doi:10.1111/j.1420-9101.2008.01609.x

Divergence in parental care, habitat selection and larval life history between two species of Peruvian poison frogs: an experimental analysis

J. L. BROWN,* V. MORALES† & K. SUMMERS*

*Department of Biology, East Carolina University, Greenville, NC, USA
†La Facultad de Ciencias Biológicas, Universidad Ricardo Palma, Lima, Peru

Keywords:

biparental care;
co-evolution;
competition;
Dendrobates imitator;
Dendrobates variabilis;
habitat preference;
male parental care;
mating strategies;
pool choice;
predation.

Abstract

Changes in the nature of the ecological resources exploited by a species can lead to the evolution of novel suites of behaviours. We identified a case in which the transition from large pool use to the use of very small breeding pools in neotropical poison frogs (family Dendrobatidae) is associated with the evolution of a suite of behaviours, including biparental care (from uniparental care) and social monogamy (from promiscuity). We manipulated breeding pool size in order to demonstrate experimentally that breeding habitat selection strategy has evolved in concert with changes in parental care and mating system. We also manipulated intra- and interspecific larval interactions to demonstrate that larval adaptation to the use of very small pools for breeding affected the evolution of larval competition and cannibalism. Our results illustrate the intimate connection between breeding pool ecology, parental care and mating strategies in Peruvian poison frogs.

Introduction

Transitions in the nature ecological resources exploited by a species can lead to the evolution of novel suites of behaviours (e.g. Breitburg, 1987; Gross, 1991; Schluter, 2000). In turn, this can have cascading effects on both adult and juvenile life-history strategies (Fincke, 1992). A useful approach to unravelling these associations is to find closely related species that differ with respect to a particular ecological trait (e.g. pool size), manipulate that trait and assess the behavioural response to the manipulation. We identified a case in which a transition to the use of very small breeding pools in neotropical poison frogs (family Dendrobatidae) is associated with the evolution of a suite of behaviours, including biparental care (from uniparental care) and social monogamy (from promiscuity) (Brown *et al.*, 2008). *Dendrobates variabilis* is a member of a widespread Amazonian group of poison frogs that breed in phytotelmata (e.g. bromeliad tanks) and show uniparental male care (Summers & McKeon, 2004; Poelman & Dicke, 2007). *Dendrobates vanzolinii* and *Dendrobates imitator* are members of this Amazonian clade, but have evolved novel parental behaviours and mating strategies in association with a transition to the use of very small phytotelm pools. Caldwell (1997) observed that *D. vanzolinii* in Brazil used very small phytotelm pools for breeding, and also showed biparental care involving the maintenance of long-term pair bonds between mates and cooperative parental care with trophic egg feeding of larvae. Brown *et al.* (2008) found this same suite of behaviours associated with the use of very small phytotelmata (e.g. pools within *Dieffenbachia* leaf axils) in *D. imitator* in Peru.

Phylogenetic analyses revealed that *D. vanzolinii* and *D. imitator* are sister taxa, and that *D. imitator* recently colonized the montane regions of northern Peru inhabited by *D. variabilis* (Symula *et al.*, 2003; Roberts *et al.*, 2006). In fact, *D. imitator* is a Müllerian mimic of *D. variabilis* in part of its range (Symula *et al.*, 2001). This colonization has placed *D. imitator* in sympatry with *D. variabilis*, and provided the opportunity to study these two species side-by-side in the same environment, minimizing confounding factors associated with environmental differences. Both *D. imitator* and *D. variabilis* breed

Correspondence: Jason L. Brown, N312 Howell Science Complex, East Carolina University, Greenville, NC 27858, USA.
Tel.: +1 252-328-6718; fax: +1 252-328-4178; e-mail: jlb0627d2@ecu.edu

in phytotelmata, and the small size and temporary nature of these pools reduce predator density and hence the risk of predation (Summers & McKeon, 2004). However, the use of small pools entails other costs. For example, small pools quickly reach carrying capacity, resulting in limited food resources and increased inter- and intraspecific competition (Menge & Sutherland, 1976, 1987).

The main difference in reproductive strategies between *D. imitator* and *D. variabilis* involves the size of phytotelmata used for breeding (see above, Fig. 1). In turn, differences in pool size are associated with differences in the relative risks of predation, competition, cannibalism and starvation (Walters, 1975; Alford, 1999; Wells, 2007). Phytotelmata typically cannot sustain multiple tadpoles, and many species that use them have extremely competitive and predatory larvae (Brust, 1993; Pramuk & Hiler, 1999; Summers, 1999). Few species of frogs are able to use very small phytotelmata, probably because these pools contain insufficient nutrients to sustain tadpoles. However, those species that are able to use these pools are likely to experience a dramatic reduction in interspecific competition, particularly when these pools represent an unexploited resource (Summers & McKeon, 2004). The species of anurans able to use these pools have evolved novel behaviours involving tadpole provisioning. In poison frogs, the transition to breeding in very small phytotelmata (from small phytotelmata) is associated with the evolution of female parental care, asymmetrical biparental care or biparental care, in which the adult females provide unfertilized eggs to their larvae (Weygoldt, 1987; Brust, 1993; Caldwell & de Oliveira, 1999; Summers & McKeon, 2004; Brown *et al.*, 2008). In Fig. 1, we illustrate this association by mapping the evolution of both pool size and parental care on a tree showing the phylogenetic relationships of dendrobatid frog species for which the requisite information is available. We expect that this transition also entailed evolutionary changes in suites of behaviours associated with parental care, and with suites of larval life-history traits involving competition for food (including cannibalism).

