Solving an evolutionary puzzle

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In a new paper published in *BMC Evolutionary Biology*, researchers found that changes in a receptor protein, called the aryl hydrocarbon receptor 2 (AHR2), may explain how killifish in New Bedford Harbor evolved genetic resistance to PCBs. Credit: Evan D'Alessandro, Rosenstiel School of Marine and Atmospheric Science

For four decades, waste from nearby manufacturing plants flowed into the waters of New Bedford Harbor—an 18,000-acre estuary and busy seaport. The harbor, which is contaminated with polychlorinated biphenyls (PCBs) and heavy metals, is one of the EPA's largest Superfund cleanup sites.

It's also the site of an evolutionary puzzle that researchers at Woods Hole Oceanographic Institution (WHOI) and their colleagues have been working to solve.

Atlantic killifish—common estuarine fishes about three inches long—are not only tolerating the toxic conditions in the harbor, they seem to be thriving there. How have they been able to adapt and live in such a highly contaminated environment?

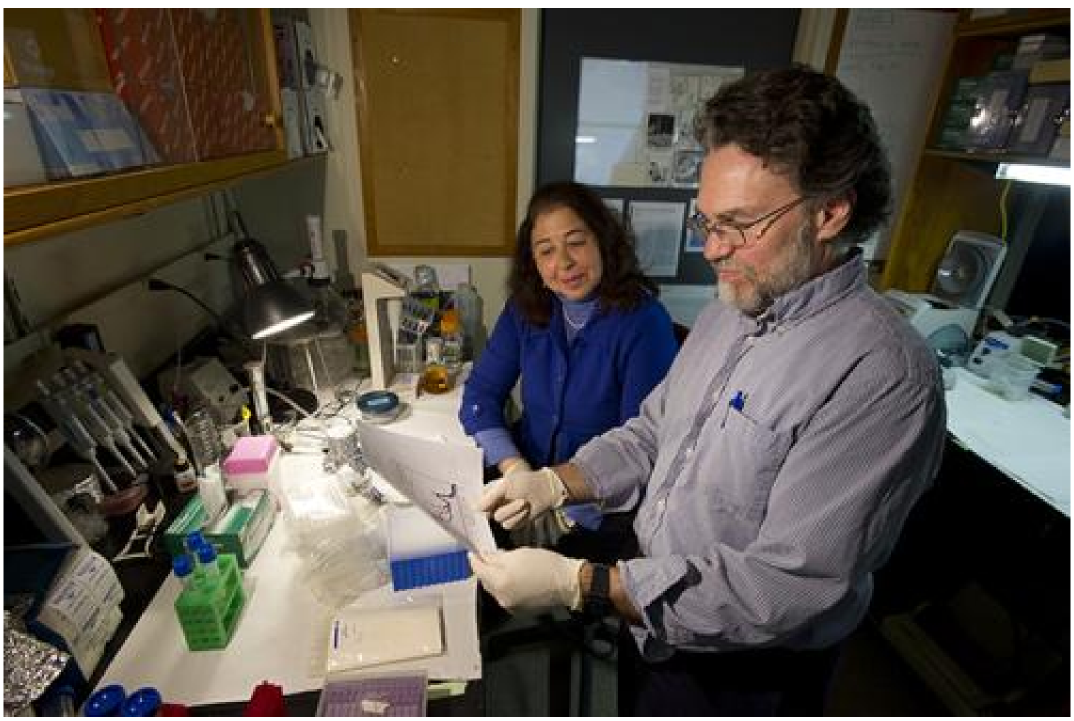
In a new paper published in *BMC Evolutionary Biology*, researchers found that changes in a receptor protein, called the aryl hydrocarbon receptor 2 (AHR2), may explain how killifish in New Bedford Harbor evolved genetic resistance to PCBs.

Killifish are prey fish that do not migrate. They live their whole lives in the same area, generally within a few hundred yards of the spot where they were hatched. Unlike fish that may come in and out of the harbor sporadically during the summer months to feed, the killifish are there year round and spend winters burrowing into the contaminated sediment.

