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Gene expression stasis and plasticity following migration into a foreign environment

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Abstract

Selection against migrants is key to maintaining genetic differences between populations linked by dispersal. However, migrants may mitigate fitness costs by proactively choosing among available habitats, or by phenotypic plasticity. We previously reported that a reciprocal transplant of lake and stream stickleback (*Gasterosteus aculeatus*) found little support for divergent selection. Here, we revisit that experiment to test whether phenotypic plasticity in gene expression may have helped migrants adjust to unfamiliar habitats. We measured gene expression profiles in stickleback via TagSeq and tested whether migrants between lake and stream habitats exhibited a plastic response to their new environment that allowed them to converge on the expression profile of adapted natives. We report extensive gene expression differences between genetically divergent lake and stream stickleback, despite gene flow. But for many genes, expression was highly plastic. Fish transplanted into the adjoining habitat partially converged on the expression profile typical of natives from their new habitat. This suggests that expression plasticity may soften the impact of migration. Nonetheless, lake and stream fish differed in survival rates and parasite infection rates in our study, implying that expression plasticity is not fast or extensive enough to fully homogenize fish performance.

KEYWORDS
convergence, gene expression, migration, plasticity, stickleback

1 | INTRODUCTION

What happens to an organism when it moves into a new habitat? Populations in disparate environments commonly exchange migrants. These migrant individuals are exposed to unfamiliar abiotic conditions and biotic communities to which their phenotypes may be poorly suited (Hereford, 2009; Kawecki & Ebert, 2004; Lenormand, 2002). Migrants can be maladapted to their new habitat because they inherited alleles that were selectively favoured in their native range but are untested by selection in their new habitat (Nosil, Vines, & Funk, 2005). Or, migrants' traits may have been shaped, during ontogeny, by their native environment (Davis & Stamps, 2004; Stamps & Davis, 2006). Either way, migrants' poor fit to their new habitat may frequently result in reduced survival, fecundity or mating success (Hereford, 2009). This selection against migrants is a key to maintaining genetic differences between populations linked by dispersal (Lenormand, 2002; Nosil et al., 2005). Yet, migrants may evade selection in two ways. First, they can proactively choose among available habitats to avoid environments to which they are mismatched (Edelaar & Bolnick, 2012; Edelaar, Siepielski, & Clobert, 2008). Or, migrants may plastically alter one or more phenotypic traits to acclimate to a new habitat (Davidson, Jennions, & Nicotra, 2011; Ghalambor, McKay, Carroll, & Reznick, 2007; López-Maury, Marguerat, & Bähler, 2008).

Plasticity is most frequently measured as change in phenotype in response to an environmental change. Reciprocal transplants or common garden experiments have been successful at partitioning the relative contributions of heritability vs. plasticity for a myriad of

ecologically relevant traits (Conover & Present, 1990; Pfennig, 1992; Schlichting & Pigliucci, 1998; West-Eberhard, 2003). A limitation of this literature, however, is a tendency to focus on readily measured phenotypic traits (e.g., morphology and size), which may not be the most crucial traits for migrants' performance and fitness, and which may not be representative of plasticity for other more subtle yet important traits (e.g., immunity, physiological homeostasis).

Gene expression profiling offers a much broader approach to assay the response of an individual to both abiotic and biotic stressors. Phenomics is often limited to the study of a few morphological traits that are identified a priori. In contrast, transcriptomics casts a broader net across many possibly relevant traits, although the choice of tissue to obtain RNA still places some constraints on the trait space being studied. Transcriptomics thus allows for more agnostic discovery of relevant traits or pathways. However, because of the substantial cost of transcriptomic analyses, there are few studies of transcriptome-wide plasticity in natural settings, and most of these have very limited biological replication (Todd, Black, & Gemmell, 2016). Other studies have achieved higher replication (and thus power) by testing for plasticity of just a few candidate genes. For example, Stutz, Schmerer, Coates, and Bolnick (2015) showed that stickleback fish transplanted between lakes converged strongly to resemble the immune gene expression profile (for seven candidate genes) of natives of their new environment, indicating strong plasticity (Stutz et al., 2015). But, is this plasticity particular to immune genes, or is it representative of gene expression across the transcriptome? We expect that the whole transcriptome may respond in one of four general patterns: (i) a large stress response, (ii) a lack of response, suggestive of a tolerance strategy, or (iii) a plastic response that may ameliorate selection (note that response 1 or 2 may be either adaptive or nonadaptive, depending on subsequent changes in fitness). (iv) Plastic responses that allow immigrants to converge on the resident phenotype may be adaptive if residents are locally adapted.

Here, we describe how the stickleback transcriptome responds to an unfamiliar environment. We recently conducted a reciprocal transplant of threespine stickleback, moved between adjacent but ecologically very different lake and stream habitats (Bolnick & Stutz, 2017; Stuart et al., 2017). The lake and stream populations are divergent with respect to morphology (Berner, Grandchamp, & Hendry, 2009; Oke et al., 2016; Stuart et al., 2017), genomic SNPs (Weber, Bradburd, Stuart, Stutz, & Bolnick, 2017) and immune gene allele frequencies (MHC IIb, Stutz and Bolnick, 2017). These differences strongly suggest that there is divergent selection, yet our reciprocal transplant experiment yielded little signal of local adaptation (Bolnick & Stutz, 2017). We hypothesized that plasticity may help migrants ameliorate the ill effects of dispersal into a foreign neighbouring habitat (Oke et al., 2016). If so, we would expect transplanted fish's transcriptomes to converge on the expression patterns of the native population. Here, we present a test of this prediction by analysing sticklebacks' transcriptomic response to transplantation between adjoining lake and stream habitats. Specifically, we tested for (i) baseline differences in gene expression between natives of each habitat, (ii) differences in gene expression associated with being moved from one habitat to another and (iii) convergence in expression profiles between native and transplanted individuals.

The threespine stickleback fish (*Gasterosteaus aculeatus*) offers an opportunity to study plasticity of both phenotypes and gene expression. Across Vancouver Island, British Columbia, there are many replicate pairs of lake and stream stickleback (Hendry, Taylor, & McPhail, 2002; Thompson, Taylor, & McPhail, 1997). These parapatric lake and stream populations are typically genetically and phenotypically divergent (Eizaguirre et al., 2011; Feulner et al., 2015; Reusch, Wegner, & Kalbe, 2001; Roesti, Hendry, Salzburger, & Berner, 2012; Weber, Bradburd et al., 2017). These phenotypic differences persist to some degree in constant laboratory settings indicating there are heritable differences (Oke et al., 2016) [for other common garden studies of phenotypic plasticity see (Berner et al., 2011; Jiang, Peichel, Ling, & Bolnick, 2017; Kalbe & Kurtz, 2006; Scharsack, Kalbe, Harrod, & Rauch, 2007)]. Adjoining lake and stream environments differ in both abiotic and biotic conditions including flow regime, oxygen concentration, habitat structure, resource availability, prey composition and parasite communities (Berner et al., 2009; Kaeuffer, Bolnick, Hendry, & Peichel, 2012; Lenz, Eizaguirre, Rotter, Kalbe, & Milinski, 2013; Stuart et al., 2017). The magnitude and direction of environmental differences between a lake and its outlet stream effectively predict the direction of phenotypic differentiation between lake and stream resident stickleback (Stuart et al., 2017). The implication, invoked by many studies of lake and stream stickleback (summarized in Weber, Bradburd et al., 2017), is that environmental differences drive divergent selection on lake and stream stickleback.

