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Arthropod predation in a dendrobatid poison frog: does frog life stage matter?

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ABSTRACT

Frogs in the family Dendrobatidae are well known for their conspicuous colors and variable alkaloid-based chemical defenses. The aposematic coloration in dendrobatid frogs appears to deter predators with color vision, but relatively little is known about how these frogs are protected and their defenses are perceived by non-color vision dominated predators. The neotropical bullet ant *Paraponera clavata* and the red-legged banana spider *Cupiennius coccineus* are predators that avoid adults of the dendrobatid *Oophaga pumilio*, but readily consume non-toxic frogs. Juvenile *O. pumilio* possess the same warning coloration as adult *O. pumilio*, but may be more palatable given that they have lower quantities of defensive chemicals. This may provide juvenile *O. pumilio* protection from color-sighted predators, while leaving them susceptible to predators that use chemoreception. To test this hypothesis, we presented juveniles and adults of both *O. pumilio* and the non-chemically defended frog *Craugastor bransfordii* to bullet ants and banana spiders. Both bullet ants and banana spiders preyed upon *C. bransfordii* significantly more than on *O. pumilio*. Adult and juvenile *C. bransfordii* experienced similar predation rates by both predators. The life stage of *O. pumilio* significantly predicted predation by bullet ants, with juveniles being consumed significantly more often than adults. However, the life stage of *O. pumilio* did not predict predation by banana spiders, as no adults or juveniles were consumed. Our study provides evidence that bullet ants can detect differences in chemical defenses between juvenile and adult *O. pumilio*, resulting in differential predation on the more palatable juvenile frogs. The avoidance of both adults and juveniles by *C. coccineus* suggests the alkaloids in *O. pumilio* act as an effective chemical deterrent to banana spiders, regardless of quantity. Overall, our results suggest that differences in alkaloid defenses among life stages in *O. pumilio* correspond to differences in relative palatability to at least one arthropod predator.

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1. Introduction

The ability to avoid predation is fundamental to the survival of all prey, a characteristic that is evident in the tremendous diversity of defensive adaptations present among organisms. Many defensive adaptations are structural, physically discouraging predators from consuming prey (e.g., spiny palms, caterpillar bristles, porcupine quills), whereas others are based upon behavior, coloration, and/or defensive chemicals (Ruxton et al., 2004; Toledo et al., 2011). Organisms that exhibit coloration similar to their environment are generally considered cryptic, and rely on being undetected by

predators for protection (Ruxton et al., 2004; Cooper et al., 2008). Some organisms utilize conspicuous signals (e.g., color, auditory, chemical) as an advertisement of their unpalatability, or toxicity, to potential predators, an evolutionary adaptation known as aposematism (Ruxton et al., 2004; Weldon et al., 2015; Weldon and Burghardt, 2015).

Brightly colored frogs in the family Dendrobatidae (poison frogs) represent a large group of organisms that utilize conspicuous colors/patterns to advertise the presence of their alkaloid-based chemical defenses to natural predators (Summers and Clough, 2001; Saporito et al., 2012). Numerous studies have provided evidence that dendrobatid coloration functions as an effective warning signal and deterrent to avian predators, which use color vision to detect prey (e.g., Darst and Cummings, 2006; Darst et al., 2006; Saporito et al., 2007a; Noonan and Comeault, 2009; Willink et al., 2013; Paluh et al., 2015; however, see Alvarado et al., 2013).

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However, organisms not always considered in the discussion of aposematic prey, such as snakes and arthropods, may also represent important natural predators of dendrobatids (Brodie and Tumbarello, 1978; Fritz et al., 1981; Szelistowski, 1985; Gray et al., 2010; Santos and Cannatella, 2011; Lenger et al., 2014; Stynoski et al., 2014a,b). It is currently unclear how these predators, especially arthropods that use mechano- and chemoreception for prey capture, perceive and respond to chemical defenses of dendrobatids.

