Non-parallel transcriptional divergence during parallel adaptation

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Abstract

Mechanisms linking genotype to phenotype must simultaneously buffer organisms from developmental noise and allow for phenotypic plasticity in response to environmental cues. How mechanistic robustness and flexibility in biological systems 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 transcriptional evolution during parallel adaptation. We observed substantial phenotypic plasticity in gene expression patterns as well as evolution of gene expression plasticity across populations. Although transcripts exhibiting expression plasticity within populations were more likely to differ in expression between populations, we found no consistent relationship between the direction of plasticity and evolutionary divergence. Similarly, while we found more overlap than expected by chance in genes differentially expressed between high- and low-predation populations from distinct lineages, the direction of expression divergence was uncoupled in the two drainages, and the majority of differentially expressed genes were not shared between lineages. Our data highlight transcriptional versatility associated with parallel phenotypic divergence in independent evolutionary lineages of a species known for rapid adaptation.

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

Introduction

Phenotypic evolution is biased by the mechanisms that link genetic variation to phenotypic variation, i.e. the genotype-phenotype map (Alberch 1991). Mechanisms linking genotype and phenotype are characterized by both robustness and flexibility. Robust developmental processes buffer phenotypes from noise such that multiple transcriptional, biochemical, or cellular network configurations may give rise to similar organismal phenotypes while flexibility in these processes nonetheless allows for phenotypic plasticity in response to environmental conditions (Wagner 2011; Gutierrez et al. 2013). The complex nature of the genotype-phenotype map makes it challenging to understand how mechanistic properties facilitate or constrain adaptive evolution. In this study, we combine studies of phenotypic plasticity and parallel adaptation across independent lineages to assess how mechanistic biases shape evolutionary trajectories across timescales.

One feature of biological networks that may channel divergence into particular paths is environmentally induced plasticity. The relationship between phenotypic plasticity and genetic divergence is of long-standing theoretical and empirical interest. On the one hand, plasticity may constrain adaptation by allowing successful phenotypes to be attained without genetic change, effectively shielding organisms from selection (Price et al. 2003; West-Eberhard 2003; Ghalambor et al. 2007). In contrast, plasticity may facilitate adaptation, either by increasing immediate survival and allowing time for adaptive divergence (Baldwin 1896; West-Eberhard 2003; Ghalambor et al. 2007; Lande 2009), or if non-adaptive plasticity increases the strength of selection in a novel environment (Grether 2005; Ghalambor et al. 2007; Conover et al. 2009; Velotta and Cheviron 2018). Alternatively, mechanisms mediating phenotypic plasticity might promote the accumulation of genetic variation in phenotypes, thereby fostering associations between plasticity and divergence in the early stages of adaptation to novel environments (Espinosa-Soto et al. 2011; Draghi and Whitlock 2012).

Parallel evolutionary transitions can also reveal biases in mechanisms of evolution. Similar mechanisms underlying parallel evolutionary transitions suggest the genotype-phenotype map is constrained by limited ways possible to construct adaptive phenotypes, whereas non-shared mechanisms suggest that mechanistic versatility may facilitate adaptation. One potential consequence of biological robustness is that variation affecting the function of most individual genes will have minimal phenotypic consequences, and only variation in a small number of key genes will alter organismal phenotypes. In this case, phenotypic evolution may rely repeatedly on a limited number of mechanistic ‘paths’, those that yield the greatest phenotypic responses. Indeed, compelling examples demonstrate that similar phenotypes share underlying neural,

physiological, molecular, and/or genetic mechanisms, even across highly divergent taxa (e.g. Insel and Young 2000; Manceau et al. 2010; Rosenblum et al. 2010). In contrast, other studies demonstrate versatility in underlying mechanisms suggesting that different biological 'solutions' can give rise to shared phenotypes in closely related species, among populations of the same species, or even among individuals of the same population (e.g. Abouheif and Wray 2002; Crawford and Oleksiak 2007; Grashow et al. 2009; Badyaev and Morrison 2018). Nevertheless, even if mechanistic versatility is a common feature of robust biological networks, shared genetic background or patterns of pleiotropy in a lineage could still direct evolutionary paths toward predictable mechanistic pathways.

In the present study, we take advantage of independent lineages of Trinidadian guppies (*Poecilia reticulata*) to explore patterns of versatility and constraint in brain gene expression during parallel phenotypic evolution. Guppies have become a model system in ecology and evolutionary biology due to repeated, independent adaptation of natural populations to distinct predation environments (Haskins et al. 1961; Endler 1995; Reznick et al. 2001). High- and low-predation population pairs from different river drainages represent independent evolutionary lineages (Gilliam et al. 1993; Barson et al. 2009; Willing et al. 2010; Fraser et al. 2015) in which colonization of low-predation environments has led to parallel, adaptive changes in life history traits, morphology, and behavior (Reznick et al. 1990, 2001; Endler 1995; Reznick 1997; Magurran 2005). Laboratory breeding designs demonstrate that the combined effects of genetic and environmental influences of predation shape life history (Torres Dowdall et al. 2012), morphology (Torres-Dowdal et al. 2012; Fischer et al. 2013; Ruell et al. 2013; Handelsman et al. 2014), physiology (Handelsman et al. 2013; Fischer et al. 2014), and behavior (Huizinga et al. 2009; Torres-Dowdal et al. 2012; Fischer et al. 2016b); however, transcriptional mechanisms underlying adaptive phenotypic differences between populations and rearing environments remain largely unexplored. A single study characterizing brain gene expression during the earliest stages of adaptation following colonization of low-predation environment found a negative relationship between phenotypic plasticity and adaptive divergence (Ghalambor et al. 2015).

