

Does alkaloid sequestration protect the green poison frog, *Dendrobates auratus*, from predator attacks?

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Abstract. An alkaloid-sequestering frog, *Dendrobates auratus*, and a non-toxic frog, *Physalaemus pustulosus* were offered as prey to theraphosid spiders, *Sericopelma rubronitens*. The spiders, which do not use visual cues when hunting, sampled all presented frogs. They rejected, or failed to fully consume, toxic frogs significantly more often than the sympatric non-toxic frogs. Alkaloid sequestration did not protect *D. auratus* from attacks, but did reduce the risk of being eaten. The spiders are one of only a few documented predators of *D. auratus* on the Panamanian island of Taboga, and may represent a strong selective force for the high toxicity in this population.

Key words. Dendrobatidae, Leiuperidae, spider predation, feeding choice study, *Physalaemus pustulosus*

Introduction

Dendrobates auratus (GIRARD, 1855) (family Dendrobatidae), the green poison frog, is a diurnal terrestrial frog from Central America and adjacent South America, with toxic skin secretions that contain many alkaloids (DALY & MYERS 1967, DALY et al. 1987). These alkaloids are diet-derived, with ants and mites being the main sources of toxic compounds identified so far (DALY et al. 2000, SAPORITO et al. 2004, DALY et al. 2005, SAPORITO et al. 2007). These alkaloidal skin secretions are thought to protect the frogs from predation (DALY et al. 1978), but this has never been tested in the field or with an identified predator. The reason for this may be that so little is known about the predators of *D. auratus*. Observed natural predators of adults, based on anecdotal accounts, include a fish, the Macabi tetra, *Brycon guatemalensis*, (HEDSTROM & BOLAÑOS 1986), a bird, the rufous motmot, *Baryphthengus marhii* (MASTER 1998), and a theraphosid spider, the Panama red rump tarantula, *Sericopelma rubronitens* (SUMMERS 1999). A grapsid crab, *Armases angustum*, is known to predate upon the tadpoles of *D. auratus* (GRAY & CHRISTY 2000).

With a series of feeding choice experiments, we sought to answer two questions. The first question we asked was whether or not *Sericopelma rubronitens* AUSSERER, 1875 was a regular predator of *D. auratus*. The second question we asked was whether alkaloid-containing secretions afford *D. auratus* greater protection against spider attack as compared to a non-toxic prey, the túngara frog, *Physalaemus pustulosus* (COPE, 1864) (family Leiuperidae). *Physalaemus pustulosus* is a sympatric, similarly sized frog that does not secrete a potent cocktail of alkaloids (DALY et al. 1987). *Physalaemus pustulosus* is palatable to a wide range of predators (RYAN 1985) including *S. rubronitens* (GRAY et al. 1999). Isla Taboga, Panama, is home to large

populations of *D. auratus*, *P. pustulosus* and *S. rubronitens*. Both species of frog, as well as the spider, are active during the day, which contrasts with the primarily nocturnal behaviour of all other studied populations of *P. pustulosus* (JAEGER & HAILMAN 1981, RYAN 1985). On Isla Taboga, both species of frog would therefore be equally exposed to potential spider predation.

Methods

Trial location and test species

Experiments were conducted on Isla Taboga, Republic of Panama (8°47'N, 79°34'W) in June of 1998 during daylight hours. The two frog species were similar in mean snout-vent length (*D. auratus*—29.1 mm ± 2.19, n = 163; *P. pustulosus*—31.8 mm ± 2.33, n = 180 (GRAY et al. 2002)). Frogs used in trials were hand-captured and measured (snout-vent length) to the nearest 0.1 mm using dial calipers so each experimental trial could use frogs that were matched for size.

