Non-parallel transcriptional divergence during parallel adaptation

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1   **Abstract**

2   Mechanisms linking genotype to phenotype must simultaneously buffer organisms from

3   developmental noise and allow for phenotypic plasticity in response to environmental cues. How

4   mechanistic robustness and flexibility in biological systems bias evolution toward predictable

5   outcomes remains an area of active debate. In this study, we leveraged phenotypic plasticity and

6   parallel adaptation across independent lineages of Trinidadian guppies (*Poecilia reticulata*) to

7   assess the predictability of transcriptional evolution during parallel adaptation. We observed

8   substantial phenotypic plasticity in gene expression patterns as well as evolution of gene

9   expression plasticity across populations. Although transcripts exhibiting expression plasticity

10   within populations were more likely to differ in expression between populations, we found no

11   consistent relationship between the direction of plasticity and evolutionary divergence. Similarly,

12   while we found more overlap than expected by chance in genes differentially expressed between

13   high- and low-predation populations from distinct lineages, the direction of expression divergence

14   was uncoupled in the two drainages, and the majority of differentially expressed genes were not

15   shared between lineages. Our data highlight transcriptional versatility associated with parallel

16   phenotypic divergence in independent evolutionary lineages of a species known for rapid

17   adaptation.

18

19   **Key words**: *Poecilia reticulata*, transcriptomics, phenotypic plasticity, adaptation, convergent

20   evolution

21 **Introduction**

22   Phenotypic evolution is biased by the mechanisms that link genetic variation to phenotypic

23 variation, i.e. the genotype-phenotype map (Alberch 1991). Mechanisms linking genotype and

24 phenotype are characterized by both robustness and flexibility. Robust developmental processes

25 buffer phenotypes from noise such that multiple transcriptional, biochemical, or cellular network

26 configurations may give rise to similar organismal phenotypes while flexibility in these processes

27 nonetheless allows for phenotypic plasticity in response to environmental conditions (Wagner

28 2011; Gutierrez et al. 2013). The complex nature of the genotype-phenotype map makes it

29 challenging to understand how mechanistic properties facilitate or constrain adaptive evolution.

30 In this study, we combine studies of phenotypic plasticity and parallel adaptation across

31 independent lineages to assess how mechanistic biases shape evolutionary trajectories across

32 timescales.

33   One feature of biological networks that may channel divergence into particular paths is

34 environmentally induced plasticity. The relationship between phenotypic plasticity and genetic

35 divergence is of long-standing theoretical and empirical interest. On the one hand, plasticity may

36 constrain adaptation by allowing successful phenotypes to be attained without genetic change,

37 effectively shielding organisms from selection (Price et al. 2003; West-Eberhard 2003; Ghalambor

38 et al. 2007). In contrast, plasticity may facilitate adaptation, either by increasing immediate

39 survival and allowing time for adaptive divergence (Baldwin 1896; West-Eberhard 2003;

40 Ghalambor et al. 2007; Lande 2009), or if non-adaptive plasticity increases the strength of

41 selection in a novel environment (Grether 2005; Ghalambor et al. 2007; Conover et al. 2009;

42 Velotta and Cheviron 2018). Alternatively, mechanisms mediating phenotypic plasticity might

43 promote the accumulation of genetic variation in phenotypes, thereby fostering associations

44 between plasticity and divergence in the early stages of adaptation to novel environments

45 (Espinosa-Soto et al. 2011; Draghi and Whitlock 2012).

46   Parallel evolutionary transitions can also reveal biases in mechanisms of evolution. Similar

47 mechanisms underlying parallel evolutionary transitions suggest the genotype-phenotype map is

48 constrained by limited ways possible to construct adaptive phenotypes, whereas non-shared

49 mechanisms suggest that mechanistic versatility may facilitate adaptation. One potential

50 consequence of biological robustness is that variation affecting the function of most individual

51 genes will have minimal phenotypic consequences, and only variation in a small number of key

52 genes will alter organismal phenotypes. In this case, phenotypic evolution may rely repeatedly on

53 a limited number of mechanistic 'paths', those that yield the greatest phenotypic responses.

54 Indeed, compelling examples demonstrate that similar phenotypes share underlying neural,

55 physiological, molecular, and/or genetic mechanisms, even across highly divergent taxa (e.g.

56 Insel and Young 2000; Manceau et al. 2010; Rosenblum et al. 2010). In contrast, other studies

57 demonstrate versatility in underlying mechanisms suggesting that different biological 'solutions'

58 can give rise to shared phenotypes in closely related species, among populations of the same

59 species, or even among individuals of the same population (e.g. Abouheif and Wray 2002;

60 Crawford and Oleksiak 2007; Grashow et al. 2009; Badyaev and Morrison 2018). Nevertheless,

61 even if mechanistic versatility is a common feature of robust biological networks, shared genetic

62 background or patterns of pleiotropy in a lineage could still direct evolutionary paths toward

63 predictable mechanistic pathways.

64   In the present study, we take advantage of independent lineages of Trinidadian guppies

65 (*Poecilia reticulata*) to explore patterns of versatility and constraint in brain gene expression during

66 parallel phenotypic evolution. Guppies have become a model system in ecology and evolutionary

67 biology due to repeated, independent adaptation of natural populations to distinct predation

68 environments (Haskins et al. 1961; Endler 1995; Reznick et al. 2001). High- and low-predation

69 population pairs from different river drainages represent independent evolutionary lineages

70 (Gilliam et al. 1993; Barson et al. 2009; Willing et al. 2010; Fraser et al. 2015) in which colonization

71 of low-predation environments has led to parallel, adaptive changes in life history traits,

72 morphology, and behavior (Reznick et al. 1990, 2001; Endler 1995; Reznick 1997; Magurran

73 2005). Laboratory breeding designs demonstrate that the combined effects of genetic and

74 environmental influences of predation shape life history (Torres Dowdall et al. 2012), morphology

75 (Torres-Dowdal et al. 2012; Fischer et al. 2013; Ruell et al. 2013; Handelsman et al. 2014),

76 physiology (Handelsman et al. 2013; Fischer et al. 2014), and behavior (Huizinga et al. 2009;

77 Torres-Dowdal et al. 2012; Fischer et al. 2016b); however, transcriptional mechanisms underlying

78 adaptive phenotypic differences between populations and rearing environments remain largely

79 unexplored. A single study characterizing brain gene expression during the earliest stages of

80 adaptation following colonization of low-predation environment found a negative relationship

81 between phenotypic plasticity and adaptive divergence (Ghalambor et al. 2015).

