ORIGINAL ARTICLE

Independently evolved pollution resistance in four killifish populations is largely explained by few variants of large effect

Jeffrey T. Miller[1] | Bryan W. Clark[2] | Noah M. Reid[3] | Sibel I. Karchner[4] |
Jennifer L. Roach[1] | Mark E. Hahn[4] | Diane Nacci[2] | Andrew Whitehead[1]

[1]Department of Environmental Toxicology, Center for Population Biology, Coastal and Marine Sciences Institute, University of California, Davis, Davis, California, USA

[2]Office of Research and Development, Center for Environmental Measurement and Modeling, Atlantic Coastal Environmental Sciences Division, US Environmental Protection Agency, Narragansett, Rhode Island, USA

[3]Department of Molecular & Cell Biology, University of Connecticut, Storrs, Connecticut, USA

[4]Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, USA

Correspondence
Andrew Whitehead, Department of Environmental Toxicology, Center for Population Biology, Coastal and Marine Sciences Institute, University of California, Davis, Davis, CA, USA.
Email: awhitehead@ucdavis.edu

Present address
Jeffrey T. Miller, Molecular, Cellular, and Biomedical Sciences, University of New Hampshire, Durham, New Hampshire, USA

Abstract

The genetic architecture of phenotypic traits can affect the mode and tempo of trait evolution. Human-altered environments can impose strong natural selection, where successful evolutionary adaptation requires swift and large phenotypic shifts. In these scenarios, theory predicts that adaptation is due to a few adaptive variants of large effect, but empirical studies that have revealed the genetic architecture of rapidly evolved phenotypes are rare, especially for populations inhabiting polluted environments. *Fundulus* killifish have repeatedly evolved adaptive resistance to extreme pollution in urban estuaries. Prior studies, including genome scans for signatures of natural selection, have revealed some of the genes and pathways important for evolved pollution resistance, and provide context for the genotype–phenotype association studies reported here. We created multiple quantitative trait locus (QTL) mapping families using progenitors from four different resistant populations, and using RAD-seq genetically mapped variation in sensitivity (developmental perturbations) following embryonic exposure to a model toxicant PCB-126. We found that one to two large-effect QTL loci accounted for resistance to PCB-mediated developmental toxicity. QTLs harbored candidate genes that govern the regulation of aryl hydrocarbon receptor (AHR) signaling. One QTL locus was shared across all populations and another was shared across three populations. One QTL locus showed strong signatures of recent natural selection in the corresponding wild population but another QTL locus did not. Some candidate genes for PCB resistance inferred from genome scans in wild populations were identified as QTL, but some key candidate genes were not. We conclude that rapidly evolved resistance to the developmental defects normally caused by PCB-126 is governed by few genes of large effect. However, other aspects of resistance beyond developmental phenotypes may be governed by additional loci, such that comprehensive resistance to PCB-126, and to the mixtures of chemicals that distinguish urban estuaries more broadly, may be more genetically complex.

1 | INTRODUCTION

Human activities can cause rapid and dramatic environmental changes that perturb the health and performance of many wild species. Examples of contemporary environmental change include habitat loss, introduction of invasive species, harvest pressure, climate change, and pollution (Vitousek et al., 1997). These perturbations can cause population declines and extinctions. Adaptive evolutionary change is one mechanism whereby populations may respond and escape local extinction (Hendry et al., 2017; Hoffmann & Sgro, 2011; Palumbi, 2001; Sih et al., 2011; Smith & Bernatchez, 2008). However, specific features of the environment and of species may influence the pace and extent of adaptive phenotypic evolution, and it is important to understand how these features interact with natural selection to predict the likelihood of species' persistence in the Anthropocene. Adaptation is likely accelerated when environmental change is quick and severe, but reduced when the complexity (dimensionality) of change is high (Bay et al., 2017; Lindsey et al., 2013; Lourenço et al., 2013; Tilman & Lehman, 2001; Whitehead et al., 2017). Adaptation is predicted to be faster in species with larger population sizes, shorter generation times, higher standing genetic variation, insufficient plasticity, and limited gene flow (Barrett & Schluter, 2008; Bell, 2013; Bell & Gonzalez, 2009; Bergland et al., 2014; Kopp & Matuszewski, 2014; Kreiner et al., 2018; Orr & Unckless, 2014). The genetic architecture of relevant traits also has bearing on the pace of adaptive change.

The genetic architecture of rapidly evolving traits has emerged as a key area of study in eco-evolutionary dynamics. Within this context we consider genetic architecture to include the number of loci, their effect sizes, their interactions, and the ways in which they fit into molecular pathways. It is generally understood that variation in most phenotypic traits is controlled by many loci, most with small effects (Barton & Keightley, 2002), and that local adaptation is therefore often polygenic, requiring shifts in allele frequencies at many loci (Pritchard & Di Rienzo, 2010). However, the architecture of adaptation in response to anthropogenic change may be atypical. This is because the pace of environmental change is so rapid (Vitousek et al., 1997), and new phenotypic optima may be distant from historical averages (Hendry et al., 2008). Furthermore, natural selection can be very strong, such that successful adaptation requires large, rapid phenotypic shifts, potentially favoring fewer loci with much larger effects (Gomulkiewicz et al., 2010; Kopp & Matuszewski, 2014). Indeed, population genetic theory is pivoting to confront these new scientific challenges (Messer et al., 2016), and simulation studies are examining how the genetic architecture of traits affects the pace of adaptation in changing environments (Jain & Stephan, 2017; Stetter et al., 2018). Our goal in this work is to understand the genetic architecture that underlies an example

of this rapid adaptation, the evolved resistance to the toxic effects of extreme environmental pollution in the Atlantic killifish (*Fundulus heteroclitus*), and to exploit this system as a model to better understand how adaptation to complex human-altered environments may proceed.

Fundulus heteroclitus is a small nonmigratory fish inhabiting estuarine habitats of eastern coastal North America. Many estuaries in this region have been heavily impacted by urbanization, which in the 20th century included the release of large quantities of a diversity of highly toxic, persistent, bioaccumulative organic pollutants, including chlorinated dibenzo-*p*-dioxins ("dioxins"), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). These pollutants are highly toxic to most vertebrates, even at very low concentrations, where the most sensitive responses to exposure include defects in cardiovascular system development (Kopf & Walker, 2009). Nevertheless, populations of *F. heteroclitus* and its sister species *F. grandis* have repeatedly evolved resistance to them, even in some of the most intensely polluted sites, where resistance of resident populations scales with local pollution levels (Nacci et al., 2010; Oziolor et al., 2019). This toxicant resistance evolved rapidly, insofar as pollution spiked in the latter half of the 20th century (Pesch et al., 2001), exceeding normally lethal levels within ~2 decades, with *F. heteroclitus* resistance first documented by the early 1990s (e.g., Prince & Cooper, 1995). Observed resistance is genetically heritable (Nacci et al., 2002, 2010; Ownby et al., 2002); however, the relative contributions of environment-induced defenses and trans-generational plasticity have yet to be quantified.

Prior work in killifish has shed some light on the genetic underpinnings of evolved toxicant resistance. Evidence has come from comparative transcriptomics (Oleksiak et al., 2011; Whitehead et al., 2010, 2012), genome scans (Oziolor et al., 2019; Reid et al., 2016; Williams & Oleksiak, 2011), and association studies (Nacci et al., 2016). Transcriptomic responses after exposure to PCB-126 (3,3′,4,4′,5-pentachlorobiphenyl; a model toxicant) showed that global desensitization of the aryl hydrocarbon receptor (AHR) signaling pathway (Figure S1) distinguished all resistant from all sensitive populations. This is consistent with other findings from killifish and other species, where AHR signaling mediates toxicity of ubiquitous pollutants such as dioxins and some PCBs and PAHs. Many dioxins, PCBs and PAHs interact directly with the AHR, and the subsequently activated molecular signaling cascade is largely responsible for the toxicity observed in vertebrates, especially fishes during early (embryo-larval) development (Clark et al., 2010; King-Heiden et al., 2012; Shankar et al., 2020). In fact, activation of AHR signaling defines a class of highly toxic, persistent, and bioaccumulative pollutants known as 'dioxin-like compounds' (DLCs). DLCs typically include dioxins and coplanar (non-*ortho*-substituted) PCBs. Though PAHs share some mechanisms of toxicity (such as AHR activation),

as a class of chemicals they are typically considered distinct from DLCs.

Association and genome scan studies have also further implicated AHR pathway elements. A microsatellite-based QTL study found that resistance in the New Bedford Harbor (MA) population was associated with a small number of genomic regions, most prominently including a region containing a key AHR signaling gene, aryl hydrocarbon receptor interaction protein (AIP) (Nacci et al., 2016). Genome-wide scans for signatures of natural selection, which included multiple resistant and sensitive populations from both *F. heteroclitus* and *F. grandis*, found loci that are key parts of the AHR signaling pathway to be major targets of selection in populations from polluted sites. Three loci harbored top-ranked signatures of selection in all four resistant *F. heteroclitus* populations studied—a strong signal of convergent evolution (Reid et al., 2016): One locus contained a tandem pair of AHR genes (there are two tandem AHR pairs in the *F. heteroclitus* genome); a second locus contained AIP, a key binding partner of AHR; and a third locus contained Cyp1A, a major downstream regulatory target of AHR. A number of other AHR signaling pathway genes were found to be targets of selection in just three or fewer resistant populations. In *F. grandis*, the same tandem AHR pair was found to be under selection at polluted sites, where the selected variant had introgressed from *F. heteroclitus* (Oziolor et al., 2019). Modeling indicated that selection coefficients for the AIP locus in *F. heteroclitus* and the AHR locus in *F. grandis* ranged from 0.3 to 0.8. This means that selected variants could rise to fixation from very low frequency on a time scale of tens of generations, which is consistent with the onset of pollution in the mid-20th century.

While genome scan studies have been successful in unveiling some critical aspects of the genetics of resistance (the AHR pathway is important), their correlational nature leaves many important questions unanswered. Genome scans can reveal suites of loci that may contribute to fitness (e.g., by revealing signatures of natural selection), but they do not provide information that directly connects genotype to phenotype. This means that genome scans cannot reveal whether the selected loci all contribute to a single adaptive phenotype or many, nor can they determine the effect size of a locus, if they are redundant, or if they interact. It is likely that multiple traits are simultaneously under selection between different environments (Langerhans, 2018). For example, in complex environments like urban estuaries (Rivkin et al., 2019), loci associated with adaptation to urban pathogens may tend to accompany loci associated with urban toxicant resistance. As well, under these intense selective conditions, it may be likely that multiple redundant alleles are favored by selection, resulting in soft selective sweeps (Messer & Petrov, 2013; for an example, see Kreiner et al., 2021). To extend our understanding of the genetic architecture of rapidly evolved pollution resistance in this system requires experimental manipulation.

In the study reported here, we used an experimental approach to further reveal the genetic architecture of adaptation to pollution in *F. heteroclitus*. We build upon a prior QTL study (Nacci et al., 2016) by sampling the genome at higher density (RAD-seq SNPs vs. microsatellites), using a highly contiguous genome assembly as reference (Miller et al., 2019; Reid et al., 2017), and by expanding the number of populations examined (from 1 to 4). We created one QTL mapping family for each of four DLC-resistant killifish populations by crossing one killifish from each of four resistant populations with a killifish from a common sensitive population, followed by full-sibling matings (F2 intercross). Embryos from mapping families were exposed to a model toxicant (PCB-126) at a dose known to distinguish sensitive from resistant individuals, and each embryo was scored for developmental abnormalities indicative of their sensitivity to toxicity. We then genotyped ~48 of the most resistant and ~48 of the most sensitive individuals from each family. Our QTL analysis of developmental abnormalities revealed a small set of loci that accounted for a large portion of phenotypic variation in each population. Some QTL were shared among populations, whereas others were not. Most but not all QTL were in regions that also showed strong signatures of selection in wild-resistant fish, and many regions that had very strong signatures selection in resistant populations were not found to be associated with resistance in this experiment. These results highlight the power of integrating quantitative and population genetic approaches to understand the genetic basis of adaptation and represent a large advance in our understanding of the genetic architecture underlying rapid parallel adaptation to extreme pollution in urban killifish.

