For poison frogs, bitter is better

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You are what you eat. Although this common phrase is one that all animals can relate to, for poison frogs, what they choose to eat could be a matter of life and death. Poison frogs are famously known for the brilliant colours and striking patterns that bring attention to themselves (Figure 1)—but attention seekers they are not. Instead, they are engaged in a complex advertisement scheme, aimed to warn predators that they might want to eat something else. You see, poison frogs are chock-full of bitter-tasting alkaloids, which most predators find distasteful and many pathogens find hard to live with. Interestingly, these defensive chemicals don't just come from anywhere—instead, poison frogs choose to eat food items that are loaded with them, and then, like kleptomaniacs, they steal them for themselves! Where they get their alkaloids and how they use them in defence, all without poisoning themselves, is a fascinating story that is beginning to unfold from decades of research on the chemistry, ecology and molecular biology of these charismatic tropical frogs.

Figure 1. An adult strawberry poison frog (*Oophaga pumilio*) from Costa Rica. Photo: J.P. Lawrence.

Where do poison frogs get their poisons?

Using unpleasant, repulsive and sometimes toxic chemicals to deter predators and protect from pathogens is common among organisms, but in most cases, these defensive chemicals are produced in-house by an organism's own metabolic machinery. Instead, poison frogs are part of a smaller group of organisms that rely on their diet to obtain defensive chemicals, which can make them pretty picky eaters. A typical poison frog meal is usually a combination of tiny leaf-litter mites, ants, millipedes and beetles, many of which contain alkaloids themselves. Coincidentally, some of the alkaloids found in these arthropods are used for defence, but these defences, of course, do not work against poison frogs. Once eaten by the frogs, these chemicals are taken-up, transported and then stored in specialized glands (called poison glands) in the skin of the frog (Figure 2).

Basically, poison frogs make a living by stealing the defences of others—and with respect to their poisonous nature, are the epitome of the phrase 'you are what you eat'! There are a handful of poison frogs that can also synthesize some of their alkaloids, but a frog is not really a poison frog unless some are stolen from arthropods. This also means that poison frogs raised in captivity and fed an artificial diet, such as those at zoos and museums, are not really poisonous at all, even though they still display their brilliant colours.

However, alkaloids are not only found in poison frogs. In fact, they are actually a diverse group of nitrogen-containing organic compounds found in many different organisms, each of which has a unique structure, its own physiological effects and its own biosynthetic pathway. From a human perspective, these chemicals have found many uses and are so well integrated into our lives that many people cannot go a single day without their favourites—caffeine and nicotine. Many alkaloids are also frequently used in human medicine, such as taxol (a cancer-fighting agent), morphine (a powerful painkiller) and quinine (an anti-malarial drug). Although these are not common to poison frogs, over 1200 have been detected in frogs worldwide (Figure 3), including epibatidine, which may one day serve as a non-opioid painkiller for humans. Given the large number of alkaloids present in poison frogs, we expect to find just as many in arthropods, which on its own is an active area of fascinating research, but at this point, the source for only about 10% have been identified.

Remarkably, the source of most frog alkaloids are mites and ants. Most of these can be classified as branched or unbranched, based on their chemical structure, and interestingly, mites largely contain branched structures, whereas ants largely contain unbranched structures. Of course, mites and ants being the major source of these defensive chemicals makes sense given the natural diet of poison frogs, which almost exclusively consists of these two arthropod groups. However, perhaps even more interesting, is the fact that this specialized diet is strongly correlated with a frog's ability to sequester

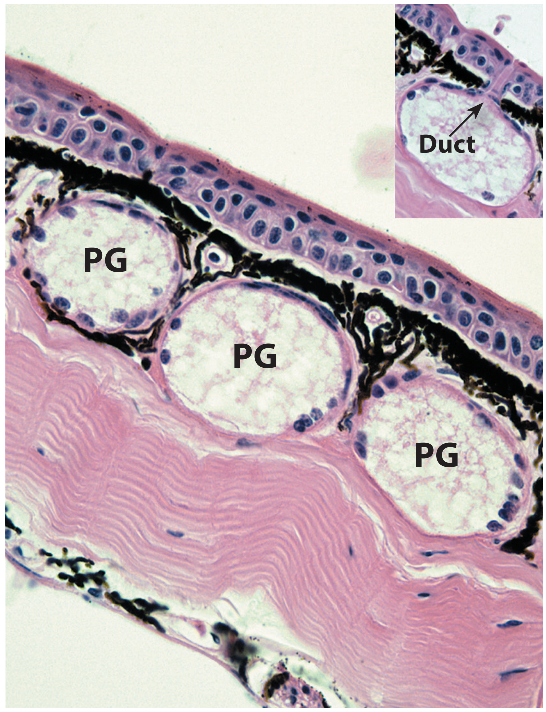


Figure 2. Microphotograph of poison glands (PG) in the skin of an adult poison frog. The smaller photo shows a poison gland and its duct, which carries alkaloids to the surface of the skin when a frog is attacked by a predator. Photo: R.A. Saporito.

