

Tactical reproductive parasitism via larval cannibalism in Peruvian poison frogs

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We report an unusual example of reproductive parasitism in amphibians. *Dendrobates variabilis*, an Amazonian poison frog, oviposits at the surface of the water in small pools in plants and deposits tadpoles within the pools. Tadpoles are highly cannibalistic and consume young tadpoles if they are accessible. Deposition of embryos and tadpoles in the same pool is common. Genetic analyses indicate that tadpoles are frequently unrelated to embryos in the same pool. A pool choice experiment in the field demonstrated that males carrying tadpoles prefer to place them in pools with embryos, facilitating reproductive parasitism via cannibalism.

Keywords: reproductive parasitism; egg cannibalism; *Dendrobates*; *Ranitomeya*; deposition strategies; anuran

1. INTRODUCTION

Brood parasitism occurs when individuals intentionally place their offspring into a context in which they can exploit the parental effort of other individuals of the same or different species. This phenomenon is common in birds and insects and also occurs in fishes (Sato 1986; Zink 2000; Yom-Tov & Geffen 2006). The broader term reproductive parasitism is used here to describe the phenomena in which individuals exploit the reproductive effort of other individuals, using a diverse array of tactics (e.g. Taborsky 1994; Dierjes *et al.* 1999). The nature of reproductive parasitism varies between taxa. In insects, for example, reproductive parasitism can occur when an individual seizes resources allocated to parental care by another individual (Vollrath 1984; Moczek & Cochrane 2006). In fishes, reproductive parasitism can include behaviours such as oophagy, brood adoption, kleptogamy, zygote dumping, nest parasitism, as well as satellite behaviour and sneak mating (Sato 1986; Baba *et al.* 1990; Taborsky 1994; Wisenden 1999). Here, we report an unusual example of reproductive parasitism in frogs, in which adult males actively facilitate the cannibalism of unrelated embryos by their tadpoles.

Observational and experimental evidence on the behaviour of a Peruvian poison frog, *Dendrobates variabilis*, and a closely related species (*Dendrobates ventrimaculatus*) in Ecuador with a similar breeding strategy (Symula *et al.* 2003), provides the context for

our experiments. These species use small pools of water in the leaf axils of plants (phytotelmata) for breeding (Summers & Amos 1997; Summers 1999; Brown *et al.* 2008a). Eggs are laid on the wall of the phytotelmata at the water's surface. After a week, the male allows each tadpole to wriggle onto his back (Brown *et al.* 2008a). The male then transports each tadpole to another phytotelma, where they are deposited into separate pools. The transporting males choose to deposit tadpoles in pools lacking both tadpole and odonate predators to minimize cannibalism or predation of their offspring (Brown *et al.* 2008b). But occasionally, the developing embryos slide into the pool below (during hatching or placement on the male's back) or the water level rises within the pool and the embryos are submerged, where they are less likely to be retrieved by the male (J. L. Brown 2006, personal observation; Brown *et al.* 2008a). This can also occur if the male does not transport them in time and the embryos hatch (Brown *et al.* 2008a).

In both species (*D. ventrimaculatus* and *D. variabilis*), individual home ranges overlap extensively and reproductive resources are not guarded (Summers & Amos 1997; Brown *et al.* 2008a; Poelman & Dicke 2008). Owing to this, the deposition of more than one clutch in a pool is common. It is also common for tadpoles to be deposited in pools that have one or more clutches of eggs at the surface (Summers 1999; Brown *et al.* 2008a).

These factors make it possible for individuals to feed earlier offspring by placing later offspring (either eggs or small tadpoles) in pools containing a previously deposited tadpole. The fact that both eggs and tadpoles can be placed in the same pools enables individuals to parasitize the reproductive effort of other individuals by deliberately placing tadpoles into pools that have previously been used for egg deposition (reproductive parasitism, figure 1). The critical test to determine whether males pursue a reproductively parasitic strategy is to provide a choice between a pool that contains unrelated eggs and a pool that does not. If males consistently prefer to deposit the tadpoles into pools containing a clutch of embryos, this would provide evidence for a deliberate parasitic strategy.