2 | METHODS

2.1 | Breeding design

We created mapping families from the same source populations as those described in Reid et al. (2016), which have previously been characterized for sensitivities to PCB-126 (Nacci et al., 2010). DLC-resistant source populations originated from three sites from the northern portion of the species' range: New Bedford Harbor, MA, USA (NBH), Bridgeport Harbor, CT, USA (BRP), Newark, NJ, USA (NEW), and one site from the southern portion of their range: the Atlantic Wood site of the Elizabeth River, Virginia, USA (ELR) (Figure 1). The DLC-sensitive source population originated from Block Island, RI, USA (BLI). Sediment DLC and/or PAH concentrations are orders of magnitude higher in the four polluted sites compared to the clean site, and magnitude of resistance correlates with the magnitude of pollution (Nacci et al., 2010) (Table S1).

We created QTL mapping families using wild killifish that were maintained in the laboratory (Narragansett EPA), where individuals from BLI (sensitive population) were mated with individuals from each resistant population (NBH, BRP, NEW, or ELR) to produce F1 hybrid families. F1 hybrid individuals were subsequently paired to produce the F2 mapping families. The specific identities of mapping families were NBH 1105, BRP 812, NEW 1413, and ELR 1433. Over the course of this study, we tried slightly differing breeding strategies to maximize the number of progeny with divergent phenotypes (Figure S2; also, see Figure S3 for the distributions of phenotypes
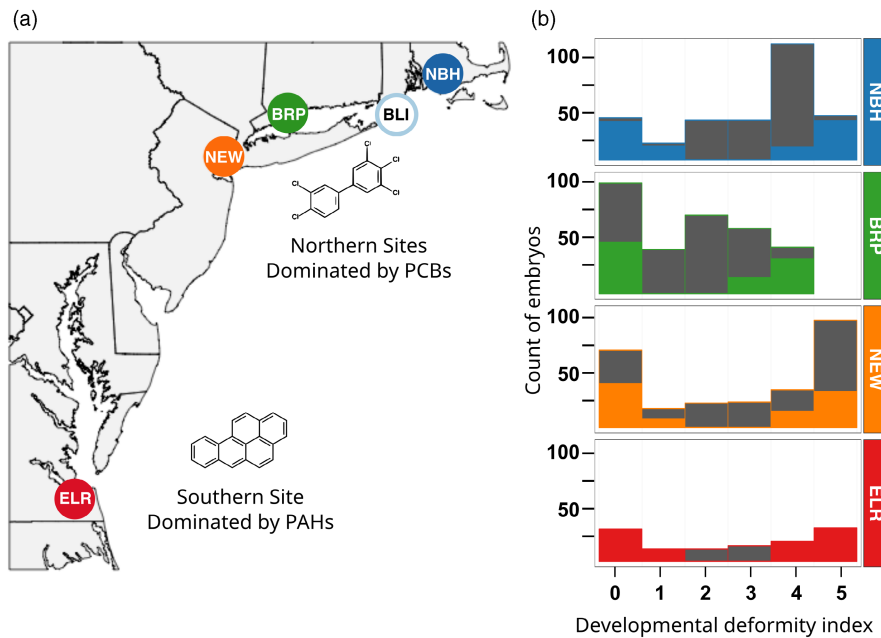
FIGURE 1 (a) Locations of source populations for founders of mapping families. Sites are characterized by complex mixtures of pollutants but dominated by either polychlorinated biphenyls (PCBs) or dioxins in the three Northern sites (New Bedford Harbor, MA [NBH], Bridgeport Harbor, CT [BRP], and Newark, NJ [NEW]), or polycyclic aromatic hydrocarbons (PAHs) in the Southern site (Elizabeth River, VA [ELR]). All polluted site populations (filled circles) have been previously shown to be resistant to PCB-126. Open blue circle is the clean reference site (Block Island, RI [BLI]) and source for one of the progenitors for each of the mapping families. (b) Bar graphs show the phenotypic distribution (developmental deformity index, *x*-axis) of embryos from each of the F2 intercross mapping families at 10 days after an exposure to a discriminating dose, (200 ng PCB-126/L in sea water). Full bars show the total count of embryos collected and assigned a deformity score, which ranged from 0 (no deformities) to 5 (most severe deformities) (the BRP families were scored on a scale where a score of 4 was maximum). Colored portion of each bar indicates the subset of embryos that were selected for genotyping.

per family). The NBH and ELR F2 hybrid mapping families descended from mating one wild-caught female killifish from the resistant population (NBH or ELR) and one wild-caught male killifish from BLI. The NEW mapping family descended from mating one laboratory-bred female killifish from BLI and one laboratory-bred male killifish from NEW, while the BRP-mapping family descended from two lines, each produced by mating one wild-caught BLI female and one wild-caught BRP male. Thus, resistance was inherited through females in the NBH and ELR families, and through males in the NEW and BRP families (Figure S2). Previous studies have demonstrated that inheritance of resistance is not sex linked (Nacci et al., 1999). Furthermore, there was a single tolerant progenitor for NBH, ELR, and NEW families (sib: sib matings), while the BRP family was derived from two tolerant progenitors (nonsib mating) (Figure S2). To characterize tolerance, we challenged embryos from each of the four F2 mapping families with a discriminating dose of the model DLC (PCB-126), scored individuals for developmental abnormalities associated with DLC toxicity, and then archived them for later genotyping (described below).

2.2 | Phenotyping

We exposed embryos from all families to acetone-solubilized PCB-126 dissolved in sea water at a concentration (200 ng/L; ~0.6 nM)

that discriminates sensitive from resistant killifish. PCB-126 was selected because it is a highly toxic PCB congener (Doering et al., 2023) that is abundant at polluted sites (Nacci et al., 2010), and exposures to PCB-126 reveal large differences in sensitivity between populations from clean and polluted sites (e.g., Nacci et al., 2010). The exposure lasted from 1 day post-fertilization (dpf) to 7 dpf, followed by transfer to clean seawater. At 10 dpf, we observed the embryos microscopically and scored them for the presence or absence of a suite of developmental deformities that are characteristic of DLC-mediated toxicity in fish embryos, where the appearance of multiple abnormalities is tightly correlated with reduced probability of larval survival and is, therefore, a good proxy for DLC sensitivity. Each abnormality is given a score of 1 (present) or 0 (absent), and each embryo was scored as the sum of the observed abnormalities, from 0 (no deformities) to 5 (most deformities), which represents the full range of sensitivity displayed by originating killifish populations (representative images of normal and severely affected individuals, Figure S4). Some aspects of phenotype scoring are subjective as to the presence or severity; phenotyping was, therefore, conducted with multiple experienced observers who had each trained together to minimize observer bias. The abnormalities commonly observed and the method of scoring each were as follows. *Hemorrhaging*: Most commonly hemorrhaging was found in the head and/or tail, occasionally in other areas such as the yolk sac or pericardial space; each occurrence of a hemorrhage in a different body area was given a

score of 1 (i.e., a hemorrhage in both the tail and the head would result in 2 points). *Reduced size*: At 10 dpf, a normally developed embryo fills the chorion such that the tail has curled around in front of the fish and overlaps the eye, possibly even extending further; body size was scored as reduced and given a score of 1 if the caudal fin did not reach to overlap the fish's eye. *Heart abnormality*: At 10 dpf, the heart of normally developing embryos sits tightly in the space between the underside of the embryo's jaw and the yolk sac; they exhibit a fully looped heart with an imaginary line between the centers of the ventricle and atrium sitting roughly parallel to a line between the centers of the embryo's eyes. A hallmark of fish embryonic DLC toxicity is the loss of this structure and occurrence of what is commonly known as a "stringy heart". Embryos were scored for the presence of a heart abnormality if they exhibited a loss of obvious differentiation of the two heart chambers, stretching of the heart such that the chambers appear vertically stacked (in a line with the inflow and outflow tracts as opposed to side by side), or in severe cases, the full loss of heart structure such that all that remained was a stringy heart tube. *Pericardial edema*: As described previously, the heart of a normally developing 10 dpf embryo sits tightly between the underside of the fish's jaw and the yolk sac and the pericardial space is small (sometimes it can be observed bulging out by the sides of the embryo's head), with the beating of the heart typically depressing the yolk sac slightly. Pericardial edema has historically been considered a sensitive hallmark of fish embryonic DLC toxicity, so it contributes to the deformity score as either 1 point for minor presence or 2 for severe edema. Embryos scored as a 1 for edema exhibited a larger space around the heart such that the embryo's head is lifted off of the yolk sac due to the visible enlargement of the pericardial space and the beating of the heart no longer depresses the yolk sac. In an observation of severe edema, scored as a 2, the pericardial space is greatly enlarged and the embryo's head is lifted far from the yolk sac. At this level of severity, the embryo can be turned on its side and the observer can typically look fully through the pericardial space and out the other side of the embryo with no other structures observed due to the amount of fluid in the pericardium. Finally, the phenotyping also included space for an "Other" observation which also resulted in a score of 1 and allowed for rare occurrences to be included in scoring (e.g., severe spinal curvature, severe developmental delay, edema of the yolk sac). Although the number of abnormalities described above could result in an individual with a higher score, the abnormality score was capped at 5 (4 for BRP); in our experience, any individual scored 4 or higher does not survive past 2 weeks post-hatch. After phenotyping, we flash froze embryos and stored them at −80°C until DNA extraction. Phenotype scores for all individuals selected for genotyping are in Appendix S1.

2.3 | Genotyping

We used balanced selective genotyping (or two-tail genotyping) (Lander & Botstein, 1989; Lebowitz et al., 1987), where we selected ~48 individuals from each of the most sensitive and most resistant ends of the phenotypic distribution for each family (we did not genotype all individuals from each family because of limited resources). We used RAD-seq (Miller et al., 2007) for genotyping these individuals from F2 intercross families and mapping family founders. DNA was extracted from frozen embryos with a proteinase-K digestion and the Qiagen DNAeasy kit. Sequencing libraries were prepared for RAD-seq by ligating individual barcodes and NEBnext Illumina oligonucleotides to genomic DNA at *SbfI* cut sites. Four lanes of paired-end (PE-100) sequence data (Illumina HiSeq 2500) were collected (one lane per plate of 96 samples) to enable genotyping of offspring and founders at RAD sites. After de-multiplexing by barcode and evaluating the quality of each sample with FASTQC, sequence reads were aligned to the linkage-mapped *F. heteroclitus* assembly (Miller et al., 2019; EBI BioStudies accession S-BSST163; this map orders scaffolds from the *F. heteroclitus* reference genome assembly Fundulus_heteroclitus-3.0.2, NCBI BioProject PRJNA177717). We assigned read group information and aligned reads with BWA-MEM using default parameters, marked duplicates with SAMBLASTER, sorted reads with SAMTOOLS, and flagged improperly paired reads with BAMTOOLS (Barnett et al., 2011; Faust & Hall, 2014). We used FreeBayes to call genotypes on all populations simultaneously (Garrison & Marth, 2012). We clustered sample genotypes with metric MDS for QAQC and to cluster genotypes at chromosome 5 to assign sex to the immature embryos (Figure S5) so that sex could be included as a covariate in the QTL search and model fit. Families were each split into separate datasets and filtered for genotypes from repetitive sequence alignments and family-specific invariant sites with PLINK 1.9 (Chang et al., 2015).

Genotypes for each mapping family were formatted to be loaded as independent crosses in R/qtl (Broman et al., 2003). In R/qtl, offspring genotypes were filtered based on the number of genotypes per locus (>12.5% missing data) and the level of segregation distortion ($p < 0.001$) from a 1:2:1 ratio following R/qtl recommendations (Broman & Sen, 2009). Outcrosses in R/qtl are assumed to be from inbred founders, so we limited our analysis to markers that were homozygous for alternate alleles in the founders (double heterozygotes in F1 parents). Our segregation distortion filter was conservative because selective genotyping is expected to lead to some distortion, particularly at QTL of large effect (Xu, 2008). After filtration, markers that were genotyped in the founders and F2 embryos were assigned to a consistent allele (A or B) within and among linkage groups. Any markers that we could not confirm with founder genotypes were filtered out so that those remaining were clearly linked with other markers in the linkage group (formLinkageGroups in R/qtl with a recombination frequency <0.1 and LOD score of 10). We initially anchored markers to their physical position along chromosomes, but then used recombination frequency to reorder (assign a different order than the initial meiotic map) markers and estimated mapping distance in this order with the Kosambi mapping algorithm (Kosambi, 1943). Differences from the initial meiotic map may be the result of genome structural variation within a mapping population or read mapping and genome assembly errors. We then checked whether the final filtered marker set sufficiently covered

the reference genome physical map and provided a sensible mapping order by visualizing the pairwise recombination frequency and LOD linkage matrix for each chromosome, as well as the relationship between genetic and physical distances along chromosomes (Figure S6). After filtering, the number of linked markers were: 37,304 for the NBH family; 16,725 for the BRP family; 27,119 for the NEW family; and 57,489 for the ELR family (Table S2).