To test for this inferred selection, multiple studies have transplanted lake and stream stickleback into their native and neighbouring habitats, measuring whether residents systematically outperform immigrants in a variety of measures (survival, growth, infection; Bolnick, 2004; Bolnick & Stutz, 2017; Hanson, Moore, Taylor, Barrett, & Hendry, 2016; Hendry et al., 2002; Kaufmann, Lenz, Kalbe, Milinski, & Eizaguirre, 2017; Moser, Frey, & Berner, 2016; Scharsack, Kalbe et al., 2007). However, these experiments yielded surprisingly inconsistent evidence for divergent selection (summarized in extended data of (Bolnick & Stutz, 2017)). Why is divergent selection rarely observed (but see (Hendry et al., 2002; Kaufmann et al., 2017), despite evidence of phenotypic divergence? Several recent studies discuss the possibility of habitat choice helping to maintain lake–stream differences (Berner & Thibert-Plante, 2015; Bolnick et al., 2009; Jiang, Torrance, Peichel, & Bolnick, 2015; Weber, Bradburd et al., 2017). Another possibility is that plasticity mitigates selection against migrants. Here, we use a reciprocal transplant experiment that found negligible support for divergent selection, to also test for plasticity. We measured both physical traits (e.g., change in mass over a given period or the value of an ecologically relevant trait) and gene expression profiles via TagSeq (Lohman, Weber, & Bolnick, 2016) for a large number of transplanted individuals. Using this data, we tested whether migrants' gene expression shifts to more closely resemble expression by the native population in their new habitat,

suggesting a role for expression-mediated phenotypic plasticity by migrants.

There is ample evidence for phenotypic plasticity in ecologically relevant traits in stickleback. For example, previous experiments reared stickleback from different habitats in a common garden setting (laboratory aquaria), and fed them alternative diets to test for plasticity in feeding morphology (Day & McPhail, 1996; Svanbäck & Schluter, 2012). These studies measured body shape, gill raker and gape traits that are both readily measured and clearly relevant to foraging. Life-history traits also show plasticity in stickleback, including breeding size, clutch size, egg size and relative clutch mass (Baker & Foster, 2002). Finally, prior studies have examined plasticity in gene expression (Gibbons, Metzger, Healy, & Schulte, 2017; Leder et al., 2014; Robertson, Bradley, & MacColl, 2016; Wang et al., 2014). One such study focused on expression of two candidate genes for osmoregulation and salinity tolerance (McCairns & Bernatchez, 2010). A larger, whole-transcriptome approach suggested that the invasion of freshwater and thermal tolerance drove the evolution of gene expression plasticity (Morris et al., 2014). However, while these studies of gene expression plasticity have sought to answer how the transcriptome may respond to a novel environment, they have been carried out in the laboratory and do not account for the diverse stressors of the wild. We therefore tested whether migrants between lake and stream habitats indeed exhibit a strong plastic response to their new environment that allows them to converge on the gene expression profile of the native population. To the extent that native gene expression is honed by previous natural selection, it is reasonable to suspect that such convergence reflects adaptive transcriptional plasticity.

2 | METHODS

2.1 | Sample acquisition

We analyse data from a reciprocal transplant experiment using stickleback from Roberts Lake and Stream (Vancouver Island, British Columbia, Canada), whose fitness effects were previously reported by Bolnick and Stutz (2017). Prior studies have documented differences between these populations, with respect to genotype and phenotype (Weber, Bradburd et al., 2017; Stutz & Bolnick, 2017; Berner et al., 2009, among others). Wild-caught stickleback were trapped, weighed, measured for length, individually marked with unique spine clips and then placed in cylindrical wire cages. Lake cages and stream cages both received a total of 60 lake fish and 60 stream fish. Each cage was ~1.6 m in diameter, placed in 1 m deep water and sealed to the substrate to prevent escape. Cages were made of wire mesh that allowed free flow of water and movement of prey items. In the lake, cages were situated along the shoreline approximately 150 m from the outlet stream. In the stream, cages were placed 150 m downstream from the lake. In an effort to reduce the influence of gene flow on stream genotypes, stream fish for the experiment were gathered from 1.5 km downstream of the lake outlet. Each of the 80 enclosures (40 in the lake and 40 in the stream) contained three fish. Half the cages received a 1:2 ratio of lake:stream fish, the other half of the cages received a 2:1 ratio. Thus, a total of 240 fish were transplanted, with 60 in each of the four treatments detailed below. Within each cage, the three fish were uniquely marked with dorsal spine clips to facilitate identification. After 8 weeks, Bolnick and Stutz recaptured the caged stickleback. As a control for the effect of caging, Bolnick and Stutz also collected wild uncaged fish from both lake and stream at the conclusion of the experiment, from habitat immediately adjoining the cages. Hereafter, here we refer to uncaged fish as the "wild" group, all fish recovered from cages are "transplanted." Within the transplanted fish, we distinguish between "natives" (same origin and destination habitats) and "immigrants" (different origin and destination).

At the conclusion of the field experiment, Bolnick and Stutz euthanized the collected fish with an overdose of MS-222. Fish were weighed, measured and dissected to remove head kidneys ("pronephros") which were stored in RNAlater (Ambion) for subsequent RNA extraction and expression analysis. Head kidney was chosen because it is the major site of hematopoiesis and the site of an immune response (Fischer, Koppang, & Nakanishi, 2013; Fischer et al., 2006; Scharsack, Kalbe, Derner, & Millinski, 2004; Scharsack, Koch, & Hammerschmidt, 2007). After dissection, specimens were preserved in ethanol for later dissection to enumerate parasites by complete dissection and examination under dissecting microscope (including body cavity, external surface and all organs). Morphological features were measured with digital callipers (pelvic width is the width of the pelvic girdle, body depth is the distance from the base of the first dorsal spine and the anterior point of the pelvic girdle, gape width is the distance between mouth corners). Because of its importance in defence against parasites, Bolnick and Stutz (2017) sequenced MHC IIb exon 2 from all caged fish, using DNA from prerelease spine clips. MHC IIb was sequenced and data were analysed as described in Stutz and Bolnick (2014). A previous clinal survey of stickleback from this lake and stream revealed population differences in MHC IIb allele frequencies and significant associations between MHC alleles and the prevalence of particular parasites (Stutz & Bolnick, 2017). The lake–stream transplant experiment revealed that transplanted foreign fish accumulated higher parasite infections than native fish, but parasites apparently exploited native MHC genotypes (Bolnick & Stutz, 2017; Stutz & Bolnick, 2014). Here, we use the MHC data to test for correlations between genotype and transcriptome profile.

2.2 | RNAseq library preparation, sequencing and bioinformatics

Following total RNA extraction (Ambion AM1830), we built 96 individual TagSeq libraries according to Lohman et al. (2016). We selected fish to construct a fully balanced design with 16 individuals in each treatment. We selected individuals that had been housed in the same cage, as available. We prioritized cage over sex ratio resulting in more males than female (48 vs. 39 in the final data set). However, the sex ratios with transplanted fish are nearly even: foreign transplants: 15/14 and native transplants: 16/14 (males/

females). TagSeq libraries were sequenced on the HiSeq 2500 with 1x100V4 chemistry at the Genome Sequencing and Analysis Facility at the University of Texas at Austin, generating an average of ~5 M raw reads per sample. This read depth is appropriate for TagSeq (because sequencing effort is targeted at the 3′ end of the mRNA) and has been shown to be successful (Dixon et al., 2015; Kenkel & Matz, 2016; Lohman et al., 2016; Meyer, Aglyamova, & Matz, 2011; Meyer et al., 2009; Wright, Aglyamova, Meyer, & Matz, 2015).