Here, we compare the effects of genetic background and rearing environment on gene expression levels in the brain across two parallel, independent evolutionary lineages of guppies that diverged at least 600,000 years ago (Fajen and Breden 1992; Willing et al. 2010). We examine transcript abundance in the brain given previous evidence for behavioral plasticity in our focal populations (Fischer et al. 2016b) and because the previous gene expression study in guppies used brain tissue (Ghalambor et al. 2015). We examined the relationship between developmental plasticity and genetic divergence and characterized the evolution of plasticity in

brain gene expression between independent guppy lineages. If only a few transcriptional configurations can give rise to shared adaptive behavioral phenotypes, we expect parallel adaptation to low-predation habitats to be characterized by parallel evolution in a set of largely shared genes. In contrast, if transcriptional mechanisms of behavior are versatile, we expect gene expression divergence in largely non-overlapping gene sets. Taken together, our results allow us to assess the predictability of transcriptional mechanisms of adaptation across timescales.

Material and Methods

Fish collection and rearing

We established lab populations of guppies from high- and low-predation populations pairs collected from the Aripo and Quare river drainages in 2012 and 2014, respectively. To maintain the genetic variation of the original wild-caught fish, we established 20 - 30 unique family lines from each population (i.e., for each generation a single female from each family was crossed to a single male from another family) (Reznick and Bryga 1987). To minimize environmental and maternal effects, we used second-generation lab born fish from these unique family lines in this study. At birth, we split second-generation siblings into rearing environments with (pred+) or without (pred-) predator chemical cues, and they remained in these environments until the completion of the experiment (as in Fischer et al. 2016b). We used only mature males in this study. To maximize the range of genetic variation captured among focal fish, all males in a given experimental group (i.e. population and rearing environment) were from distinct families. Figure S1 shows an overview of our experimental design and interpretation of comparisons.

All guppies were individually housed in 1.5 liter tanks on a 12:12 hour light cycle at Colorado State University. Fish were fed a measured food diet once daily, receiving Tetramin™ tropical fish flake paste and hatched *Artemia* cysts on an alternating basis. Prior to tissue collection for this study, behavioral and hormone data were collected in an identical fashion for all males and these results are reported elsewhere (Fischer et al. 2016b). All experimental methods were approved by the Colorado State University Animal Care and Use Committee (Approval #12-3818A).

Tissue collection and processing

We collected brain tissue from the Aripo and Quare lineage males described above in 2013 and 2015, respectively. To standardize any effects of recent experience, behavior, and circadian rhythm on gene expression, we collected whole brains within 10 minutes of lights-on. We interpret our transcriptional data as baseline, in the sense that fish were minimally stimulated

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

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

Transcriptome construction and transcript abundance estimation

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

Our initial assembly contained 411,043 transcripts (N50 = 2,025). To improve assembly quality, we filtered the assembly by clustering overlapping transcripts and retained only those 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

differential expression analysis using a modified pipeline. We normalized read counts using DESeq2 in R (Love et al. 2014) and performed differential expression analysis using the lme4 package in R (github.com/lme4). Count data were modeled using a generalized linear mixed model with negative binomial distribution. We included population of origin, rearing environment, and their interaction as fixed effects. In addition, we included family (siblings split across rearing environments) and week (tissue was collected from animals in balanced blocks across multiple weeks) as random effects. A Wald's test was performed gene-wise to obtain p-values for main effects (Lehmann and Romano 2005). We adjusted p-values for multiple hypothesis testing using the Benjamini-Hochberg procedure and called all transcripts with an adjusted p-value <0.05 differentially expressed (DE). To examine whether differential expression calls were influenced by transcript abundance, we compared mean and median counts of DE and non-DE genes using two sample t-tests and Wilcoxon rank sum tests. We also compared overall patterns of gene-wise variance between DE and non-DE groups with respect to either population or rearing effect using Siegel-Tukey tests and Kolmogorov-Smirnov tests. We performed GO term enrichment analysis for all sets of DE transcripts using annotation information for 'Biological Processes' in the topGO package in R (Alexa and Rahnenfuhrer 2016).

Overlap and expression concordance in DE gene sets

To evaluate overlap in DE transcript sets and concordance in their expression direction, we used chi-square tests of independence to test for greater than chance overlap in the number and expression concordance of transcripts based on (1) population and rearing effects within a single drainage, and (2) differentially expressed transcript sets between the two datasets. Within drainage, we compared the direction of genetic and plastic expression changes in all transcripts differentially expressed based on population of origin, even if these transcripts were not differentially expressed based on rearing environment. We reasoned that even subthreshold expression changes in response to rearing environment could affect expression divergence propensity. We used log-fold expression changes in transcript abundance to determine whether transcript expression differences were in the same (e.g. upregulated in high-predation and in response to rearing with predator cues, or down-regulated in response to genetic and environmental exposure to predation) or opposite directions (e.g. upregulated in high-predation populations, but down-regulated in response to rearing with predators, or vice versa).

Between drainages we compared those transcripts significantly differentially expressed based on population of origin in both drainages (i.e. the intersection of population DE lists) as well as those transcripts significantly differentially expressed in either drainages (i.e. the union of

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

Analysis of transcripts with significant interaction effects

Based on post hoc differences between rearing environments within a population ($p<0.05$), we grouped transcripts with statistically significant interaction effects into categories outlined by Renn and Schumer (Renn and Schumer 2013): (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 not 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; and (5) Unclassified: all remaining transcripts which had a significant main interaction effect but no significant *post hoc* rearing differences, as the lack of pairwise rearing effects meant transcripts could not be unambiguously classified into one of the other categories. All statistical tests and data visualization were performed in R (version 3.5.1; The R Foundation for Statistical Computing). Additional details of statistical procedures are in the Supplemental Methods.

Results

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

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

analyses for population of origin, rearing, and interaction effects are in the Supplemental Materials (Tables S4 & S5).