In addition to genome-wide genotyping with RAD-seq, we also sought to confirm genotypes at two discrete loci: at the loci that encode aryl hydrocarbon receptor 2a/1a (AHR2a/1a) on chromosome 1 and aryl hydrocarbon receptor-interacting protein (AIP) on chromosome 2. These loci encode proteins that are core components of the signaling pathway that is adaptively de-sensitized in resistant fish (Whitehead et al., 2012), were implicated as a QTL in the NBH population (Nacci et al., 2016), and show among the strongest signatures of natural selection in resistant populations (Reid et al., 2016). Since the adaptive haplotypes are not fixed in resistant populations (Reid et al., 2016), we sought to confirm whether adaptive haplotypes were segregating in our mapping families. We genotyped ELR offspring with sensitive (scores 4 and 5) and resistant (scores 0 and 1) phenotypes for a deletion that spans the last exon of AHR2a and the first six exons of neighboring AHR1a (99.7 kb deletion; Figure S7) which exists at 81% frequency in wild ELR fish but is absent in sensitive fish from a nearby reference population (methods described in Reid et al., 2016). The genotypes at the AHR2a/1a locus (presence/absence of a deletion; Table S3) were added to the QTL analysis with RAD-Tag markers to test for linkage with other chromosomal markers and for an association with the resistant phenotype. Two AIP nonsynonymous SNPs at amino acid positions 224 and 252 were genotyped in a subset of mapping family individuals ($n = 8$ for each family, 4 each sensitive/resistant, except for $n = 35$ in ELR for SNP252; Tables S4 and S5) and in the founders of each cross. We amplified a 1.4 kb genomic fragment with PCR primers AIP3F (5′-GGCGCTATACCCGCTCGTGTCC-3′) and AIP5R2 (5′-CTTCATATTTG AAGACGAGGGAGG-3′) using 10 ng genomic DNA and Advantage DNA polymerase (Clontech) with the following cycling conditions: [94°C, 1 min]; [94°C, 5 s; 68°C, 2 min] 35×; [68°C, 5 min]. The amplified product was direct-sequenced with the AIP5R2 primer for SNP analysis.

2.4 | QTL analysis

Prior to interval mapping, we performed a marker regression on unfiltered markers to set the priors for exploring QTL model-space for interval mapping (Figure S8). We then performed interval mapping on the filtered markers (see above) in R/qtl to test the single QTL model on each chromosome via multiple imputation ($n = 500$) and Haley–Knott regression, which estimates the most likely interval genotypes and QTL position between RAD markers from the log posterior distribution. If the LOD score for the single QTL test exceeded our permuted threshold (top 15% of the null distribution) in a single QTL scan, the position of the highest LOD score was added to the full QTL model. We compared multiple single and full-QTL models between methods (normal, binary, transformed data with parametric and nonparametric models). We examined models that included only the initial two major QTL discovered by marker regression, as well as more inclusive models that included the minor effect QTL that were discovered by interval mapping. QTL were dropped if they did not significantly change the fit of the full-model in the drop-one-qtl analysis. The full model in R/qtl estimated the additive and dominance effect of each of the QTL in the model. We compared their relative effect sizes to illuminate the genetic architecture of resistance in each of the mapping families.

2.5 | Comparison of methods to previous QTL experiment

Our methods were similar but not identical to those of (Nacci et al., 2016). Here, we contrast the approaches of these two studies to facilitate interpretation of overlap between our findings. The 2016 study created multiple families with different progenitor individuals from one resistant population (NBH), whereas the current study created one family from each of four resistant populations. The 2016 study genotyped individuals with microsatellites and SNPs resulting in ~240 genetic markers (Waits et al., 2016), whereas the current study genotyped using between 16,000 and 57,000 SNP markers (RAD-seq) depending on the family. Both studies used the same breeding design (F2 intercross), the same exposure and phenotype scoring regime, both used two-tailed genotyping, and similar statistical models. Note that the genetic map upon which the 2016 QTL study was based (Waits et al., 2016) had some assembly mistakes (Appendix S2); most notably it was missing chromosome 18, thereby precluding the 2016 study from revealing the large-effect QTL that we observe on Chr18 (see Section 3).

2.6 | Comparison to selection scan data

To further refine our understanding of the genetic variation that contributes to resistance, we tested for overlap between previously identified signatures of selection from population genomics studies (Reid et al., 2016) and the QTL identified here by aligning both datasets to the same chromosomal coordinates. This approach has been effective for connecting QTLs to strong selection from domestication and breeding (Rubin et al., 2012). Signatures of selection were identified from whole-genome scans of diversity and divergence between each of the four resistant populations and nearby nonresistant populations (Reid et al., 2016). In the population genomics studies, genomic scaffolds were scanned for extreme values of p_i, F_{ST}, and Tajimas D in 5 kb windows to identify signatures of strong recent natural selection. The selection signature thresholds for each statistic were set using a demographic model to simulate a null distribution of each statistic. These thresholds were used to convert the statistics into an aggregate z-score. The Z-scores (which included the

width and magnitude of the signature region) formed a composite rank for each signature of selection in each polluted population. F_{ST}, delta p_i, and composite ranks on the scaffold-level genome assembly were lifted over to a physical position on the killifish chromosomal assembly (Miller et al., 2019, *Fundulus_heteroclitus-3.0.2*, NCBI *BioProject PRJNA177717*) with liftover using a chain-file generated by ALLMAPS (Hinrichs et al., 2006; Tang et al., 2015). QTL intervals were evaluated against the 50 and 200 top-ranked composite signatures of selection from Reid et al. (2016).

3 | RESULTS

3.1 | Phenotypes

We observed the full spectrum of sensitive to resistant phenotypes in mapping families, indicating that the founders from each of the populations harbored genetic variation for resistance to PCB-126. Frequency distributions of phenotypic variation for the families that were chosen for mapping (Figure 1b) were representative of phenotype distributions from other F2 intercross families created from these same populations (Figure S3). In our analyses, we grouped individuals with phenotypes of 4 and 5 as sensitive and individuals with phenotypes of 0 and 1 as resistant and modeled the resistance phenotype as binary.

3.2 | QTL analysis

We identified a small number of loci that account for a large proportion of phenotypic variation in PCB sensitivity in QTL mapping families from all four resistant populations. Genome-wide marker regression and QTL interval mapping of RAD-Tag markers identified one or two large-effect QTL in each of the families and these QTL tended to be shared between multiple mapping families (Figure 2a,b). A large-effect QTL on CHR18 was shared among all four resistant populations. This CHR18 QTL accounted for a large fraction of resistance variation in each family (11% in NBH, 27% in BRP, 40% in NEW, and 34% in ELR). Another large-effect QTL on CHR2 was shared among the three northern resistant populations. This CHR2 QTL accounted for a large fraction of resistance variation in each northern family (40% in NBH, 22% in BRP, and 18% in NEW). Thus, families from the three northern populations shared the same set of two major-effect QTLs on chromosomes 2 and 18 (CHR2 and CHR18, respectively), despite a different breeding design and phenotypic distribution for the BRP family. Together, this set of two QTLs accounted for a very large fraction of variation in sensitivity to PCB-induced toxicity in northern families (70% in NBH, 53% in BRP, and 55% in NEW). We also found that, at least for the NBH mapping family, the QTL on CHR2 appears to be recessive, and the few heterozygous individuals that are phenotypically resistant carry the resistant genotype on CHR18 (Figure 2c). Parameter estimates for the large-effect QTL models are included in Table S6 and File S1.

We detected support for medium to small effect QTL that do not overlap between families. When included in the full model, these additional QTL explain a relatively small proportion of variation (variance explained <6%, drop-one-qtl analysis estimates). We included parameter estimates for large effect loci with the more inclusive models as shown in Table S6 and File S1.

3.3 | QTL and signatures of selection

We tested for an overlap between our QTL peaks and regions of the genome showing signatures of recent strong natural selection from genome-wide scans (Reid et al., 2016), such that overlaps between QTL and selection signatures could narrow the interval for inferring candidate genes. We found that both major QTLs contain genomic regions encoding core components of the AHR signaling pathway (Appendix S3). The two large-effect QTL intervals found on CHR2 and CHR18 (Figure 2) include AIP and AHR genes, respectively. As defined by high allele frequency differentiation between resistant and reference populations (F_{ST}) and reduced nucleotide diversity in the resistant population (delta-p_i), the AIP genomic region of CHR2 ranks among the strongest signatures of natural selection in all resistant populations (Reid et al., 2016) (Figure 3). This region overlaps with the large-effect QTL for the three northern-population crosses (NBH, BRP, NEW; Figure 3), but not for the ELR mapping family (Figures 2 and 3). The large-effect QTL for all four resistant populations on CHR18 (Figure 2) contains genes AHR1b and AHR2b. This region included the 20th ranked signature of selection in ELR, 164th in NEW, but did not include highly ranked signatures of selection for NBH or BRP (Figure 3).

In addition to the large-effect QTL, we detected support for smaller effect QTL. Markers on chromosomes 8,11,13, and 24 exceeded the permuted threshold in the single QTL scans. However, we found nominal support for the effect and position of these QTL in the full-model with drop-one-qtl analysis. Of the moderate/low effect QTL, the CHR8 QTL had the highest individual LOD score in NBH, but only explained <2.3% variation when included in the full model. None of these smaller-effect QTL coincide with genomic regions showing strong signatures of selection in the wild-resistant populations from which mapping families were derived.

