LETTER

doi:10.1038/nature15256

Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature

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Phenotypic plasticity is the capacity for an individual genotype to produce different phenotypes in response to environmental variation[1]. Most traits are plastic, but the degree to which plasticity is adaptive or non-adaptive depends on whether environmentally induced phenotypes are closer or further away from the local optimum[2–4]. Existing theories make conflicting predictions about whether plasticity constrains or facilitates adaptive evolution[4–12]. Debate persists because few empirical studies have tested the relationship between initial plasticity and subsequent adaptive evolution in natural populations. Here we show that the direction of plasticity in gene expression is generally opposite to the direction of adaptive evolution. We experimentally transplanted Trinidadian guppies (*Poecilia reticulata*) adapted to living with cichlid predators to cichlid-free streams, and tested for evolutionary divergence in brain gene expression patterns after three to four generations. We find 135 transcripts that evolved parallel changes in expression within the replicated introduction populations. These changes are in the same direction exhibited in a native cichlid-free population, suggesting rapid adaptive evolution. We find 89% of these transcripts exhibited non-adaptive plastic changes in expression when the source population was reared in the absence of predators, as they are in the opposite direction to the evolved changes. By contrast, the remaining transcripts exhibiting adaptive plasticity show reduced population divergence. Furthermore, the most plastic transcripts in the source population evolved reduced plasticity in the introduction populations, suggesting strong selection against non-adaptive plasticity. These results support models predicting that adaptive plasticity constrains evolution[6–8], whereas non-adaptive plasticity potentiates evolution by increasing the strength of directional selection[11,12]. The role of non-adaptive plasticity in evolution has received relatively little attention; however, our results suggest that it may be an important mechanism that predicts evolutionary responses to new environments.

A long-standing problem in evolutionary biology is to understand the relationship between environmentally induced variation observed within a generation, and genetically-based evolutionary changes between generations[1–5]. It has long been recognized that the expression of traits is plastic—the same genotype can produce a range of phenotypes in response to different environmental cues. However, the causal relationship between a trait's plasticity and that trait's evolution remains an unresolved and contentious problem[1]. Traditional models of adaptive evolution ignored any role for plasticity, because environmentally induced plasticity was viewed as non-heritable variation[1–3]. Current models recognize that environments can cause predictable patterns of plasticity that are either adaptive or non-adaptive with respect to the local phenotypic optimum; such plasticity may influence evolutionary change by altering the distribution of phenotypes upon which selection acts. For example, plasticity is adaptive when the phenotype is altered in the same direction favoured by natural selection in that environment[4–12]. Some models predict that adaptive plasticity weakens the strength of directional selection and slows adaptive evolution[6–8,13]. Other models suggest that adaptive plasticity is a critical first step in the process of adaptive evolution (for example, via genetic assimilation or accommodation)[1,14], for instance by increasing population persistence in new environments (the Baldwin effect) and allowing more time for selection to act on heritable variation[4–10]. In contrast, plasticity is non-adaptive when a population encounters an environment that induces the production of phenotypes further away from the local optimum[4–12], resulting in a negative relationship between the direction of plasticity and the direction of adaptive evolution. Non-adaptive plasticity reduces relative fitness and is predicted to increase the strength of directional selection because traits are further from the phenotypic optimum, resulting in an evolutionary response sometimes referred to as 'genetic compensation' or 'counter-gradient variation'[11,12]. Laboratory selection experiments have found support for a positive (adaptive)[14,15] and negative (non-adaptive)[16] relationship between the direction of plastic responses and the direction of evolution. However, testing such relationships in natural populations has been challenging because comparisons between ancestral and derived populations typically occur long after the populations have diverged[17–20]. Here, we test the relationship between plasticity and the early stages of evolutionary divergence using experiments in nature. We assess both ancestral plasticity in the source population and evolved changes in replicated derived populations by comparing plastic and evolved patterns of gene expression.

We quantified gene expression in Trinidadian guppies derived from natural populations and from populations undergoing early divergence following an experimental translocation. Individuals from a population that experiences high mortality from fish predators (high-predation, denoted as HP), particularly the pike cichlid (*Crenicichla frenata*), were introduced into each of two low-predation sites lacking cichlids: 'Intro1' and 'Intro2' (Extended Data Fig. 1). Thirty-eight gravid females and 38 mature males were introduced into each stream. One year after the introduction (3–4 guppy generations), guppies were collected from the ancestral HP source population, descendant introduction populations (Intro1 and Intro2), and a naturally colonized low-predation guppy population (denoted as LP) from the same drainage (Methods). The natural LP population represents an older evolutionary descendant of the HP source population[21] adapted to the same predation regime as the experimental populations. It thus provides an a priori prediction for the expected direction of evolutionary change.

To assess plastic and evolved changes in transcription, we bred wild-caught fish under common laboratory conditions for two generations and generated unique family lines within each of the four populations. Two generations of rearing in a common environment controls for environmental, maternal and other non-heritable sources of variation. Within 24 h of birth, second generation full-siblings of each family were randomly split between tanks that differed in exposure to chemical predator cues. Siblings reared with predator cues were raised in recirculating units that housed a cichlid within the water supply[22]. Cichlids were fed two guppies per day. Predator cues included both

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predator kairomones from the cichlid as well as any alarm pheromones from guppies, simulating the ancestral olfactory environment[22]. Guppies reared without predator cues were housed in identical recirculating units without the cichlid predator, simulating the derived environment. Differences in transcription between siblings reared in these two environments represent predator-induced plasticity in gene expression, while differences between populations measured under the same conditions for multiple generations represent heritable differences[23].

To determine whether the introduction populations showed evidence for adaptive evolutionary divergence, we measured patterns of transcription in all four populations under the derived rearing environment. We measured the abundance of 37,493 messenger RNA transcripts expressed in whole brains of mature males reared without predator cues (mean age = 124.03 days old, range = 118–154), using high-throughput RNA sequencing. We used multivariate between-group principal components analysis (Methods) to visualize overall transcription differences among the four populations (Fig. 1). Two major axes explained 74.5% of the variation. Principal component 1 (PC1; 44.4% of variation) separated the naturally occurring LP population from the natural HP and introduction populations, and thus appears to reflect long-term divergence between these populations. PC2 (30.1% of variation) separated the HP source population from the two introduction populations and the natural LP population, thus capturing a signal of rapid and parallel evolutionary divergence to the LP environment (Fig. 1). Whereas genetic drift, founder effects, and unique attributes of each of the introduction streams would be expected to produce independent genetic changes in the introduction populations[24], the parallel change of Intro1 and Intro2 in the same direction as the natural LP population supports the interpretation that PC2 describes rapid adaptive evolution. Indeed, the rate of evolutionary divergence in gene expression between the source population and introduction populations for the top 500 transcripts loading on PC2 (median Haldanes (a change in phenotypic standard deviations per generation) in Intro1 = 0.256 and Intro2 = 0.226) are comparable to rapid rates of evolution observed in life history and morphology during previous experimental introductions of guppies[25,26] (Methods and Extended Data Fig. 2a, b).

