

To hatch and hatch not: similar selective trade-offs but different responses to egg predators in two closely related, syntopic treefrogs

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Abstract Risk-sensitive hatching is adaptive for species facing a trade-off between egg-stage and post-hatching risks, and environmental variation in one or both stages. Such plasticity has been found in amphibians, fishes, reptiles and spiders, with red-eyed treefrogs (*Agalychnis callidryas*) being the best-studied case. We assessed hatching plasticity and egg- and larval-stage risks in a closely related, syntopic species, the gliding leaf-frog (*Agalychnis spurrelli*). We found a lower hatching response to egg-eating snakes in *A. spurrelli* (9–28% of embryos escaped) than in *A. callidryas* (59–80% escaped). Levels of snake predation were similarly high for clutches of both species monitored at a pond in Costa Rica, and in fish predation experiments early-hatched *A. spurrelli* tadpoles were more vulnerable than later hatchlings, as has been shown for *A. callidryas*. *A. spurrelli* thus face a risk trade-off similar to *A. callidryas*, and likely would benefit from predator-induced hatching; their lower responsiveness to snakes appears nonadaptive. *A. spurrelli* embryos showed a stronger hatching response (57% hatched in 1 h) to submergence underwater than to snake attacks even though submergence is a less frequent risk. This suggests they have a greater capacity for early hatching than is expressed in the context of snake attacks, but have much lower sensitivity to snake cues than to flooding cues. Development in *A. spurrelli* is accelerated compared to syntopic *A. callidryas*, and spontaneous hatching is earlier and more synchronous. This is

congruent with predictions based on selection by egg predators in the absence of a strong escape hatching response.

Keywords Embryo behaviour · Hatching · Inducible defence · Phenotypic plasticity · Predation

Introduction

Most animals begin life as eggs. Both eggs and newly hatched young often have weak defences, compared with more developed stages, and consequently suffer high levels of predation (Fuiman and Magurran 1994; Vonesh 2005). Life history theory suggests that hatching, like other ontogenetic switch points, should evolve to balance these risks, as well as the opportunities, in adjacent life stages (Shine 1978; Werner and Gilliam 1984; Sih and Moore 1993). For instance, egg predators can select for earlier hatching, while predators of newly hatched young can select for later hatching.

If stage-specific risks and opportunities are stable, selection will favour a single fixed hatching stage. However, such stability may be unusual. Environmental heterogeneity in either egg or post-hatching stage can preclude a single optimum, and favour phenotypic plasticity. In general, phenotypic plasticity is favoured by selection if three conditions are met: environmental heterogeneity, a cue that indicates conditions, and a fitness trade-off that makes different phenotypes most fit under different conditions, (West-Eberhard 1989; Via et al. 1995; DeWitt and Scheiner 2004). Adaptive evolution of plasticity is widespread, including divergence between closely related species exposed to different regimes of environmental heterogeneity (Schlichting 1986; Leips and Travis 1994; Doughty 1995; Gotthard and Nylin 1995; Dudley 1996) and convergence

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between distantly related species under similar conditions (Reader et al. 1992; Relyea 2001; Van Buskirk 2002).

Adaptive plastic changes in hatching timing in response to predators and/or pathogens of eggs or larvae are known from several taxa, but have not been found in others. Delayed hatching in response to post-hatching risks has been demonstrated in two amphibians (Sih and Moore 1993; Moore et al. 1996; Laurila et al. 2002) and is suspected in crustaceans (Blaustein 1997). However, eggs of eight amphibian species exposed to chemical cues from known or putative larval predators did not delay hatching (reviewed in Orizaola and Braña 2004). Responses to egg-stage predators and pathogens seem more consistent with predictions, with two fishes (Wedekind 2002; Kusch and Chivers 2004), a lizard (Moreira and Barata 2005), a spider (Li 2002) and eight amphibians (Warkentin 1995; Chivers et al. 2001; Laurila et al. 2002; Vonesh 2005; Touchon et al. 2006; Gomez-Mestre et al. 2006) hatching early, one amphibian hatching smaller, but not earlier (Vonesh 2000), and one amphibian showing no response (Laurila et al. 2002).

To begin to understand the variation across species in embryo responses to risk, we need further comparative studies (Laurila et al. 2002), complete with information on environmental heterogeneity and fitness trade-offs, as well as the responses themselves. For instance, in some cases, predators feed on both egg and larval stages. Without assessment of relative, stage-specific risk it is difficult to predict how embryos should respond, or to assess the adaptive value of particular responses (Laurila et al. 2001; Schalk et al. 2002; Johnson et al. 2003; Saenz et al. 2003). Moreover, responses to subsets of cues in experiments may not always reflect responses to natural predation.

Adaptive plasticity in hatching has been most thoroughly studied in the red-eyed treefrog, *Agalychnis callidryas* (Phyllomedusinae, Hylidae) (Warkentin 1995; Warkentin 1999a, b, 2000a, b, 2002, 2007; Warkentin et al. 2001, 2005, 2006a, b). Here we assess the plastic hatching response to two egg-stage risks in a closely related, syntopic species, the gliding leaf-frog *Agalychnis spurrelli*, and compare it with that of *A. callidryas*. We quantify the egg-stage risk for both species in nature and experimentally test for a fitness trade-off across hatching in *A. spurrelli*, to contextualize variation in hatching responses between the two species. Our null hypotheses were: (1) both species experience similar levels of egg predation; (2) they have the same pattern of undisturbed or spontaneous hatching; (3) their escape hatching responses to snake attack and flooding are similar; and (4) early hatching increases vulnerability to aquatic predators in *A. spurrelli*, as it does in *A. callidryas*.