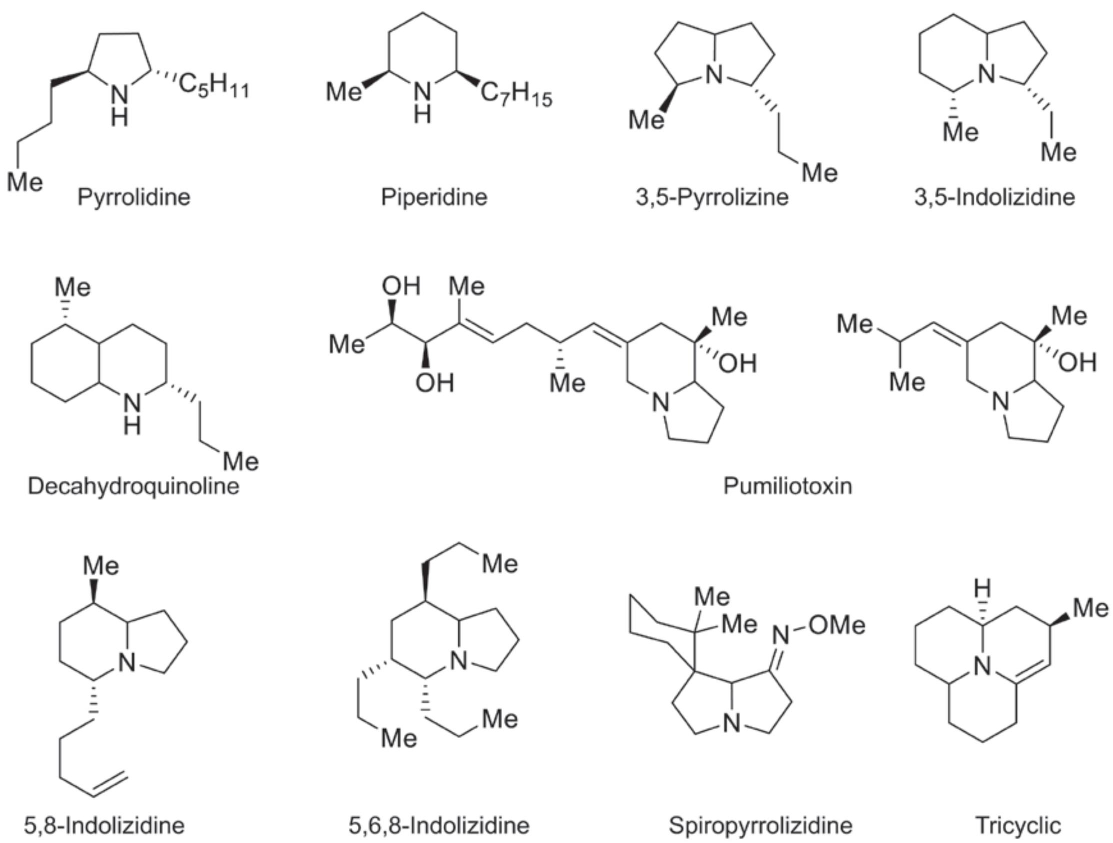


Figure 3. Ten of the major alkaloid classes found in the skin of poison frogs. More than 1200 alkaloids have been detected in poison frogs worldwide, which are arranged into about 24 classes based on their chemical structure.

alkaloids—in fact, this specialization is so important that it has evolved *independently* several times among different groups of poison frogs.

Poison frogs never get bored of the same old food because, in addition to ants and mites, they can select from an ever-changing menu that includes alkaloid-containing millipedes and beetles, but also other arthropods without alkaloids. A bitter diet is key, but that's not all they will eat. Poison frogs are entirely dependent on the arthropods available to them. In other words, their defences are partly based on what they choose to eat, and also on what food is available on the menu when and where they set the table. Each species, population or even individual frog has its own unique alkaloid cocktail, which can change over time, but is presumably adapted to the suite of predators and pathogens that pose the greatest threat; however, there are also other factors at play. For one, older frogs tend to have more types and a greater quantity of defences than younger frogs, simply because they have had more time to live and eat arthropods. In some species, female frogs have more alkaloids than males, possibly because some females share them with their offspring. There is also evidence that species differ in their ability to sequester alkaloids, with some species being better than others. Whatever way you dice it, variation in defence is a common theme among poison frogs, and a diverse arsenal might just provide better protection against the numerous array of predators, such as birds, snakes and spiders, as well as different diseases and pathogens.