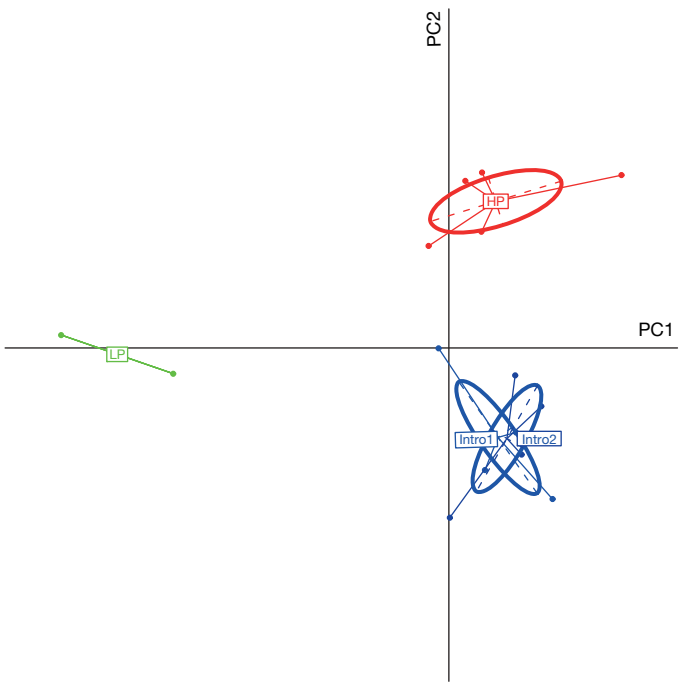

Figure 1 | Rapid evolutionary divergence in gene expression as measured in second-generation laboratory-born guppies derived from the wild. Shown is a principal components analysis of all 37,493 expressed genes in the four populations. HP is a naturally occurring high-predation population that is the source population for the two experimentally introduced populations, Intro1 and Intro2. LP is a naturally occurring low-predation population. Points represent individual families within each population, and are connected by solid lines. Dashed lines represent the major and minor axes of the confidence ellipse for each population.

To distinguish transcripts that exhibited evolution in the introduction populations as a result of selection from those that exhibited changes as a result of other processes, we identified transcripts that exhibited highly significant parallel evolutionary change in both introduction populations and that diverged in the same direction in the natural LP population. Permuted data sets ($n = 250$) were generated by randomly reassigning population labels to individual samples. We then used general linear statistical models to assess divergence in the two introduction populations and the natural LP population (that is, HP versus Intro1 and HP versus Intro2 and HP versus LP) for each transcript (Methods). If the test statistic for each of the three contrasts fell in the extreme 5% of the distribution of the permutation test statistics, and the contrasts all had the same sign, we called the transcript concordantly differentially expressed (CDE). We found 135 transcripts that met these stringent criteria, which was many more than observed in the permuted data sets (median = 6, interquartile range = 3–14; Methods and Supplementary Table 1). By contrast, only one transcript diverged significantly in opposite directions in the two descendant introduction populations, consistent with expectations based on the distribution of permuted values (median = 1, interquartile range = 1–2). These 135 CDE transcripts loaded highly on PC2 (the median rank of the PC2 loadings for the CDE transcripts was 361 out of 37,493 total transcripts). The prevalence of these parallel changes suggests that this subset of transcripts evolved through the direct or indirect effects of natural selection, because genetic drift would have produced discordant as well as concordant evolution in the descendant introduction populations. Indeed, divergence in transcription between the ancestral and introduction populations greatly exceeded allele frequency divergence in putatively neutral microsatellite loci[24] (Extended Data Table 1). Collectively, these results demonstrate rapid and repeatable patterns of adaptive evolutionary divergence in transcription, similar to what has been observed for other fitness-related guppy traits following the colonization of low-predation environments[22,26–28].

Given the evidence for rapid evolution of transcription, we determined if the pattern of ancestral plasticity in the HP source population predicted adaptive evolution in the descendant introduction populations. We assessed plasticity in the HP population by measuring the change in transcript abundance of full siblings reared with and without the predator cue (that is, simulating the ancestral high-predation and derived low-predation environments). If plasticity in transcript abundance was in the same direction as the parallel divergence observed in CDE transcripts, we considered plasticity to be adaptive. If the plastic changes were in the opposite direction as the evolved changes in CDE transcripts, we considered the plasticity to be non-adaptive (see Extended Data Fig. 3). We found a robust pattern of non-adaptive plasticity predicting evolutionary change in CDE transcripts; when HP fish were reared without the predator cue, the change in transcript abundance was overwhelmingly in the opposite direction to that of evolved changes in the descendant introduction populations (Fig. 2). The negative association between the direction of plasticity and the direction of evolution was highly significant ($\chi^2 = 89.9$, d.f. = 1), which is outside the range of all 250 permuted χ^2 values (range = 0.0–55.9), with 89% (120 of 135) of all transcripts exhibiting a plastic response opposite to the direction of evolution (see grey points in Fig. 2). Of the remaining 11% (15 of 135) of transcripts, when the direction of plasticity and evolution aligned, the degree of plasticity was negligible (see black points in Fig. 2). The correlation between ancestral plasticity and evolution ($r = -0.82$) is substantially more negative than correlations generated from a randomization test

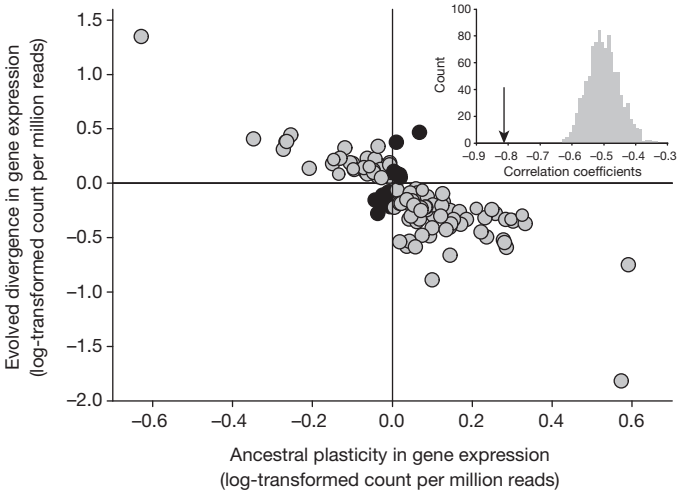

Figure 2 | Rapid evolutionary divergence is highly correlated with non-adaptive plasticity. Shown is a scatter plot of ancestral plasticity (change in transcript abundance to the absence of cichlid predator cues) against adaptive evolutionary divergence (135 concordantly differentially expressed transcripts) in the descendent populations transplanted to streams lacking cichlid predators. Grey points denote transcripts exhibiting non-adaptive plasticity, and black points denote adaptive plasticity. Inset shows the distribution of the Spearman rank correlations between evolutionary divergence and ancestral plasticity from 1,000 permutated correlation values for the 135 concordantly differentially expressed transcripts, with the arrow indicating the observed correlation, which is substantially more negative than all permuted values.

