

SHORT COMMUNICATION

Bufadienolide and alkaloid-based chemical defences in two different species of neotropical anurans are equally effective against the same arthropod predators

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Abstract: Defensive chemicals in anuran skin secretions function in protection against potential predators. Although studies have demonstrated that particular chemicals are effective against certain predators, very little is known about how different chemicals from different species function against the same predators. Understanding how different chemicals function as a defence against similar predators is fundamental to the ecology and evolution of chemical defences in frogs. In the present study, the defensive function of bufadienolide-based defences in adult *Rhaebo haematiticus* (Bufonidae) were compared with alkaloid-based defences in adult and juvenile *Dendrobates auratus* (Dendrobatidae) against the same predators. Most bufonids contain synthesized bufadienolides, whereas dendrobatids contain dietary-derived alkaloids. Predation trials were performed with two potential invertebrate predators, *Paraponera clavata* (bullet ant) and *Cupiennius coccineus* (ctenid spider), to determine how these predators respond to two different types of frog chemical defence. The non-chemically defended frog *Craugastor fitzingeri* served as a control in all predation trials. Our results suggest that bufadienolide defences of *R. haematiticus* and alkaloid defences of *D. auratus* are equally effective towards bullet ant and ctenid spider predators. The similar avoidance and cleaning behaviours exhibited by these ants and spiders after contact with bufadienolides and alkaloids suggest that both types of defence are unpalatable to these arthropod predators.

Key Words: alkaloid, bufadienolide, bufonid, bullet ant, ctenid spider, *Cupiennius coccineus*, dendrobatid, palatability, *Paraponera clavata*

Anurans contain a diversity of defensive chemicals in their skin secretions, which function in protection against pathogens, parasites and/or predators (Conlon 2011, Mina *et al.* 2015). These defensive chemicals include amines, peptides, proteins and steroids, which are manufactured by anurans, as well as lipophilic alkaloids, which are obtained from dietary arthropods (reviewed in Saporito *et al.* 2009). Although several studies have examined the defensive function of particular chemicals among anuran species (Formanowicz & Brodie 1982, Fritz *et al.* 1981, Szelistowski 1985, Weldon *et al.* 2006), very little research has compared the effectiveness of

different defensive chemicals between sympatric species exposed to the same predators. Given the diversity of defensive chemicals present in anuran skin, it is possible that some frog species are more or less protected from certain types of predators than others. Understanding how different defensive chemicals function against similar predators will provide important insight into the ecology and evolution of chemical defences in frogs. The aim of our study was to gain an understanding of how bufadienolide and alkaloid-based defences protect anurans from similar predators in their natural habitats.

Bufonid toads (Bufonidae) are typically characterized by their cryptic colouration and behaviour, which provides camouflage in their leaf-litter habitats (Heinen 1985). Most bufonids produce a variety of steroids (e.g. bufadienolides; Erspamer 1994) that are stored in a few localized parotoid glands on their dorsum (Hostetler & Cannon 1974), which defend these toads

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and their tadpoles from predation by both invertebrates and vertebrates (Brodie *et al.* 1978, Formanowicz & Brodie 1982, Shine 2010, Toledo *et al.* 2007). Unlike bufonids, certain dendrobatid frogs (Dendrobatidae) possess alkaloid-based chemical defences, which are acquired entirely from a diet mainly consisting of mites and ants (reviewed in Saporito *et al.* 2009, 2012). Alkaloids are stored in thousands of poison (granular) glands that are distributed throughout the dorsum of these frogs (Saporito *et al.* 2010). Alkaloid defences are unpalatable, and in some cases toxic, to a variety of invertebrate and vertebrate predators (Brodie & Tumbarello 1978, Fritz *et al.* 1981, Murray *et al.* 2016, Szelistowski 1985). Chemically defended dendrobatids have conspicuous colours, which serve as warning (aposematic) signals to colour-visioned predators such as birds (Maan & Cummings 2012, Paluh *et al.* 2014, Summers & Clough 2001; however, see Alvarado *et al.* 2013).