Materials and methods

Study species

Both *A. spurrelli* and *A. callidryas* are found in humid tropical lowland to premontane forests of Central America (Duellman 2001; Savage 2002). The distribution of *A. callidryas*, from central Veracruz, Mexico, to northern Colombia, includes most of that of *A. spurrelli* (southeastern Costa Rica to northwestern Ecuador). *A. callidryas* is more abundant than *A. spurrelli*, sharing all of the *A. spurrelli* breeding ponds we know in Central America. The species also overlap temporally, both breeding during the rainy season, although *A. callidryas* breed on most rainy nights while *A. spurrelli* breed only a few times each rainy season (Kubicki 2004).

Both species attach their eggs to vegetation over ponds. They use an overlapping range of oviposition sites, mostly leaves and sometimes branches, tree trunks, or other structures; *A. spurrelli* clutches are occasionally embedded in moss, whereas *A. callidryas* eggs are not. The clutches are morphologically distinct. *A. callidryas* lays eggs in smooth, often elliptical, gelatinous masses 10–15 mm deep, whereas *A. spurrelli* eggs are laid in an irregularly shaped flat monolayer (4–5 mm deep), or rarely in a partial bilayer, with little associated jelly. Hatchlings fall into the water to continue their development to metamorphosis, and the tadpoles are behaviourally and morphologically similar (Duellman 2001; Savage 2002). Development of *A. callidryas* embryos is highly synchronous within and among egg clutches at a site (Warkentin 1999b, 2002) but external gill regression, which is used as a developmental marker in standard staging tables (Gosner 1960), is highly plastic. Therefore, we use age within the plastic hatching period as a proxy for developmental stage, and consider it relative to the timing of spontaneous hatching for each species.

A. callidryas embryos hatch prematurely to escape from egg-eating snakes (Warkentin 1995, 2005), wasps (Warkentin 2000b; Warkentin et al. 2006a), a pathogenic fungus (Warkentin et al. 2001) and flooding (Warkentin 2002). Vibrations serve as a cue in snake-induced hatching (Warkentin 2005; Warkentin et al. 2006b) but other cues must mediate the premature hatching of flooded and fungus-infected eggs (Warkentin 2002). This species experiences a risk trade-off at hatching, as early hatchlings are at higher risk of predation by aquatic predators (Warkentin 1995, 1999a). Since *A. spurrelli* share egg and larval environments and much of their phylogenetic history with *A. callidryas* (Wiens et al. 2006; I. G.-M., J. J. Wiens and K. M. W., unpublished data), we hypothesized that they would also share plastic hatching responses to egg-stage risks.

Field survey

We monitored 338 individually marked *A. callidryas* clutches and 30 *A. spurrelli* clutches at a pond (*Agalychnis* pond) near Sirena Field Station in Corcovado National Park, Costa Rica, from 22 June to 14 July 1993, and from 9 June to 5 July 1994. Clutch locations were marked with flagging tape and plastic cups (9 cm diameter \times 7 cm deep, drain holes at 1 cm above the bottom) were hung under each clutch to collect hatchlings (following Hayes 1983; Warkentin 1995). Each clutch was checked twice daily (early morning and late afternoon), and we made additional opportunistic observations of predation during the day and night. Eggs were counted and staged based on easily visible external morphology (Gosner 1960; Pyburn 1963; Warkentin 1999b, 2002), hatchlings were counted and released, and the state of the clutch was noted. Egg predation was assessed as animals that disappeared between censuses.

Predator type was determined by the pattern of egg removal and condition of clutch remains, corroborated by many direct observations of predation (Warkentin 1995, 2000b). Wasps, *Polybia rejepta*, take one embryo at a time repeatedly during the day, and leave broken egg capsules, smeared yolk, and injured embryos and hatchlings. Snakes take both embryos and jelly, consuming entire clutches or large portions thereof, but do not damage eggs that they leave. The most common egg-eating snake, the cat-eyed snake, *Leptodeira septentrionalis*, is strictly nocturnal, but parrot snakes, *Leptophis ahaetulla*, which are active day and night, also prey on *Agalychnis* eggs. White-faced capuchin monkeys, *Cebus capucinus*, consume entire or sometimes partial clutches, sometimes breaking leaves off plants to do so and leaving teeth marks on clutch remains. Fly larvae are visible in and on the egg clutches (Villa 1980; Villa and Townsend 1983).