Aposematic dendrobatids possess a large diversity of skin alkaloids that are sequestered from a diet of alkaloid-containing arthropods (for review, see Saporito et al., 2009) and are stored in dermal poison glands (Neuwirth et al., 1979; Saporito et al., 2010a). The composition of these defenses is largely dependent on the availability of alkaloid-containing arthropods and has been shown to vary among species, individuals, between sexes, and among life stages (Saporito et al., 2007b, 2012; Stynoski et al., 2014a). In the dendrobatid frog *Oophaga pumilio*, poison gland number and size increases with frog size, and adults contain significantly more and larger-sized glands when compared to juveniles (Saporito et al., 2010a). As a function of differences in diet, and in certain cases gland morphology, individuals of *O. pumilio* differ in the type, number, and quantity of alkaloid defenses (Saporito et al., 2012). In particular, tadpole and juvenile *O. pumilio* contain much smaller quantities of alkaloids than adults (see Daly et al., 2002; Stynoski et al., 2014a), potentially making them more palatable to predators and prone to higher predation. Previous studies on chemically defended anurans that manufacture defenses have demonstrated that differences in ontogenetic stages and gland morphology play an important role in the defense against predators, where some life stages are more protected than others (Formanowicz and Brodie, 1982; Lawler and Hero, 1997; Weldon and Carroll, 2007; Hayes et al., 2009). It is therefore possible that risk of predation will also differ among the life stages of dendrobatid frogs.

Like frogs, chemically defended invertebrates that sequester chemical defenses from dietary sources also exhibit variation in defense (Bowers, 1993; Opitz and Müller, 2009; Savitzky et al., 2012). In some cases, this variation can result in a 'palatability spectrum' of prey, whereby individuals exhibit differences in palatability that are perceived by natural predators (Brower et al., 1968). In these cases, predators are able to detect differences in palatability and preferentially consume prey with fewer chemical defenses (Brower et al., 1968; Bowers, 1993; Saporito et al., 2012). Arthropod predators that largely use chemoreception in prey detection may be especially sensitive to variation in alkaloid defenses, which could result in differential predation upon life stages of *O. pumilio*.

The neotropical bullet ant, *Paraponera clavata* (Formicidae), and the red-legged banana spider, *Cupiennius coccineus* (Ctenidae), have both been experimentally shown to reject most adult *O. pumilio*, despite consuming other non-toxic leaf-litter frogs as prey (Fritz et al., 1981; Szelistowski, 1985). Recently, these same predators have also been shown to avoid alkaloid-containing tadpoles of *O. pumilio*, but to a much lesser extent when compared to adults (Stynoski et al., 2014a,b). Fritz et al. (1981) and Szelistowski (1985) suggested that bullet ants and banana spiders, respectively, might use contact chemoreception to determine the palatability of prey. Although bullet ants and banana spiders appear to exhibit differences in the degree of predation between tadpole and adult *O. pumilio* (Fritz et al., 1981; Szelistowski, 1985; Stynoski et al., 2014a,b), it is not known how they respond to juveniles of *O. pumilio*, which have lower levels of chemical defenses when compared to the adult life stage (Stynoski et al., 2014a).

Therefore, in the present study, we examined differences in predation upon juveniles and adults of the dendrobatid frog *O. pumilio* as compared to the non-chemically defended leaf-litter frog *Cra-*

gastor bransfordii by two different arthropod predators, the bullet ant, *P. clavata* and the red-legged banana spider, *C. coccineus*. If chemical defenses in *O. pumilio* effectively deter arthropod predation, we expect to see lower predation rates on *O. pumilio* when compared to *C. bransfordii*. Furthermore, if bullet ants and banana spiders are able to detect differences in chemical defenses (i.e., differences in palatability) between juvenile and adult *O. pumilio*, then we expect juveniles to experience higher predation rates when compared to adults.

2. Materials and methods

2.1. Sample and data collection

The present study was conducted at La Selva Biological Research Station in north-eastern Costa Rica (10° 26' N, 83° 59' W) during two different time periods, representing two independent experiments. We conducted the first experiment from 22 June 2011 to 20 July 2011 and the second experiment from 06 June 2015 to 01 July 2015.

