# The palatability of Neotropical poison frogs in predator-prey systems: do alkaloids make the difference?

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## ABSTRACT

Neotropical poison frogs sequester alkaloid-based chemical defenses from their diet, which seem to repel invertebrate predators after contact. In this study, we tested the reaction of predatory ants toward skin secretions of captive-bred poison frogs without alkaloids. Although the secretions had a smell and taste similar to those of wild-caught frogs, the ants did not avoid them, but actually preferred them. We infer that amphibian secretions are attractive to invertebrate predators (probably due to palatable nutritive components), but that this attraction is overridden when unpalatable alkaloids are present.

CHEMICAL DEFENSES ARE WIDESPREAD THROUGHOUT THE ANIMAL KINGDOM and are found in a variety of invertebrate and vertebrate species where they play an important role in the predator-prey interactions (e.g., Pasteels et al. 1983). An example of this kind of chemical defense is the skin of amphibians. It contains an array of bioactive compounds, such as amines, peptides, bufadienolides, or alkaloids, which are thought to deter potential predators and/or pathogens (Mina et al. 2015). Alkaloid-based skin defenses are well known from poison frogs, a group of approximately 150 species of conspicuously colored anurans that are distributed globally among five distinct families (Saporito et al. 2012). Poison frogs sequester alkaloids from an arthropod diet and accumulate them into dermal granular glands (commonly referred to as poison glands; Saporito et al. 2009, Hantak et al. 2013). The family Dendrobatidae, found in Neotropical rain forests of Central and South America, is the most species-rich group of poison frogs. Of the over 800 alkaloids identified in poison frogs worldwide, more than 520 have been identified in dendrobatids. Alkaloid defenses are believed to protect the frogs from potential bird, reptile, mammal, and arthropod predators (see Willink et al. 2014, Murray et al. 2016).

While certain vertebrate predators can learn to avoid dendrobatid frogs due to their aposematic coloration (e.g., Stuckert et al. 2014a), there are several studies that indicate invertebrate predators avoid frogs based largely on chemoreception. For example, experiments with spiders (ctenid: Cupiennius coccineus; tarantula: Sericopelma rubronitens) and bullet ants (Paraponera clavata) have revealed that dendrobatids are rejected immediately after initial contact, while frogs without alkaloid defenses are more likely to be attacked and consumed by spider and ant predators (Fritz et al. 1981, Szelistowski 1985, Gray & Kaiser 2010, Stynoski et al. 2014, Hantak et al. 2016, Murray et al. 2016). However, Yeager (2013) observed swarming army ants (Eciton hamatum) in Panama avoid dendrobatids without any direct contact. A recent field study in Costa Rica discovered that ants (Ectatomma ruidum) tend to avoid sugar solutions that contain skin extracts of wild-caught dendrobatid frogs of the species Oophaga pumilio and Dendrobates auratus (Blanchard et al. 2014, Bolton and Saporito, unpubl. data). Furthermore, Bolton et al. (2015) found that fruit flies (Drosophila melanogaster) avoid sugar solutions with alkaloid-containing skin extracts from the dendrobatid frog O. pumilio. The avoidance of dendrobatids by arthropods is likely due to the unpalatability or distastefulness of the alkaloids present in their skin, but may also be related to the strong effects that some alkaloids have on insects after direct contact. For example, mosquitoes that landed on membranes treated with dendrobatid alkaloids frequently autotomized their legs and sometimes died, although in most cases they simply avoided landing on the membranes (Weldon et al. 2006).

