 drift, by calculating P_{ST} (a measure of phenotypic divergence between populations) from
349 phenotypic variance components and comparing it to published estimates of F_{ST} (a measure of
350 neutral genetic divergence between populations) for the focal populations, using the method of
351 Leinonen, Cano, Mäkinen, & Merilä (2006). For these calculations, we made the conservative
352 assumption that heritability (h^2) of transcript abundance = 0.5 as we have done previously
353 (Ghalambor et al., 2015), and obtained estimates of within and between population variance from
354 general linear mixed models (see Supplemental Methods). We then restricted DE gene sets in
355 both lineages to those in which $P_{ST} > F_{ST}$ and compared the number of overlapping genes and
356 their relative expression direction using chi-square tests as above. We used F_{ST} estimates for our
357 focal populations (Aripo = 0.225; Quare = 0.340) reported by Willing et al. (2010).

358

359 **Results**

360 *Differential gene expression and GO term enrichment*

361 Before testing our specific hypotheses, we first summarized differential expression
362 patterns in both datasets. In the Aripo drainage, 766 genes were differentially expressed (DE)
363 between high- and low-predation populations, 1,118 genes were differentially expressed between
364 pred- and pred+ fish, and 825 genes had interaction effects (Fig. 2; Table S2). DE genes were
365 enriched for GO categories including DNA integration, immune function, and morphogenesis. In
366 the Quare drainage, 3,831 genes were differentially expressed between high- and low-predation
367 populations, 791 genes were differentially expressed between pred- and pred+ fish, and 586
368 genes had interaction effects (Fig. 2; Table S3). DE genes were enriched for GO categories

369 including immune function, developmental processes, and neurogenesis. Complete results of GO
370 enrichment analyses for population of origin, rearing, and interaction effects are in the
371 Supplemental Materials (Tables S4 & S5).

372 Given the marked difference in the total number of DE genes between datasets, we looked
373 for evidence that this pattern might arise from technical variation or expression bias. While we
374 cannot rule out some influence of technical variation, we found no evidence for differences in
375 sequence quality, read alignment statistics, or variance in transcript abundance to suggest that
376 variation in the number of DE transcripts between drainages was of technical origin. Nor did we
377 find evidence that identification of DE transcripts was biased by transcript expression level: mean
378 and median gene expression were significantly lower in DE (population, rearing, and/or interaction
379 effects) as compared to non-DE transcripts in both the Aripo (Fig. S1; mean: DE=263, non-
380 DE=344, $p<0.0001$; median: DE=65, non-DE=107, $p<0.0001$), and Quare (Fig. S1; mean:
381 DE=264, non-DE=334, $p<0.0001$; median: DE=86, non-DE=101, $p<0.0001$) datasets. This
382 suggests that identification of DE transcripts was not simply a side effect of relatively greater
383 expression of these transcripts.

384

385 *Expression plasticity biases the propensity for and direction of genetic expression divergence*

386 Evolutionary theory and empirical studies suggest that environmentally induced plasticity
387 may facilitate adaptation to novel environments. To test this prediction, we first asked whether
388 genes with significant ancestral expression plasticity were more likely to show genetic divergence
389 in expression. To compare expression changes upon colonization of low-predation environments
390 by high-predation fish to those present after generations of adaptation to low-predation
391 environments, we examined the extent of overlap in gene sets differentially expressed between
392 high-predation fish reared with and without predators and high- versus low-predation fish reared
393 in a shared, predator-free environment. In both drainages, the DE genes with significant ancestral
394 plasticity were more likely to show significant population divergence than expected by chance

395 (Aripo: 264 transcripts, 95% CI for differences in conditional probability of significant divergence
396 for plastic genes compared to conditional probability of divergence for non-plastic genes: [0.125,
397 0.150]; Quare: 409 transcripts, 95% CI: [0.211,0.239]), supporting an association between plastic
398 responses and genetic divergence.

399 We next asked whether the directions of expression plasticity and genetic divergence were
400 associated. We did so for those genes with both significant ancestral plasticity and population
401 differences, as well as the larger set of all transcripts with significant population differences (i.e.
402 including those without significant expression plasticity). We performed the second, more
403 conservative comparison because we reasoned that even subthreshold expression plasticity
404 could influence the direction of adaptive divergence. Within both drainages, we found a signature
405 of non-adaptive plasticity associated with genetic expression divergence. Approximately 84% of
406 DE genes exhibited population and rearing expression changes in non-concordant directions in
407 the Aripo drainage (95% CI for difference in probability of same versus opposite expression
408 direction: [-0.564,-0.499]), and 68% of DE genes exhibiting non-concordant expression in the
409 Quare drainage (95% CI: [-0.520,-0.456]) (Fig. 3). Patterns were consistent in both drainages
410 when we considered the larger set of all genes with significant population expression differences
411 (Aripo: 80% of genes with non-concordant expression, 95% CI: [-0.811,-0.752]; Quare: 66% of
412 genes with non-concordant expression, 95% CI: [-0.803,-0.733]).

413

414 *Evolution of expression plasticity*

415 We next explored the evolution of expression plasticity itself. We identified genes with
416 evolved expression plasticity as those in which the direction and/or degree of plasticity depended
417 on population of origin, i.e. those genes with significant population-by-rearing interaction effects.
418 The number of genes with significant expression plasticity that evolved (i.e. significant rearing and
419 interaction effects) was approximately a quarter to a third of the number of genes with significant

420 expression plasticity that did not evolve (i.e. significant rearing effect but not interaction; 263/1,118
421 in Aripo, 286/791 in Quare) (Fig. 2).

422 To characterize how expression plasticity evolved, we further subdivided genes with
423 significant population-by-rearing interaction effects into one of five categories: assimilated,
424 accommodated, reversed, evolved plastic, or unclassified (Fig. 4A; based on Renn and Schumer,
425 2013). We found many transcripts that exhibited evolution of plasticity, with all five categories
426 represented in both datasets (Fig. 4B). In the Aripo drainage 263 (32%) transcripts showed
427 patterns of expression assimilation, 81 (10%) transcripts showed patterns of expression
428 accommodation, 113 (14%) transcripts exhibited reversed plasticity, and 182 (22%) transcripts
429 evolved plasticity in the derived population. In the Quare drainage, 164 (28%) transcripts showed
430 patterns of expression assimilation, 110 (19%) transcripts showed patterns of expression
431 accommodation, 128 (22%) transcripts exhibited reversed plasticity, and 95 (16%) transcripts
432 evolved plasticity in the derived population (Fig. 4B).

433

434 *Parallelism in gene expression patterns across drainages*

435 To test for shared mechanisms mediating parallel phenotypic adaptation across
436 drainages, we asked whether differentially expressed genes within drainages were overlapping
437 in identity and expression direction between drainages. Of the genes that diverged between high-
438 and low-predation populations within a drainage (i.e., population main effect), 174 were
439 overlapping between drainages (Table S6), more than expected by chance (χ^2=8.02, p=0.0046).
440 However, the direction of expression divergence among these overlapping genes was not
441 associated across the two drainages (χ^2=0.16, p=0.686): 91 genes had concordant expression
442 changes and 83 genes had non-concordant expression changes between drainages (Fig. 5). To
443 control for the influence of false negatives on our conclusions, we repeated this comparison at
444 less stringent significance cut-offs. At a p=0.1, we found marginally significant overlap in gene
445 identify (347 overlapping genes; χ^2=3.38, p=0.06), but no association in expression direction

446 (χ^2=0.65, p=0.42), and at p=0.2 we found no significant overlap or association in expression
447 direction. As a final, more conservative test, we compared the subset of genes with strong
448 evidence for adaptive population divergence due to selection by restricting our comparison to
449 population DE genes for which $P_{ST} > F_{ST}$. We found 534 genes (mean P_{ST} = 0.38, F_{ST} = 0.225) in
450 the Aripo and 1163 genes (mean P_{ST} = 0.496, F_{ST} = 0.340) in the Quare drainage that met this
451 criterion. Of these, 53 were overlapping between drainages, again more than expected by chance
452 (χ^2=12.7, p=0.0004), but without any association in expression direction (28 genes had
453 concordant expression changes and 25 genes had non-concordant expression changes between
454 drainages). P_{ST} values for all genes are in Table S8.