O. pumilio is a conspicuously colored, chemically defended, diurnal frog species, whereas *C. bransfordii* is a cryptically colored, non-chemically defended, diurnal frog. Both species are similar in size and are commonly found in the same leaf litter habitats at La Selva (Guyer and Donnelly, 2005).

2.2. Experiment 1: red-legged banana spider predation

We collected 40 *O. pumilio* and 40 *C. bransfordii* from the leaf litter throughout the main trail system at La Selva. For each species, half of the frogs were juveniles (snout-vent length (SVL) < 18.0 mm; although 19 mm SVL is the cut-off size between juveniles and adults of *O. pumilio*, we chose to only consider frogs as juveniles if they were < 18 mm and did not use any frogs that were between 18 and 19 mm) and the other half were adults (SVL > 19.0 mm). Equal numbers of male and female adult *O. pumilio* were used to control for potential differences in defenses between sexes (see Saporito et al., 2010b). Male frogs were identified by the presence of a darkened throat patch (Donnelly, 1989). We collected frogs during the day and housed them individually in plastic bags with a small amount of leaf litter at ambient temperature for no more than three days prior to an experiment.

The red-legged banana spider *C. coccineus* actively hunts at night on vegetation, and retreats during the day to hiding places in the same vegetation (Barth et al., 1988a). We conducted predation trials with 80 individual spiders found along the Camino Experimental Sur Trail between 0 m and 550 m and between 1900 h and 0000 h. We located the spiders on tree trunks or leaves, and identified the species and sex of each individual (Barth et al., 1988a). Female *C. coccineus* were more commonly encountered, and therefore we conducted the majority of the trials with female spiders ($n = 79$), and only one trial with a male spider. We did not perform trials in rainy conditions to avoid the potential confounding effects on spider behavior (Barth et al., 1988b). *C. coccineus* often remain in the same location over time, and therefore we flagged the location of each spider following each trial and did not use the same individual in more than one experimental trial.

For each trial, we presented a randomly chosen frog to a single spider while holding the base of a back leg of the frog with a pair of 26 cm long forceps (similar to the methods of Fritz et al., 1981). To avoid the possibility of alkaloid contamination during trials with *C. bransfordii*, we used one pair of forceps for presenting *O. pumilio* and a different pair for *C. bransfordii*. Once secured by the forceps, each frog was positioned upright, facing the spider, and was slowly moved toward the spider until about 1 cm of distance remained between the frog and the spider. If the spider did

not immediately attack the frog at this distance, we moved the frog forward to gently touch one of the spider's front legs, following the methods of Szelistowski (1985). Occasionally, a spider's position made it difficult to avoid human interference, such as on a flexible palm leaf. In these cases, we dropped the frog in front of the spider from about 15 cm above. After allowing 2 min for each trial, we assessed spider behavior using the same categories as Szelistowski (1985): (1) attacked and consumed; (2) attacked but rejected; and (3) not attacked. Each experimental trial consisted of a new spider being presented with a new individual frog.

In trials where the spider attacked and rejected a frog, we recorded the approximate time the spider held onto the frog before rejection. If the spider was disrupted by human interference at any point during a trial or the interaction category was unclear, we deemed the trial unfit for inclusion in the final analysis and did not use the spider in the study. If this occurred during the presentation of *O. pumilio*, we did not use the frog in any further trials in order to control for potential variation in alkaloid secretion. If the spider attacked and consumed *O. pumilio*, we caught the spider and held it in the laboratory for 72 h to determine if the interaction was fatal to the spider. Additionally, if the spider attacked and rejected *O. pumilio*, we re-captured the frog and held it in the laboratory for 72 h to determine if the interaction was fatal to the frog. We returned all surviving frogs and spiders to their original point of capture.

2.3. Experiment 2: bullet ant predation

We collected 40 *O. pumilio* and 40 *C. bransfordii* individuals from the leaf litter throughout the main trail system at La Selva. As in experiment 1, half of the 40 frogs of each species were juveniles (SVL < 18.0 mm) and half were adults (SVL > 19.0 mm). We used an equal number of male and female adult *O. pumilio* and housed all frogs under the same conditions as outlined in experiment 1.