Collectively, these findings suggest that invertebrate predators are able to detect and avoid dendrobatid frogs, probably due to the presence of alkaloid skin secretions. Wild-caught dendrobatids have a disagreeable and bitter taste for humans, presumably associated with the presence of alkaloid defenses in the frogs' skin (e.g., Myers & Daly 1976). We recently observed that the captive-bred dendrobatid frog Ranitomeya imitator also has a disagreeable taste (and smell) to humans, especially when stressed. This was observed by smelling our hands as well as smelling and (lightly) tasting the frogs themselves after handling them (L. M. Schulte and K. Summers, pers. obs.; A. M. M. Stuckert and E. Twomey, pers. comm.). Captive-bred frogs do not contain skin alkaloids, as they have not fed on alkaloid-containing arthropods (Saporito et al. 2009). Therefore, it is not clear if the

non-alkaloid-based tastes/smells associated with *R. imitator* also function as a defense against potential predators, independent of alkaloids.

To answer this question, we designed an experiment similar to that of Weldon *et al.* (2013), where they showed that fire ants (*Solenopsis invicta*) that came into direct contact with individual dendrobatid alkaloids exhibited a negative behavioral reaction (*i.e.,* convulsions and reduced ambulation). Fire ants naturally occur in tropical and subtropical regions of South America, but are highly invasive in other areas, especially in the southern United States. Using the fire ant *Solenopsis invicta*, we tested for avoidance behavior toward non-alkaloid-containing skin secretions of captive-bred poison frogs of the species *R. imitator*. Due to the observed taste and smell, we hypothesized that ants would avoid the skin secretions of *R. imitator*, even though they do not contain alkaloids.

We conducted our experiments in the laboratory at East Carolina University with 30 adult *R. imitator* in January 2015. These frogs occur naturally in tropical rain forests of northern Peru; however, the animals used for our experiments (and the parental generation) were bred in captivity and housed in tanks with up to seven conspecifics. Frogs were fed a regular diet of fruit flies (*Drosophila melanogaster*) throughout their lives, which are not a source of alkaloids. Skin secretions were obtained from individual frogs by applying weak electrical impulses to their skin using a Transcutaneous Amphibian Stimulator (TAS, Grant *et al.* 2002, frequency: 50 Hz, pulse width: 2 ms, amplitude: 9V, length: 10 sec; Fig. 1). The electrical current stimulates the smooth muscle surrounding the poison glands in the frog skin, causing them to expel their contents. This method is not invasive and has been used to successfully extract defensive chemicals in other studies (*e.g.,* Clark *et al.* 2006, Hantak *et al.* 2013, Bolton and Saporito, unpubl. data). Following stimulation, we swabbed each frog with two circular pieces of filter paper (diameter: 6 mm; Bunn-o-Matic
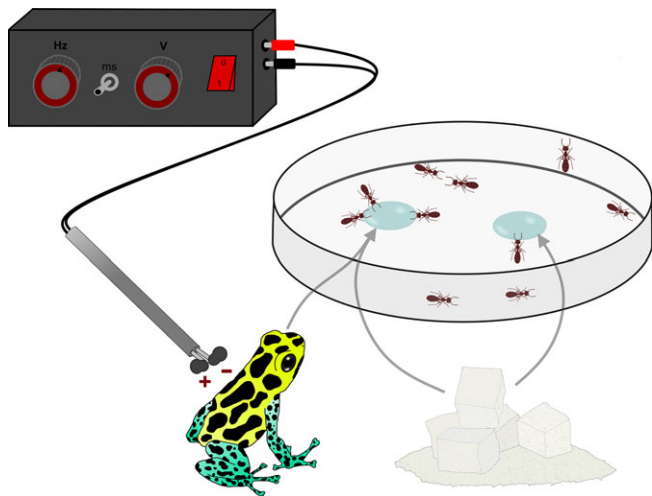


FIGURE 1. Scheme of the methodological setup, showing the choice given to the ants: (1) control solution with only sugar/ethanol and (2) treatment solution with frog skin secretions (obtained after electrical stimulation with the transcutaneous amphibian stimulator) with sugar/ethanol.

