

Getting on top, genetically

Study shows rapid genetic response to social opportunity in cichlid fish

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Sudden change in social status triggers genetic response in male fish, study finds

Throughout the animal kingdom, rival males routinely challenge one another for the right to reproduce. From the head-on collisions between bighorn rams to the ritualized wrestling matches of male rattlesnakes, combat is often the key to reproductive success.

But now scientists studying a species of African cichlid fish have discovered that low-ranking male cichlids can quickly become leading men without even putting up a fight. In fact, a drab subordinate male cichlid will begin physically transforming into a colorful dominant male as soon as he notices that his competition is no longer around, according to a new study conducted at Stanford University.

"We show for the first time that subordinate males can become dominant within minutes of an opportunity to do so, displaying dramatic changes in body coloration and behavior," write neurobiologists Sabrina Burmeister, Erich Jarvis and Russell Fernald, co-authors of the study published in the Oct. 17 edition of the journal *PloS Biology*. During this radical makeover, the low-ranking male undergoes a rapid metamorphosis. His body color changes from dull gray to flashy blue or yellow, and a prominent black stripe ("eyebars") appears across his face. This physical transformation signals to males and females alike that he's the top fish now and will vigorously defend his newly acquired breeding grounds.

"We found that when the dominating male is removed, the subordinate male perceives an opportunity to advance in social status and responds both behaviorally and by turning on genes that ultimately make him capable of reproducing," notes Fernald, the Benjamin Scott Crocker Professor of Human Biology at Stanford. This finding offers the first direct evidence that changes in social status also trigger cellular and molecular changes in the brain, he adds, which could have significant implications for understanding how other vertebrates, including humans, respond to social information.

"I think there could be parallels in human social status change," Fernald says. "For example, if you're in a situation that's socially awkward, it may influence how well you can speak, or your sense of yourself may be altered. Those reactions have to have some kind of cellular underpinnings."

The study also contributes to a growing body of scientific evidence that fish are more than mindless creatures that instinctively swim about in search of food and mates. "Our study shows that the male cichlid is obviously interacting with the world around it," says Burmeister, a former postdoctoral fellow in the Fernald lab, now assistant professor of biology at the University of North Carolina-Chapel Hill. "The subordinate male is responding to the absence of another individual, so he has to have some kind of

understanding of what their relationship was in the past and what it is now. This implies a cognitive ability to process complex information, which is much more than we usually think of in fish."

Reversible dominance

The PLoS Biology study is the latest in a series of experiments from the Fernald lab on the behavior of *Astatotilapia (Haplochromis) burtoni*--one of hundreds of cichlid species that inhabit Lake Tanganyika and other freshwater lakes in East Africa. Like all cichlids, *A. burtoni* lives in a hierarchical social system where showy dominant males defend small territories that are used for courtship and breeding. However, dominance among male cichlids is reversible: If a subordinate successfully challenges a dominant male in a face-to-face confrontation, the dominant fish will lose his status and with it his vibrant coloring, black eyebar and the ability to produce sperm.

In earlier studies, Fernald and his colleagues discovered that a change in social status also causes a change in a group of brain cells that produce gonadotropin-releasing hormones--chemical signals from the brain to the gonads that regulate sexual development in all vertebrates, including people. As the male fish ascends toward dominance, these brain cells grow eight times bigger in volume and begin producing large amounts of the hormone. As a result, the fish becomes more aggressive, his appearance changes dramatically and his gonads mature. When the male descends in status, the opposite occurs--the hormone-producing brain cells decrease in size, the ostentatious colors and stripes disappear, and the testes shrink and the male becomes infertile.

"Previously we did an experiment in which we moved the subordinate male out of the tank and put him in a situation where he could rise and become dominant," Fernald says. "Because he was in a new situation, it took several days before he began to change physically and behaviorally."

But how would a subordinate male respond if the dominant fish were to suddenly disappear? To find out, Burmeister designed an experiment in which the dominant male was surreptitiously removed from the tank after dark. "Cichlids have great vision, but like all animals, they can't see in total darkness and basically do nothing," Fernald explains, "In some ways, this experiment was more like nature, where a predator comes in and picks off a dominant fish opening up the field for non-dominant animals."

Nighttime behavior

To observe nighttime behavior of the cichlids, Burmeister donned infrared night-vision goggles and kept close watch on the experimental fish tanks. She soon discovered that dominant males undergo a temporary change in the dark--their bright color turns pale, and their eyebar fades. "But when the lights come on, the dominant male slowly reactivates," she says. "He goes through a warm-up period in which his coloration and dominant behaviors return. Everyone in the tank accepts this process." Burmeister and her colleagues were the first to observe this diurnal transformation among dominants.

"During the experiment, I would remove the dominant male one hour before the lights came on," she says. "We used this nighttime-kidnap-the-bully approach as a gentle way

of not disturbing other fish in the tank."

Burmeister observed that after the lights came on, the subordinate male quickly determined that no dominant rival was in the tank and rapidly began his ascent toward domination. In minutes his coloring changed, his eyebar appeared and he began making threatening displays and chasing other fish. "Once they start changing, there's no question they'll become dominant within 2 to 10 minutes," Burmeister says. "It's like they decide to go for it: They know their place in the hierarchy, and they make a decision to change."

After 20 minutes, the ascending males were sacrificed and their brain cells prepared for laboratory analysis. The researchers wanted to see if they could identify the genes responsible for triggering the ascent to sexual dominance. They were particularly interested in a gene called *egr-1*, which produces a regulatory protein that activates other genes. The research team hypothesized that, when a male encounters an opportunity to climb the social ladder, the *egr-1* genes in his brain send an immediate signal to the gonadotropin-releasing hormones cells to start growing. As these cells get bigger, they crank out more and more hormones, transforming the male from doormat to dominant within 10 minutes.

In fact, laboratory analysis confirmed that there was twice as much *egr-1* gene expression in the brain cells of ascending cichlids than in either dominant or subordinate males, suggesting that the *erg-1* gene plays a crucial role in jumpstarting the whole physical transformation.

"This is the first study where I have seen gene activation due to not purely sensory or motor aspects of the behavior, but to a social opportunity," says co-author Jarvis, an associate professor of neurobiology at Duke University. "What I think is striking about this paper is how rapid the social change, the physiological sign of dominance and the associated genomic response occur."

Opportunity knocks

"These results show that the rapid behavioral responses to social opportunity were matched by a rapid genomic response in the brain," Fernald says. "We didn't expect to get changes in gene expression in the brain so early in the process, just 20 minutes after the male has seen the prospect for social change."

These results suggest that subordinate males are always looking for opportunities to change, Burmeister adds. "They must constantly be ready, because they make the shift in no time," she says. "They keep track of who's who and who's the biggest so they can take the opportunity to reproduce. An animal that goes through that kind of transition has to recall how he relates to other males, and also has to be aware that he can change. This raises a bigger question about sophisticated social awareness in fish. After all, socialization requires more brainpower, and all vertebrates--fish, amphibians, birds, humans--use a variety of cues in the environment."

The difficult question that remains, according to Fernald, is to figure out exactly how social information can trigger a genetic response that results in such a profound change in an animal's appearance and behavior.

"All we did was to change his social status, and yet it's reading from his brain right down to his gonads in a way that has to have a cellular and molecular underpinning," Fernald says. "In other vertebrates, it's the same process. Human males go through puberty, although it's not reversible, of course. However, we are social animals, and we respond to social situations. We may be seeing in cichlids the evolutionary basis of social systems, including our own."