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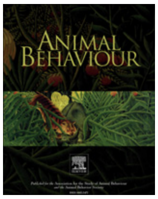

The smell of success: choice of larval rearing sites by means of chemical cues in a Peruvian poison frog

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Parental care is a common strategy among vertebrates to ensure successful reproduction. Anuran amphibians have evolved a remarkable diversity of reproductive methods including advanced levels of parental care. Among the most derived strategies are those of the Neotropical poison frogs (Dendrobatidae). These amphibians exhibit a wide array of behavioural traits such as egg guarding, larval transport by parental frogs and larval feeding with trophic (unfertilized) eggs. *Ranitomeya variabilis* from the upper Amazon basin in Peru deposits both eggs and tadpoles in phytotelmata. The exploitation of these small pools is advantageous as it lowers the risk of predation, but it is more costly because of limited resource availability. Additionally, poison frog larvae are often cannibalistic, so the identification and avoidance of conspecifics represents an adaptive behaviour for these amphibians. While studies have shown that poison frogs actively avoid depositing with conspecifics, the mechanism for assessing pool quality remains unknown. In field experiments, we found that parental *R. variabilis* frogs used chemical cues to recognize the presence of tadpoles in phytotelmata. Furthermore, they distinguished between cannibalistic and noncannibalistic tadpoles, a behaviour that supports the survival of their own offspring.

Reproductive behaviour can be influenced by biotic interactions, or by a limitation in resources necessary for reproduction (Sih 1994; Jönsson 1997). Amphibians can mitigate predation threats to their larvae by exploiting novel resources (Magnusson & Hero 1991; Wells 2007; Indermaur et al. 2009), as well as avoiding competitors (Hardin 1960; Chesson 2000). There are 39 reproductive modes recognized among the approximately 5900 known anuran amphibians, which display remarkable diversity in oviposition and egg development strategies (Haddad & Prado 2005). Complex reproductive strategies are often associated with parental care such as egg guarding and tadpole transport (Summers & McKeon 2004).

Numerous studies have demonstrated high levels of parental care in poison frogs (Aromobatidae, Dendrobatidae) from Central and South America (e.g. Summers 1990; Brust 1993; Pröhl & Hödl 1999; Caldwell & de Araújo 2004; Summers & McKeon 2006; Lötters et al. 2007; Poelman & Dicke 2007; Brown et al. 2008a, b). Among them, at least four distinct strategies of parental care have evolved (Summers & McKeon 2004; Lötters et al. 2007). The more advanced of these strategies involve the parental transport of single tadpoles from egg deposition sites to phytotelmata (small water bodies in plants such as leaf axils of bromeliads; Fig. 1; Zimmermann & Zimmermann 1988; Summers & McKeon 2004; Wells 2007). This behaviour minimizes the risk of predation associated with larger water bodies (Aspbury & Juliano 1998) as well as competition for the already scarce food resources (Summers 1990). In the species *Ranitomeya variabilis*, older larvae have been shown to feed strategically on younger siblings in times of low resource availability (Brown et al. 2009b). Cannibalistic behaviour benefits the tadpole both by eliminating competitors and by compensating for the limited food resources in phytotelmata (Summers 1990; Caldwell 1993; Wells 2007).

To avoid predation by previously deposited tadpoles, poison frog species of the genera *Adelphobates*, *Dendrobates*, *Oophaga* and

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Figure 1. The poison frog *Ranitomeya variabilis*, carrying its larvae. In this species male parents transport their offspring to phytotelmata (typically water-filled leaf axils of bromeliad plants). Tadpoles are usually transported singly but sometimes two or three tadpoles can be found on the back of the male parent. Photo: J.L. Brown.

