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 (1). 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.

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(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 *F. grandis* 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*<sub>ST</sub>), population branch statistic (*D*), 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*<sub>ST</sub> = 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 almost 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 (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). Aaryl 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
generations before sampling) and strong selective advantage ($s = 0.055$) (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. S3D). 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.

REFERENCES AND NOTES

15. See supplementary materials.

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Data and materials availability: Sequence data are archived at the National Center for Biotechnology Information (PRJNA437462); physiology and chemistry data are archived at the National Center for Biotechnology Information (PRJNA437462); and additional code (https://github.com/oziolor/grandis_introgression) is archived on GitHub.

SUPPLEMENTAL MATERIALS

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Materials and Methods

Figs. S1 to S32

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Adaptive introgression enables evolutionary rescue from extreme environmental pollution

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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.

Science, this issue p. 455; see also p. 433
Supplementary Materials for

Adaptive introgression enables evolutionary rescue from extreme environmental pollution

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Materials and Methods

1. Animal Experiments

1.1. Fish collection and care

All animal collections and experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at Baylor University. For animal exposure experiments we collected fish from 12 locations along Galveston Bay using minnow traps between the years of 2012 and 2014 (Fig 1A; Table S1). Sample sizes varied between 30 to 120 fish per population. Fish were transported to IACUC approved fish facilities at Baylor University and placed in 40-gallon tanks within a recirculating system including biological, mechanical and UV filtration. Newly captured fish were prophylactically treated with 2.5 mg/L praziquantel (PraziPro CD-22968, Aquarium Solutions, Spokane Valley, WA, USA) twice, with two weeks in between. Temperature was maintained at 25°C, light cycle was set to 14/10 light/dark, salinity was held at 10‰ and fish were fed twice daily with pellet feed (Aquamax®, Fingerling Starter 200, PMI Nutritional International, LLC, Brentwood, MO, USA) and Tetramin® Tropical Fish Food (Tetra Systems, Blacksburg, VA, USA). We allowed fish to acclimate for 60 days before they were used for any experiments.

1.2. PCB exposure experiments to determine population differences in sensitivity

We spawned fish from each population manually (fish from the same population mated together) by pooling milt and oocytes, and fertilized embryos were treated with antiseptic (0.3% hydrogen peroxide) for 1 minute. All individuals from a population sample were spawned at each experiment. Due to natural variability in spawning success, we only conducted an experiment if at least 5 females contributed to the genetic pool. We placed embryos in a 28°C incubator overnight. At 24 h post fertilization (hpf) we exposed embryos to a range of concentrations of polychlorinated biphenyl (PCB) 126 (0, 0.01, 0.1, 0.5, 1, 5, 10, 50, 100 µg/L; Absolute Standards, Hamden, CT, USA) and 0.21 µg/L ethoxyresorufin (Sigma-Aldrich, St. Louis, MO, USA) in 100-mL hexane rinsed glass vessels using 0.1% dimethyl sulfoxide (DMSO) as carrier. Each container housed 5 embryos in 50 mL of dosing solution. A minimum of 10 embryos were exposed for each exposure level per experiment. Each final end-point is represented by a minimum of three independent experiments for each population and each end-point. During the six-day exposure, we incubated embryos at 28°C at 14/10 light/dark cycle and screened them for deformities and CYP1A activity at 144 hpf. Cardiac deformities were scored (blind to treatment) on a scale from 0 (normal) to 2 (highly deformed) as described in (9). CYP1A activity was determined with the ethoxyresorufin-O-deethylase assay as originally described by (10) and modified as described in (7). Briefly, embryos were screened for fluorescence of resorufin under an epifluorescent microscope (AZ-100, Nikon, Melville, NY, USA). CYP1A activity was expressed as total fluorescent signal in a representative region of the urinary bladder with subtracted background fluorescence from a representative region of the remainder of the embryo. We derived 50% effective concentration (EC50) values for cardiac deformities with a three-parameter log-linear model fit in the drc package in R (26). We used exponential and power regressions to relate the increase of EC50 concentrations to a repression of the maximal inducibility of CYP1A activity and to the level of total PCB contamination, as determined by sediment, fish tissue and passive samplers (4) (Fig.1, Fig. S1, S2, S3). Cardiac deformities and CYP1A activities were estimated from a minimum of three replicate exposure experiments for each dose/population combination.
1.3. Test for heritability of the resistance phenotype

We spawned fish populations manually (fish from the same population mated together) and incubated embryos for 10 days on damp western blot paper in conditions described above. At 10 dpf embryos were hatched after re-submersion in 10‰ artificial salt water and shaking of 55 rpm for 30 minutes. We fed larvae with hatched Artemia and performed water renewals daily for 1 month, after which larvae were moved to recirculating tanks. We spawned fish after they reached sexual maturity (~1.5 years), to obtain the F2 generation and screened for cardiac deformity and CYP1A activity as described above. Cardiac deformity and CYP1A activity dose-response were analyzed with 2-way ANOVAs in JMP12 statistical software (Fig. S1).

2. Mapping the distribution of chemical contamination in Galveston Bay

We obtained PCB chemistry data for sediment, fish tissue and water from Oziolor et al. (4). We created a composite contamination score for each matrix by scoring sites (0-100) and taking mean contamination percentile over the three matrices. Final total contamination was expressed as percent of the most contaminated site. The contaminant index map (Fig. 1A) was made in ArcMap 10.6 (ESRI; Redlands, CA). An outline of Galveston Bay and the Houston Ship Channel was extracted from a coastal salinity map available online through the National Oceanic and Atmospheric Association (NOAA) (27). The bay footprint was edited to include all sampling sites within its boundary aligning new edges with satellite imagery from an ESRI base map. Contamination index values were interpolated using the kernel smoothing interpolation with barriers method in the Geostatistical Analyst extension. A prediction surface was produced using the exponential method with a zero-order polynomial. Bandwidth was set to 0.5 to cover the entire bay footprint. The smoothing factor was set to 0.6 and the search radius to 0.27 to reduce root-mean-square error.

3. Population Genomics

3.1. DNA extraction, library building and sequencing

We obtained fin clips from 7 out of the 12 populations (populations denoted with a circle in Fig. 1A) through non-lethal fin clipping, snap froze the samples in liquid nitrogen and stored them at -80°C until extraction. We extracted DNA with QIAGEN DNeasy Blood and Tissue extraction kits, including RNA removal, and confirmed DNA integrity and concentration (minimum of 20 ng/µL) using a NanoDrop. DNA samples were stored at -20°C until library preparation. We sheared DNA in samples to ~500bp fragments (Covaris220) and used NEBnext Ultra DNA library preparation kits (New England Biotechnologies, MA, USA) creating three sets of 96 unique combinations of indexes for a total of 288 individual fish. Proportion of males was between 30-50% per population. We confirmed adaptor lengths through TapeStation High sensitivity T1000 (Agilent Technologies, CA, USA) and gel electrophoresis. We equalized DNA concentrations per sample, confirmed with qPCR and merged individuals in three pools of 96 individuals as follows: S1 (48) + S2 (48), R3 (49) + R2 (47), IH2 (48) + IH1 (24) + R1 (24). We ran each pool on one lane of Illumina HiSeq 2500 (125 bp paired-end; Brigham Young University) with version 4 chemistry in two separate runs for an average genomic coverage of 0.6x per individual.
3.2. Sequence read mapping and variant calling

We removed any remaining adapter sequences and trimmed low quality headers of base sequences using Trimmomatic v0.33 (28). We mapped reads to our reference assembly using Burrows-Wheeler aligner with high mapping rates (>96%) (29). We ordered non-coordinant read files and removed duplicates using samtools v1.3 (30). We excluded ~52 Mb (~5%) of our reference assembly that had abnormally high coverage as possible repeated low complexity or poorly mapped regions. We used Freebayes v0.9 to call reads, while discarding low quality reads <30, bases <20, unmapped and duplicate reads (31). We filtered the obtained variant calls with vcftools v0.1.13 to allow only for bi-allelic SNPs, with a minimum of 8 samples/population, minor allele frequency of > 0.05 and quality scores of >30 (32).