455 Of the genes that showed significant expression plasticity (i.e. main effect of rearing), 64
456 transcripts were overlapping between drainages (Table S7), more than expected by chance
457 (χ^2=14.06, p=0.0001). Of these 43 had expression changes in the same direction and 21 had
458 expression changes in opposite directions between drainages, a significant association between
459 drainages in the direction of expression plasticity associated with rearing environment (χ^2=7.56,
460 p=0.006) (Fig. 5). At less stringent p-value cut-offs for differential expression we found no more
461 overlap in gene identity than expected by chance (p<0.1: 108 overlapping genes; χ^2=0.05, p=0.81;
462 p<0.2: 276 overlapping genes; χ^2=1.36, p=0.24).

463

464

465 **Discussion**

466 We examined transcriptomic differences associated with adaptive divergence and rearing
467 environment across repeated, independent evolutionary lineages in guppies. Within lineages, we
468 observed phenotypic plasticity in gene expression patterns as well as the evolution of gene
469 expression plasticity itself. Plastic genes were more likely to exhibit population differences in
470 expression, and these population differences were more likely to be in the opposite, rather than
471 the same, direction as ancestral expression plasticity. Comparing across lineages, gene sets

472 differentially expressed were largely non-overlapping (i.e. >75% non-shared identity) both for
473 genes that diverged between populations and for plastic genes. Nonetheless, the small number
474 of DE genes that did overlap between drainages was more than expected by chance for both
475 population divergence and plasticity. Taken together our findings suggest that a small number of
476 core genes may be repeatedly targeted during colonization of low-predation environments, but
477 that largely non-shared, alternative transcriptional solutions are associated with parallel
478 phenotypic adaptation across lineages.
479
480 *Impacts of gene expression plasticity on expression evolution*
481 A growing number of studies are using transcriptomic and proteomic surveys to address
482 the long-standing debate on the role of plasticity in evolution, with contrasting results favoring the
483 alternative hypotheses that adaptive (Fraser et al., 2014; Gleason & Burton, 2015; Li et al., 2018;
484 Mäkinen, Papakostas, Vøllestad, Leder, & Primmer, 2016; Scoville & Pfrender, 2010; Shaw et al.,
485 2014; Wang & Althoff, 2019) versus non-adaptive (Dayan, Crawford, & Oleksiak, 2015;
486 Ghalambor et al., 2015; W. Ho & Zhang, 2018; Pespeni et al., 2013; Schaum, Rost, Millar, &
487 Collins, 2013) plasticity facilitates adaptation. We recently proposed that non-adaptive plasticity
488 dominates during the earliest stages of rapid evolution, and that adaptive plasticity may contribute
489 to subsequent fine-tuning of phenotypes (Fischer et al., 2016a). In line with this prediction and
490 our previous findings, we report here that the strong signature of non-adaptive plasticity in brain
491 gene expression (89% of transcripts) observed in guppy populations in the earliest stages of
492 adaptation to low-predation environments (Ghalambor et al., 2015) is present but weaker in long-
493 established low-predation populations. Furthermore, within our data set, this signature of non-
494 adaptive plasticity was more apparent in the more recently diverged Aripo lineage (80% of genes)
495 as compared to the older Quare lineage (65% of genes). Our findings highlight the need to
496 explicitly consider how plasticity relates to divergence throughout successive stages of adaptation
497 in order to build a more holistic understanding of the role of plasticity in adaptation.

498

499 *Evolution of plasticity in gene expression*

500 Diverse transcriptional patterns could accompany evolved differences in behavioral
501 plasticity (Renn & Schumer, 2013), but relevant data characterizing the evolution of gene
502 expression plasticity in the brain has been lacking. In both lineages, we found approximately one
503 third magnitude evolution of plasticity as we did conserved plasticity. As was observed for
504 transcriptomic evolution of gill tissue in stickleback fish (Gibbons, Metzger, Healy, & Schulte,
505 2017), plasticity evolution showed no consistent pattern, with genes gaining, losing, and switching
506 the direction of expression plasticity between ancestral and derived populations in both lineages.
507 The lack of consistency in these patterns and the dearth of studies that have characterized the
508 evolution of gene expression plasticity make these patterns difficult to interpret at present. Some
509 of the diversity is likely associated with adaptive phenotypic divergence between high- and low-
510 predation populations, in which initially plastic behavioral shifts may become fixed, eliminated, or
511 altered over time. For example, evolution in gene expression plasticity could reflect the gains,
512 losses, and switches in plasticity of different behaviors in these populations (Fischer et al., 2016b).
513 Alternatively, compensatory and homeostatic mechanisms could promote diversity among plastic
514 responses in gene expression without altering higher-level phenotypic traits such as morphology
515 and behavior (Badyaev, 2018; Fischer, Ghalambor, & Hoke, 2016a; Renn & Schumer, 2013).
516 Finally, because fish in low-predation habitats experience relaxed selection on predator-induced
517 plasticity in conjunction with low effective populations sizes, some of the evolution of
518 transcriptional plasticity we report likely arose as a product of genetic drift, rather than selection
519 for altered plastic responses (Lynch, 2007). Documenting the evolution of expression plasticity is
520 an important first step, and additional studies are needed to understand the ubiquity and
521 evolutionary sources of these patterns.

522

523 *Transcriptomic signatures of population divergence in two independent evolutionary lineages*

524 We identified many genes differentially expressed between high- and low-predation
525 populations in each river drainage. The absolute number of population DE transcripts was smaller
526 in the Aripo drainage as compared to the Quare drainage, but the number of developmental and
527 interaction differences was greater in the Aripo drainage. The Quare dataset was 1.5X larger than
528 the Aripo dataset (40 versus 60 total samples) and the identification of a greater number of DE
529 genes in the Quare dataset is therefore likely in part related to greater statistical power. This
530 difference may also be biological in origin, as high- and low-predation populations in the Quare
531 drainage show greater genetic divergence than those in the Aripo drainage (Willing et al., 2010).
532 Indeed, we note that the larger number of genes in the Quare dataset come from a large difference
533 the number of genes with population, but not rearing or interaction, effects. In sum, we suggest
534 that the difference in the number of differentially expressed transcripts between datasets is likely
535 a combination of greater statistical power in the larger Quare dataset and the greater degree of
536 genetic divergence in the Quare as compared to the Aripo river drainage (Willing et al., 2010).
537 When we compared DE gene sets between drainages, we found a small, but significant,
538 number of population and rearing DE genes shared across lineages. However, the majority of DE
539 genes were non-shared across drainages for both population (Aripo: 78%; Quare: 96%) and
540 rearing (Aripo: 94%; Quare: 92%) effects. Only rearing genes showed a significant association in
541 expression direction, with 67% of genes concordantly differentially expressed between lineages.
542 In contrast, only 52% of the genes that diverged when low-predation fish colonized high-predation
543 habitats in both drainages were concordantly differentially expressed. We suggest that these
544 patterns across drainages point to a small number of core genes that exhibit predictable, plastic
545 expression responses upon colonization of low-predation environments, but that lineage-specific
546 selection pressures, differences in genetic background, non-adaptive processes (e.g. drift,
547 inbreeding, founder effects), and alternative compensatory gene expression responses give rise
548 to largely non-overlapping, non-concordant expression differences associated with parallel
549 phenotypic adaptation across drainages.