Raw reads were processed (removal of adapter contamination, poly-A and PCR duplicates followed by quality filtering, *n*—20) according to the iRNAseq pipeline (Dixon et al., 2015; Lohman et al., 2016; Meyer et al., 2011), producing a total of 19,556 genes. The stickleback genome contains 20,787 genes, so we conclude our read depth was sufficient, especially considering that we sampled a single tissue at a single time point. We further filtered these genes by removing all genes for which the mean among all samples was less than one, resulting in 9,748 genes for further analysis. Due to a machine error during the HiSeq run, BaseSpace was unable to convert cycle 35 to a base call, and thus base 35 is N in every read. We adjusted for this by adding the *–n* option to all calls to fastx_clipper in the iRNAseq pipeline. Mapping with Bowtie2 should not be influenced by this error (~53.3% alignment rate (min = 14.07%, max = 61.3%), postquality filtering, adaptor trimming and poly-A removal). GO enrichment was conducted according to Wright et al. (2015) using transcriptome annotation built with the UNIPROTKB database and following previously described procedures (Dixon et al., 2015; Lohman, Steinel, Weber, & Bolnick, In review).

2.3 | Statistical analysis

We analysed gene expression using a series of linear models in DESeq2 (Love, Huber, & Anders, 2014), limma (Ritchie et al., 2015) and base R (R Development Core Team 2007). All raw *p* values were multiple test corrected (10% FDR, Benjamini–Hochberg). We sought to estimate three effects.

2.3.1 | What are the differences between wild fish from Roberts Lake and Stream?

We tested for differences in gene expression between wild (uncaged) fish from Roberts Lake vs. Roberts Stream by modelling gene count as a function of origin (lake or stream). We tested for GO enrichment within the main effect of origin with a Mann–Whitney *U* via GO_MWU (Dixon et al., 2015). We used weighted gene coexpression network analysis (WGCNA; Langfelder & Horvath, 2008) to estimate correlations between suites of coexpressed genes and traits, including morphology, parasite burdens and genotypes (e.g., MHC allelic diversity). WGCNA is an unbiased, data-driven method to cluster groups of genes with similar expression patterns. We removed batch effects and normalized counts using limma (Ritchie et al., 2015) before starting WGCNA. We followed the tutorial of Langfelder and Horvath (2008), and constructed a signed network with a soft thresholding power of 6 and a minimum module size of 30 genes. We used dynamic tree cut and merged modules with greater than 80% similarity, producing a total of 11 modules. We plot the FDR-corrected Pearson correlation coefficient between module eigengenes and trait values. We have assumed that lake and stream fish have similar gene expression networks and combined both to generate a single coexpression network. However, merging lake and stream fish may confound correlations between modules and traits with an effect of origin. To ensure that this hidden variable problem did not obscure our results, we also generated population-specific networks (thereby eliminating the hidden variable) with identical parameters and recalculated correlations between modules and traits.

2.3.2 | What is the effect of being transplanted into a novel environment?

We tested for changes in gene expression of transplanted (caged) fish as function of origin habitat, destination habitat and the interaction between origin and destination. Using our estimated gene network, we calculated the FDR-corrected Pearson correlation coefficient between module eigengenes and traits unique to the transplant design (treatment, origin, destination, delta mass and delta length). A main effect of origin indicates stable gene expression differences between native lake vs. native stream fish. These expression differences can be stable because they are heritable, or because they are environmentally induced only early in ontogeny but remain canalized in adults, which we used for this experiment. A main effect of destination indicates genes that respond plastically to recently experienced environments. An interaction between origin and destination would indicate ecotype differences in how they respond to a given environment. Such interactions could entail G*E effects on expression, but we point out they could also arise from ecotype differences in the extent of canalization of early plasticity.

2.3.3 | How well do immigrants converge on the expression profile of natives?

We conducted a PCA of expression of all genes in all fish and then selected only transplanted fish and used the leading 57 PC axes (explaining 90% of the total variance) for subsequent linear discriminant analysis. The original expression matrix has too much collinearity for LDA. Dropping higher-order PCA axes reduces this collinearity, enabling LDA. This approach is sometimes called DAPC (Jombart, Devillard, & Balloux, 2010; Kenkel & Matz, 2016). We plotted these results in LDA space, adding vectors connecting each ecotype's expression at home to the same ecotype's expression in the foreign habitat. These vectors represent the magnitude and direction of expression plasticity along DAPC axes. Convergence in expression would result in an angle of 180° between the vectors for lake and stream ecotypes. Moreover, we compared the lengths of these vectors to evaluate whether lake and stream ecotypes are equally plastic.

Lastly, if plasticity effectively recreates lake–stream differences, then we would expect that genes that are more highly expressed in lake natives would also be more highly expressed in fish transplanted into the lake. This can be tested by measuring the correlation, across genes, between the origin effect sizes and destination effect sizes estimated in analysis (2) above. Adaptive plasticity generating convergence on the native expression profile should result in a positive correlation.

3 | RESULTS

3.1 | What are the differences between wild fish from Roberts Lake and Stream?

Our linear model revealed that 647 genes were differentially expressed between wild Roberts Lake and Roberts Stream stickleback (Wald, $p < .1$ after 10% FDR, or 306 when $p < .05$). GO analysis showed that these genes are enriched both for a variety of categories including (but not limited to) genes regulating macrophage differentiation (biological processes, Mann–Whitney U, $p < .05$ after 10% FDR correction, Figure 1), and genes involved in the MHC Class II protein complex (cellular components, Mann–Whitney U, $p < .1$ after 10% FDR). Both of these GO groups have known functions in parasite defence and have been previously implicated in the response to selection and parasite prevalence in stickleback (Bolnick & Stutz, 2017; Eizaguirre, Lenz, Kalbe, & Milinski, 2012; Lohman et al., In review). Previous studies revealed that Roberts Lake and Stream stickleback populations harbour significantly different parasite communities (Bolnick & Stutz, 2017), with corresponding differences in MHC Class II allele frequencies (Stutz & Bolnick, 2017).

In addition to gene-by-gene linear modelling, we also tested for correlations between modules of coexpressed genes and various traits, including morphology, infection by parasites and MHC Class IIb genotype (Figure 2). We found morphology to be correlated with many different modules, each with modest correlation but highly significant p values. It is noteworthy that all modules except the turquoise module have a negative correlation with morphology (coexpressed gene modules are given arbitrary colour names). There are correlations between MHC allele number and several modules, including greenyellow, blue, magenta and pink. Interestingly, MHC allele number and two measures of parasite diversity have equal strength but opposite sign in their correlation to the greenyellow module (Figure 3a). This is consistent with previous experimental and theoretical data that animals with more diverse MHC genotypes should have fewer parasites (Wegner, Kalbe, Kurtz, Reusch, & Milinski, 2003). Finally, we also considered linear discriminant axes of MHC II genotypes from a prior analysis of these same fish. We find that LDA1 and LDA3 of MHC II are correlated with turquoise and purple modules. The turquoise expression module is also correlated with fish origin ($r = .36$, $p \ll .001$), so these correlations are likely a result of differences in MHC genotype between the two ecotypes. Infection by several functional groups of parasites is significantly correlated with particular modules. For instance, the purple module is correlated with infection by nematodes ($r = -.22$, $p < .04$, Figure 3b) and genes in the red module are correlated to infection by any species of *Proteocephalus* (Figure 3c, $r = .28$, $p < .01$). In addition to population-independent correlations between modules and traits, we also observe correlations which are population specific. There are a larger number of significant correlations between infection by parasites and modules in this population-specific setting, suggesting that lake and stream fish may respond differently to different kinds of parasites (see Supporting information).

3.2 | What is the effect if being transplanted into a novel environment?