While we cannot rule out some influence of technical variation, we found no evidence for differences in sequence quality, read alignment statistics, or variance in transcript abundance to suggest the greater number of differentially expressed transcripts in the Quare drainage was of technical origin. Nor did we find evidence that identification of DE transcripts was biased by transcript expression level: mean and median gene expression were significantly lower in DE (population, rearing, and/or interaction effects) as compared to non-DE transcripts in the Aripo dataset (Fig. S2; mean: DE=365.86, non-DE=451.91, $p<0.0001$; median: DE=97, non-DE=150, $p<0.0001$), whereas they were significantly higher in the Quare dataset (Fig. S2; mean: DE=460.76, non-DE=422.38, $p<0.0001$; median: DE=170, non-DE=131, $p<0.0001$). Opposing patterns between drainages suggest that identification of DE transcripts was not a side effect of relatively greater or lesser expression values of these transcripts.

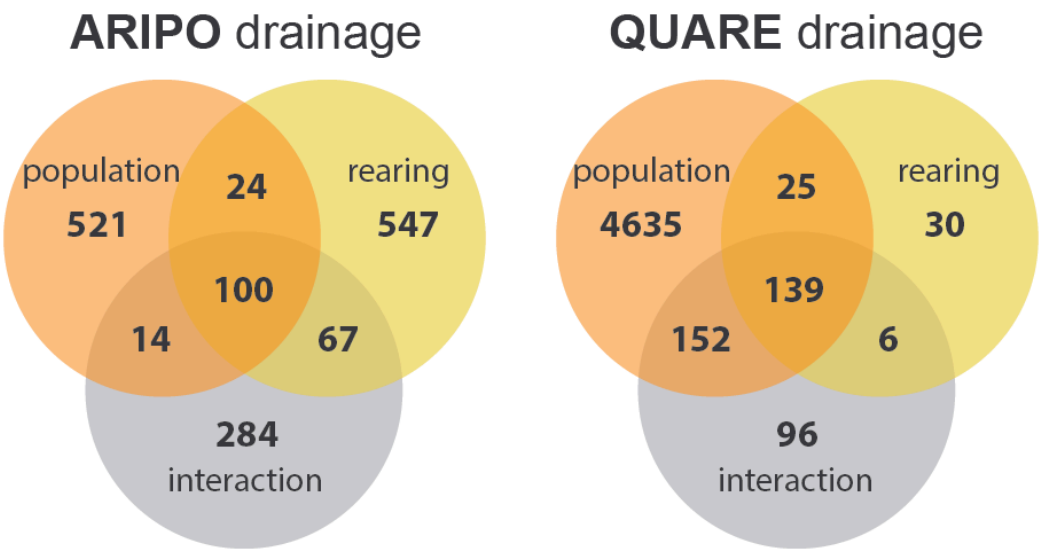

Figure 1. Summary of differential expression analyses. We identified many transcripts in both the Aripo (left) and Quare (right) drainages whose expression levels differed based on evolutionary history of predation (population of origin; orange), developmental experience of predation (rearing; yellow), 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.

Relationship between phenotypic plasticity and genetic divergence in transcript abundance

In both drainages, the sets of transcripts with main effects of population of origin and of rearing environment overlapped more than expected by chance (Fig. 1; Aripo: 24 transcripts, χ^2=24.109, p=0.0065; Quare: 25 transcripts, χ^2=66.413, p=0.0005; transcripts with interaction effects excluded from analysis). Given this non-independence of population and rearing expression differences, we asked whether the direction of expression plasticity predicted gene expression divergence between populations. We did so for all population DE transcripts regardless of whether plastic expression differences were significant or not, reasoning that even small expression changes in response to rearing environment could influence divergence propensity. We found no significant relationship between the direction of population divergence and rearing expression in either drainage, with approximately 50% of transcripts showing population and rearing expression changes in the same direction and 50% showing genetic and plastic expression changes in opposite directions in both drainages (Fig. 2).