82   Here, we compare the effects of genetic background and rearing environment on gene

83 expression levels in the brain across two parallel, independent evolutionary lineages of guppies

84 that diverged at least 600,000 years ago (Fajen and Breden 1992; Willing et al. 2010). We

85 examine transcript abundance in the brain given previous evidence for behavioral plasticity in our

86 focal populations (Fischer et al. 2016b) and because the previous gene expression study in

87 guppies used brain tissue (Ghalambor et al. 2015). We examined the relationship between

88 developmental plasticity and genetic divergence and characterized the evolution of plasticity in

89   brain gene expression between independent guppy lineages. If only a few transcriptional

90   configurations can give rise to shared adaptive behavioral phenotypes, we expect parallel

91   adaptation to low-predation habitats to be characterized by parallel evolution in a set of largely

92   shared genes. In contrast, if transcriptional mechanisms of behavior are versatile, we expect gene

93   expression divergence in largely non-overlapping gene sets. Taken together, our results allow us

94   to assess the predictability of transcriptional mechanisms of adaptation across timescales.

95

96   **Material and Methods**

97   *Fish collection and rearing*

98   We established lab populations of guppies from high- and low-predation populations pairs

99   collected from the Aripo and Quare river drainages in 2012 and 2014, respectively. To maintain

100   the genetic variation of the original wild-caught fish, we established 20 - 30 unique family lines

101   from each population (i.e., for each generation a single female from each family was crossed to

102   a single male from another family) (Reznick and Bryga 1987). To minimize environmental and

103   maternal effects, we used second-generation lab born fish from these unique family lines in this

104   study. At birth, we split second-generation siblings into rearing environments with (pred+) or

105   without (pred-) predator chemical cues, and they remained in these environments until the

106   completion of the experiment (as in Fischer et al. 2016b). We used only mature males in this

107   study. To maximize the range of genetic variation captured among focal fish, all males in a given

108   experimental group (i.e. population and rearing environment) were from distinct families. Figure

109   S1 shows an overview of our experimental design and interpretation of comparisons.

110   All guppies were individually housed in 1.5 liter tanks on a 12:12 hour light cycle at

111   Colorado State University. Fish were fed a measured food diet once daily, receiving Tetramin™

112   tropical fish flake paste and hatched *Artemia* cysts on an alternating basis. Prior to tissue

113   collection for this study, behavioral and hormone data were collected in an identical fashion for all

114   males and these results are reported elsewhere (Fischer et al. 2016b). All experimental methods

115   were approved by the Colorado State University Animal Care and Use Committee (Approval #12-

116   3818A).

117

118   *Tissue collection and processing*

119   We collected brain tissue from the Aripo and Quare lineage males described above in

120   2013 and 2015, respectively. To standardize any effects of recent experience, behavior, and

121   circadian rhythm on gene expression, we collected whole brains within 10 minutes of lights-on.

122   We interpret our transcriptional data as baseline, in the sense that fish were minimally stimulated

123   prior to tissue collection such that expression levels should reflect genetic background and
124   developmental experience more strongly than responses to immediate environmental context.
125   Fish were anesthetized by immersion in ice water followed by rapid decapitation. Whole brains
126   were removed, flash frozen in liquid nitrogen, and stored at -80°C until further processing. Tissue
127   collection took <2 minutes, rapid enough to minimize changes in gene expression from handling
128   and dissection.

129   We extracted total RNA from brain tissue using the Qiagen RNeasy Lipid Tissue Mini Kit
130   (Qiagen, Germany) following manufacturer guidelines. We prepared separate sequencing
131   libraries for each individual using the NEBNext Ultra RNA Library Prep Kit for Illumina (New
132   England Biolabs, Massachusetts, USA) following manufacturer instructions. Libraries were
133   sequenced on an Illumina HiSeq 2000 at the Florida State University College of Medicine
134   Translational Science Laboratory (Tallahassee, Florida) in May 2014 (Aripo dataset) and January
135   2016 (Quare dataset). For the Aripo dataset, 40 samples (N=10 per group) were combined with
136   unique barcodes into eight samples per pool and each pool was sequenced on a single lane. For
137   the Quare dataset, 60 samples (N=12-14 per group) were combined into three pools with 20
138   samples per pool and each pool was sequenced in two separate lanes. Experimental groups were
139   balanced across lanes.

140

141   *Transcriptome construction and transcript abundance estimation*

142   Given rapid advancements in sequencing technology and our larger, more deeply
143   sequenced dataset, we chose to construct a new transcriptome for the present study rather than
144   using that used in Ghalambor et al. (2015). We received 465 million 100-bp paired-end reads that
145   passed the HiSeq quality filter, averaging 11 million reads per sample. We used Rcorrector to
146   amend Illumina sequencing errors (Song and Florea 2015) and removed adapter sequences and
147   trimmed reads for high-quality sequence using Trim Galore! (Babraham Bioinformatics,
148   Babraham Institute). Following developer recommendations, we used a quality score of 33, a
149   stringency of 5, and a minimum read length of 36bp. We pooled corrected, trimmed reads from
150   all individuals prior to transcriptome construction using Trinity (Grabherr et al. 2011; Haas et al.
151   2013). We used only the initial Aripo dataset to construct our high-quality transcriptome but note
152   that all populations had a similarly high percentage of transcripts mapping back to the final
153   transcriptome (see below).