Bullet ants (*P. clavata*) commonly nest in the base of trees and actively forage in the canopy, returning to their nests to consume prey (Young and Hermann, 1980). We conducted all experimental trials on tree trunks along the main foraging trail of an active colony located in the La Selva Arboretum. For each experimental trial, following the methods of Szelistowski (1985), each frog was tied at the waist to the end of a brown piece of thread ca. 35 cm in length and lowered slowly into the foraging trail of bullet ants. To control for potential variation in ant behavior within and between nests, we presented one of each frog type to a single nest in a random order. We used each individual frog for a single trial and did not use any single nest for more than five trials in a single day ($n = 11$ nests). After allowing 4 min for each trial, we assessed predation similarly to Fritz et al. (1981). We used the categories (1) touched, but rejected; (2) attacked, but rejected; and (3) attacked, not rejected. Touch included any physical contact that was not a bite or sting, and usually consisted of an ant investigating the frog with its antennae. We considered a bite or sting on any part of the body to be an attack. From the time of the attack, we waited 1 min for the ant to reject the frog. In the case of multiple attacks, we considered the frog to be rejected only if all attacking ants released it. Frogs that were attacked and not rejected were pulled toward the nest by one or multiple ants, which we interpreted as an attempt by the ant(s) to feed upon the frog. This interpretation is based upon a pilot study in which we presented and observed 10 adult *C. bransfordii* that were taken into a bullet ant nest. In all other cases, attacked frogs were rescued from bullet ants and held for 24 h to determine if the attack was fatal. All surviving frogs were returned to their original point of capture.

Table 1

Behavioral responses of *C. coccineus* (banana spiders) when presented adult and juvenile individuals of the chemically defended frog *O. pumilio* and the non-chemically defended frog *C. bransfordii*. Data are presented as totals ($n = 80$).

Life stage and species	Not attacked	Attacked, rejected	Attacked, consumed
Adult <i>O. pumilio</i>	1	19	0
Juvenile <i>O. pumilio</i>	0	20	0
Adult <i>C. bransfordii</i>	1	0	19
Juvenile <i>C. bransfordii</i>	0	3	17

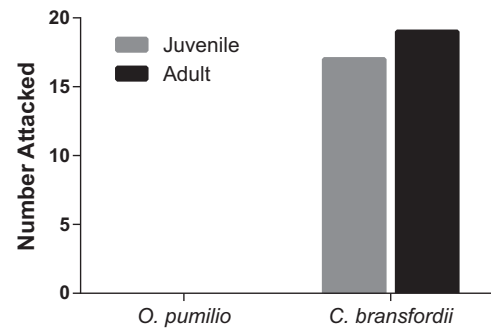


Fig. 1. Number of attacks on adult and juvenile *O. pumilio* (chemically defended) and *C. bransfordii* (non-chemically defended) frogs by *C. coccineus* (banana spider). The attacks reported are the number of frogs attacked without rejection ($n = 80$).

2.4. Statistical analysis

We used binary logistic regression to determine if frog species and frog life stage were significant predictors of spider and ant predation. All statistical analyses were conducted in SPSS version 18.0 and graphs were constructed in Prism version 6.0 g (GraphPad Software, San Diego, CA, USA).

3. Results

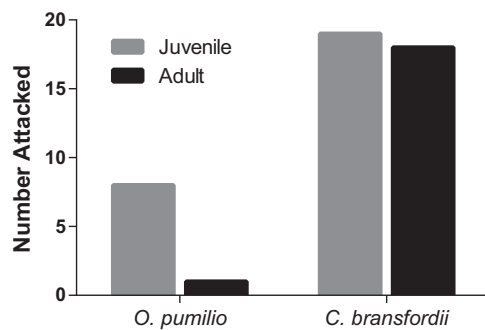
3.1. Experiment 1: red-legged banana spider predation