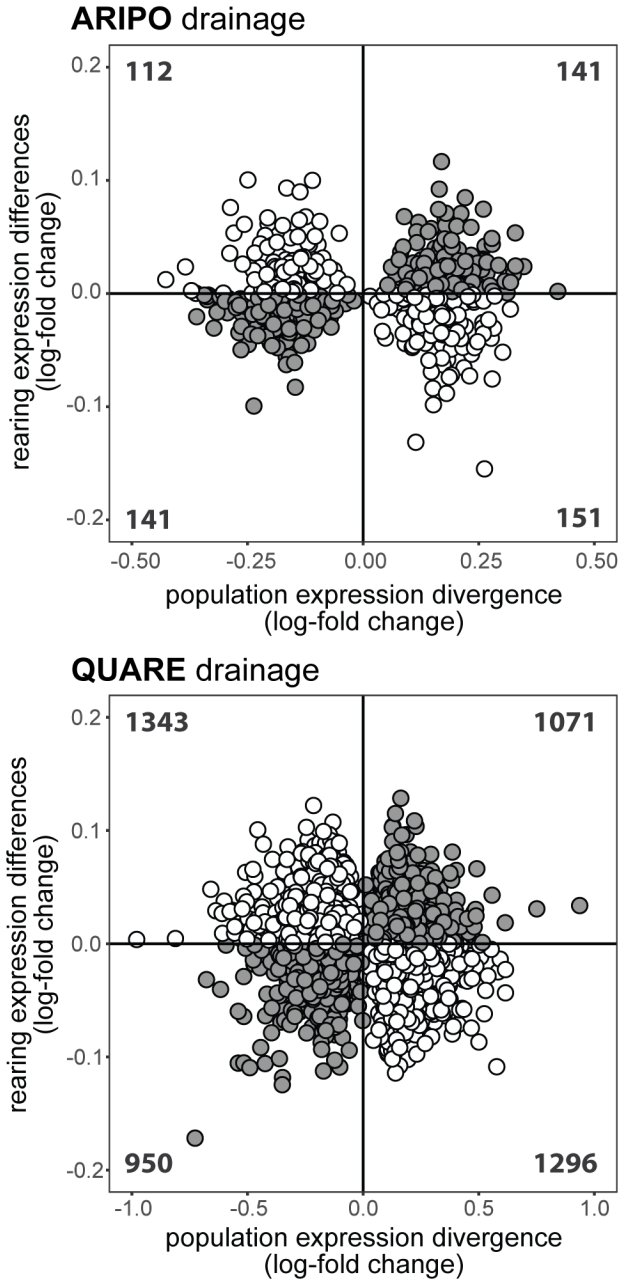

Figure 2. Relationship between plasticity and expression divergence. We found no relationship between evolved expression divergence and the direction of plastic expression differences: approximately half of genes differentially expressed between high- and low-predation populations have the same directions 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).

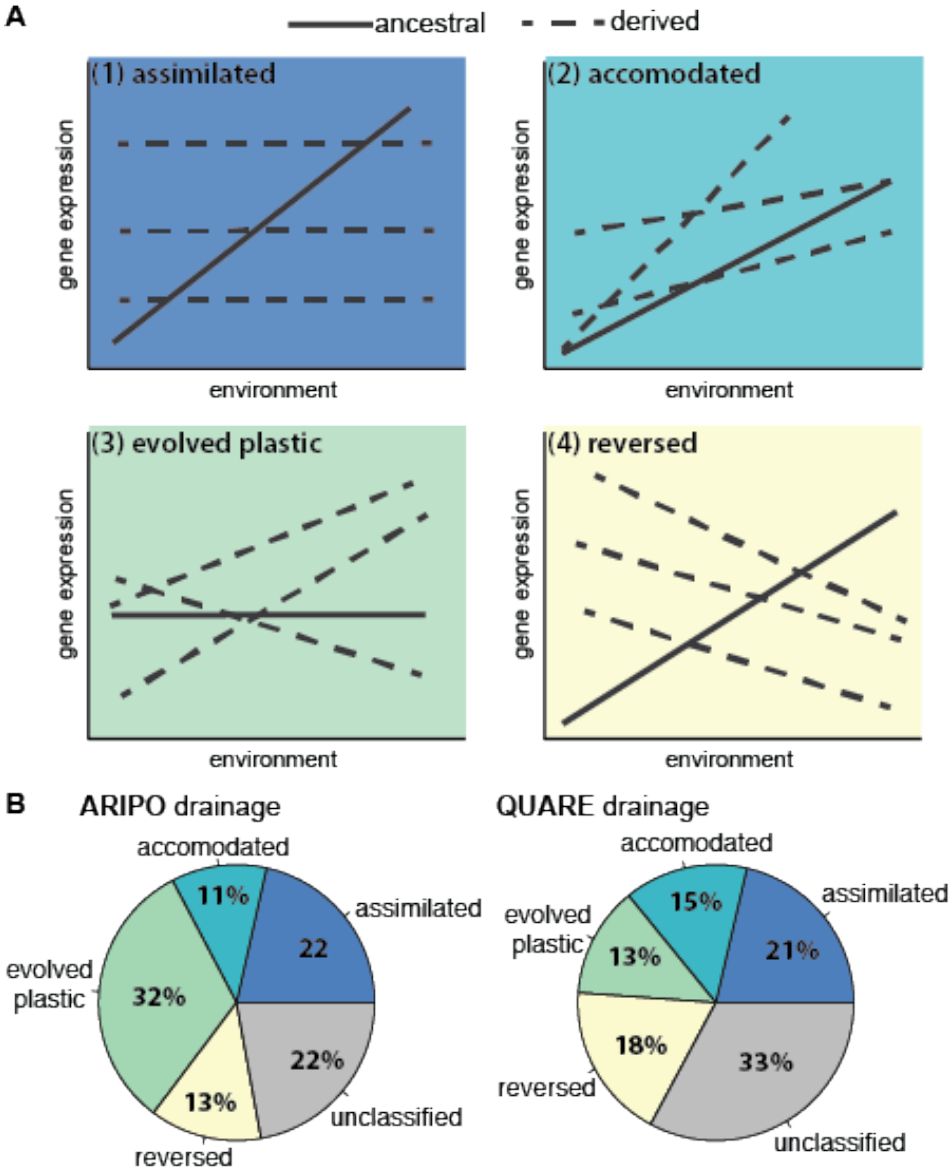

Figure 3. Evolution of transcript expression plasticity. (A) We grouped transcripts with significant interaction effects into one of four categories based on patterns of evolution in expression plasticity: (1) Assimilated: plasticity in the ancestral high-predation population but a loss of plasticity 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.

