Amazon poison frogs (Ranitomeya amazonica) use different phytotelm characteristics to determine their suitability for egg and tadpole deposition

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ORIGINAL PAPER

Amazon poison frogs (*Ranitomeya amazonica*) use different phytotelm characteristics to determine their suitability for egg and tadpole deposition

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Abstract Parents have to assess the multivariate characteristics of their reproductive sites to maximize their reproductive success through offspring performance. In addition, they may provide care to ensure optimal performance of their offspring. In poison frogs it has been identified that ecological characteristics of reproductive sites may underlie transitions in the involvement of parental sexes in care for offspring. To elucidate the ecological factors that may drive these transitions, it is important to understand which characteristics poison frogs use to assess the quality of their reproductive site. We studied the use of small water bodies in leaf axils of bromeliads, phytotelmata, for egg and tadpole deposition by Amazon poison frogs (*Ranitomeya amazonica*). We compared phytotelm quality characteristics for preferred egg and tadpole deposition sites and used two choice tests with plastic cups to study the causal relationship with tadpole deposition for the identified characteristics. The differences among quality characteristics of deposition sites were largest among bromeliad species, and for egg or tadpole deposition different bromeliad species were preferred. However, males were also selective in the leaf axils within a bromeliad species that they used for egg or tadpole deposition. Eggs were deposited in small, resource limited water bodies that were close to the forest floor. Tadpoles were deposited in leaf axils holding resource-rich phytotelmata with larger water volumes. Preference of detritus containing water over clear water in choice tests confirmed that Amazon poison frogs assess quality of their tadpole deposition sites on food availability. We conclude that preference for large water volume and resource rich phytotelmata plays an important role in determining male involvement in parental care and speculate that distribution of preferred resources may bring about selection on female involvement in parental care.

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Keywords Phytotelm quality · Poison frog · Parental care · Oviposition · Tadpole deposition

Introduction

Divergence in reproductive strategies of organisms has intrigued biologists for centuries. The most elaborate reproductive strategies are found in vertebrates which parents invest in the quality of few offspring by providing nourishment, protection or other types of parental care (Trivers 1972; Clutton-Brock 1991). What determines whether a parent is involved in providing care and what brings about transitions in the mode of parental care of a species are central questions in evolutionary ecology. The primary driving forces behind the involvement of one of the sexes in parental care are their initial investment in gamete size, the certainty of paternity, the chances of leaving offspring first (for example by the order of gamete release), and the balance between deserting and caring in terms of fitness gain by additional mating versus loss of offspring when leaving the current brood (Trivers 1972; Dawkins and Carlisle 1976; Clutton-Brock 1991). Although most of these characteristics and thus mode of parental care are strongly defined by the ancestral state of a species, ecological factors may drive transitions in modes of parental care by for example shaping the costs of deserting offspring. Although ecological factors are now considered capable of bringing about fast transitions of complex species traits (West-Eberhard 2003), few studies have provided empirical evidence of a causal relationship between an ecological parameter and a transition in a mode of parental care (but see Johnson et al. 2007 for an example).

How ecological parameters may drive reproductive decisions is well studied in insects that use distinct resources for the development of their offspring (Mayhew 1997). Many insect species desert their offspring upon laying eggs and confine their offspring to a food source such as a single fruit in fruit flies (Mock and Parker 1997), a caterpillar host in parasitic wasps (Godfray 1994; Harvey et al. 2013) or a small body of water in a tree hole for damselflies (Fincke 1994). The success of development of offspring in such distinct resources depends on many ecological factors such as the quantity, quality and stability of the food source as well as the risk of predation and competition (Fincke 1994; Lehtinen 2004; Refsnider and Janzen 2010; Harvey et al. 2013). This implies that mothers need to assess the multivariate quality characteristics of a distinct resource and adapt their oviposition strategy to this information. Factors shaping oviposition decisions may be hierarchical in their effect size, for example predator avoidance may be prioritized above avoidance of competition or preference for pool size. The hierarchy may depend on the variation in the quality characteristic a female can choose from and the associated variation in reproductive success for a female making her choice based on the quality characteristic (Crump 1991). Each of these ecological factors could potentially drive a shift in reproductive decisions that may include decisions on parental investment in single offspring (Ekbom and Popov 2004).