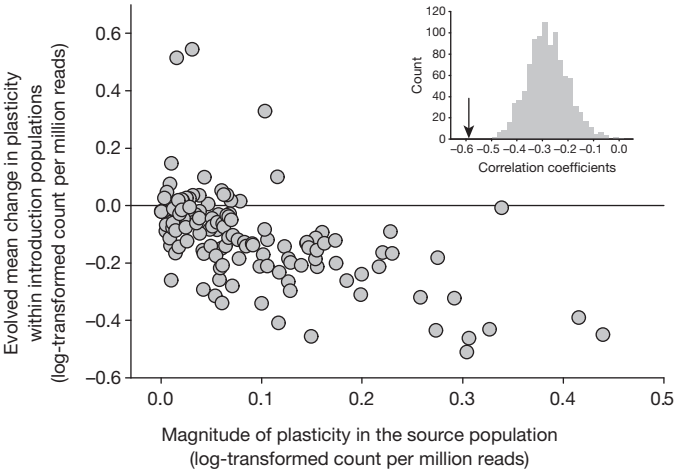

Figure 3 | Rapid evolution of reduced plasticity. Shown is a scatter plot of the absolute values for the magnitude of ancestral plasticity (the normalized difference in transcript abundance between the presence and absence of cichlid cues) against the change in plasticity between the source and introduction populations. Inset shows the distribution of the Spearman rank correlations between the magnitude of plasticity in the ancestral population and the change in plasticity in the introduction populations from 1,000 permutated correlation values for the 135 concordantly differentially expressed transcripts, with the arrow indicating the observed correlation, which is substantially more negative than all permuted values.

$P < 0.0001$; Fig. 2). These results suggest that plasticity potentiates rapid adaptive evolution, but not because plasticity is adaptive, as is assumed in many evolutionary models, but rather because it is non-adaptive and under stronger selection to change (Fig. 2). The same pattern is observed when we restrict the analysis to a separate data set that included 565 transcripts exhibiting significant plasticity to the rearing treatments in the HP source population (Supplementary Table 2 and Extended Data Fig. 4)

The magnitude of plasticity can also evolve in response to selection[1–10]. If natural selection acts most strongly on transcripts exhibiting non-adaptive plasticity, we predicted plasticity should evolve to be reduced in the descendant introduction populations. We tested this prediction by comparing plasticity in the ancestral source population to that in the derived introduction populations for the subset of transcripts that were CDE. The magnitude of plasticity decreased in the introduction populations (median change $= -11\%$, sign test $M = -45.5$, $P < 0.001$). Moreover, the decline in plasticity in these descendant populations was negatively associated with the magnitude of ancestral plasticity ($P < 0.001$ based on a randomization test; Fig. 3), in accord with the idea that selection acts more strongly to decrease plasticity in those transcripts showing the greatest non-adaptive plasticity. Thus, traits exhibiting initially non-adaptive plastic responses to new environments may be a transient phenomenon, because selection may act to rapidly reduce their magnitude.

Attempts to model the effects of plasticity on subsequent adaptive evolution often assume that plasticity is adaptive. However, when populations experience novel environments, as when we experimentally transplanted guppies, many of the initial plastic responses are likely to be non-adaptive, because selection has not had an opportunity to act on the genetic variation for plasticity[2–4]. In such cases, both adaptive and non-adaptive plastic responses would be expected by chance, but traits exhibiting adaptive plasticity should be under weaker directional selection relative to traits exhibiting non-adaptive plasticity and further from the new phenotypic optimum[11,12]. Indeed, both theoretical and empirical studies show that adaptive plasticity reduces directional selection[7,13,27]. While we were unable to directly estimate the strength of selection on transcript abundance phenotypes, previous introduction experiments have demonstrated strong directional selection and rapid adaptation in response to low-predation environments[25,26]. If traits exhibiting non-adaptive plasticity are under stronger directional selection, then newly established populations will probably face a dual challenge if they are to persist and avoid extinction. First, they must overcome the fitness costs associated with strong directional selection on non-adaptive responses, including declines in population size; and second, they must harbour enough genetic variation to rapidly respond to selection[9,10,28]. Because heritable genetic variation for transcription appears to be common[24], the potential for rapid adaptation may ameliorate one set of costs. However, other costs may be more difficult to avoid, as models suggest that population size, the distance a non-adaptive trait is from the local optimum, and the relationship of that trait to fitness will ultimately determine whether populations persist[10,28]. In the case of the introductions here, such costs may have been reduced, because individuals were transplanted to relatively more 'benign' conditions, such that high predator-induced mortality was replaced with increased competition, reduced food availability, and other environmental factors characterizing the low-predation streams[29].

Understanding the role of phenotypic plasticity in adaptive evolution remains a contentious problem in evolutionary biology, in part because few studies have been able to capture the initial patterns of plasticity and subsequent adaptive divergence of traits in natural populations. Nevertheless, it is during the early stages of adaptive divergence that selection in new environments is likely to be strongest[9,10,28], and when plasticity will either reduce or exacerbate the initial mismatch between the mean and optimal phenotypic responses[6–10]. Recent work in these same guppy populations documents a similar pattern in which non-adaptive plasticity potentiates a rapid evolution of growth rate[22], suggesting a general pattern that extends to other phenotypic traits. While such results are consistent with many models of how selection acts on phenotypes[6–10], the role of non-adaptive plasticity in adaptive evolution remains understudied, despite arguments that it may be a common, but cryptic, form of evolution[11,12]. More generally, understanding when and how plasticity affects evolutionary response is critical for predicting the short- and long-term effects of environmental change on organisms. Predictive evolutionary models of

phenotypic plasticity also have practical importance. For example, disease states within organisms respond plastically to treatments and also evolve, thus gene expression profiles can be used (as was done here) to predict how response to treatment influences disease progression[30]. Additional experimental evolution studies, especially those conducted in natural environments, will be critical for validating and parameterizing future models of how plasticity influences evolutionary change.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 4 April; accepted 3 August 2015.
Published online 2 September 2015.

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Supplementary Information is available in the online version of the paper.