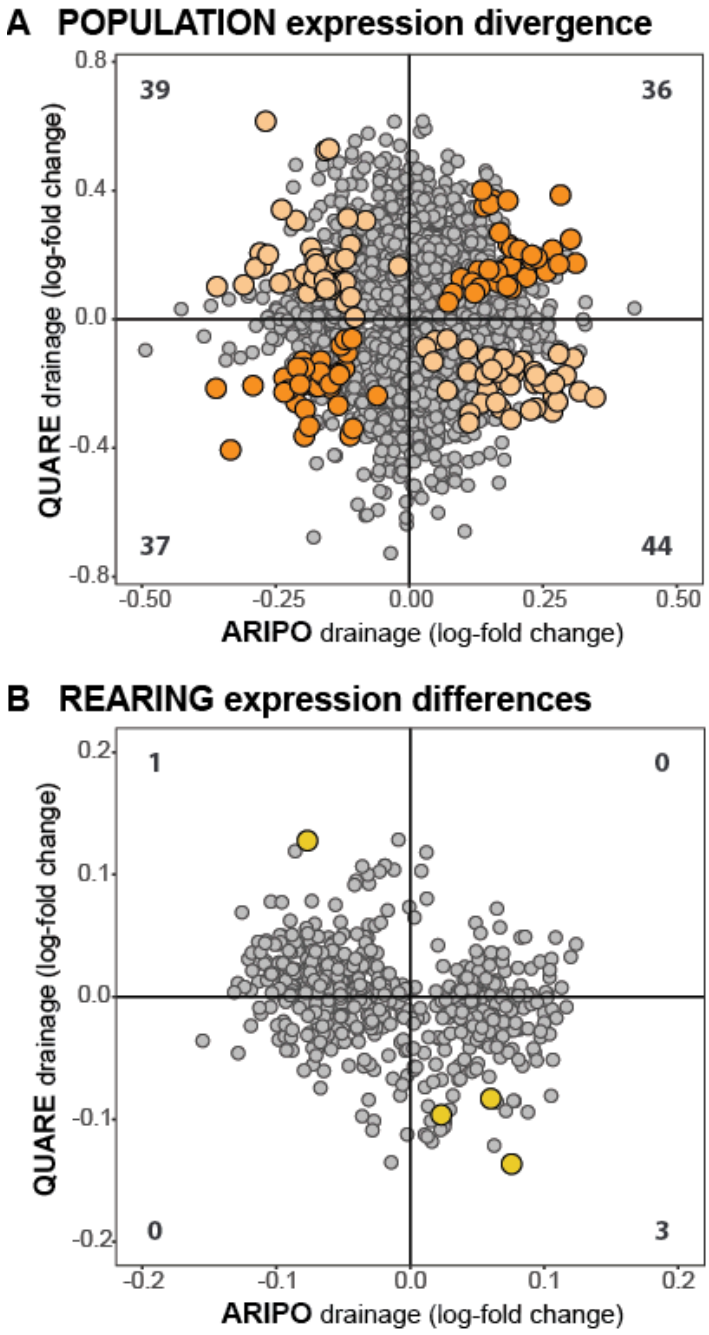

Figure 4. Overlap in population and rearing expression differences across evolutionary lineages. (A) Of the transcripts differentially expressed based on population of origin, 156 were overlapping between drainages. Of these, 73 were concordantly differentially expressed (i.e. genetic expression divergence 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.

Discussion

We examined transcriptomic differences associated with adaptive divergence and rearing environment across repeated, independent evolutionary lineages in guppies. Within lineages, we observed phenotypic plasticity in expression patterns as well as the evolution of gene expression plasticity between high- and low-predation populations. Although plastic transcripts were more likely to exhibit population differences in expression, we found no relationship between the direction of plastic change and evolutionary divergence. Comparing across lineages, we found largely non-overlapping gene sets and no evidence for an association in the direction of expression divergence associated with parallel phenotypic divergence, suggesting that transcriptional versatility is associated with parallel phenotypic adaptation in guppies.

Impacts of plasticity in transcript expression on evolution

A growing number of studies have used transcriptome and proteome surveys to address the long-standing debate on the role of plasticity in evolution, with contrasting results favoring alternative hypotheses that adaptive (Scoville and Pfrender 2010; Fraser et al. 2014; Shaw et al. 2014; Gleason and Burton 2015; Mäkinen et al. 2016; Li et al. 2018; Wang and Althoff 2019) or non-adaptive (Pespeni et al. 2013; Schaum et al. 2013; Dayan et al. 2015; Ghalambor et al. 2015; Ho and Zhang 2018) plasticity facilitates adaptation. We recently proposed that non-adaptive plasticity dominates during the earliest stages of rapid evolution, and that adaptive plasticity may contribute to subsequent fine-tuning of phenotypes (Fischer et al., 2016). In line with this prediction and our previous findings, we report here that the strong signature of non-adaptive plasticity in brain gene expression observed in guppy populations recently introduced to low-predation environments (Ghalambor et al., 2015) is not present in either of our natural, long-established low-predation populations. Experimental design differences preclude a direct comparison, as we considered patterns in all transcripts exhibiting significant expression divergence, while Ghalambor et al. (2015) focused their analyses on those transcripts for which evidence of selection was strongest (i.e. those with concordant differential expression in three low-predation populations from the same drainage, all assayed simultaneously and sharing a common ancestral population). The lack of association between the direction of plasticity and genetic divergence in our study highlights the need to directly test how ancestral plasticity relates to divergence throughout successive stages of adaptation to novel environments.

Evolution of plasticity in transcript expression

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

variation, founder effects and bottlenecks, and drainage-specific environmental conditions result in distinct transcriptional trajectories to arrive at shared organisms level phenotypes. Nonetheless, transcriptional patterns may become more similar over time (i.e. environmentally plastic versus evolved responses) as the funneling effects of selection push populations towards those transcriptional solutions with the smallest pleiotropic load (Stern & Orgogozo, 2008; Gompel & Prud'homme, 2009). Because transcriptional network configuration – which emerges from a combination of adaptive, non-adaptive, and neutral divergence – will shape evolutionary outcomes, a holistic understanding of convergent phenotypic evolution will ultimately require an understanding of the origins and impacts of concordant and lineage-specific transcript divergence across timescales.

