RESEARCH ARTICLE

EVOLUTIONARY RESCUE

Adaptive introgression enables evolutionary rescue from extreme environmental pollution

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Radical environmental change that provokes population decline can impose constraints on the sources of genetic variation that may enable evolutionary rescue. Adaptive toxicant resistance has rapidly evolved in Gulf killifish (*Fundulus grandis*) that occupy polluted habitats. We show that resistance scales with pollution level and negatively correlates with inducibility of aryl hydrocarbon receptor (AHR) signaling. Loci with the strongest signatures of recent selection harbor genes regulating AHR signaling. Two of these loci introgressed recently (18 to 34 generations ago) from Atlantic killifish (*F. heteroclitus*). One introgressed locus contains a deletion in *AHR* that confers a large adaptive advantage [selection coefficient (*s*) = 0.8]. Given the limited migration of killifish, recent adaptive introgression was likely mediated by human-assisted transport. We suggest that interspecies connectivity may be an important source of adaptive variation during extreme environmental change.

Human alterations of the environment can be swift and severe, and thereby result in population declines and increased selective pressures. Adaptation requires genetic variation from mutations, standing variation, migration, or interspecific hybridization (introgression). However, declining population size coupled with immediate threats to fitness may constrain the sources of genetic variation that enable evolutionary rescue (*1*, *2*); new mutations arise slowly, standing variation may be insufficient, habitat fragmentation may limit migration, and hybridization may be rare and deleterious. Little is known of the relative importance of these sources of variation in the context of evolutionary rescue.

Gulf killifish (*Fundulus grandis*) are common in coastal estuaries along the northern Gulf of Mexico. They occupy the Houston Ship Channel, much of which is heavily polluted with halogenated and polycyclic aromatic hydrocarbons (HAHs and PAHs, respectively) resulting from more than 60 years of industrial activity (*3*), forming a gradient of contamination through Galveston Bay (*4*) (Fig. 1A). Many HAHs cause cardiac deformities in developing vertebrates that directly impair fitness (*5*, *6*). Nevertheless, *F. grandis* persist at these sites and are resistant to the normally lethal effects of these toxicants (*7*). We examine the molecular underpinnings of apparently evolved resistance to extreme pollution among populations densely sampled across the pollution gradient, using experimental and population genomic approaches. We integrate prior work on the sister taxon of *F. grandis*, the Atlantic killifish (*F. heteroclitus*), which has also adapted to similar chemicals (*8*), to uncover the evolutionary history of key adaptive variants.

We sampled fish from 12 sites spanning the pollution gradient (Fig. 1A and table S1), spawned them separately in the laboratory, and exposed their embryos to a range of concentrations of polychlorinated biphenyl 126 (PCB126, a model HAH). At the population level, resistance to toxicity, as measured by rates of cardiac teratogenesis, is correlated with pollution along this gradient, where fish from the most polluted sites are resistant to concentrations of HAHs 1000 times higher than normally teratogenic levels (Fig. 1, B and C). Resistance is retained through at least two generations in a clean environment (fig. S1) and is intermediate in hybrids from crosses between sensitive and resistant populations (*7*), indicating a genetic basis.

Aryl hydrocarbon receptor (AHR) signaling mediates HAH-induced cardiac teratogenesis (*9*). We compared AHR pathway function (CYP1A activity in response to PCB126 exposure) (*10*) (fig. S2) among populations across the pollution gradient. Similar to resistance, desensitization of the AHR pathway scales with levels of pollution