Ranitomeya examine phytotelmata before the deposition of a new larva and avoid already occupied pools (Weygoldt 1980; Zimmermann & Zimmermann 1984; Summers 1990; Brust 1993; Caldwell & de Araújo 1998; Caldwell & de Oliveira 1999; Summers 1999; Poelman & Dicke 2007; Brown et al. 2008a, b; Stynoski 2009). Furthermore, it may also be advantageous to distinguish between tadpoles with different feeding strategies. Deposition of tadpoles with noncannibalistic tadpoles could be advantageous as these could serve as prey items. On the other hand, competition risk could also increase within the small phytotelmata. In terms of egg deposition, noncannibalistic tadpoles may pose a potential threat, as larvae of several anuran species have been shown to feed on anuran eggs (Jungfer & Weygoldt 1999). Poelman & Dicke (2007) showed that *Ranitomeya ventrimaculata* typically deposited eggs away from tadpoles at the beginning of the rainy season to protect them against cannibalism, but as the dry season advanced, they placed clutches within reach of previously deposited tadpoles to speed their development. Several congeners are known to feed their offspring trophic eggs to offset low resource levels of small phytotelmata (Caldwell & de Oliveira 1999; Brown et al. 2008a). This egg-feeding strategy is seen in some nondendrobatids as well (Jungfer & Weygoldt 1999).

Potential cues that frogs may use to identify occupied pools may include movement, visual identification and/or chemical cues. In the dendrobatines *Adelphobates castaneoticus*, *Oophaga pumilio* and *R. ventrimaculata*, visual inspection of phytotelmata has been observed (Weygoldt 1980; Caldwell 1993; Poelman & Dicke 2007), presumably used to identify the presence of larvae. *Oophaga* tadpoles facilitate detection by displaying distinct vibration behaviour to beg for a food egg (Weygoldt 1980; Zimmermann & Zimmermann 1984; Stynoski 2009); however, cannibalistic larvae of nonegg-feeding poison frogs do not display vibration behaviour. *Ranitomeya variabilis* larvae even tend to hide at the bottom of phytotelmata (L. M. Schulte, personal observation). Because the tadpoles of many species lack a visual display, it may be hypothesized that in (at least some) dendrobatine species chemical cues play a role in the identification and avoidance of predatory tadpoles in phytotelmata.

Chemical signalling and communication is well documented in salamanders and newts (Marco et al. 1998; Gillette et al. 2000; Jaeger & Peterson 2002; Verrell 2003; Secondi et al. 2005; Gautier et al. 2006), but several studies have identified chemical communication in anurans as well (see Brizzi & Corti 2007; Belanger & Corkum 2009). Examples include pheromone-based sex recognition in adult anurans (Wabnitz et al. 1999, 2000; Pearl et al. 2000; Brizzi & Corti 2007) or kin recognition in tadpoles (Blaustein & O'Hara 1982; Pfennig 1990). Chemical communication between adult frogs and tadpoles has so far been documented in the Taiwanese tree frog, *Chirixalus eiffingeri*, whose tadpoles respond more strongly to chemical than visual cues of their maternal attendant (Kam & Yang 2002). No study about chemical communication has investigated whether male frogs respond to their tadpoles. We investigated for the first time the capacity of the poison frog species *R. variabilis* to base deposition decisions on larval chemical cues. This species is from the lower Amazonian versant of the northern Andes of Peru and displays advanced levels of parental care. It oviposits in phytotelmata (just above the edge of the water). After hatching, larvae are transported singly on the back of the male parent into different phytotelmata (Brown et al. 2008a). We hypothesized that adult *R. variabilis* are able to recognize larval cues and use them to avoid deposition with cannibalistic tadpoles. We additionally proposed that frogs that are able to identify noncannibalistic tadpoles may continue to deposit larvae in the same phytotelmata, as there is not a direct threat to their larval survival, and these larvae could represent a potential food source. With regard to egg deposition, we hypothesized that *R. variabilis* avoids pools containing conspecific tadpoles or tadpoles of related species and other (nondendrobatid) tadpoles since these may feed on eggs as well (Jungfer & Weygoldt 1999).

METHODS

Study Site

Field work was carried out in a late-stage secondary forest near Tarapoto in the upper Cainarachi Valley, Departamento San Martín, Peru; a laboratory was erected in a nearby field station of the INIBICO organization from Tarapoto. Permits for the work with *R. variabilis* in this forest were issued by the National Institute of Natural Resources (INRENA). Four different study sites were established between July and November 2008, each covering 1000 m^2. Sites 1–3 were located between 540 and 580 m above sea level on the western side and site 4 at 530 m above sea level on the eastern side of the Cainarachi River, respectively. As site 4 was separated from the other sites by the river, there is little doubt about its independence from the other three sites. A similar level of independence was expected for the remaining sites, as the shortest distance between them was 30 m, which exceeds the known home range size in *R. variabilis* (Brown et al. 2009a). The partitioning of sites was intended to avoid pseudoreplication from repeated depositions by the same male in different study plots.