Egg predation experiments

Hatchling counts from clutches attacked by snakes in the field underestimate escape rate because snakes often displace clutches from their position over the cups, allowing hatchlings to fall directly into the pond. Thus, we experimentally exposed clutches of both species (16 *A. spurrelli* and 49 *A. callidryas*) to snake attacks to assess embryo responses (as in Warkentin 1995). We used cat-eyed snakes, *L. septentrionalis*, which were by far the most common *Agalychnis* egg predator at the pond. We caught snakes at night at the pond and kept them individually in mesh cages in the forest. We exposed hatching-competent clutches of both species (ages 5–8 days and 4–5 days for *A. callidryas* and *A. spurrelli*, respectively) to snakes in their cages, with individual water containers placed below the clutches to collect hatchlings. The snakes are arboreal and

do not feed on tadpoles in the water. Escape hatching success was calculated as the fraction of eggs present at the start of an attack that hatched during the attack. We checked clutches and snakes frequently during the night (every 15 min to 1 h while snakes were active) so that any eggs that hatched spontaneously prior to contact with the snake could be excluded from escape success calculations. We used a dim flashlight and approached cages cautiously to minimize disturbance to snakes. In most snake attacks all eggs either hatched or were eaten, so escape hatching success and survival were the same. In four *A. spurrelli* trials, the snake left one or a few unhatched eggs; these hatched much later and are not included in the number hatched during the attack. Trials with *A. callidryas* were run in 1992 and 1993 using seven individual *L. septentrionalis*. A subset of trials at age 5 and 6 days in 1992 were run concurrently with snake-free controls and reported in Warkentin (1995). Trials with *A. spurrelli* were run in 1993–1995 using six *L. septentrionalis*. Individual snakes were used in experiments with multiple egg clutches, offered individually on different nights, and on some nights multiple predation trials were run concurrently with different snakes. In 1993 the same individual snakes were used, on different nights, with egg clutches of both frog species. In the field snakes can consume several clutches in a single night, and return on multiple sequential nights to forage on eggs (K. M. W., unpublished data).

Embryo response to flooding

To test whether submergence induces hatching in *A. spurrelli* we conducted flooding experiments in 2004 at EARTH University using 15 clutches collected from a pond near Guayacán, Limón, Costa Rica. The developmental stage and age at hatching competence, and the modal age of spontaneous hatching in this population are the same as that in Sirena (I. G.-M. and K. M. W., unpublished data). We randomly assigned ten clutches to flooding and five to control treatments (clutch size 45.8 ± 4.1 , mean \pm SE). Clutches were hung in 250-ml plastic cups and tested in the morning at age 4 days. Experimental clutches were completely submerged by pouring pond water into their cups. We poured the same volume of pond water into control cups but drain holes prevented flooding of the clutches (Warkentin 2002). We counted the number of embryos hatched every 5 min for 1 h.

Hatchling predation experiments

To test if premature hatching of *A. spurrelli* increases hatchling vulnerability to an aquatic predator, we carried out 24-h predation trials with a poeciliid fish, *Poecilia gillii*, and newly hatched tadpoles of different ages. This experiment

was run in 2004 at La Selva Biological Station, Costa Rica, using egg clutches collected from Guayacán, as above. *P. gilli* is the most common freshwater fish in Costa Rica (Bussing 2002) and co-occurs with tadpoles of *A. spurrelli*. Trials used ten hatchlings of the same age exposed to a single fish, in round plastic containers with 3.25 l of pond water and leaf litter to half the water depth (16 cm), and were replicated 6 times each for animals hatched at embryonic ages 4 and 5 days. Individual fish were starved for 48 h prior to the trials to standardize hunger. Embryos were mechanically induced to hatch in the evening, when there is a natural diel peak in hatching (K. M. W. and I. G.-M., unpublished data), and placed immediately into containers with fish. Survivors were counted after 24 h. All hatchlings were in Gosner stage 23, with the operculum developed and bilateral external gills (Gosner 1960).

Statistical analyses

All analyses were carried out in SAS version 8.00 (SAS Institute, Cary, N.C.). We tested for differences in the hatching patterns of undisturbed clutches in the field using a log-likelihood ratio test. The overall proportion of monitored clutches predated in the field, escape hatching success in snake predation experiments, and proportion of hatchlings surviving fish predation were all analysed using generalized linear models with an underlying binomial distribution and a logit link function in PROC GENMOD.

A. spurrelli bred on a subset of the nights when *A. callidryas* bred, so that only a subset of *A. callidryas* clutches overlapped temporally with *A. spurrelli* clutches. Temporal variation in predator activity might therefore confound species effects on the overall incidence of egg predation. Therefore to assess predator selectivity or bias we also tested if the proportion of clutches predated by the most common predator, snakes, differed between *A. callidryas* and *A. spurrelli* on the subset of nights when clutches of both species were present. Cat-eyed snakes are strictly nocturnal predators. We thus considered each night an independent opportunity for the snakes to consume a subset of the clutches present at the pond. We performed a log-linear analysis of the number of clutches of each species that were predated and undisturbed on each of 16 nights when both were present. Model parameters were estimated through maximum likelihood using PROC CATMOD, assuming an underlying Poisson distribution.

We tested for effects of embryo development on escape success in snake attacks using generalized linear models. Because some experiments with *A. callidryas* and *A. spurrelli* were done in different years, we first tested for age and year effects within each species separately. In both cases year effects were non-significant (both $P > 0.39$). We therefore pooled across years and used a single model to test for

effects of species, developmental stage (days relative to the peak of spontaneous hatching), and species by stage interaction. Also, because development is faster in *A. spurrelli* than *A. callidryas*, neither chronological age nor the absolute difference from modal hatching age are strictly comparable. We therefore specified two contrasts between species: (1) at the peak of spontaneous hatching (modal hatching age), and (2) early in the period of hatching competence, 1 day before modal hatching for *A. spurrelli* versus 2 days before for *A. callidryas*.