One key behavioural trait that should be associated with parental care type is reproductive habitat selection. Many anurans gauge the quality (or 'risk') of a reproductive resource using visual and chemical cues (Spieler & Linsenmair, 1997; Halloy & Fiaño, 2000; Binckley & Resetarits, 2003). For example, Resetarits & Wilbur (1989) demonstrated that *Hyla chrysoscelis* avoided cattle tanks containing black-banded sunfish (*Enneacanthus chaetodon*) and would actively deposit eggs in tanks that lacked predators. Crump (1991) conducted similar choice experiments using *Hyla pseudopuma* in Costa Rica and found that females laid more eggs in pools without predators and conspecific tadpoles. Downie *et al.* (2001) reported that male *Mannophyryne trinitatis* avoided depositing tadpoles in pools containing fish (*Rivulus*) or shrimp (*Macobrachium*). In some populations of *M. trinitatis*, males were only provided pools with predators and in turn deposited their tadpoles in wet leaf litter.

In an Ecuadorian population of *Dendrobates ventrimaculatus* s.s., Summers (1999) demonstrated that males avoided pools which contained conspecific tadpoles (that are cannibalistic) for egg and tadpole deposition. In French Guiana, where breeding seasons are more exaggerated and shorter, *D. ventrimaculatus* s.l. used a similar strategy early in the reproductive season, avoiding pools with tadpoles (Poelman & Dicke, 2007). The research described above investigated reproductive habitat

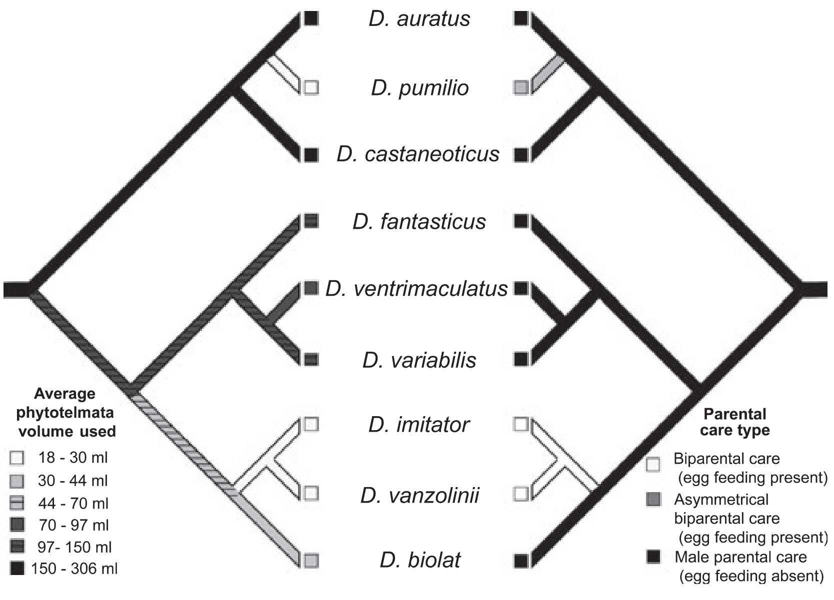

Fig. 1 Ancestral state reconstruction of average phytotelm size and parental care strategy in the poison frog genus *Dendrobates*.

selection and tadpole deposition strategies in the context of predator or cannibal avoidance; fewer studies have addressed the role of competition on deposition strategies, and (to our knowledge) no study has investigated breeding habitat choice from a comparative perspective in the context of parental care evolution.

The mating system of *D. variabilis* is promiscuous, and pools commonly receive multiple depositions (Brown *et al.*, 2008). This results in high levels of larval competition for food within phytotelmata, and all but one of the tadpoles deposited in the pool at the same time are typically cannibalized. In this study, we presented paired pools that differed in size and observed adult pool preferences for tadpole deposition in *D. variabilis* and *D. imitator* in northern Peru. We predicted that adults of *D. variabilis* would prefer to deposit tadpoles in relatively large pools (within the confines of the normal sizes of phytotelmata used by this species), because smaller pools lack adequate food to sustain a tadpole. By contrast, *D. imitator* appears to have a monogamous mating strategy (Brown *et al.*, 2008), in which males and females form pair bonds and mutually defend a territory containing breeding pools. Tadpoles are fed trophic eggs regularly, and hence there is no lack of nutrients and no need to use large pools. Because of this, we predicted that this species would show a preference for very small pools, in order to minimize the risk of multiple depositions (by other adults of their own species and by *D. variabilis* adults) and associated competition and cannibalism. *Dendrobates variabilis* also uses phytotelmata for egg deposition, placing eggs at the surface of the water. This provided an opportunity to look at the effects of pool size and predators on egg deposition strategies.