550 A previous study in guppies performed a similar comparison of gene expression changes
551 associated with adaptation to low-predation environments (Ghalambor et al., 2015) and found a
552 strong signal of concordant differential expression in genes differentially expressed based on
553 population of origin. Whereas the present study compared long-term natural population
554 divergence across drainages, Ghalambor et al. (2015) characterized early stages of adaptation
555 of experimentally introduced low-predations populations derived from founders from a single high-
556 predation source population within the same drainage. These contrasting findings in comparisons
557 of population pairs within the same drainage versus across drainages highlight the impacts of
558 standing genetic variation within the source population on mechanisms of divergence (Feiner,
559 Rago, While, & Uller, 2017; Thompson, Osmond, & Schluter, 2019), particularly at early stages
560 of evolution (Barrett & Schluter, 2008): while alternative transcriptional 'solutions' are possible,
561 shared genetic background appears to bias evolutionary outcomes toward shared patterns.

562 Both adaptive and non-adaptive processes may contribute to the combination of shared
563 and distinct transcriptional mechanisms we find associated with parallel, adaptive life-history,
564 morphological, and behavioral phenotypes across lineages in guppies. First, as described above,
565 differences in standing genetic variation likely influence which mechanisms are available to
566 selection in response to common environmental conditions in different drainages (Barrett &
567 Schluter, 2008; Thompson et al., 2019). Second, low-predation populations are typically
568 established by a very small number of individuals (Barson et al., 2009; Fraser et al., 2015; Willing
569 et al., 2010), making them susceptible to the unpredictable, non-adaptive influences of founder's
570 effects, genetic drift, and/or inbreeding on gene expression divergence – although we note that
571 we found no more evidence for shared mechanisms among genes most likely under selection
572 than among all diverged genes (i.e. genes with $P_{ST} > F_{ST}$). Third, the large number of significantly
573 evolved genes that did not overlap between drainages may also represent adaptive responses to
574 drainage- or site-specific environmental factors other than predation (Fitzpatrick, Torres-Dowdall,
575 Reznick, Ghalambor, & Chris Funk, 2014; Zandonà et al., 2011). Finally, alternative

576 compensatory or homeostatic gene expression responses may arise in response to any of the
577 above factors, leading to alternative transcriptional configurations associated with similar higher-
578 level phenotypes. In other words, genetic similarity among ancestral populations may channel
579 low-predation populations within the same drainage toward shared transcriptional solutions (as in
580 Ghalambor et al. 2015), while differences in standing genetic variation, drainage-specific
581 environmental conditions, founder effects, and alternative compensatory changes could result in
582 distinct mechanistic paths to arrive at shared organism-level phenotypes. We cannot definitely
583 distinguish causal from non-adaptive and compensatory gene expression differences under these
584 scenarios – indeed, it is likely a combination of these factors that contribute to distinct
585 transcriptional patterns associated with parallel adaptation. Nonetheless, in either case,
586 alternative transcriptional patterns suggest that mechanistic flexibility and 'many-to-one' mapping
587 of gene expression to organism level phenotypes may facilitate adaptation.
588
589 *Conclusions*
590 We assessed the extent to which adaptation to common environments targets predictable
591 changes in transcript abundance across independent evolutionary events. Within lineages, genes
592 with greater expression plasticity were more likely to diverge in expression between populations,
593 but in the direction opposite of plastic changes. Despite a small number of differentially expressed
594 genes shared across drainages, parallel adaptation to low-predation environments in independent
595 lineages was associated with divergence in largely non-overlapping transcripts. While
596 identification of shared genes is generally used as the starting point for work exploring
597 mechanisms of parallel adaptation – and our work here indeed suggests that a small number of
598 core genes may indeed be critical during the earliest stages of adaptation – we propose that
599 parallel evolutionary transitions are not limited to a small set of possible transcriptional
600 mechanisms in guppies. Instead, our results highlight the potential for alternative transcriptional
601 solutions associated with parallel, adaptive trait evolution even within a single species.

602 Transcriptional network versatility, in which diverse alterative network configurations can produce
603 common network outputs and behavioral phenotypes, may allow underlying networks to
604 simultaneously accommodate the influences of selection, drift, and genetic background and
605 thereby facilitate evolution in a species known for rapid adaptation to novel environments.
606

607 **Acknowledgements**: We thank the members of the Colorado State University Guppy Group for
608 fish care, especially Sarah E Westrick and Kimberly E Dolphin for help with tissue collection. We
609 thank Laura R Stein for assistance with tissue processing and Cameron K Ghalambor for fruitful
610 discussions and comments on earlier versions of the manuscript. All high-powered computing
611 was performed on the Odyssey computing cluster supported by the FAS Science Division
612 Research Computing Group at Harvard University. This work was supported by the National
613 Science Foundation DDIG-1311680 (to EKF), RCN IOS-1256839 (to EKF), IOS-1354755 (to
614 KLH), IOS-1354775 (to KAH), IOS-0934451 (to KAH), DEB-0846175 (to CKG), IIS-1545994 (to
615 WZ), and US Department of Energy DE-SC0018344 (to WZ).
616

617 **Data Accessibility**: Raw sequencing reads will be made available through the NCBI SRA
618 repository upon publication. R code for statistical analyses will be made available on GitHub
619 upon publication.
620

621 **Author contributions**: EKF and KLH conceived of the study; EKF collected samples and
622 performed molecular work; EKF and KAH performed gene expression mapping, transcript
623 abundance estimation, and preliminary differential expression analyses; YS and WZ devised
624 and performed statistical analyses with input from EKF and KLH; EKF and KLH wrote the
625 manuscript with contributions from all authors. All authors gave final approval for publication.
626

627 **References**

628 Abouheif, E., & Wray, G. (2002). Evolution of the gene network underlying wing polymorphism in
629 ants. *Science*, *297*(2002), 249–252. doi: 10.1126/science.1071468

630 Alberch, P. (1991). From genes to phenotype: dynamical systems and evolvability. *Genetica*, *84*,
631 5–11. doi: 10.1007/BF00123979

632 Alexa, A., & Rahnenfuhrer, J. (2016). *topGO: Enrichment Analysis for Gene Ontoloty*. R package
633 version 2.30.1.

634 Badyaev, A. V. (2018). Evoulutionary transitions in controls reconcile adaptation with continuity
635 of evolution. *Seminars in Cell and Developmental Biology*, *88*, 36–45. doi:
636 10.1016/j.semcdb.2018.05.014

637 Badyaev, A. V, & Morrison, E. S. (2018). Emergent buffering balances evolvability and robustness
638 in the evolution of phenotypic flexibility. *Evolution*, *72*(3), 647–662. doi: 10.1111/evo.13441

639 Baldwin, J. M. (1896). A New Factor in Evolution. *The American Naturalist*, *30*, 441–451; 536–
640 553.

641 Barrett, R. D. H., & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends in*
642 *Ecology and Evolution*, *23*(1), 38–44. doi: 10.1016/j.tree.2007.09.008

643 Barson, N. J., Cable, J., & Van Oosterhout, C. (2009). Population genetic analysis of microsatellite
644 variation of guppies (*Poecilia reticulata*) in Trinidad and Tobago: Evidence for a dynamic
645 source-sink metapopulation structure, founder events and population bottlenecks. *Journal of*
646 *Evolutionary Biology*, *22*(3), 485–497. doi: 10.1111/j.1420-9101.2008.01675.x

647 Conover, D. O., Duffy, T. A., & Hice, L. A. (2009). The covariance between genetic and
648 environmental influences across ecological gradients: Reassessing the evolutionary
649 significance of countergradient and cogradient variation. *Annals of the New York Academy*
650 *of Sciences*, *1168*, 100–129. doi: 10.1111/j.1749-6632.2009.04575.x