154   Our initial assembly contained 411,043 transcripts (N50 = 2,025). To improve assembly
155   quality, we filtered the assembly by clustering overlapping transcripts and retained only those
156   transcripts longer than 250bp. We then annotated transcripts by blastx queries against SwissProt

157 and restricted our assembly to those transcripts with annotations to known vertebrate proteins.

158 We used default parameters for our blastx queries with an e-value cutoff of 10^{-10}. We further

159 annotated this filtered assembly using Trinotate (trinotate.github.io). Our final assembly contained

160 54,608 transcripts (N50 = 4,106) representing 23,619 presumptive genes. We used BUSCO

161 (Simão et al. 2015) to assess assembly completeness based on conserved ortholog content

162 across highly conserved vertebrate genes. BUSCO analysis estimated assembly completeness

163 at 86%. We aligned corrected, trimmed reads from both datasets to our final assembly and

164 estimated their abundance using Kallisto (Bray et al. 2016). On average, 70% of sequences per

165 individual mapped back to our final reference transcriptome. We performed all subsequent

166 analyses at the gene, rather than transcript, level to avoid issues introduced by our incomplete

167 understanding of sequence and regulatory variation among populations.

168

169 *Data filtering and screening*

170 Preliminary cluster analyses revealed retinal contamination in a subset of our Aripo

171 dataset brain samples. While opsins are expressed at low levels in the brain, the very high

172 expression levels (>10,000 copies) in three samples pointed to retinal contamination. To deal with

173 this issue, we devised a sample filtering and screening procedure to remove genes in which

174 expression differences between samples were likely dominated by retinal contamination. Briefly,

175 we used contigs annotated as known retinal genes (Rhodopsin, red/green-sensitive opsins, blue-

176 sensitive opsins) as seed contigs to identify other contamination-related transcripts based on high

177 positive correlations of expression levels with seed genes. We calculated the gene-wise sum of

178 correlations between candidate genes and seed genes and performed multiple hypothesis testing

179 based on a false discovery rate (FDR) controlling procedure. The nominal level of FDR was set

180 to α=0.2 to remove presumptive contaminant contigs. Using this approach, we identified 1,151

181 contigs as presumptive retina-enriched genes (~ 3% of all contigs in our final assembly) which we

182 removed from all subsequent analyses in both datasets (Table S1). More detailed descriptions of

183 statistical procedures are in the Supplemental Methods.

184

185 *Differential expression analysis*

186 Due to differences in timing of fish rearing, sample processing, and sequencing, we did

187 not combine Aripo and Quare datasets for statistical analysis but instead performed analysis in

188 an identical fashion for both drainages and conducted separate analyses to explicitly compare

189 patterns across drainages. Because standard differential expression analysis packages could not

190 accommodate the random effects in our experimental design (see below), we performed

191   differential expression analysis using a modified pipeline. We normalized read counts using

192   DESeq2 in R (Love et al. 2014) and performed differential expression analysis using the lme4

193   package in R (github.com/lme4). Count data were modeled using a generalized linear mixed

194   model with negative binomial distribution. We included population of origin, rearing environment,

195   and their interaction as fixed effects. In addition, we included family (siblings split across rearing

196   environments) and week (tissue was collected from animals in balanced blocks across multiple

197   weeks) as random effects. A Wald's test was performed gene-wise to obtain p-values for main

198   effects (Lehmann and Romano 2005). We adjusted p-values for multiple hypothesis testing using

199   the Benjamini-Hochberg procedure and called all transcripts with an adjusted p-value <0.05

200   differentially expressed (DE). To examine whether differential expression calls were influenced

201   by transcript abundance, we compared mean and median counts of DE and non-DE genes using

202   two sample t-tests and Wilcoxon rank sum tests. We also compared overall patterns of gene-wise

203   variance between DE and non-DE groups with respect to either population or rearing effect using

204   Siegel-Tukey tests and Kolmogorov-Smirnov tests. We performed GO term enrichment analysis

205   for all sets of DE transcripts using annotation information for 'Biological Processes' in the topGO

206   package in R (Alexa and Rahnenfuhrer 2016).

207

208   *Overlap and expression concordance in DE gene sets*

209   To evaluate overlap in DE transcript sets and concordance in their expression direction,

210   we used chi-square tests of independence to test for greater than chance overlap in the number

211   and expression concordance of transcripts based on (1) population and rearing effects within a

212   single drainage, and (2) differentially expressed transcript sets between the two datasets. Within

213   drainage, we compared the direction of genetic and plastic expression changes in all transcripts

214   differentially expressed based on population of origin, even if these transcripts were not

215   differentially expressed based on rearing environment. We reasoned that even subthreshold

216   expression changes in response to rearing environment could affect expression divergence

217   propensity. We used log-fold expression changes in transcript abundance to determine whether

218   transcript expression differences were in the same (e.g. upregulated in high-predation and in

219   response to rearing with predator cues, or down-regulated in response to genetic and

220   environmental exposure to predation) or opposite directions (e.g. upregulated in high-predation

221   populations, but down-regulated in response to rearing with predators, or vice versa).

222   Between drainages we compared those transcripts significantly differentially expressed

223   based on population of origin in both drainages (i.e. the intersection of population DE lists) as well

224   as those transcripts significantly differentially expressed in either drainages (i.e. the union of

225 population DE lists). We again used log-fold expression changes to call expression differences
226 as being in the same of opposite direction in high-predation as compared to low-predation
227 populations in both drainages. We excluded all transcripts with significant interaction effects from
228 the lists of population and rearing DE transcripts used in these analyses, as characterizing simple
229 effects of population and rearing is inappropriate when an interaction is present and we therefore
230 explored transcripts with significant interaction effects separately (see below).

