Motherly poison frogs shed light on maternal brain

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A Climbing Mantella tadpole with a recently laid unfertilized egg. Credit: Alexandre Roland

For most frogs, motherhood begins and ends with the release of hundreds of eggs into a sizable body of water and then hopping or swimming away.

Two curious exceptions are the Little Devil frog of Ecuador and the Climbing Mantella of Madagascar. The females of both poison frog species lay only a few eggs at a time, depositing each one into its own tiny pool formed from the cupped leaves of native rainforest plants. The mothers then spend months painstakingly feeding each tadpole unfertilized eggs until they are ready to leave their aquatic nests.

Why all the devotion? A team led by Stanford biologist Lauren O'Connell has discovered an intriguing possible answer.

In a new study published Nov. 21 in the journal *Current Biology*, the scientists report that the nutritious eggs the frogs feed their hatchlings are also laced with poisons, likely as a way of passing chemical defenses on to the next generation.

"This egg provisioning strategy is a way for these frogs to chemically defend their offspring sooner," said O'Connell, who is an assistant professor of biology in the Stanford School of Humanities and Sciences.

By comparing the brains of the two frogs with each other and with mammals, the researchers also discovered new clues that have bearing on an even greater biological mystery: the neural basis of motherhood itself.

A female Little Devil frog. Credit: Vincent Prémel

Convergent evolution

Despite their convergent reproductive strategies, the Little Devil and the Climbing Mantella are about as different as two frog species can be. They each split from a common ancestor some 140 million years ago, around the time the first amphibians appeared, and have since evolved independently on opposite ends of the globe.

This early evolutionary schism, combined with their very un-froglike nursing strategy, makes them ideal for investigating the evolution of maternal care, O'Connell said. "Maternal behavior has evolved only once

in mammals," she added. "We wanted to know, are there different ways to build the maternal brain? Or do they all use the same mechanisms and molecules? These frogs allow us to investigate these questions."

Another reason these frogs are valuable case studies for maternal care is that only the mothers are involved in rearing the tadpoles. "Some frogs do have bi-parental care, but the neural mechanism behind that is complicated because you can't really disentangle the relationship with the tadpole from pair-bonding between the parents," said study co-author Nora Asher Moskowitz, a graduate student in O'Connell's lab. "These frogs allow you to study maternal care in isolation."

Waiting for mom

Members of O'Connell's team spent months in the rainforests of Ecuador and Madagascar documenting the mother frogs nursing their tadpoles in the wild. This involved many early mornings spent loitering near known nests, waiting for the frogs to make their weekly feeding rounds.

"The hardest part was just sitting really, really still. It's really hard to not scratch your face or move around," Moskowitz said.

A Little Devil tadpole. Credit: Vincent Prémel

Upon arriving at a nest, the mother frogs would wait for a sign from their tadpole before depositing unfertilized eggs into the pool. For the Little Devil frog, this signal took the form of an intense wriggle. "The tadpoles do this very short but intensive vibration right next to the mom," O'Connell said.

The researchers also collected brain tissue from the mothers and some of the unfertilized eggs the mothers had laid, samples of tadpole skin and water from their nesting pools for analysis.

A mother's brain

Back in the lab, the researchers found traces of alkaloid toxins on both the eggs and the tadpole skin, leading them to conclude that the tadpoles were obtaining their poisons primarily from the eggs the mothers provided. Previous studies had found poisons on the mother frogs and on the tadpoles, but not on the eggs themselves.

"Our finding is consistent with the idea that maternal provisioning evolved as a way to transfer nutrients but also various other goodies to your offspring," said study first author Eva Fischer, a postdoctoral fellow in O'Connell's lab. "We mammals transfer a lot of things that are important for immune function via breast milk. These frogs are transferring toxins through their eggs."

An examination of the collected frog brains turned up other surprises. The frogs employed two brain regions known to be active in nursing mammals, birds and fish.

"Both within frogs and also across vertebrates more generally, there seem to be shared brain regions that are being used to build the maternal brain," Fischer said.

However, when the scientists looked for neurons that respond to oxytocin, a brain chemical or neurotransmitter that is important for promoting maternal care in mammals, only the Climbing Mantella frog tested positive. Oxytocin did not seem to be important for nursing in the Ecuadorian frogs.

"This study tells us that there is more than one way to promote maternal behavior in the brain because these frog species are using different sets of neurons to do it," O'Connell said. "Their behaviors are similar, but under the hood, the neural mechanisms are very different."

More information: Eva K. Fischer et al. Mechanisms of Convergent

Egg Provisioning in Poison Frogs, *Current Biology* (2019). DOI: 10.1016/j.cub.2019.10.032