Chemical Cues of Cannibalistic Tadpoles

We established our experiments using pairs of plastic cups (200 ml volume, 10 cm height, 7 cm in diameter, made of polypropylene) as artificial phytotelmata on trees at 0.5–1.5 m above ground, which are quickly adopted by wild *R. variabilis* (Brown et al. 2008a). Cups within a pair were hung up directly next to each other on the same tree while the different pairs were spaced at least 4 m apart to reduce the likelihood of recording multiple deposition events for a single male to avoid pseudoreplication (Brown et al. 2008a). In each of the four study sites we put 20 pairs of dark, opaque pairs of plastic cups (a total of 80 pairs). Two-thirds of the opening of each plastic cup was covered with a dark plastic membrane to impair visual inspection by frogs. The pairwise set-up consisted of one cup with 25 ml of clean water and one cup with

25 ml of tadpole-treated water. Clean water was produced at the laboratory. For this purpose, rainwater was collected in a rain barrel and run through one 50 μm prefilter, one 20 μm prefilter, one 1 μm main filter and one activated carbon filter (Spena Fish, Lima, Peru).

Forty-five tadpoles were held in captivity in originally clean water to produce tadpole-treated water. Each specimen was housed singly in a standardized 50 ml plastic tank in the field station under ambient temperature. While contact with plastic containing toxic chemicals leads to high tadpole mortality within 24 h (McDonald et al. 2008; Greer et al. 2009), the polypropylene cups used in our study did not show any negative effect on the development of eggs or larvae (L. M. Schulte, personal observation). Water of all tadpoles was taken and mixed for use in the field every other day and tadpoles were fed afterwards with flaked fish food (Tetra) ad libitum. Tadpoles were collected in phytotelmata at a nearby site and were mostly *R. variabilis*. Once captive tadpoles had reached Gosner developmental stage 40 (Gosner 1960), they were released and replaced with new tadpoles from the wild (which were also later released). A small number (1–2; < 5%) belonged to the closely related *Ranitomeya fantastica*. In addition, less than 10% of the larvae belonged to the noncannibalistic hyloxaline poison frog *Hyloxalus azureiventris*. This was surprising considering that this species was not previously known to use phytotelmata (Lötters et al. 2007). To ensure that the conditions stayed the same during the whole experiment, we always included three or four larvae of *H. azureiventris* with the 45 larvae being used to treat water.

Cups in the field were checked with a small flashlight for newly deposited clutches and tadpoles every other day and both clean and tadpole-treated water were changed afterwards. All depositions by *R. variabilis* were scored (the correct identification of the species was verified some weeks later if possible) and a new pair of cups was put on another tree at least 4 m away and, when possible, alternated with the cups of the other experiments. To minimize pseudoreplication, we scored just one randomly chosen deposition for any same-day deposition in adjacent pools within the same site (Brown et al. 2008b).

Presence of Cannibalistic Tadpoles

To determine whether the results were similar when the cannibalistic tadpoles were present and when just their chemical cues were used, we placed 60 pairs of transparent plastic cups in study sites 1–3 (20 per site). Each cup was equipped with a cage made of a smaller, cracked plastic cup (80 ml volume, 5 cm height, 5.75 cm in diameter) and a net and filled with 100 ml of clean water (less water was not possible because the cages had to be covered). In one cage of each pair we placed a tadpole of *R. variabilis*, while the other one stayed empty. Tadpoles were collected outside each experimental site to ensure they were not the offspring of the individuals from the test site. Cups were checked every other day for egg and tadpole depositions. If a new tadpole was found, a new pair of cups was placed in the aforementioned manner.