Poison frogs do not generally alter the chemical make-up of the alkaloids they sequester, but there are a few species that can slightly modify the structure of some dietary alkaloids. How and why some are modified, while others are not, remains a mystery to be solved. One interesting example revolves around two different alkaloids, called a pumiliotoxin and an allopumiliotoxin. It turns out that when some species eat arthropods that have a specific pumiliotoxin, they are able to selectively hydroxylate it by adding an -OH group, and in doing so, form an allopumiliotoxin instead. The hydroxylation step is so specific that it only happens to the positive enantiomer of the pumiliotoxin, which is one of the two forms that this alkaloid is found. Although we are still unsure of where and how this modification happens—it could happen in the liver, soon after the frog eats, when the alkaloids are being transported through the body or even long after they are already packed away in the poison glands—we do know that hydroxylation of this pumiliotoxin likely involves a specific enzyme and leads to an approximate five-fold increase in the toxicity of the alkaloid, which might serve as better protection for the frog.

As poison frogs are what they eat and sequester alkaloids from food, one of the most vulnerable periods of their lives is before they are able to eat the right food on their own. However, some members of this group of frogs have found an ingenious way around this problem. Mother frogs in the genus *Oophaga*, which translates into 'egg eater', actually feed their tiny tadpoles unfertilized eggs that are laced with small amounts of alkaloids so that they too are chemically defended from anything looking to grab a quick bite or maybe cause an infection. Keeping a tadpole alive and well in the rainforest is no easy feat, but poison frogs make great parents and are excellent caretakers. Similar to how primate mothers care for their young as they develop, or how mother birds care for their chicks, poison frogs do the same. Generally speaking, adult poison frogs mate, not in a pond or a puddle, but on land and females lay their eggs so that they are hidden in the layers of the leaf litter on the forest floor. Once the eggs hatch into tadpoles, one parent returns and carries the tadpoles like a very wriggly backpack to small pools of rainwater that collect in the crevices of large tropical plants (Figure 4).

Figure 4. An adult *Andinobates dorisswansonae* from Colombia transporting a tadpole to a small pool of water for development. Photo: T. Grant.

In the jungles of Central and South America, some species of poison frog go one step further in fulfilling their parental duties. Amazingly, some mother poison frogs are able to remember exactly where in the dense, tangled jungle they left their tadpoles and return every couple of days to feed them with unfertilized eggs that she lays. There are several different species that feed their tadpoles eggs, but for strawberry poison frog (*Oophaga pumilio*) tadpoles, eating a full serving of eggs is especially important because they are gifted with chemical defences from their mother (Figure 5).

Besides giving tadpoles the nutrients they need to grow, alkaloids sequestered from these maternally provided eggs turn the tadpoles bitter—just like their mother and father. Nutritive eggs, each of which is only slightly bigger than the head of a pin, have only a few nanograms of alkaloid compared with the hundreds of micrograms found in adults. With each egg-filled breakfast, tadpoles gather more and more defences until they too have their own collection of alkaloids. Tiny tadpoles are able to take their chemical defences with them when they transform into juveniles and start to eat arthropods of their own. In the same way that adult frogs specialize in eating ants and mites, strawberry poison frog tadpoles specialize in eating eggs—and in fact, are *only* able to eat the eggs from their mother until they grow legs and leave the safety of their little tropical plant pools. This interesting way of life may be the reason this unique type of maternal care has only been found in this group of frogs and not any others.

Figure 5. An adult strawberry poison frog (*Oophaga pumilio*) mother sitting on a bromeliad axil that is filled with a small amount of water and her developing tadpoles. Mother frogs back into axils and deposit unfertilized eggs as tadpole food. Photo: R.A. Saporito.

How do poison frogs not poison themselves?

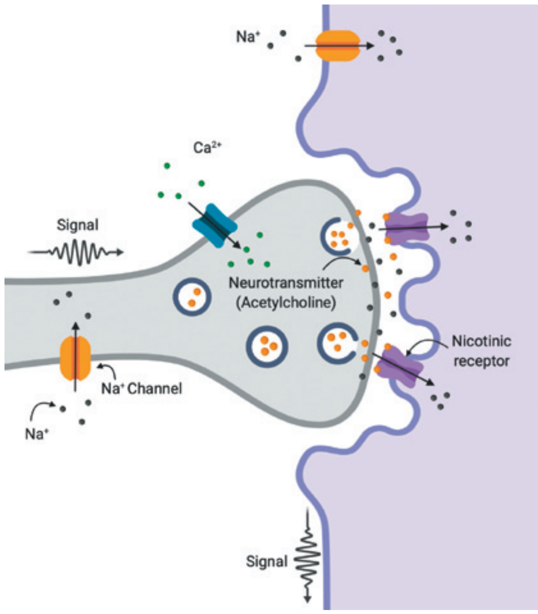
Poison frogs are what they eat, but how do they not poison themselves in the process? Any plant or animal that uses chemical compounds for defence must have a way of preventing those same defensive chemicals from harming themselves. This often means that the defensive chemicals must be packed away somewhere within the body and isolated from critical bodily functions like muscle movement. In poison frogs, alkaloids are transported to and stored in poison glands in their skin where they are ready to defend the frogs against sneaking predators and pathogens.