We performed targeted genotyping of the mapping families to verify the presence or the absence of putatively adaptive haplotypes that were discovered by the whole-genome scans for selection. In ELR, a highly ranking signature of recent natural selection on CHR1 was previously discovered to contain a 99.7-kb deletion affecting coding sequences of AHR1a and AHR2a (Figure S7A), and this deletion had swept from low frequency in a nearby reference population (not detected) to high frequency (81%) in the resistant ELR population (Reid et al., 2016). PCR tests, using primers inside and outside of the deletion region (Figure S7B), verified that the AHR1a/AHR2a deletion on CHR1 is segregating within our ELR mapping family (Table S3). However, in ELR we detect no evidence for a QTL associated with variable sensitivity to PCB-126 toxicity anywhere
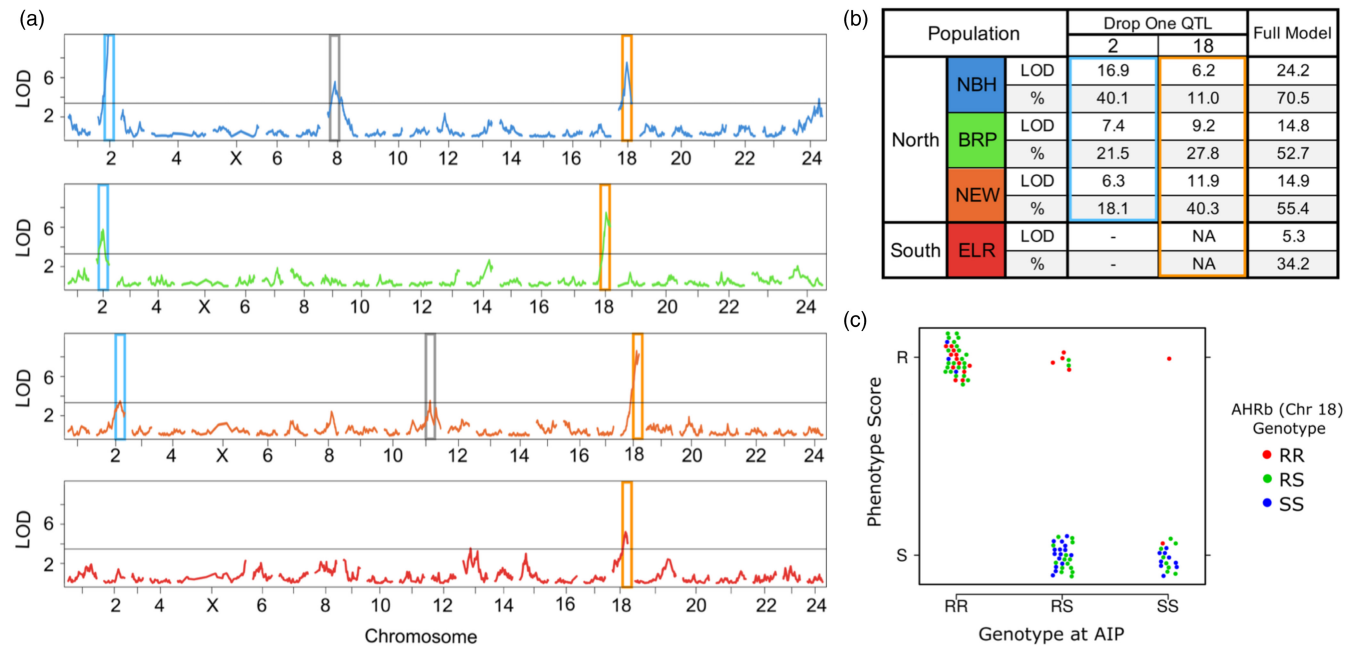
FIGURE 2   QTL and their contribution to variation in sensitivity to PCB-induced toxicity. (a) LOD score results along each chromosome (*x*-axis numbers) for single QTL scans of RAD-Tag markers in each mapping family. Blue, green, orange, and red series correspond to mapping families from NBH, BRP, NEW, and ELR, respectively. Tall colored boxes (blue and gold) indicate the QTL that fall on the same chromosome/ position in multiple families. Grey boxes indicate minor effect QTL that explain a relatively minor proportion of the resistance phenotype and are not shared between populations. Note that the vertical alignment of homologous chromosomes between families is not perfect, because estimated mapping distances for the same chromosome can vary between mapping families because of variation in recombination rates between families and genotyping error. (b) Summary of the contribution (drop one QTL analysis) of large-effect QTL (blue and gold boxes) to the full QTL model (LOD and percent variance explained) in each mapping family. (c) Genotype-by-phenotype plot for the NBH family at the AIP candidate locus (QTL on chromosome 2) and AHR1b/2b candidate locus (QTL on chromosome 18). The *Y*-axis groups individuals (points) as either resistant (R: phenotype malformation scores 0–1 following PCB-126 exposure) or sensitive (S: phenotype malformation scores 4–5 following PCB-126 exposure). The *X*-axis distinguishes individuals based on their genotype at the AIP locus (QTL on chromosome 2), where individuals homozygous for the resistant allele, heterozygous, or homozygous for the sensitive allele, are represented by RR, RS, or SS, respectively. The color of points distinguishes individuals based on their genotype at the AHR1b/2b locus (QTL on chromosome 18), where individuals homozygous for the resistant allele, heterozygous, or homozygous for the sensitive allele, are represented by red, green, or blue, respectively. All individuals that carry the RR genotype at the AIP locus are phenotypically resistant (top left group). Those that carry one or no copies of the resistant allele at the AIP locus tend to be phenotypically sensitive (bottom middle and right groups), unless they are also homozygous for the resistant allele at the AHR1b/2b locus (red dots) in which case they tend to be phenotypically resistant (top middle and right groups).

on CHR1 (Figure S8). The PCR genotypes of 40 resistant individuals from our ELR mapping family indicated that eight are homozygous for the deletion, 22 are heterozygous, and 10 are homozygous intact. Genotyping of 47 sensitive individuals from our mapping family indicated six homozygous for the deletion, 25 heterozygous, and 16 homozygous intact (Table S3). The phenotype association LOD score

of the PCR deletion genotype and RAD markers near the deletion haplotype do not support a QTL on CHR1 for ELR or any of the mapping families (LOD of 0.051 for the deletion genotype in ELR).

Additional targeted genotyping of nonsynonymous SNPs in the coding sequence for AIP (CHR2) was used to confirm whether the adaptive genotype at that locus (inferred from results of selection

FIGURE 3   Large-effect QTL (first row) on CHR2 (left column) and CHR18 (right column) aligned with signatures of selection from genome scans for each resistant population (remaining rows). *X*-axis tick marks represent Mb intervals along the entire length of the two chromosomes. QTL LOD scores (top row) are the results from a single-QTL scan (marker regression) in each of the QTL mapping families (the blue series represents LOD scores from NBH mapping families, green from BRP, orange from NEW, and red from ELR). Panels in the remaining rows include F_{ST} and delta p_i between each of the resistant populations and their nearby reference populations (original genome scan data were collected by Reid et al., 2016). Elevated F_{ST} and reduced delta p_i are signatures of recent strong natural selection. Tall boxes highlight the position and rank of the top-ranked signatures of selection that co-localize to a large-effect QTL. The large effect QTL on CHR2 (which contains AIP) coincides with a highly ranked selection signature region; although this region shows a strong signature of selection in all four populations, it includes a QTL for only three of the populations (NBH, BRP, and NEW). The large effect QTL on chromosome 18 (which contains AHR1b and AHR2b) is found in all four populations, but coincides with highly ranked selection signature regions in only two of those populations (ELR and NEW).
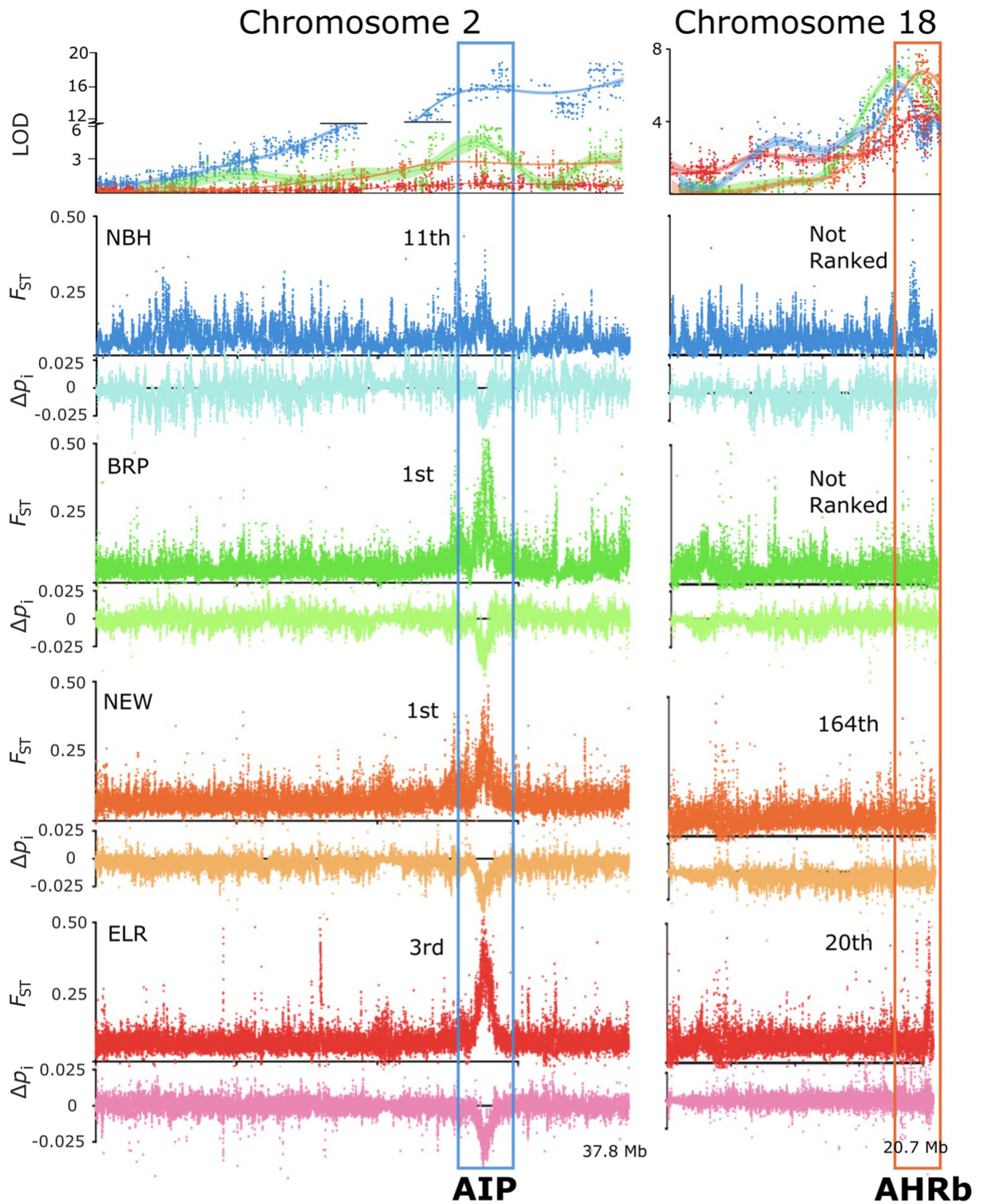
scans from Reid et al., 2016) was segregating within our mapping families. We were particularly keen to determine why the ELR mapping family was the only one where the genomic region containing the AIP locus was not a QTL, even though the AIP locus is within one of the highest ranked signatures of natural selection in all populations including ELR (the AIP locus was within the third highest ranked signature of natural selection detected in ELR; Reid et al., 2016). We examined two nonsynonymous SNPs in AIP that

have high allele frequency differences between resistant populations and their paired sensitive reference populations (Tables S3 and S4). These variants fall within the region containing the adaptive haplotype on CHR2 (Figure 3), and we hereby refer to these variants as "adaptive" insofar as they are indicative of large haplotype frequency differences (and they are in a candidate gene). The Trp→Leu variant in AIP amino acid position 224 (aa224) shows large frequency differences between northern resistant populations (where the Leu residue is between 70% and 74% frequency) and their paired reference populations (where the Leu residue is between 0% and 4% frequency) (Table S4). This suggests that the variant is linked to a variant(s) that promotes fitness advantage in polluted estuaries. The Northern resistant founders (NBH, BRP, NEW) of mapping families are all homozygous for the aa224 Leu variant, which is consistent with the support of a QTL at AIP in the Northern mapping families (NBH, BRP, NEW). However, the Trp residue at aa224 is fixed in both the ELR population and its paired reference population (Table S4). In contrast, a different amino acid variant (Ile→Val at aa252) shows large allele frequency differentiation at the AIP locus between ELR and its paired reference population, where the Val residue is absent in the reference population, but at 48% frequency in ELR (Table S5). We also found that both the Ile and Val residues were segregating within our ELR mapping family at equal frequency, but variation at this locus was not predictive of resistance (Table S5). This indicates that putatively adaptive variation at the AIP locus was captured in all of our mapping families but was only predictive of resistance to PCB-126 in the three northern populations and not predictive of resistance in the ELR population.

4 | DISCUSSION

We evaluated the genetic basis for adaptation to a particularly toxic class of pollutants in four populations of killifish that have independently evolved resistance to extreme pollution in urban estuaries of the US Atlantic coast. This study was founded on knowledge that killifish populations from all four polluted sites have converged on a similar level of evolved resistance to the developmental toxicity of a model DLC toxicant, PCB-126 (Nacci et al., 2010). Furthermore, mechanistic evidence suggested a convergently evolved transcriptional response to PCB-126 exposure in these four populations, which implicated shared adaptive de-sensitization of the AHR signal transduction pathway (Whitehead et al., 2012), which is normally activated by DLC exposure and mediates toxicity (Clark et al., 2010). Subsequent population genetic analyses, including those that specifically examined AHR pathway genes (Proestou et al., 2014; Reitzel et al., 2014) and genome-wide approaches (Osterberg et al., 2018; Reid et al., 2016), revealed AHR pathway genes (among many others) showing signatures of natural selection. An earlier QTL mapping experiment (Nacci et al., 2016) also implicated AHR pathway elements in the NBH population, and provided the foundation for the current studies. We anticipated that genetic variation in AHR pathway elements, and other loci, would be associated with adaptive resistance

to PCB-126 toxicity, but sought to reveal how many loci contributed to resistance, and whether they were shared among families from different populations. We have interpreted results within the context of the comprehensive population genomic study that included all four resistant killifish populations and their nearby sensitive killifish pairs (Reid et al., 2016).