3.3. Summary statistics

We estimated $F_{ST}$ values for populations using vcflib and the Weir and Cockerham’s calculation (33) both in 1 kb non-overlapping windows. We noticed that there were inflated levels of pairwise $F_{ST}$, particularly when our lowest sample size populations were included. We re-ran the calculations with all populations subsampled to the same number of individuals and found that patterns of $F_{ST}$ were much more consistent with isolation by distance. We suspected that since Weir and Cockerham’s $F_{ST}$ uses a correction factor for sample size, this could be over-correcting for our smallest sample sized populations, which have 24 versus 47-49 individuals, which could be inflating the $F_{ST}$ estimates for those pairs. We calculated pairwise divergence instead using Hudson’s $F_{ST}$, which does not correct for population size with full population samples. Estimates of Hudson’s $F_{ST}$ matched isolation by distance patterns much more closely and resembled the sub-sampled estimates of Weir and Cockerham’s statistic. Thus, we made a choice to use Hudson’s divergence estimates for outlier approaches (Fig. S4). To identify outlier loci we compared $F_{ST}$ between each resistant population and both reference populations by calculating the population branch statistic (PBS) (11).

To estimate $\pi$ and Watterson’s $\theta$, we calculated folded site frequency spectra for a randomly selected 49 Mb subsample from every population using the Analysis of Next Generation Sequencing Data (ANGSD) software (34). Based on those site frequency spectra we estimated per base site allele frequencies using ANGSD (34, 35). We chose to use the same filters on individual representation, read quality, base quality and high coverage regions as described for variant calling. Watterson’s $\theta$ and $\pi$ were estimated on a per SNP basis with ANGSD and summed into 20kb windows with 1kb slide and represented them as an average per window. Tajima’s D was calculated from those estimates as:

$$D = \frac{\pi - \theta}{\sqrt{\text{var}(\pi - \theta)}}$$

For Hudson’s $F_{ST}$ statistic, we calculated $\pi$ and $D_{xy}$ statistics per site with a custom script (https://github.com/eoziolor/fgfh_post/blob/master/scripts/combined.Rmd). Those were averaged in a similar fashion over 20kb windows with 1kb slide.

3.4. Selecting outliers

To focus on regions of the genomes that represent the expected strong selective sweeps, we chose an outlier approach that ranked genomic regions based on the PBS (11). To eliminate artificially elevated divergence estimates derived from sites with low coverage, we eliminated any windows with less than 2000 bases estimated across our populations. We only selected the top 1% of outliers among comparisons. We consolidated outlier windows within 50kb of each other into outlier regions using BEDtools (36). We ranked the selection signals of outlier regions by combining the size and the level of divergence observed across that region: we summed the
divergence statistics for each region, which placed at highest rank the largest and most divergent regions. We tested whether those outlier regions were shared between all adapted populations (Fig. 2C).

We confirmed that regions of high divergence also have low diversity, characteristic of recent selective sweeps. We obtained regions with low neutral diversity in resistant populations, estimated by the pairwise differences (π). We also observed that regions of high divergence more frequently fell within regions of low diversity in resistant populations compared to sensitive (Fig. S5). To obtain regions with both high divergence and low diversity, we converted both $F_{ST}$ statistics and π estimates into Z scores to evaluate them on the same scale. We subtracted the diversity estimate Z score from the divergence estimate Z score to obtain high scores for windows with only high diversity and low divergence ($Z_{SUM}$) (Fig. S6). We used the same method as above to merge these windows within 50kb into regions of interest and rank regions with highest total $Z_{SUM}$ score.

Compared to intermediate populations, resistant populations have stronger divergence from reference populations. This is likely driving the 1% outlier threshold up and limiting the ability to detect the less divergent regions, which we identify in intermediate populations. To confirm the lack of distinct outlier peaks that define intermediate populations, we performed a PBS with R1 and S1 as background for each of the intermediately resistant populations (Fig. S7). The lack of shared divergence outliers between the two intermediate populations confirms the lack of genomic regions that distinguish intermediately resistant populations from highly resistant populations.

### 3.5. Genetic distance between populations

We explored the background genome-wide substructure through 20 random megabases (~2%) of our genome and calculated allele frequencies to estimate neighbor-joining tree with Rphylip (37) (Fig. 2A). The genome-wide substructure is consistent with the IBD pattern observed in $F_{ST}$ pairwise relationships (Fig. S4). We repeated this procedure for regions of interest including our top-ranking outliers (Fig. S8-S12).

### 3.6. Comparison of a deletion in the AHR2a/1a region between F. grandis populations and with a homologous region in F. heteroclitus

We found the top ranked divergence outlier to contain an approximately 77kb deletion, which spans exons from both AHR2a and AHR1a. We estimated per base depth of coverage over the scaffold containing AHR using Samtools 1.3 (30). We plotted this region over all individuals and compared this region to the read coverage in a population of F. heteroclitus, which also has a deletion (~83kb) in the same region (Fig. S13-S14) but with different break points.

### 4. Model-based inference of introgression: DMC-MYAdIDAS (DMC Modified for Young Adaptive Introgression Distinguished from Allele Standing)

#### 4.1. Method overview

In two genomic regions, we confirmed and characterized adaptive introgression using and extending the inference method DMC ([https://github.com/kristinmlee/dmc](https://github.com/kristinmlee/dmc)) (14) that aims to distinguish among types of convergent adaptation. This composite likelihood-based approach utilizes genomic data to detect cases of convergent adaptation at the genetic level and the loci involved, taking advantage of the signature that selection has on linked neutral variation. We
compared the composite likelihoods estimated under various modes of convergent adaptation, including those due to gene flow and selection on shared ancestral standing variation. Here, we extended this method by i) modifying the migration model to include staggered sweeps, ii) allowing for extremely strong selection regimes, and iii) allowing for differences in effective population sizes among populations. We also slightly modified this method to fit patterns observed to be unique to each region. In our analysis for chromosome 1, we assigned the selected site to be the deletion midpoint instead of making it a free parameter. In our analysis for chromosome 10, we modified the models to exclude selection in the source population of the putatively introgressed haplotype. Details for these extensions can be found in Section 4.4. The R code and documentation for our approach are provided in https://github.com/kristinmlee/dmc/myAdidas.