Interestingly, Neotropical poison frogs (Dendrobatoidea) have both the use of distinct resources and parental care combined in their reproductive biology, offering a system in which ecological parameters can be studied for their effect on transition of modes of parental care. Some clades of poison frogs use small water bodies in leaf axils or tree holes, i.e. phytotelmata (Kitching 2000), as rearing sites for their tadpoles (Summers and McKeon 2004). These phytotelmata are distinct resources that may vary in water volume, nutrient composition, stability and risk of competition or predation (Lehtinen 2004). Phytotelm volumes may typically be in the range of a few milliliters to less than 100 ml (Kitching 2000). A derived state of parental care in poison frogs is that males care for the egg clutch and transport tadpoles on their back to deposit them individually in phytotelmata (Summers et al. 1999). Similar to larvae of solitary parasitic wasps that develop individually inside caterpillars and kill other parasitoid larvae that share the caterpillar (Godfray 1994; Harvey et al. 2013), the tadpoles are cannibalistic and a single phytotelm can yield only a single offspring at a time (Summers and Amos 1997; Summers and Symula 2001; Poelman and Dicke 2007). Males have been shown to be discriminative for different characteristics of the phytotelm such as preference for a certain volume, height of the phytotelm above the forest floor or avoidance of competition and predation (Downie et al. 2001; Brown et al. 2008a, b; von May et al. 2009a; Ryan and Barry 2011). Interestingly, female involvement in parental care has evolved in parallel to the use of smaller more resource limited phytotelmata (Brown et al. 2010). In several lineages this has resulted in species that exhibit bi-parental care or uni-parental female care in which females provision their offspring with nutritive eggs (Weygoldt 1980; Caldwell 1997; Caldwell and de Oliveira 1999; Summers et al. 1999; Pramuk and Hiler 1999). Although water volume may correlate with the mode of parental care, little is known about the characteristics of phytotelmata that poison frogs use to assess the quality of the pool and that determines their type of care. Some studies have reported on the water volume preferences and avoidance of cannibalistic conspecific tadpoles in separate tests (Schulte et al. 2011), few studies have evaluated which of these characteristics most likely explains reproductive site choice by poison frogs. Moreover, most comparisons have been made across bromeliad species, not looking at preference for phytotelmata within single bromeliad species and thereby cannot rule out that poison frogs choose for reproduction in certain bromeliad species rather than choosing for a certain qualitative trait.

We studied phytotelm use for oviposition and tadpole deposition by Amazon poison frogs (*Ranitomeya amazonica*) under rainy season conditions. We compared phytotelm quality characteristics for preferred egg and tadpole deposition sites. We specifically addressed whether preference for phytotelmata by *R. amazonica* differs for egg and tadpole deposition, and what are the underlying characteristics of the preferred phytotelmata in each context. For deposition of eggs, we hypothesized no preference for nutrients and water volume since egg development takes on average only 14 days and needs a moist environment. Since tadpoles of *R. amazonica* require about 3 months of development before the metamorphs can leave the phytotelm they were deposited in (Poelman and Dicke 2007), we hypothesized that tadpoles would be deposited in phytotelmata that have relatively large water volumes that are less likely to desiccate. Furthermore, these pools should offer tadpoles a stable food source for that length of time and therefore should be rich in detritus and have a high algal growth. The characteristics that were found to correlate with deposition preferences were studied in two-choice tests for their causal relationship. We discuss the implications of differences in egg and tadpole deposition sites for the reproductive ecology and evolution of parental care in poison frogs.