Focusing next on transplanted (caged) stickleback, we observed significant effects of both origin and destination for many genes (507 and 111, respectively when $p < .05$, see Supporting information for full list, after 10% FDR). Here, the effect of origin represents genotype effects that persisted after transplantation (because the effect of transplantation is averaged). Approximately 94% of the genes with significant ($p < .1$, after 10% FDR) origin effect in transplanted fish were also significantly different between wild fish ecotypes. This overlap of origin effects in caged and wild fish suggests that stickleback exhibit realistic lake–stream expression differences when placed in lake or stream cages.

Destination effects represent plasticity that was independent of genotype (genotype effects are averaged in our model). Most notably, this list of genes includes *hsp90* (lower in fish transplanted into the stream, Wald, $p \ll .001$ after 10% FDR), a stress response protein which has been studied in many different animals (Queitsch, Sangster, & Lindquist, 2002; Rutherford & Lindquist, 1998). In addition, *stat1* (lower in fish transplanted into the stream, Wald, $p < .09$ after 10% FDR) was also significantly different between fish transplanted in alternate environments. This transcription factor has a rich history of study for its critical role in multiple signalling cascades throughout the immune system (Murphy, 2011).

We found only 10 genes whose expression depended on the interaction of origin and destination (Wald, $p < .1$ after 10% FDR, or 4 when $p < .05$, Figure 4; Table S1, Fig. S1). Such interactions can loosely be interpreted as genotype by environment interactions (e.g., genetic differences in plasticity), with the caveat that we are studying wild-caught fish, so we cannot infer heritable differences with certainty. Of these 10 genes, two candidates are possibly involved in defence against parasites: *cyp24a1*, a cytochrome p450 variant (Annalora et al., 2010), and *dhx58*, an antiviral gene about which little is known (Leavy, 2012). In both cases, lake natives have higher expression in the lake than do stream fish, but decreased expression when moved into the stream. Stream fish have higher expression in their native habitat, but only higher than foreign lake fish for *dhx58*. Furthermore, it is noteworthy that for all 10 interaction genes, the genes are more highly expressed in lake than in stream fish (all in lake cages). And, for all 10 genes, the lake natives decrease expression when moved into the stream (Figure 4).

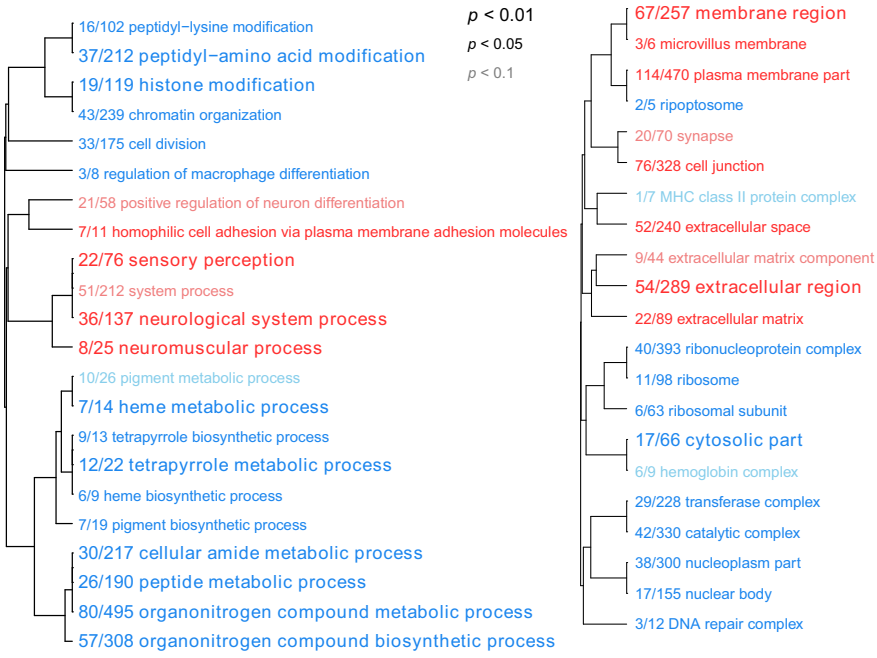

FIGURE 1 GO analysis results on Lake vs. Stream wild fish. Blue terms are underexpressed while red terms are overexpressed relative to the lake baseline. *p* values are Mann–Whitney U. Dendrograms indicate similarity of GO groups. Left group is from the biological processes cluster while the right group is from cellular components

We used DESeq2 to estimate caging effects by comparing wild fish to natives within each environment. We found a moderate number of differentially expressed genes; 35 genes were differentially expressed between wild lake fish and caged lake natives. Somewhat more genes (79) were differentially expressed between wild stream fish and stream natives (all Wald, $p < .1$ after 10% FDR, or 19 and 52 when $p < .05$, respectively. See Supporting information for full list). There are very few notable differences due to caging in lake genotypes. Lake natives have higher expression of *cyp24a1* than wild lake fish (log2 fold change = 3.8, $p = .065$ after 10% FDR correction). Lake transplants also have higher expression of *ebf4*, an early B-cell factor (log2 fold change = 4.3, $p = .049$ after 10% FDR correction) than wild lake fish. In contrast, when we make the same contrast but in stream genotypes, almost all differentially expressed genes (76 of 79 passing $p < .1$ after 10% FDR correction) exhibit a pattern of lower expression in transplants than in wild fish (see Supporting information for full list of genes and statistics). Stream transplants have lower expression of immune genes with known function including the complement system (complement 3, 8 and 9), a leucocyte-derived chemotaxin (*lect2l*) and three fibrinogen genes (alpha, beta and gamma). In addition, two coagulation factor genes are lower in natives (factor 13 and 7i) than wild stream fish. The cage effect for stream fish is partially confounded, however, with genotype. The stream transplants were from 1.5 km downstream of the cage site, whereas the wild fish were collected among the cages, 100 m downstream from the lake. So, differences between wild stream and transplanted stream fish may be genetic rather than exclusively a plastic response to caging. There were almost no genes (only 2) that showed significant effects of caging in both the lake and in the stream, indicating that there is no generic transcriptomic response to caging (Fig. S2).

Our coexpression analysis of transplanted fish revealed significant correlations between traits unique to this subset of fish and modules of gene expression. For example, treatment (transplanted into foreign or native environment) and origin are both correlated with the turquoise module. In contrast, destination is only weakly correlated to the pink and magenta modules. The red module has a negative correlation to origin and a positive correlation to change in length over the course of the experiment. Change in mass is correlated with both the magenta and purple modules. Interestingly, there is no overlap between change in mass and change in length. This difference suggests a change in condition within individuals (Figure 5).

3.3 | How well do immigrants converge on the expression profile of wild controls?

We tested for convergence between natives and immigrants in the entire expression profile. Within a bivariate discriminant function space, we found that LDA1 separates fish by origin (lake vs. stream, explains 86% of variance). LDA2 separated fish based on their transplant destination (explains 10% of variance). LDA3 roughly separates native/non-native status (explains 3.5% of variance, Figure 6; Fig. S3). We plotted a vector from the mean of each resident ecotype at home, to the mean expression of the same ecotype when moved into a new environment. The vector showing the expression change of lake fish is almost in exactly the opposite direction from the expression change of stream fish (~180°, visually). In each case, fish moved into a new habitat converged on the expression profile of their new neighbours along LD2 (but not along LD1 or LD3). Lake fish moved into the stream actually overshot the stream expression profile, resulting in a much larger reaction norm vector than stream fish moved into the lake (LD2 ~origin + destination + origin:destination) and found a significant effect of the interaction ($p \ll .001$). Because of this overshooting, both the lake-to-stream migrants and stream-to-lake migrants were significantly different (for LD2) from the resident "target." We conclude that immigrant stickleback partially converge on native expression profiles after emigration to a