Specifically, we sought to (1) use genotype–phenotype (QTL) mapping to determine the genetic architecture of one component of evolved pollution resistance (developmental toxicity), (2) test whether that architecture is similar or different between our four focal populations, and (3) examine overlap between phenotype-associated loci and loci showing strong signatures of recent natural selection in wild populations. We found that (1) a small number of large-effect QTL loci accounted for resistance to DLC-mediated developmental toxicity, (2) QTLs harbored candidate genes that govern the regulation of AHR signaling, (3) some (but not all) of these QTL loci were shared across all populations, and (4) some (but not all) of these QTL loci showed signatures of recent natural selection in the corresponding wild population (Reid et al., 2016). Reciprocally, some top-ranked genes that were considered strong candidates for PCB resistance inferred from genome scans in wild populations were identified as QTL, but some key candidate genes were not. In what follows, we discuss the nature and implications of each of these key findings.

4.1 | Genetic complexity of evolved PCB resistance

Two major large-effect QTL were identified among the four killifish families, with the full model that included these two QTL accounting for a very large proportion of phenotypic variation in developmental sensitivity to our model toxicant (34.2%–70.1%) (Figure 2). One major QTL was shared among the four mapping families: the QTL on CHR18, which includes candidate genes AHR1b/2b paralogs, accounted for 11%–43% of the variation in DLC sensitivity. The other major QTL was shared among the Northern resistant killifish but was not identified as a QTL in ELR: the QTL on CHR2, which includes candidate gene AIP, accounted for 18.1%–45.4% of the variance in DLC sensitivity. A couple of other loci, private to some of the families, accounted for a small fraction of phenotypic variation in some families. For example, minor QTL were noted on CHR8 in the NBH family and on CHR11 in the NEW family. These results are largely congruent with the prior NBH QTL study, where a small number of QTL accounted for 69% of phenotypic variation, with the largest effect loci associated with AHR pathway genes and a region on CHR8 (Nacci et al., 2016). We conclude that variants in a small number of key genes that regulate AHR signaling largely achieve the desensitization of AHR signaling that is protective of DLC-induced development toxicity in urban killifish populations. These results indicate that the genetic basis of evolved variation in developmental sensitivity to PCB-126 is not complex or highly polygenic. Resistance evolved rapidly through increased frequency of large-effect loci, where the adaptive alleles are found at very low frequency (or are

undetectable) in sensitive populations from clean habitats (Reid et al., 2016). This core finding is consistent with expectations when adaptation requires a rapid and large shift in the phenotypic optimum (Stetter et al., 2018).

It is possible that variants in AHR and/or AIP may result in similar phenotypic outcomes, by effecting adaptive desensitization of PCB-induced AHR signaling. Variation at the DLC-binding site of the AHR is highly predictive of DLC sensitivity in bird species (e.g., Farmahin et al., 2013). Similarly, evolved adaptive DLC resistance in Hudson River (NY, USA) tomcod is largely explained by variants in AHR localized near the site where AIP complexes with AHR (Wirgin et al., 2011). Moreover, AIP is an evolutionarily conserved AHR chaperone that influences the stability of the AHR complex and eventual nuclear translocation of AHR (Bell & Poland, 2000; Lecoq et al., 2016; Meyer et al., 2000). AIP loss-of-function mutations in mice protect against some forms of DLC toxicity (Nukaya et al., 2010). However, the role of AHR–AIP interactions in fish, and whether there are ligand-specific differences in AHR–AIP interactions, are not well understood. Convergent evolution that is underpinned by convergent genetic changes, as we observe with these two AHR pathway genes, is more likely when selection is strong and genes have large phenotypic effects (MacPherson & Nuismer, 2017). Our results are consistent with these expectations, insofar as selection has been strong, and AHR and AIP mutations have large effect sizes. The likelihood of genetic convergence also increases when functional constraints on relevant pathways are high (Feldman et al., 2012; Weinreich et al., 2006). We hypothesize that changes to either AHR or AIP locus may result in an altered AHR-AIP-agonist complex that modifies the molecular events that would normally initiate AHR signal transduction. Whether this convergence emerges from pleiotropic constraints, bias in the likelihood of certain types of mutations (e.g., Martin & Orgogozo, 2013), shared selective environments, or shared genetic background, or some combination of these, remains to be experimentally determined.

Few studies have revealed the genomic basis of evolved pollution resistance (but see, e.g., Wright et al., 2013, 2015). However, many studies have examined adaptations to environmental pollutants that are toxic by design, such as pesticides, which may offer relevant lessons. Often few loci of major effect are implicated in evolved resistance to pesticides, for example, loci that are the biochemical targets of toxicity or loci that more broadly govern metabolic detoxification (Ffrench-Constant, 2013). Although the literature on the genetic basis of pesticide resistance is relatively large, most studies focus on candidate genes; genome-wide approaches are rare (e.g., Kreiner et al., 2019, 2021; Pélissié et al., 2022; Van Etten et al., 2020). Using knowledge from pesticide resistance to make predictions about the genomic basis of pollution resistance may be fraught, partly because genome-wide understanding of the relevant genetics is thin, and partly because the nature of selection in polluted environments may be different from that in agricultural settings. For example, pesticides and antibiotics are typically designed to target very specific biochemical processes, and they are usually encountered in high concentrations as single chemicals or simple mixtures. In contrast, pollution can present fitness challenges that are more complex, because these chemicals may perturb multiple biochemical pathways (Gerstein et al., 2012, 2015), and multiple pollutants often co-occur in the environment (Whitehead et al., 2017). Toxic chemicals are often encountered in human-modified environments where multiple co-occurring habitat alterations may contribute additional selective pressures (e.g., altered competitive interactions, thermal and flow regimes, hypoxia), which further increases the dimensionality of fitness challenges and the required adaptations (Iriart et al., 2021; Rivkin et al., 2019).

Many more loci than those few QTL reported here appear to be important for fitness in urban populations of killifish (Reid et al., 2016). It is, therefore, important to consider whether more broadly defined resistance to PCB toxicity, and fitness in polluted urban estuaries even more generally, has a simple or complex genetic basis. In this study, we only examined resistance to a limited suite of toxic effects caused by a single dose of a single PCB congener during one life stage. Different sets of mutations may underlie sensitivity to different doses (Wang & Kruglyak, 2014), or at different life stages (Everman et al., 2021). PCB-126 is only one representative of the many DLCs and other pollutant classes that may affect fitness of urban fish. We currently do not know whether other aspects of DLC toxicity in embryos or later life stages (e.g., immune dysfunction, endocrine dysfunction, neurological dysfunction, or cancer [White & Birnbaum, 2009]) are resolved in resistant fish by the same or different QTL as those identified here. Similarly, our model toxicant PCB-126 is only one of a complex mixture of chemicals that pollute these urban estuaries and threaten fitness. Populations inhabiting these sites have likely evolved resistance to many of those chemicals (e.g., Bugel et al., 2014; Celander et al., 2021; Grans et al., 2015; Greytak et al., 2010). Resistance to some of those chemicals may be underpinned by the same loci as we identified here, but other loci are also likely to contribute, especially for chemicals with unique mechanisms of toxicity. Furthermore, it is plausible that initial costs of adaptive AHR desensitization caused by mutations of large effect may have prompted compensatory adaptive fine-tuning (MacLean et al., 2010) to restore some function to AHR signaling and connected pathways. By extension of the above reasoning, the many environmental changes that distinguish urban from natural estuaries include diverse nonchemical perturbations (e.g., biotic interactions, flow regimes, pathogens, etc.) that may drive adaptation through entirely unique suites of traits, pathways, and genes. We conclude that evolved resistance to the developmental toxicity induced by PCB-126 has a relatively simple genetic basis in resistant individuals, while acknowledging that the genetic changes required for adaptation to urban estuaries are likely much more complex.

4.2 | Variation in QTL among resistant populations

Our mapping families derived from four focal populations share some similarities and also some differences in the loci that contribute to resistance. Mapping families from the three northern

populations share each of the two major-effect QTL (on CHR2 and CH18), whereas the mapping family from the southern population (ELR) is most different and shares just one QTL (on CHR18) with those from the northern populations. A lack of parallelism at the genetic level following parallel resistance to toxicants has been observed in many systems including pesticide resistance in weeds (Van Etten et al., 2020) and beetles (Pélissié et al., 2022), and heavy metal resistance in sea campion (Papadopulos et al., 2021). It is often concluded that this is consistent with a complex genetic architecture for resistance, but this is rarely tested experimentally (e.g., with quantitative genetics). However, for copper resistance in fruit flies, an extensive overlap was observed between QTL and selection scan outliers, although QTL were clearly a small subset of the selection scan outliers (Everman et al., 2021, 2023). For killifish, where the genetic architecture is not complex, similarities and differences between QTL for different populations may be driven by any of three factors: (1) the presence of functionally redundant variants, (2) differences in genetic background, or (3) differences between selective environments; we consider these in sequence.

First, for many adaptations, the genetic basis is polygenic. This could manifest as many variants, each of small effect, contributing to phenotypic differences between two individuals (one resistant, one sensitive). However, this does not appear to be the case for the data presented here. Alternatively, polygenic adaptation could manifest from multiple redundant large-effect variants contributing to adaptive fitness among different pairs of individuals (e.g., Goldstein & Holsinger, 1992). Indeed, there was limited overlap in PCB resistance QTL among the three mapping families created with different NBH progenitors (Nacci et al., 2016). This type of polygenic adaptation, perhaps better termed "oligogenic," is likely relevant for toxicant-resistant killifish populations, insofar as the resistance phenotype is fixed but almost no SNPs are fixed in those populations (Reid et al., 2016). Therefore, individuals selected as QTL mapping family progenitors may harbor different combinations of adaptive loci (e.g., as observed in Nacci et al., 2016). If adaptation is polygenic or oligogenic, then QTL studies (because they include only one or very few progenitors) may not reveal the complete sets of loci that contribute to evolved phenotypes. However, we do not consider this a good explanation for the QTL differences on CHR2 presented here. Variants on CHR2 have been subject to strong recent natural selection in all four populations (Figure 3; Osterberg et al., 2018; Reid et al., 2016), yet association with resistance was found only in NBH, BRP, and NEW families but not the ELR family. Perhaps the adaptive haplotype was missing from the ELR individual that we selected as the mapping family progenitor? We consider this unlikely since both putatively adaptive and sensitive genotypes at this locus were segregating in the ELR mapping family (Table S5). However, it is still possible that these variants are not closely linked with the adaptive variant(s). Ongoing high-density genetic mapping should resolve this.

The second factor that could contribute to inter-family variation in QTL is genetic background. Convergent phenotypic evolution is more likely to be underpinned by convergent genetic evolution when genetic variation is ancestrally shared and selection is strong (e.g., Alves et al., 2019; Jones et al., 2012; Therkildsen et al., 2019). The three northern populations are genetically quite similar to each other (average pairwise $F_{ST} = 0.09$), whereas the southern population is genetically distinct (average pairwise F_{ST} between ELR and each of the northern populations $= 0.19$) (Reid et al., 2016). Accordingly, the genetic variation available for contemporary natural selection was different between the northern populations and the southern population, such that the genotypes underlying parallel adaptation were shared in the north but different in the south. However, we do not consider this sufficient to explain the QTL differences presented here. Although the adaptive haplotypes were different between northern and southern populations, the genomic region surrounding the CHR2 QTL was among the very top-ranked signatures of natural selection in all four resistant populations (Figure 3; Osterberg et al., 2018; Reid et al., 2016). If this adaptive variation supported resistance to PCBs, then the region on CHR2 should be a QTL in all four populations.

The third factor that could contribute to inter-family variation in QTLs is differences between the environments that select for variants at different adaptive loci. The northern populations are all predominantly polluted by halogenated DLCs (PCBs and dioxins), whereas the southern ELR site is primarily polluted by PAHs. Although DLCs and PAHs may exert some aspects of developmental toxicity in part through a common mechanism (AHR signaling; Clark et al., 2010), the detailed mechanisms of action of these two chemical classes differ (Billiard et al., 2008; Denison et al., 2011; Incardona, 2017). One important difference is that PAHs are readily metabolized by AHR-regulated proteins (potentially creating many reactive metabolites), whereas PCBs are not. Another difference is that dioxins and dioxin-like PCBs mediate cardiac toxicity largely through the AHR, but many PAHs exert their cardiac toxicity through AHR and AHR-independent mechanisms (Brette et al., 2014; Incardona et al., 2005). Clearly, variation at CHR2 has been subject to strong selection in all four populations (Figure 3). Perhaps PCB-induced selection in northern estuaries favored variation at CHR2 that enables PCB-resistant phenotypes, whereas PAH-induced selection in ELR also favored variation at CHR2 but those variants promote aspects of adaptation (e.g., PAH resistance) that are independent of PCB resistance. We consider this a plausible explanation for our data. Ongoing experiments are testing for QTL for multiple classes of chemical resistance (including PAHs) in multiple killifish individuals and populations.