2. MATERIAL AND METHODS

(a) Embryo and tadpole cannibalism experiments

Embryos were collected and allowed to hatch, and then raised for two weeks at a field station. Tadpoles were fed ad libitum spirulina flakes. Water was changed weekly. After two weeks, feeding was discontinued and a single embryo was placed in each pool. The pools were monitored daily for the presence of the embryos or the newly hatched tadpoles.

(b) Genetic relatedness

Between May and August 2007, we surveyed the pools for the presence of both embryos and a larger tadpole (too large to be an early hatchling from the same clutch of embryos). When found, the embryos and the tadpole were collected. Tissues were collected from putatively unrelated adults from four research sites that were within 1 km of each other (Brown *et al.* 2008a,b). Genetic relatedness was assessed using three polymorphic tetra-repeat microsatellite loci cloned from *D. variabilis* genomic DNA. Fluorescently labelled primer pairs were amplified using standard methods. PCR products were multiplexed and run on an ABI 3100 capillary sequencer. Results were analysed using GENEMAPPER v. 4.0 (ABI). Relatedness was calculated with RELATEDNESS v. 5.0 (Queller & Goodnight 1989).



Figure 1. Photo illustration of breeding strategies in *D. variabilis*: a male transporting tadpoles, a tadpole and two embryos in a bromeliad tank.

(c) Reproductive parasitism experiments

Between May and August 2006, we carried out pool choice experiments with two artificial pools presented side by side: one with and one without embryos. The pools were 160 ml opaque plastic cups, wrapped in leaves, wired to trees, filled with 120 ml of water and matched to appear identical except for the presence of embryos. Paired pools ($n=15$) were dispersed haphazardly throughout the site (10 m $\times$ 20 m) at 1.3 m height. All experimental sites were in the Cainarachi Valley, San Martín, Peru (6°25'41.34" S, 76°14'21.18" W) within the secondary premontane rainforest. Both pools contained equal amounts of detritus to simulate natural pools. One of the paired pools contained a clutch of *D. variabilis* embryos that were laid on the wall of the artificial pool at the surface of the water. Each clutch contained between two to six embryos between Gosner stages 1–21 (Gosner 1960). Embryos were harvested from a separate plot 500 m away to ensure they were not the offspring of frogs from the test site. Deposition events were scored by the presence of a tadpole in either of the pools.

3. RESULTS

(a) Embryo and tadpole cannibalism experiments

Embryo cannibalism was not observed ($n=26$); however, within a week 85 per cent (22 of 26) of the newly hatched tadpoles were cannibalized (figure 2a).

(b) Genetic relatedness

Relatedness was estimated as $R=0.004$ (s.e.=0.005) among putatively unrelated individuals ($n=61$) and $R=0.58$ (s.e.=0.31) among embryos from a single clutch ($n=4$ clutches). Relatedness between tadpoles and separate embryos from the same pool ($n=6$ pools, 7 tadpoles, 13 embryos and 19 tadpole–embryo pairwise comparisons) was $R=0.18$ (s.e.=0.36). Figure 2c shows the distribution of relatedness estimates for each tadpole–embryo comparison. The distribution shows a wide range of values for relatedness, indicating that some comparisons are likely to be between siblings, whereas others are likely to be between unrelated individuals.

(c) Reproductive parasitism experiments

Males consistently deposited tadpoles into the pool containing the embryos (figure 2b; $n=16$, $\chi^2_1=3.462$, $p=0.031$).