Acknowledgements This work was supported by grants from the National Science Foundation (DEB-0846175 to C.K.G., EF-0623632 to D.N.R., and IOS-0934451 and IOS-1354775 to K.A.H.). We thank C. Handelsman, K. Langin, D. Broder, E. Duval, I. Janowitz, E. Lange, A. Shah, J. Havrid, E. Kane and L. Angeloni for helpful comments on the study. Computing for this project was performed on the Spear cluster at the Research Computing Center at the Florida State University

Author Contributions C.K.G., K.L.H. and K.A.H. planned and executed the study, E.W.R. reared the fish, E.K.F. collected the tissues K.A.H. analysed the gene expression data, D.N.R. planned and oversaw the field introduction experiments, and C.K.G. oversaw the laboratory experiments. All authors participated in writing the manuscript.

Author Information The sequence data are available at the Sequence Reads Archive (SRA) under accession number SRP06236. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to C.K.G. (cameron1@colostate.edu) or K.A.H. (kahughes@bio.fsu.edu).

METHODS

No statistical methods were used to predetermine sample size. The investigators were not blinded to allocation during experiments and outcome assessment.

Study system and populations. Guppies are a model system in evolutionary biology because they provide an opportunity to study rapid adaptive evolution in the wild[25,26]. In lowland rivers, guppies occur in diverse fish communities where they experience high mortality from a number of fish predators. In small upstream tributaries, guppies occur in simpler communities, typically co-existing only with the killifish (*Rivulus hartii*), which poses little risk to adult guppies resulting in a low predator-induced mortality rate[25,26]. Past research has shown that numerous life history, behavioural, and morphological traits vary between these contrasting environments, and that these differences can evolve rapidly following experimental introductions[25,26]. We sampled four populations of guppies within the Guanapo River drainage in the Northern Range Mountains of Trinidad, West Indies (Extended Data Fig. 1). The first population, hereafter referred to as HP, is a naturally occurring population subject to high predation in the lower Guanapo river drainage that contains a variety of predator species, including the common predator on guppies, the pike cichlid[25,26]. The second population, hereafter referred to as LP, represents a native low-predation population from the same drainage and was sampled from the upstream Taylor tributary of the Guanapo river, where guppies co-exist with only *R. hartii*. *R. hartii* are gape-limited omnivores that prey primarily on juvenile guppies[25,26]. The remaining two populations were experimentally established in two low-predation tributaries (the Lower Lalaja, and the Upper Lalaja) within the Guanapo drainage.

Introduction experiments. In March 2008, HP guppies were introduced into the Lower Lalaja (denoted as Intro1) and Upper Lalaja tributaries (denoted as Intro2) of the Guanapo drainage[22]. The two introduction populations were established in 100-m reaches of these small, first-order tributaries. The upper limit of the introduction reach on the Lower Lalaja was bounded by a waterfall, which was artificially enhanced to prevent emigration and the establishment of populations above the streams receiving introductions. The upper limit of the Upper Lalaja introduction reach had a natural barrier. The lower limit of both introduction sites had natural barriers, which blocked immigration from downstream populations of guppies. The streams below these downstream barriers were also guppy-free before our introduction and were separated from the main river by additional barriers.

Each stream was stocked with 38 gravid females and 38 mature males. These fish had been collected as juveniles, reared to maturity in single sex groups, and then mated in groups of 4–5 males and 4–5 females per breeding group before introduction. To minimize the potential for founder effects and equalize genetic diversity in each stream, males and females from each breeding group were introduced into alternate streams. Doing so increased the effective population size of each population, because females retained the sperm from mating with one set of 38 males, then were introduced and subsequently mated with a second set of 38 males. As part of a separate experiment the riparian forest canopy was experimentally thinned in the Intro2 stream before the introductions[31], but the two introduction streams were similar in all other respects.

Laboratory breeding experiments. Laboratory populations used for the gene expression assays were second-generation laboratory fish that were originally derived from 30 adult females and 30 adult males collected from each of the HP, LP and two introduction populations (Intro 1, Intro 2) in March of 2009. This time period represented one year or 3–4 generations after the establishment of introduction populations. Fish were kept in 1.5-l tanks (Aquatic Habitats) connected to a custom-made recirculating system and maintained on a 12-h light cycle at $25 \pm 1\ ^{\circ}C$[22,32,33]. Fish were reared on standardized food levels adjusted weekly for age and number of individuals per tank (morning, Tetramin tropical fish flakes, Spectrum Brands, Inc.; afternoon, brine shrimp (nauplii of *Artemia spp.*), Brine Shrimp Direct). The quantity of food offered daily approximated *ad libitum* and was comparable to the high level of food administered in other studies[34].

We reared all wild-caught guppies for two generations under common garden conditions using a breeding design that retains the genetic variation of the original population, prevents inbreeding, and minimizes maternal and other environmental effects[34]. The first generation (G1) line in the laboratory was derived from wild-caught juveniles and reared to maturity in the lab. Wild-caught gravid females were housed individually until parturition and their offspring were used to create G1 family lines. Females that did not give birth within about 30–35 days of capture were randomly crossed with a wild-caught male; however, no two females were crossed with the same male. The G1 offspring from each brood were housed separately until sexed, and then separated into single-sex tanks. Juvenile females (28–56 days) can be identified by the presence of melanophores in a triangular patch that appears on their ventral abdomens, which is absent in males[34]. Sexing was accomplished by anaesthetizing guppies in buffered MS-222 ($0.85\ mg\ ml^{-1}$; ethyl 3-aminobenzoate methane sulfonic acid salt) (Sigma-Aldrich) and observing the melanophores under a microscope. Males are considered to be sexually mature when the apical hood grows even with the tip of their gonopodium; females usually mature within ±1–2 days of males[34]. Mature males and females from each family line were then randomly chosen and crossed to other families to produce the second generation (G2). Each G2 family was the product of a unique cross, to minimize inbreeding and maximize the genetic variation within each population.

Within 24 h of birth, G2 full-sibling broods were randomly assigned to two 1.5-l tanks (2–10 full siblings per tank) that differed in exposure to chemical cues from a predator (reared with or without cues from a predator) using a split-brood design. Siblings reared with cues from predators were reared in recirculating units that housed a pike cichlid within the sump that supplied water to the tanks[22,32,33]. Chemical predation cues included both kairomones from the cichlid predator and alarm pheromones from the two guppies consumed daily by the cichlid. Guppies reared without cues from predators were housed in identical recirculating units without predators in the water supply. G2 juveniles were anaesthetized and sexed at 29 days (see above). From each population, we randomly selected 5–6 families to raise pairs of male siblings within each rearing treatment.