To demonstrate that these species respond in the same manner to an experimental manipulation when they are expected to, we investigated the response of these species to the presence of a potential predator (a larval insect or large tadpole). The impact of a large potential predator on the fitness of parents of either species should be the same (the loss of any tadpole placed in a pool containing the predator). Hence, we predicted that the response of each species would be the same: to avoid the pool with the potential predator.

The contrasting parental strategies of *D. imitator* and *D. variabilis* combined with the distinct larval habitats employed by these two species should profoundly affect the evolution of their larval life-history strategies. In *D. variabilis*, the frequent occurrence of multiple depositions, the intensity of larval competition for food and the risk of larval cannibalism should select for tadpoles with the ability to compete vigorously, to cannibalize conspecifics (and incidentally heterospecifics) and to resist cannibalism. By contrast, *D. imitator* tadpoles are protected from competition by utilizing very small phytotelmata that cannot be used by most other anurans, and these pools are defended aggressively by their parents (minimizing conspecific interactions). Selection on competitive and predatory capabilities of larvae should be weak or absent. Hence, we predicted that *D. variabilis* tadpoles would show superior competitive and predatory capabilities relative to *D. imitator* tadpoles. To test these predictions experimentally, we measured the competitive abilities of both species in a combination of tadpole competition treatments. The size of experimental pools used reflects the volume of pools probably used by the common ancestor of *D. imitator* and *D. variabilis* (Fig. 1), and this is also the size of pools currently used by *D. variabilis*. We predicted that *D. imitator* would be an inferior competitor and predator relative to *D. variabilis*. We also expected the lag time to cannibalism to be shorter in treatments that contained *D. variabilis*. The differences in parental care and mating strategies between these species provide a unique opportunity to look at the association of larval predation and competition with the evolution of parental care strategies.

Materials and methods

Experiments 1 and 2 – pool choice experiments

Experiments 1 and 2 were performed in four premontane rainforest sites in the upper Cainarachi Valley, Cordillera Escalera, San Martin, Peru. Sites 1 and 2 were located in late-stage secondary forests ranging between 520 and 550 m above sea level; site 1 (6°25′37″S, 76°17′42″W) was located about 50 m south from site 2. Sites 3 and 4 were located in old growth forests about 540 m above sea level; site 3 (6°25′29″S, 76°17′36″W) was located about 200 m east from site 4. Sites 1 and 2 were about 0.8 km south-west from sites 3 and 4. The four sites separated by at least 50 m were chosen to reduce pseudo-replication from repeated depositions by the same male between sites. To reduce pseudoreplication within sites, if depositions occurred on the same day at adjacent treatments, only one randomly chosen deposition was scored. All sites contained populations of *D. imitator* and *D. variabilis.*

We conducted two types of paired pool choice experiments to evaluate male deposition strategies based on presence/absence of predators and differences in size/volume of pools. Pools were established in 2005. For both experiments, two clear plastic cups were wired to trees at 1 m height (separated by 5 cm). A second pair of plastic cups (the reservoir cups) were surrounded with leaves and placed within the plastic cups wired to trees; this allowed for easy removal of pools for inspection and minimized visual cues from the exterior of the cups. Detritus (i.e. leaves) and water were added to each pool; excessive, accumulated detritus was removed and water levels in pools were maintained. Pool pairs were placed haphazardly throughout each site from 4 to 8 m apart. When possible, pools from experiments 1 and 2 were alternated.

For experiment 1, paired pools consisted of two 100-ml cups filled to 80 mL: one with a predator and the other without. The ‘predator pool’ contained one *Ameerega* *trivittata* tadpole or a damselfly larva (Zygoptera spp.) with mandibles removed (as described in Wissinger & McGrady, 1993). *Ameerega trivittata* tadpoles were similar in appearance and size to cannibalistic tadpoles of *Dendrobates*. These tadpoles are primarily herbivores and were not observed to cannibalize tadpoles of *Dendrobates* (Silverstone, 1976; J.L. Brown, unpublished data). However, because of the morphological similarities, we assumed that the *A. trivittata* tadpoles would be perceived as potential predators by adult frogs. Damselfly naiads (larvae) have repeatedly been cited as predators of tadpoles (Caldwell *et al.*, 1980; Gascon, 1992; Resetarits, 1998), able those with modified mandibles were unable to feed on *Dendrobates* tadpoles (J.L. Brown, unpublished data). Both predators were chosen because they represented the most commonly observed types of predators encountered in natural phytotelmata in the upper Cainarachi valley (J.L. Brown, unpublished data). Experiment 1 used a total of 40 paired pools (site 1: 10, site 2: 10, site 3: 10 and site 4: 10) containing 14 damselfly larvae and 26 *A. trivittata* tadpoles.

