Maternally derived chemical defences are an effective deterrent against some predators of poison frog tadpoles (*Oophaga pumilio*).

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Parents defend their young in many ways, including provisioning chemical defences. Recent work in a poison frog system offers the first example of an animal that provisions its young with alkaloids after hatching or birth rather than before. But it is not yet known whether maternally derived alkaloids are an effective defence against offspring predators. We identified the predators of *Oophaga pumilio* tadpoles and conducted laboratory and field choice tests to determine whether predators are deterred by alkaloids in tadpoles. We found that snakes, spiders and beetle larvae are common predators of *O. pumilio* tadpoles. Snakes were not deterred by alkaloids in tadpoles. However, spiders were less likely to consume mother-fed *O. pumilio* tadpoles than either alkaloid-free tadpoles of the red-eyed treefrog, *Agalychnis callidryas*, or alkaloid-free *O. pumilio* tadpoles that had been hand-fed with *A. callidryas* eggs. Thus, maternally derived alkaloids reduce the risk of predation for tadpoles, but only against some predators.

1. Introduction

Parents defend offspring from predators with a variety of mechanisms, including supplying young with chemical defences [1–5]. Parental provisioning of chemical defences generally occurs before offspring are born or hatch, so the ability of offspring to deter predators diminishes with development [3,5]. Recent research shows that strawberry poison frog (*Oophaga pumilio*) mothers provision tadpoles with alkaloids for many weeks after hatching, and that the quantity of alkaloids increases with development. Currently, little is known about whether maternally derived alkaloids are an effective defence against tadpole predators [6].

Dendrobatid frogs such as *O. pumilio* are a model system for understanding chemical defence and aposematism [7]. *Oophaga pumilio* sequesters alkaloids from a diet of certain mites, ants, beetles and millipedes and stores them in granular skin glands [7]. Alkaloids have recently been detected in tadpoles, juveniles and adults [6]. The aposematic signal of adult *O. pumilio* deters avian predators [8,9]. Also, while mechano- and chemosensory hunting bullet ants (*Paraponera clavata*) and wandering spiders (*Cupiennius coccineus*) readily eat alkaloid-free *Craugastor bransfordii* frogs (which are sympatric and similar in size to *O. pumilio*), these predators avoid eating juvenile and adult *O. pumilio* [10–12].

*Oophaga pumilio* demonstrates unique and complex parental care. Males defend and hydrate fertilized egg clutches until females return to transport...
tadpoles individually to rainwater collected in small pools (i.e. phytotelmata), such as in the axils of bromeliad plants [13]. Then, for approximately six weeks until tadpoles metamorphose, mother frogs return every few days to feed tadpoles with unfertilized eggs [13–15]. The eggs that mothers provide contain arthropod-derived alkaloids that accumulate in tadpoles over time; alkaloids were not detected in very young tadpoles but were detected and increased in quantity from stage 34 through metamorphosis [6].

Predation of *O. pumilio* tadpoles is high (67% [14]). The alleged predator is a common wandering spider (*Cupiennius* sp.) that coexists in the bromeliad habitat with *O. pumilio* tadpoles and eats other anuran adults and larvae [11,14,16]. However, there is not yet definitive evidence of spiders eating phytotelm-dwelling tadpoles. Herein, we hypothesized that the alkaloids that mother frogs provision to tadpoles are an effective defence against their natural predators. We first used motion-activated video cameras in the field to identify natural predators. Then, we conducted laboratory and field preference tests with two primary predators to determine whether maternally derived alkaloids deter predators and thus serve as an effective chemical defence.

2. Material and methods

We completed this study at La Selva Biological Station, Costa Rica from October 2010 to October 2013. Artificial phytotelmata made from 15 ml clear plastic cups were hung on trees to simulate the natural rearing environment. Mother frogs deposit and care for tadpoles in cups just as they do in natural bromeliads [15].