RNA-sequencing. Focal animals were euthanized by immersion in ice water followed by rapid decapitation (IACUC approved protocol #12-3818A). Whole brains were collected by cutting the head sagittally down the centre line and removing all brain tissue. Brains were then flash frozen in liquid nitrogen and stored at $-80\ ^{\circ}C$ until further processing. Tissue collection took <2 min per fish, fast enough to minimize changes in gene expression due to handling. Whenever possible, we combined brains from two full-siblings in the same treatment group to ensure we could obtain sufficient RNA for sequencing, while minimizing variation among pooled individuals. To minimize temporal and circadian variation, we performed all dissections within 15 min after lights-on in the morning (fish were all kept on a 12:12 h light–dark cycle). In addition, gene expression levels at lights-on minimized expression differences in response to recent experiences. Our data thus represent baseline transcription levels. The age of the fish (118–154 days) and the timing of sampling were randomly distributed across populations. Because all dissections occurred within 15 min, no more than 8 individuals (1–2 families distributed in both treatments) could be sampled per day, and the order in which populations were sampled was randomized.

RNA was extracted from whole brain tissue using Qiagen RNAeasy lipid extraction kit. A separate sequencing library was prepared for each pooled family, using unique index sequences from the Illumina Tru-Seq RNA kit following manufacturer's instructions. Sequencing libraries were constructed and sequenced on three lanes of an Illumina HiSeq 2000 at the HudsonAlpha Genomic Services Laboratory (Huntsville, Alabama) in April 2012. In total, 32 samples were sequenced in 5 lanes (sample sizes that passed quality filters: $n = 5$ for HP reared with and without predators, $n = 4$ for Intro1 and Intro2 reared with and without predators, $n = 3$ for LP reared with predators, and $n = 2$ reared without predators). We obtained 736,693,718 100-base pair (bp) reads that passed the machine quality filter, with 17,517,493 to 28,265,561-bp reads per sample, and average quality >35.6 for all samples.

Sequencing reads were mapped to a high-quality brain-specific reference transcriptome for *P. reticulata*. We constructed the reference from a data set containing >450 million 100-bp paired-end reads, which were filtered for high-quality sequences and normalized *in silico* to compress the range in *k*-mer abundance. We used SeqMan NGEN 4.1.2 to perform the assembly, which contained 41,347 contigs, N50 = 2,548, and recovered 63% of Tilapia (*Oreochromis niloticus*) Ensembl proteins (Release 70). Contigs from the assembly were annotated by blastx queries against SwissProt (database downloaded 6 October 2012), UniProt/Trembl (28 November 2012), and nr (11 December 2012). Default parameters were used in the blastx queries, with e-value cut-off of 1×10^{-4}.

Reads were mapped to the reference assembly using Bowtie 2 v2.0.0 on a server running Red Hat Enterprise Linux version 6.5. We used a seed size of 20 bp, with no mismatches allowed in the seed (run options: -D 15 -R 2 -N 0 -L 20 -i S,1,0.75). We retained mappings with quality scores >30 (<0.001 probability that the read maps elsewhere in the reference) and kept only contigs represented by ≥1 count per million reads in at least three samples. After removing low-abundance transcripts, 628,797,716 reads (85.3%) mapped to 37,493 unique contigs in the reference transcriptome. We used the number of reads mapping to each of those contigs along with TMM-normalized library sizes[35] to analyse differential expression.

Data analysis. Between-group analysis (BGA) was conducted[36] as implemented in the R package made4 (ref. 37). BGA is a multivariate discriminant approach that is appropriate when the number of variables exceeds the number of cases; it is carried out by ordinating groups of samples and projecting the individual sample locations on the resulting axes. We used principal components analysis (PCA) as the ordination method (Fig. 1). To quantify the rate of evolution along the axis separating the HP source population from the introduction populations, we cal-

culated evolutionary divergence in Haldanes[38]. We assumed a time of 3.5 generations, and used the difference in mean transcript abundance in the no predator treatment with a pooled standard deviation[38] (see Extended Data Figs 2a, b).

We used random permutation tests to evaluate differential expression across populations and treatment groups. Permuted data sets were generated by randomly reassigning entire RNA-seq samples among population and treatment categories to produce an empirical null distribution against which to test hypotheses. This approach preserves any non-independence among transcripts that could bias inferences if the non-independence were not taken into account. We first computed transcript-specific test statistics from the actual data (see below) and compared that statistic to the distribution of the same statistic derived from 250 permuted data sets. If the statistic for the real data fell within the extreme tails of the permuted values for that transcript, we called the transcript differentially expressed (DE). To determine if more transcripts were called DE than expected, we compared the number of DE transcripts in the real data set to the distribution of that number in the 250 permuted data sets.

To determine if transcripts were significantly evolved in each introduction population we restricted the analysis to samples collected from fish reared without predator cues. For both the actual and the permuted data sets, a general linear model was applied separately to each transcript, with the normalized transformed number of reads as the dependent variable and population (HP and Intro1 or HP and Intro2, depending on the analysis) as a fixed effect. We then used general linear statistical models to assess divergence in the two introduction populations and the natural LP population (that is, HP versus Intro1 and HP versus Intro2 and HP versus LP) for each transcript. If the test statistic for each of the three contrasts fell in the extreme 5% of the distribution of the permutation test statistics, and the contrasts all had the same sign, we called the transcript concordantly differentially expressed (CDE). To calculate the number of transcripts expected to be called CDE in the two introduction populations under random expectations, we conducted this same analysis in each of the 250 permuted data sets, and calculated the number of transcripts meeting the same criteria. This permutation analysis accounts for any spurious associations that might result from comparing both introduction populations to the same ancestral HP population[39].

To test if the divergence in gene expression is greater than would be expected by neutral processes, we calculated P_{ST} (a measure of phenotypic divergence between populations) from phenotypic variance components as in ref. 40, assuming $h^2 = 0.5$ (where h = heritability of a trait) for transcript expression level. This h^2 estimate is substantially higher than the average estimate from a recent analysis in sticklebacks[24], making our comparison of P_{ST} with published F_{ST} estimates (F_{ST} is a measure of genetic divergence between populations) conservative with respect to the hypothesis that divergence is greater than expected under genetic drift.

We assessed the association between evolutionary divergence and ancestral plasticity in gene expression by conducting likelihood ratio tests of independence and comparing the resulting χ^2 value to the distribution of χ^2 values produced by conducting the same test on the 250 permuted data sets. Similarly, for the CDE transcripts, we calculated the Spearman rank correlation between evolution (mean change in expression level between HP and introduction populations in the no-predator-cue environment) and plasticity (mean change in expression in the HP ancestral population reared in the two predator-exposure environments), and compared that value to the distribution of values obtained from 1,000 random permutations of the population and treatment group labels. This permutation analysis accounts for any spurious correlation that can result because the calculations for evolutionary divergence and plasticity share a common term (mean expression level in the HP source population reared without predator cues)[39].