The hatching response of *A. spurrelli* to underwater submergence was tested against its unflooded controls by fitting a Cox regression model using PROC PHREG. The effects of hatching age on the proportion of hatchlings surviving in the fish predation experiments was analysed with a generalized linear model, including fish size as a covariate.

Results

Field survey

Clutch fates and hatching success for the 338 *A. callidryas* and 30 *A. spurrelli* clutches monitored are summarized in Table 1. Clutch sizes ranged from 12 to 231 eggs (mean 76.8 ± 64.5 SD) for *A. spurrelli* and from ten to 101 eggs for *A. callidryas* (40.5 ± 15.5). Half of the clutches of each species were neither disturbed by predators nor flooded. Their spontaneous hatching patterns are presented in Fig. 1. Eggs were laid at night, *A. callidryas* mostly from 2200–0200 hours, and *A. spurrelli* later, often near dawn (K. M. W., personal observation). *A. spurrelli* began hatching spontaneously during the fourth night after oviposition, i.e. at embryonic age 3 days or just barely 4 days. Their peak of spontaneous hatching was during the night they were age 5–6 days, and clutches were completely hatched the following night. *A. callidryas* started hatching during the fifth night after oviposition (age 4–5 days), with a gradual increase in the proportion of eggs hatched each night until the peak of spontaneous hatching on the eighth night (age 7–8 days). Spontaneous hatching in *A. spurrelli* began earlier than in *A. callidryas* ($n_{AS} = 16$, $n_{AC} = 171$ Mann–Whitney $U = 573.5$, $P < 0.0001$), and also peaked earlier ($n_{AS} = 16$, $n_{AC} = 171$ Mann–Whitney $U = 705.5$, $P < 0.001$) and more strongly than in *A. callidryas* (*A. spurrelli* kurtosis, $G_2 = 9.42$; *A. callidryas*, $G_2 = 3.29$), resulting in different distribution shapes (Fig. 1).

Development was also faster in *A. spurrelli* than in *A. callidryas*. In the afternoon at age 2 days, *A. callidryas* embryos were in the tail bud stage (stage 17; Gosner 1960), while *A. spurrelli* already had a heartbeat and external gills (Gosner stage 19). Embryos of both species became hatching

Table 1 Fates of egg clutches of *Agalychnis callidryas* ($n = 338$) and *Agalychnis spurrelli* ($n = 30$) monitored from soon after oviposition until hatching at a pond in Corcovado National Park, Costa Rica, during June–July 1993 and June–July 1994

Fate	<i>A. callidryas</i>		<i>A. spurrelli</i>	
	Proportion of clutches ^a	Hatching success ^b	Proportion of clutches ^a	Hatching success ^b
Undisturbed	0.506 (171)	0.944 ± 0.017	0.533 (16)	0.968 ± 0.059
Snake predation ^c	0.426 (144)	0.277 ± 0.019	0.400 (12)	0.208 ± 0.083
Young clutches	(103)	0.181 ± 0.026	(6)	0.177 ± 0.085
Hatchable clutches	(41)	0.504 ± 0.037	(6)	0.255 ± 0.126
Monkey predation	0.027 (9)	0.037 ± 0.075 ^d	–	–
Fly larvae	0.015 (5)	0.736 ± 0.101	–	–
Flooded	0.012 (4)	0.259 ± 0.123 ^e	0.067 (2)	0.206 ± 0.206 ^e
Wasps	0.009 (3)	0.315 ± 0.131	–	–
Ants	0.006 (2)	0.868 ± 0.160	–	–

^a Data shown are proportion (number) of clutches affected by each fate

^b Hatching success is mean proportion hatched, out of initial clutch size, across affected clutches ± SE

^c Total hatching success of snake-predated clutches (both young clutches attacked before hatching competence and older hatching-competent clutches) includes eggs left by the snake that hatched later, tadpoles that hatched before the snake attacked, and tadpoles hatched during attacks that fell into the hatchling-catching cup. The last category is an underestimate, as some tadpoles fall out of the cup when attacking snakes displace leaves. Clutches of *A. callidryas* ≥ 5 days old and those of *A. spurrelli* ≥ 4 days old were considered hatching competent

^d These survivors had hatched before the monkey attacked their clutch

^e Fates of flooded clutches vary with developmental stage. Submergence is lethal before hatching competence (Gosner stage 23), but non-lethal later in development. Some submerged eggs may have been eaten by aquatic predators, but we assume here that normally developed, hatching-competent eggs hatched after flooding

competent in Gosner stage 23 (Gosner 1960), the last stage with bilateral external gills, but *A. spurrelli* reached this stage a day earlier than *A. callidryas*. Like *A. callidryas* (Warkentin 2000b, 2002), *A. spurrelli* embryos retained external gills and remained in stage 23 until they hatched, although other structures continued developing. Also like *A. callidryas*, their gills regressed rapidly after hatching (I. G.-M. and K. M. W., personal observation).