For experiment 2, paired pools consisted of two cups that differed in size. The following sizes of plastic cups were used: small (water 40 mL, total volume 60 mL), medium (80 mL, 100 mL) and large (275 mL, 370 mL). Holes were made in the cups to maintain water levels. We used two treatments: small vs. medium and medium vs. large. Paired pools contained equal amounts of detritus. Experiment 2 used a total of 80 paired pools: 40 of S vs. M (site 1: 20, site 3: 20) and 40 of M vs. L (site 2: 20, site 4: 20). Between 20 May to 27 July 2006 and 25 May to 1 August 2007 pools for both experiments were surveyed three to five times per week by pouring contents of each pool through a fine mesh.

For experiments 1 and 2, field identification of tadpoles was accomplished using the following characteristics: grey to dark pigmentation (*D. variabilis*) vs. transparent to cream pigmentation (*D. imitator*); if coloured (> Gosner stage 30), single nose spot present (*D. variabilis*) vs. paired spots (*D. imitator*); head shape round and interorbital distance large (*D. imitator*). After deposition, tadpoles were transferred to the field station and were raised until the nose spot and patterns were evident. *Ameerega trivitatta* tadpoles posses white spots on the corners of the mouth and upper lip; species of *Dendrobates* lack this character.

Experiment 3 – tadpole competition experiments

Experiment 3 was performed between 20 May and 1 August 2006 within the ASPRAVEP laboratory at San Jose, San Martin, Peru. These experiments contained five tadpole treatments and a total of 48 replicates: (A) one *D. imitator* tadpole ($n = 8$); (B) one *D. variabilis* tadpole ($n = 8$); (C) two *D. imitator* tadpoles (the *D. imitator* intraspecific treatment, $n = 10$); (D) two *D. variabilis* tadpoles (the *D. variabilis* intraspecific treatment, $n = 10$); and (E) one *D. imitator* tadpole and one *D. variabilis* tadpole (the interspecific treatment, $n = 12$). Tadpole treatments were placed in 370-mL cups filled with 100 mL of water and contained 1.5 g of detritus (sterile *Cecropia* leaves boiled for 20 min). Tadpole treatments were fed equal, small amounts of spirulina-enriched fish flakes two times a week and received a 50% water change weekly. Each week (for 8 weeks), tadpoles were weighed to the nearest 0.01 g using an Ohaus–Scout scale and were measured to the nearest 0.1 mm using calipers, measuring total length (TL) and body length (BL). If a treatment contained two tadpoles, at the start of each replicate, tadpoles were matched by size by ranking the following measurements in order: mass (M), TL and BL. If tadpoles differed by more than 2 mm TL, 1 mm BL or 0.01 g, they were not paired. In most instances, paired tadpoles were almost identical in size, especially in treatment E.

All tadpoles in experiment 3 were collected as embryos and hatched in the laboratory, and after hatching, tadpoles were kept for at least 3 days before they were placed in treatments. Embryos of each species were collected from breeding plants that were only used by one of the two species. For example, *D. imitator* is only known to deposit eggs on the basal sheathing leaf of *Heliconia* plants, and conversely only *D. variabilis* deposit their eggs at the water's edge of a pool within bromeliads. Identity was also confirmed by egg/embryo colour: eggs of *D. imitator* are whitish grey and eggs of *D. variabilis* are dark grey to black. Individuals from the same clutch were placed in separate treatments.

Statistical analysis

In the pool choice experiments (1 and 2), only single depositions were recorded and frequencies of deposition events in each pool type were compared using a one-way *G*-test under the null hypothesis that the frequency of deposition events would be random (0.5 in either pool). Due to problems with calculating the multinomial distribution associated with small observations, chi-squared analyses were not performed and *G*-tests were used (Gotelli & Ellison, 2004). If an experiment had an expected frequency of less than five, the *G*-statistic was adjusted using the method proposed by Williams (1976). Given the matching effects, predator treatments in experiment 1 were combined and analysed together. Chi-squared analyses were used for comparisons within *D. variabilis* and chi-squared contingency tests were used for comparisons between species (calculated by hand). When comparing the chi-squared contingencies between species in experiment 2B, because of limited observations, a Yates' correction was applied (Gotelli & Ellison, 2004). *G*-tests and chi-squared analysis were performed in SPSS 15 (SPSS Inc., 2007).

Comparative analysis of phytotelmata volume and parental care

The ancestral state reconstructions were carried out in MESQUITE 2.0 (Maddison & Maddison, 2007) under parsimony (Fig. 1) using a simplified phylogeny from Roberts *et al.* (2006). Data on average phytotelmata water volume (mL) and parental care were from the following sources: Summers & McKeon (2004): data on parental care; Summers (1990): *D. auratus* (280 mL); Brown *et al.* (2008): *D. imitator* (24 mL) and *D. variabilis* (112 mL); Caldwell & de Oliveira (1999): *D. vanzolinii* (18 mL); McDade *et al.* (1994): *D. pumilio* (25 mL); Caldwell & Myers (1990) and J.L. Brown (unpublished data): *D. castaneoticus* (ca. 225 mL); J.L. Brown (unpublished data): *D. fantasticus* (125 ± 27 mL, $n = 4$); K. Summers (unpublished data): *D. ventrimaculatus* from Pompeya, EC (85 ± 41 mL, $n = 28$); and R. von May (unpublished data): *D. biolat* (36 ± 21 mL, $n = 8$ and observed male parental care).