4.3 | Coincidence between QTL and signatures of natural selection

Some QTLs for PCB resistance showed signatures of selection in wild populations, but some did not. The QTL on CHR2 in mapping families from NBH, BRP, and NEW overlaps with a top-ranked signature of selection in genome scans of those same populations. In

contrast, the QTL on CHR18 found in all four mapping families was a ranked signature of selection in only two of the four wild populations; this locus was ranked 20th and 164th in ELR and NEW populations, respectively, but no evidence for recent strong selection was detected in genome scans of BRP and NBH populations (Figure 3). One possible explanation is that multiple functionally redundant adaptive variants at this locus contributed to resistance in wild killifish populations, or a single adaptive variant embedded in multiple genetic backgrounds contributed to resistance. The soft sweeps or sets of partial sweeps that would emerge to underlie adaptation are notoriously hard to detect because of diminished signal of linked selection (Pritchard et al., 2010; Yeaman, 2015). Additional studies that, for example, take advantage of linkage-disequilibrium based tests (Pennings & Hermisson, 2006) would be necessary to detect a soft sweep on CHR18 and test this hypothesis.

Many loci that showed strong signatures of selection in wild populations (Reid et al., 2016) were not QTL in this study. Genome scans may identify loci that affect fitness in wild populations, but do not directly implicate the agent of selection. Urban estuaries likely pose not only many fitness challenges for killifish, including chemical pollution, but also changes in biotic interactions and in nutrient, temperature, hypoxia, and flow regimes. The full suite of adaptive solutions to these complex and multifarious fitness challenges can imprint in genome scans. It is, therefore, not surprising that loci that are QTLs for PCB resistance are only a small subset of loci that are subject to selection in urban estuaries (e.g., as observed in evolved copper resistance in fruit flies [Everman et al., 2021, 2023]). However, we were surprised that a large deletion that spans AHR2a and AHR1a on CHR1 (Figure S7A) was not associated with PCB resistance in the ELR mapping family. This is surprising because AHR desensitization through AHR knockout/knockdown is protective of PCB (or dioxin) toxicity in rodents (Fernandez-Salguero et al., 1996), zebrafish (Garcia et al., 2018; Goodale et al., 2012; Prasch et al., 2003; Souder & Gorelick, 2019), and killifish (Clark et al., 2010), and an AHR2a deletion had swept to high frequency in resistant populations of Atlantic tomcod (Wirgin et al., 2011), Gulf killifish (Oziolor et al., 2019), and the ELR population of Atlantic killifish (Reid et al., 2016). Since the deletion was segregating in the ELR mapping family (Table S3), we conclude that it must be associated with some other aspect of evolved pollution sensitivity.

One locus that showed among the strongest signatures of selection in all wild populations was also strongly associated with PCB resistance but not in all populations. The locus on CHR2 was the top ranked signature of selection in all four wild resistant populations. Adaptive variants at this locus were segregating in all four mapping families (Tables S3 and S4). However, this locus was a major-effect QTL in only the three northern populations but not in the southern ELR family (Figure 2). We speculate that strong selection at AIP confers resistance to PCB toxicity in northern populations, but selection at this locus contributes to other aspects of adaptation in the ELR population. Since PAH pollution distinguishes the ELR site from the others, and AHR signaling is important for PAH toxicity, perhaps variation at AIP is important for evolved resistance to PAH toxicity

that is independent of the mechanisms that drive resistance to PCB toxicity.

Conspicuously, two tandem AHR paralogs show strong signatures of selection in the wild, whereas two other AHR paralogs are QTL. The AHR paralogs AHR1a and AHR2a are found on CHR1. This locus is under strong selection in all four urban killifish populations (Proestou et al., 2014; Reid et al., 2016; Reitzel et al., 2014). However, variation on CHR1 was not associated with PCB resistance in any of our mapping families. This was unexpected because AHR2a was shown to mediate developmental toxicity of PCBs and PAHs in killifish (Clark et al., 2010). However, in contrast, the QTL region on CHR18 harbors the other two AHR paralogs AHR1b and AHR2b. This region is a QTL in all populations, but shows only a moderately ranked signature of selection in only two of the wild populations (Reid et al., 2016). In zebrafish, AHR2b mediates the developmental toxicity of PCB-like chemicals (Garcia et al., 2018; Goodale et al., 2012; Prasch et al., 2003; Souder & Gorelick, 2019) and AHR1b protein binds with DLCs (Karchner et al., 2005). It is, therefore, plausible that the AHR1b/2b locus plays a role in PCB toxicity, but the relative roles of each of the four killifish AHR paralogs remain to be resolved. All four AHR proteins exhibit high-affinity binding to [^{3}H] TCDD in vitro and TCDD-inducible transcriptional activation in cell culture (Karchner, Franks, Hahn, unpublished results), suggesting that DLC effects in killifish could involve multiple AHRs. Evidence for strong selection at AHR1a/2a but no QTL for PCB resistance at that locus, and QTL for PCB resistance at AHR1b/2b with evidence for weaker and perhaps polygenic selection at that locus, suggests that evolved pollution resistance is underlain by complex interactions among AHR paralogs and their protein partners (e.g., AIP). In any case, it is evident from both genotype–phenotype mapping and scans for signatures of recent natural selection that genetic variation in core regulatory components of the AHR signaling pathways contributes to evolved resistance to pollution in urban estuaries.

4.4 | Caveats and considerations

Selective genotyping may have limited power to detect epistatic or small effect loci. We chose selective genotyping because of the power and efficiency (costs savings) that are afforded (Lander & Botstein, 1989). However, modeling studies suggest that if there are loci of large effect then the effectiveness of selective genotyping becomes unpredictable (Sen et al., 2009). Clearly, our results reveal loci of large effect, but small effect loci and nonadditive or epistatic loci may also be present (e.g., as in Nacci et al., 2016). Indeed, our data suggest that the QTL on CHR2 is recessive (Figure 2c). Ongoing studies by our group involve genotyping and phenotyping entire families that span the entire range of sensitive to resistant phenotypes, which should resolve this potential issue.

Although our results indicate that resistance to PCB-126-induced developmental toxicity is underlain by few variants of large effect, one should be cautious in concluding that this evolved resistance is not more polygenic. This is because our experiments included only

one resistant individual as the progenitor for each mapping family (except for BRP which had two resistant progenitors), so sampling of within-population variation in resistance was far from thorough. By sampling many more individuals, GWAS studies could reveal more loci that contribute to resistance, especially if resistance is underlain by multiple redundant variants that are not fixed within resistant populations. For example, GWAS for evolved herbicide resistance has revealed many loci, even when large-effect loci were present, and these additional loci were segregating across a range of allele frequencies, suggesting a complex and polygenic architecture (Kreiner et al., 2021). For killifish, some QTL varied between three NBH mapping families in a previous study (Nacci et al., 2016). However, our three mapping families derived from the three resistant northern populations (NBH, BRP, and NEW) all implicated the same major-effect QTL (Figure 2). It is plausible that minor-effect and epistatic QTL, which we had little power to detect in our study, may be variable among individuals, so that the complexity of resistance to PCB-126-induced developmental toxicity during development may be greater than it currently appears.

One should also be cautious about concluding that all aspects of resistance to PCB-126, or adaptation to pollution more broadly, or adaptation to urban estuaries even more broadly, is not highly polygenic. This is simply because multiple traits, each with a simple or complex genetic basis, likely contribute to fitness in these highly altered urban environments (as we discuss in more detail in the section above "Genetic complexity of evolved PCB resistance"). Additional quantitative genetic studies are needed to draw genotype–phenotype associations for the diversity of phenotypes induced by exposure to the array of stressors that distinguish urban estuaries.

ACKNOWLEDGMENTS

CONFLICTS OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

A GitHub repository including scripts for the analyses presented here can be found at https://github.com/WhiteheadLab/killifish-RADseq-4popQTL. Sequence reads have been deposited at NCBI, and are linked with the NCBI BioProject Accession PRJNA944691.

ORCID

Mark E. Hahn [ID] https://orcid.org/0000-0003-4358-2082

Andrew Whitehead [ID] https://orcid.org/0000-0002-5457-6449

REFERENCES

Alves, J. M., Carneiro, M., Cheng, J. Y., Lemos de Matos, A., Rahman, M. M., Loog, L., Campos, P. F., Wales, N., Eriksson, A., Manica, A., Strive, T., Graham, S. C., Afonso, S., Bell, D. J., Belmont, L., Day, J. P., Fuller, S. J., Marchandeau, S., Palmer, W. J., … Jiggins, F. M. (2019). Parallel adaptation of rabbit populations to myxoma virus. *Science*, *363*, 1319–1326.

Barnett, D. W., Garrison, E. K., Quinlan, A. R., Stromberg, M. P., & Marth, G. T. (2011). BamTools: A C++ API and toolkit for analyzing and managing BAM files. *Bioinformatics*, *27*, 1691–1692.

Barrett, R. D. H., & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends in Ecology & Evolution*, *23*, 38–44.

Barton, N. H., & Keightley, P. D. (2002). Understanding quantitative genetic variation. *Nature Reviews. Genetics*, *3*, 11–21.

Bay, R. A., Rose, N., Barrett, R., Bernatchez, L., Ghalambor, C. K., Lasky, J. R., Brem, R. B., Palumbi, S. R., & Ralph, P. (2017). Predicting responses to contemporary environmental change using evolutionary response architectures. *The American Naturalist*, *189*, 463–473.

Bell, D. R., & Poland, A. (2000). Binding of aryl hydrocarbon receptor (AhR) to AhR-interacting protein. The role of hsp90. *Journal of Biological Chemistry*, *275*, 36407–36414.

Bell, G. (2013). Evolutionary rescue and the limits of adaptation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *368*, 20120080.

Bell, G., & Gonzalez, A. (2009). Evolutionary rescue can prevent extinction following environmental change. *Ecology Letters*, *12*, 942–948.

Bergland, A. O., Behrman, E. L., O'Brien, K. R., Schmidt, P. S., & Petrov, D. A. (2014). Genomic evidence of rapid and stable adaptive oscillations over seasonal time scales in Drosophila. *PLoS Genetics*, *10*, e1004775.

Billiard, S. M., Meyer, J. N., Wassenberg, D. M., Hodson, P. V., & Di Giulio, R. T. (2008). Nonadditive effects of PAHs on early vertebrate development: Mechanisms and implications for risk assessment. *Toxicological Sciences*, *105*, 5–23.

Brette, F., Machado, B., Cros, C., Incardona, J. P., Scholz, N. L., & Block, B. A. (2014). Crude oil impairs cardiac excitation-contraction coupling in fish. *Science*, *343*, 772–776.

Broman, K. W., & Sen, S. (2009). *A guide to QTL mapping with R/qtl*. Springer.

Broman, K. W., Wu, H., Sen, S., & Churchill, G. A. (2003). R/qtl: QTL mapping in experimental crosses. *Bioinformatics*, *19*, 889–890.

Bugel, S. M., Bonventre, J. A., White, L. A., Tanguay, R. L., & Cooper, K. R. (2014). Chronic exposure of killifish to a highly polluted environment desensitizes estrogen-responsive reproductive and biomarker genes. *Aquatic Toxicology*, *152*, 222–231.

Celander, M. C., Goldstone, J. V., Brun, N. R., Clark, B., Jayaraman, S., Nacci, D., & Stegeman, J. J. (2021). Resistance to Cyp3a induction by polychlorinated biphenyls, including non-dioxin-like PCB153, in gills of killifish (*Fundulus heteroclitus*) from New Bedford Harbor. *Environmental Toxicology and Pharmacology*, *83*, 103580.

Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M., & Lee, J. J. (2015). Second-generation PLINK: Rising to the challenge of larger and richer datasets. *GigaScience*, *4*, 7.

Clark, B. W., Matson, C. W., Jung, D., & Di Giulio, R. T. (2010). AHR2 mediates cardiac teratogenesis of polycyclic aromatic hydrocarbons and PCB-126 in Atlantic killifish (*Fundulus heteroclitus*). *Aquatic Toxicology*, *99*, 232–240.