231

232 *Analysis of transcripts with significant interaction effects*

233   Based on post hoc differences between rearing environments within a population
234 ($p<0.05$), we grouped transcripts with statistically significant interaction effects into categories
235 outlined by Renn and Schumer (Renn and Schumer 2013): (1) Assimilated: plasticity in the
236 ancestral high-predation population but a loss of plasticity in the derived low-predation population;
237 (2) Accommodated: a change in the degree, but not the direction, of plasticity in the derived as
238 compared to the ancestral population; (3) Reversed: opposing directions of plasticity in high-
239 versus low-predation populations; (4) Evolved plastic: no plasticity in the ancestral high-predation
240 population but an emergence of plasticity in the derived low-predation population; and (5)
241 Unclassified: all remaining transcripts which had a significant main interaction effect but no
242 significant *post hoc* rearing differences, as the lack of pairwise rearing effects meant transcripts
243 could not be unambiguously classified into one of the other categories. All statistical tests and
244 data visualization were performed in R (version 3.5.1; The R Foundation for Statistical
245 Computing). Additional details of statistical procedures are in the Supplemental Methods.

246

247

248 **Results**

249 *Impacts of population of origin and rearing conditions on brain transcript abundance*

250   In the Aripo drainage, 659 transcripts were differentially expressed (DE) between high-
251 and low-predation populations, 738 genes were differentially expressed between pred- and pred+
252 fish, and 465 transcripts had interaction effects (Fig. 1; Table S2). Differentially expressed
253 transcripts were enriched for GO categories related to metabolic processes, immune function,
254 and nervous system development. In the Quare drainage 4,951 transcripts were differentially
255 expressed between high- and low-predation populations, 200 genes were differentially expressed
256 between pred- and pred+ fish, and 393 transcripts had interaction effects (Fig. 1; Table S3).
257 Differentially expressed transcripts were enriched for GO categories related to metabolic
258 processes, immune function, and macromolecule transport. Complete results of GO enrichment

9

259   analyses for population of origin, rearing, and interaction effects are in the Supplemental Materials

260   (Tables S4 & S5).

261   While we cannot rule out some influence of technical variation, we found no evidence for

262   differences in sequence quality, read alignment statistics, or variance in transcript abundance to

263   suggest the greater number of differentially expressed transcripts in the Quare drainage was of

264   technical origin. Nor did we find evidence that identification of DE transcripts was biased by

265   transcript expression level: mean and median gene expression were significantly lower in DE

266   (population, rearing, and/or interaction effects) as compared to non-DE transcripts in the Aripo

267   dataset (Fig. S2; mean: DE=365.86, non-DE=451.91, p<0.0001; median: DE=97, non-DE=150,

268   p<0.0001), whereas they were significantly higher in the Quare dataset (Fig. S2; mean:

269   DE=460.76, non-DE=422.38, p<0.0001; median: DE=170, non-DE=131, p<0.0001). Opposing

270   patterns between drainages suggest that identification of DE transcripts was not a side effect of

271   relatively greater or lesser expression values of these transcripts.

272

273
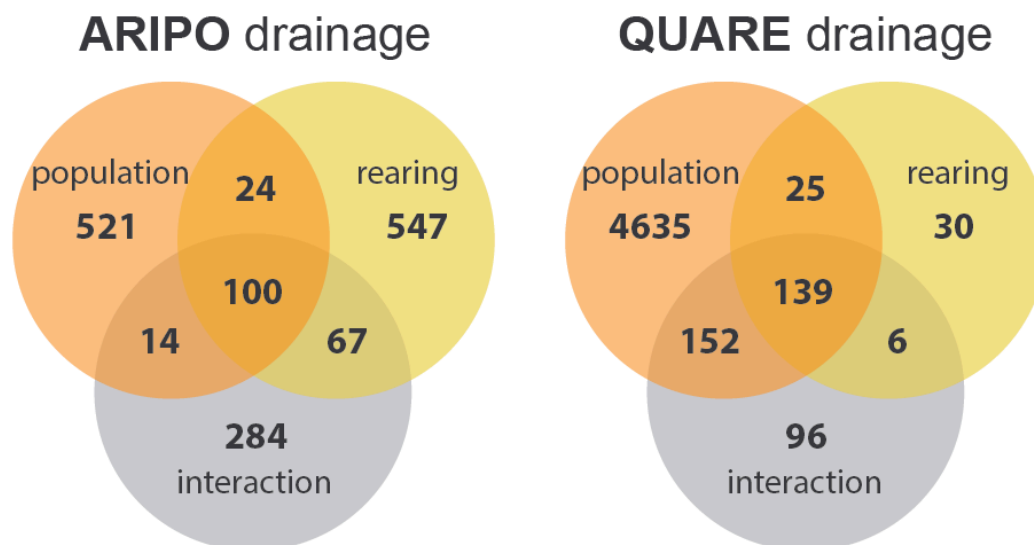
Figure 1. Summary of differential expression analyses. We identified many transcripts in both the Aripo (left) and Quare (right) drainages whose expression levels differed based on evolutionary history of predation (population of origin; orange), developmental experience of predation (rearing; yellow), and their interaction (grey). Transcripts with significant interaction effects were removed from analyses of population and rearing effects and analyzed separately for patterns in the evolution of plasticity.

274   *Relationship between phenotypic plasticity and genetic divergence in transcript abundance*

275   In both drainages, the sets of transcripts with main effects of population of origin and of

276   rearing environment overlapped more than expected by chance (Fig. 1; Aripo: 24 transcripts,

277   χ^2=24.109, p=0.0065; Quare: 25 transcripts, χ^2=66.413, p=0.0005; transcripts with interaction

278   effects excluded from analysis). Given this non-independence of population and rearing

279   expression differences, we asked whether the direction of expression plasticity predicted gene

280   expression divergence between populations. We did so for all population DE transcripts

281   regardless of whether plastic expression differences were significant or not, reasoning that even

282   small expression changes in response to rearing environment could influence divergence

283   propensity. We found no significant relationship between the direction of population divergence

284   and rearing expression in either drainage, with approximately 50% of transcripts showing

285   population and rearing expression changes in the same direction and 50% showing genetic and

286   plastic expression changes in opposite directions in both drainages (Fig. 2).

