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Home / Blogs / March 27th, 2010; Vol.177 #7 / Science & the Public / [Blog entry](#)

Frogs: Weed killer creates real Mr. Moms

Some "chemically castrated" guys develop into egg-laying gals

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ENLARGE

BERKELEY'S AMPHIBIAN LAB 'RAT'

When this species is raised in the lab with water tainted with what EPA considers acceptable water levels of atrazine, males change — sometimes into apparent females.

Furryscaly/Flickr

Several months back, a Berkeley undergraduate began witnessing distinctly odd behavior in frogs she was caring for in the lab. At about 18-months old, some frisky guys began regularly mounting tank mates, as if to copulate. Except that their chosen partner was invariably male. He had to be. Because genetically, every animal in the tank was male.

What Ngoc Mai Nguyen didn't know at the time was that something in the water had reproductively altered several of the frogs to the point that they not only became submissive, like females, but also apparently sent out come-hither signals to the bro's in their tank.

"We were conducting a double-blind study, so I didn't know what treatment [if any] they were getting," Nguyen recalls. She did know, however, that each animal had been bred to ensure it was genetically male.

So she informed her boss, biologist Tyrone Hayes. "I told Tyrone, 'I don't know what's going on, but I don't think this is normal.'" He asked her to log the guys' behavior on a daily basis. And this confirmed some frogs definitely behaved like gals. Those who did even looked like females.

These frogs had been raised from hatching in tanks of water containing low levels of atrazine, a widely used weed killer. "What Mai discovered," Hayes says, "is that when they're in these tanks, atrazine-exposed males will copulate with other males." Indeed, he

notes, among the atrazine-exposed animals that Mai was caring for, some 30 percent exhibited this girly behavior. It's something Hayes previewed last Tuesday (Feb. 23) in a briefing before the Illinois legislature, in Springfield — and will describe in a paper that his group plans to submit for publication later this week.

In some regions of the country, Hayes says, year-round atrazine pollution of surface waters downstream of crop fields can match the concentrations that he used — 2.5 parts per billion in water. The Environmental Protection Agency allows up to 3 ppb of the herbicide in drinking water.

The new observations, outrageous as they may sound, expand upon findings to be published online by Hayes' team this week (ahead of print) in the *Proceedings of the National Academy of Sciences*. Those data demonstrate that chronic 2.5 ppb atrazine exposures can fully reassign the gender of some frogs. Indeed, the researchers report raising three generations of *Xenopus laevis* — African clawed frogs that serve as the amphibian equivalent of lab rats (from colonies that never contained a real female, one whose DNA contains the gene to develop into a mom).



ENLARGE



MORE THAN FRIENDS

Atrazine-treated males, seen here, pair up — but only when a fully feminized male (bottom) is present.

T.B. Hayes/UC Berkeley

Other research groups had seen evidence that atrazine can demasculinize — and in some instances feminize — exposed animals. But frogs don't have readily visible sex chromosomes, so it was hard to tell sometimes if feminized males were actually masculinized females. Or vice versa.

To focus on effects in males only, Hayes' team reared animals, crossing them in ways to get a line of pure males. Genetic males, anyway. Then he raised some of them in clean water. Others grew from larval stage to three-years post metamorphosis in tanks laced with trace concentrations of atrazine.

And compared to males growing in clean water, those chronically exposed to the weed killer developed few sperm, produced very low testosterone levels and failed to "sing" the calls that should invite females or repel competing would-be suitors.

But some of the males didn't just have diminished levels of the male sex hormone. Four out of 40 described in the new *PNAS* paper also produced elevated levels of estrogen, the primary female sex

hormone (not as high as true females would develop, Hayes notes, but far higher concentrations than a male should ever have). These animals also developed external reproductive organs typical of females and exhibited female behaviors.

Two were opened up and their internal reproductive organs were characteristic of females as well. The remaining two transgender animals were introduced to males who had grown up in tanks of clean water. The would-be females accepted the advances of the males, allowing them to fertilize their eggs. Which grew into healthy frogs.

Those offspring were, of course, all male. And if housed in clean water, they developed into robust normal males, Hayes says. If reared in atrazine-laced water, however, they showed the same demasculinization of the earlier generation. Some also mirrored their Mr. Mom, becoming a fertile female able to bear viable eggs.

But why did the Mr. Mom effects show up in only 10 percent of treated frogs? It appears to trace to genetic susceptibility, Hayes says. In subsequent studies, "we now see that the proportion of completely feminized males varies from family to family. We have some where 45 percent of the animals come up 'females'. . . . In others, more than half will be 'female.'"

One valuable aspect of the work reported in the new *PNAS* paper is that Hayes' group used a new genetic marker to confirm that the feminization they were seeing was not due to misidentified real females, observes Caren Helbing of the University of Victoria in British Columbia. Female frogs carry a gene known as DMW, the molecular biologist notes. But the apparent female frogs in the Berkeley study lacked this gene, confirming they were genetic males.

Owing to the Berkeley group's clever use of all-male starting populations, "any sex-reversal effects are unequivocal," says Vance Trudeau of the University of Ottawa in Ontario, Canada. Any behavioral or fertility changes become equally clear, he says.

His Ottawa group has recently been investigating the hormonal tinkering by which atrazine perturbs reproductive development in frogs. But without the DMW marker, his group has had to infer sex reversal based on whether a frog grows up to look like a male or female. Hayes' new study "is the type of experiment I would have liked to have conducted," Trudeau says. Bottom line, Trudeau argues: Between his team's outdoor studies with wild leopard frogs and the Berkeley group's controlled experiments in lab frogs, "the feminization effects of atrazine are clear" — and show up at concentrations of the pollutant currently found in the environment.

Syngenta, atrazine's primary North American manufacturer, disagrees. Said Steven Goldsmith, Syngenta's senior communications manager, in Greensboro, N.C., "this issue was resolved long ago. EPA's website says about amphibians [and atrazine]: 'EPA believes that no additional testing is warranted to address this issue.'"

Tim Pastoor, principal scientist at Syngenta Crop Protection, contends in an e-mail that in the *PNAS* paper, "Hayes repeatedly cites other researchers incorrectly, distorting their findings in order to bolster his own claims." Those citations do not, however, appear to directly affect the Berkeley team's interpretations of their new data. Pastoor also argues that data in the new paper contradict those in Hayes' earlier studies. "Either his current study discredits his previous work, or his previous work discredits this study," Pastoor charges. Pastoor does not, however, specify what those contradictions are.

Throughout the world, amphibians are on the decline. Probably for a long list of reasons. If common chemical pollutants, like atrazine, can depress the libido and behavior of males in the wild — as the Berkeley team reports that it is doing in their lab — then it could pose a risk to the survival of wild populations. Contends Helbing: "I definitely think that's a fair conclusion."

NEXT: Research finds cues to atrazine's amphibian tinkering

SUGGESTED READING :

CITATIONS & REFERENCES :

Hayes, T.B., et al. 2010. Atrazine Induces Complete Feminization and Chemical Castration in Male African Clawed Frogs (*Xenopus laevis*). *Proceedings of the National Academy of Sciences* (in press). DOI: 10.1073/pnas.0909519107