However, isolating alkaloids to one part of the body is not the only way to prevent self-poisoning. In order to avoid the toxic effects of their own secretions, poison frogs have gone so far as to make special improvements to their own genetic make-up. Ion channels in the nervous and muscular systems are the most common target of frog alkaloids and it is within these protein channels that poison frogs have unlocked the ability to eat toxic prey. However, eating toxic prey and living to tell the tale isn't so easy when each and every alkaloid has its own physiological effects that have to be avoided. For instance, some alkaloids, like batrachotoxins, affect nervous and muscle tissue by permanently binding to sodium channels on the cell membrane and keeping them open. This causes a depolarization of the cell and prevents any further signals from being sent. Similarly, pumiliotoxins can affect the heart by prolonging the opening of sodium channels in cardiac muscle. Other alkaloids, like 5,8-indolizidnes, decahydroquinolines and spiropyrrolizidines are non-competitive blockers of nicotinic receptors, named after the alkaloid nicotine, and this has a cascading effect that disrupts the normal function of nerve and muscle cells. If you are a bird trying to make a meal out of a poison frog, it could mean risking paralysis or even death. Most animals that have defensive chemicals, like pufferfish with tetrodotoxin or toads with bufadienolides, only have a few compounds that they have to worry about protecting themselves from. Poison frogs, on the other hand, have to protect themselves from many different types of alkaloids and all at the same time. So how do they do it?

We know surprisingly little about how poison frogs avoid the toxic effects of their own alkaloids, but we do know a little about how they affect sodium channels and nicotinic receptors and what that might mean for these little frogs. When a bird or a snake tries to eat a poison frog, the alkaloids immediately find their way to the predator's sodium channels or nicotinic receptors, which are incredibly important for keeping muscles moving and neurons firing. However, poison frogs appear to have found a clever way around the effects of their own poison. While some details are still murky, it seems that these frogs have made specific modifications to their own sodium channels and nicotinic receptors in nerve and muscle cells (Figure 6). The sodium channels and nicotinic receptors of poison frogs are proteins, and all organisms have their own channels and receptors built with a unique combination of amino acids. By replacing or substituting a few specific amino acids for others, poison frogs are able to avoid the effects of their own alkaloids because they are no longer able to bind to the channels and wreak havoc. However, even within poison frogs, not every species of frog has the same substitutions. What a frog eats throughout its lifetime and the alkaloids it comes across may play a role in shaping the evolution of sodium channels, nicotinic receptors and their amino acids. Discovering how poison frogs avoid poisoning themselves will require more study but it seems likely to involve a similar mechanism to that already uncovered.

Poison frogs are not only able to sequester their defensive chemicals entirely from their diet, but they've also evolved several means to protect themselves from the very alkaloids they ingest. From the time they are tadpoles and all the way through to adulthood, these frogs specialize in foods that provide them a way to defend themselves against the dangers lurking in the jungles that they live in. Despite the fact that these frogs live all over the world, they have all converged on a unique meal plan composed of mites and ants. These

Figure 6. A normally functioning neuromuscular junction. Many poison frog alkaloids interfere with the normal operation of Na+ channels or nictonic receptors in the nervous and muscular system. Image created with BioRender.



arthropods provide the basis for many of the alkaloids used by the frogs for defence, and because poison frogs can only eat food that lives nearby, the strength of their defences is determined almost entirely by where they live and what they eat. Alkaloids obtained from arthropods are so good at providing effective defensive chemicals for the frogs that they mostly leave them alone and sequester the alkaloids unchanged. However, there is a least one case where some frogs can form a new and improved defensive compound. Even though poison frogs spend all day eating toxic prey, they never have to face the consequences because they appear to have evolved their own unique ion channels that are not as susceptible to the detrimental effects of their alkaloid cocktails. However, we still have much to learn about how these frogs do not poison themselves and where exactly these frogs get their poisons from. As we continue to discover more about poison frogs and the chemistry of their defences, we continue to unravel the story of alkaloids and their role in the everyday lives of many different organisms. ■

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