We ran this method on two regions that show the greatest signals of adaptive introgression in the dxy analysis (Fig 3A): the AHR deletion on chromosome 1 and the ARNT region on chromosome 10. To investigate the origin of the putatively introgressed haplotypes in F. grandis, we distinguished between models in which the deletion was found in both F. grandis and F. heteroclitus due to incomplete lineage sorting or introgression. These models are consistent with the evidence that the AHR deletion in resistant F. grandis lies on a haplotype most closely related to the resistant F. heteroclitus population sampled from the Elizabeth River (hT4) and that, for an F. heteroclitus population with whole-genome resequencing data (8), hT4 is geographically closest to the species range of F. grandis. DMC can be used to dissect the mode of convergence between any group of populations and controls for background population structure. Here, we ran the method using population allele frequency data from three pairs of resistant and sensitive populations, respectively: i) hT2 and hS2 – from the northern clade of F. heteroclitus, ii) hT4 and hS4 – from the southern clade of F. heteroclitus, and iii) gR2 and gS2 – from F. grandis. We chose gR2 as the representative for resistant F. grandis because it has the AHR deletion at high frequency (~85%) and the highest number of individuals sampled among the resistant F. grandis populations. The F. grandis gS2 population was used as the representative sensitive population because no deletions were sampled in it. In the analysis for the AHR deletion, for each resistant and sensitive population used for inference, we included only individuals that were either homozygous for a deletion (resistant) or homozygous for intact haplotypes (sensitive). In the analysis of the ARNT region, we included only F. grandis individuals in gR2 that had an introgressed haplotype (at 30-45% frequency), and only individuals in gR2 that did not have an introgressed haplotype. Haplotypes were estimated with a custom script (https://github.com/eoziolor/fgfh_post/blob/master/scripts/combined.Rmd), assigning individuals to groups of introgressed vs. non-introgressed haplotype in this region based on genetic similarity.

We tested four models (Fig. S15) to further investigate the similar haplotypes observed between hT4 and gR2. First, we assumed a model of selection on ancestral standing variation (Fig. S15A), (here referred to as the incomplete lineage sorting model). This model specifies that the selected variant was standing at very low frequency in the ancestor of the two adapted populations (here the two species) and remained standing after the species split until it recently swept independently in both populations. The other three models invoke migration of the selected variant from hT4 to gR2. First, the standing variant source model (see Appendix A.4 in Lee and Coop 2017 (14)) in which the selected variant was standing at low frequency in hT4 and then was introduced to gR2 via migration (Fig. S15B). The selected variant may still be standing for some time post-migration before it sweeps in both populations when the selective pressure
begins. Next, we assumed the migration model (Fig. S1C), in which the selected variant migrates from \( h_{T4} \) to \( g_{R2} \) during the sweep in \( h_{T4} \) and immediately begins to sweep in \( g_{R2} \). This model assumes that migration occurs during the sweep and may be more relevant for populations that are more closely related and experience constant rates of gene flow. We call this case the concurrent sweeps model since both populations are sweeping at once. Lastly, we specified a new model of migration (Fig. S1D) that is a modification of the concurrent sweeps model and may be more relevant for our distantly related populations. Since contemporary migration from the Atlantic coast to the Gulf coast is extremely rare, we allowed the sweep to finish in \( h_{T4} \) before migration to \( g_{R2} \). In this case, we also allow for some amount of “standing time” (a free parameter) between when migration ended and the sweep began in \( g_{R2} \). This model assumes that migration occurs during the sweep and may be more relevant for populations that are more closely related and experience constant rates of gene flow. We call this case the concurrent sweeps model since both populations are sweeping at once. Lastly, we specified a new model of migration (Fig. S1D) that is a modification of the concurrent sweeps model and may be more relevant for our distantly related populations. Since contemporary migration from the Atlantic coast to the Gulf coast is extremely rare, we allowed the sweep to finish in \( h_{T4} \) before migration to \( g_{R2} \). In this case, we also allow for some amount of “standing time” (a free parameter) between when migration ended and the sweep began in \( g_{R2} \). This model may be relevant if \( g_{R2} \) does not immediately experience a large selective advantage of having the migrant haplotype (i.e. pollution has not started yet or is weaker). Additionally, while \( h_{T4} \) is our southernmost sampled \( F. \) heteroclitus population, it is likely that the deletion actually migrated from a different southern-clade population of \( F. \) heteroclitus. The standing time can then represent some time that allows for more independent recombination to occur between \( h_{T4} \) and \( g_{R2} \) that may be a better fit to the data if the haplotypes in \( h_{T4} \) and \( g_{R2} \) look slightly different at the edges. We refer to this model as the staggered sweeps model and outline the details below. Here, the standing variant source, concurrent sweeps, and staggered sweeps models are all cases of adaptive introgression that we aimed to distinguish between.

For the analysis of the AHR deletion and ARNT region, we used SNPs from the first 15 Mb of chromosome 1 and last 16 Mb of chromosome 10, respectively. We assumed a recombination rate of \( 1.48 \times 10^{-8} \) M/bp/gen, estimated from a linear fit in our regions of interest from the \( F. \) heteroclitus recombination map (EBI BioStudies Accession S-BSST163). Genome-wide SNP data from regions not showing signs of selection and only individuals included in the respective analysis (565,528 SNPs for chromosome 1 analysis and 594,086 SNPs for chromosome 10 analysis) were used to estimate the neutral coancestry matrix, following Lee and Coop 2017 (14), to summarize neutral population structure. We used SNPs that had data across all populations, effectively excluding the deletion region from our analysis of chromosome 1. Since the approach utilizes signatures in neutral loci flanking the putatively selected site (their population allele frequencies and the recombination distance from the selected site), there is no effect of removing the deletion apart from fixing the location of the selected site, which is normally a free parameter in the models. We expand on this in Section 4.4.3. The parameters in which we tested our models on for each genomic region are shown in Tables S2 (AHR) and S3 (ARNT).

### 4.2. AHR Deletion Analysis

The profile-likelihood surfaces for each parameter are shown for each model in Figures S16 and S17. The standing variant source and staggered sweeps models consistently have higher likelihoods than the concurrent sweeps model. All models have their highest likelihoods at extremely strong selection pressures, between 60% and 92% reduction in fitness for individuals homozygous for intact haplotypes compared to individuals homozygous for deletions (Fig. S16). The models that include a frequency of the deletion haplotype, the standing variant source and staggered sweeps models, both have maximum composite-likelihood estimates of fraction of migrants in \( g_{R2} \) that imply the initial deletion haplotype was very rare (Figure S17D). The inferred standing time between introgression and selection is 18 generations in the standing variant source model and 16 generations in the staggered sweeps model (Fig. S17B).
consider this an upper bound because the inferred standing time may have actually been shorter had we sampled a more southern *F. heteroclitus* population. Under the incomplete lineage sorting model, the inferred minimum age of the deletion is 10,000 generations (Fig. S17C). This time specifies the time between the splitting of *h*T4 and *g*R2 and the onset of selection.