Normally when fish are exposed to harmful chemicals, the body steps up production of enzymes that break down the pollutants, a process controlled by the AHR2 protein. Some of the PCBs are not broken down in this way, and their continued stimulation of AHR2 disrupts cellular functions, leading to toxicity. In the New Bedford Harbor killifish, the AHR2 system has become resistant to this effect.

"The killifish have managed to shut down the pathway," said Mark Hahn, a biologist at WHOI and coauthor of the paper. "It's an example of how some populations are able to adapt to changes in their environment—a snapshot of evolution at work."

The research team, which includes colleagues from the Atlantic Ecology Division of the U.S. Environmental Protection Agency, the Boston University School of Public Health, and the University of North Carolina at Charlotte, used a "candidate gene" approach, sequencing the protein-coding portion of three candidate resistance genes (AHR1, AHR2, AHRR) in fish from the New Bedford site and six other locations, both clean and polluted, along the northeast coast.



"It's a fascinating example of how human activities can drive evolution," said Mark Hahn, shown here at work in the lab with biologist Diana Franks. Credit: Photo by Tom Kleindinst, Woods Hole Oceanographic Institution

Looking for single nucleotide polymorphisms (SNPs) or subtle variations in the DNA sequence, they found differences in AHR2, which plays an important role in mediating toxicity in early life stages.

"The function of this receptor is what mediates the toxic effects," said Sibel Karchner, a coauthor and biologist in Hahn's lab. "If you don't have a functional receptor, then you're not going to get the toxic effects as much as a fish that does."

AHR2 in killifish has 951 amino acids and nine of those vary among individuals. The different combinations of amino acid variants lead to 26 different forms of the protein.

"We see that the pattern of variants present in the New Bedford Harbor killifish is much different from the patterns at nearby sites, which is unexpected under normal circumstances," Hahn said. "There are a few protein variants that are common in New Bedford Harbor killifish, but uncommon elsewhere. Similarly, the protein variants that are most common at the nearby reference sites are much less common in the New Bedford Harbor killifish."

A companion paper published in BMC Evolutionary Biology by colleagues at the EPA lab in Narragansett, RI, that used a "candidate gene scan" approach—examining SNPs from 42 genes associated with the AHR pathway—also identified AHR2 as a gene that appears to be under selection and is likely to be involved in the resistance. The results suggest that evolution of resistance in independent populations of killifish converges on the same target gene.

"The results of these studies and the genetic tools developed in the course of these studies are helping to dissect how evolution occurs on a contemporary (rather than geological) scale and why some species are more likely to adapt to a rapidly changing world," said Diane Nacci, a research biologist at EPA and coauthor on both papers.

AHR2 is also the same gene identified in a 2011 Science paper by WHOI biologists and colleagues from New York University and NOAA on PCB-resistant tomcod from the Hudson River. AHR2 proteins in the Hudson River tomcod are missing two of the 1,104 amino acids normally found in this protein.

"Even though the specific molecular changes that are found in PCB-resistant tomcod and killifish are different, in both species AHR2 seems to be one of the genes—possibly the major gene—that is responsible for the resistance," Hahn said.

While the killifish themselves seem to be immune to the toxic effects of the PCBs, they can still transfer contaminants up the food chain. They're a major source of food for bluefish, striped bass and other fish eaten by humans.

Despite their healthy appearance, there could be unknown negative costs for the New Bedford Harbor killifish associated with the resistance to PCBs. Researchers will look next at whether the adaptation affects how the killifish are able to respond to other kinds of stressors in their environment, such as low oxygen levels.

"Obviously, the fact that they are resistant to PCBs allows them to survive in this really polluted environment, but what will happen once the harbor gets cleaned up? There could be costs that make it no longer adaptive for these fish to live there," Hahn said.

"It's a fascinating example of how human activities can drive evolution," he added. "The ability to adapt to changing conditions is going to become even more important as humans impact the environment, whether it's from ocean acidification or increasing temperatures or other types of global changes that are occurring."

Provided by Woods Hole Oceanographic Institution

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