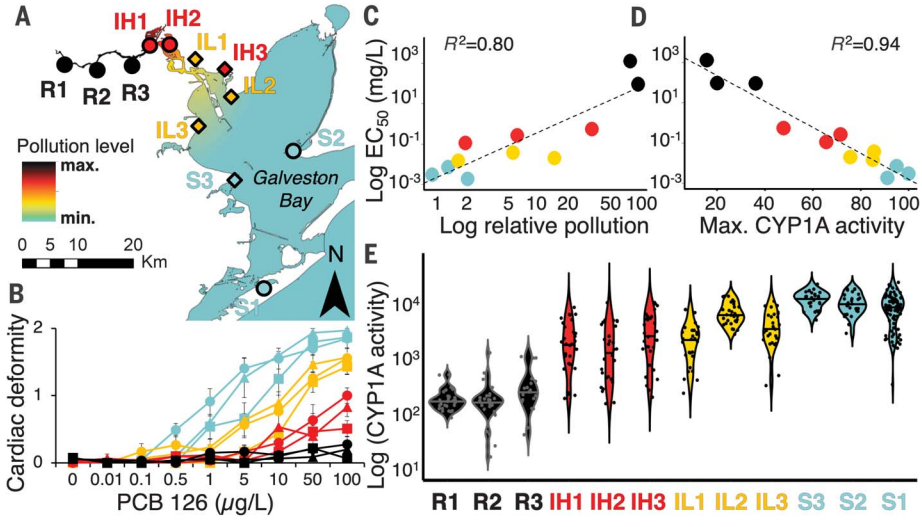

Fig. 1. Variation in sensitivity to pollution among *F. grandis* populations distributed along a steep pollution gradient in Galveston Bay (USA). (**A**) Pollution gradient is scaled by color, from low (blue) to high (black). Populations include resistant (black, R1 to R3), intermediate-high resistance (red, IH1 to IH3), intermediate-low resistance (gold, IL1 to IL3), and sensitive (blue, S1 to S3). Genomics data were collected for populations denoted with circles. (**B**) Population variation in cardiac deformities in embryos exposed to PCB126 (error bars indicate standard error of the mean). Population variation in sensitivity to PCB-induced cardiac deformities [log median effective concentration (EC_{50})] correlates with (**C**) habitat pollution and (**D**) AHR pathway inducibility (CYP1A activation by PCB126). (**E**) PCB-induced CYP1A activity varies among individuals and populations.

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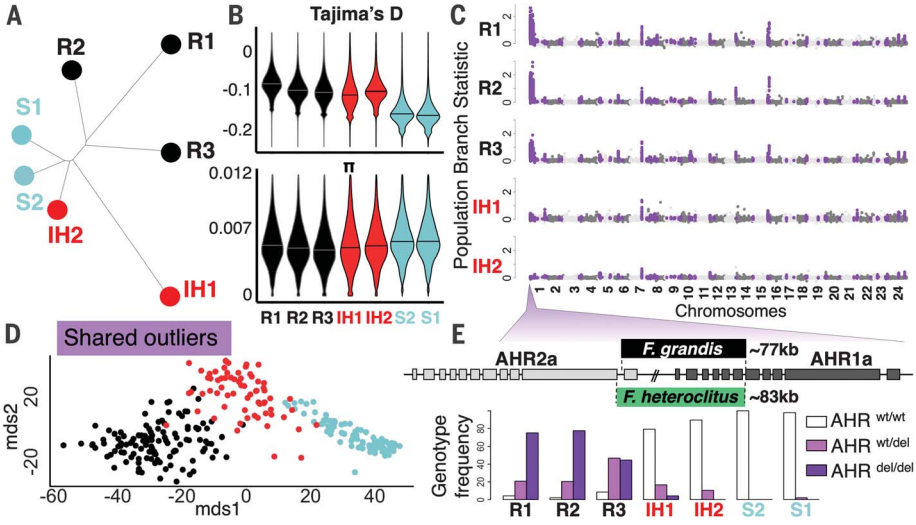

Fig. 2. Genetic variation within and among populations. (**A**) Neighbor-joining tree (from genome-wide data) indicates population clustering along the pollution gradient. (**B**) Consistent with a demographic bottleneck, populations from polluted sites have an excess of rare variants (Tajima's D, top panel) and reduced nucleotide diversity genome wide (π, bottom panel) compared with clean site populations. (**C**) Genetic differentiation for each resistant and intermediate population (five panels) compared with both sensitive populations (population branch statistic). Background divergence is low, and purple indicates outlier regions (1% most divergent) that are shared among all adapted populations. (**D**) Multidimensional scaling (mds) of genetic variation for shared outlier regions clusters individuals and populations by resistance level. (**E**) A deletion in *F. grandis* (~77 kb spanning *AHR2a* and *AHR1a* on chromosome 1) has swept to high frequency in resistant populations (wt, wild type; del, deletion).

