

Study reveals genes that 'don't play well together' in swordtail fish hybrids drive speciation

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The sheephead swordtail fish species (pictured left) has a reduced sword, a large body, and unique body patterning and coloration compared to its cousin, the highland swordtail (pictured center). The hybrid of these two species (pictured right) displays a blend of these characteristics. Credit: Schumer Lab

Stanford researchers have gained new insights into how the rise of reproductive barriers between organisms creates new species and drives the incredible diversification of life on Earth.

One major way that creatures become distinct from each other as separate species is through "hybrid incompatibility"—a phenomenon in which the offspring of two intermingling and still closely [related species](#) have health problems or reduced reproductive capabilities. A familiar

example of hybrid incompatibility is a mule, the sterile offspring of a male donkey and a female horse.

Pinpointing how hybrid incompatibility operates at the genetic level, however, has long eluded scientists and left fundamental questions unanswered at the heart of the origin of species.

Now, working with populations of swordtail fish, Stanford researchers have discovered a set of genes, known as Complex I genes, involved in major developmental issues and early deaths of hybrid fish sired by two swordtail species. The findings bolster the theory that troublesome genetic mismatches are likeliest to crop up in rapidly evolving genes, especially in cases where multiple genes must operate in sync for an organism to survive.

The paper summarizing this research was [published](#) Jan. 10 in the journal *Nature*.

"We have identified combinations of genes that don't play well together when they are inherited by hybrid offspring," said Ben Moran, a doctoral student in the lab of Molly Schumer, an assistant professor of biology in the Stanford School of Humanities and Sciences. "Studying this model system in swordtail fish can help us learn more about what forms [new species](#) and keeps them separate."

"As biologists, we're really interested in the diversity of life we see around us, and we want to understand how that diversity is happening at the [genetic level](#)," Schumer said. "But it's been very difficult to get empirical data on individual genes that cause the problems of hybrid incompatibility. With this swordtail study, we had the opportunity to understand the whole arc of genes impacting an organism."

Coming back together after having gone their

separate ways

The fish at the center of the research are two closely related species, *Xiphophorus birchmanni* (also known as sheephead swordtails) and *Xiphophorus malinche* (or highland swordtails).

As their common names suggest, many swordtail males have a pointy ornamental extension to their tail fins. *X. birchmanni*, however, have evolved to lack this feature. Compared to *X. malinche*, they are also larger bodied and display different body pattern colorations as compared to their *X. malinche* cousins, among other differences.

Befitting their differences in appearance, the two fish species have likewise evolved to occupy different areas in the rivers they share in central Mexico; *X. malinche* opts for higher-elevation headwaters and *X. birchmanni* prefers the lower-elevation downstream regions.

Starting in the 1990s, researchers began documenting unusual-looking blended versions of these fish living in rivers, suggesting hybridization, which was later confirmed through genetic testing. Scientists believe the ongoing *X. birchmanni* and *X. malinche* reproductive rendezvous are actually cases of mistaken identity ultimately due to the increased human presence in the area. Contaminant runoff from farms and other industry into river waters has possibly scrambled the olfactory and visual cues the respective fish species have normally relied on to tell each other apart.

"This breaking down of reproductive barriers for naturally hybridizing swordtails has offered us a unique means of studying the genetics of hybrid incompatibility," said Schumer, who has been traveling to the Hidalgo, Mexico, area for 10 years to investigate the piscine populations.

On the hunt for clashing genes

To identify potential genes of interest, Moran, Schumer, and colleagues sequenced swordtail genomes to establish the DNA fingerprints associated with each species. Sequencing the genomes also gave them access to the genes in the swordtail mitochondria.

Mitochondria harbor their own distinct genome, a relic of when these once-free-living microbes formed a symbiosis with ancient cells more than a billion years ago. Compared to the bulk of an organism's genes, which are located in the nuclei of cells, mitochondrial genes undergo far more rapid evolution. This occurs for several reasons including their use of different DNA repair pathways and their lack of other genetic processes like recombination (the shuffling and exchange of genetic material to form new genetic combinations).

Prior research by Schumer suggested that combinations of mitochondrial genes and nuclear genes that must work together to perform a critical function could be good candidates for causing hybrid incompatibilities in these fish. Accordingly, the researchers zeroed in on the genes that code for the proteins that make up Complex I—a highly intricate enzyme needed for producing cellular "fuel" in mitochondria.

Following this lead, the Stanford team and colleagues saw that, sure enough, hybrid swordtails with certain combinations of these Complex I genes displayed hybrid incompatibility. The affected fish suffered from significant developmental issues, especially developmental delays and cardiovascular defects, which often led to death before or shortly after birth.

"When combined from parents from two different swordtail species, these genes did not make compatible proteins for making a well-functioning Complex I, and the hybrid children failed to thrive," Moran said.

"The hybrid incompatibility we see for some offspring of *X. birchmanni* and *X. malinche* with these genes is a rare, empirical case where we really know the [genes](#) that are causing a problem and how they evolve," Schumer said.

Looking ahead, the Stanford researchers plan to dig more deeply into the nitty-gritty of the hybridized protein complex. "From a molecular biology standpoint, there are a lot of interesting questions about why this big molecule [Complex I] doesn't work that we hope to figure out," Moran said.

On a broader scale, the study also adds to the accumulating body of evidence that hybridization is more common between closely related species groups than had been thought. Many aspects of the evolutionary impacts and dynamics of this gene exchange, however, remain poorly understood.

"Understanding the link between evolutionary processes and what's going on in actual [species](#) is really exciting to me," Schumer said.

More information: Benjamin M. Moran et al, A lethal mitonuclear incompatibility in complex I of natural hybrids, *Nature* (2024). [DOI: 10.1038/s41586-023-06895-8](https://doi.org/10.1038/s41586-023-06895-8)

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