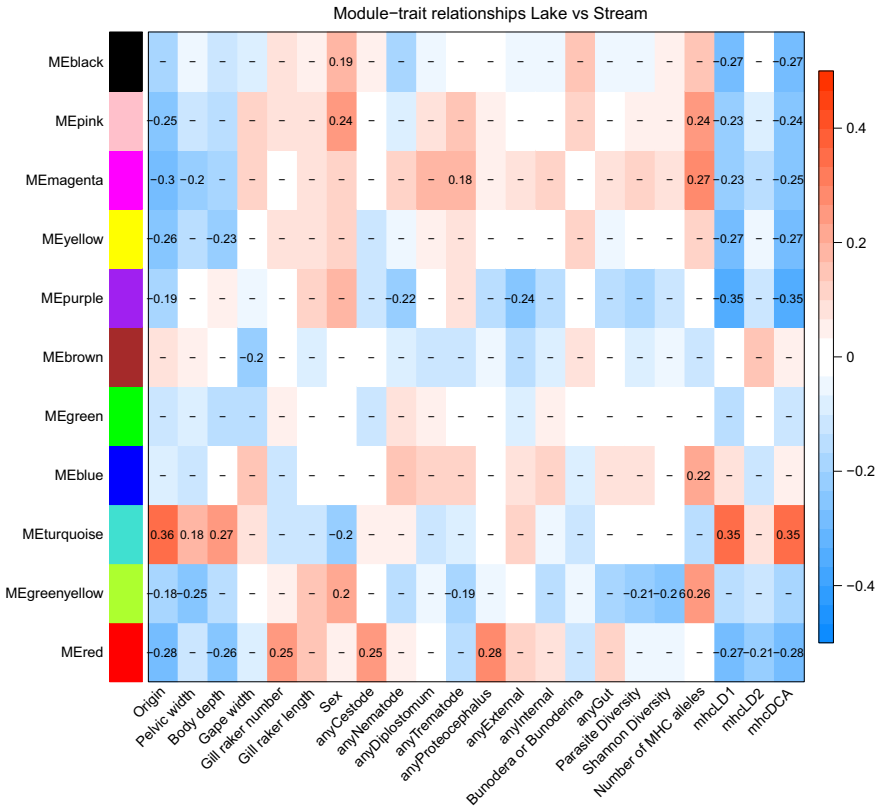

FIGURE 2 WGCNA reveals correlations between modules of coexpressed genes and traits in wild lake and stream fish. Cell values are Pearson correlation coefficients. Only correlations with p values <.1 are presented. Modules shown are the same as Figure 5. Pelvic width is the width of the pelvic girdle, body depth is the distance from the base of the first dorsal spine and the anterior point of the pelvic girdle, gape width is the distance between mouth corners. Columns labelled "Any" refer to any species of a broad group

new habitat and that lake fish exhibit stronger plasticity. The latter finding matches the greater plasticity of lake fish in our gene-by-gene analysis with DESeq2 (above; Figure 7).

We also considered convergence at the individual gene level. Using the DESeq2 linear model estimates, we found that destination effects were positively correlated with origin effects (r = .67, Figure 7). That is, transcripts that were more abundant in lake natives were also more abundant in fish placed in lake cages, and vice versa for stream-biased transcripts (Figure 7). This implies that for many genes, expression differences between the native populations are recapitulated by plastic responses to animals' recent environment. The observed destination origin relationship has a slope less than 1 (0.42, $p \ll .001$) indicating that the plasticity is not, however, complete, which fits with the fact that the major LDA axis still separates lake vs. stream natives and explains more variation than the second LDA axis that measured plasticity.

4 | DISCUSSION

Organisms' adaptation to their native habitats means that migrants will often be maladapted to novel environments. One way that migrants may be able to ameliorate stressors of new habitats is by modulating gene expression. Prior studies have used reciprocal transplants to uncover plasticity in select candidate genes, but this approach could miss a myriad of responses to the environment (although see (Ghalambor et al., 2015; Kenkel & Matz, 2016) for a transcriptome-wide approach). Transcriptional plasticity could be adaptive in several ways. First, generic stress responses could be used to protect immigrants' from unfamiliar environmental conditions, parasites, low energy income, etc. Such genes might be upregulated for all migrants regardless of origin or destination. Second, organisms may adjust their expression to better match the local environment, converging on residents of the migrants' new habitat. Such genes would exhibit transcriptional convergence, a main effect of destination habitat. Of course, we also must acknowledge that transcriptional plasticity can be maladaptive: a signal of stress or poor condition, or a misguided response to an unfamiliar environmental cue.

To look for static and plastic responses in gene expression associated with emigration, we tested for differences in gene expression among stickleback reciprocally transplanted between two adjoining habitats containing genetically divergent populations. We found expression differences between these populations, and changes in response to emigration, at the level of individual genes, gene coexpression and the whole transcriptome [see Jones et al., (2012) for similar discussion contrasting observations of wild marine and freshwater stickleback gene expression and (Ishikawa et al., 2017) for an eQTL study testing for the relative contribution of *cis* and *trans* regulatory elements and their connection with genomic islands of adaptation in adaptation to freshwater].

4.1 | There are constitutive differences in gene expression between lake and stream stickleback

Although Roberts Lake and Stream are adjoining habitats that permit easy movement of stickleback between sites, the resident stickleback