Materials and methods

Study sites

The field study was conducted during two field seasons (10 April–27 July 2002 and 2 April–28 April 2008) at three sites in French Guiana. The weather conditions at these sites are characterized by seasonal rainfall with a dry period from the end of July until mid-November (Grimaldi and Riéra 2001). Two of the study sites are located in the Nouragues

Nature Reserve (4°05N, 52°41W). The first and main study site, "Inselberg", is situated on top of a granite outcrop 411 m above sea level. The patches of forest, of up to a few hundred square meters in size are dominated by trees of the genus *Clusia* and are separated by bare granite rock. The bromeliad community at this site is dominated by the epiphytic *Catopsis berteroniana* and the two terrestrial bromeliads, *Pitcairnia geykessi* and *Aechmea aquilega* (see Sarthou 2001 for a detailed description of the study area). The second study site "Campsite" was situated near the campsite of Nouragues. Here a semi-natural habitat is formed by patches of diverse bromeliad species that have been collected for research purpose and attached to trees around the campsite (see Poelman and Dicke (2008) for the genera of bromeliads found here). The third study site "Emerald Jungle Village" was located in the primary forest behind the lodge at Emerald Jungle Village (4°48N, 52°22W). Here a semi-natural habitat is formed by a large private collection of bromeliads. The two semi-natural sites were only used for experiments on tadpole deposition preference of *R. amazonica* based on two-choice tests with plastic cups.

Study species

Amazon poison frogs, *R. amazonica* (Brown et al. 2011) [*Ranitomeya ventrimaculata* sensu Grant et al. 2006 and *Dendrobates ventrimaculatus* sensu Shreve 1935, the latter name we used in former reports on the populations studied here (Poelman and Dicke 2007, 2008)] (Fig. 1), belong to a complex of closely related species that can be found from the Guyana Shield through the Amazon basin of Brazil into the East Andean lowlands of Colombia, Ecuador, and Peru (Brown et al. 2011). Males are territorial and both males and females can be found around bromeliads or other plants that hold phytotelmata in their leaf axils (*Heliconia* and aroids) (Poelman and Dicke 2008). The clutch of 1 up to 6 eggs is laid on the edge of the phytotelm partly submerged below the water line. Here we refer to egg laying in a phytotelm as oviposition. Tadpoles that hatch are transported one or two at the time on the back of the male and are deposited in other phytotelmata (Bechter and Lescure 1982; Summers and Amos 1997; Poelman and Dicke 2007). Here the tadpoles feed on detritus, algae and cannibalize on conspecific eggs or tadpoles. Metamorphosis takes place after 3 months (Poelman and Dicke 2007). None of the six other species of Dendrobatoidae present in French Guiana, *Dendrobates tinctorius*, *Ameerega hahneli*, *Allobates femoralis*, *Allobates granti*, *Anomaloglossus baeobatrachus*, and *Anomaloglossus degranvillei* (Born and Gaucher 2001; Kok et al. 2006), use similar phytotelmata as *R. amazonica*.

Assessing phytotelm quality

To determine whether *R. amazonica* prefers certain bromeliad species for reproduction, we counted the number of bromeliads and their number of phytotelmata for the most common bromeliad species (*C. berteroniana*, *A. aquilega*, *P. geykessi*) on nine forest patches located on the natural study site "Inselberg". Over the study period in 2002, the nine patches of a 100 m^2 each were visited in series of three consecutive days followed by a day without observations. We monitored all leaf axils for the presence of eggs or tadpoles and noted the bromeliad species for each encounter.

In 2008 at Inselberg, we studied which qualitative characteristics make phytotelmata or the leaf axils that hold them preferred for oviposition or tadpole deposition. To determine the characteristics of an average leaf axil and its phytotelm of *C. berteroniana* and *A. aquilega*, we non-selectively sampled a single leaf axil, in which no eggs or tadpoles were found, of fourty bromeliads for each of the two bromeliad species. We also searched