Conclusions

We assessed the extent to which adaptation to common environments targets predictable changes in transcript expression across independent evolutionary events. Within lineages, genes with a greater expression plasticity were more likely to diverge in abundance between populations. At the same time, parallel adaptation to low-predation environments in independent lineages was associated with divergence in largely non-overlapping transcripts. While identification of shared genes is generally used as the starting point for work exploring mechanisms of parallel adaptation, we propose that parallel evolutionary transitions are not limited to a small set of possible transcriptional mechanisms in guppies. Instead, our results highlight the potential for extensive transcriptional versatility associated with parallel, adaptive trait evolution even within a single species. Transcriptional network versatility, in which diverse alterative network configurations can produce common network outputs and behavioral phenotypes, may allow underlying networks to simultaneously accommodate the influences of selection, drift, and genetic background and thereby facilitate evolution in a species known for rapid adaptation to novel environments.

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464 **References**

465 Abouheif, E., and G. Wray. 2002. Evolution of the gene network underlying wing polymorphism
466 in ants. Science. 297:249–252.
467 Alberch, P. 1991. From genes to phenotype: dynamical systems and evolvability. Genetica.
468 84(5):5–11.
469 Alexa, A., and J. Rahnenfuhrer. 2016. topGO: Enrichment Analysis for Gene Ontoloty. R
470 package version 2.30.1.
471 Badyaev, A. V. 2018. Evolutionary transitions in controls reconcile adaptation with continuity of
472 evolution. Semin. Cell Dev. Biol. 88:36-45.
473 Badyaev, A. V, and E. S. Morrison. 2018. Emergent buffering balances evolvability and
474 robustness in the evolution of phenotypic flexibility. Evolution. 72:647–662.
475 Baldwin, J. M. 1896. A New Factor in Evolution. Am. Nat. 441–451.
476 Barrett, R. D. H., and D. Schluter. 2008. Adaptation from standing genetic variation. Trends
477 Ecol. Evol. 23:38–44.
478 Barson, N. J., J. Cable, and C. Van Oosterhout. 2009. Population genetic analysis of
479 microsatellite variation of guppies (*Poecilia reticulata*) in Trinidad and Tobago: Evidence for
480 a dynamic source-sink metapopulation structure, founder events and population
481 bottlenecks. J. Evol. Biol. 22:485–497.
482 Bray, N. L., H. Pimentel, P. Melsted, and L. Pachter. 2016. Near-optimal probabilistic RNA-seq
483 quantification. Nat. Biotechnol. 34:525–527.
484 Conover, D. O., T. A. Duffy, and L. A. Hice. 2009. The covariance between genetic and
485 environmental influences across ecological gradients: Reassessing the evolutionary
486 significance of countergradient and cogradient variation. Ann. N. Y. Acad. Sci. 1168:100–
487 129.
488 Crawford, D. L., and M. F. Oleksiak. 2007. The biological importance of measuring individual
489 variation. J. Exp. Biol. 210:1613–1621.
490 Dayan, D. I., D. L. Crawford, and M. F. Oleksiak. 2015. Phenotypic plasticity in gene expression
491 contributes to divergence of locally adapted populations of *Fundulus heteroclitus*. Mol.
492 Ecol. 24:3345–3359.
493 Draghi, J. A., and M. C. Whitlock. 2012. Phenotypic plasticity facilitates mutational variance,
494 genetic variance, and evolvability along the major axis of environmental variation. Evolution
495 (N. Y). 66:2891–2902.
496 Endler, J. A. 1995. Multiple-trait coevolution and environmental gradients in guppies. Trends
497 Ecol. Evol. 10:22–29.

498 Espinosa-Soto, C., O. C. Martin, and A. Wagner. 2011. Phenotypic plasticity can facilitate
499 adaptive evolution in gene regulatory circuits. BMC Evol. Biol. 11:1–14.
500 Fajen, A. V. A., and F. Breden. 1992. Mitochondrial DNA sequence variation among natural
501 populations of the Trinidad guppy, *Poecilia reticulata*. Evolution. 46:1457–1465.
502 Feiner, N., A. Rago, G. M. While, and T. Uller. 2017. Signatures of selection in embryonic
503 transcriptomes of lizards adapting in parallel to cool climate. Evolution. 72:67–81.
504 Fischer, E. K., C. K. Ghalambor, and K. L. Hoke. 2016a. Can a Network Approach Resolve How
505 Adaptive vs Nonadaptive Plasticity Impacts Evolutionary Trajectories? Integr. Comp. Biol.
506 56:877–888.
507 Fischer, E. K., C. K. Ghalambor, and K. L. Hoke. 2016b. Plasticity and evolution in correlated
508 suites of traits. J. Evol. Biol. 29:991–1002.
509 Fischer, E. K., R. M. Harris, H. A. Hofmann, and K. L. Hoke. 2014. Predator exposure alters
510 stress physiology in guppies across timescales. Horm. Behav. 65:165–172.
511 Fischer, E. K., D. Soares, K. R. Archer, C. K. Ghalambor, and K. L. Hoke. 2013. Genetically and
512 environmentally mediated divergence in lateral line morphology in the Trinidadian guppy
513 (*Poecilia reticulata*). J. Exp. Biol. 216:3132–3142.
514 Fitzpatrick, S. W., J. Torres-Dowdall, D. N. Reznick, C. K. Ghalambor, and W. Chris Funk. 2014.
515 Parallelism Isn't Perfect: Could Disease and Flooding Drive a Life-History Anomaly in
516 Trinidadian Guppies? Am. Nat. 183:290–300.
517 Fraser, B. A., I. Janowitz, M. Thairu, J. Travis, K. A. Hughes, B. A. Fraser, I. Janowitz, M.
518 Thairu, J. Travis, and K. A. Hughes. 2014. Phenotypic and genomic plasticity of alternative
519 male reproductive tactics in sailfin mollies. Proc. R. Soc. B Biol. Sci. 281:20132310.
520 Fraser, B. A., A. Künstner, D. N. Reznick, C. Dreyer, and D. Weigel. 2015. Population genomics
521 of natural and experimental populations of guppies (*Poecilia reticulata*). Mol. Ecol. 24:389–
522 408.
523 Ghalambor, C. K., K. L. Hoke, E. W. Ruell, E. K. Fischer, D. N. Reznick, and K. A. Hughes.
524 2015. Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in
525 nature. Nature 525:372–375.
526 Ghalambor, C. K., J. K. McKay, S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-
527 adaptive phenotypic plasticity and the potential for contemporary adaptation in new
528 environments. Funct. Ecol. 21:394–407.
529 Gibbons, T. C., D. C. Metzger, T. M. Healy, and P. M. Schulte. 2017. Gene expression plasticity
530 in response to salinity acclimation in threespine stickleback ecotypes from different salinity
531 habitats. Mol. Ecol. 26:2711–2725.