11
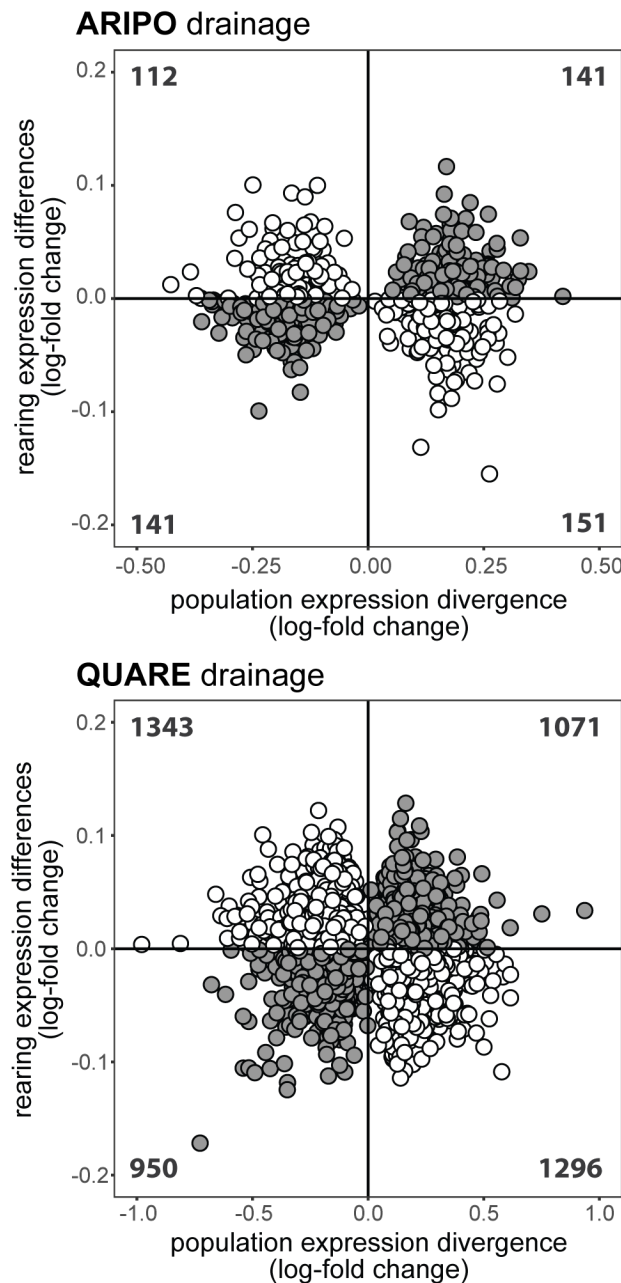
Figure 2. Relationship between plasticity and expression divergence. We found no relationship between evolved expression divergence and the direction of plastic expression differences: approximately half of genes differentially expressed between high- and low-predation populations have the same directions of plasticity and divergence (grey circles) and half have opposite directions (white circles) in both the Aripo (top) and Quare (middle) lineages. The number of transcripts in each quadrant is indicated on the graphs. Only those transcripts with statistically significant expression differences based on population of origin and rearing environment are plotted.

287   *Evolution of expression plasticity*

288   To examine the evolution of expression plasticity, we grouped transcripts with significant

289   interaction effects into one of five categories: assimilated, accommodated, reversed, evolved

290   plastic, or unclassified. We found many transcripts that exhibited plasticity evolution, with all five

291   categories represented in both datasets (Fig. 3). In the Aripo drainage 100 (28%) transcripts

292   showed patterns of expression assimilation, 149 (41%) transcripts showed patterns of expression

293   accommodation, 61 (17%) transcripts exhibited reversed plasticity, and 52 (14%) transcripts

294   evolved plasticity in the derived population. In the Quare drainage, 84 (32%) transcripts showed

295   patterns of expression assimilation, 51 (19%) transcripts showed patterns of expression

296   accommodation, 57 (22%) transcripts exhibited reversed plasticity, and 72 (27%) transcripts