651 Crawford, D. L., & Oleksiak, M. F. (2007). The biological importance of measuring individual
652 variation. *Journal of Experimental Biology*, *210*(9), 1613–1621. doi: 10.1242/jeb.005454

Dayan, D. I., Crawford, D. L., & Oleksiak, M. F. (2015). Phenotypic plasticity in gene expression contributes to divergence of locally adapted populations of *Fundulus heteroclitus*. *Molecular Ecology*, *24*(13), 3345–3359. doi: 10.1111/mec.13188

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., … Gingeras, T. R. (2013). STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics*, *29*(1), 15–21. doi: 10.1093/bioinformatics/bts635

Draghi, J. A., & Whitlock, M. C. (2012). Phenotypic plasticity facilitates mutational variance, genetic variance, and evolvability along the major axis of environmental variation. *Evolution*, *66*(9), 2891–2902. doi: 10.1111/j.1558-5646.2012.01649.x

Drion, G., O'Leary, T., & Marder, E. (2015). Ion channel degeneracy enables robust and tunable neuronal Firing rates. *PNAS*. doi: 10.1073/pnas.1516400112

Efron, B., & Tibshirani, R. J. (1993). An introduction to the bootstrap. Monographs on Statistics and Applied Probability, No. 57. Chapman and Hall, London, 436 p. In *Monographs on Statistics and Applied Probability*.

Elvidge, C. K., Ramnarine, I., & Brown, G. E. (2014). Compensatory foraging in Trinidadian guppies: Effects of acute and chronic predation threats. *Current Zoology*, *60*(3), 323–332. doi: 10.1093/czoolo/60.3.323

Endler, J. A. (1995). Multiple-trait coevolution and environmental gradients in guppies. *Trends in Ecology & Evolution*, *10*(1), 22–29. doi: 10.1016/S0169-5347(00)88956-9

Espinosa-Soto, C., Martin, O. C., & Wagner, A. (2011). Phenotypic plasticity can facilitate adaptive evolution in gene regulatory circuits. *BMC Evolutionary Biology*, *11*(5), 1–14. doi: 10.1186/1471-2148-11-5

Fajen, A. V. A., & Breden, F. (1992). Mitochondrial DNA sequence variation among natural populations of the Trinidad guppy, *Poecilia reticulata*. *Evolution*, *46*(5), 1457–1465.

Feiner, N., Rago, A., While, G. M., & Uller, T. (2017). Signatures of selection in embryonic transcriptomes of lizards adapting in parallel to cool climate. *Evolution*, *72*(1), 67–81. doi:

10.1111/evo.13397

Fischer, E. K., Ghalambor, C. K., & Hoke, K. L. (2016a). Can a Network Approach Resolve How Adaptive vs Nonadaptive Plasticity Impacts Evolutionary Trajectories? *Integrative and Comparative Biology*, *56*(5), 877–888. doi: 10.1093/icb/icw087

Fischer, E. K., Ghalambor, C. K., & Hoke, K. L. (2016b). Plasticity and evolution in correlated suites of traits. *Journal of Evolutionary Biology*, *29*(5), 991–1002. doi: 10.1111/jeb.12839

Fischer, E. K., Harris, R. M., Hofmann, H. A., & Hoke, K. L. (2014). Predator exposure alters stress physiology in guppies across timescales. *Hormones and Behavior*, *65*(2), 165–172. doi: 10.1016/j.yhbeh.2013.12.010

Fischer, E. K., Soares, D., Archer, K. R., Ghalambor, C. K., & Hoke, K. L. (2013). Genetically and environmentally mediated divergence in lateral line morphology in the Trinidadian guppy (*Poecilia reticulata*). *Journal of Experimental Biology*, *216*(16), 3132–3142. doi: 10.1242/jeb.081349

Fitzpatrick, S. W., Torres-Dowdall, J., Reznick, D. N., Ghalambor, C. K., & Chris Funk, W. (2014). Parallelism Isn't Perfect: Could Disease and Flooding Drive a Life-History Anomaly in Trinidadian Guppies? *The American Naturalist*, *183*(2), 290–300. doi: 10.1086/674611

Fraser, B. A., Janowitz, I., Thairu, M., Travis, J., Hughes, K. A., Fraser, B. A., … Hughes, K. A. (2014). Phenotypic and genomic plasticity of alternative male reproductive tactics in sailfin mollies. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20132310.

Fraser, B. A., Künstner, A., Reznick, D. N., Dreyer, C., & Weigel, D. (2015). Population genomics of natural and experimental populations of guppies (*Poecilia reticulata*). *Molecular Ecology*, *24*(2), 389–408. doi: 10.1111/mec.13022

Ghalambor, C. K., Hoke, K. L., Ruell, E. W., Fischer, E. K., Reznick, D. N., & Hughes, K. A. (2015). Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature*, *525*(7569), 372–375. doi: 10.1038/nature15256

Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-

adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, *21*(3), 394–407. doi: 10.1111/j.1365-2435.2007.01283.x

Ghalambor, C. K., Reznick, D. N., & Walker, J. A. (2004). Constraints on adaptive evolution: The functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). *American Naturalist*, *164*(1), 38–50. doi: 10.1086/421412

Gibbons, T. C., Metzger, D. C., Healy, T. M., & Schulte, P. M. (2017). Gene expression plasticity in response to salinity acclimation in threespine stickleback ecotypes from different salinity habitats. *Molecular Ecology*, *26*, 2711–2725. doi: 10.1111/mec.14065

Gilliam, J. F., Fraser, D. F., & Alkins-Koo, M. (1993). Structure of a Tropical Stream Fish Community : A Role for Biotic Interactions. *Ecology*, *74*(6), 1856–1870.

Gleason, L. U., & Burton, R. S. (2015). RNA-seq reveals regional differences in transcriptome response to heat stress in the marine snail *Chlorostoma funebralis*. *Molecular Ecology*, *24*(3), 610–627. doi: 10.1111/mec.13047

Gompel, N., & Prud'homme, B. (2009). The causes of repeated genetic evolution. *Developmental Biology*, *332*(1), 36–47. doi: 10.1016/j.ydbio.2009.04.040

Grashow, R., Brookings, T., & Marder, E. (2009). Reliable neuromodulation from circuits with variable underlying structure. *PNAS*, *106*(28), 11742–11746. doi: 10.1073/pnas.0905614106

Grether, G. F. (2005). Environmental Change, Phenotypic Plasticity, and Genetic Compensation. *The American Naturalist*, *166*(4), E115–E123. doi: 10.1086/432023

Handelsman, C. A., Ruell, E. W., Torres-Dowdall, J., & Ghalambor, C. K. (2014). Phenotypic Plasticity Changes Correlations of Traits Following Experimental Introductions of Trinidadian Guppies (*Poecilia reticulata*). *Integrative and Comparative Biology*, *54*(5), 794–804. doi: 10.1093/icb/icu112

Handelsman, Corey A., Broder, E. D., Dalton, C. M., Ruell, E. W., Myrick, C. A., Reznick, D. N.,

& Ghalambor, C. K. (2013). Predator-induced phenotypic plasticity in metabolism and rate of growth: Rapid adaptation to a novel environment. *Integrative and Comparative Biology*, *53*(6), 975–988. doi: 10.1093/icb/ict057

Harris, S., Ramnarine, I. W., Smith, H. G., & Pettersson, L. B. (2010). Picking personalities apart: Estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. *Oikos*. doi: 10.1111/j.1600-0706.2010.18028.x

Haskins, C., Haskins, E., McLaughlin, J., & Hewitt, R. (1961). Polymorphism and population structure in Lebistes reticulatus, an ecological study. In F. Blair (Ed.), *Vertebrate speciation* (pp. 320–395). Austin: University of Austin Press.