Bufonids and dendrobatids are largely sympatric in the neotropics, and many species share microhabitats, and very likely, the same predators; however, it is not currently known how bufadienolide and alkaloid defences compare against the same predators. Many arthropods utilize chemoreception as a dominant sense by which to detect their prey, and therefore represent an important group of predators to study differences in the effectiveness of chemical defences. Experimental studies with the dendrobatid frog *Oophaga pumilio* in Costa Rica have demonstrated that adult frogs are protected from predation by the predatory bullet ant, *Paraponera clavata*, and the ctenid spider, *Cupiennius coccineus* (Fritz *et al.* 1981, Szelistowski 1985). Murray *et al.* (2016) recently demonstrated that lower quantities of alkaloid defences in juvenile *O. pumilio* lead to increased predation by the bullet ant *P. clavata*, suggesting that these predators can detect differences in chemical defences. A study by Gray *et al.* (2010) demonstrated that alkaloid defences in adult *Dendrobates auratus* in Panama were effective against the red rump tarantula, *Sericopelma rubronitens*. Many studies have demonstrated that the bufonid toad, *Rhinella marina*, is protected from predators (reviewed in Shine 2010); however, little is known about the predator defence in other toad species. The common litter toad, *Rhaebo haematiticus* (Bufonidae), is sympatric with both *O. pumilio* and *D. auratus* in Central and South America (Guyer & Donnelly 2005) and is likely exposed to the same arthropod predators. As a bufonid, *R. haematiticus* contains bufadienolide-based defences (Ferreira *et al.* 2013), which probably provides chemical protection from predators.

In the present study, the effectiveness of bufadienolide-based defences in *R. haematiticus* and alkaloid-based defences in *D. auratus* were compared against two potential predators – the bullet ant (*P. clavata*) and ctenid spider (*C. coccineus*). In addition, predation between adult and juvenile *D. auratus* was compared using bullet ant predators.

Predation trials took place from 9 June to 30 July 2012 at the Organization for Tropical Studies (OTS), La Selva Biological Research Station, Costa Rica (10°26'N, 83°59'W). *Dendrobates auratus* is a diurnal, conspicuously coloured, alkaloid-containing poison frog that is present in leaf-litter, and *Rhaebo haematiticus* is a diurnal, cryptically coloured, steroid-containing bufonid frog that also occurs in leaf-litter at La Selva (Guyer & Donnelly 2005). *Craugastor fitzingeri* is a common leaf-litter frog that occurs in the same microhabitat as *D. auratus* and *R. haematiticus*, and is similar in size to both of the experimental frogs; however, it is not chemically defended, and therefore served as a negative control in all of the predation experiments. The predatory invertebrates used in this study were the bullet ant, *P. clavata*, and the ctenid spider, *C. coccineus*, both of which are abundant at the study site. All individuals of *C. fitzingeri*, *R. haematiticus*, 16 adult *D. auratus* and six juvenile *D. auratus* were collected from La Selva. The number of *D. auratus* at La Selva was fewer than expected, and therefore a second location (Chilamate, Costa Rica, 10°26'N, 84°05'W) was used to acquire an additional 15 *D. auratus*. The *D. auratus* obtained from this population were only presented to ctenid spiders, and therefore no *D. auratus* from La Selva were presented to ctenid spiders. All frogs were held in individual containers with leaf-litter for no more than 3 d preceding an experiment.