Corporation), which were then placed into 50 $\mu$l of ethanol (100%). The ethanol was mixed with 50 $\mu$l of a sugar-water solution (1 g white refined sugar per 1 ml filtered water), which constituted our treatment solution. As a control, ethanol (without frog secretions) was mixed 1:1 with the same sugar-water solution. To control for the presence of filter paper in the treatment, two pieces of clean filter paper were added for every 100 $\mu$l of the control solution. For each experimental trial with *S. invicta*, 25 $\mu$l of the treatment solution and 25 $\mu$l of the control solution were haphazardly placed *ca.* 3 cm apart from each other in a 9 cm diameter plastic Petri dish. Trials were conducted in an artificially illuminated room at 22°C. Fire ants (*S. invicta*) were collected the day before the trials from two mounds (>1 km apart from each other), using a trowel to disturb the nests causing the ants to come to the surface. Ants were kept without feeding for one day in the laboratory, separated by mounds to avoid aggression between colonies. For each trial, ten individual *S. invicta* were added to each petri dish and allowed to choose between feeding on the treatment or control solutions (Fig. 1). Each frog extract was tested in triplicate (*i.e.* 90 trials, representing skin secretions of 30 frogs), and a total of 900 ants were used in the entire experiment (10 ants per trial). Some ants became trapped in the sugar-water solutions during a few of the trials (see below), and therefore the total number of ants within each experimental trial varied (range: 4–10 ants per trial). Trials were recorded with digital cameras (Sony Handycam HDR-CX-220, Go-Pro Hero 3) for 15 min; however, only the first 5 minutes of each trial was analyzed because ants generally became disoriented (probably due to feeding on ethanol) after *ca.* 10–15 min. When analyzing the videos, we counted each instance of ant contact with the different solutions, and classified these encounters into: (1) drinking events (ants remained at the solution for longer than 3 sec) and (2) nondrinking events (ants kept moving after brief contact with the solution or were repelled by the solution, *i.e.,* moved backwards rapidly). Of the 90 trials, five could not be analyzed because of strong reflections in the video or because the different liquids ran together.

We performed a generalized linear mixed-effect model of the relationship between ant-drinking behavior (response variable) and the treatment (explanatory variable) with a binomial error distribution, using the Automatic Differentiation Model Builder package glmmADMB (Skaug *et al.* 2013) in R 3.1.3 (R-Core-Team 2014). We entered the IDs of the individual frogs whose secretions we used as random effects into the model. Because our data were overdispersed, and in order to deal with variation in the number of active ants across the trials (see above), observation-level random effects (OLRE) were fitted to the model (Browne *et al.* 2005). Due to an excess of zeroes in our data set, we modified the model to account for zero inflation (Bolker *et al.* 2013). P-values were obtained by likelihood ratio tests of the full model with the effect in question (*i.e.,* the different solution types) against the model without the effect in question (*i.e.,* without the different solution types). In order to confirm that the captive-bred *R. imitator* did not contain alkaloids, we killed three adult individuals and collected their skins in individual vials

containing 1 ml of methanol. Alkaloid analyses were conducted at the John Carroll University in April 2014 following the methods of Stuckert *et al.* (2014b), who showed that skins of wild-caught *R. imitator* do contain alkaloids.

Of the 85 trials examined in our study, ants came into contact with each solution type (treatment vs. control) over 500 times. Only a small percentage of the ants remained at one of the solutions long enough to be considered a drinking event (Fig. 2). Our analysis of the linear mixed-effects model revealed a significant effect of solution type on ant behavior (testing vs. drinking; $\chi^2_{(1,85)}$ = 9.974, $P < 0.01$), and ants chose to drink the treatment solution containing frog secretions significantly more often than the sugar/ethanol-water solution. No alkaloids were detected in the skin extracts of the three captive-bred *R. imitator*.