4. DISCUSSION

Dendrobates variabilis tadpoles were highly cannibalistic and typically consumed any tadpole of smaller

size, but were never observed consuming embryos. In an Ecuadorian population of *D. ventrimaculatus*, a closely related species, tadpoles also consumed smaller tadpoles, but additionally consumed eggs and embryos (Summers & Amos 1997; Summers 1999). In French Guiana, Poelman & Dicke (2007) manipulated the availability of breeding pools in a population of *D. ventrimaculatus* and demonstrated that adults deposited eggs and tadpoles in the pools containing previously deposited tadpoles. They reasoned that this was a late-season feeding strategy to enhance the growth of the offspring produced early at the expense of the offspring produced late in the breeding season (Poelman & Dicke 2007).

Our microsatellite analyses of *D. variabilis* reveal that the tadpole–embryo relatedness represents a mix of related and unrelated individuals (figure 2b). These results are very similar to the results on Ecuadorian *D. ventrimaculatus* (Summers & Amos 1997), and suggest that reproductive parasitism also occurs in this species, but not in French Guiana *D. ventrimaculatus*. This French Guiana population is a member of a clade that is sister to a clade containing both *D. variabilis* and *D. ventrimaculatus* from Ecuador (Noonan & Gaucher 2006). Owing to this, we are unsure if the transition to the late-season feeding evolved independently, or is a modification of reproductive parasitism that derived from a shared common ancestor (or vice versa).

Prior observations and experiments demonstrated that adults recognize the presence of tadpoles in the pools and strongly avoid the pools containing predators and heterospecific competitors (Brown *et al.* 2008b). Our experiments revealed that *D. variabilis* males prefer pools with unrelated eggs and actively deposit their tadpoles within these pools. This provides an unusual example of tactical reproductive parasitism in an amphibian.

The nature of reproductive parasitism observed in *D. variabilis* is not as complex as some forms of parasitism seen in other taxa. In particular, in classical brood parasitism in birds and insects, the parasitized parents (or alloparents) repeatedly feed the brood parasite throughout its development, but this is not the case in *D. variabilis*. Nevertheless, this simple form of reproductive parasitism could be a step towards the more complex forms of parasitism seen in other taxa. *Dendrobates imitator*, a related species of poison frog that can occur sympatrically with *D. variabilis* in Peru, exhibits biparental care and trophic egg feeding of tadpoles in the context of monogamous pair bonds (Brown *et al.* 2008a). Field experiments have demonstrated that *D. variabilis* tadpoles typically kill and consume *D. imitator* tadpoles if they are placed in the same pool (Brown *et al.* 2008b). Hence, if a male *D. variabilis* were able to place a tadpole into a pool used by a *D. imitator* pair for tadpole feeding, the *D. variabilis* tadpole would probably eliminate the *D. imitator* tadpole. This could lead to feeding of the *D. variabilis* tadpole by the *D. imitator* parents, which would be equivalent to interspecific brood parasitism as seen in avian brood parasites. However, despite intensive sampling over several years, we have not observed any *D. variabilis* tadpoles in natural pools used by *D. imitator* parents.

