

When boy fish build castles to impress girls, boy genes get 'turned on' and 'tuned in'

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This particular cichlid in an aquarium at Georgia Tech is a hybrid cross of two species, one that makes pits and one that makes castles. The two behaviors combined in one animal allows researchers to connect gene regulation with either specific behavior. Credit: Georgia Tech / Rob Felt

Call it instinct, but something compels some animals to behave in certain ways, perhaps programs in their genes. Researchers have directly



connected activities of genes with instinctive behavior in little male fish that make patterns in the sand to attract their mates.

Scientists at the Georgia Institute of Technology and Stanford University who led the new study hope in the future to see if some behaviors are indeed genetic programs. If they are clicking off via gene regulation to fire neuronal patterns thus creating behavior.

"We're not there yet, but we're beginning to get a handle on gene regulation patterns that drive the neuronal patterns," said Todd Streelman, a professor in Georgia Tech's School of Biological Sciences and also its chair. "We were able to see that there's a clear connection between gene expression and behavior."

Better understanding autism

The research also may contribute someday to a better understanding of autism because the genes behind the fish behavior have human cousins that are implicated in autism spectrum disorder. And some typical autism behaviors like "stacking," in which a child compulsively arranges objects into neat rows or towers, has parallels in how the fish, cichlids, repetitively pile up sand to make symmetrical formations.

But for now, the researchers are exploring male cichlids who are trying to attract a mate in Lake Malawi in Africa. In their study, they found that the regulation of specific genes and the occurrence of repetitive behavior associated with them went together nearly hand-in-glove, a novel discovery.

They published their results in the journal *Proceedings of the National Academy of Sciences*. The research was funded by the National Institute of Neurological Disorders and Stroke, the National Institute on Aging, and the National Institute of General Medicine, all part of the National



Institutes of Health.

Additionally, little is known about how genes underlying behavior evolve over time, and the researchers found signs that their evolution may center around gene regulation in response to what's going on in the animal's environment. In the case of male cichlids, the gene regulation and the behavior are triggered when females ready to mate show up.

Dig my castle

Let's start with the behavior then go to the gene activity.

Boy cichlids knock themselves out building stuff out of sand to impress girl fish ready to mate. Most cichlid <u>species</u> build a pit, or crater, which appears to be the evolutionarily older and better-established behavior, and other species build a <u>castle</u>, which is widely accepted as being the newer evolutionary development.

Both pits and castles are known as "bowers" and require the fish to swim in the same circle, scooping up sand in one place and spitting it out somewhere else.

The difference is that the pit builders scoop up the sand from inside of the circle they're swimming in and deposit it outside. That leaves a hole in the middle of the bower with a raised rim surrounding it that makes it resemble a crater.

Castle builders scoop the sand from outside the circle and deposit it inside, which creates a raised structure in the middle of the bower so that it resembles a volcano.





Todd Streelman studies genes in cichlid fish that are connected to their mating behavior. Cousins of these genes are implicated in autism spectrum disorder in humans, and there are similarities in behavior. Streelman is chair of the School of Biological Sciences at the Georgia Insitute of Technology. Credit: Georgia Tech / Rob Felt

Turning him on

"A switch goes on once the females become reproductively active. Suddenly, the males begin scooping and spitting thousands of times to build their structure," said Zachary Johnson, a postdoctoral researcher in Streelman's Lab. Johnson was a co-author on the new study; Streelman co-principal investigator.

Scooping and spitting are so incessant that two-inch fish shovel up two-



foot-wide structures: pit bowers for some species, castle bowers for others. The difference serves in attracting the right mate.

"Various species make their pits and castles in a common area, so structures have to be very specific, so the right female species can see, 'This is the guy that I want' compared to the other guys from other species that build the other thing. And she then has to pick the specific guy she wants from her own species," said Chinar Patil, a co-first author of the study and a graduate research assistant in Streelman's lab.

Cross-breeding cichlids

To observe the genes connected to either of these building behaviors, researchers have cross-mated pit-building species with castle-building species to make hybrid cichlids that have both sets of genes. These hybrids have delivered a lucky surprise.

The hybrid fish performed both behaviors neatly in sequence: first the pit making, then the castle making, always in that order.

"That's amazing," Johnson said. "You might expect hybrid behavior to be jumbled, or take on some intermediate form. Instead, they perform one species-specific behavior and then transition to performing the other species-specific behavior."

Bower genes power up

This is useful to research because the hybrids have one full copy of genes from the pit parent and one from the castle parent. The cleanly separated behaviors have allowed for matching each behavior with increased and decreased activation in either set of genes in the fish's brains.



The Georgia Tech and Stanford researchers were able to clearly match pit gene activation with pit behavioral mode as well as castle gene activation with castle behavioral mode.

"A lot of genes in the pit copy got up-regulated while the fish was in pit-making mode and the castle copy got up-regulated during castle-making mode," Patil said. The genes and the behavior got "turned on" and "tuned in" in tandem.

The difference in expression of either pit vs. castle genes was less of an absolute click-clack-on-off switch and more like inching one set of levers down on an audio mixer and tuning up the other set to a dominant level.

Gene-behavior evolution

Finding that was the study's big achievement. That almost sounds like genes directly creating behavior, but that's unconfirmed as of yet and possibly the topic of future study.

Then there was that insight about the genetic evolution connected to behavioral evolution:

Pit and castle species have very similar genomes. When the team sequenced the DNA of pit and castle species, evolutionary differences appeared to lie in regulatory genes and they were many of the same regulatory genes that turn on and tune in for specific bower building behavior that happens in the mating context.

More information: Ryan A. York et al, Behavior-dependent cis regulation reveals genes and pathways associated with bower building in cichlid fishes, *Proceedings of the National Academy of Sciences* (2018). DOI: 10.1073/pnas.1810140115



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