For CDE transcripts, we quantified plasticity in the source population and in the introduced populations as the difference in the mean expression values (normalized log-transformed number of reads mapping to a given transcript) for each transcript in the two predator-cue treatment groups within each population. We then calculated the change in these plasticity values between the source and introduction populations and used a nonparametric sign test to determine if that change was significant. We evaluated the association between ancestral and descendant plasticity in the CDE transcripts using a Spearman's rank correlation, and determined significance of that correlation using a random permutation test. Starting with the mean expression levels for each transcript within each population/treatment group, we randomly permuted the population/treatment labels 1,000 times, recalculated ancestral and derived plasticity values for each transcript in each permutation, and calculated Spearman's rank correlation of the permuted values. All statistical analyses were implemented in SAS 9.4 (SAS 2011) running in a Linux environment.

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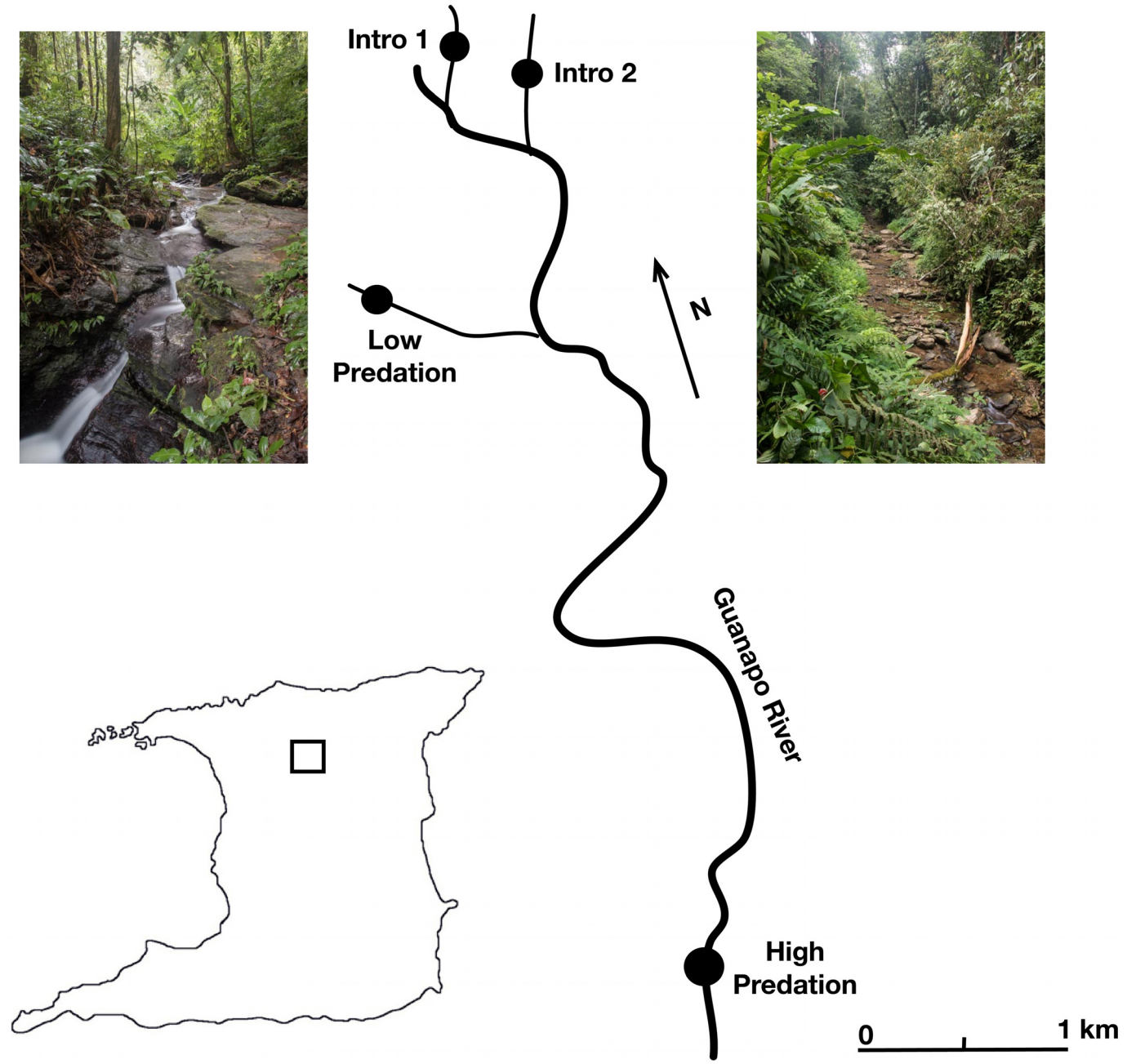
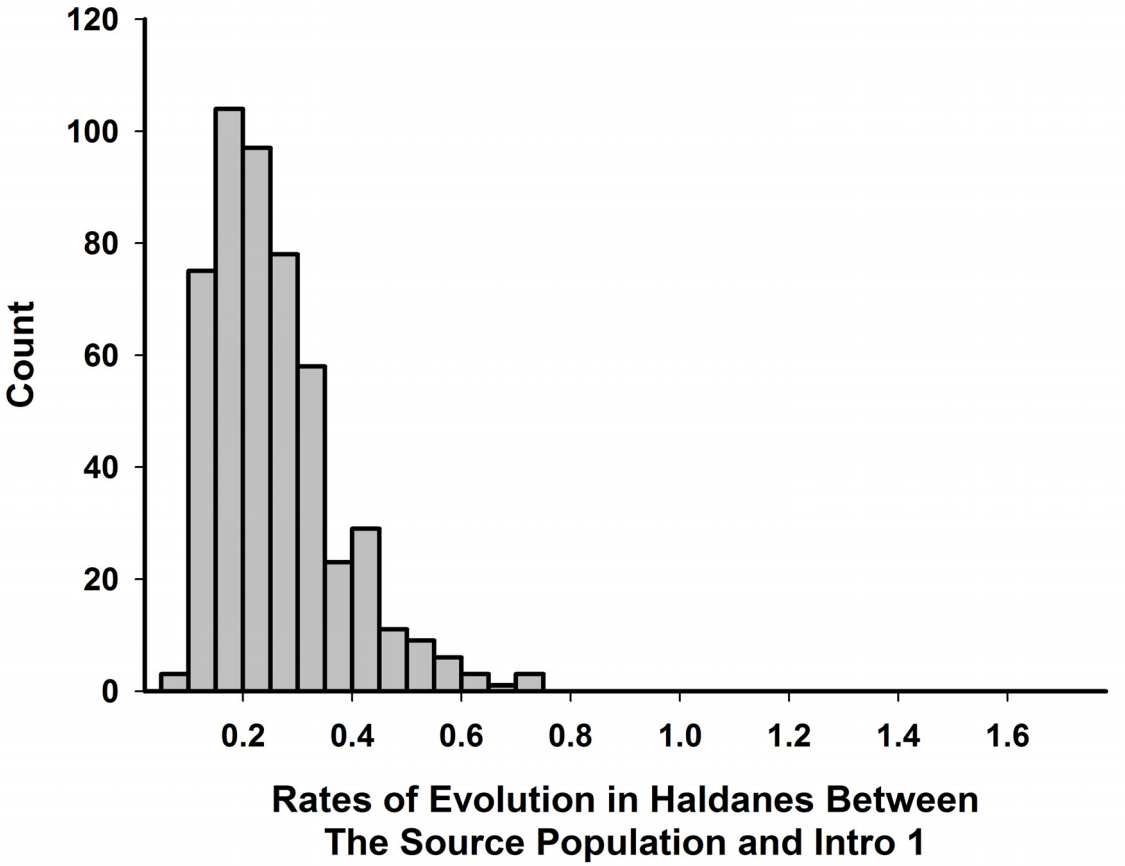
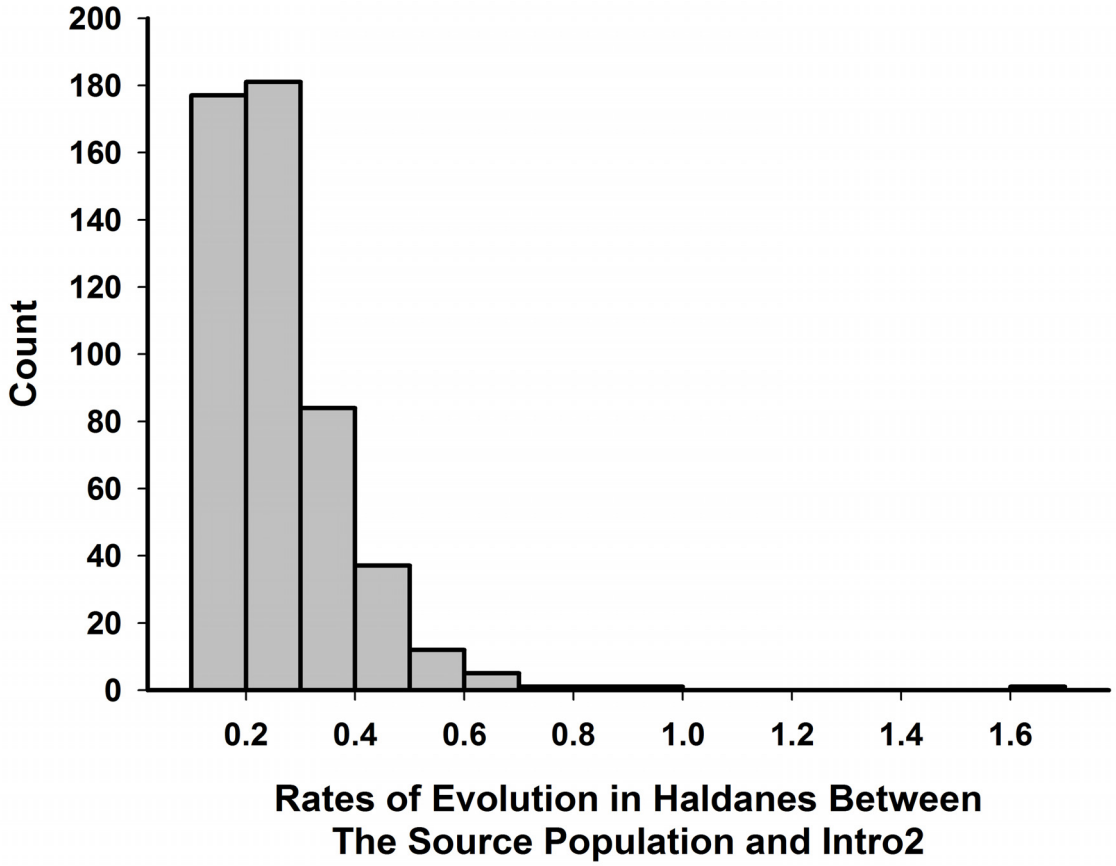
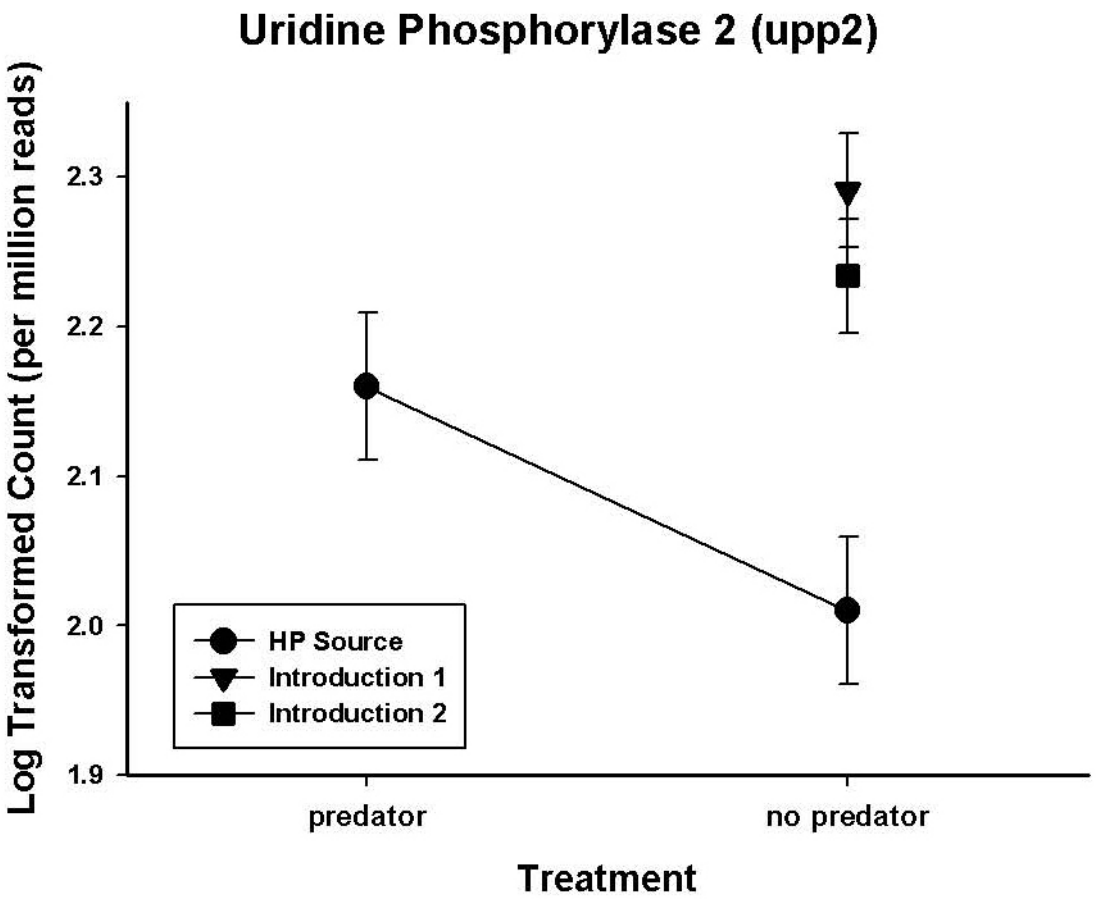


Extended Data Figure 1 | Map of Trinidad where the experimental transplants took place. Guppies were moved from a high-predation (HP) locality where they coexist with cichlid predators and introduced into two streams that lacked cichlids and guppies, Intro1 (left photograph) and Intro2 (right photograph). A naturally occurring guppy population without cichlids, low-predation (LP), was sampled to provide a low-predation reference.