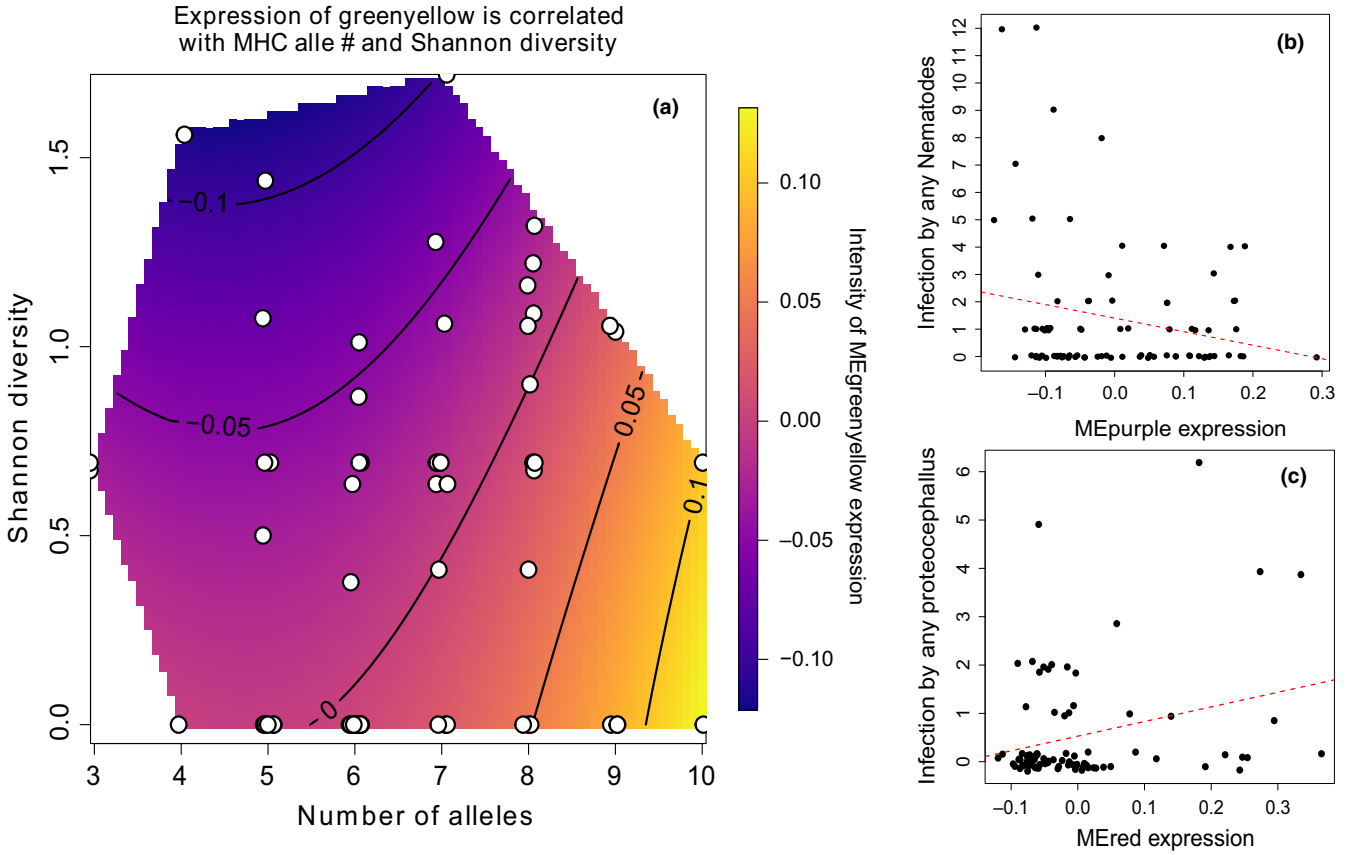

FIGURE 3 Module trait correlations from WGCNA. (a) Heat map of MEgreenyellow expression shows negative correlation with Shannon diversity of parasite infection and positive correlation with MHC allele number. (b) Increased expression of MEpurple is correlated with decreased infection by nematodes. (c) Increased expression of MEred is correlated with increased infection by proteocephalus

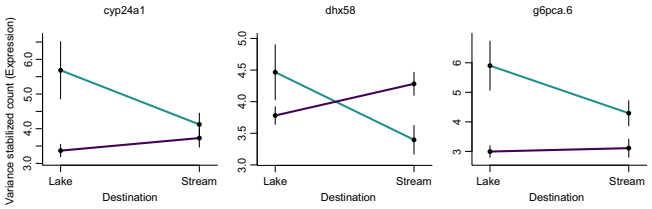

FIGURE 4 Reaction norm plots of genes significant for interaction between origin and destination. *X*-axis is destination, teal line is lake and magenta line is stream origin/genotype. Vertical line indicates standard error

populations are genetically distinct. Fish from this lake and stream differ in a range of morphological and parasitological traits (Berner et al., 2009; Bolnick & Stutz, 2017; Oke et al., 2016; Stutz & Bolnick, 2017; Weber, Bradburd et al., 2017), as is true for many such lake–stream pairs (Stuart et al., 2017). Given these genetic and phenotypic differences, we expected to find differences in gene expression between these populations. Approximately 7% of the 9,748 genes in our transcriptome data set exhibited between-population differences in relative abundance.

Some of these differences fit well within the existing literature of lake–stream divergence. For example, our GO enrichment results suggest that macrophage differentiation is different between lake and stream fish. Macrophages contribute to initiation of immune defences against a variety of parasites including but not limited to the tapeworm *Schistocephalus solidus* (Kurtz et al., 2006), whose infectious procercoids are deposited by loons and mergansers (which prefer lakes over streams) and carried by zooplankton (which are more abundant in lakes than streams). MHC class II is another parasite defence-related GO category which is different between lake and stream. Prior work in the Roberts Lake stickleback has revealed that MHC II allele frequencies differ between this particular lake and stream (Stutz & Bolnick, 2014), as well as many other lake–stream pairs (Eizaguirre et al., 2010; Kurtz et al., 2006; Wegner et al., 2006). Furthermore, individuals who carry local MHC alleles are more heavily infected with parasites than individuals carrying foreign MHC alleles (Bolnick & Stutz, 2017). Our WGCNA results suggest that MHC allele diversity and parasite diversity are negatively correlated with each other and jointly associated with a set of coexpressed genes. Specifically, the greenyellow module has a negative correlation with parasite diversity and a positive correlation with the number of MHC alleles (Figures 2 and 3a). While this result stands out as support for a large body of theory (Eizaguirre & Lenz, 2010; Spurgin & Richardson, 2010) and agrees with prior empirical evidence (Piertney, Telfer, & Oliver, 2009; Wegner et al., 2003), we would have expected greater correlations between modules and parasite infection. However, this lack of correlation is likely due to

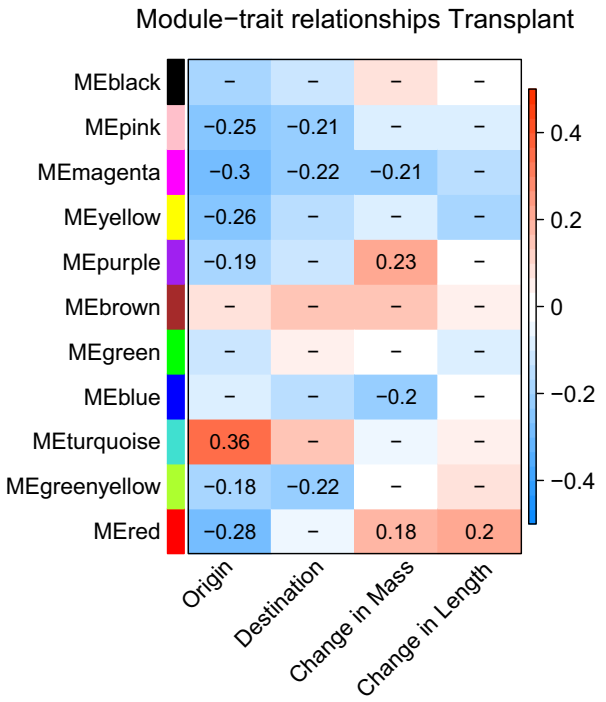

FIGURE 5 WGCNA reveals correlations between suites of coexpressed genes and traits in transplanted fish. Cell values are Pearson correlation coefficients. Only correlations with p values <.1 are presented. Modules shown are the same as Figure 2

sparse and overdispersed parasite infections, which make correlations difficult to estimate well.

4.2 | Transplantation into alternate habitats reveals static and plastic gene expression

For our experimentally transplanted fish, individuals' origin accounted for more expression variation (507 genes) than did destination (111 genes, Figure 7). The main effect of origin represents persistent between-population differences no matter which habitat the fish were caged in. Thus, we interpret the main effect of origin as a probable signal of static genetic differences in expression, insensitive to the environment. However, we also found significant destination effects for a subset of genes, indicating appreciable plasticity in gene expression in response to sticklebacks' recent (cage) environment. That is, expression of certain genes was higher in lake-caged fish than stream-caged fish, regardless of their origin. We infer that shifts in gene expression are the result of plasticity rather than selection because the expression profile of immigrants falls outside that of the natives in PCA space (Figure 6). This plasticity is consistent with prior evidence that morphological plasticity contributes to phenotypic differences between the Roberts Lake and Stream stickleback (Oke et al., 2016).

Notably, there was a positive correlation between origin effect and destination effect (r = .67). We therefore infer that the heritable lake-to-stream differences were at least partly recapitulated by plasticity. Genes more highly expressed in lake (stream) natives were also upregulated in all fish placed in lake (stream) cages (Figure 7). If expression was exclusively plastic (on the time-scale of our experiment), we would expect to see no origin effect at all, which is not the case. So, this correlation between origin and destination effects suggests that heritable and plastic differences jointly contribute, in the same direction, to between-ecotype differences in expression. The fact that the origin–destination effect correlation has a slope less than 1 confirms the statement, above, that heritable (origin) effects were somewhat stronger than the environmental (destination) effects. Moreover, the paucity of genes in the top-left and bottom-right quadrants of Figure 7 suggests that remarkably few genes exhibited plastic responses that opposed the heritable lake–stream differences.