297   evolved plasticity in the derived population (Fig. 3).
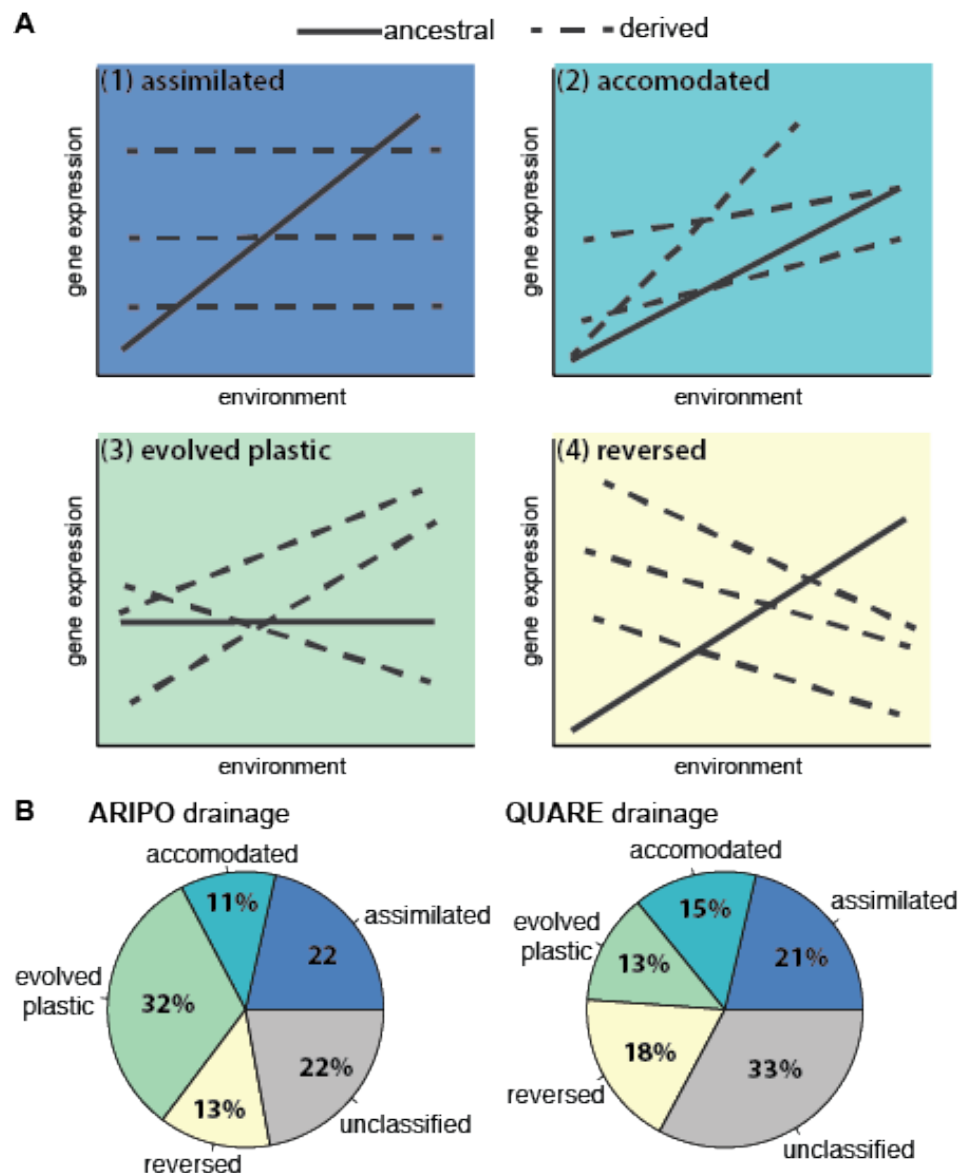
Figure 3. Evolution of transcript expression plasticity. (A) We grouped transcripts with significant interaction effects into one of four categories based on patterns of evolution in expression plasticity: (1) Assimilated: plasticity in the ancestral high-predation population but a loss of plasticity 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) Reversed: opposing directions of plasticity in high- versus low-predation populations; (4) Evolved plastic: no plasticity in the ancestral high-predation population but an emergence of plasticity in the derived low-predation population. 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 datasets.

298   *Parallelism across drainages in divergence and plasticity of transcript abundance*

299   Of the transcripts that diverged between high- and low-predation populations within a

300   drainage (i.e., population main effect but no interaction effect), 156 were overlapping between

301   drainages (Table S6), more than expected by change (χ^2=12.705, p<0.0001). However, the

302   direction of expression divergence was not predictably associated in the two drainages (χ^2=0.909,

303   p=0.422): 47% had expression changes in the same direction and 53% had expression changes

304   in opposite directions between the two drainages (Fig. 4A). Nor did we find an association of

305   expression direction between lineages when we considered the larger collection of transcripts

306   differentially expressed between populations in either drainage (χ^2=2.583, p=0.109) (Fig. 4A).

307   We performed the same analysis for the rearing DE transcripts in both datasets and found

308   only four transcripts overlapping between drainages (Table S7), marginally more than expected

309   by chance (χ^2=3.992, p=0.069), all with expression in opposite directions (Fig. 4B). When we

310   considered the larger collection of transcripts differentially expressed between rearing

311   environments in either drainage, we found an overrepresentation of transcripts expressed in

312   opposite (64%) as compared to the same (36%) direction (χ^2=53.90, p<0.0001) (Fig. 4B). Finally,

313   because parallel adaptation could repeatedly target similar cellular processes and pathways even

314   if individual transcripts don't overlap, we also compared overlap in GO terms enriched in

315   population and rearing comparisons across drainages. We found four overlapping GO terms

316   among those enriched in population DE transcripts in both lineages: biosynthetic processes

317   (GO:0009058), cellular response to abiotic stimulus (GO:0071214), cellular response to toxic

318   substance (GO:0097237), and regulation of lipid kinase activity (GO:0043550). We found no GO

319   terms overlapping between lineages among those GO terms enriched in rearing DE transcripts.

