Legless Frog Mystery Solved

Ballengée and Sessions (2009. *Journal of Experimental Zoology (Molecular and Developmental Evolution)* 312B: Published online at www.interscience.wiley.com. DOI: 10.1002/jez.b.21296) presented evidence that the most commonly found deformities in wild-caught amphibians, those featuring missing limbs and missing limb segments, may be the result of selective predation. Predatory dragonfly nymphs can severely injure and even fully amputate developing hind limbs of anuran tadpoles. Dragonflies apparently like to eat the hind legs because, as tadpoles mature, poison glands develop elsewhere on their bodies much earlier than in the skin of their hind legs, rendering the hind legs a far more palatable meal. Developmental responses of the injured/amputated tadpole limbs range from complete regeneration to no regeneration, with intermediate conditions represented by various idiosyncratic limb deformities, depending mainly on the developmental stage of the tadpole at the time of injury/amputation. These findings were reinforced by experimental amputations of anuran tadpole hind limbs that resulted in similar deformities. These studies suggest that selective predation by dragonfly nymphs and other aquatic predators may play a significant role in the most common kinds of limb deformities found in natural populations of amphibians.

THE MYSTERY OF DEFORMED FROGS

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The reason we set out to research this question is a long story, dating back more than 15 years to when I published my first paper on deformed amphibians with supernumerary limbs. We discovered that those had been caused by parasites (i.e., parasitic flatworms, or trematodes). However, since 1995, deformed (or "malformed") frogs became one of the most contentious environmental controversies of all time (at least in the United States), with the parasite researchers on one side and the "chemical company" (as I call it) on the other. A veritable media firestorm ensued, and millions of dollars of grant money were at stake, almost all of it going to research on chemical pollution.

By now, however, I am pleased to say that the evidence that frogs with extra limbs (the oddest of the deformities) are caused by parasites is widely accepted among scientists. We have had publications in both the *Journal of Experimental Zoology* (summarized previously) and *Science*, and a couple of my colleagues (Pieter Johnson of the University of Colorado and Andrew Blaustein of Oregon State University) summarized the whole thing in an article in *Scientific American* in February 2003 (www.scientificamerican.com/article.cfm?id=explaining-frog-deformiti).

However, that's not the end of the story. Frogs with extra limbs may have had the most dramatic-looking deformities (indeed, these frogs have been used by everyone as the "poster child" of the deformed frog problem), yet everyone has always realized that they are by far the least common deformities found. The most commonly found deformities occur in frogs with missing or truncated limbs and limb segments (as in the paper summarized here), and, although parasites can occasionally cause limblessness in a frog, these deformities are almost never associated with trematode infections (or at least not with the trematode species known to cause extra limbs). So, this has remained a mystery, and again the controversy has continued to brew between those who suspect chemical pollution (or even UV-B irradiation) and those who suspect (in this case) some kind of predation.

Supporters of the chemical pollution hypothesis as a cause of limbless frogs point to the range of deformities seen in these

A cleared and stained multi-legged Pacific Treefrog (*Pseudacris regilla*; left). The deformity was caused by parasites, specifically a trematode in the genus *Ribeiroia*. The Northern Leopard Frog (*Lithobates pipiens*; right) has a missing left hindlimb (the left forelimb is normal, just bent back). The spike indicates that the limb was bitten off in the course of a selective predation event.

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frogs and argue that it is unlikely that predation is involved because it is difficult to imagine a predator that would remove the hindlimbs with surgical precision and allow the tadpole to survive. This is where Brandon Ballengée comes in. Brandon (an ecoartist) and I began collaborating some years back on a "SciArt" project to make artistically beautiful and scientifically interesting images of deformed amphibians. Brandon took this one step further and went to England to do graduate work (with me as scientific adviser) focused on limbless deformed amphibians — in this case toads. Imagine my surprise when he told me that he had discovered a predator that surgically removes the developing hindlimbs of tadpoles, thus creating limbless frogs! We spent the next few months analyzing the data and writing it up for publication, knowing that at least one other laboratory had independently discovered the same thing (we offered to collaborate or cooperate in some way, but they weren't interested).