(Fig. 1D and fig. S3). Within intermediate populations, interindividual variation in AHR inducibility ranges between sensitive and resistant populations (Fig. 1E). Our experimental results suggest that desensitization of AHR to HAHs underlies resistance in *F. grandis*.

To understand the genetic basis of this phenotype, we sequenced whole genomes of 24 to 49 fish (~0.6× coverage) from three resistant (R1, R2, and R3), two intermediate (IH1 and IH2), and two sensitive (S1 and S2) populations. We searched for signatures of selection that covary with pollution through pairwise genetic differentiation (F_{ST}), population branch statistic (*11*), and differences in nucleotide diversity (π) between polluted and sensitive populations in 20-kb windows. Neighboring 20-kb windows were merged into regions, which were ranked by their size and overall divergence. We defined the 1% most differentiated windows as outliers for each polluted population.

Genome-wide differentiation between populations is low (pairwise F_{ST} = 0.002 to 0.029; fig. S4) and genetic structure is consistent with geography (Fig. 2A). Compared with sensitive populations, resistant populations have reduced nucleotide diversity and increased Tajima's D (Fig. 2B and fig. S5), possibly reflecting a recent reduction in population size. Evolved resistance (Fig. 1), accompanied by strong recent selection (Fig. 2C and fig. S6) and population decline (Fig. 2B), is consistent with evolutionary rescue in polluted populations (*1*).

Top-ranked signatures of selection are shared across all pollution-adapted populations (Fig. 2C). We detected 88 shared outlier regions distributed across 23 of 24 chromosomes. Differentiation in shared regions is higher between resistant and sensitive populations than between intermediate and sensitive populations (Fig. 2D), and intermediate populations show no consistent signatures of selection relative to both resistant and sensitive populations (fig. S7). We conclude that intermediate populations are distinguished from resistant populations, not by selection on different loci, but rather by weaker selection at the same loci.

Genomic regions showing the strongest signatures of selection harbor genes encoding key regulators of the AHR signaling pathway. *AHR1a* and *AHR2a* are centered in the most highly ranked shared outlier region (chromosome 1; Fig. 2C and fig. S8). Two paralogs of aryl hydrocarbon receptor nuclear translocator (*ARNT*), which dimerize with AHR to activate transcription (fig. S2), are within the second- and third-ranked shared outlier regions on chromosomes 8 and 10, respectively (Fig. 2C and figs. S9 to S11). Aryl hydrocarbon receptor interacting protein (AIP) regulates nuclear translocation of pollutant-activated AHR and is within the R1–R2 shared outlier region on chromosome 2 (Fig. 2C and fig. S12). Signatures of selection in multiple AHR pathway elements, coupled with additional selection signatures throughout the genome, indicate that AHR pathway modification is an important component of polygenic adaptation to the fitness challenges present in this urban estuary.

The top-ranked outlier region spans ~2 Mb and encompasses the tandem paralogs *AHR1a* and *AHR2a* (Fig. 2E). We detected a 77-kb deletion spanning these genes at high frequency in resistant populations (85, 83, and 68% in R1, R2, and R3, respectively), at moderate frequency in intermediate populations (25% and 5% in IH1 and IH2, respectively), and in only one heterozygous individual from a sensitive population (Fig. 2E and figs. S13 and S14). Given that the AHR pathway is profoundly desensitized in resistant populations (Fig. 1, D and E, and fig. S3) and moderately desensitized in intermediate populations and that its experimental knockdown is protective of toxicity (*9*), we propose that this mutation is important for the adaptive phenotype. Five different deletions in AHR genes have now been associated with rapidly evolved resistance to HAHs across three species of wild fish (*8*, *12*). Consistent with the "less-is-more" hypothesis (*13*), this suggests that rapid adaptation by disabling mutations may be common. We speculate that sweeps of such large-effect loci may quickly recover fitness after extreme environmental change, whereas smaller-effect loci, perhaps with reduced negative pleiotropy, advance more slowly.