Of the 80 frogs presented to *C. coccineus*, just 3 individuals (8%) of *C. bransfordii* were rejected after attack, whereas 39 (98%) of *O. pumilio* were rejected. Overall, frog species was a significant predictor of predation (attack and consume), such that *C. bransfordii* were significantly more likely to be preyed upon by the spiders when compared to *O. pumilio* ($P \leq 0.001$, odds ratio = 140.4; Table 1 and Fig. 1). Between adult frogs ($P \leq 0.001$, odds ratio = 190.0) and juvenile frogs ($P \leq 0.001$, odds ratio = 85.0), species was a significant predictor of predation, with *C. bransfordii* significantly more likely to be preyed upon than *O. pumilio* in their respective life stages (Fig. 1). Life stage (adults vs. juveniles) did not significantly predict predation in *C. bransfordii* or *O. pumilio*, with both stages of each species experiencing similar predation rates ($P = 0.194$ for *C. bransfordii*; spiders did not attack and consume any *O. pumilio*; Fig. 1). Often after attacking and rejecting *O. pumilio*, the spider retreated from the site and performed cleaning behaviors on its chelicerae and pedipalps, which had previously been noted by Szelistowski (1985).

Of the 42 interactions in which a frog was attacked and rejected, the spiders rejected 24 frogs (57%) instantaneously after contact and rejected 16 frogs (38%) within 1–20 s of contact. In the remaining two trials, one with an adult male *O. pumilio* and one with a juvenile *O. pumilio*, the spider held each of the frogs for approximately 4 min before rejecting the frog. After rejecting the adult *O. pumilio*, the spider moved around in a slow and uncoordinated fashion before resuming its previous hunting position. This disoriented behavior was not observed in any other trials, and after 72 h

Table 2
Behavioral responses of *P. clavata* (bullet ants) when presented adult and juvenile individuals of the chemically defended frog *O. pumilio* and the non-chemically defended frog *C. bransfordii*. Data are presented as totals ($n = 80$).

Life stage and species	Touched, rejected	Attacked, rejected	Attacked, consumed
Adult <i>O. pumilio</i>	14	5	1
Juvenile <i>O. pumilio</i>	8	4	8
Adult <i>C. bransfordii</i>	1	1	18
Juvenile <i>C. bransfordii</i>	1	0	19

**Fig. 2.** Number of attacks on adult and juvenile *O. pumilio* (chemically defended) and *C. bransfordii* (non-chemically defended) frogs by *P. clavata* (bullet ant). The attacks reported are the number of frogs attacked without rejection ($n = 80$).

of laboratory observation the spider did not demonstrate adverse side effects. The adult frog had three visible puncture marks, one on the central dorsal region and two just below its right front leg. The juvenile *O. pumilio* held by the banana spider died during the interaction, and we observed two puncture marks on its dorsum. Aside from these two trials, we did not observe puncture marks on any other rejected frogs, and all other rejected frogs and spiders survived the interaction.

3.2. Experiment 2: bullet ant predation

Of the 80 frogs presented, bullet ants (*Paraponera clavata*) attacked (i.e., preyed upon) 9 individuals (23%) of *O. pumilio* and 37 (93%) of *C. bransfordii*. Frog species was a significant predictor of predation, and *C. bransfordii* was significantly more likely to be preyed upon by bullet ants when compared to *O. pumilio* ($P \leq 0.001$, odds ratio = 42.5; Table 2 and Fig. 2). Among adult frogs (SVL > 19 mm), predation differed significantly between frog species, and *C. bransfordii* were more likely to be preyed upon by bullet ants when compared to *O. pumilio* ($P \leq 0.001$, odds ratio = 171.0; Table 2 and Fig. 2). Frog species significantly predicted predation among juveniles (SVL < 18 mm), with juvenile *C. bransfordii* more likely to be preyed upon by bullet ants when compared to juvenile *O. pumilio* ($P = 0.003$, odds ratio = 28.5; Table 2 and Fig. 2). Adults and juveniles of *C. bransfordii* experienced similar predation rates ($P = 0.556$); however, bullet ants preyed upon juvenile *O. pumilio* significantly more often than on adult *O. pumilio* ($P = 0.024$, odds ratio = 12.7; Table 2 and Fig. 2). Rejection after touch or attack by bullet ants also differed by species, with *O. pumilio* rejected 78% of the time, and *C. bransfordii* only 8% of the time (Table 2). In all observed interactions of bullet ants with both species of frogs, the ant made initial contact to the frog with antennae prior to attack.