Chemical Cues of Noncannibalistic Tadpoles

For treatment with noncannibalistic larvae, tadpoles of the bufonid toad *Rhinella poeppigii* were collected from a nearby road ditch and reared in the field station. As these tadpoles do not prey upon conspecifics, they were housed together in a tank to simplify the water change. The amount of water in the tank was calculated by a per capita volume of 50 ml. Water change and feeding were done in the same way as for the cannibalistic tadpoles in the laboratory. We installed 30 paired nontransparent cups in study sites 1–3 (10 per site) and filled them with 25 ml of water, either clean or treated with toad larvae. Other procedures were identical to those described for the experiment with chemical cues of cannibalistic tadpoles.

Presence of Noncannibalistic Tadpoles

To test whether the physical presence of noncannibalistic tadpoles elicited a similar response as chemical cues alone, 21 pairs of transparent cups were placed in study sites 1–3 (seven per site). Each was filled with 50 ml of clean water and either left empty or stocked with an *R. poeppigii* tadpole. Because this treatment used noncannibalistic tadpoles, protective cages for the newly deposited *R. variabilis* larvae were not necessary here. Other procedures followed the experiments described previously.

Control

To control for any effect the fish food may have had on the chemical cues present in our tadpole-treated water, we placed 21 pairs of plastic cups in study sites 1–3 (seven per site). Each cup was filled with 50 ml of clean water, and in one cup 0.2 ml of powdered fish food was added. Cups were checked every other day for tadpole and egg depositions by parental frogs. Every fourth day both water and fish food were replaced to prevent the fish food rotting. Furthermore, the food was placed inside the cups for the same time span as in the experiments, with the only difference being that the food was not digested throughout the study.

Data Analysis

In all experiments, only single deposition events were recorded to ensure that the experimental treatment and not previous depositions had an influence on the deposition choice of parental frogs. Frequencies of egg and tadpole depositions in each artificial pool type were compared using a *G* test (Woolf 1957). This was favoured over the chi-square test as it is a more exact method for limited observations (Sachs 1974; Gotelli & Ellison 2004). Since we had fewer than 200 observations, we adjusted the *G* test in the manner of Williams (1976). As a null hypothesis, we assumed that the frequency of deposition events was random (0.5 in either pool). For comparisons of clutch and tadpole depositions within the same treatment, contingency tables were calculated and the null hypothesis that all distributions were the same was tested using the Fisher's exact test (Fisher 1922; Townend 2002). The same method was used to compare the results of the different treatments to each other. To test whether in control treatments decisions of frogs were influenced by the size of the tadpoles (i.e. both body and total lengths), a logistic regression (McFadden 1973) was used (covariates: size of dendrobatine tadpole inside the cage or the bufonid tadpole in the cup, size of the tadpole transported by the frog).

RESULTS

Chemical Cues of Cannibalistic Tadpoles

When we used water treated with cannibalistic tadpoles, 41 larval depositions by parent *R. variabilis* were recorded, 30 of which were in clean water indicating a significant avoidance of tadpole-treated water (*G* test: $G_1 = 9.040$, $P = 0.003$; Figs 2, 3a). Results for egg depositions were similar. Thirty-two of 34 clutches were placed in clean water versus water treated with cannibalistic larvae (*G* test: $G_1 = 31.459$, $P = 0.001$; Figs 2, 3b). The avoidance of water treated with cannibalistic larvae was even stronger for egg than for tadpole depositions (Fisher's exact test: $P = 0.029$).