Trial setup

Female spiders indicate a readiness for hunting by sitting in the open with the head pointing away from their burrow. A total of 31 female spiders (> 100 mm in total length) were presented with one adult *D. auratus* and one adult *P. pustulosus* simultaneously. A 1.5 m length of black thread was tied around the waist of each frog to tether it. Tethering in this manner has no effect on the frog's mobility (FORMANOWICZ et al. 1981) and was necessary to confirm the fate of the frog in case the spider carried it into its burrow. The two frogs were lowered simultaneously to

the ground in front of the spider, a maximum distance of 20 cm away. The two frogs were never more than 10 cm apart. Prey preference was determined by which frog was consumed by a spider that had been handled both potential prey types. The fate of all frogs used in these trials was recorded to determine if the spiders differentially killed or released the two species of frog after handling. Any spider that interacted with at least one frog was not used in subsequent trials. Although the spiders were not marked, they displayed site fidelity and it was assumed that only one female used any particular burrow. A Chi-square Test was performed to test the expectation that spiders had no prey preference (ZAR 1999).

Results

Of the 31 spiders presented with two frogs, 12 handled only one frog before retreating to their burrows. These spiders did not display any preference for which species was grabbed first ($\chi^2 = 0.3103$, $0.5 < P > 0.25$, $df = 1$, $n = 29$). In two trials, the spider was directly over both frogs and although it handled each frog separately, it was ambiguous which frog the spider actually touched first.

There were 19 successful trials during which both frogs were handled and a choice could be identified. In ten of these trials (53%), at least one frog was killed by the spider. In two of the trials (11%), both frogs were killed although only one of the two frogs was ultimately eaten. Overall, in the 19 trials, three *D. auratus* (16%) and nine *P. pustulosus* (47%) were killed (Figure 1). Of the twelve dead frogs, the spiders consumed two *D. auratus* and eight *P. pustulosus*. In nine trials, both frogs were handled and then released. All released *P. pustulosus* were unharmed ($n = 10$), but four of the 16 released *D. auratus* (25%) received minor scratches. These four *D. auratus* were observed for 24 hours and then released, as they did not appear to be affected by their injuries.

Overall, significantly more *P. pustulosus* than *D. auratus* were killed by spiders ($\chi^2 = 4.3846$, $0.05 < P < 0.025$, $df = 1$, $n = 19$) and significantly more *P. pustulosus* than *D. auratus* were consumed ($\chi^2 = 4.8857$, $0.05 < P < 0.025$, $df = 1$, $n = 19$).

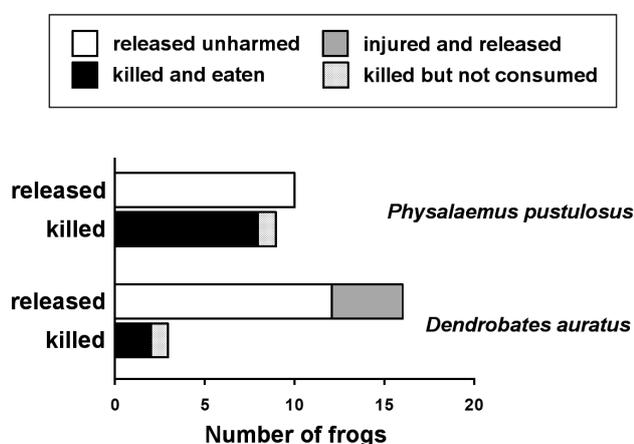


Figure 1. Fate of frogs, *Dendrobates auratus* and *Physalaemus pustulosus*, presented simultaneously to the theraphosid spider, *Sericopelma rubronitens*.

Discussion

A wide variety of spiders predate on frogs in the Neotropics (DE ARMAS 2001, MENIN et al. 2005, JOVANOVIĆ et al. 2009, PONTES et al. 2009) and spider predation on frogs is probably more common than available documentation attests. *Sericopelma rubronitens* is a non-visual hunter who uses substrate vibrations to detect potential prey (DEN OTTER 1974). In addition to vibrational cues, *S. rubronitens* has receptors on its legs and pedipalps, which are sensitive to minute air currents (DEN OTTER 1974). Even when all visual cues are removed by covering the spider's eyes, the form and effectiveness of the prey capture behaviour of *S. rubronitens* continues unchanged (DEN OTTER 1974). Experiments show that olfaction does not contribute to the decision to capture prey (DEN OTTER 1974). Therefore, as was observed, any frog moving about in the leaf litter risks attack and handling by a spider. The decision to consume captured prey is made while the captive is underneath the spider and is in contact with chemoreceptors on the distal portion of the spider's legs and pedipalps (DEN OTTER 1974, FOELIX 1982, COCROFT & HAMBLER 1989). This sampling process allows the spider to reject distasteful prey.