Bullet ant predation trials consisted of 16 adult *D. auratus* (mean SVL = 32.8 mm), 15 adult *R. haematiticus* (mean SVL = 33.6 mm), and 16 adult *C. fitzingeri* (mean SVL = 32.9 mm). In addition six trials were conducted with juvenile *D. auratus* (mean SVL = 22.6 mm) and juvenile *C. fitzingeri* (mean SVL = 22.6 mm). *Paraponera clavata* (mean length = 23.3 mm) actively nests between buttresses of trees, and forages during the day (Young & Hermann 1980). *Paraponera clavata* is a generalist predator and has been found to prey on plant parts, arthropods and small vertebrates (Fritz *et al.* 1981, Young & Hermann 1980). Predation experiments involving *P. clavata* were conducted at nests between 16h00 and 17h00. Trials were conducted along foraging trails on tree trunks. Following Fritz *et al.* (1981), individual frogs were presented to ants in the foraging line by holding each frog by its right hind limb with a pair of 30.5-cm forceps. Individual frogs of each species were randomly presented to a single nest of ants and individual frogs were used only for a single trial. Ant nests were not used more than once a day, and the same nest was used an average of six times during the study. Each bullet ant trial ran for 5 min, and began when an ant made contact with the frog. Modifying the methods of Fritz *et al.* (1981), frogs were scored as: (1) preyed upon (not released after attack) or (2) not preyed upon (touched with antennae and avoided). Following trials, all surviving frogs were held for 24 h to allow recovery

from any attacks, and then returned to their original site of capture.

Ctenid spider predation trials consisted of 15 adult *D. auratus* (mean SVL = 35.0 mm), 15 adult *R. haematiticus* (mean SVL = 34.0 mm) and 15 adult *C. fitzingeri* (mean SVL = 35.0 mm). *Cupiennius coccineus* (mean length = 24.4 mm) is typically found on vegetation at night and is a sit-and-wait predator (Barth *et al.* 1988). *Cupiennius coccineus* has been found to prey on large invertebrates and small frogs (Szelistowski 1985, MMH and RAS, pers. obs.). Predation experiments with *C. coccineus* were conducted at night between 20h30 and 24h00. Trials were conducted on the vegetation upon which the spiders were found. Following Fritz *et al.* (1981), individual frogs were randomly presented to an individual spider using 30.5-cm forceps in the same manner as described above. Individual frogs were only used for a single trial, and individual spiders were not utilized more than once in the entire experiment. Ctenid spider trials were conducted for 2 min, beginning when the frog was presented to the spider. Modified from the methods of Szelistowski (1985), frogs were scored as: (1) preyed upon (not released after attack) or (2) not preyed upon (not attacked or released after an attack). Following trials, all surviving frogs were held for 24 h, and then returned to their original site of capture.

Binary logistic regression was used to determine if frog species and frog size (adult vs. juvenile) were significant predictors of bullet ant and ctenid spider predation. All statistical analyses were conducted in SPSS version 18.0.

Bullet ants preyed upon zero (0/16) adult *D. auratus*, one (1/15) adult *R. haematiticus* and 12 (12/15) adult *C. fitzingeri*. Frog species was a significant predictor of bullet ant predation, and adult *C. fitzingeri* were 45 times more likely to be preyed upon when compared with *D. auratus* ($P = 0.001$; odds ratio = 45.0; $CI_{0.95} = 4.4$ – 457.5) and 20 times more likely when compared with *R. haematiticus* ($P = 0.002$; odds ratio = 19.5; $CI_{0.95} = 3.0$ – 126.5). There was no difference in bullet ant predation between adult *D. auratus* and *R. haematiticus* ($P = 0.571$). Bullet ants preyed upon zero (0/6) juvenile *D. auratus* and five (5/6) juvenile *C. fitzingeri*. Frog species was a significant predictor of bullet ant predation, and juvenile *C. fitzingeri* were 25 times more likely to be preyed upon when compared with juvenile *D. auratus* ($P = 0.038$; odds ratio = 25.0; $CI_{0.95} = 1.2$ – 521.0). Frog age/size class was not a significant predictor of predation between juvenile and adult *D. auratus* ($P = 0.466$).