The overall proportion of predated clutches was high for both species, and not significantly different (0.40 and 0.48 for *A. spurrelli* and *A. callidryas*, respectively; $X^2_{1,366} = 0.09$, $P = 0.76$). Most predation was by snakes, mainly *Leptodeira septentrionalis* (K. M. W., personal observation), but we also observed *Leptophis ahaetulla* and *Imantodes inornatus* feeding on clutches. A few *A. callidryas* clutches were also eaten by white-faced capuchin monkeys (*C. capucinus*), preyed on by wasps (*P. rejecta*) or ponerine ants, and infested by fly larvae after partial dehydration. A few clutches of both species were submerged by rising pond water, some before hatching competence, which is lethal (Pyburn 1970) and some after, which is not (Warkentin 2002). The proportion of concurrently present clutches that was predated by snakes per night was also not significantly different between *A. callidryas* and *A. spurrelli* (species × predation interaction term in a log-linear model, $X^2 = 1.18$, $P = 0.28$). Snakes did not always completely consume clutches, so that even for young clutches mortality was not 100% in snake attacks. However, the survival of clutches attacked before hatching

competence did not differ between *A. callidryas* and *A. spurrelli* ($X^2_{1,107} = 0.38$, $P = 0.535$; average survival ± SE, 0.181 ± 0.026, $n = 103$, 0.177 ± 0.085, $n = 6$, respectively).

Egg-predation experiments

Escape success increased with development in both species (*A. callidryas*, $X^2_{3,44} = 25.22$, $P < 0.0001$; *A. spurrelli*, $X^2_{1,12} = 12.56$, $P = 0.0004$; Fig. 2). The hatching response of embryos to snake attacks was higher overall for *A. callidryas* than it was for *A. spurrelli* ($X^2_{1,59} = 234.5$, $P < 0.0001$). This result does not change if we restrict the analysis just to the 1993 data, when both species were tested using the same individual snakes ($X^2_{1,21} = 62.71$, $P < 0.0001$). There was also a significant species by stage interaction; escape success increased more between 1 day premature and the modal hatching age for *A. spurrelli* than it did for *A. callidryas* ($X^2_{1,59} = 15.50$, $P < 0.0001$; Fig. 2). Escape success differed between the species both near the onset of hatching competence (*A. callidryas*, 26% premature, 59 ± 3% hatched; *A. spurrelli*, 17% premature, 9 ± 4% hatched; $X^2_{1,22} = 67.08$, $P < 0.0001$) and at the modal hatching age (80 ± 3.5 vs. 28 ± 4% hatched; $X^2_{1,19} = 72.64$, $P < 0.0001$). The low escape success in *A. spurrelli* held even in clutches that had already begun hatching spontaneously. For instance in one clutch attacked at the modal hatching age over half of the eggs had hatched spontaneously prior to snake contact, and yet only 28% of the remaining embryos hatched when attacked.

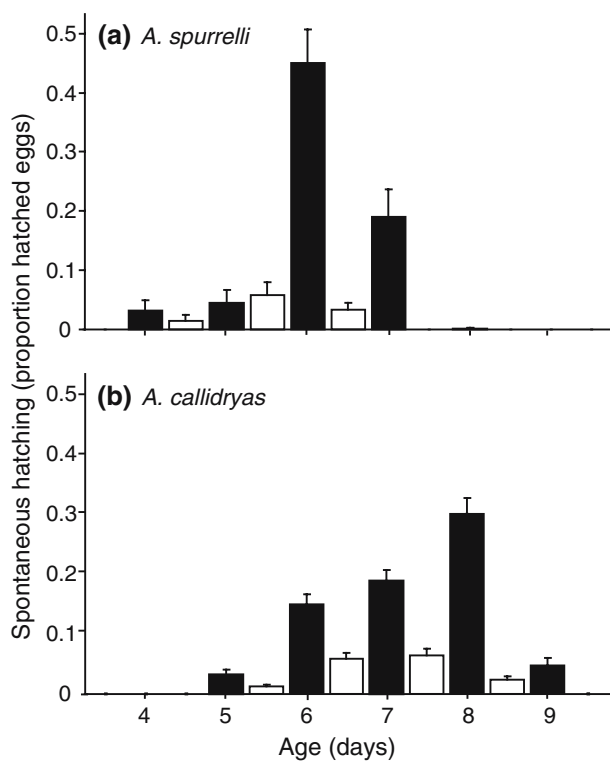


Fig. 1 Spontaneous hatching of undisturbed **a** *Agalychnis spurrelli* ($n = 16$) and **b** *Agalychnis callidryas* ($n = 171$) clutches monitored at Sirena Field Station in Corcovado National Park, Costa Rica, during June–July 1993 and June–July 1994. Data are mean (+SE) proportion hatched at each age out of initial number of eggs in each clutch. Dark bars indicate hatching at night, light bars indicate hatching during the day. Eggs are laid at night, therefore age (days) increments during the period between evening and morning egg checks. No eggs hatched before the night they turned 4 days old

Embryo response to flooding

A higher proportion of embryos hatched within the 1-h observation period in flooded *A. spurrelli* clutches than in control clutches (57 ± 7 vs. $2 \pm 1\%$, respectively, mean \pm SE; $X^2_{1,13} = 37.09$, $P < 0.0001$; Fig. 3). These flooded clutches were tested, and hatched, 26% prematurely, compared with the modal hatching age.