15
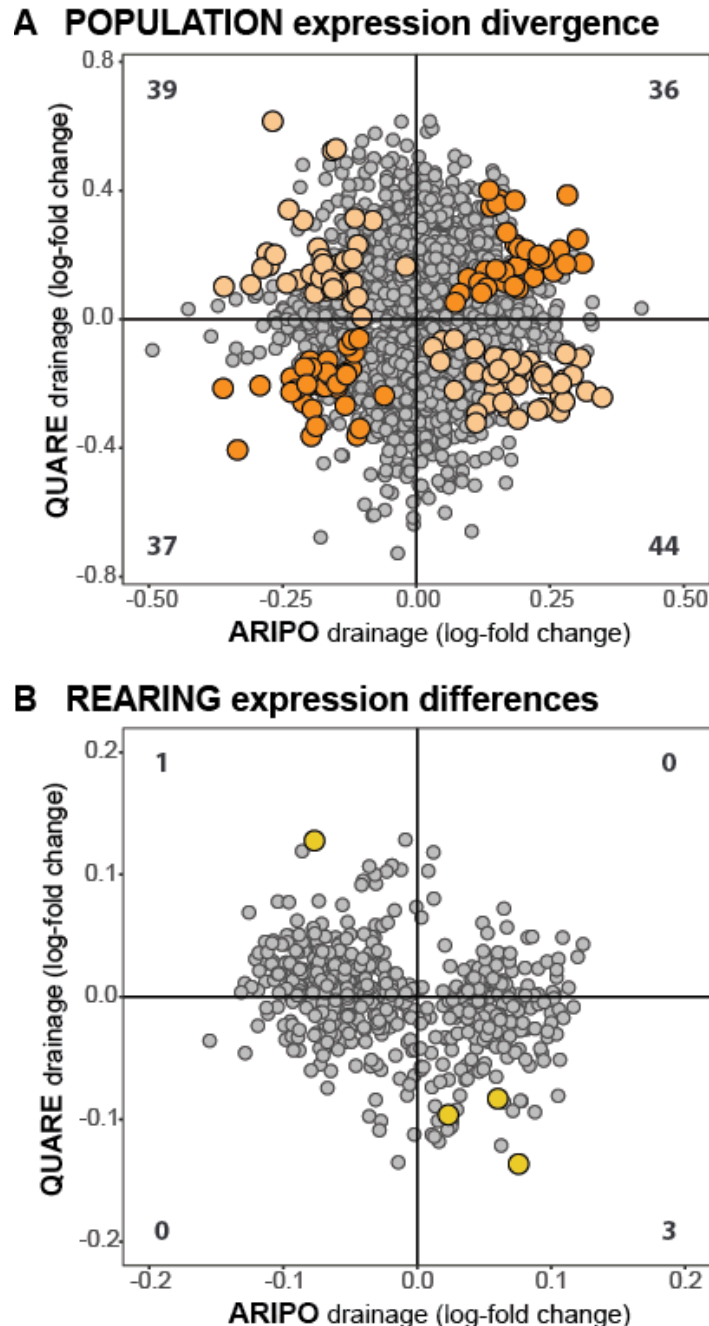
Figure 4. Overlap in population and rearing expression differences across evolutionary lineages. (A) Of the transcripts differentially expressed based on population of origin, 156 were overlapping between drainages. Of these, 73 were concordantly differentially expressed (i.e. genetic expression divergence in the same direction; dark orange circles) and 83 were not (i.e. genetic expression divergence in opposite directions; light orange circles), no different than expected by chance. (B) Of the transcripts differentially expressed based on rearing environment, four were overlapping in the two drainages. Of these all were non-concordantly differentially expressed (yellow circles), marginally 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.

16

Discussion

320

321   We examined transcriptomic differences associated with adaptive divergence and rearing

322   environment across repeated, independent evolutionary lineages in guppies. Within lineages, we

323   observed phenotypic plasticity in expression patterns as well as the evolution of gene expression

324   plasticity between high- and low-predation populations. Although plastic transcripts were more

325   likely to exhibit population differences in expression, we found no relationship between the

326   direction of plastic change and evolutionary divergence. Comparing across lineages, we found

327   largely non-overlapping gene sets and no evidence for an association in the direction of

328   expression divergence associated with parallel phenotypic divergence, suggesting that

329   transcriptional versatility is associated with parallel phenotypic adaptation in guppies.

330

331   *Impacts of plasticity in transcript expression on evolution*

332   A growing number of studies have used transcriptome and proteome surveys to address

333   the long-standing debate on the role of plasticity in evolution, with contrasting results favoring

334   alternative hypotheses that adaptive (Scoville and Pfrender 2010; Fraser et al. 2014; Shaw et al.

335   2014; Gleason and Burton 2015; Mäkinen et al. 2016; Li et al. 2018; Wang and Althoff 2019) or

336   non-adaptive (Pespeni et al. 2013; Schaum et al. 2013; Dayan et al. 2015; Ghalambor et al. 2015;

337   Ho and Zhang 2018) plasticity facilitates adaptation. We recently proposed that non-adaptive

338   plasticity dominates during the earliest stages of rapid evolution, and that adaptive plasticity may

339   contribute to subsequent fine-tuning of phenotypes (Fischer et al., 2016). In line with this

340   prediction and our previous findings, we report here that the strong signature of non-adaptive

341   plasticity in brain gene expression observed in guppy populations recently introduced to low-

342   predation environments (Ghalambor et al., 2015) is not present in either of our natural, long-

343   established low-predation populations. Experimental design differences preclude a direct

344   comparison, as we considered patterns in all transcripts exhibiting significant expression

345   divergence, while Ghalambor et al. (2015) focused their analyses on those transcripts for which

346   evidence of selection was strongest (i.e. those with concordant differential expression in three

347   low-predation populations from the same drainage, all assayed simultaneously and sharing a

348   common ancestral population). The lack of association between the direction of plasticity and

349   genetic divergence in our study highlights the need to directly test how ancestral plasticity relates

350   to divergence throughout successive stages of adaptation to novel environments.

351

352   *Evolution of plasticity in transcript expression*

17

353   Diverse transcriptional patterns could accompany evolved differences in behavioral

354   plasticity (Renn and Schumer 2013), but relevant data characterizing the evolution of gene

355   expression plasticity in the brain has been lacking. We observed a substantial number of

356   transcripts with evolved differences in expression plasticity in both lineages. However, as was

357   observed for transcriptomic evolution of gill tissue in stickleback fish (Gibbons et al. 2017), these

358   transcripts showed no consistent pattern, with transcripts gaining, losing, and switching direction

359   of expression plasticity between the ancestral and derived populations in both lineages. Part of

360   this diversity in transcriptional plasticity could reflect the gains, losses, and switches in plasticity

361   of different behaviors in these populations (Fischer et al. 2016b). Alternatively, compensatory and

362   homeostatic mechanisms could promote diversity among evolutionary and plastic responses in

363   transcript expression that do not alter higher-level phenotypic traits such as morphology and

364   behavior (Renn and Schumer 2013; Fischer et al. 2016a; Badyaev 2018). In either case, because

365   fish in low-predation habitats may experience relaxed selection on predator-induced plasticity,

366   some of the evolution of transcriptional plasticity we report likely arose as a byproduct of genetic

367   drift and adaptation to low-predation environments, rather than selection for altered plastic

368   responses (Lynch 2007). Although we cannot distinguish between adaptive and non-adaptive

369   causes, our data demonstrate evolution in gene expression plasticity accompanying adaptation

370   within lineages.