Results

Comparative analyses

Figure 1 shows the reconstruction of the evolution of pool size and parental care in the dendrobatid frogs for which data are available. Although sample size of evolutionary transitions is too small for statistical analysis, the results are consistent with the hypothesis that there is an association between the evolution of very small phytotelmata use and the evolution of egg feeding. In turn, egg feeding is associated with biparental care.

Experiment 1 – predator pool choice

Both *D. imitator* and *D. variabilis* avoided depositing tadpoles in pools containing predators ($\Lambda_1 = 5.35$, $P < 0.01$; $\Lambda_1 = 10.52$, $P < 0.001$ respectively). Further, *D. variabilis* avoided depositing its eggs in pools that contained predators ($\Lambda_1 = 9.77$, $P < 0.001$, see Table 1 and Fig. 2 for results).

Experiment 2A – small vs. medium size pool choice

Dendrobates imitator and *D. variabilis* displayed opposite pool-size preferences for tadpole deposition ($\chi^2 = 14.89$, $P < 0.001$): *D. imitator* preferred small pools (marginally significant) for depositing tadpoles ($\Lambda_1 = 2.68$, $P = 0.051$), whereas *D. variabilis* strongly preferred medium pools ($\Lambda_1 = 6.89$, $P < 0.005$). Further, the tadpole and egg deposition strategies of *D. variabilis* were significantly different ($\chi^2 = 5.75$, $P < 0.01$), having no preference for small or medium pools for oviposition ($\Lambda_1 = 0.04$, $P = 0.42$).

Experiment 2B – medium vs. large size pool choice

There was no statistical difference in pool preference between *D. imitator* and *D. variabilis* for tadpole deposition ($\chi^2 = 0.97$, $P = 0.33$): *D. imitator* had no preference for medium or large pools for tadpole deposition ($\Lambda_1 = 0.10$, $P = 0.37$) and *D. variabilis* strongly preferred large pools

Table 1 Deposition data from experiments 1 and 2.

	Experiment 1				Experiment 2			
	Damselfly treatment		Tadpole treatment		Treatment A		Treatment B	
	With predator	W/O predator	With predator	W/O predator	Small	Medium	Medium	Large
Dendrobates imitator								
Site 1	0	0	0	1	7	2	NA	NA
Site 2	0	1	0	0	NA	NA	2	2
Site 3	0	1	0	2	6	2	NA	NA
Site 4	0	0	0	1	NA	NA	0	1
Total	0	2	0	4	13	4	2	3
D. variabilis – tadpole								
Site 1	0	1	0	3	0	6	NA	NA
Site 2	0	0	1	6	NA	NA	1	9
Site 3	0	3	0	2	1	7	NA	NA
Site 4	0	2	0	2	NA	NA	1	13
Total	0	6	1	13	1	13	2	24
D. variabilis – egg								
Site 1	0	0	0	0	4	2	NA	NA
Site 2	0	0	0	4	NA	NA	6	1
Site 3	0	0	0	3	3	4	NA	NA
Site 4	0	2	0	4	NA	NA	8	4
Total	0	2	0	11	7	6	14	5

Each cell represents the number of observations.