We analyzed whole-genome sequences from *F. grandis* and *F. heteroclitus* to contrast variants associated with resistance. In resistant *F. grandis*, the region containing the *AHR* deletion on chromosome 1, as well as the region from chromosome 10 encompassing *ARNT*, was more similar to *F. heteroclitus* haplotypes than to other *F. grandis* haplotypes (Fig. 3, A and B, and fig. S10), which could result from incomplete lineage sorting or admixture. The species divergence time estimated from elsewhere in the genome predated the divergence of the selected haplotypes (Fig. 3C), suggesting admixture as the source.

To formally test for introgression and to estimate the timing and strength of selection, we extended a coalescent theory–based inference method that distinguishes among modes of adaptation (*14*). Introgression of the deletion-bearing haplotype was much more likely than incomplete lineage sorting (Fig. 3E and figs. S15 to S17), consistent with divergence-time estimates (Fig. 3C). We inferred a large fitness advantage for individuals carrying the AHR deletion haplotype (s = 0.8), and very recent gene flow (16 generations before onset of selection; Fig. 3E), with the sweep occurring over 18 generations. This model estimates that introgression happened ~34 generations before the sampling events in 2014–2015. As we are unlikely to have sampled the true *F. heteroclitus* source population (resistance is widespread in this species), this time may be even shorter (*15*). For the adaptive ARNT region, we infer similar timing of gene flow (36 to 37