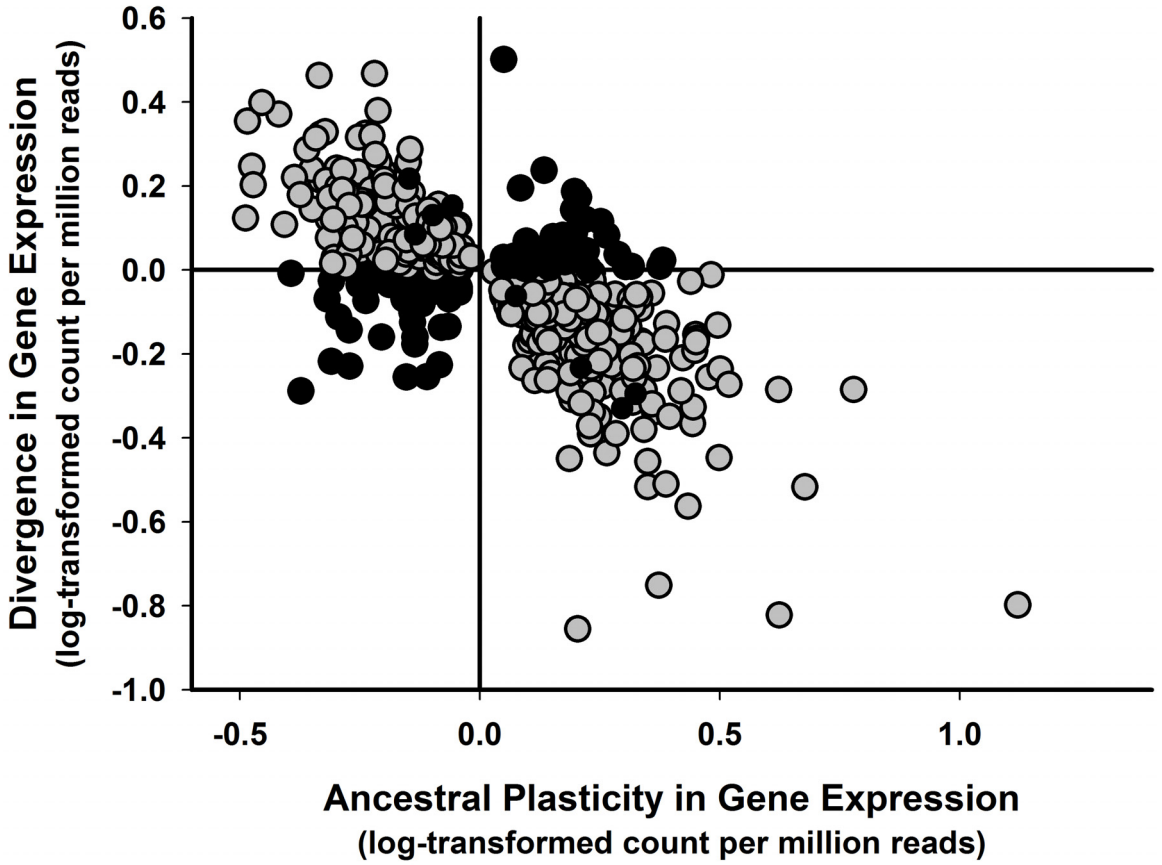




Extended Data Figure 2 | Frequency histogram of Haldanes for the top 500 transcripts loading on PC2—the axis representing rapid evolutionary divergence between the source and introduction populations. **a**, Intro1 (median Haldane = 0.256, range = 0.07–0.74). **b**, Intro2 (median = 0.226, range = 0.10–1.68).



Extended Data Figure 3 | Ancestral plasticity and evolution in patterns of gene expression for a representative gene: uridine phosphorylase 2 (*upp2*). Shown is the plastic response of the high-predation source population and the evolved responses in the two experimental introduction populations (Intro1 and Intro2). In this case the plastic response results in a decrease in expression, whereas the evolved response in the introduction populations is to increase expression, thus illustrating non-adaptive plasticity.



Extended Data Figure 4 | Scatter plot of ancestral plasticity (change in transcript abundance to the absence of cichlid predator cues) and population divergence. Shown are the 565 transcripts that exhibited significant differences in expression between the predator and non-predator rearing treatments in the HP source population. We found a similar pattern as was found for the CDE transcripts (Fig. 2): 75% (424 out of 565) of the significantly plastic genes exhibited population divergence in the introduction populations in the opposite direction of plasticity ($\chi^2 = 284.2$, d.f. = 1). This result falls in the upper percentile of the 250 permuted χ^2 values; median permuted values = 19.1, interquartile range = 6.7–50.8. Only eight transcripts were common to the data sets that were significantly evolved (CDE; Figs 2, 3) and significantly plastic, suggesting that short-term plastic responses and longer-term evolutionary responses involve largely different sets of genes.

Extended Data Table 1 | Comparison of gene expression divergence (P_{ST}) with divergence of putatively neutral microsatellite loci (F_{ST})

	Intro1[c]	Intro2[d]	
P_{ST}[a]	0.32 (0.21)	0.27 (0.21)	Only CDE transcripts
	0.05 (0.11)	0.05 (0.12)	Only non-CDE transcripts
	0.05 (0.11)	0.05 (0.10)	All transcripts
F_{ST}[b]	0.01	N/A	10 microsatellite loci

[a]Quantitative divergence estimated by P_{ST}, a phenotypic proxy for quantitative genetic divergence Q_{ST}[40], calculated under the conservative assumption that half the within-population variation was heritable. Numbers in parentheses are standard deviations.
[b]Neutral divergence estimated from 10 microsatellite loci[41].
[c]Divergence between the ancestral HP site (Guanapo) and the Intro1 site (Lower Lalaja).
[d]Divergence between the ancestral HP site (Guanapo) and the Intro2 site (Upper Lalaja).