Ho, W. C., & Zhang, J. (2019). Genetic Gene Expression Changes during Environmental Adaptations Tend to Reverse Plastic Changes Even after the Correction for Statistical Nonindependence. *Molecular Biology and Evolution*, *36*(3), 604–612. doi: 10.1093/molbev/msz002

Ho, W., & Zhang, J. (2018). Evolutionary adaptations to new environments generally reverse plastic phenotypic changes. *Nature Communications*, *9*(350), 1–11. doi: 10.1038/s41467-017-02724-5

Huizinga, M., Ghalambor, C. K., & Reznick, D. N. (2009). The genetic and environmental basis of adaptive differences in shoaling behaviour among populations of Trinidadian guppies, *Poecilia reticulata*. *Journal of Evolutionary Biology*, *22*(9), 1860–1866. doi: 10.1111/j.1420-9101.2009.01799.x

Insel, T. R., & Young, L. J. (2000). Neuropeptides and the evolution of social behavior. *Current Opinion in Neurobiology*, *10*(6), 784–789. doi: 10.1016/S0959-4388(00)00146-X

Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology*, *22*(7), 1435–1446. doi: 10.1111/j.1420-9101.2009.01754.x

Lehmann, E. L., & Romano, J. P. (2005). *Testing Statistical Hypotheses* (Third Edit). New York:

Springer.

Leinonen, T., Cano, J. M., Mäkinen, H., & Merilä, J. (2006). Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. *Journal of Evolutionary Biology*, *19*, 1803–1812. doi: 10.1111/j.1420-9101.2006.01182.x

Li, L., Li, A., Song, K., Meng, J., Guo, X., Li, S., … Xu, F. (2018). Divergence and plasticity shape adaptive potential of the Pacific oyster. *Nature Ecology Evolution*, *2*, 1751–1760. doi: 10.1038/s41559-018-0668-2

Losos, J. B. (2011). Convergence, adaptation, and constraint. *Evolution*, *65*(7), 1827–1840. doi: 10.1111/j.1558-5646.2011.01289.x

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 1–21. doi: 10.1186/s13059-014-0550-8

Lynch, M. (2007). The frailty of adaptive hypotheses for the origins of organismal complexity. *PNAS*, *104*, 8597–8604. doi: 10.17226/11790

Magurran, A. E. (2005). Evolutionary ecology: the Trinidadian guppy. In *Oxford Series in Ecology and Evolution* (Vol. 19). doi: 10.1093/acprof

Magurran, A. E., & Seghers, B. H. (1990a). Population differences in predator recognition and attack cone avoidance in the guppy *Poecilia reticulata*. *Animal Behaviour*, *40*(3), 443–452. doi: 10.1016/S0003-3472(05)80524-X

Magurran, A. E., & Seghers, B. H. (1990b). Population Differences in the Schooling Behaviour of Newborn Guppies, Poecilia reticulata. *Ethology*, *84*, 334–342. doi: 10.1111/j.1439-0310.1990.tb00807.x

Magurran, A. E., & Seghers, B. H. (1991). Variation in Schooling and Aggression Amongst Guppy (*Poecilia Reticulata*) Populations in Trinidad. *Behaviour*, *188*(3–4), 214–234. doi: 10.1163/156853991X00292

Magurran, A. E., & Seghers, B. H. (1994). Predator Inspection Behaviour Covaries with Schooling

Tendency Amongst Wild Guppy, *Poecilia Reticulata*, Populations in Trinidad. *Behaviour*, *128*(1–2), 121–134. doi: 10.1163/156853994X00073

Mäkinen, H., Papakostas, S., Vøllestad, L. A., Leder, E. H., & Primmer, C. R. (2016). Plastic and evolutionary gene expression responses are correlated in European grayling (*Thymallus thymallus*) subpopulations adapted to different thermal environments. *Journal of Heredity*, *107*(1), 82–89. doi: 10.1093/jhered/esv069

Manceau, M., Domingues, V. S., Linnen, C. R., Rosenblum, E. B., & Hoekstra, H. E. (2010). Convergence in pigmentation at multiple levels: mutations, genes and function. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1552), 2439–2450. doi: 10.1098/rstb.2010.0104

Mandic, M., Ramon, M. L., Gerstein, A. C., Gracey, A. Y., & Richards, J. G. (2018). Variable gene transcription underlies phenotypic convergence of hypoxia tolerance in sculpins. *BMC Evolutionary Biology*, *18*, 163. doi: 10.1186/s12862-018-1275-1

Marder, E., & Goaillard, J. M. (2006). Variability, compensation and homeostasis in neuron and network function. *Nature Reviews Neuroscience*, *7*, 563–574. doi: 10.1038/nrn1949

Pankey, M. S., Minin, V. N., Imholte, G. C., Suchard, M. A., & Oakley, T. H. (2014). Predictable transcriptome evolution in the convergent and complex bioluminescent organs of squid. *PNAS*, *111*(44), E4736–E4742. doi: 10.1073/pnas.1416574111

Pespeni, M. H., Sanford, E., Gaylord, B., Hill, T. M., Hosfelt, J. D., Jaris, H. K., … Palumbi, S. R. (2013). Evolutionary change during experimental ocean acidification. *PNAS*, *110*(17), 6937–6942. doi: 10.1073/pnas.1220673110

Pigliucci, M. (2010). Genotype-phenotype mapping and the end of the “genes as blueprint” metaphor. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*, 557–566. doi: 10.1098/rstb.2009.0241

Renn, S. C. P., & Schumer, M. E. (2013). Genetic accommodation and behavioural evolution : insights from genomic studies. *Animal Behaviour*, *85*(5), 1012–1022. doi:

10.1016/j.anbehav.2013.02.012

Reznick, D. A., Bryga, H., & Endler, J. A. (1990). Experimentally induced life-history evolution in a natural population. *Nature*, *346*(6282), 357–359. doi: 10.1038/346357a0

Reznick, D., Butler IV, M. J., & Rodd, H. (2001). Life-History Evolution in Guppies. VII. The Comparative Ecology of High- and Low-Predation Environments. *The American Naturalist*, *157*(2), 126–140. doi: 10.1086/318627

Reznick, D. N. (1997). Life history evolution in guppies (*Poecilia reticulata*): Guppies as a model for studying the evolutionary biology of aging. *Experimental Gerontology*, *32*(3), 245–258. doi: 10.1016/S0531-5565(96)00129-5

Reznick, D. N., & Bryga, H. (1987). Life-History Evolution in Guppies (*Poecilia reticulata*): 1. Phenotypic and Genetic Changes in an Introduction Experiment. *Evolution*, *41*(6), 1370. doi: 10.2307/2409101

Rice, T. K., Schork, N. J., & Rao, D. C. (2008). Methods for Handling Multiple Testing. *Advances in Genetics*, *60*, 293–308. doi: 10.1016/S0065-2660(07)00412-9

Rosenblum, E. B., Rompler, H., Schoneberg, T., & Hoekstra, H. E. (2010). Molecular and functional basis of phenotypic convergence in white lizards at White Sands. *PNAS*, *107*(5), 2113–2117. doi: 10.1073/pnas.0911042107

Ruell, E. W., Handelsman, C. A., Hawkins, C. L., Sofaer, H. R., Ghalambor, C. K., & Angeloni, L. (2013). Fear, food and sexual ornamentation: plasticity of colour development in Trinidadian guppies. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1758), 20122019–20122019. doi: 10.1098/rspb.2012.2019

Schaum, E., Rost, B., Millar, A. J., & Collins, S. (2013). Variation in plastic responses of a globally distributed picoplankton species to ocean acidification. *Nature Climate Change*, *3*(3), 298–302. doi: 10.1038/nclimate1774

Scoville, A. G., & Pfrender, M. E. (2010). Phenotypic plasticity facilitates recurrent rapid adaptation to introduced predators. *PNAS*, *107*(9), 4260–4263. doi:

835 10.1073/pnas.0912748107

836 Shaw, J. R., Hampton, T. H., King, B. L., Whitehead, A., Galvez, F., Gross, R. H., … Stanton, B.
837 A. (2014). Natural selection canalizes expression variation of environmentally induced
838 plasticity-enabling genes. *Molecular Biology and Evolution*, *31*(11), 3002–3015. doi:
839 10.1093/molbev/msu241

840 Siegal, M. L., & Leu, J.-Y. (2014). On the Nature and Evolutionary Impact of Phenotypic
841 Robustness Mechanisms. *Annual Review of Ecology, Evolution, and Systematics*, *45*(1),
842 495–517. doi: 10.1146/annurev-ecolsys-120213-091705

843 Song, L., & Florea, L. (2015). Rcorrector: Efficient and accurate error correction for Illumina RNA-
844 seq reads. *GigaScience*, *4*(1), 1–8. doi: 10.1186/s13742-015-0089-y

845 Stern, D. L., & Orgogozo, V. (2008). The loci of evolution: How predictable is genetic evolution?
846 *Evolution*, *62*(9), 2155–2177. doi: 10.1111/j.1558-5646.2008.00450.x

847 Storey, J. D. (2002). A direct approach to false discovery rates. *Journal of the Royal Statistical*
848 *Society. Series B: Statistical Methodology*, *64*, 479–498. doi: 10.1111/1467-9868.00346

849 Strimmer, K. (2008). fdrtool: A versatile R package for estimating local and tail area-based false
850 discovery rates. *Bioinformatics*, *24*(12), 1461–1462. doi: 10.1093/bioinformatics/btn209

851 Thompson, K. A., Osmond, M. M., & Schluter, D. (2019). Parallel genetic evolution and speciation
852 from standing variation. *Evolution Letters*, *3*(2), 129–141. doi: 10.1002/evl3.106

853 Torres-Dowdal, J., Handelsman, C. a, Reznick, D. N., & Ghalambor, C. K. (2012). Local
854 adaptation and the evolution of of phenotypic plasticity in Trinidadian guppies (*Poecilia*
855 *reticulata*). *Evolution*, *66*(11), 3432–3443. doi: 10.5061/dryad.84gf5

856 Torres Dowdall, J., Handelsman, C. A., Ruell, E. W., Auer, S. K., Reznick, D. N., & Ghalambor,
857 C. K. (2012). Fine-scale local adaptation in life histories along a continuous environmental
858 gradient in Trinidadian guppies. *Functional Ecology*, *26*(3), 616–627. doi: 10.1111/j.1365-
859 2435.2012.01980.x

860 Velotta, J. P., & Cheviron, Z. A. (2018). Remodeling Ancestral Phenotypic Plasticity in Local

Adaptation : A New Framework to Explore the Role of Genetic Compensation in the Evolution of Homeostasis. *Integrative and Comparative Biology*, *58*(6), 1098–1110. doi: 10.1093/icb/icy117

Wagner, A. (2011). *The origins of evolutionary innovations: a theory of transformative change in living systems*. Oxford: Oxford University Press.

Wang, S. P., & Althoff, D. M. (2019). Phenotypic plasticity facilitates initial colonization of a novel environment. *Evolution*, *73*(2), 303–316. doi: 10.1111/evo.13676

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. doi: 10.2002/ajpa.20219

Willing, E. M., Bentzen, P., Van Oosterhout, C., Hoffmann, M., Cable, J., Breden, F., … Dreyer, C. (2010). Genome-wide single nucleotide polymorphisms reveal population history and adaptive divergence in wild guppies. *Molecular Ecology*, *19*(5), 968–984. doi: 10.1111/j.1365-294X.2010.04528.x

Yang, J. R., Maclean, C. J., Park, C., Zhao, H., & Zhang, J. (2017). Intra and interspecific variations of gene expression levels in yeast are largely neutral. *Molecular Biology and Evolution*. doi: 10.1093/molbev/msx171

Zandonà, E., Auer, S. K., Kilham, S. S., Howard, J. L., López-Sepulcre, A., O'Connor, M. P., … Reznick, D. N. (2011). Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. *Functional Ecology*, *25*(5), 964–973. doi: 10.1111/j.1365-2435.2011.01865.x

Figures

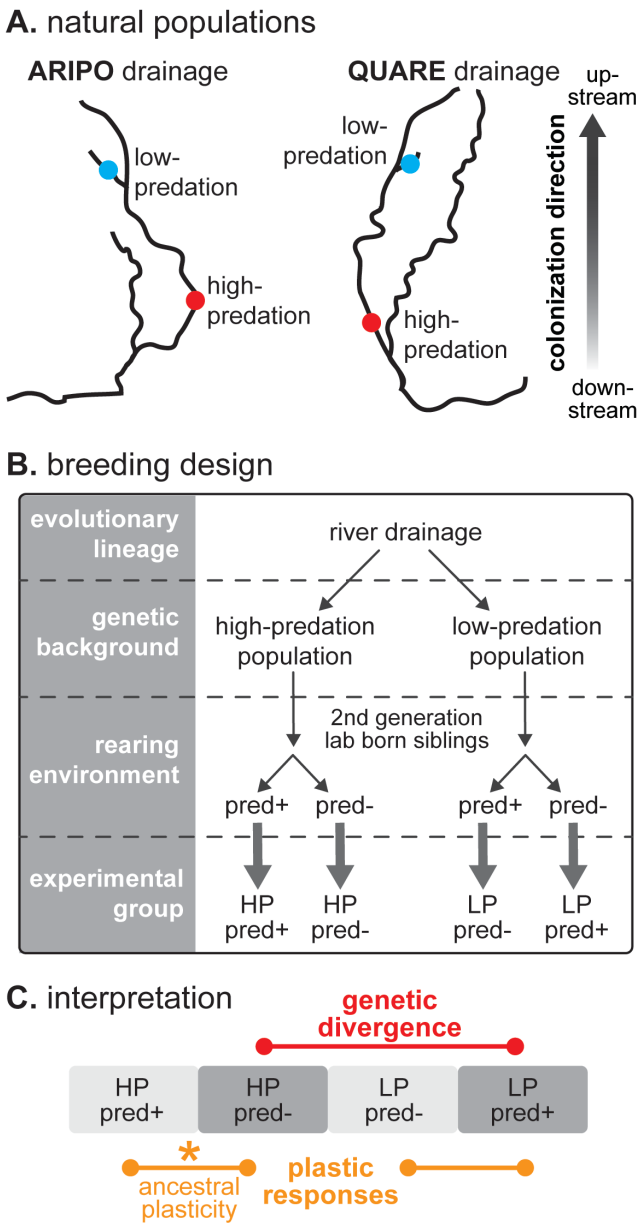

Figure 1. Overview of experimental design and interpretation. (A) Schematic shows Aripo and Quare river drainages in the Northern Range Mountains of Trinidad. We sampled population pairs from these two drainages, which represent distinct evolutionary lineages. (B) Wild-caught fish from each high- and low-predation population pair were reared to the second generation in the lab to control for maternal and environmental effects. At birth, second generation siblings from each population were split into rearing environments with (pred+) and without (pred-) predation cues. (C) This rearing design allows us to discern lineage effects (contrast between drainages), genetic divergence (contrast between different populations in the same environment), phenotypic plasticity (contrast between genetically similar fish in different rearing condition), and their interactions.