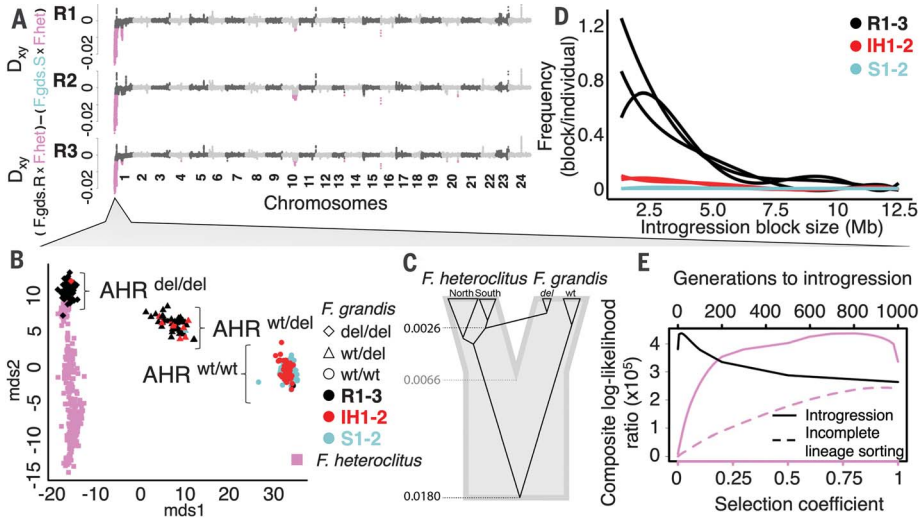

Fig. 3. Evidence for adaptive introgression from *F. heteroclitus*. (**A**) Sequence similarity (D_{xy}) calculated between each resistant *F. grandis* population and *F. heteroclitus*, contrasted with similarity between a sensitive *F. grandis* population and *F. heteroclitus*. Negative values indicate similarity with *F. heteroclitus* that is higher in resistant than sensitive *F. grandis* populations (pink). (**B**) Genetic variation flanking the AHR deletion is more similar between resistant *F. grandis* and *F. heteroclitus* than between resistant and sensitive *F. grandis*, suggesting introgression. (**C**) Mean time to coalescence of 1 Mb surrounding the AHR deletion between *F. grandis* and southern *F. heteroclitus* is 0.0026 mutations per site, and between intact *F. grandis* alleles and all of *F. heteroclitus* is 0.018. Interspecific coalescence times that predate population divergence (0.0066) is evidence of gene flow. (**D**) Large introgression blocks are restricted to resistant populations. (**E**) Maximum composite log-likelihood ratios under introgression and incomplete lineage-sorting models, as a function of strength of selection (pink, bottom axis) and time between introgression and onset of selection (top axis) for the AHR deletion region, relative to a neutral model. Introgression is likely the source of the adaptive haplotype in *F. grandis*, which rapidly swept because of large selective advantage (s = 0.8).

generations before sampling) and strong selective advantage (s = 0.55) (figs. S18 and S19). These alleles clearly have a large effect on fitness and so likely explain a large proportion of the genetic variance in pollution tolerance. We conclude that recent introgression from a few successful *F. heteroclitus* migrants (figs. S17D and S19D) provided crucial genetic variation to rescue *F. grandis* populations from rapid environmental change.

We evaluated the footprint of introgression in *F. grandis* genomes by scanning for genomic segments (>400 kb) showing evidence of *F. heteroclitus* ancestry. These ancestry blocks collapse into 15 discrete regions totaling 70 Mb on nine chromosomes (Fig. 3A and figs. S20 and S21). Most large introgressed blocks are rare (11 of the 15 regions are found in fewer than five individuals) and are found exclusively in polluted populations (Fig. 3D). More than 30 Mb of the 40 Mb comprising chromosome 1 showed evidence of introgression in at least one individual, representing *F. heteroclitus* ancestry that "hitchhiked" with the deletion sweep. Mean haplotype block size also suggests that the introgression event was recent (~65 to 155 generations before sampling; fig. S21). A recent discrete hybridization event occurred in the Houston Ship Channel, introducing highly advantageous adaptive haplotypes from *F. heteroclitus* and leaving behind evidence of *F. heteroclitus* ancestry scattered across the genome. Because *F. heteroclitus* have small home ranges (*16*) and the nearest populations are in Florida, >2500 km away, human-mediated transport is the likely mechanism of introduction (*17*), possibly through ballast water transfer (*18*) or baitfish transport (*19*).

The importance of hybridization in conservation biology is contentious (*20*, *21*). Recipient populations are at risk for negative ecological interactions with invaders, introduction of locally maladaptive or deleterious alleles, or genetic incompatibilities (*22*, *23*). We propose that *F. heteroclitus* introgression into Galveston Bay was sufficiently rare to preclude extensive accumulation of deleterious variation in *F. grandis*, and that the adaptive advantage afforded by introgressed loci was sufficient to overcome maladaptation imposed by linked loci. Although a growing body of work shows that ancient hybridization can contribute adaptive genetic variation [e.g., (*24*, *25*)], our work shows that hybridization can provide variation crucial for adaptation following swift and extreme environmental change.

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SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/364/6439/455/suppl/DC1
Materials and Methods
Figs. S1 to S21
Tables S1 to S3
References (*26*–*41*)

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An unexpected advantage

Human activities are altering Earth's environment in many ways. Will other species be able to adapt in the face of rapid change? Adaptation requires genomic variability, but declining populations lose diversity, which casts doubt on adaptation as a survival mechanism in today's world. Oziolor *et al.* report a case of rapid adaptation to pollution in killifish, apparently enabled by introduction of a non-native congener within the last 30 generations (see the Perspective by Pfennig). This related species, possibly carried in ship ballast water, appears to have provided advantageous genetic variability that has allowed the native fish to adapt to its increasingly polluted environment.

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