In many cases, after bullet ants made contact with adult *O. pumilio*, ants were observed to perform cleaning behaviors, which had previously been noted by Fritz et al. (1981). These behaviors consisted of bringing the antennae to their mouths to clean, or wiping antennae and/or mouthparts repeatedly on the tree bark. Disorientation behavior sometimes accompanied cleaning, characterized by changes in direction and/or continuing along a

non-linear path. These behaviors were never observed when bullet ants made contact with juvenile *O. pumilio*.

4. Discussion

Organisms that sequester chemical defenses from diet typically exhibit variation in their defenses (Daly and Myers, 1967; Opitz and Müller, 2009; Saporito et al., 2009; Savitzky et al., 2012), and studying how predators respond to this variation is fundamental to understanding predator–prey interactions. Some chemically defended arthropods exhibit ‘palatability spectra’, which can result in differential predation by certain predators (Brower et al., 1968; Bowers, 1993; Savitzky et al., 2012). Dendrobatids sequester their alkaloid-based chemical defenses from an arthropod diet, resulting in variable defenses (see Saporito et al., 2012 for review), one aspect of which involves differences between juvenile and adult frogs (Stynoski et al., 2014a). The results of the present study support the hypothesis that alkaloid defenses in the skin of the dendrobatid frog *O. pumilio* are effective at deterring natural arthropod predators. The effectiveness of alkaloid defenses in *O. pumilio*, however, appears to be related to predator type as well as differences in defense between frog life stages.

Juvenile *O. pumilio* possess fewer and smaller-sized poison glands than adults, and these contain significantly smaller quantities of alkaloid defenses (Saporito et al., 2010a; Stynoski et al., 2014a). Our study demonstrates that both adult and juvenile *O. pumilio* are equally protected from predation by the banana spider *C. coccineus*, even though juveniles contain significantly less alkaloids than adults. The bullet ant *P. clavata*, however, preyed upon *O. pumilio* juveniles significantly more often than on adult frogs, which suggests that the lower levels of alkaloid defenses in juvenile *O. pumilio* are detectable by bullet ants. Late-stage tadpoles of *O. pumilio* contain similar quantities of alkaloids as juveniles, providing them greater protection from predation than a non-chemically defended tadpole, but bullet ants also prey upon them more frequently than on adults (Stynoski et al., 2014a). Collectively, these findings suggest that bullet ants (and possibly other arthropod predators) perceive variation in alkaloid defenses between life stages of *O. pumilio* as a spectrum of palatability and preferentially feed upon less defended frogs. This protection is likely due to the differences in alkaloid defenses, although it remains possible that other or additional chemical defenses (not described or known to be present) could be acting as a deterrent in these individuals (see Weldon and Carroll, 2007 for other possible deterrents). Despite containing significantly less alkaloid defenses than adults, juvenile *O. pumilio* (and late-stage tadpoles; see Stynoski et al., 2014a) possess similar coloration to adult frogs, and therefore may be protected from visual predators as a type of automimicry among life stages (see Saporito et al., 2010a and Stuckert et al., 2014 for further discussion), but are not as well defended against certain arthropod predators.

Differences in feeding mechanisms between arthropod predators may explain the ability of bullet ants, but not banana spiders, to prey upon some *O. pumilio* despite the presence of chemical defenses. Bullet ants can be cooperative foragers (Young and Hermann, 1980) and exhibit graded recruitment, in which varying

amounts of workers respond to the capture of different prey items (Breed et al., 1987). This feeding mechanism suggests that individual bullet ants share large food items and therefore may ingest smaller portions of potentially harmful alkaloids when compared to an individual banana spider that hunts alone and would consume the entire frog. The specific feeding behavior exhibited by spiders may increase their exposure to a greater amount of alkaloid defenses, thus leading to greater avoidance. Bullet ants and banana spiders may also suffer different physiological consequences from alkaloid ingestion. The slow, uncoordinated behavior observed in the spider that attacked and punctured an adult male *O. pumilio* suggests that exposure to alkaloids may have a temporary negative effect on spider behavior. During this period of slow behavior, the spider did not return to its hunting position, potentially costing it foraging opportunities or other fitness-enhancing activities. Both of these consequences represent a behavioral trade-off for these spiders. Although we also observed disoriented behavior in bullet ant individuals after attacking *O. pumilio*, this likely confers a negligible fitness loss to the colony.