532 Gilliam, J. F., D. F. Fraser, and M. Alkins-Koo. 1993. Structure of a Tropical Stream Fish
533 Community : A Role for Biotic Interactions. Ecology 74:1856–1870.
534 Gleason, L. U., and R. S. Burton. 2015. RNA-seq reveals regional differences in transcriptome
535 response to heat stress in the marine snail *Chlorostoma funebralis*. Mol. Ecol. 24:610–627.
536 Gompel, N. & Prud'homme, B. 2009. The causes of repeated genetic evolution. *Developmental*
537 *Biology* 332: 36–47.
538 Grabherr, M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, L.
539 Fan, R. Raychowdhury, Q. Zeng, Z. Chen, E. Mauceli, N. Hacohen, A. Gnirke, N. Rhind, F.
540 Di Palma, B. W. Birren, C. Nusbaum, K. Lindblad-Toh, N. Friedman, and A. Regev. 2011.
541 Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat.
542 Biotechnol. 29:644–652.
543 Grashow, R., T. Brookings, and E. Marder. 2009. Reliable neuromodulation from circuits with
544 variable underlying structure. Proc. Natl. Acad. Sci. 106:11742–11746.
545 Grether, G. F. 2005. Environmental Change, Phenotypic Plasticity, and Genetic Compensation.
546 Am. Nat. 166:E115–E123.
547 Gutierrez, G. J., T. O. Leary, and E. Marder. 2013. Multiple Mechanisms Switch an Electrically
548 Coupled, Synaptically Inhibited Neuron between Competing Rhythmic Oscillators. Neuron
549 77:845–858. Elsevier Inc.
550 Haas, B. J., A. Papanicolaou, M. Yassour, M. Grabherr, P. D. Blood, J. Bowden, M. B. Couger,
551 D. Eccles, B. Li, M. Lieber, M. D. Macmanes, M. Ott, J. Orvis, N. Pochet, F. Strozzi, N.
552 Weeks, R. Westerman, T. William, C. N. Dewey, R. Henschel, R. D. Leduc, N. Friedman,
553 and A. Regev. 2013. De novo transcript sequence reconstruction from RNA-seq using the
554 Trinity platform for reference generation and analysis. Nat. Protoc. 8:1494–1512.
555 Handelsman, C. A., E. D. Broder, C. M. Dalton, E. W. Ruell, C. A. Myrick, D. N. Reznick, and C.
556 K. Ghalambor. 2013. Predator-induced phenotypic plasticity in metabolism and rate of
557 growth: Rapid adaptation to a novel environment. Integr. Comp. Biol. 53:975–988.
558 Handelsman, C. A., E. W. Ruell, J. Torres-Dowdall, and C. K. Ghalambor. 2014. Phenotypic
559 Plasticity Changes Correlations of Traits Following Experimental Introductions of
560 Trinidadian Guppies (*Poecilia reticulata*). Integr. Comp. Biol. 54:794–804.
561 Haskins, C., E. Haskins, J. McLaughlin, and R. Hewitt. 1961. Polymorphism and population
562 structure in *Lebistes reticulatus*, an ecological study. Pp. 320–395 *in* F. Blair, ed.
563 Vertebrate speciation. University of Austin Press, Austin.
564 Ho, W., and J. Zhang. 2018. Evolutionary adaptations to new environments generally reverse
565 plastic phenotypic changes. Nat. Commun. 9:1–11.

566 Huizinga, M., C. K. Ghalambor, and D. N. Reznick. 2009. The genetic and environmental basis
567 of adaptive differences in shoaling behaviour among populations of Trinidadian guppies,
568 *Poecilia reticulata*. J. Evol. Biol. 22:1860–1866.
569 Insel, T. R., and L. J. Young. 2000. Neuropeptides and the evolution of social behavior. Curr.
570 Opin. Neurobiol. 10:784–789.
571 Lande, R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity
572 and genetic assimilation. J. Evol. Biol. 22:1435–1446.
573 Lehmann, E. L., and J. P. Romano. 2005. Testing Statistical Hypotheses. Third Edit. Springer,
574 New York.
575 Li, L., A. Li, K. Song, J. Meng, X. Guo, S. Li, C. Li, P. De Wit, H. Que, F. Wu, W. Wang, H. Qi,
576 and F. Xu. 2018. Divergence and plasticity shape adaptive potential of the Pacific oyster.
577 Nat. Ecol. Evol. 2:1751–1760.
578 Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and
579 dispersion for RNA-seq data with DESeq2. Genome Biol. 15:1–21.
580 Lynch, M. 2007. The frailty of adaptive hypotheses for the origins of organismal complexity.
581 Proc. Natl. Acad. Sci. 104:8597–8604.
582 Magurran, A. E. 2005. Evolutionary ecology: the Trinidadian guppy. Oxford University Press,
583 Oxford.
584 Mäkinen, H., S. Papakostas, L. A. Vøllestad, E. H. Leder, and C. R. Primmer. 2016. Plastic and
585 evolutionary gene expression responses are correlated in European grayling (*Thymallus*
586 *thymallus*) subpopulations adapted to different thermal environments. J. Hered. 107:82–89.
587 Manceau, M., V. S. Domingues, C. R. Linnen, E. B. Rosenblum, and H. E. Hoekstra. 2010.
588 Convergence in pigmentation at multiple levels: mutations, genes and function. Philos.
589 Trans. R. Soc. B Biol. Sci. 365:2439–2450.
590 Pespeni, M. H., E. Sanford, B. Gaylord, T. M. Hill, J. D. Hosfelt, H. K. Jaris, M. LaVigne, E. A.
591 Lenz, A. D. Russell, M. K. Young, and S. R. Palumbi. 2013. Evolutionary change during
592 experimental ocean acidification. Proc. Natl. Acad. Sci. 110:6937–6942.
593 Price, T. D., A. Qvarnström, and D. E. Irwin. 2003. The role of phenotypic plasticity in driving
594 genetic evolution. Proc. R. Soc. B Biol. Sci. 270:1433–1440.
595 Renn, S. C. P., and M. E. Schumer. 2013. Genetic accommodation and behavioural evolution :
596 insights from genomic studies. Anim. Behav. 85:1012–1022.
597 Reznick, D. A., H. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in
598 a natural population. Nature 346:357–359.
599 Reznick, D., M. J. Butler IV, and H. Rodd. 2001. Life-History Evolution in Guppies. VII. The

