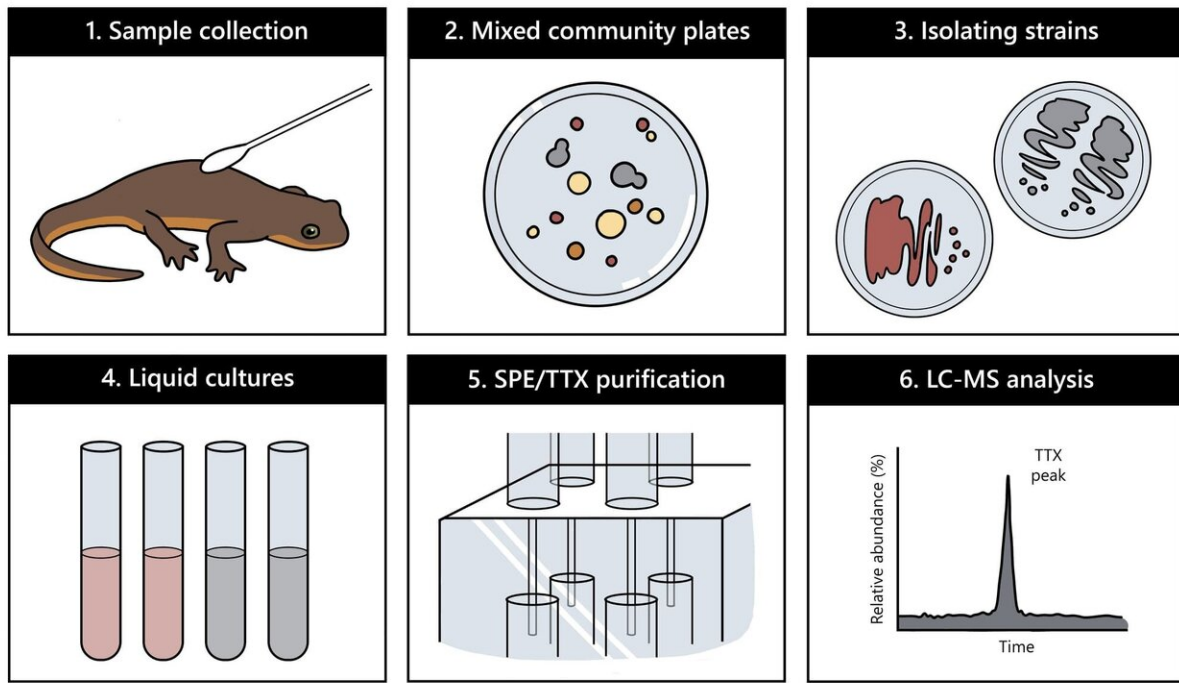


Skin microbiome of poisonous newts facilitates adaptive tetrodotoxin production

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The poisonous rough-skinned newt and the garter snake are locked in an evolutionary arms race, but the newt has a secret, invisible weapon, TTX producing bacteria on its skin. Credit: Ayley L. Shortridge

The textbook example in ecology, literally, goes like this: The poisonous rough-skinned newt and the garter snake are locked in an evolutionary arms race. The more resistant the snake becomes to the newt's

neurotoxic defense, the more deadly toxin the newt produces—in some newts, enough to kill two dozen humans.

But in a paper published in the open-access journal *eLife*, Michigan State University College of Natural Science, or NatSci, graduate Patric Vaelli, now conducting postdoctoral studies at Harvard, just made the story of the rough-skinned newt a lot more complex.

It turns out the arms race has an invisible, and highly influential, third party: neurotoxin-producing bacteria living on the newt's skin.

The BEACON

Seven years ago, NatSci's Heather Eisthen, a neuroethologist in the MSU Department of Integrative Biology and Vaelli's future advisor, gave a presentation at the BEACON Center for the Study of Evolution in Action.

"I was giving a talk about rough-skinned newts—charming, but deadly poisonous amphibians loaded with the neurotoxin tetrodotoxin, or TTX," said Eisthen, who has studied evolutionary changes in animal nervous systems and behavior at MSU for more than 23 years. "I explained that no one had ever identified the source of TTX on newts."

Vaelli came to MSU as a graduate student in Eisthen's lab around the same time, and the environment was perfect for cultivating the study. MSU is world-renowned for microbial ecology and evolution, from Richard Lenski's [E. coli Long-term Experimental Evolution Project](#) to the digital, evolving organisms of Charles Ofria, who helped land the National Science Foundation grant that founded the BEACON Center.

Vaelli's search for the source of the newts' TTX germinated with Eisthen's talk and grew with the help of two initial grants from the

BEACON Center.

The Bacteria

Vaelli knew from the literature that bacteria in puffer fish produced TTX, and he set out to find if it was the same with newts. He had little experience in microbiology, so Kevin Theis, assistant professor of [microbial ecology](#) at Wayne State University and co-author on the paper, gave him guidance on how to sequence the 16S ribosomal RNA gene, a kind of bar code used to identify bacteria.

"I bought some [petri dishes](#), read about culture media, poured plates, swabbed newts and began building a culture collection of bacteria that I stored in a deep freeze," Vaelli said.

Then Vaelli had to determine how the neurotoxin was produced, which turned out to be a major complication.

"I had to culture all of the bacteria and analyze the media for TTX production," Vaelli said. "Replicating the environment of a newt's skin and getting the bacteria to produce natural molecules was challenging."

After three years of screening hundreds of bacterial cultures at MSU's Mass Spectrometry Core Facility, Vaelli was able to identify TTX-producing bacterial strains from four genera—one of which turned out to be significantly correlated with the level of newt toxicity, *Pseudomonas*.

It was the first time TTX-producing bacteria had been identified on an amphibian, or any terrestrial animal.

The rough-skinned newt

After culturing [bacteria](#) for three years with little result, Vaelli was worried he may not have anything for his dissertation, so he began to look at how newts developed resistance to TTX.

Discovered in Japan in 1909, TTX is a well-studied molecule. It binds to a protein in the [nervous system](#) called voltage-gated sodium channels, or Nav, the very first proteins involved in generating neural electrical signals. TTX blocks Nav and prevents sodium from flowing into the cell, silencing neurons and causing muscle paralysis.

Complicating matters is the fact that the newts possessed not just one Nav protein, but six.

"I thought this was a cool molecular evolution question because we could ask what parts of the protein change and if the six proteins differed in how they evolved resistance," Vaelli said. "I sequenced five of the six genes (one gene had been previously sequenced) and found that all six had mutations, and they all evolved resistance independently."

Vaelli's results were a beautiful example of convergent evolution—where two different species evolve the same structure to achieve the same purpose—like wings on a bird and a butterfly.

"What is coming together is a whole new way of understanding natural selection," Eisthen said. "You cannot understand the whole picture of the [newt](#)/garter snake arms race unless you look at how microbes are directly intersecting with physiology."

More information: Patric M Vaelli et al. The skin microbiome facilitates adaptive tetrodotoxin production in poisonous newts, *eLife* (2020). [DOI: 10.7554/eLife.53898](https://doi.org/10.7554/eLife.53898)

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