



Customer case study
Field of study: Evolutionary Biology

Discovery of Gene Network Linked to Shifting Phenotype Offers Lessons in Evolution



New research from Dartmouth bioinformatician Thomas Hampton and collaborators used a dual-stressor system to discover important aspects of evolution in a fish with unique phenotypic plasticity. QIAGEN's Ingenuity Pathway Analysis helped them make the connection.

Thomas Hampton is no stranger to making sense of massive data sets. In fact, he's built his career around solving mega-scale information problems. As a senior bioinformatics analyst at the Geisel School of Medicine at Dartmouth College and part of the Dartmouth Toxic Metals Superfund Research Program, Hampton deploys his expertise for a range of studies, from cancer to epithelial cells' pathogen response to mercury in seafood. He focuses largely on gene expression, and uses Ingenuity Pathway Analysis (IPA) from QIAGEN to accelerate his research.

In one recent study, Hampton and his collaborators used IPA to characterize genes underlying an important biological mechanism in *Fundulus heteroclitus*, a type of killifish. Their results were published in *Molecular Biology and Evolution* in a paper entitled "Natural Selection Canalizes Expression Variation of Environmentally Induced Plasticity-Enabling Genes."

The paper sheds light on a poorly understood but critical trait in killifish, and would not have been possible without conducting a dual-stressor study, Hampton says. Such studies are rare in science because of concerns about confounding variability, but Hampton and his team were able to carefully craft their experiments and use IPA to determine the meaning of the gene expression data they generated. The result is a new look at the scale of evolution in killifish, which may contribute to a better appreciation of how evolution functions in other organisms too.

“

That's what Ingenuity helped us figure out. It's an interesting result that suggests that evolution has acted not just on genes but on gene networks to make phenotypic plasticity happen.

Morphing mummichog

Hampton and his collaborators, including Joseph Shaw at Indiana University and John Colbourne at the University of Birmingham, chose to study killifish for a most unusual trait: the fish is equally happy in fresh water or in salt water, due to its ability to change the morphology and function of its gills as needed to adjust for salinity levels in the water. "This is really special. They can remodel their gills on a week-to-week basis," Hampton says. "This would be like if anytime we needed to fly, we just sprouted a pair of wings."

The on-demand ability to remodel its gills makes this particular killifish, known as the mummichog, interesting for research related to wound healing or biological regeneration. It is also an intriguing model for understanding effects of climate change, since it can tolerate changes such as shifting salinity levels that are associated with global warming. At its

most basic level, remodeling a gill is a stress response, so elucidating this mechanism in killifish could also have a broader impact on figuring out stress reactions in general. The work was funded by the National Institute of Environmental Health Sciences.

To study gill remodeling, the scientists had to use two different stressors: first, the fish were moved from fresh water to salt water; second, they were exposed to trace amounts of arsenic in the water to inhibit the mummichog's ability to transform. While killifish, like many organisms, can tolerate arsenic well, previous work had shown that even small amounts of the toxin could prevent the gill remodeling process. As the killifish were exposed to both stressors, Hampton and his team interrogated gene expression with custom arrays to reveal what was going on.

Of course, seeing the expression levels from 135,000 probes is not inherently helpful. "If you measure all the genes in something, some are going to go up and some are going to go down and you're going to have a big problem on your hands trying to characterize what it means biologically," Hampton says. That's why he uses IPA: "Ingenuity really jump-starts that process."

In the killifish experiment, even gene expression analysis was not terribly insightful. "The kinds of genes engaged in this plasticity response were not that interesting, viewed from a distance," Hampton says. "It looks like a bunch of different genes that are not related to anything that makes a tremendous amount of sense."

Fortunately, the team used IPA to dig deeper, looking at the genes on a network level. "We found that the genes that facilitate phenotypic plasticity seem to be less connected to other genes, almost as if they're in their own little world, ready to be activated in one direction or another to facilitate this process without a lot of cross-talk from other systems," Hampton says. "That's what Ingenuity helped us figure out. It's an interesting result that suggests that evolution has acted not just on genes but on gene networks to make phenotypic plasticity happen."

In the paper, Hampton and his co-authors write, "These findings also provide a generalized, conceptual framework of how genes might interact with the environment and evolve towards the development of plastic traits."

"Let Ingenuity Do Its Thing"

Hampton's lab covers a lot more than killifish. Stress response is a common theme among his current projects, which include the evaluation of pathogen response from airway epithelial cells in humans; studies of *Daphnia* water fleas; and cancer research, including melanoma and renal cell carcinoma. He also hopes to follow up the mummichog experiment with a project analyzing these fish going from salt water to fresh to see whether the same patterns of reduced connectivity emerge.

“

When you get to these dynamically derived networks, you're going to see things [with Upstream Regulator Analysis] that you never would have imagined because you don't know the genes and you don't know their relationships to each other. That's hugely important, because you can't be an expert on every gene.

Through it all, Hampton is glad to have IPA in his toolbox. He uploads expression data from arrays as well as from RNA-seq experiments and “we let Ingenuity do its thing,” he says. Reports from the application yield both expected and unexpected findings, allowing Hampton to get back to the lab more quickly to test new hypotheses.

IPA's intuitive interface is one reason Hampton uses it regularly. He remembers that even the first time he used it, “within a couple of hours, without any explanation whatsoever, I was actually making progress.” That's not the case with most software. “You can learn the IPA interface on an as-needed basis. You don't

have to take courses or read vast manuals to get started,” he says.

Recently, Hampton has been making frequent use of IPA's Upstream Regulator Analysis tool to get the bigger picture of what's going on. He also focuses on network analysis, which has proven valuable for putting findings in context. “When you get to these dynamically derived networks, you're going to see things that you never would have imagined because you don't know the genes and you don't know their relationships to each other,” he says. “That's hugely important, because you can't be an expert on every gene.”

QIAGEN Silicon Valley

1700 Seaport Blvd. Third Floor
Redwood City, CA 94063

Tel. +1 650 381 5100
Fax. +1 650 381 5190

info@ingenuity.com
www.ingenuity.com

© QIAGEN 2015. All Rights Reserved.