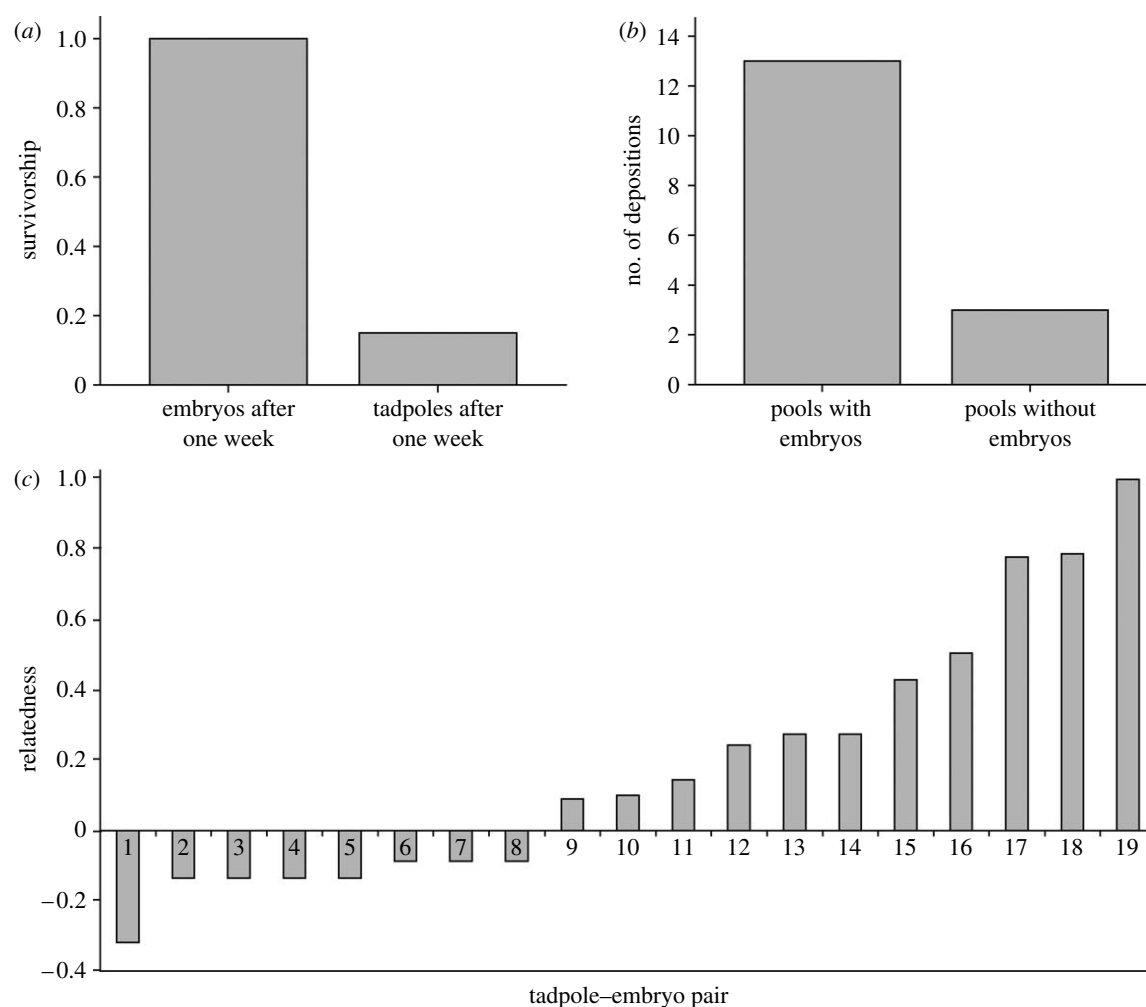


Figure 2. (a) The survivorship of embryos or tadpoles after one week of being housed with a larger tadpole. (b) The results of the pool choice experiment (pools with and without clutches of embryos). (c) Distribution of relatedness estimates for 19 pairwise comparisons between tadpoles and embryos collected simultaneously from the same pool.

Hence, although the potential for more complex forms of reproductive parasitism to evolve exists in this system, that potential does not seem to have been realized at this point in time. One reason that interspecific brood parasitism has not evolved in *D. variabilis* may be because the tadpoles of this species do not eat eggs (figure 2a), the only source of food provided to *D. imitator* tadpoles.

The methods used were approved by the East Carolina University Animal Care and Use Committee (AUP 202), and by the Peruvian Ministry of Natural Resources (permit no. 050-2006-INRENA-IFFS-DCB).