The cleaning of chelicerae and pedipalps after rejecting *O. pumilio* in banana spiders, as well as a similar wiping behavior observed in bullet ants suggests that both of these predators use contact chemoreception to determine prey palatability and find *O. pumilio* distasteful (Fritz et al., 1981; Szelistowski, 1985). Poison glands are widely distributed throughout the skin of *O. pumilio*, and any contact with frogs would presumably result in direct contact with alkaloids (Saporito et al., 2010a). Fang wiping has also been observed in the spider *Sericopelma rubronitens* after coming into contact with *Dendrobates auratus*, further suggesting that contact with alkaloids is perceived as distasteful (Gray et al., 2010). Alkaloids in *O. pumilio* (and other dendrobatids) likely function as a chemical deterrent and may provide an evolutionary benefit against predation by predators that use contact chemoreception, even if alkaloids are present at low levels. Ants and spiders typically made contact with frogs before attacking, and it is likely that they use contact chemoreception to differentiate palatable from unpalatable prey. Several dendrobatid alkaloids have been reported as toxic to mosquitoes and ants (*Solenopsis invicta*) based simply on contact (Weldon et al., 2006, 2013). The initial contact by banana spiders and bullet ants to the frogs also suggests that predators did not use color or pattern recognition in avoidance of *O. pumilio*. Studies have demonstrated that members of the genus *Cupiennius* cannot distinguish differences in color patterning of moving objects (Orlando and Schmid, 2011) and lack color vision (Barth, 2002), both of which may be expected of a nocturnal predator such as these spiders. For these reasons, contact chemoreception may be critically important to these predators. Using contact chemoreception as a mechanism to differentiate prey may provide these invertebrates with an advantage in identifying palatable prey. These predators are able to perceive the chemical signal rather than a potentially misleading visual signal, such as the conspicuous coloration exhibited by juvenile *O. pumilio*, despite their relative palatability.

The findings of the present study also have implications for the evolution of chemical defenses in dendrobatid frogs. While avian predation is almost certainly a driving evolutionary force behind aposematic coloration in these frogs (Summers and Clough, 2001; Darst et al., 2006; Noonan and Comeault, 2009), arthropod predators have likely played an important role in the evolution of chemical defenses. Aposematic theory suggests that selection should favor automimics (i.e., palatable, yet aposematically colored individuals) because they circumvent the potentially high cost of maintaining secondary defenses while still avoiding predation (Brower et al., 1970; Guilford, 1994). Color-sighted predators are thought to regulate the prevalence of automimics using sample-and-reject strategies (Fink and Brower, 1981; Gamberale-Stille and

Guilford, 2004; Ruxton and Speed, 2006); however, invertebrate predators may also play a large and currently underappreciated role in this regulation. Understanding how often invertebrate predators naturally prey upon dendrobatid frogs may yield further insight into the maintenance of automimicry in natural aposematic systems.

The presence of alkaloids in *O. pumilio* appears to complement warning coloration and may allow for protection against not only color-sighted predators, but predators that use chemoreception as well. On the basis of our experiment, predators using chemoreception likely avoid *O. pumilio* based solely on the presence of alkaloids, suggesting that alkaloids are an effective warning signal to these predators. However, the degree to which alkaloid defenses protect *O. pumilio* from predation depends on both the predator they encounter and the relative alkaloid defenses an individual may contain. Differences in alkaloid defenses among life stages in *O. pumilio* correspond to differences in relative palatability, whereby ant predators are able to prey on more palatable juvenile frogs that contain fewer defenses than adults.

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