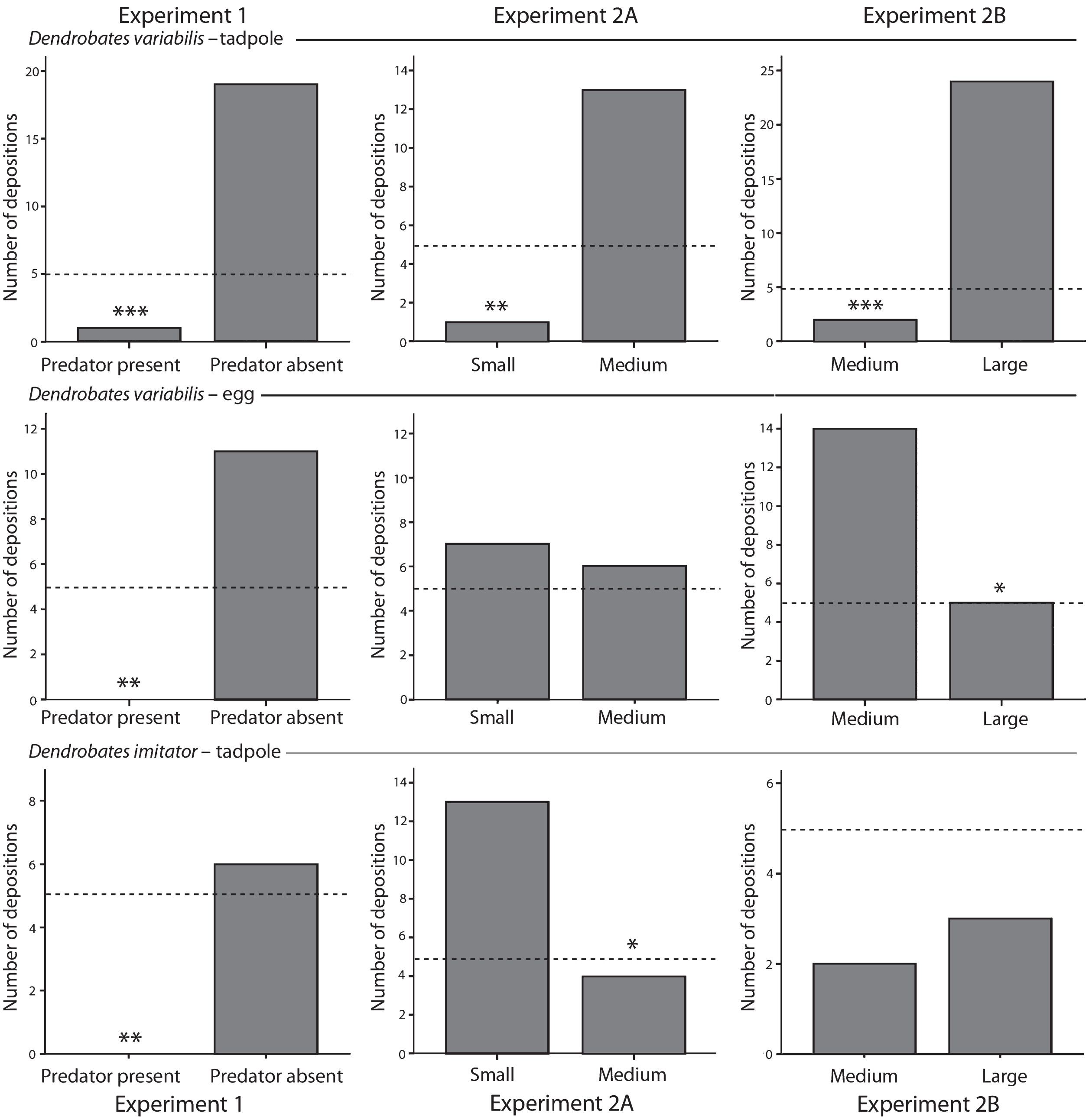

Fig. 2 Tadpole and egg deposition frequencies of *Dendrobates imitator* and *Dendrobates variabilis* in experiments 1 and 2. Top row: *D. variabilis* tadpole depositions; middle row: *D. variabilis* egg depositions; bottom row: *D. imitator* tadpole depositions. *$P < 0.1$, **$P < 0.01$, ***$P < 0.001$. Note: the scale of the number of depositions is not standardized (to show the differences in the number of observations between treatments, a dashed line was placed at five depositions).

for tadpole deposition ($\Lambda_1 = 12.34$, $P < 0.001$). However, the tadpole and egg deposition strategies of *D. variabilis* were significantly different ($\chi^2 = 22.57$, $P < 0.001$) and *D. variabilis* exhibited a trend towards medium pool use for oviposition ($\chi^2 = 2.34$, $P = 0.06$) and large pool use for tadpole deposition.

Experiment 3 – competition experiments

The 40 *D. imitator* tadpoles used in the experiments were on average 12.4 ± 1.2 mm TL, 4.7 ± 0.7 mm BL and 0.018 ± 0.007 g at the start of the experiments. The 40 *D. variabilis* tadpoles were on average 12.4 ± 1.2 mm TL,

Table 2 Tadpole competition experiments (experiment 3): growth rates (values at week 8 − values at week 1; mean ± SD) and the time to cannibalism (in paired treatments).

	Dendrobates variabilis solitary (A)	*Dendrobates imitator* solitary (B)	*D. variabilis* paired (C)	*D. imitator* paired (D)	*D. imitator* and *D. variabilis* (E)
Mass (g)	0.080 ± 0.021	0.089 ± 0.033	0.085 ± 0.025	0.076 ± 0.029	0.076 ± 0.019
Total length (mm)	7.04 ± 2.02	7.55 ± 1.32	7.36 ± 1.70	7.07 ± 1.06	5.70 ± 2.21
Body length (mm)	2.18 ± 0.53	2.55 ± 0.94	2.81 ± 0.52	2.6 ± 0.81	2.11 ± 0.74
Weeks to cannibalism	NA	NA	1.5 ± 0.8	1.4 ± 0.5	2.2 ± 1.1