Denison, M. S., Soshilov, A. A., He, G., DeGroot, D. E., & Zhao, B. (2011). Exactly the same but different: Promiscuity and diversity in the

molecular mechanisms of action of the aryl hydrocarbon (dioxin) receptor. *Toxicological Sciences*, 124, 1–22.

Doering, J. A., Tillitt, D. E., & Wiseman, S. (2023). Reevaluation of 2,3,7,8-Tetrachlorodibenzo-p-dioxin equivalency factors for dioxin-like polychlorinated Dibenzo-p-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls for fishes. *Environmental Toxicology and Chemistry*, 42, 2215–2228.

Everman, E. R., Cloud-Richardson, K. M., & Macdonald, S. J. (2021). Characterizing the genetic basis of copper toxicity in Drosophila reveals a complex pattern of allelic, regulatory, and behavioral variation. *Genetics*, 217, iyaa020.

Everman, E. R., Macdonald, S. J., & Kelly, J. K. (2023). The genetic basis of adaptation to copper pollution in *Drosophila melanogaster*. *Frontiers in Genetics*, 14, 1144221.

Farmahin, R., Manning, G. E., Crump, D., Wu, D., Mundy, L. J., Jones, S. P., Hahn, M. E., Karchner, S. I., Giesy, J. P., Bursian, S. J., Zwiernik, M. J., Fredricks, T. B., & Kennedy, S. W. (2013). Amino acid sequence of the ligand binding domain of the aryl hydrocarbon receptor 1 (AHR1) predicts sensitivity of wild birds to effects of dioxin-like compounds. *Toxicological Sciences*, 131, 139–152.

Faust, G. G., & Hall, I. M. (2014). SAMBLASTER: Fast duplicate marking and structural variant read extraction. *Bioinformatics*, 30, 2503–2505.

Feldman, C. R., Brodie, E. D., Brodie, E. D., & Pfrender, M. E. (2012). Constraint shapes convergence in tetrodotoxin-resistant sodium channels of snakes. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 4556–4561.

Fernandez-Salguero, P. M., Hilbert, D. M., Rudikoff, S., Ward, J. M., & Gonzalez, F. J. (1996). Aryl-hydrocarbon receptor-deficient mice are resistant to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced toxicity. *Toxicology and Applied Pharmacology*, 140, 173–179.

Ffrench-Constant, R. H. (2013). The molecular genetics of insecticide resistance. *Genetics*, 194, 807–815.

Garcia, G. R., Bugel, S. M., Truong, L., Spagnoli, S., & Tanguay, R. L. (2018). AHR2 required for normal behavioral responses and proper development of the skeletal and reproductive systems in zebrafish. *PLoS One*, 13, e0193484.

Garrison, E., & Marth, G. (2012). Haplotype-based variant detection from short-read sequencing. *arXiv*, 1207, 3907.

Gerstein, A. C., Lo, D. S., & Otto, S. P. (2012). Parallel genetic changes and nonparallel gene–environment interactions characterize the evolution of drug resistance in yeast. *Genetics*, 192, 241–252.

Gerstein, A. C., Ono, J., Lo, D. S., Campbell, M. L., Kuzmin, A., & Otto, S. P. (2015). Too much of a good thing: The unique and repeated paths toward copper adaptation. *Genetics*, 199, 555–571.

Goldstein, D. B., & Holsinger, K. E. (1992). Maintenance of polygenic variation in spatially structured populations: Roles for local mating and genetic redundancy. *Evolution*, 46, 412–429.

Gomulkiewicz, R., Holt, R. D., Barfield, M., & Nuismer, S. L. (2010). Genetics, adaptation, and invasion in harsh environments. *Evolutionary Applications*, 3, 97–108.

Goodale, B. C., La Du, J. K., Bisson, W. H., Janszen, D. B., Waters, K. M., & Tanguay, R. L. (2012). AHR2 mutant reveals functional diversity of aryl hydrocarbon receptors in zebrafish. *PLoS One*, 7, e29346.

Grans, J., Wassmur, B., Fernandez-Santoscoy, M., Zanette, J., Woodin, B. R., Karchner, S. I., Nacci, D. E., Champlin, D., Jayaraman, S., Hahn, M. E., Stegeman, J. J., & Celander, M. C. (2015). Regulation of pregnane-X-receptor, CYP3A and P-glycoprotein genes in the PCB-resistant killifish (*Fundulus heteroclitus*) population from New Bedford Harbor. *Aquatic Toxicology*, 159, 198–207.

Greytak, S. R., Tarrant, A. M., Nacci, D., Hahn, M. E., & Callard, G. V. (2010). Estrogen responses in killifish (*Fundulus heteroclitus*) from polluted and unpolluted environments are site- and gene-specific. *Aquatic Toxicology*, 99, 291–299.

Hendry, A. P., Farrugia, T. J., & Kinnison, M. T. (2008). Human influences on rates of phenotypic change in wild animal populations. *Molecular Ecology*, 17, 20–29.

Hendry, A. P., Gotanda, K. M., & Svensson, E. I. (2017). Human influences on evolution, and the ecological and societal consequences. *Philosophical Transactions of the Royal Society B*, 372, 20160028.

Hinrichs, A. S., Karolchik, D., Baertsch, R., Barber, G. P., Bejerano, G., Clawson, H., Diekhans, M., Furey, T. S., Harte, R. A., Hsu, F., Hillman-Jackson, J., Kuhn, R. M., Pedersen, J. S., Pohl, A., Raney, B. J., Rosenbloom, K. R., Siepel, A., Smith, K. E., Sugnet, C. W., ... Kent, W. J. (2006). The UCSC genome browser database: Update 2006. *Nucleic Acids Research*, 34, D590–D598.

Hoffmann, A. A., & Sgro, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, 470, 479–485.

Incardona, J. P. (2017). Molecular mechanisms of crude oil developmental toxicity in fish. *Archives of Environmental Contamination and Toxicology*, 73, 19–32.

Incardona, J. P., Carls, M. G., Teraoka, H., Sloan, C. A., Collier, T. K., & Scholz, N. L. (2005). Aryl hydrocarbon receptor-independent toxicity of weathered crude oil during fish development. *Environmental Health Perspectives*, 113, 1755–1762.

Iriart, V., Baucom, R. S., & Ashman, T.-L. (2021). Herbicides as anthropogenic drivers of eco-evo feedbacks in plant communities at the agro-ecological interface. *Molecular Ecology*, 30, 5406–5421.

Jain, K., & Stephan, W. (2017). Rapid adaptation of a polygenic trait after a sudden environmental shift. *Genetics*, 206, 389–406.

Jones, F. C., Grabherr, M. G., Chan, Y. F., Russell, P., Mauceli, E., Johnson, J., Swofford, R., Pirun, M., Zody, M. C., White, S., Birney, E., Searle, S., Schmutz, J., Grimwood, J., Dickson, M. C., Myers, R. M., Miller, C. T., Summers, B. R., Knecht, A. K., ... Kingsley, D. M. (2012). The genomic basis of adaptive evolution in threespine sticklebacks. *Nature*, 484, 55–61.

Karchner, S. I., Franks, D. G., & Hahn, M. E. (2005). AHR1B, a new functional aryl hydrocarbon receptor in zebrafish: Tandem arrangement of *ahr1b* and *ahr2* genes. *Biochemical Journal*, 392, 153–161.

King-Heiden, T. C., Mehta, V., Xiong, K. M., Lanham, K. A., Antkiewicz, D. S., Ganser, A., Heideman, W., & Peterson, R. E. (2012). Reproductive and developmental toxicity of dioxin in fish. *Molecular and Cellular Endocrinology*, 354, 121–138.

Kopf, P. G., & Walker, M. K. (2009). Overview of developmental heart defects by dioxins, PCBs, and pesticides. *Journal of Environmental Science and Health, Part C*, 27, 276–285.

Kopp, M., & Matuszewski, S. (2014). Rapid evolution of quantitative traits: Theoretical perspectives. *Evolutionary Applications*, 7, 169–191.

Kosambi, D. D. (1943). The estimation of map distances from recombination values. *Annals of Eugenics*, 12, 172–175.

Kreiner, J. M., Giacomini, D. A., Bemm, F., Waithaka, B., Regalado, J., Lanz, C., Hildebrandt, J., Sikkema, P. H., Tranel, P. J., Weigel, D., Stinchcombe, J. R., & Wright, S. I. (2019). Multiple modes of convergent adaptation in the spread of glyphosate-resistant *Amaranthus tuberculatus*. *Proceedings of the National Academy of Sciences of the United States of America*, 116, 21076–21084.

Kreiner, J. M., Stinchcombe, J. R., & Wright, S. I. (2018). Population genomics of herbicide resistance: Adaptation via evolutionary rescue. *Annual Review of Plant Biology*, 69, 611–635.

Kreiner, J. M., Tranel, P. J., Weigel, D., Stinchcombe, J. R., & Wright, S. I. (2021). The genetic architecture and population genomic signatures of glyphosate resistance in *Amaranthus tuberculatus*. *Molecular Ecology*, 30, 5373–5389.

Lander, E. S., & Botstein, D. (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, 121, 185–199.

Langerhans, R. B. (2018). Predictability and parallelism of multitrait adaptation. *Journal of Heredity*, 109, 59–70.

Lebowitz, R. J., Soller, M., & Beckmann, J. S. (1987). Trait-based analyses for the detection of linkage between marker loci and quantitative trait loci in crosses between inbred lines. *Theoretical and Applied Genetics*, 73, 556–562.

Lecoq, A.-L., Viengchareun, S., Hage, M., Bouligand, J., Young, J., Boutron, A., Zizzari, P., Lombès, M., Chanson, P., & Kamenický, P. (2016). AIP mutations impair AhR signaling in pituitary adenoma patients fibroblasts and in GH3 cells. *Endocrine-Related Cancer*, 23, 433–443.

Lindsey, H. A., Gallie, J., Taylor, S., & Kerr, B. (2013). Evolutionary rescue from extinction is contingent on a lower rate of environmental change. *Nature*, 494, 463–467.

Lourenço, J. M., Glémin, S., & Galtier, N. (2013). The rate of molecular adaptation in a changing environment. *Molecular Biology and Evolution*, 30, 1292–1301.

MacLean, R. C., Hall, A. R., Perron, G. G., & Buckling, A. (2010). The population genetics of antibiotic resistance: Integrating molecular mechanisms and treatment contexts. *Nature Reviews. Genetics*, 11, 405–414.

MacPherson, A., & Nuismer, S. L. (2017). The probability of parallel genetic evolution from standing genetic variation. *Journal of Evolutionary Biology*, 30, 326–337.

Martin, A., & Orgogozo, V. (2013). The loci of repeated evolution: A catalog of genetic hotspots of phenotypic variation. *Evolution*, 67, 1235–1250.

Messer, P. W., Ellner, S. P., & Hairston, N. G. (2016). Can population genetics adapt to rapid evolution? *Trends in Genetics*, 32, 408–418.

Messer, P. W., & Petrov, D. A. (2013). Population genomics of rapid adaptation by soft selective sweeps. *Trends in Ecology and Evolution*, 28, 659–669.

Meyer, B. K., Petrulis, J. R., & Perdew, G. H. (2000). Aryl hydrocarbon (Ah) receptor levels are selectively modulated by hsp90-associated immunophilin homolog XAP2. *Cell Stress & Chaperones*, 5, 243–254.

Miller, J. T., Reid, N. M., Nacci, D. E., & Whitehead, A. (2019). Developing a High-Quality Linkage Map for the Atlantic Killifish *Fundulus heteroclitus*. *G3: Genes, Genomes, Genetics*, 9, 2851–2862.

Miller, M. R., Dunham, J. P., Amores, A., Cresko, W. A., & Johnson, E. A. (2007). Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. *Genome Research*, 17, 240–248.

Nacci, D., Champlin, D., & Jayaraman, S. (2010). Adaptation of the estuarine fish *Fundulus heteroclitus* (Atlantic Killifish) to polychlorinated biphenyls (PCBs). *Estuaries and Coasts*, 33, 853–864.

Nacci, D., Coiro, L., Champlin, D., Jayaraman, S., McKinney, R., Gleason, T. R., Munns, W. R., Specker, J. L., & Cooper, K. R. (1999). Adaptations of wild populations of the estuarine fish *Fundulus heteroclitus* to persistent environmental contaminants. *Marine Biology*, 134, 9–17.