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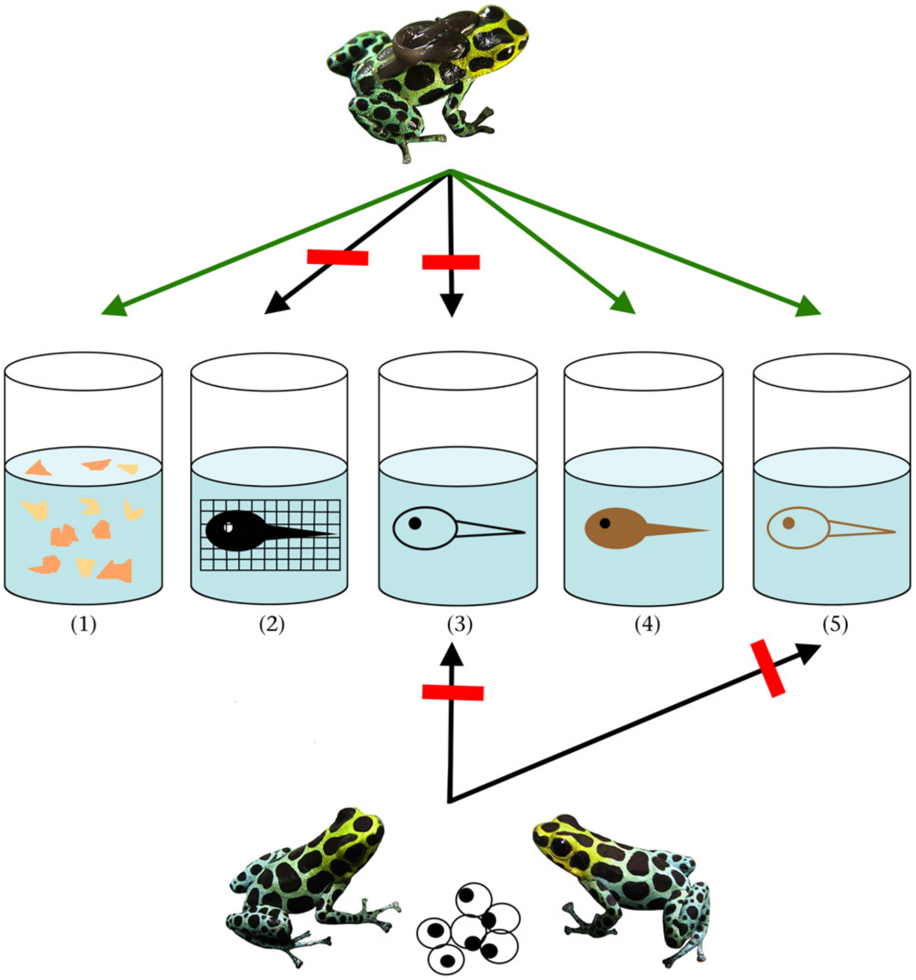

Figure 2. Pool choice experiments for tadpole and egg deposition in *Ranitomeya variabilis*. Each of the cups shown was paired with a cup containing clean water. Contents of the cups: (1) fish food, (2) cannibalistic tadpole in a cage, (3) chemical cues of cannibalistic tadpoles (cups wrapped in opaque barrier), (4) noncannibalistic tadpole present and (5) chemical cues of noncannibalistic tadpoles (cups wrapped in opaque barrier). The green arrows show what kind of cups were used by the frogs for tadpole (above) and egg depositions (below) besides the clean water, while the red-crossed arrows show cups avoided by the frogs.

Presence of Cannibalistic Tadpoles

When parent frogs could choose between clean water and water in which tadpoles were physically present, we saw a similar trend as in the experiment with only chemical cues of cannibalistic tadpoles. Of the 36 larvae deposited, 26 were deposited in clean water versus pools containing cannibalistic larvae (*G* test: $G_1 = 7.265$, $P = 0.007$; Fig. 2). There was no significant association between the avoidance of occupied cups and tadpole size (logistic regression, Wald test: total length: resident tadpoles: $T_{W1,35} = 0.285$, $P = 0.594$; transported

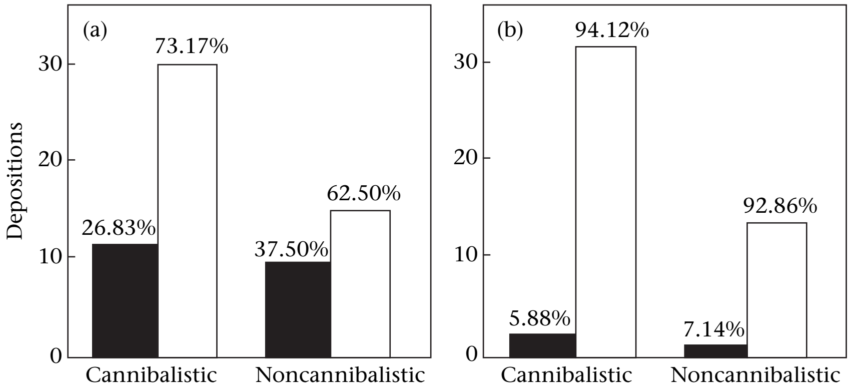

Figure 3. Number of (a) tadpoles and (b) clutches deposited in relation to presence of chemical cues of cannibalistic and noncannibalistic tadpoles. Percentage of depositions is given above each bar. Black bars: tadpole-treated water; white bars: clean water.