The culprits, as you know, are dragonfly nymphs, which have mouthparts adapted to grab their prey, almost like a mechanical arm with a claw on the end that they can shoot out. Once they grab a tadpole, they use their front legs to turn it around and around, searching for the tender bits, in this case the hindlimb buds, which they then snip off with their mandibles (http://blip.tv/file/1418583). Often the tadpole is released and is able to swim away to live another day. If the tadpole survives, it metamorphoses into a toad with missing or deformed hindlimbs, depending on the developmental stage of the tadpole (at early stages, the tadpoles can completely regenerate their limbs, but this ability diminishes as they grow older). We think the dragonflies select the hindlimbs because toad tadpoles have poison glands in mature skin, and the developing hindlimbs have immature glands. We call this phenomenon "selective predation." Other selective predators include stickleback fishes and even other tadpoles.

What do these results mean for the role of chemical pollution in amphibian deformities? We have purposefully focused our research over the years on specific kinds of deformities, especially those that involve the limbs (especially the hindlimbs), mainly because these are by far the most frequently observed deformities in wild-caught amphibians. I think these also have caught people's attention, because everyone remembers the horrible limb deformities caused by thalidomide, which have come to be seen as the quintessential congenital birth defects in humans. Furthermore, we all are primed for some kind of environmental catastrophe, be it ozone depletion, global warming, or some kind of toxic pollution. Amphibians are seen as "indicator species" for environmental toxins because they have a thin skin that can absorb almost anything from an aquatic (or even a merely moist) environment, and the mysterious "amphibian declines" appear to be happening in many corners of the world. So, I am not surprised at all that so many people suspect chemical pollution as the cause for deformities in frogs — and perhaps these pollutants do play a role, even if indirectly, in some kinds of deformities. Endocrine disruptors, for example, could compromise the immune system of tadpoles, making them more vulnerable to parasites (although no compelling evidence exists for this scenario at the moment). Organic pollutants (nitrogen and phosphorus) could enhance eutrophication, leading to abnormal population densities of aquatic vegetation, snails, parasites, and aquatic insects — but our research over the years has shown that the definitive cause of supernumerary limbs in wild populations of amphibians is a specific species of trematode — and now we have strong evidence that the remaining major type of limb deformity, missing or truncated limbs, is caused by selective predation by aquatic insect larvae.

These results do not completely eliminate the potential role of chemical pollutants, rather we see them as the leading current hypotheses to be excluded when confronted with deformed amphibians, at least those featuring extra limbs or missing limbs. Are parasites (i.e., the specific species of trematode) sufficient to cause extra limbs? Yes. Is selective predation sufficient to cause loss or reduction of limbs? Yes. Are chemical pollutants necessary to understand either of these phenomena? No (in fact, one could argue that the parasites and small predators would be just as, if not more — because they are smaller — vulnerable to chemical pollutants than the frogs!).

NEWSBRIEFS

Europe's Amphibians and Reptiles Under Threat

One fifth of Europe's reptiles and nearly a quarter of its amphibians are threatened, according to new studies carried out by the IUCN for the European Commission. The studies, released on International Biodiversity Day, are the first European Red Lists for amphibians and reptiles, and reveal alarming population trends. More than half of all European amphibians (59%) and 42% of reptiles are in decline, which means that amphibians and reptiles are even more at risk than European mammals and birds.

For 23% of amphibians and 21% of reptiles the situation is so severe that they are classified as threatened in the European Red List. Most of the pressure on these declining species comes from mankind's destruction of their natural habitats, combined with climate change, pollution and the presence of invasive species. "Southern Europe is particularly rich in amphibians, but climate change and other threats are placing its freshwater habitats under severe stress," says Dr. Helen Temple, co-author of the study and Program Officer for the IUCN Red List Unit. "Natural habitats across Europe are being squeezed by growing human populations, agricultural intensification, urban sprawl, and pollution. That is not good news for either amphibians or reptiles."