Overall, the standing variant source and staggered sweeps models have the highest likelihoods. We are unable to distinguish between them (Fig. S17A). This is not surprising because these two models leave very similar signatures on haplotype patterns. Even though we are not able to distinguish between whether the deletion had already swept in *h*T4 or not prior to migration, the staggered sweeps model is more plausible. In the standing variant source model, the deletion haplotype migrated into *F. grandis* from *F. heteroclitus* where it was standing at frequency 2x10^{-6}. Given the high estimates for the strength of selection, it is not unlikely for the deletion haplotype to sweep from a very low initial frequency. However, with such rare migration between the species, the probability of that deletion haplotype migrating would be very low. It is more probable to have arrived from a source population where the haplotype is at very high frequency as is the case in the staggered sweeps model.

4.3. ARNT Region Analysis

We ran separate analyses for a sweep that finishes at both 30% and 45% frequency, the range of the observed putatively introgressed haplotype frequency. For both final frequencies, the profile-likelihood surfaces for each parameter are almost identical. This is because when the sweep ends at intermediate frequencies, the same selection coefficient will lead to a very similar number of generations that the sweep takes to reach each final frequency. Our method uses this sweep duration to describe patterns of haplotype similarity among populations, and therefore the results for each final frequency are indistinguishable. We therefore present results for a sweep that ends at 45% frequency and note when results differ for a sweep that ends at 30% frequency.

The results from the ARNT region are largely consistent with the results from the AHR deletion region. Like the AHR deletion region, the adaptive introgression models have much higher likelihoods than the incomplete lineage sorting model (Fig. S18). In addition, the standing variant source and staggered sweeps models consistently have higher likelihoods than the concurrent sweeps model. All models have their highest likelihoods at strong selection pressures, though weaker than in the AHR deletion region (Fig. S19A). The standing variant source and staggered sweeps models both have the highest likelihood for a 55% reduction in fitness for individuals without any copies of the selected variant. This is consistent with a sweep that lasts 30-31 generations, the lower bound of this estimate corresponding to a final frequency of 30% and the upper bound, 45%. Inferred migration rates in the concurrent sweeps model are the same as in the AHR deletion region, at approximately 10^{-5} proportion migrants per generation. For the models that include the fraction of migrants in *g*R2, the standing variant source and staggered sweeps models, the former has a maximum composite-likelihood estimate at 5x10^{-6} and the latter at 10^{-6} (Figure S19D). The inferred standing time between introgression and selection is 10 generations in the standing variant source model and 6 generations in the staggered sweeps model (Fig. S19B). Thus, introgression was extremely recent and occurred approximately 36-37 generations ago in the staggered sweeps model (upon inclusion of an estimate of the duration of the sweep). This is consistent with results from the AHR deletion region, in which introgression was inferred to occur 34 generations ago. Under the incomplete lineage sorting model, the inferred minimum age of the deletion is 5000 generations, shorter than in the AHR deletion.
region (Fig. S19C). This time specifies the time between the splitting of $h_{T4}$ and $g_{R2}$ and the onset of selection.

4.4. Details of modifications to DMC

We begin with a brief description of the model framework to provide some background for this section. The inference method, DMC, uses coalescent theory to model how a sweep acts to modify the variance and covariance in mean-centered neutral allele frequencies among populations at linked loci, relative to neutral population structure (14). The variance in these allele frequencies at a locus within a population can be modeled as a function of the ancestral allele frequency at the locus and the probability that a pair of alleles sampled within a population coalesce before reaching the ancestral population. The ancestral population is the common ancestor of all populations that are considered. The covariance of allele frequencies between populations at a locus is the same as the variance within a population except it instead considers the probability that a pair of alleles, one sampled from each of the two populations of interest, coalesce before reaching the ancestral population. The ancestral allele frequency used to estimate the variances and covariances at a locus is the same for each model; it is the probabilities of coalescing that differ among models and allow us to distinguish among and characterize scenarios of convergent adaptation. The modifications and new models that we introduce all inform these probabilities of coalescing.

The models that we outline are concerned with defining a matrix of probabilities of coalescing due to a sweep, $F^{(s)}$, which is a function of the neutral probabilities of coalescing due to population structure ($F$) the recombination distance between the locus of interest and the selected site, and free parameters such as the location of the selected site, the strength of selection, and others specific to the models. The rows and columns of $F^{(s)}$ and $F$ represent the different populations we consider.

4.4.1. Modification of the probability that a lineage does not recombine out of the sweep ($y$):

Previously, the probability that a lineage stays linked to the selected variant during the sweep used approximations that assumed the strength of selection was very low ($s \ll 1$). However, the length of haplotype similarity within resistant $F. grandis$ populations implies that selection was extremely strong and thus violates these assumptions. Instead of using this analytical approximation, we used numerical analysis as outlined by Charlesworth and Charlesworth 2010 (38). We calculated the probability that a lineage stays linked to the selected variant during the sweep by taking the product of the probabilities that the lineage does not recombine out each generation, which change according to the frequency of the deletion. To get the frequency of the deletion each generation, we simulated a deterministic sweep from starting frequency value $1/2N_e$ or low standing frequency (depending on the model) to frequency 0.85 in AHR and 0.3 or 0.45 in ARNT. We used an additive model of selection in which the homozygote of the deletion has fitness 1, the heterozygote $1 - s / 2$, and the homozygote of the intact haplotype $1 - s$.

$$y = \prod_{i=1}^{\tau}[1 - r(1 - x_i)],$$

where $\tau$ is the number of generations the sweep lasts, and $x_i$ is the frequency of the selected variant in generation $i$. 
4.4.2. Allowing for different effective population sizes between species:

Our two species have different effective population sizes, with *F. heteroclitus* being larger than *F. grandis*. Two terms in the model are parameterized by the effective population size: *i*) the probability that a lineage does not recombine out of the sweep (*y*) and *ii*) the rates of coalescence and recombination during standing phase. While the written model assumes that these terms don’t vary according to effective population size, in practice we estimate and apply them separately for each population. Effective population sizes for each species were inferred from estimates of π, using the Atlantic herring mutation rate (39). There are few estimates of mutation rate existing in fish and this mutation rate is on the same order of magnitude, and within the confidence interval of other known rates in fishes, such as the estimated cichlid mutation rate (40). More importantly, the mutation rate itself is not a parameter in these models, and the effective population sizes estimated from it play a small role in them. The models are robust to effective population sizes changing within an order of magnitude. The effective population size estimate for *F. heteroclitus* is $2.25 \times 10^6$ and for *F. grandis* is $8.00 \times 10^5$.

4.4.3. Assignment of selected site to be the AHR deletion

In the analysis of the AHR deletion on chromosome 1, we used the midpoint of the deletion breakpoints that are furthest from each other and therefore did not vary the position of the selected site in the inference. We defined the breakpoints as specified in Figure S14. Therefore, we set the locus experiencing selection to be 762,684 kb and assumed normal recombination outside the breakpoints as we did not consider SNPs falling inside our defined breakpoints. Additionally, this allowed us to utilize only the SNPs with reads in all populations.