First, we identified the natural predators of *O. pumilio* tadpoles using video cameras (high-resolution colour weather-proof cameras with 3.5–8 mm zoom vari-focal lens and infrared night vision, ACC-P07N ActiveVision Inc., Ronkonkoma, NY, USA) and the motion-activated setting on digital video recorders (4-channel H.264 DVR, Advance Security, Belleville, IL, USA). We attached cameras to trees above artificial or natural phytotelmata containing early stage mother-fed tadpoles. When a tadpole disappeared, we reviewed the video to identify the predator and note the time of predation. We chose two common predators from videos: the southern cat-eyed snake (*Leptodeira septentrionalis*) and the wandering spider (*C. coccineus*), and tested whether tadpole alkaloids were an effective deterrent against those predators. With snakes, which are rarely sighted (i.e. it took three years to capture 14 snakes and seven snake predation videos), we conducted choice tests in laboratory aquariums in which unresolved number of tadpole baits would be needed to conduct the study in the field. With spiders, we conducted predation trials in the field on 14 nights during July, August and November 2012, because spiders are easily found in their territories and would not eat prey in captivity.

With *L. septentrionalis*, we placed each snake (*n* = 14) in the centre chamber of a three-chambered aquarium (with two parallel dividers creating chambers) in an outdoor, screened laboratory with leaf litter from the capture site and rainwater ad libitum to acclimate for one week. Then, we placed one tadpole of *O. pumilio* stage 37–42 (those that very likely have alkaloids but not yet any dorsal coloration [6]) in a cup of recently collected rainwater in a randomly chosen side chamber and a tadpole of *Agalychnis callidryas* in another cup of rainwater in the other side chamber. We used stage 25 *A. callidryas* tadpoles because they are approximately the same size as stage 37–42 *O. pumilio* tadpoles. We allowed the snake and tadpoles to acclimate for 24 h in the aquarium before removing the two dividers. Then we checked tadpole presence every 12 h for five days or until the snake ate one of the tadpoles. If a snake ate a tadpole, we removed the other tadpole and casually observed the snake for two days to determine whether tadpole ingestion affected snake condition or behaviour. Each animal was used in only one trial. The aquarium and cups were thoroughly cleaned between trials.

With spiders, we conducted predation trials within natural territories. Each spider was presented with a single tadpole in one of three groups: (i) mother-fed *O. pumilio* stage 37–42 that very likely contain alkaloids (*n* = 12) [6], (ii) *O. pumilio* fed for five weeks with eggs of *A. callidryas* (*n* = 9) or (iii) *A. callidryas* tadpoles of a similar size as late-stage *O. pumilio* tadpoles (*n* = 9). Tadpoles hand-reared with the eggs of *A. callidryas* do not contain alkaloids [6]. Trials were conducted between 19.30 and 22.30 h when spiders come out of their daytime retreats for sit-and-wait hunting and perch on the vegetation [17]. We attached a tadpole to a probe and slowly presented it to the spider at a practised and approximately equal speed for each trial (as in [12]). We used identical but separate probes for each group of tadpoles to prevent alkaloid contamination. We recorded in each trial whether the spider attacked and/or consumed the tadpole. We flagged spider territories after each test to avoid testing the same spider twice; spiders do not change location frequently [12,17].

We used Fisher’s exact test to determine whether tadpole species influenced predation by snakes. We used binomial logit general linear models to test whether tadpole type was a significant predictor of attack or consumption by spiders in the field. We conducted all analyses in R v. 3.0.2.

3. Results

Field cameras captured 13 predation events: two by click beetle larvae (family Elateridae) in bromeliads, two by centid spiders (*C. coccineus*) in bromeliads, seven by northern cat-eyed snakes (*L. septentrionalis*; six in cups and one in a bromeliad) and two by pink-bellied litter snakes (*R. decorata*). Field cameras captured 13 predation events: two by click beetle larvae (family Elateridae) in bromeliads, two by centid spiders (*C. coccineus*) in bromeliads, seven by northern cat-eyed snakes (*L. septentrionalis*; six in cups and one in a bromeliad) and two by pink-bellied litter snakes (*R. decorata*). All predation events occurred in the dark (beetle larvae at 3.38 and 23.53 h; *R. decorata* at 19.56 h; *L. septentrionalis* at 1.59, 2.37, 3.11, 8.56, 17.49, 19.27 h) or at dusk or dawn (*C. coccineus* at 18.03 and 18.11 h; *R. decorata* at 5.26 h; *L. septentrionalis* at 18.10 h). Video recorders also detected events such as mother frogs provisioning tadpoles and ants skirring around cups, so the videos captured likely provide a reliable record of the primary predators of *O. pumilio* predators at this site. Videos are available from the authors upon request.