600 Comparative Ecology of High- and Low-Predation Environments. Am. Nat. 157:126–140.
601 Reznick, D. N. 1997. Life history evolution in guppies (Poecilia reticulata): Guppies as a model
602 for studying the evolutionary biology of aging. Exp. Gerontol. 32:245–258.
603 Reznick, D. N., and H. Bryga. 1987. Life-History Evolution in Guppies (*Poecilia reticulata*): 1.
604 Phenotypic and Genetic Changes in an Introduction Experiment. Evolution. 41:1370.
605 Rosenblum, E. B., H. Rompler, T. Schoneberg, and H. E. Hoekstra. 2010. Molecular and
606 functional basis of phenotypic convergence in white lizards at White Sands. Proc. Natl.
607 Acad. Sci. 107:2113–2117.
608 Ruell, E. W., C. A. Handelsman, C. L. Hawkins, H. R. Sofaer, C. K. Ghalambor, and L. Angeloni.
609 2013. Fear, food and sexual ornamentation: plasticity of colour development in Trinidadian
610 guppies. Proc. R. Soc. B Biol. Sci. 280:20122019–20122019.
611 Schaum, E., B. Rost, A. J. Millar, and S. Collins. 2013. Variation in plastic responses of a
612 globally distributed picoplankton species to ocean acidification. Nat. Clim. Chang. 3:298–
613 302.
614 Scoville, A. G., and M. E. Pfrender. 2010. Phenotypic plasticity facilitates recurrent rapid
615 adaptation to introduced predators. Proc. Natl. Acad. Sci. 107:4260–4263.
616 Shaw, J. R., T. H. Hampton, B. L. King, A. Whitehead, F. Galvez, R. H. Gross, N. Keith, E.
617 Notch, D. Jung, S. P. Glaholt, C. Y. Chen, J. K. Colbourne, and B. A. Stanton. 2014.
618 Natural selection canalizes expression variation of environmentally induced plasticity-
619 enabling genes. Mol. Biol. Evol. 31:3002–3015.
620 Simão, F. A., R. M. Waterhouse, P. Loannidis, E. V. Kriventseva, and E. M. Zdobnov. 2015.
621 BUSCO: Assessing genome assembly and annotation completeness with single-copy
622 orthologs. Bioinformatics 31:3210–3212.
623 Song, L., and L. Florea. 2015. Rcorrector: Efficient and accurate error correction for Illumina
624 RNA-seq reads. Gigascience 4:1–8.
625 Stern, D.L. & Orgogozo, V. 2008. The loci of evolution: How predictable is genetic evolution?
626 *Evolution* 62: 2155–2177.
627 Torres-Dowdal, J., C. a Handelsman, D. N. Reznick, and C. K. Ghalambor. 2012. Local
628 adaptation and the evolution of of phenotypic plasticity in Trinidadian guppies (*Poecilia*
629 *reticulata*). Evolution. 66:3432–3443.
630 Torres Dowdall, J., C. A. Handelsman, E. W. Ruell, S. K. Auer, D. N. Reznick, and C. K.
631 Ghalambor. 2012. Fine-scale local adaptation in life histories along a continuous
632 environmental gradient in Trinidadian guppies. Funct. Ecol. 26:616–627.
633 Velotta, J. P., and Z. A. Cheviron. 2018. Remodeling Ancestral Phenotypic Plasticity in Local

Adaptation : A New Framework to Explore the Role of Genetic Compensation in the Evolution of Homeostasis. Integr. Comp. Biol. 58:1098–1110.

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

Wang, S. P., and D. M. Althoff. 2019. Phenotypic plasticity facilitates initial colonization of a novel environment. Evolution. 73:303–316.

West-Eberhard, M. J. 2003. Developmental plasticity and evolution. Oxford University Press, New York.

Willing, E. M., P. Bentzen, C. Van Oosterhout, M. Hoffmann, J. Cable, F. Breden, D. Weigel, and C. Dreyer. 2010. Genome-wide single nucleotide polymorphisms reveal population history and adaptive divergence in wild guppies. Mol. Ecol. 19:968–984.

Zandonà, E., S. K. Auer, S. S. Kilham, J. L. Howard, A. López-Sepulcre, M. P. O'Connor, R. D. Bassar, A. Osorio, C. M. Pringle, and D. N. Reznick. 2011. Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. Funct. Ecol. 25:964–973.