Very few genes (10) were significant for the interaction of origin and destination. This is consistent with prior observations that there are no interactions effects between origin and destination for parasite load, survival, growth or condition in this experiment (Bolnick & Stutz, 2017). The few interactions that do exist follow two distinct patterns. First, some genes were downregulated after individuals were placed in a foreign habitat. Second, other genes were more highly expressed by lake fish, but also showed stronger plastic downregulation in lake fish placed in the foreign stream habitat. The absence of genes which were more highly expressed by stream fish, regardless of habitat, is notable. Some of the interaction genes we do detect may be involved in ROS production and antiviral response, both of which may be potentially important to fitness. For example, ROS production was recently shown to be a heritable response to infection by *S. solidus* (Weber, Steinel, Shim, & Bolnick, 2017).

We observed no cases where expression was higher in foreign habitats. While this could be a product of the low number of interaction genes, this pattern is surprising and worth considering in future work. Intuitively, we would have expected transplanted fish in either direction to upregulate stress genes, but this apparently did not occur. Perhaps the absence of interaction effects on genes here is because plasticity reinforced between-ecotype differences. The paucity of interaction effects may also be a consequence of our analytical technique: log transformation of expression levels converts multiplicative (interaction) effects into additive effects, which can reduce power or even completely obscure our ability to detect significant interactions between origin and destination effects. Nevertheless, other reciprocal transplant studies using large-scale RNAseq have found more interaction genes and this seems to be relatively common (Lovell et al., 2016; Reid et al., 2016).

Our WGCNA analysis revealed only weak correlations between origin and phenotypes unique to transplanted fish. For example, change in mass and length. However, it is interesting to note that changes in mass and length are most correlated with different modules. This may suggest a change in condition (loss of mass but increase in length due to growth but poor foraging efficiency, Figure 5).

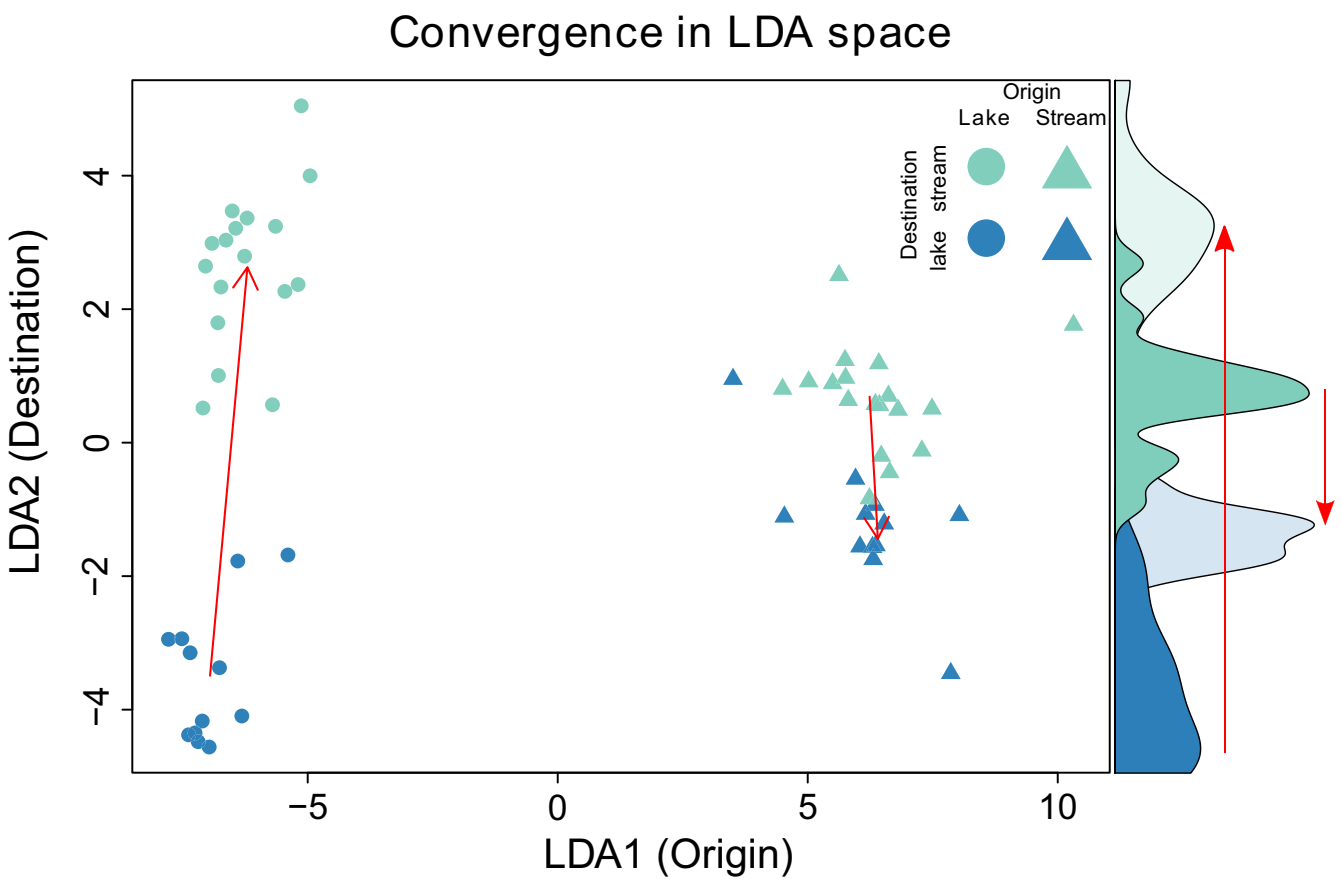

FIGURE 6 Convergence of immigrant expression profiles towards native expression profiles in transplanted fish. Red arrows are drawn between the means of each distribution. Fish originating from the lake move farther along LD2 than stream fish (two-factor ANOVA, $p \ll .001$). LDA1 explains 86% of the total variance while LDA2 explains 10%

4.3 | On the whole-transcriptome level, lake fish are more plastic than stream fish

At the whole-transcriptome level, we again observe substantial and persistent differences between the expression profiles of lake and stream fish, captured by LD axis 1. However, along LD2, we observe substantial plastic convergence of immigrant fish towards the expression profile of their new population (Figure 6). Interestingly, we also observed convergence in parasite community composition in this same experiment. Lake and stream natives carried distinct parasite communities, and individuals transplanted to the neighbouring habitat exhibited an intermediate parasite community (Bolnick & Stutz, 2017).

Our analysis suggests that fish from the lake exhibit a more plastic response to being transplanted into the stream, compared to stream fishes' more limited plasticity when placed in the lake. This transcriptome-wide analysis is consistent with our single-gene analyses which also found that lake natives tended to show greater plasticity in response to transplantation. Assuming fish caged in their native habitat adopt a locally optimal expression profile, we infer that lake sticklebacks' strong plastic response is actually excessive, overshooting the stream profile along LD2. In contrast, stream fish placed in lake cages fall short of the optimum expression in the lake (Figure 6). We therefore conclude that transcriptomic plasticity is incomplete (LD1 remains intact and explains the most variance), and differs between lake and stream ecotypes. This result implies that sticklebacks' transcriptional reaction norms may be evolving as they adapt to different habitats. However, because we used wild-caught rather than laboratory-raised fish for this experiment, we cannot rule out effects of early rearing environment, and hence cannot definitively ascribe a genetic cause to the different reaction norms of lake and stream fish.