Nacci, D., Proestou, D., Champlin, D., Martinson, J., & Waits, E. R. (2016). Genetic basis for rapidly evolved tolerance in the wild: Adaptation to toxic pollutants by an estuarine fish species. *Molecular Ecology*, 25, 5467–5482.

Nacci, D. E., Champlin, D., Coiro, L., McKinney, R., & Jayaraman, S. (2002). Predicting the occurrence of genetic adaptation to dioxinlike compounds in populations of the estuarine fish *Fundulus heteroclitus*. *Environmental Toxicology and Chemistry*, 21, 1525–1532.

Nukaya, M., Lin, B. C., Glover, E., Moran, S. M., Kennedy, G. D., & Bradfield, C. A. (2010). The aryl hydrocarbon receptor-interacting protein (AIP) is required for dioxin-induced hepatotoxicity but not for the induction of the Cyp1a1 and Cyp1a2 genes. *The Journal of Biological Chemistry*, 285, 35599–35605.

Oleksiak, M. F., Karchner, S. I., Jenny, M. J., Franks, D. G., Mark Welch, D. B., & Hahn, M. E. (2011). Transcriptomic assessment of resistance to effects of an aryl hydrocarbon receptor (AHR) agonist in embryos of Atlantic Killifish (*Fundulus heteroclitus*) from a marine superfund site. *BMC Genomics*, 12, 263.

Orr, H. A., & Unckless, R. L. (2014). The population genetics of evolutionary rescue. *PLoS Genetics*, 10, e1004551.

Osterberg, J. S., Cammen, K. M., Schultz, T. F., Clark, B. W., & Di Giulio, R. T. (2018). Genome-wide scan reveals signatures of selection related to pollution adaptation in non-model estuarine Atlantic killifish (*Fundulus heteroclitus*). *Aquatic Toxicology*, 200, 73–82.

Ownby, D. R., Newman, M. C., Mulvey, M., Vogelbein, W. K., Unger, M. A., & Arzayus, L. F. (2002). Fish (*Fundulus heteroclitus*) populations with different exposure histories differ in tolerance of creosote-contaminated sediments. *Environmental Toxicology and Chemistry*, 21, 1897–1902.

Oziolor, E. M., Reid, N. M., Yair, S., Lee, K. M., Guberman VerPloeg, S., Bruns, P. C., Shaw, J. R., Whitehead, A., & Matson, C. W. (2019). Adaptive introgression enables evolutionary rescue from extreme environmental pollution. *Science*, 364, 455–457.

Palumbi, S. R. (2001). Evolution – Humans as the world's greatest evolutionary force. *Science*, 293, 1786–1790.

Papadopulos, A. S. T., Helmstetter, A. J., Osborne, O. G., Comeault, A. A., Wood, D. P., Straw, E. A., Mason, L., Fay, M. F., Parker, J., Dunning, L. T., Foote, A. D., Smith, R. J., & Lighten, J. (2021). Rapid parallel adaptation to anthropogenic heavy metal pollution. *Molecular Biology and Evolution*, 38, 3724–3736.

Pélissié, B., Chen, Y. H., Cohen, Z. P., Crossley, M. S., Hawthorne, D. J., Izzo, V., & Schoville, S. D. (2022). Genome resequencing reveals rapid, repeated evolution in the Colorado potato beetle. *Molecular Biology and Evolution*, 39, msac016.

Pennings, P. S., & Hermisson, J. (2006). Soft sweeps III: The signature of positive selection from recurrent mutation. *PLoS Genetics*, 2, e186.

Pesch, C. E., Voyer, R. A., Copeland, J., Morrison, G., & Lund, J. (2001). *Imprint of the past: Ecological history of New Bedford Harbor*. U.S. Environmental Protection Agency, Region 1.

Prasch, A. L., Teraoka, H., Carney, S. A., Dong, W., Hiraga, T., Stegeman, J. J., Heideman, W., & Peterson, R. E. (2003). Aryl hydrocarbon receptor 2 mediates 2,3,7,8-tetrachlorodibenzo-p-dioxin developmental toxicity in zebrafish. *Toxicological Sciences*, 76, 138–150.

Prince, R., & Cooper, K. R. (1995). Comparisons of the effects of 2,3, 7,8-tetrachlorodibenzo-p-dioxin on chemically impacted and non-impacted subpopulations of *Fundulus heteroclitus* .1. Tcdd toxicity. *Environmental Toxicology and Chemistry*, 14, 579–587.

Pritchard, J. K., & Di Rienzo, A. (2010). Adaptation – Not by sweeps alone. *Nature Reviews. Genetics*, 11, 665–667.

Pritchard, J. K., Pickrell, J. K., & Coop, G. (2010). The genetics of human adaptation: Hard sweeps, soft sweeps, and polygenic adaptation. *Current Biology*, 20, R208–R215.

Proestou, D. A., Flight, P., Champlin, D., & Nacci, D. (2014). Targeted approach to identify genetic loci associated with evolved dioxin tolerance in Atlantic Killifish (*Fundulus heteroclitus*). *BMC Evolutionary Biology*, 14, 7.

Reid, N. M., Jackson, C. E., Gilbert, D., Minx, P., Montague, M. J., Hampton, T. H., Helfrich, L. W., King, B. L., Nacci, D. E., Aluru, N., Karchner, S. I., Colbourne, J. K., Hahn, M. E., Shaw, J. R., Oleksiak, M. F., Crawford, D. L., Warren, W. C., & Whitehead, A. (2017). The landscape of extreme genomic variation in the highly adaptable Atlantic killifish. *Genome Biology and Evolution*, 9, 659–676.

Reid, N. M., Proestou, D. A., Clark, B. W., Warren, W. C., Colbourne, J. K., Shaw, J. R., Karchner, S. I., Hahn, M. E., Nacci, D., Oleksiak, M. F., Crawford, D. L., & Whitehead, A. (2016). The genomic landscape of rapid repeated evolutionary adaptation to toxic pollution in wild fish. *Science*, 354, 1305–1308.

Reitzel, A. M., Karchner, S. I., Franks, D. G., Evans, B. R., Nacci, D., Champlin, D., Vieira, V. M., & Hahn, M. E. (2014). Genetic variation at aryl hydrocarbon receptor (AHR) loci in populations of Atlantic killifish (*Fundulus heteroclitus*) inhabiting polluted and reference habitats. *BMC Evolutionary Biology*, 14, 6.

Rivkin, L. R., Santangelo, J. S., Alberti, M., Aronson, M. F. J., de Keyzer, C. W., Diamond, S. E., Fortin, M., Frazee, L. J., Gorton, A. J., Hendry, A. P., Liu, Y., Losos, J. B., MacIvor, J. S., Martin, R. A., McDonnell, M. J., Miles, L. S., Munshi-South, J., Ness, R. W., Newman, A. E. M., …

Johnson, M. T. J. (2019). A roadmap for urban evolutionary ecology. *Evolutionary Applications*, 12, 384–398.

Rubin, C.-J., Megens, H.-J., Barrio, A. M., Maqbool, K., Sayyab, S., Schwochow, D., Wang, C., Carlborg, Ö., Jern, P., Jørgensen, C. B., Archibald, A. L., Fredholm, M., Groenen, M. A. M., & Andersson, L. (2012). Strong signatures of selection in the domestic pig genome. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 19529–19536.

Sen, Ś., Johannes, F., & Broman, K. W. (2009). Selective genotyping and phenotyping strategies in a complex trait context. *Genetics*, 181, 1613–1626.

Shankar, P., Dasgupta, S., Hahn, M. E., & Tanguay, R. L. (2020). A review of the functional roles of the zebrafish aryl hydrocarbon receptors. *Toxicological Sciences*, 178, 215–238.

Sih, A., Ferrari, M. C. O., & Harris, D. J. (2011). Evolution and behavioural responses to human-induced rapid environmental change: Behaviour and evolution. *Evolutionary Applications*, 4, 367–387.

Smith, T. B., & Bernatchez, L. (2008). Evolutionary change in human-altered environments. *Molecular Ecology*, 17, 1–8.

Souder, J. P., & Gorelick, D. A. (2019). ahr2, but not ahr1a or ahr1b, is required for craniofacial and fin development and TCDD-dependent cardiotoxicity in zebrafish. *Toxicological Sciences*, 170, 25–44.

Stetter, M. G., Thornton, K., & Ross-Ibarra, J. (2018). Genetic architecture and selective sweeps after polygenic adaptation to distant trait optima. *PLoS Genetics*, 14, e1007794.

Tang, H., Zhang, X., Miao, C., Zhang, J., Ming, R., Schnable, J. C., Schnable, P. S., Lyons, E., & Lu, J. (2015). ALLMAPS: Robust scaffold ordering based on multiple maps. *Genome Biology*, 16, 3.

Therkildsen, N. O., Wilder, A. P., Conover, D. O., Munch, S. B., Baumann, H., & Palumbi, S. R. (2019). Contrasting genomic shifts underlie parallel phenotypic evolution in response to fishing. *Science*, 365, 487–490.

Tilman, D., & Lehman, C. (2001). Human-caused environmental change: Impacts on plant diversity and evolution. *Proceedings of the National Academy of Sciences United States of America*, 98, 5433–5440.

Van Etten, M., Lee, K. M., Chang, S.-M., & Baucom, R. S. (2020). Parallel and nonparallel genomic responses contribute to herbicide resistance in Ipomoea purpurea, a common agricultural weed. *PLoS Genetics*, 16, e1008593.

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science*, 277, 494–499.

Waits, E., Martinson, J., Rinner, B., Morris, S., Proestou, D., Champlin, D., & Nacci, D. (2016). Genetic linkage map and comparative genome analysis for the Atlantic killifish (*Fundulus heteroclitus*). *Open Journal of Genetics*, 6, 28–38.

Wang, X., & Kruglyak, L. (2014). Genetic basis of haloperidol resistance in Saccharomyces cerevisiae is complex and dose dependent. *PLOS Genetics*, 10, e1004894.

Weinreich, D. M., Delaney, N. F., DePristo, M. A., & Hartl, D. L. (2006). Darwinian evolution can follow only very few mutational paths to fitter proteins. *Science*, 312, 111–114.

White, S. S., & Birnbaum, L. S. (2009). An overview of the effects of dioxins and dioxin-like compounds on vertebrates, as documented in human and ecological epidemiology. *Journal of Environmental Science and Health, Part C*, 27, 197–211.

Whitehead, A., Clark, B. W., Reid, N. M., Hahn, M. E., & Nacci, D. (2017). When evolution is the solution to pollution: Key principles, and lessons from rapid repeated adaptation of killifish (*Fundulus heteroclitus*) populations. *Evolutionary Applications*, 10, 762–783.

Whitehead, A., Pilcher, W., Champlin, D., & Nacci, D. (2012). Common mechanism underlies repeated evolution of extreme pollution tolerance. *Proceedings of the Royal Society B: Biological Sciences*, 279, 427–433.

Whitehead, A., Triant, D. A., Champlin, D., & Nacci, D. (2010). Comparative transcriptomics implicates mechanisms of evolved pollution tolerance in a killifish population. *Molecular Ecology*, 19, 5186–5203.

Williams, L. M., & Oleksiak, M. F. (2011). Ecologically and evolutionarily important SNPs identified in natural populations. *Molecular Biology and Evolution*, 28, 1817–1826.

Wirgin, I., Roy, N. K., Loftus, M., Chambers, R. C., Franks, D. G., & Hahn, M. E. (2011). Mechanistic basis of resistance to PCBs in Atlantic tomcod from the Hudson River. *Science*, 331, 1322–1325.

Wright, K. M., Hellsten, U., Xu, C., Jeong, A. L., Sreedasyam, A., Chapman, J. A., Schmutz, J., Coop, G., Rokhsar, D. S., & Willis, J. H. (2015). Adaptation to heavy-metal contaminated environments proceeds via selection on pre-existing genetic variation. *bioRxiv* 029900.

Wright, K. M., Lloyd, D., Lowry, D. B., Macnair, M. R., & Willis, J. H. (2013). Indirect evolution of hybrid lethality due to linkage with selected locus in *Mimulus guttatus*. *PLoS Biology*, 11, e1001497.

Xu, S. (2008). Quantitative trait locus mapping can benefit from segregation distortion. *Genetics*, 180, 2201–2208.

Yeaman, S. (2015). Local adaptation by alleles of small effect. *The American Naturalist*, 186(Suppl 1), S74–S89.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.