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tadpoles: $T_{W1,35} = 0.434$, $P = 0.510$; body length: resident tadpoles: $T_{W1,35} = 0.264$, $P = 0.608$; transported tadpoles: $T_{W1,35} = 0.626$, $P = 0.429$). The strength of preference for clean water was not significantly different between chemical-cue and visual-cue experiments (Fisher's exact test: $P = 1.000$). There were no clutches deposited in the trials with transparent cups: egg deposition preferences appear to favour small dark pools (Lötters et al. 2007; Brown et al. 2008a).

Chemical Cues of Noncannibalistic Tadpoles

Water treated with noncannibalistic tadpoles was not avoided for tadpole deposition, as only nine of 24 larvae were deposited in clean water (*G* test: $G_1 = 1.485$, $P = 0.223$; Figs 2, 3a). This result is significantly distinct from that of the experiment testing chemical cues of cannibalistic tadpoles (Fisher's exact test: $P = 0.008$). However, *R. variabilis* reacted differently with regard to oviposition preferences. Here, 13 of 14 clutches were deposited in clean water (*G* test: $G_1 = 11.782$, $P = 0.001$; Figs 2, 3b). These deposition preferences between tadpoles and egg clutches were significantly different (Fisher's exact test: $P = 0.002$). Egg deposition preferences between water treated with noncannibalistic versus cannibalistic tadpoles were not significantly different (Fisher's exact test: $P = 1.000$).

Presence of Noncannibalistic Tadpoles

There was no significant preference between empty pools and pools containing noncannibalistic tadpoles (23 of 39 larvae depositions in clean water; *G* test: $G_1 = 1.247$, $P = 0.264$; Fig. 2). A comparison of the two treatments with noncannibalistic tadpoles (tadpole present versus tadpole treated) similarly revealed that the preference strengths were not significantly different (Fisher's exact test: $P = 0.123$,). An influence of the body size of the tadpoles can be excluded here as well (logistic regression, Wald test: total length: resident tadpoles: $T_{W1,35} = 0.294$, $P = 0.588$; transported tadpoles: $T_{W1,35} = 0.322$, $P = 0.570$; body length: resident tadpoles: $T_{W1,35} = 0.401$, $P = 0.526$; transported tadpoles: $T_{W1,35} = 0.193$, $P = 0.660$).

Control

The fish food (Tetra) given to the tadpoles in the experiments did not have an influence on the decisions made by frogs. Of 43 *R. variabilis* tadpoles deposited, 22 were in clean water and 21 were in water containing fish food ($G_1 = 0.023$, $P = 0.880$; Fig. 2).

DISCUSSION

We have experimentally demonstrated that wild *R. variabilis* were able to recognize the presence or absence of anuran larvae in phytotelmata using chemical cues. Moreover, they reacted differently to cannibalistic versus noncannibalistic tadpoles. Even though we did not compare cannibalistic and noncannibalistic tadpoles directly, a clear difference between the reactions to the different tadpoles, each compared to clean water, could be shown. The presence of cannibalistic larvae is clearly disadvantageous for both egg and tadpole depositions. However, the presence of noncannibalistic larvae could be beneficial to tadpoles given that they represent a potential food source, although they are detrimental to eggs if the tadpoles are capable of consuming developing embryos. Because of the unambiguous deposition decisions, we can conclude that, in *R. variabilis*, chemical cues play a role in the identification and avoidance of predatory tadpoles in phytotelmata. Our findings strongly suggest that these frogs act by means of chemical cues and that chemical recognition of predators is more effective than identification by physical or visual cues because even hidden predators can be detected.