Our results lend additional support to an emerging insight that transcriptomic plasticity may play a substantial role in migrants' adaptation to novel environments. This has been very extensively explored in experimental settings in the laboratory, where organisms may be exposed to alternative environmental conditions (often a single variable such as salinity, temperature or a toxin). Many studies find plastic responses in candidate genes, or a subset of the transcriptome, in response to such experimental treatments (Morris et al., 2014; Reid et al., 2016; Velotta et al., 2017; Whitehead, Roach, Zhang, & Galvez, 2011). Often, these plastic responses are genotype dependent, with one population exhibiting a stronger response than another (e.g., PCB-tolerant killifish are less plastic than

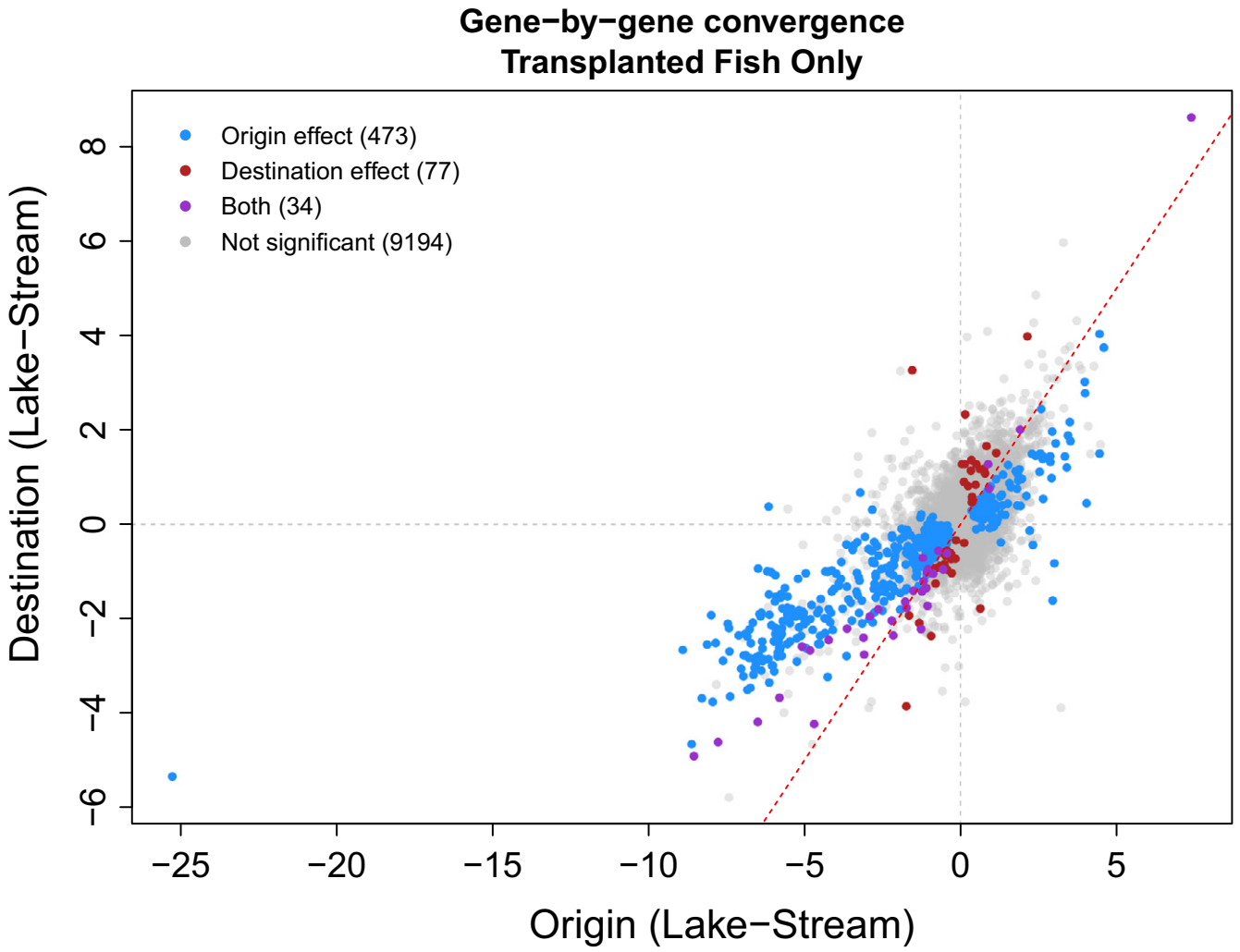

FIGURE 7 Gene-by-gene convergence among transplanted fish. We included only transplanted fish in a linear model in DESeq2: with expression of each focal gene as a function of origin + destination + origin:destination. X- and *Y*-axis are Log2 fold changes between lake and stream fish by origin, and destination, respectively. Points are coloured when *q* value <.05, and colour-coded based on which effect(s) were significant. The red dashed line is 1:1, helping to visualize that the main trend has a slope <1, indicating that plasticity (destination) effects are weaker than origin effects

PCB-susceptible populations (Reid et al., 2016)). Fewer studies have examined transcriptomic plasticity of migrants in natural settings. Kenkel and Matz (2016) subjected corals to a reciprocal transplant experiment across a temperature gradient, and also found transcriptomic convergence of migrants towards residents, as we do. They also found that one genotype was more transcriptionally plastic than the other, as we do. In a similar design to ours (Ghalambor et al., 2015) transplanted fish between two locations in streams and measured subsequent changes in gene expression. Their results highlighted that adaptive plasticity decreases the impact of directional selection, and thus the pace of evolution. Under this paradigm, we might expect that expression profiles of stream fish might evolve more rapidly than lake fish because of their reduced plasticity.

A large body of existing empirical and theoretical studies suggest that increased plasticity should evolve in more temporally or spatially heterogeneous habitats (Auld & Relyea, 2011; Baythavong, 2011; Davidson et al., 2011; Dudley & Schmitt, 1996; Murren et al., 2015; Van Buskirk, 2002). Our result is thus somewhat puzzling, in that we observe greater transcriptomic plasticity in lake fish, which inhabit the more temporally stable habitat. While stream habitats are generally very diverse (flow regime, overhead foliage, substrate, spatial distribution of prey), lake habitats generally have large and smooth transitions between any variation in environmental variables (and in most cases very little variation (Ahmed, Thompson, Bolnick, & Stuart, 2017; Stuart et al., 2017). However, lakes may be less predictable in other ways. For instance, lake stickleback consistently harbour more diverse parasite communities (Bolnick & Stutz, 2017; Stutz & Bolnick, 2017), and so may have evolved greater immunological plasticity to handle an unpredictable suite of pathogens and helminths.

In conclusion, we see extensive gene expression differences between genetically divergent stickleback populations inhabiting adjoining habitats but connected by gene flow (Weber, Bradburd et al., 2017). But, for many genes, transcript abundance is highly plastic. Fish that disperse into the adjoining foreign habitat will

partially converge on the gene expression profile typical of their new habitat. This suggests that expression plasticity can soften the impact of immigration into an unfamiliar habitat. Nonetheless, lake and stream fish differed in survival rates and parasite infection rates in our study, implying that this expression plasticity is not fast or extensive enough to fully homogenize the lake and stream fish performance.

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DATA ACCESSIBILITY

Meta data, parasite data, code for processing raw reads, code for statistical analysis and plotting are located in DRYAD entry https://doi.org/10.5061/dryad.mk8ns. Raw reads are available for download via "wget http://web.corral.tacc.utexas.edu/Lohman_et_al_2017_MolecularEcology/" from the terminal. The iRNAseq pipeline is available here: https://github.com/z0on/tag-based_RNAseq. The GO_MWU software is available here: https://github.com/z0on/GO_MWU.

AUTHOR CONTRIBUTIONS

BKL built and sequenced TagSeq libraries, and performed gene expression data analysis. WES performed the experiment which generated the samples. BKL and DIB wrote the manuscript. All authors approved the final version. The authors declare no conflict of interests.

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SUPPORTING INFORMATION

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