Hatchling predation experiment

Younger *A. spurrelli* hatchlings were more vulnerable than older hatchlings to fish predation ($X^2_{1,9} = 23.08$, $P < 0.0001$; Fig. 4).

Discussion

A. callidryas and *A. spurrelli* embryos differ markedly in their hatching response to snake attacks. Effective snake-induced escape hatching has been demonstrated previously

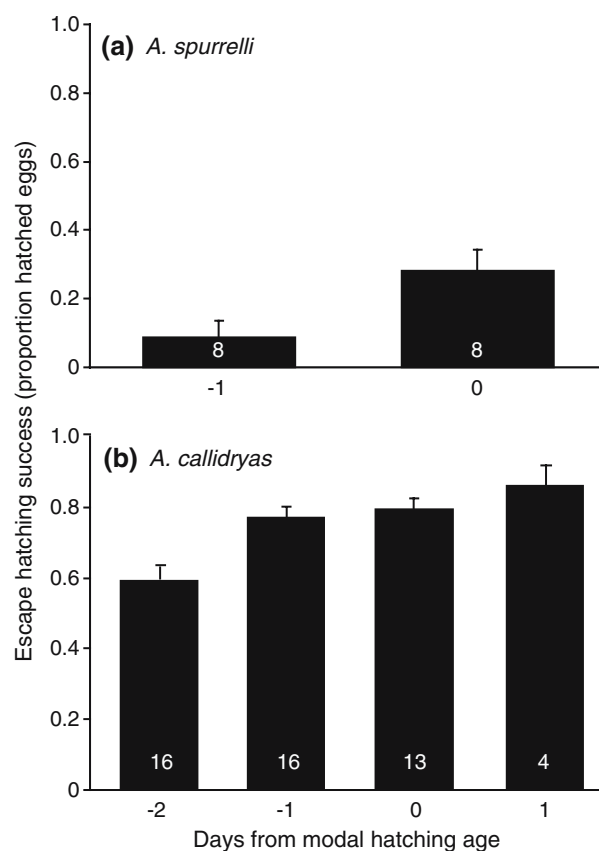


Fig. 2 Escape hatching success of **a** *A. spurrelli* and **b** *A. callidryas* in attacks by cat-eyed snakes, *Leptodeira septentrionalis*, at different ages relative to the modal hatching age (0) for each species. Data are mean proportion hatched (+SE) of eggs present at the start of the attack; sample size at each age is indicated. Modal hatching ages were 5 and 7 days, respectively, for *A. spurrelli* and *A. callidryas*

in *A. callidryas* (Warkentin 1995). Here we show that the escape rate is age-specific, improving as embryos approach the peak of spontaneous hatching. However, even in attacks 2 days before the peak of spontaneous hatching (26% premature) the escape success of embryos was $\sim 60\%$. This escape ability is not shared by *A. spurrelli*. Even at the age of peak spontaneous hatching only 28% of embryos hatched during snake attacks, and consequently most were eaten. Embryos of *A. callidryas* respond rapidly to snake attack, performing vigorous hatching movements to exit the egg. This behavioural response occurs within seconds to a few minutes, and can also be elicited by playbacks of vibrations recorded from clutches in snake attacks (Warkentin 1995, 2005; Warkentin et al. 2007). In contrast, *A. spurrelli* embryos in attacked clutches remain largely motionless, even in eggs close to the snake's mouth (I. G.-M. and K. M. W., personal observation from videotapes). Thus the escape hatching response to snakes has clearly changed in the lineage leading to *A. callidryas* and/or *A. spurrelli* since their last common ancestor.

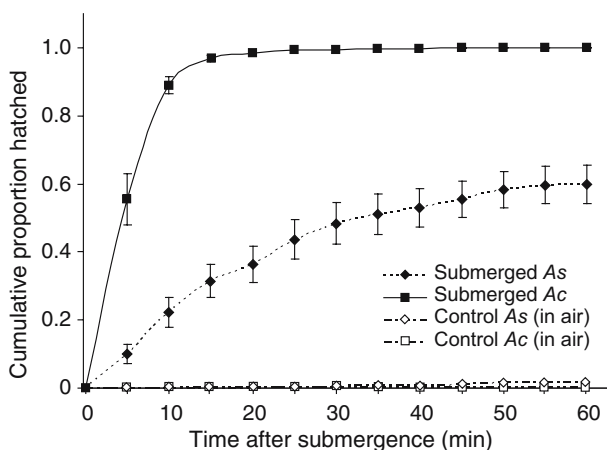


Fig. 3 Hatching pattern of egg clutches of *A. spurrelli* (As, diamonds) and *A. callidryas* (Ac, squares) submerged in pond water at age 4 and 5 days, respectively (filled symbols), and simultaneously monitored unflooded controls (open symbols). Data are mean (\pm SE) cumulative proportion hatched. Samples sizes are ten and five clutches for submerged and control *A. spurrelli* and seven clutches each for submerged and control *A. callidryas*. Data for *A. callidryas* are redrawn from Warkentin (2002)