The mechanism of cue detection is not yet fully understood. One possibility is the use of the olfactory system. Dawley (1998) showed that the detection of chemosignals in anurans takes place primarily at the epithelium of the olfactory chamber and at the vomeronasal organ. Communication by olfaction has been suggested to be the most primitive sensory system in anuran amphibians (Waldman & Bishop 2004). Freitag et al. (1995) showed that anurans possess both fish-like receptor genes that allow them to detect soluble odorants and mammalian-like receptor genes for the detection of volatile odours. Examples of reactions to different chemical cues in anurans of different developmental stages are given in Table 1.

In our experimental trials, the influence of the diet of tadpoles can be excluded as all animals were fed with the same fish food. Because diet was fixed in our trials, the chemical signals triggering the frogs' decisions may have been uniquely produced by cannibalistic larvae. Another possibility is that frogs are able to detect familiar cues of conspecific larvae. With this strategy, frogs are able to protect their newly deposited larvae from competitors, as cannibalism in poison frogs is facultative behaviour evolved primarily as an emergency solution to eliminate potential competitors (Crump 1992) and to capitalize on already limited resources.

A possible effect of chemicals leaching from the plastic cups (or the rain barrel) into the water used for our experiments cannot be excluded and it would have been useful to test how the frogs react to the cups compared to real phytotelmata. However, these cups are commonly used as artificial phytotelmata and are generally well accepted among poison frogs (Poelman & Dicke 2007; Brown et al. 2008a, 2009a; Stynoski 2009), so such an effect may be negligible. The frogs on which we conducted our experiments have become used to polypropylene cups over several years (Brown et al. 2008a, 2009a). For that reason, they may be accustomed to possible cues of the plastic and therefore be able to discriminate between these and different cues. We can also exclude an effect of differential housing of cannibalistic and noncannibalistic tadpoles in the laboratory (singly or together). Chemical cues released because of a possible crowding effect (Glennemeier & Denver 2002; Gromko et al. 2005) would probably have a negative effect on the decision of the parents (telling them that the water is already full of tadpoles). A crowding effect caused by too many tadpoles in a small amount of water could only have occurred with the noncannibalistic tadpoles reared together in a tank. However, in contrast to the water of the singly reared cannibalistic tadpoles, the chemical cues released by noncannibalistic toad tadpoles were not avoided for tadpole deposition.

We have also made observations on a closely related species, *Ranitomeya imitator*, whose deposition decisions are similar to those we observed in *R. variabilis*. In the treatment containing chemical cues of cannibalistic larvae, six cups in total were used for tadpole deposition by parental *R. imitator*, and all avoided the tadpole-treated water ($G_1 = 7.678$, $P = 0.006$). There was no statistical difference between deposition decisions made by *R. imitator* and *R. variabilis* (Fisher's exact test: $P = 0.312$). Despite differences in reproductive strategies (*R. imitator* feeds its offspring with trophic eggs; Brown et al. 2008a, b) both *R. imitator* and *R. variabilis* make similar decisions when faced with the risk of tadpole cannibalism. This means that *R. imitator* can detect chemical cues of the congeneric species *R. variabilis*, which probably indicates a common ancestry for cue detection in this species. For that reason, we suspect that our observations of *R. variabilis* can probably be extrapolated to other species in the genus *Ranitomeya*, which consists of approximately 30 species (15 throughout the

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Table 1
Examples of the recognition and reaction to chemical cues in anurans in different species and developmental stages