371

372   *Transcriptomic signatures of population divergence in two evolutionary lineages*

373   We identified many transcripts that were differentially expressed between high- and low-

374   predation populations in each river drainage. The absolute number of differentially expressed

375   transcripts was smaller in the Aripo drainage as compared to the Quare drainage, but the

376   proportion of developmental and interaction differences was greater in the Aripo drainage. Natural

377   populations differ in their degree of genetic divergence and hence may differ in their degree of

378   gene expression divergence. Indeed, previous SNP analyses document less genetic divergence

379   in the Aripo as compared to the Quare river drainage (Willing et al. 2010), consistent with our

380   differential expression patterns.

381   When we compared differentially expressed transcript sets between drainages, the

382   majority of differentially expressed transcripts were non-shared across drainages for both

383   population effects (Aripo: 474/659; Quare: 4,766/4,951) and rearing effects (Aripo: 734/738;

384   Quare: 196/200). Nonetheless, there was more overlap than excepted by chance in population

385   differentially expressed transcripts between lineages. Of the 156 transcripts with population main

386   effects that were overlapping between lineages, approximately half were differentially expressed

387   in the same direction (i.e. concordantly differentially expressed) and half were differentially
388   expressed in opposite direction. Thus, while the number of overlapping transcripts was more than
389   expected by chance, there was no association in the direction of expression divergence between
390   lineages.

391   A previous study in guppies performed a similar comparison of gene expression changes
392   associated with adaptation to low-predation environments (Ghalambor et al. 2015) and found a
393   strong signal of concordant differential expression. Whereas the present study compared long-
394   term population divergence across drainages, Ghalambor et al. (2015) characterized early stages
395   of adaptation of populations within the same lineage. Our contrasting results highlight the impacts
396   of standing genetic variation within the source population on mechanisms of divergence (Feiner
397   et al. 2017), particularly at early stages of evolution (Barrett and Schluter 2008): while alternative
398   transcriptional 'solutions' are possible, shared genetic background appears to bias evolutionary
399   outcomes toward shared patterns.

400   In contrast to patterns based on population effects, overlap across lineages in transcripts
401   differentially expressed based on rearing environment was only marginally significant. Due to the
402   very small number of rearing differentially expressed transcripts overlapping between lineages,
403   we additionally compared patterns of expression direction in the larger collection of transcripts
404   differentially expressed based on rearing environment in either drainage. Here we found a
405   signature of non-concordant expression across lineages. Thus, while there was little overlap in
406   the identity of differentially expressed transcripts, differentially expressed transcripts were
407   nonetheless likely to show opposing expression patterns across drainages suggesting alternative
408   transcriptional solutions.

409   Both adaptive and non-adaptive processes may contribute to largely non-overlapping
410   transcriptional mechanisms giving rise to parallel life-history, morphological, and behavioral
411   phenotypes across lineages in guppies. First, differences in standing genetic variation likely
412   influence which mechanisms are available to selection in response to common environmental
413   conditions in different drainages (Barrett and Schluter 2008), as described above. Second, low-
414   predation populations are typically established by a very small number of individuals (Barson et
415   al. 2009; Willing et al. 2010; Fraser et al. 2015), and founder effects in these populations make
416   them susceptible to expression divergence resulting from genetic drift and inbreeding. Third,
417   differential expression in non-overlapping transcripts may also represent adaptive responses to
418   drainage- or site-specific environmental factors other than predation (Zandonà et al. 2011;
419   Fitzpatrick et al. 2014). In other words, genetic similarities may channel populations within a
420   drainage toward shared transcriptional configurations, while differences in standing genetic

19

421   variation, founder effects and bottlenecks, and drainage-specific environmental conditions result

422   in distinct transcriptional trajectories to arrive at shared organisms level phenotypes. Nonetheless,

423   transcriptional patterns may become more similar over time (i.e. environmentally plastic versus

424   evolved responses) as the funneling effects of selection push populations towards those

425   transcriptional solutions with the smallest pleiotropic load (Stern & Orgogozo, 2008; Gompel &

426   Prud'homme, 2009). Because transcriptional network configuration – which emerges from a

427   combination of adaptive, non-adaptive, and neutral divergence – will shape evolutionary

428   outcomes, a holistic understanding of convergent phenotypic evolution will ultimately require an

429   understanding of the origins and impacts of concordant and lineage-specific transcript divergence

430   across timescales.

431

432   *Conclusions*

433   We assessed the extent to which adaptation to common environments targets predictable

434   changes in transcript expression across independent evolutionary events. Within lineages, genes

435   with a greater expression plasticity were more likely to diverge in abundance between

436   populations. At the same time, parallel adaptation to low-predation environments in independent

437   lineages was associated with divergence in largely non-overlapping transcripts. While

438   identification of shared genes is generally used as the starting point for work exploring

439   mechanisms of parallel adaptation, we propose that parallel evolutionary transitions are not

440   limited to a small set of possible transcriptional mechanisms in guppies. Instead, our results

441   highlight the potential for extensive transcriptional versatility associated with parallel, adaptive

442   trait evolution even within a single species. Transcriptional network versatility, in which diverse

443   alterative network configurations can produce common network outputs and behavioral

444   phenotypes, may allow underlying networks to simultaneously accommodate the influences of

445   selection, drift, and genetic background and thereby facilitate evolution in a species known for

446   rapid adaptation to novel environments.

447

448

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