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Baba, R., Nagata, Y. & Yamagishi, S. 1990 Brood parasitism and egg robbing among three freshwater fish. *Anim. Behav.* **40**, 776–778. (doi:10.1016/S0003-3472(05)80707-9)

Brown, J. L., Twomey, E. M., Morales, V. & Summers, K. 2008a Phytotelm size in relation to parental care and

mating strategies in two species of Peruvian poison frogs. *Behaviour* **145**, 1139–1165. (doi:10.1163/156853908785387647)

Brown, J. L., Morales, V. & Summers, K. 2008b Divergence in parental care, habitat selection and larval life history between two species of Peruvian poison frogs: an experimental analysis. *J. Evol. Biol.* **21**, 1534–1543. (doi:10.1111/j.1420-9101.2008.01609.x)

Dierjes, P., Taborsky, M. & Kohler, U. 1999 Reproductive parasitism of broodcare helpers in a cooperatively breeding fish. *Behav. Ecol.* **10**, 510–515. (doi:10.1093/beheco/10.5.510)

Gosner, K. L. 1960 A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183–190.

Moczek, A. P. & Cochrane, J. 2006 Intraspecific female brood parasitism in the dung beetle *Onthophagus taurus*. *Ecol. Entomol.* **31**, 316–321. (doi:10.1111/j.1365-2311.2006.00773.x)

Noonan, B. P. & Gaucher, P. 2006 Genetic diversity, population structure and the origin of variation in the Dyeing Poison Frog: *Dendrobates tinctorius* (Anura: Dendrobatidae). *Mol. Ecol.* **15**, 4425–4435. (doi:10.1111/j.1365-294X.2006.03074.x)

Poelman, E. H. & Dicke, M. 2007 Offering offspring as food to cannibals: oviposition strategies of Amazonian poison frogs (*Dendrobates ventrimaculatus*).

- Evol. Ecol.* **21**, 215–227. (doi:10.1007/s10682-006-9000-8)
- Poelman, E. H. & Dicke, M. 2008 Space use of Amazonian poison frogs: testing in the reproductive resource defense hypothesis. *J. Herpetol.* **42**, 270–278. (doi:10.1670/07-1031.1)
- Queller, D. C. & Goodnight, K. F. 1989 Estimation of genetic relatedness using allozyme data. *Evolution* **43**, 258–275. (doi:10.2307/2409206)
- Sato, T. 1986 A brood parasitic catfish of mouthbrooding cichlid fishes in Lake Tanganyika. *Nature* **323**, 58–59. (doi:10.1038/323058a0)
- Summers, K. 1999 The effects of cannibalism on Amazonian poison frog egg and tadpole deposition and survivorship in Heliconia axil pools. *Oecologia* **119**, 557–564. (doi:10.1007/s004420050819)
- Summers, K. & Amos, W. 1997 Behavioral, ecological, and molecular genetic analyses of reproductive strategies in the Amazonian dart-poison frog, *Dendrobates ventrimaculatus*. *Behav. Ecol.* **8**, 260–267. (doi:10.1093/beheco/8.3.260)
- Symula, R., Schulte, R. & Summers, K. 2003 Molecular systematics and phylogeography of Amazonian poison frogs of the genus *Dendrobates*. *Mol. Phylogenet. Evol.* **26**, 452–475. (doi:10.1016/S1055-7903(02)00367-6)
- Taborsky, M. 1994 Sneakers, satellites, and helpers: parasitic and cooperative behavior behavior in fish reproduction. *Adv. Study Behav.* **23**, 1–100. (doi:10.1016/S0065-3454(08)60351-4)
- Vollrath, F. 1984 Kleptobiotic interactions in invertebrates. In *Producers and scroungers: strategies of exploitation and parasitism* (ed. C. J. Barnard), pp. 61–94. London, UK: Chapman & Hall.
- Wisenden, B. D. 1999 Alloparental care in fishes. *Rev. Fish Biol. Fish.* **9**, 45–70. (doi:10.1023/A:1008865801329)
- Yom-Tov, Y. & Geffen, E. 2006 On the origin of brood parasitism in altricial birds. *Behav. Ecol.* **17**, 196–205. (doi:10.1093/beheco/arj013)
- Zink, A. G. 2000 The evolution of intraspecific brood parasitism in birds and insects. *Am. Nat.* **155**, 395–405. (doi:10.1086/303325)

NOTICE OF CORRECTION

Figure legend 2 is now presented in the correct form.

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