Species	Stage, sex	Olfactory cues of	Reaction	Source
Bufo americanus	Tadpoles	Siblings	Preference	Waldman 1985
B. americanus	Tadpoles	Predator	Decreased activity, hiding	Gallie et al. 2001
Bufo boreas	Tadpoles	Predators	Decreased activity, hiding	Kiesecker et al. 1996
B. boreas	Tadpoles	Injured conspecifics	Increased activity, avoidance	Hews 1988
Bufo cognatus	Froglets	Conspecifics	Orientation towards cue	Graves et al. 1993
B. cognatus, Bufo microscaphus	Froglets	Predators	Avoidance	Flowers & Graves 1997
Bufo marinus	Tadpoles	Injured conspecifics	Decreased activity	Summey & Mathis 1998
Bufo valliceps	Adults	Own home range	Orientation towards cue	Grubb 1973, 1976
Dendrobates auratus	Males/females	Females/males	Preference of other sex	Korbeck & McRobert 2005
Hymenochirus sp.	Females	Males	Orientation towards cue	Pearl et al. 2000
Leiopelma hamiltoni	Adults	Faeces of different individuals	Preference of own faeces	Lee & Waldman 2002
L. hamiltoni	Adults	Different individuals	Preference of own mark	Waldman & Bishop 2004
Leiopelma paheha, Leiopelma hochstetteri	Adults and froglets	Conspecifics of different stages	Attraction to juveniles	Delaney & Bishop 2007
Leptodactylus fallax	Males	Males	Aggressive behaviour	King et al. 2005
Litoria splenditda	Females	Males	Orientation towards cue	Wabnitz et al. 1999, 2000
Oophaga hamiltoni	Adults	Own home range	Orientation towards cue	Forester & Wisnieski 1991
Pipa pipa	Males	Females	Agitation, calling	Rabb & Rabb 1963
Rana aurora	Tadpoles	Predators fed with tadpoles	Decreased activity	Wilson & Lefcort 1993
R. aurora	Tadpoles	Predator	Decreased activity, hiding	Kiesecker & Blaustein 1997
R. aurora	Tadpoles	Injured conspecifics	Decreased activity, hiding	Kiesecker et al. 1999
Rana cascadae	Tadpoles	Siblings	Preference	Blaustein & O'Hara 1982
Rana clamitans	Adults	Own home range	Orientation towards cue	Oldham 1967
Rana pipiens	Adults	Own home range	Orientation towards cue	Dole 1968
Rana sylvatica	Embryos, tadpoles	Predators	Decreased activity (after hatching)	Mathis et al. 2008
Scaphiopus multiplicatus	Tadpoles	Breeding water (+siblings)	Preference	Pfennig 1990
Xenopus laevis	Adults	Food	Hunting behaviour	Altner 1962

Amazon basin), many of which display similar reproductive behaviours. Other dendrobatines that develop in phytotelmata also have similar reproductive behaviours. Thus, it is likely that chemical recognition occurs in other species of dendrobatines, particularly ones that use small phytotelmata where the risk of competition and cannibalism is increased. Some *Oophaga* species recognize the general presence of tadpoles by a larval begging behaviour (see Introduction), and can detect their own offspring by remembering their physical location (Stynoski 2009). However, these behaviours of tadpoles and adults do not exclude the possibility that these species are also able to recognize tadpoles by chemical cues, as most studies do not exclude the possible concomitant use of visual and chemical cues (Brizzi & Corti 2007). Dendrobatines are strict *K*-strategists (Lötters et al. 2007) with a limited number of offspring and high costs of egg maintenance and larval development. Therefore, chemical detection in these species, in terms of phytotelmata inspection, is advantageous, as tadpoles already occupying phytotelmata can remain unrecognized when only visual inspections are carried out (Poelman & Dicke 2007; L. M. Schulte, personal observation). Failing to identify resident larvae would negate the generally reduced predation risk of phytotelmata use (Aspbury & Juliano 1998), as newly deposited eggs and tadpoles may be eaten by tadpoles already present in the pool (Summers 1990; Caldwell 1993; Wells 2007).

The use of small pools may also facilitate chemical recognition, as larvae may be easier to detect in small pools in which a chemical cue would be relatively concentrated. In this study, we demonstrated that *R. variabilis* has the capacity to avoid cannibalistic tadpoles using only chemical cues. The study adds to the growing understanding of parental care in poison frogs and, more generally, the functions of chemical cues in amphibians. It adds a unique component to the general knowledge of reproductive habitat selection in anurans, specifically those with advanced parental care of a well-studied group of poison frogs (Dendrobatinae). Our results raise questions regarding the nature of the chemical cues themselves. What compounds are involved and which species are able to produce them? Are species adapted only to recognize cues of sympatric species, or are cues generalized across close relatives? Furthermore, the mechanism of cue recognition is unknown. Answering these questions would represent a substantial advancement in our understanding of the evolution and utilization of chemical communication in amphibians.

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