4.4.4. Staggered Sweeps Model

4.4.4.1. Prelude

The resistant *F. heteroclitus* population from which the haplotype migrated is called the ‘source’ (*j*) and the resistant *F. grandis* population that received migrants is called the ‘recipient’ (*i*). In this model, there is a sweep in the source. After the sweep finishes, there is migration from the source to the recipient. We specify migration as a single pulse of admixture (lasts one generation), in which a proportion *g* of alleles in the recipient population are migrants from the source. After some standing time $t_B$, there is a sweep at that locus in the now resistant *F. grandis* recipient population (*i*).

The sections below describe the modified probability of coalescing within or between pairs of populations due to migration and selection. Since we know *g* should be very low, we can assume that the probability a lineage in the recipient population that is not a migrant coalesces with lineages in other populations or lineages in its own population is the same as the neutral probability of coalescing estimated from the data.

The following terms are used multiple times in the model specification and are therefore described here:

- $r$ is the recombination rate between the selected site and the neutral site of interest in a single generation.
• $y = \prod_{i=1}^{\tau}[1 - r(1 - x_i)]$ is the probability that a lineage does not recombine out of the sweep (i.e. remains linked to the selected variant). $\tau$ is the number of generations the sweep lasts, and $x_i$ is the frequency of the selected variant in generation $i$.
• $e^{-\rho t_m}$ is the probability a lineage does not recombine out of its background during the standing time ($t_m$) between migration and the sweep
• $p_m$ is the probability a lineage sampled from the recipient (resistant) population migrates back to the source population. $p_m = y(e^{-\rho t_m} + (1 - e^{-\rho t_m})g) + (1 - y)g$. A lineage does not migrate back with probability $1 - p_m = y(1 - e^{-\rho t_m})(1 - g) + (1 - y)(1 - g)$.

Below, each subsection describes independent probabilities. Nested ones are conditional on the statement of the overarching subsection heading.

4.4.4.2. Within resistant, source $F.\ heteroclitus$ population ($f$)

We can treat this case the same as independent sweep model (14).

$$f_{jj}^{(s)} = y^2 + (1 - y^2)f_{ij}$$

4.4.4.3. Within recipient population ($i$)

First we define a term $z$ that is used multiple times in this case. Conditional on both lineages recombining out at some point before migration, the probability that they coalesce is $z = g^2f_{jj}^{(s)} + 2g(1 - g)f_{ij} + (1 - g)^2f_{ii}$. Here, whether the lineages coalesce or not depends on whether they still migrate back or not.

• Neither lineage recombines out of the sweep with probability $y^2$. Now, they enter standing phase that lasts time $t_m$ before migration.
  – A coalescence or recombination event occurs before migration with probability $w = 1 - e^{-\rho t_m}$. This event is coalescence with probability $\frac{1}{1 + 4Ngr}$. Otherwise, one of the lineages recombines off of the introgressed ‘haplotype’ with probability $\frac{4Ngr}{1 + 4Ngr}$. We call the lineage that recombined off L1, and the other lineage that is still linked L2. In the case that the first event is recombination:
    • L2 does not recombine out with probability $e^{-\rho t_m}$, meaning it must migrate back to the source population ($f$). The lineage that did recombine out also migrates back with probability $g$ or does not migrate back with probability $1 - g$. Thus they coalesce with probability $gf_{jj}^{(s)} + (1 - g)f_{ij}$.
    • L2 also recombines out with probability $1 - e^{-\rho t_m}$. Conditional on this, the lineages coalesce with probability $z$ (defined earlier).
  – A coalescence or recombination event does not occur before migration with probability $1 - w$. Therefore both lineages migrate back to the source population, and coalesce there with probability $f_{jj}^{(s)}$.
• Both lineages recombine out of the sweep with probability $(1 - y)^2$. Conditional on this, the lineages coalesce with probability $z$ (defined earlier).
• One lineage recombines out of the sweep while the other doesn’t with probability $2y(1 - y)$. We call the lineage that recombined out L1, and the other lineage that is still linked L2.
L2 does not recombine out with probability $e^{-rt_m}$, meaning it must migrate back to the source population ($j$). The lineage that did recombine out also migrates back with probability $g$ or does not migrate back with probability $1 - g$. Thus, they coalesce with probability $gf_{jj}^{(s)} + (1 - g)f_{ij}$.

L2 recombines out during standing phase with probability $1 - e^{-rt_m}$. Conditional on this, the lineages coalesce with probability $z$ (defined earlier).

Altogether:

$$f_{ii}^{(s)} = y^2 \left[ w \left( \frac{1}{1 + 4Ng\gamma} + \frac{4Ng\gamma}{1 + 4Ng\gamma} (e^{-rt_m} (gf_{jj}^{(s)} + (1 - g)f_{ij}) + (1 - e^{-rt_m})z) \right) + (1 - w)f_{ij}^{(s)} \right] + (1 - y)^2z + 2y(1 - y) \left[ e^{-rt_m} (gf_{jj}^{(s)} + (1 - g)f_{ij}) + (1 - e^{-rt_m})z \right]$$

4.4.4.4. Between recipient ($i$) and source ($j$)

Both the recipient and the source experience a sweep. To get the probability that one lineage sampled from each coalesce, we condition on whether the lineage from $i$ migrates to $j$ or not ($p_m$ and $1 - p_m$, respectively).

$$f_{ij}^{(s)} = p_mf_{jj}^{(s)} + (1 - p_m)f_{ij}$$

4.4.4.5. Between recipient ($i$) and non-source populations ($k$)

The probability that a lineage sampled from $i$ (recipient) and a lineage sampled from $k$ (not source) coalesce depends on whether the lineage in $i$ migrates to $j$ (source population).

$$f_{ik}^{(s)} = p_mf_{jk} + (1 - p_m)f_{ik}$$

4.4.5. Modification of models to exclude selection in the source population of the putatively introgressed haplotype

In the ARNT region of chromosome 10, there is no signal of selection in $h_{T4}$ or any other $F. heteroclitus$ population we included in our model. Therefore, the following describes the probability of coalescing for the terms that are altered in each model.

For all models, the probability of coalescing within the source population ($j$) is no longer modified by selection, and is rather the neutral probability of coalescing:

$$f_{jj}^{(s)} = f_{jj}$$

In the incomplete lineage sorting model, there is no migration modeled between populations. Therefore, the probability of coalescing for every pair of lineages sampled from different populations is the neutral probability estimated for each population pair.

The probability of coalescing within the recipient population ($g_{R2}$) changes for both the staggered sweeps and concurrent sweeps models. This is because lineages sampled in the recipient population that migrate back to the source population were previously considered to experience another sweep in the source population. The change we make in the staggered sweeps model is more straightforward: replace all $f_{jj}^{(s)}$ terms with $f_{jj}$ (where $j$ is the source population).