Ctenid spiders preyed upon one (1/15) adult *D. auratus*, one (1/15) adult *R. haematiticus* and seven (7/15) adult *C. fitzingeri*. Frog species was a significant predictor of ctenid spider predation, and adult *C. fitzingeri* were 12 times more likely to be preyed upon when compared with *D. auratus* ($P = 0.030$; odds ratio = 12.3; $CI_{0.95} = 1.3$ – 118) and *R. haematiticus*

($P = 0.030$; odds ratio = 12.3; $CI_{0.95} = 1.3$ – 118). There was no difference in ctenid spider predation between adult *D. auratus* and *R. haematiticus* ($P = 0.999$).

Bufo and dendrobatid frogs are both considered chemically defended anurans, yet they utilize different types of chemicals in their defence against predators – bufonids possess synthesized bufadienolides, whereas dendrobatids contain sequestered alkaloids. The present field-based study demonstrates that both *R. haematiticus* (bufonid) and *D. auratus* (dendrobatid) are similarly protected from predation by the predatory ant *P. clavata* and ctenid spider *C. coccineus*. These results provide evidence that bufadienolide-based and alkaloid-based chemical defences are equally effective at deterring predation by the same arthropod predators. In both cases, predator avoidance was likely associated with the presence of chemical defences, whereas the control frogs were palatable (Brodie *et al.* 1978, Formanowicz & Brodie 1982, Fritz *et al.* 1981, Szelistowski 1985).

Bullet ants always made contact with frogs before deciding to prey upon them. In several experimental trials, bullet ants would wipe their mandibles on a substrate after coming into contact with *R. haematiticus* or *D. auratus*, suggesting that the defensive chemicals were distasteful (i.e. unpalatable). Interestingly, this behaviour never occurred after a bullet ant came into contact with *C. fitzingeri* (control frogs). Similar results were reported by Fritz *et al.* (1981) and Murray *et al.* (2016), both of which demonstrated that *O. pumilio* are chemically defended from *P. clavata*. In several instances throughout the experiment, after coming into contact with *D. auratus*, *C. coccineus* wiped their pedipalps with their anterior appendages, suggesting that alkaloids were considered unpalatable, as has been observed in other studies (Murray *et al.* 2016, Szelistowski 1985). Gray *et al.* (2010) observed a similar behaviour (fang wiping) in the tarantula *Sericopelma rubronitens* after it was presented to adult *Dendrobates auratus*. These behaviours never occurred after *C. coccineus* came in contact with *C. fitzingeri* or *R. haematiticus*; however, the spiders always retreated similarly after coming in contact with *D. auratus* or *R. haematiticus*. The cleaning behaviours exhibited by each arthropod predator after making contact with *R. haematiticus* (bullet ants) and *D. auratus* (bullet ants and ctenid spiders) provide evidence that both bufadienolides and alkaloids are considered unpalatable and are equally avoided.

Juvenile poison frogs are smaller in size, and have smaller poison glands and reduced quantities of alkaloids (Saporito *et al.* 2010, Stynoski *et al.* 2014). Therefore, it may be possible for predators to detect this reduced amount of alkaloids and more successfully prey on juvenile frogs. Murray *et al.* (2016) reported that juvenile *O. pumilio* were preyed on significantly more often than adults by *P. clavata*, which was attributed to the smaller

quantities of alkaloids in juveniles. The results of the present study, however, demonstrate that juvenile *D. auratus* appear to be as equally protected against predation by *P. clavata* when compared with adults. The differences observed between the two studies are likely due to differences in alkaloid quantities between juvenile *D. auratus* and *O. pumilio*. Although juvenile *D. auratus* and *O. pumilio* are both smaller than adults of their respective species, the juvenile *D. auratus* used in the present study were approximately the same size as adult *O. pumilio*. Therefore, it is likely that juvenile *D. auratus* contain relatively similar quantities of alkaloids when compared with adult *O. pumilio*, which appears to provide them equal protection from bullet ant predators.

Overall, the findings of the present study provide evidence that although the defensive chemicals present in *D. auratus* and *R. haematiticus* are different, they are both equally effective at deterring predation by the same invertebrate predators.

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