When confronted by a spider during our experiment, *D. auratus* would produce visible amounts of skin secretions. These toxic skin secretions appear unpalatable to *S. rubronitens*. One spider during our trials envenomed a *D. auratus*, then dropped it and vigorously wiped its fangs. The same spider subsequently proceeded to envenom and consume a *P. pustulosus*. Fang wiping has also been observed in other spiders when presented with unpalatable prey (FOELIX 1982). The distastefulness of another dendrobatid frogs (*Oophaga pumilio*) to invertebrate attackers has been demonstrated in the ant, *Paraponera clavata* (FRITZ et al. 1981) and the ctenid spider, *Cupiennius coccineus* (SZELISTOWSKI 1985).

As the toxins in dendrobatid skin secretions are diet-derived, this generates individual and population-level variation in toxicity (DALY et al. 1994a, b, 2002, SAPORITO et al. 2010). Such individual variation could account for spiders rejecting some *D. auratus* while consuming others that may be less toxic. *Sericopelma rubronitens* represents the only confirmed predator of adult *D. auratus* on Taboga and through constant sampling of the *D. auratus* population, these predators may exert strong selective pressure to maintain high levels of toxicity. Indeed, *D. auratus* from Taboga have a relatively greater toxicity than other species of *Dendrobates* (DALY et al. 1978). Within *D. auratus*, the Taboga population has a greater quantity and variety of alkaloids than found in other populations (DALY et al. 1987). In the predator-prey interactions of the spider-dendrobatid system on Isla Taboga, spiders indiscriminately attack frogs, irrespective of their toxicity. The more toxic frogs may escape injured but alive, whereas the less toxic frogs will perish.

Dendrobatid frogs are classically considered to be aposematic (COTT 1940, DUNN 1941, DALY & MYERS 1967, ENDLER 1986, DALY et al. 1987). Aposematic or warning colouration in animals is the co-occurrence of conspicuous colouration and toxicity favored by a single agent of natural selection: predation pressure (COTT 1940). In the case of *S. rubronitens*, the bold green and brown patterns

of *D. auratus* are unlikely to serve as a warning of distastefulness. *Sericopelma rubronitens* is a non-visual hunter and the decision to consume a prey item is made outside of the spider's field of vision (DEN OTTER 1974). As no visually-hunting predators of *D. auratus* have been identified on Taboga, we are led to propose that the bright colours and toxicity in this system are not evolutionarily coupled, and that colouration must be maintained by something other than predation.

Although anurans commonly rely upon vocalizations for communication, visual signaling is also found in a variety of diurnal species (WELLS 1980, HADDAD & GIARETTA 1999). The colours of other diurnal dendrobatids have been shown to be involved in intraspecific communication. For example, mate selection and aggressive behaviour in *Oophaga pumilio* (DUELLMAN 1966, SUMMERS et al. 1999) and territorial defense in both sexes of *Colostethus trinitatis* (WELLS 1980) depend to a great degree on colour displays. *Dendrobates auratus* is known to have a complex social system that includes male territorial defense and parental care (SUMMERS 1989, SUMMERS 1990). Male *D. auratus* produce low-volume calls directed at one or more nearby frogs that were first visually located (H. M. GRAY unpubl. data). Females, which do not vocalize, guard their mates and aggressively chase away encroaching females (SUMMERS 1989). Bright colours, present in both sexes, would facilitate identification of nearby frogs and serve as an important signal used to maintain the frog's complex social system.

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