4.5 ± 0.7 mm BL and 0.018 ± 0.007 g at the start the experiments. Tadpoles paired in treatments C–E differed on average by 0.70 mm (5.7%, 2.1–0 mm) TL, 0.28 mm (5.2%, 0.9–0 mm) BL and 0.0032 g (17.3%, 0.01–0 g). In the interspecific cannibalism treatments (E), there was no significant association between the larger tadpole and occurrence of cannibalism ($\Lambda_1 = 0.168$ $P = 0.68$). Due to the lack of morphological differences of tadpoles in intraspecific treatments (C and D), we were unable to keep track of the tadpoles' identities in those treatments. At the end of 8 weeks, there was no difference in the mass, TL or BL of tadpoles between all treatments (mass: $F_{4,47} = 0.38$, $P = 0.82$; TL: $F_{4,47} = 1.28$, $P = 0.30$; and BL: $F_{4,47} = 1.28$, $P = 0.30$; Table 2).

In all paired tadpole treatments, after 1–5 weeks (average 1.7 week) only one tadpole survived. There was no difference in time to cannibalism between paired tadpole treatments ($F_{2,31} = 2.38$, $P = 0.11$). In treatment E, in all cases but one, the *D. variabilis* tadpoles survived ($\Lambda_1 = 5.45$, $P < 0.05$, Fig. 3) and were superior competitors.

Discussion

In the first experiment, we predicted that both *D. variabilis* and *D. imitator* would show the same response to the risk of predation, i.e. they would avoid placing tadpoles (and eggs in the case of *D. variabilis*) in pools with potential predators. These predictions were confirmed in each case. This demonstrates that these two species make similar choices when confronted with ecological situations that affect their fitness in similar ways (in each case, the effect of depositing a tadpole with a potential predator is likely to reduce the probability of survival). It is possible that the pools containing *A. trivitatta* tadpoles were perceived as competitors, rather than predators. In either case, we would expect both species to make similar choices and avoid pools that contained competitors or predators.

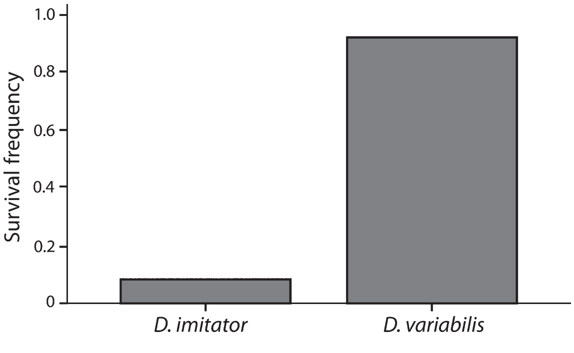

Fig. 3 Interspecific paired competition experiments. Frequency of survival in paired *Dendrobates imitator* and *Dendrobates variabilis* tadpole competition experiments ($P = 0.02$).

In the second experiment, we predicted that these species would show opposite preferences with regard to pool size. The promiscuous mating system and lack of female care and trophic egg feeding in *D. variabilis* probably imposes a trade-off between safety from predation and nutrient availability, with pools that are too small conferring a high risk of starvation (J.L. Brown, unpublished data). Hence, we predicted that *D. variabilis* males would show a strong preference for large pools within the size range normally used by this species. By contrast, the mating system of *D. imitator* involves pair bonding and biparental care, including trophic egg feeding. Hence, tadpoles face little risk of starvation even in very small phytotelmata, and we predicted that this species would actually prefer small phytotelmata in order to minimize the risk of competition.

The results of the second experiment generally agree with our predictions. *Dendrobates imitator*, the biparental care species, preferred small artificial pools for tadpole deposition (Fig. 1), but had no preference between medium and large pools, typically avoiding them. This lack of preference may be the result of a size threshold, causing the larger pools to be treated equally. The avoidance of larger pools is supported by three observations of tadpoles placed beneath the medium reservoir cup, within the bottom cup (the cup wired to the tree that the reservoir cup was placed into) in less than 20 mL of water. The preference of small pools coincides with observations on natural phytotelmata use, where individuals used pools that were on average 24 mL for tadpole deposition (Brown *et al.*, 2008).

Because *D. variabilis* used phytotelmata for both egg and tadpole deposition, we were able to compare these deposition strategies. Surprisingly, their preferences were not the same and *D. variabilis* overwhelmingly preferred larger pools for tadpole deposition, but smaller pools for egg deposition in experiment 2B. *Dendrobates variabilis* had no preference between the small and medium pools for egg deposition (experiment 2A). This lack of preference may be a result of a size threshold, so that the medium and small pools were treated equivalently for egg deposition. The preference for larger pools for tadpole deposition is probably related to nutrient availability. Given that this species has male-only parental care and males are not able to supplement their tadpoles with trophic eggs, tadpoles are probably constrained by the abundance of food. The observed preference for large pools (275 mL) exceeded the size of natural pools used by this species (averaging 112 mL; Brown *et al.*, 2008) and reinforces the notion that smaller pools contain limited resources. However, because larger pools were actively sought out for tadpole deposition, eggs were probably deposited in smaller pools to avoid cannibalism. Once embryos developed into tadpoles, they were usually transported to larger pools.