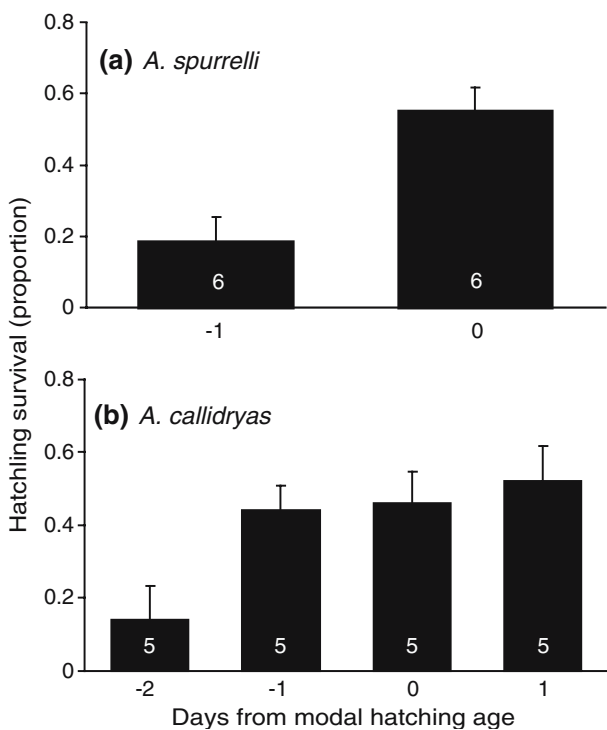


Fig. 4 **a** Survivorship of *A. spurrelli* hatchlings over 24 h with a poeciliid fish predator, *Poecilia gillii*, at different ages relative to the modal hatching age (0). **b** Survivorship of *A. callidryas* hatchlings with the poeciliid *Brachyraphis rhabdophora*, redrawn from Warkentin (1995) for comparison. Modal hatching ages were 5 and 7 days, respectively, for *A. spurrelli* and *A. callidryas*. Data are mean (\pm SE) proportion surviving; sample sizes are indicated

Given a similar risk trade-off across hatching and a high level of egg predation the optimal fixed hatching stage, for a species lacking plasticity, will be earlier than the optimal spontaneous (uninduced) hatching age for a species with adaptively plastic hatching. We observed such a difference in the spontaneous hatching pattern of *A. spurrelli* compared with *A. callidryas*. Hatching in *A. spurrelli* is more synchronous, and the modal hatching age is 2 days (25%) earlier. This earlier hatching is achieved by an acceleration of development; based on external morphological criteria, the two species hatch at the same developmental stage (Gosner 1960).

One hypothesis that could explain the difference in hatching plasticity between these syntopic, closely related species is that natural selection favours plasticity in one species and not in the other. Plasticity in life history switch points is favoured when there is a trade-off of stage-specific risks or opportunities, and environmental variation in one or both stages (Werner 1986; Werner 1988). Although they breed at the same ponds, differences in oviposition site choice or the temporal pattern of breeding activity could result in different predation pressures on eggs. If *A. spurrelli* experience little egg predation, there would be little selection for escape hatching behaviour in predator attacks. Our field monitoring of clutch fates does not support this hypothesis. Despite their different development rates, and thus duration of exposure to egg predators, clutches of *A. callidryas* and *A. spurrelli* experienced very similar predation pressures (Table 1). Snakes were the main egg predators for both species, attacking ~40% of the clutches in both cases. Moreover, we found no evidence that snakes discriminate between *A. callidryas* and *A. spurrelli* clutches when they have a choice. Nonetheless, snakes sometimes leave uneaten eggs. If this is more likely for *A. spurrelli* it could reduce selection imposed by snakes, compared with that on *A. callidryas*. However, we found no difference in survival rates between *A. callidryas* and *A. spurrelli* attacked before hatching competence, suggesting that no such amelioration occurs. It therefore seems that *A. spurrelli* embryos would also benefit from an effective response to snakes.

If there were no trade-off of risks across hatching, so that hatching early entailed no post-hatching survival cost, then egg-stage risks would select for consistently earlier hatching, not for hatching plasticity. A larval-stage predation cost of early hatching could occur in two ways. Hatching earlier may require spending more time with aquatic predators before metamorphosis. This time-based increment of risk may, however, be small for species with a long larval period, such as these two *Agalychnis* (2–3 months, Warkentin 1999b; Kubicki 2004; Vonesh and Warkentin 2006). A stronger cost occurs if embryonic development alters the ability of hatchlings to avoid or evade aquatic predators, as

occurs for *A. callidryas* with fish and several invertebrate predators (Warkentin 1995, 1999a). Our fish predation experiment tested for and found such a cost in *A. spurrelli*, suggesting that selection by aquatic predators would favour later hatching.

Both requirements for the conditions that favour switch point plasticity are met for hatching in *A. spurrelli*. There is a risk trade-off across hatching (egg vs. tadpole predators), and there is environmental variation (snakes attack only some clutches). Moreover these conditions appear very similar to those for *A. callidryas*, which shows highly plastic, risk-sensitive hatching timing and a much stronger response to snake attack (Warkentin 1995, 2000b, 2002; Warkentin et al. 2001). Thus the low responsiveness of *A. spurrelli* embryos to egg-eating snakes appears nonadaptive, and potentially maladaptive. Embryos eaten by snakes will never reproduce whereas if those embryos, instead, hatched prematurely they would have at least a chance of surviving to reproduce.