The 10 snakes that ate a tadpole in the laboratory did not show a preference for *O. pumilio* (six eaten) or *A. callidryas* (four eaten; odds ratio = 1.47; *p* = 1.0). No ill effects were noted when snakes ate tadpoles.

Spiders were over 33 times more likely to consume alkaloid-free *O. pumilio* (*p* = 0.01; Exp(B2) = 33.02) and over 87 times more likely to consume *A. callidryas* (*p* = 0.003; Exp(B1) = 87.97) than they were to consume mother-fed *O. pumilio* (figure 1). On the other hand, spiders were not more likely to attack alkaloid-free *O. pumilio* (*p* = 0.11; Exp(B2) = 7.03) or *A. callidryas* (*p* = 0.09; Exp(B1) = 8.00) than they were to attack mother-fed *O. pumilio*.

4. Discussion

Field videos identified four natural predators of tadpoles in bromeliads and artificial phytotelmata: southern cat-eyed...
snakes, pink-bellied litter snakes, ctenid spiders and elaterid beetle larvae. The alkaloids that mother frogs provision to tadpoles appear to be an effective defence against spider predators, yet do not appear to deter snake predators.

Snakes were the primary predators identified in videos and they were not deterred by the presence of maternally derived alkaloids in tadpoles. Snakes are well-known predators of many species of non-arboreal tadpoles in aquatic habitats [18]. Colubrid snakes and spiders also predate adult poison frogs [19]. In this study, snakes did not demonstrate obvious harm from eating alkaloid-containing tadpoles. Other colubrid snakes are able to sequester defensive chemicals from their prey [2], and future studies could explore that ability in L. septentrionalis or R. decorata.

Spiders rely heavily on mechanical signals to hunt prey [17], but once they attacked alkaloid-containing O. pumilio tadpoles, they were deterred from consuming them. Spiders demonstrated a strong aversion to consuming alkaloid-containing tadpoles relative to both alkaloid-free O. pumilio and A. callidryas. In a similar study, bullet ants (P. clavata) avoided eating late-stage O. pumilio tadpoles but not A. callidryas tadpoles [6]. Because young tadpoles contain little to no alkaloid compared with the late-stage tadpoles used in this experiment (both overall and per unit mass [6]), our findings suggest that there is selective pressure for tadpoles to acquire alkaloids from their mothers quickly to deter predation by spiders.

In this study, we demonstrate that poison frog tadpoles living in phytotelmata have various predators that enter their aquatic habitat from the external ecosystem. Protection against spiders in later tadpole stages may sufficiently benefit tadpole survival to allow for selection of maternal provisioning of chemical defences. However, predation is still a threat from snakes and in young tadpoles that have not yet sequestered alkaloids from their mothers’ eggs.

This study was conducted with IACUC approval (University of Miami 11-075) and permit from MINAET of Costa Rica (101-2010-SINAC).

Acknowledgements. We thank A. Bremes and M. Arguedas for field assistance, M. Sasa and C. Guyer for snake identification, R. Leschen for beetle larvae identification, R. Saporito, E. Murray and the La Selva maintenance staff.

Funding statement. This study was financially supported by a fellowship from the University of Miami to J.L.S. and a DDEP award (NSF-OISE-1114218) to J.L.S.

References

12. Hantak M. 2013 Dietary alkaloid sequestration and chemical defense in poison frogs: an experimental test of alkaloid uptake in Melanophryniscus steltneri (Bufonidae) and the role of chemical compounds in defense against predation in Dendrobates auratus (Dendrobatidae) and Rhaebo haematomus (Bufonidae). Master’s thesis, John Carroll University, New York, OH, USA.

14. Maple M. 2002 Maternal effects on offspring fitness in Dendrobates pumilio, the strawberry poison frog. PhD dissertation, University of Kentucky, Lexington, KY, USA.