The egg deposition strategies of *D. variabilis* are complicated by mixed male parental care strategies (Brown *et al.*, 2008), where in most cases males transport their tadpoles to separate pools to be reared individually, but in some cases males abandon their clutch allowing all the embryos to hatch in the same pool (usually resulting in one tadpole cannibalizing the others). Although the latter strategy is infrequent (20%, 7/35), males that utilize this strategy may achieve higher reproductive returns by placing their egg clutches in larger pools, providing the tadpoles with increased food and reducing competitive interactions. Alternatively, because cannibalism usually eliminates all but one member of a clutch, the added nutrition from the 'trophic' tadpoles may reduce the food constraints of smaller pools. The desertion strategy opens the opportunity for brood parasitism and males could place their tadpoles in pools containing unrelated embryos. We do not know if egg desertion is a conditional strategy used when pools and receptive females are abundant, or simply the result of poor temporal or geographic memory.

The observed association between the preference for larger pools for tadpole deposition and male parental care is supported by observations of another male parental care species, *D. fantasticus* (a rare syntopic poison frog), that also preferred to deposit its tadpoles only in large pools ($\Lambda_1 = 4.57$, $P = 0.032$, $n = 5$) at sites 2 and 4. This species was observed at all sites; however, tadpole depositions were never observed in any test pools at sites 1 and 3 (sites that lacked large pools). The pool preferences of all three species agree with the observed pattern of phytotelmata use of the other male parental care and biparental care species (Fig. 1). Further, the results from our comparative analysis suggest that the two instances where egg feeding evolved are connected with the transition to very small pool use.

The results from the third experiment support the hypothesis that very small pool use reduces intraspecific competition and hence that *D. imitator* have evolved to be inferior competitors relative to *D. variabilis* tadpoles. Contrary to our original prediction, there were no significant differences in growth rates between any treatments (including solitary tadpoles). It is possible that during the processes of killing and consuming the other tadpole, tadpoles of the same size expended a great deal of energy and the nutrients gained from the cannibalism simply replenished the energy used. Given the limited nutrients in small phytotelmata, the benefits of cannibalizing a tadpole are probably much greater than sharing a pool (where the limed resources are shared throughout development vs. shared for a brief period). These benefits should be even greater if the sizes of paired tadpoles are asymmetrical, because the larger tadpole would quickly cannibalize the smaller tadpole (J.L. Brown, unpublished data).

There is another reason to predict that *D. imitator* tadpoles might evolve to be less aggressively competitive than *D. variabilis* tadpoles. The highly promiscuous nature of the *D. variabilis* mating system and the opportunistic nature of their tadpole deposition strategy suggest that tadpoles placed together in the same pool will frequently be half-sibs or unrelated. Genetic analysis of the relatedness between tadpoles in a species very closely related to *D. variabilis* (*D. ventrimaculatus s.s.*, a species that shows a breeding strategy very similar to that of *D. variabilis*) is consistent with this prediction (Summers & Amos, 1997). By contrast, in *D. imitator*, when tadpoles are placed in the same pool (a rare occurrence) they would probably be full-sibs. Hence, kin selection could favour lower levels of aggression in this species (Hamilton, 1964; Pfennig & Collins, 1993).

Our results illustrate the intimate connections between breeding pool ecology, parental care and mating strategies in Peruvian poison frogs. Few studies demonstrated that interspecific competition at larval stages influences adult mating systems or vice versa. Fincke (1992) demonstrated that interspecific larval competition for reproductive resources affected the evolution of mating behaviours in three species of damselflies. The two species of *Mecistogaster* (*M. ornata* and *M. linearis*) were inferior competitors and exhibited a scramble mating system, bred explosively, displayed an increased colonizing ability and used smaller tree holes for larval rearing, whereas the superior competitor, *Megaloprepis coerulatus*, bred year round and used larger tree holes (defended by males) for larval rearing. Fincke's results and the results of this study support the hypothesis that differences in larval ecology influence differences in adult parental strategies and mating systems and vice versa. These features of life history are likely to coevolve in a mutually reinforcing manner.

Acknowledgments

We are grateful to E. Twomey and B. Richardson for field assistance. We are thankful to R. Schulte and ASPRAVEP for the use of their field station and assistance in the Cainarachi Valley. We are very grateful to C. Aguilar, J. Córdova, K. Siu Ting and M. Muller Medina for their help at the Museo de Historia Natural, San Marcos, Peru, and Karina Ramirez for help at the Ministry of Natural Resources. This research was funded by grants from the National Science Foundation (IOB-0544010), the National Geographic Society (7658-04) and an East Carolina University Research and Development Grant (2006). Research permits were obtained from the Ministry of Natural Resources (INRENA) in Lima, Peru (authorization no. 050-2006-INRENA-IFFS-DCB, no. 067-2007-INRENA-IFFS-DCB).

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Received 9 May 2008; revised 15 July 2008; accepted 16 July 2008