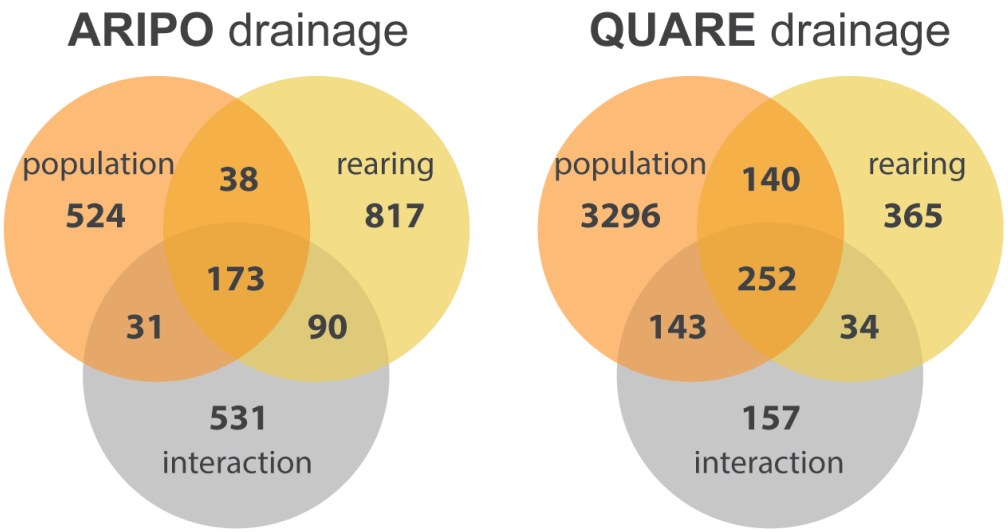

Figure 2. Summary of differential expression analyses. We identified many transcripts in both the Aripo (left) and Quare (right) drainages whose expression levels differed based on evolutionary history of predation (population of origin; orange), developmental experience of predation (rearing; yellow), and their interaction (grey).

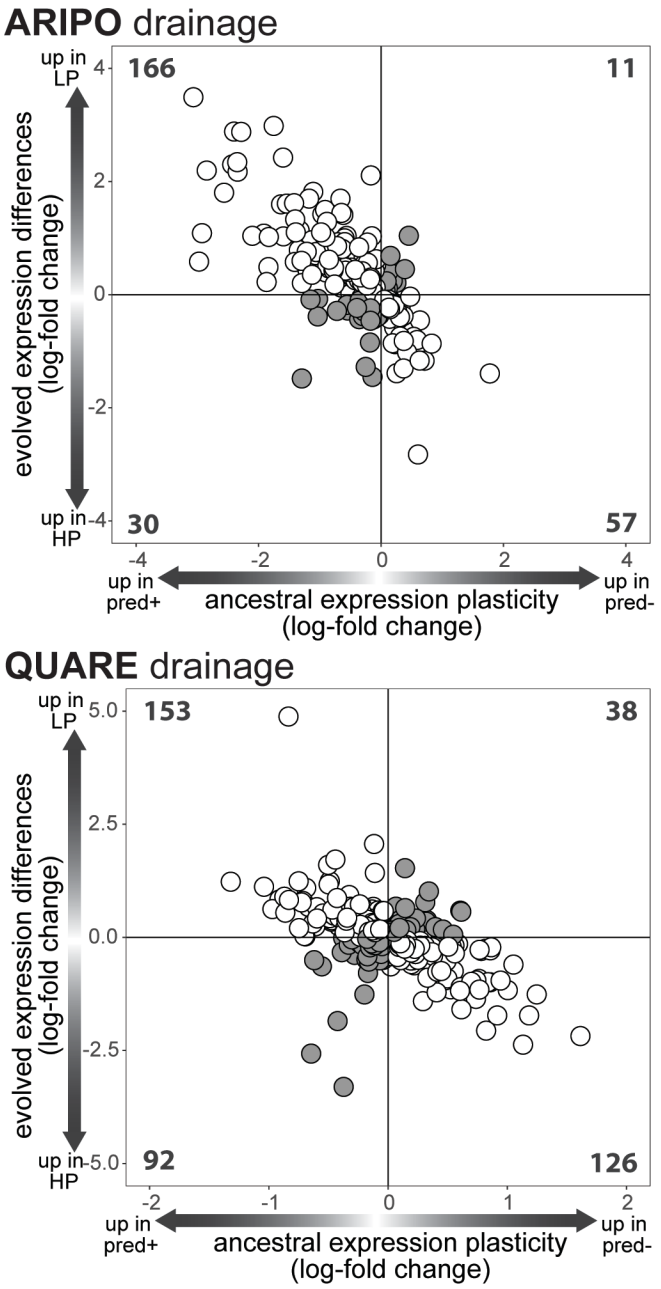

Figure 3. Relationship between ancestral plasticity and evolved expression divergence. Genes exhibiting significant ancestral expression plasticity (i.e. different between high-predation fish reared with and without predator cues) were more likely to also differ in expression between high- and low-predation populations reared without predator cue exposure in both the Aripo (top) and Quare (bottom) lineages. Each circle represents expression differences in a single transcript with statistically significant ancestral plasticity in the high-predation population as well as significant expression differences between high- and low-predation populations. Plastic expression changes were more likely to be in the opposite (white circles) than in the same (grey circles) direction as genetic expression divergence. The number of transcripts in each quadrant is indicated on the graphs. Note that axes differ between plots in order to best visualize variation within each dataset.