The following describes the modification we make to the concurrent sweeps model:
As described in Lee and Coop 2017 (14), Q describes the probability that two lineages sampled at the selected site coalesce in the recipient population (i), such that a single lineage migrates back to the source population (j). 1 – Q is therefore the probability that two lineages sampled at the selected site do not coalesce before migrating back to the source population. We can condition on these two scenarios to get the probability of coalescing for a pair of lineages sampled at a neutral site a given distance away from the selected site.

\[ f_{il}^{(s)} = Q(y^2 + (1 - y)^2 f_{li} + 2y(1 - y)f_{ij}) \]

\[ + (1 - Q)(y^2 f_{jj} + (1 - y)^2 f_{li} + 2y(1 - y)f_{ij}) \]

This reduces to:

\[ f_{il}^{(s)} = y^2(Q + (1 - Q)f_{jj}) + (1 - y^2)f_{li} + 2y(1 - y)f_{ij} \]

For all adaptive introgression models, the probability of coalescing between the recipient population (\( g_{R2} \)) and all other populations depends on whether the lineage sampled in the recipient population migrates back to the source populations. This probability of migrating to the source (\( p_m \)) varies by model and is described below:

For the standing variant source and staggered sweeps models, \( v \) is the probability that a lineage does not recombine out of the selected background during standing phase, if it didn’t recombine out during the sweep. As a reminder, \( t_m \) is the amount of standing time between migration and the sweep and \( g \) is the standing frequency of the selected variant.

\[ v = e^{-r \cdot t_m} \]

Standing variant source and staggered sweeps:

\[ p_m = y(v + (1 - v)g) + (1 - y)g \]

Concurrent sweeps:

\[ p_m = y \]

According to the different probabilities of migrating to the source for each model, the probability of coalescing between the source and recipient populations for all adaptive introgression models is as follows:

\[ f_{lk}^{(s)} = p_m f_{jk} + (1 - p_m)f_{lk} \]

5. Introgression scans

To identify potential blocks of recent \( F. heteroclitus \) ancestry, we scanned the genomic data by calculating a statistic related to \( G_{\text{min}} \) of Geneva et al. (41). Their statistic is defined as

\[ G_{\text{min}} = \min(D_{xy}) / D_{xy} \]

where \( \min(D_{xy}) \) is the minimum distance between phased haplotypes between two populations, and \( D_{xy} \) is the mean pairwise distance between the two populations. There are many other statistics in use for this purpose, but most require data from more than just the two focal populations. We modified this approach due to the lack of phased haplotypes.
We first “haploidized” individual genotypes, sampling single alleles for each variant site when more than one was sequenced. Because of the low coverage, very little data were lost this way. We then calculated the mean number of pairwise differences between haploidized individual genotypes from each *F. grandis* individual to the entire sample of *F. heteroclitus* individuals in 100kb windows. We standardized values in each 100kb window by the median of all these values, rather than the mean D<sub>x,y</sub> between species. We filtered out windows containing less than 50kb of genomic sequence. An approach like this should be effective because D<sub>x,y</sub> between *F. grandis* and *F. heteroclitus* is ~ 3.5%, while pi within *F. grandis* is 0.65% and within *F. heteroclitus* is < 1.8%. This large overall divergence relative to within species diversity should result in large deviations in this statistic where large tracts of *F. heteroclitus* ancestry persist.

To set a threshold to identify putatively introgressed windows, we examined the empirical distribution of statistics. We set this value to 0.93, which is equivalent to the 0.6% quantile (Fig. S20). The 0.6% quantile marks an inflection point in the distribution of statistics. Shifting the threshold up or down by 0.01 does not alter the general conclusions.

We plotted our analog of G<sub>min</sub> (the minimum statistic across all individuals) for two sets of individuals, those from contaminated sites and those from clean reference sites, excluding the single S1 individual bearing the deletion haplotype (Fig. S20). There are large deviations in resistant populations that are not present in sensitive populations.

In order to identify blocks of introgressed *F. heteroclitus* ancestry on an individual basis, we modified the statistic again; we calculated mean genetic distance for each 100kb window for each *F. grandis* individual relative to all *F. heteroclitus* individuals and divided by the window median. Within each individual, we merged windows within 400kb of one another. Examining small blocks of introgression, reveals a pattern with the highest numbers of blocks having a hump-shaped distribution across geography, with R3 having the highest numbers. This signal may localize the hybridization event to that population (Fig. S21).

In order to estimate the age of admixture, we used a method relying on the size of introgressed blocks as an indicator of the number of recombination events (24). This method is as follows, with block lengths in Morgans:

\[
1/\text{mean(block length)}
\]

Nearly every large introgressed ancestry block is located in contaminated sites in the Houston Ship Channel, which we take as evidence that a recent hybridization event within the Ship Channel introduced the beneficial alleles found there. Proceeding from this, we assume that many small blocks of putatively introgressed ancestry found in the clean sites are either false positives or a result of an on-going background process of introgression between the species. To estimate the mean introgression block length in contaminated sites owing to the recent local hybridization event, we subtract the expected contribution of background processes and noise as estimated from the clean sites as follows:

\[
\mu_h = \frac{(\mu_r * n_r) - (\mu_s * n_s)}{(n_r - n_s)}
\]

Where \(\mu\) is a given population’s mean block length, \(n\) is a given population’s mean number of blocks per individual, and the subscripts \(h\), \(r\), and \(s\) denote blocks due to recent ship channel hybridization, blocks in resistant individuals, and blocks in sensitive individuals respectively. We calculated this for each resistant population assuming a mean recombination rate of 1.5 x 10<sup>-8</sup>, and admixture times ranged from 65 to 115 generations ago, depending on which resistant population was analyzed.
Resistance to high levels of contamination is inherited across at least two generations following field sampling. A) The most resistant populations of *F. grandis* (e.g., R2 and R3) can withstand PCB126-induced cardiac teratogenesis at concentrations more than 1200x higher than those that induce cardiac teratogenesis in reference sensitive populations (B). Populations (e.g., R3 and S1) vary in resistance to PCB126-induced cardiac teratogenesis (B) and CYP1A activation (C), and these differences are retained between first generation (f1) and second generation (f2) animals raised in a common clean environment, indicating that population differences in resistance are heritable.
Fig. S2.
Diagram of the aryl hydrocarbon receptor (AHR) signaling pathway. In the cytoplasm, AHR is associated with proteins heat shock protein 90 (HSP90), aryl hydrocarbon receptor interacting protein (AIP, also known as XAP2), and HSP90 co-chaperone (p23). When entering the cell, halogenated aromatic hydrocarbons (HAHs) act as ligands that bind to, and activate, AHR which then translocates to the nucleus. This translocation is mediated in part by AIP. In the nucleus, ligand-activated AHR forms a complex with the aryl hydrocarbon receptor nuclear translocator (ARNT). This complex binds xenobiotic response elements (XREs) and activates transcription of a battery of genes. One of the most strongly up-regulated genes within this battery is cytochrome P450 1A (CYP1A).
Fig. S3.
Multi-population dose-response curves of CYP1A activity (relative fluorescent units, EROD assay) following embryonic exposure to PCB126. Fish from polluted sites (black curves) were resistant to the dose-dependent induction of the AHR pathway observed in fish from clean sites (blue curves), whereas fish from sites of intermediate pollution (yellow and red curves) exhibited intermediate levels of CYP1A induction. The de-sensitization of the AHR pathway in fish from polluted sites is characterized by a lower total expression, and therefore activity, of CYP1A, as well as activation at higher concentrations of PCB126. Variation among populations in maximal CYP1A induction and the dose that causes significant up-regulation is scaled by level of pollution.
Fig. S4.