The *A. spurrelli* lineage either diverged from that of *A. callidryas* before the strong hatching response to snakes evolved, or else its responsiveness to snakes has weakened. Fully resolving this will require a more complete phylogeny of the genus *Agalychnis* than is currently available (Faivovich et al. 2005; Wiens et al. 2005), as well as information about the hatching plasticity of its other members. However, current information can narrow the range of possible explanations for the divergence in hatching responses. Although the response of *A. spurrelli* embryos to snake attack is weak, particularly compared to that of *A. callidryas* embryos, it is not completely absent; 9% hatch in attacks 1 day (17%) before the peak of spontaneous hatching, and 28% hatch in attacks at the modal hatching age. The fact that they show any response at all suggests that there might be variation in the response on which selection could act. A simple lack of genetic variation for hatching traits is possible, but it seems unlikely, suggesting that there might be another constraint on the evolution of escape hatching.

A. callidryas hatch prematurely in response to several egg-stage risks: snake and wasp attack, fungal infection, and flooding (Warkentin 1995, 2000b, 2002; Warkentin et al. 2001). The responses of *A. spurrelli* embryos to wasps and fungus are unknown but their hatching response to flooding at age 4 days, 26% prematurely, is over sixfold stronger than their response to snakes ~12 h later, at 17% premature. This difference suggests two possibilities. First, perhaps *A. spurrelli* embryos have a slower escape response than *A. callidryas*, and the higher hatching success in flooding versus snake attacks reflects the fact that slow-hatching eggs are eaten by snakes. Videotapes of snakes eating *A. spurrelli* clutches suggest otherwise: the few embryos that performed hatching movements escaped within seconds,

while snakes took as long as 55 min to finish eating a clutch (I. G.-M. and K. M. W., unpublished data). Direct observations of hatching in submerged clutches corroborate that hatching is a rapid behavioural process in *A. spurrelli*, once initiated. Second, perhaps the difference in hatching response to snakes and flooding is due to the different cues available from these two risks.

A. callidryas embryos use specific vibrational cues in snake attacks to assess danger, and distinguish between vibrations from snakes and those from benign disturbances (Warkentin 2005; Warkentin et al. 2006b, 2007). Snakes cause vibrations in egg masses by physically deforming the clutch as they feed. Viscoelastic models predict increased stretching both as the thickness of an object increases and as the material flexibility increases (Snowdon 1968). Thus if snakes apply similar forces to *A. callidryas* and *A. spurrelli* clutches, the thinner, stiffer *A. spurrelli* clutches are expected to deform less. Indeed, we have seen much larger, more obvious deformations of clutches in videotapes of attacks on *A. callidryas*, compared with *A. spurrelli* (I. G.-M. and K. M. W., unpublished data). A smaller deformation of the clutch translates into a lower amplitude vibration, and thus reduced stimulus to the *A. spurrelli* embryos, compared with *A. callidryas*. The weaker hatching response of *A. spurrelli* embryos in snake attacks could therefore be due to a biomechanical constraint on vibrational information. Flooding-induced hatching, in contrast, is likely cued by oxygen stress (Petranka et al. 1982; Warkentin 2002). In both *A. callidryas* and *A. spurrelli* all hatchable eggs have part of their egg capsule exposed (Warkentin et al. 2005; K. M. W. and I. G.-M., personal observation), so the effects of flooding on gas exchange across the capsule are likely similar. Indeed, the somewhat lower response of *A. spurrelli* embryos to flooding (Fig. 3), compared with *A. callidryas* (Warkentin 2002), might have been due to different, unmeasured, levels of oxygen in the pond water in the two experiments.

If the limited hatching response to snakes in *A. spurrelli* is mechanistically explained by constraints on risk cues imposed by clutch structure, then understanding the evolution of hatching plasticity will also require understanding other factors that may drive the evolution of clutch structure. *Agalychnis saltator* has a similar clutch structure to *A. spurrelli*, while *Agalychnis annae* and *Agalychnis moreletti* have clutches similar to *A. callidryas*; the responses of these species to egg-eating snakes could therefore be informative. It is also possible that the weak hatching response of *A. spurrelli* to snakes evolved or is maintained due to a correlation with some other, unknown trait that is favoured by selection. If so, such a trait must confer a benefit greater than the selective cost incurred when hatching-competent embryos are eaten by snakes.

Two-species comparisons cannot tell us how plasticity, or any other traits, have evolved (Garland and Adolph 1994; Doughty 1996). However, comparisons of two closely related species can tell us if plasticity has evolved. Such studies can thus focus attention on taxa that merit further study to elucidate evolutionary patterns. Moreover, ecologically and mechanistically contextualized studies of plasticity narrow the range of hypotheses that are plausible explanations for evolutionary changes. Without data on both environmental variation and trade-offs between stage-specific risks or opportunities, we cannot interpret a lack of switch point plasticity in response to a single stage-specific factor to be maladaptive, nor can we assume plasticity per se to be adaptive, although induced traits may be. Likewise, without a mechanistic understanding of cues and response pathways, we cannot identify what constrains the evolution of plasticity that is favoured by selection.

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