The positive response of the ants toward the solution containing frog skin secretions contradicts the prediction that *S. invicta* would avoid the alkaloid-free *R. imitator* skin extracts. This might suggest that the unpleasant smell/taste we detected in the secretions of these frogs is not considered unpleasant to the ants. In contrast, the clear preference for feeding upon the solution with frog secretions suggests that predatory ants actually prefer alkaloid-free *R. imitator*. This could be explained by the fact that omnivorous fire ants prefer solutions containing sugar, along with lipids and proteins (Glunn *et al.* 1981). A variety of lipids, including fatty acid methyl esters, were identified in the skin secretions of *R. imitator*, and glycoproteins were also likely present in the secretion (Lillywhite 2006). A similar reaction was shown in Neotropical bullet ants (*Paraponera clavata*) that are more likely to grasp liquid mixtures that contain high concentrations of both sugars and proteins (Jandt *et al.* 2013). Therefore, it is possible

that *S. invicta* in our present study were more attracted to the skin secretions because they contained sugars and proteins, rather than the control solution that only contained sugar.

We cannot, however, completely exclude the possibility that our sampling methods prevented our treatment solutions from containing the unknown chemicals responsible for the disagreeable smell and taste observed in the captive-bred *R. imitator*. For example, Smith *et al.* (2003) showed that the odor of the Australian green tree frog (*Litoria caerulea*) consists of chemical compounds different from the defensive toxins in their parotoid glands. Based on these results, Smith *et al.* (2003) suggest that the odor acts as an aposematic signal, advertising the presence of chemical defenses in the frog skin. It is possible that the smell and taste observed in the captive-bred *R. imitator* of the present study has a similar function, but that this unknown chemical was not captured in our test solution. Future research will be necessary to identify the chemical(s) responsible for the disagreeable smell/taste present in captive-raised *R. imitator*. Nevertheless, comparisons of our results with similar studies that tested alkaloid avoidances by ants (*e.g.*, Weldon *et al.* 2013, Blanchard *et al.* 2014) confirm that the presence of alkaloids can result in avoidance behavior, whereas poison frogs that lack alkaloids are likely palatable to predatory invertebrates. Our study contributes to the slowly accumulating body of evidence that dendrobatids are not only confronted by vertebrate predators that detect frogs based on vision but also a diversity of invertebrate predators that use chemoreception for detecting and deciding whether to prey upon frogs. Accordingly, we infer that, in order to repel chemically oriented predators, the unpalatability of the chemical defenses of a prey species has to outweigh the attraction of any nutritive chemicals the predators may detect.

## ACKNOWLEDGMENTS

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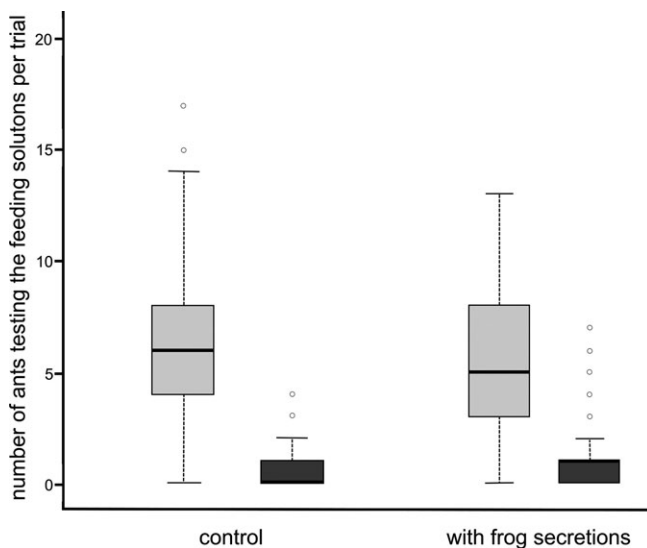


FIGURE 2. Box-and-whisker plots of the number of ants that tested the different feeding solutions per trial (control and treatment with frog secretions). The dark gray boxes illustrate the number of 'drinking decisions' for each solution, while the light gray boxes illustrate the number of 'contacts' with the solution that the ants chose not to drink. Whiskers span the range of the data, except for the outliers (open circles).

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