Genome-wide differentiation ($F_{ST}$) between all pairs of populations (colored cells), including nucleotide diversity for each population ($\pi$; diagonal). Genome-wide differentiation between populations tends to be low (<0.03), whereas nucleotide diversity within populations is high. Nucleotide diversity tends to be lower in polluted site populations (red and black population labels) compared to clean site populations (blue population labels).
Fig. S5.
Genome wide nucleotide diversity ($\pi$; x and y axes) between pairs of polluted (red and black labels) and clean site (blue labels) populations. Genome-wide nucleotide diversity tends to be reduced in pollution adapted populations (skew to below 1:1 dotted line). Loci most differentiated ($F_{ST}$) from sensitive populations (red dots) tend to coincide with regions of low $\pi$. 

in pollution adapted populations compared to sensitive populations. $F_{ST}$ and $\pi$ are calculated in 5kb sliding windows (1kb slide).

**Fig. S6.**

Genome-wide signatures of selection for each polluted site population (consecutive panels) compared to both reference clean site populations. Y-axis is the z-score statistic that combines two signals of natural selection: high divergence ($F_{ST}$) and low nucleotide diversity ($\pi$) in the polluted site population relative to clean site populations, which are each calculated in 20kb sliding windows (1kb slide). Outliers (1% highest z-score) are colored for each population. Those colored purple are outliers that are shared across all polluted site populations.
**Fig. S7.**
Differentiation between each intermediate population (IH1, IH2; consecutive panels) and both resistant and sensitive populations (R2 and S1; population branch statistic) indicate no strong signatures of selection. Outliers (highlighted in red) are the 1% most highly differentiated regions (5kb windows), and indicate no consistent or highly differentiated regions that are unique to intermediate populations.
Fig. S8.
Patterns of differentiation surrounding key genes in the aryl hydrocarbon receptor (AHR) signaling pathway reveal signatures of selection in polluted site populations. Highlighted genes include tandem paralogs of the aryl hydrocarbon receptor (AHR), a xenobiotic-activated transcription factor. A) Population divergence measured by population branch statistic between each adapted population and both sensitive reference populations. B) Neighbor-joining tree groups individuals with deletion haplotype together (top), heterozygotes for AHR deletion in the middle, and wild type haplotypes together (bottom).
Fig. S9.
Patterns of differentiation surrounding key genes in the aryl hydrocarbon receptor signaling pathway reveal signatures of selection in polluted site populations. Highlighted gene is the aryl hydrocarbon receptor nuclear translocator-like 2a (ARNT-L2a), which is a nuclear dimerization partner of aryl hydrocarbon receptor (AHR), required for activation of the xenobiotic response pathway. A) Population divergence measured by population branch statistic between each adapted population and both sensitive reference populations. The ARNT gene is located within or near the region of high differentiation. Neighbor-joining tree (B) and MDS plot (C) reveal clustering of haplotypes from sensitive and resistant populations.
Figure S10. Genetic differentiation encompassing a key AHR binding partner, AHR nuclear translocator-like 2a (ARNT-L2a) in the aryl hydrocarbon receptor (AHR) signaling pathway in individuals from resistant populations reveal higher similarity to *F. heteroclitus* than reference *F. grandis*. A neighbor joining tree (A) and MDS plot (B) reveal clustering of haplotypes suggesting an introgression pattern, later confirmed in population genetic models.
Fig. S11.
Patterns of differentiation surrounding key genes in the aryl hydrocarbon receptor (AHR) signaling pathway reveal signatures of selection in polluted site populations. Highlighted gene is the aryl hydrocarbon receptor nuclear translocator 1c (ARNT), which is a nuclear dimerization partner of AHR, required for initiation of the xenobiotic response pathway. A) Population divergence measured by population branch statistic between each adapted population and both sensitive reference populations. The ARNT gene is located within the region of high differentiation. Neighbor-joining tree (B) and MDS plot (C) reveal clustering of haplotypes from sensitive and resistant populations.
Fig. S12.

Patterns of differentiation surrounding key genes in the aryl hydrocarbon receptor (AHR) signaling pathway reveal signatures of selection in polluted site populations. Highlighted gene is the aryl hydrocarbon receptor interacting protein (AIP, also known as XAP2) which regulates AHR signaling. A) Population divergence measured by population branch statistic between each adapted population and both sensitive reference populations. The AIP gene is located within the region of high differentiation. Neighbor-joining tree (B) and MDS plot (C) reveal clustering of haplotypes from sensitive and resistant populations.
Fig. S13.
A deletion, spanning the last exon of AHR2a and first 6 exons of AHR1a, is present at high frequency in resistant populations (R1, R2) and at low frequency in intermediate populations (IH1, IH2). Each horizontal line represents a single individual and the intensity of green is amount of raw sequence coverage over the represented region. Dark coloring indicates regions where few or no reads mapped to the reference genome from that individual. Though deletions are found in the same genomic region in both *F. heteroclitus* (resistant population Elizabeth River, top panel) and in *F. grandis* polluted populations, the deletion break points appear to be different between *F. heteroclitus* and *F. grandis*.
Fig. S14.
The deletion that encompasses portions of genes AHR1a and AHR2a on chromosome 1 is apparent from plots of smoothed sequence coverage from individuals that represent the three genotypic variants (homozygous wildtype, homozygous deletion, heterozygote). The blue line is from an individual from a reference sensitive population (S2) that does not carry the deletion (homozygous wildtype), where sequence coverage is relatively continuous across the region. The black line is from an individual from a resistant population (R1) that is homozygous for the deletion, where sequence coverage drops very low (effectively zero). The red line is from an individual from an intermediate population (IH1) that is heterozygous for the deletion, where reduced sequence coverage is intermediate. Purple dashed lines bracket the most continuous portion of the deletion, which is preceded by a region of likely high mis-mapping, which is common for deletion breakpoints.
Models tested on three pairs of sensitive (black) and resistant (red) populations (one from *F. grandis* and two from *F. heteroclitus* representing Northern and Southern clade). We allowed for selection in *h*\textsubscript{T4} and *g*\textsubscript{R2} for the analysis of the AHR deletion region (chromosome 1) and selection only in *g*\textsubscript{R2} for the ARNT region (chromosome 10). A) Incomplete lineage sorting model: selected variant arose (orange star) in ancestor of *F. heteroclitus* and *F. grandis*, and stood until the onset of selection (blue triangles). B) Standing variant source model: selected variant arose in *h*\textsubscript{T4} (could be along ancestral branch between *h*\textsubscript{S4} and *h*\textsubscript{T4} in this cartoon as well). Haplotype was introduced into *g*\textsubscript{R2} and may have stood for some time in both populations (free parameter) prior to selection. C) Concurrent sweeps model: deletion haplotype arose in *h*\textsubscript{T4} and migrated into *g*\textsubscript{R2} during the sweep in *h*\textsubscript{T4} and began to sweep immediately. D) Staggered sweeps model: Selected variant arose in *h*\textsubscript{T4} and was immediately selected. After fixation, the haplotype migrated into *g*\textsubscript{R2}, where it may have stood for some time (free parameter) prior to the onset of selection in *g*\textsubscript{R2}.
Fig. S16.
Likelihood results from AHR deletion region (chromosome 1). Composite log-likelihood ratio of given model relative to the neutral model of no selection as a function of the selection coefficient (s). The standing variant source and staggered sweeps models have the highest maximum likelihood. For the standing variant source model, the maximum-composite-likelihood estimate (MCLE) is at $s = 0.82$. For the staggered sweeps model, the MCLE is at $s = 0.8$. 
Fig. S17.
Likelihood results from AHR deletion region (chromosome 1). Composite log-likelihood ratio (CLR) of given model relative to the neutral model of no selection as a function of various parameters. A) CLR as function of selection coefficient zoomed in for the top two models. B) CLR as a function of time between introgression and selection for models that include this parameter. The standing variant source model has a maximum-composite-likelihood estimate (MCLE) of 18 generations while the staggered sweeps model has a MCLE of 16 generations. C) CLR as a function of the duration of the standing time in the incomplete lineage sorting model. This time represents the minimum age of the standing variant and specifically is the time (in generations) between the splitting of $h_{T4}$ and $g_{R2}$ and the onset of selection. The MCLE of this time is 10,000 generations but there is little change in likelihood as the parameter increases past this value. D) CLR as a function of the frequency of variant prior to selection. In the top two introgression models, this represents the frequency of the allele upon introgression. For the incomplete lineage sorting model, this parameter represents the frequency at which the variant is standing. We find the MCLE for the staggered sweeps model is $1/2Ne=6.8 \times 10^{-7}$, where $Ne$ is the effective population size estimate for $F.\ grandis$. The MCLE for the standing variant source model is $2 \times 10^{-6}$ and for the incomplete lineage sorting model is $10^{-6}$. 
Fig. S18.
Likelihood results from ARNT region (chromosome 10). Composite log-likelihood ratio of given model relative to the neutral model of no selection as a function of the position of the selected site. All models have their maximum-composite-likelihood estimate (MCLE) at 26,019,105 bp, or 2 Mb downstream of the ARNT region (~24 Mbp).
Fig. S19.