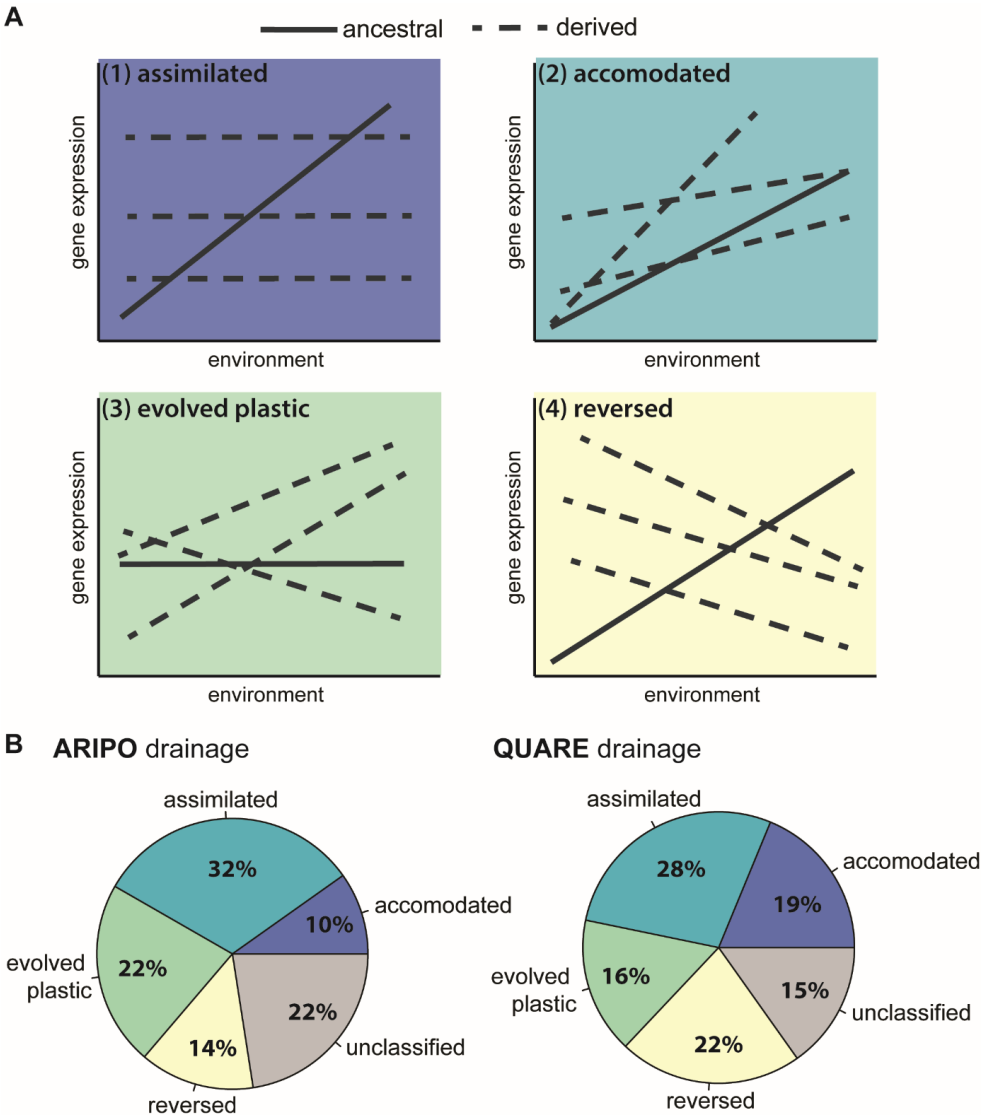

Figure 4. Evolution of transcript expression plasticity. (A) We grouped transcripts with significant interaction effects into one of four categories based on patterns of evolution in expression plasticity: (1) Assimilated: plasticity in the ancestral high-predation population but a loss of plasticity in the derived low-predation population; (2) Accommodated: a change in the degree, but no the direction, of plasticity in the derived as compared to the ancestral population; (3) Evolved plastic: no plasticity in the ancestral high-predation population but an emergence of plasticity in the derived low-predation population; (4) Reversed: opposing directions of plasticity in high- versus low-predation populations. We categorized remaining transcripts that had a significant main interaction effect, but no significant *post hoc* rearing differences as unclassified. Adapted from Renn & Schumer (2013). (B) All categories were represented in both Aripo and Quare datasets.

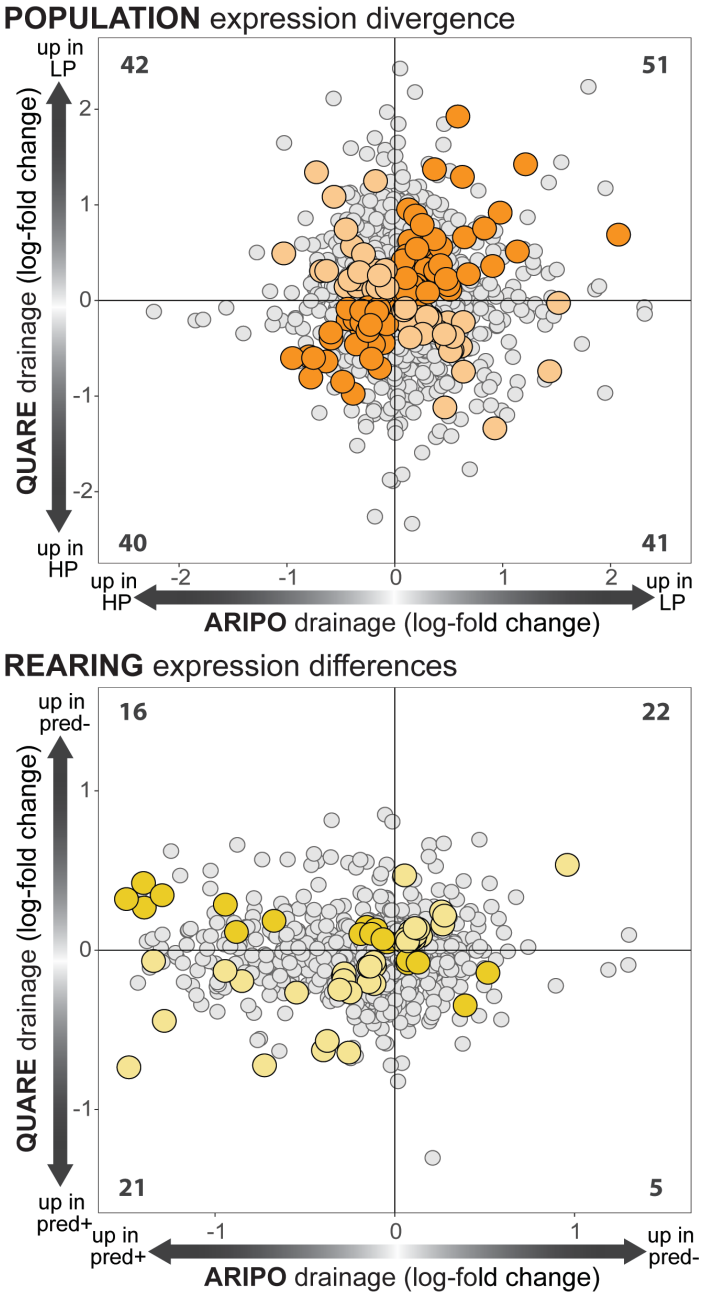

Figure 5. Overlap in population and rearing expression differences across evolutionary lineages. Of the transcripts differentially expressed based on population of origin (top), 124 were overlapping between drainages. Of these, 66 were concordantly differentially expressed (i.e. expression divergence in the same direction in both drainages; dark orange circles) and 58 were not (i.e. expression divergence in opposite directions between drainages; light orange circles), no different than expected by chance. Of the transcripts differentially expressed based on rearing environment (bottom), 35 were overlapping in the two drainages. Of these, 19 were concordantly differentially expressed (dark yellow circles) and six were not (light yellow circles), more non-concordance than expected by chance. The number of differentially expressed transcripts in each quadrant is indicated on the graphs and transcripts differentially expressed in one but not both drainages are shown in grey.

Supplemental Materials

Table S1. Transcripts filtered from the analysis following identification as retinal contaminants. Provided as Excel file.

Table S2. Transcripts significantly differentially expressed between high- and low-predation populations in the Aripo drainage. Provided as Excel file.

Table S3. Transcripts significantly differentially expressed between high- and low-predation populations in the Quare drainage. Provided as Excel file.

Table S4. Results of GO enrichment analyses for population, rearing, and interaction effects in the Aripo drainage. Provided as Excel file.

Table S5. Results of GO enrichment analyses for population, rearing, and interaction effects in the Quare drainage. Provided as Excel file.

Table S6. Population DE transcripts overlapping between Aripo and Quare drainages. 185 transcripts were DE in both drainages, of these 89 had expression differences in the same direction in both datasets (concordant differential expression). Provided as Excel file.

Table S7. Rearing DE transcripts overlapping between Aripo and Quare drainages. 16 transcripts were DE in both drainages, of these 7 had expression differences in the same direction in both drainages (i.e. concordant differential expression). Provided as Excel file.

Table S8. Pst estimates for the Aripo and Quare drainages.

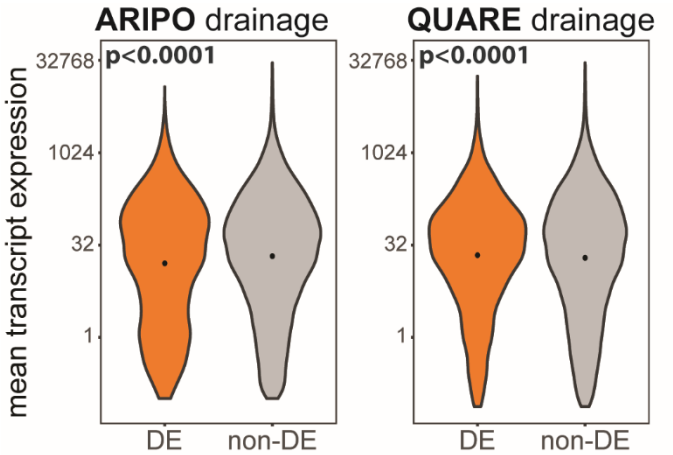

Figure S1. Differences in means but not variance of differentially expressed (DE) and non-differentially expressed (non-DE) transcripts. DE transcripts had significantly higher means in the Aripo drainage and lower means in the Quare drainage as compared to non-DE transcripts. Due to the large range of expression values, mean differences are plotted on a log scale (y-axis).