Likelihood results from ARNT region (chromosome 10). Composite log-likelihood ratio (CLR) of given model relative to the neutral model of no selection as a function of various parameters. A) CLR as function of selection coefficient for all models. The maximum-composite-likelihood estimate (MCLE) for the standing variant source and staggered sweeps model is $s=0.55$, for the incomplete lineage sorting model is $s=0.8$, and for the concurrent sweeps model is $s=0.45$. B) CLR as a function of time between introgression and selection for models that include this parameter. The standing variant source model has an MCLE of 10 generations and the staggered sweeps model has an MCLE of 6 generations. C) CLR as a function of the duration of the standing time in the incomplete lineage sorting model. This time represents the minimum age of the standing variant and specifically is the time (in generations) between the splitting of $h_{T4}$ and $g_{R2}$ and the onset of selection. The MCLE of this time is 9,000 generations. D) CLR as a function of the frequency of variant prior to selection. In the top two introgression models, this represents the frequency of the allele upon introgression. The standing variant source model has an MCLE of $5\times10^{-6}$ and the staggered sweeps model has an MCLE of $10^{-6}$. For the incomplete lineage sorting model, this parameter represents the frequency at which the variant is standing. This model's MCLE is $8.5\times10^{-7}$. 
Fig. S20.
Modified Gmin statistic reveals regions of introgression present among polluted sites. A) Histogram of window scores with selected outlier threshold, resulting in 0.6% of windows being labeled as introgressed. B) Genome-wide distribution of modified Gmin statistic (100kb windows) for 5 resistant populations (top panel) and 2 sensitive populations (bottom panel). Large regions of introgressed *F. heteroclitus* ancestry are restricted to resistant populations located in the Houston Ship Channel.
Fig. S21.
The distribution of introgression blocks. A) Length distribution of introgression blocks $\geq 400$kb across all *F. grandis* individuals. Most introgression blocks were $< 3$mb, with few larger blocks found in few individuals. B) Number of introgression blocks $\geq 100$kb per individual, by population. The largest number of introgression blocks are found in R3, which suggests that, among the sampled populations, R3 may closest to the location of the initial hybridization event between *F. grandis* and *F. heteroclitus* that introduced beneficial alleles.
**Table S1.**
Field site locations and sampling

<table>
<thead>
<tr>
<th>Site name</th>
<th>Sequenced fish (n)</th>
<th>Coordinate</th>
<th>Sequencing coverage per individual (x)</th>
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<td></td>
<td>Latitude</td>
<td>Longitude</td>
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Table S2.
Parameters that coalescent-based models were tested on in the AHR deletion region.

<table>
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<tr>
<th>Parameter</th>
<th>Model(s) that include it</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strength of selection (s)</td>
<td>All</td>
<td>0.001, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.08, 0.1, 0.12, 0.14, 0.16, 0.18, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.82, 0.84, 0.86, 0.88, 0.9, 0.92, 0.94, 0.96, 0.98, 1</td>
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<tr>
<td>Standing time between migration and selection ($t_m$)</td>
<td>Standing variant source, Staggered sweeps</td>
<td>0, 5, 10, 15, 16, 18, 20, 22, 24, 26, 28, 30, 35, 40, 60, 80, 100, 200, 500, 1000, 5000, $10^7$</td>
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<tr>
<td>Minimum age of standing variant / Standing time between species split and selection (t)</td>
<td>Incomplete lineage sorting</td>
<td>3000, 4000, 5000, 7500, 9000, 10000, 25000, 50000, $10^5$, $10^6$, $10^7$, $10^8$</td>
</tr>
<tr>
<td>Frequency of variant before selection / fraction of migrants in R2 (g)</td>
<td>Standing variant source, Staggered sweeps, Incomplete lineage sorting</td>
<td>$6.878 \times 10^{-7}$ ($=1/2N_e$), $8.5 \times 10^{-7}$, $10^{-6}$, $2 \times 10^{-6}$, $5 \times 10^{-6}$, $8 \times 10^{-5}$, $10^{-5}$, $10^{-4}$, $10^{-3}$, $10^{-2}$</td>
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<tr>
<td>Migration rate per generation (m)</td>
<td>Concurrent sweeps</td>
<td>$10^{-5}$, $10^{-4}$, $5 \times 10^{-4}$, 0.001, 0.005, 0.01, 0.1, 0.3</td>
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Table S3.
Parameters that coalescent-based models were tested on in the ARNT region.

<table>
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<tr>
<th>Parameter</th>
<th>Model(s) that include it</th>
<th>Values</th>
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<td>Position of selected site</td>
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<td>18000003, 18572796, 19145589, 19718382, 20291175, 20863968, 21436761, 22009554, 22582347, 23155140, 23727933, 24300726, 24873519, 25446312, 26019105, 26591897, 26735096, 26878294, 27021492, 27164690, 27355621, 27546552, 27737483, 28310276, 28883069, 29455862, 30028655, 30601448, 31174241, 31747034, 32319827, 32892620, 33465413, 34038206, 34610999</td>
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<tr>
<td>Strength of selection (s)</td>
<td>All</td>
<td>0.001, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.9, 1</td>
</tr>
</tbody>
</table>
References and Notes


15. See supplementary materials.