Fig. 1 A male *R. amazonica* at Inselberg in the Nouragues nature reserve

for leaf axils of *C. berteroniana* and *A. aquilega* that contained eggs or tadpoles by visually inspecting phytotelmata. For each of the leaf axils that had received oviposition, tadpole deposition and those that were non-selectively sampled and not in use for reproduction we measured 17 characteristics (Fig. 2): the bromeliad species (1), the height of the bromeliad above the forest floor (2), the geographic orientation of the leaf axil (3), the number of leaf rosettes of the bromeliad (4), the leaf rosette the axil is part of (5), the width of the leaf axil (6), the depth of the leaf axil (7), the water volume of the phytotelm (8). The water quality of the phytotelm was measured for its temperature (9), pH (10), K^+ in mol/m^3 (11), NO_3^-–NO_2^- in mg/l (12) and phosphate PO_4^{3-} in mg/l (13). The phytotelm was examined for the presence and amount of detritus (14) that we scored on a scale of 0–5 and the presence of algal growth (15) that we scored on a scale of 0–3. On both scales a 0 means absence of the characteristic (detritus or algal growth). For detritus we defined 1–5 by rating how murky the water of the phytotlem was after the water was extracted from the leaf axil and gently shaken in a plastic vial. We rated each water sample for transparency [1 = 20 %, 2 = 40 %, 3 = 60 %, 4 = 80 % with a maximum of 5 (100 % murky, non-transparent)]. Algal growth was determined by visually inspecting the leaf axil for presence of algae. Leaf axils that were covered for up to 1/3 with algae were scored as 1, 2/3 as 2 and fully covered scored as 3. Furthermore, we counted the number of insect larvae (16) and tadpoles of other species (17) present in the phytotelm. Height of bromeliads above the forest floor was measured to the nearest centimeter using a tape measure and all other size measurements were taken with dial calipers to the nearest millimeter. The leaf rosettes were counted as estimate of bromeliad size by counting from the center of the plant, increasing number to the outer rosettes (Fig. 2). The position of the measured leaf axil within the bromeliad was calculated as the rosette number it was part of divided by the total number of rosettes of that bromeliad. The geographic orientation of the leaf axil was assessed using the degrees derived from the north as pointed by a compass that was held above the bromeliad. The geographic orientation was computed into eight segments of the 360° circle (Fig. 2). Position and geographic orientation of the leaf axils were assessed because we hypothesized that bromeliads would be heterogeneous in their axil quality, and that across individual bromeliads the distribution of leaf axil quality may be similar when caused by the amount of sun light or exposure to wind. Water volume was assessed by extracting the water from the leaf axil with a 50-ml syringe. Water temperature was measured using a digital thermometer (Checktemp 1 Pocket Thermometer, Hanna Instruments BV). We used indicator tests for pH (Duotest pH 3.5–6.8 and 7.0–10.0, Macherey–Nagel), K^+ (Aquadur 0–4.5 mol/m^3, Macherey–Nagel), NO_3^-–NO_2^- (Quantofix Nitrate 10–500 mg/l Nitrite 1–80 mg/l, Macherey–Nagel) and PO_4^{3-} (Quantofix Phosphat(e) 3–100 mg/l, Macherey–Nagel).

Two choice experiments

We experimentally tested whether water volume or presence of detritus affected tadpole deposition preference of *R. amazonica*. At the start of our field season in 2008, we attached two-choice tests consisting of a pair of plastic coffee cups to bromeliads at the three study sites. The plastic cups were exposed to natural tadpole deposition by Amazon poison frogs for 3 weeks and were monitored weekly for the presence of tadpoles. We tested whether water volume affected tadpole deposition by offering a cup with 10 ml paired to a cup containing 50 ml. Whether detritus of leaf litter affected tadpole deposition, was tested with paired cups of similar water volume (50 ml). One cup contained collected rain water only, the other cup contained water with small pieces of leaf litter with a summed size of 4 cm^2. Water levels were kept at their volume by puncturing a hole in the plastic cup that functioned as drain for excessive water and the daily rainfall kept water levels at the maximum holding capacity of the drained cups. At each study site 20 paired cups of each of the two tests were offered with a minimum distance of 10 m between pairs of cups.

Statistical analyses

To test whether certain bromeliad species were preferred by *R. amazonica* for egg or tadpole deposition, we counted the number of egg and tadpole depositions found in the three bromeliad species. We used χ^2 tests to test whether the observed distribution differed from an expected distribution of the availability of phytotelmata of the three bromeliads in the study site as assessed by counting. Differences in the multivariate characteristics of the two bromeliad species, and differences among non-selectively sampled leaf axils and those used by *R. amazonica* for egg or tadpole deposition were analyzed using redundancy analysis (RDA). We used this constrained multivariate ordination analysis to seek the leaf axil characteristics that best predicted the difference among bromeliads, or use for reproduction by *R. amazonica*. Characteristics were evaluated for their significance using forward selection and Monte Carlo Permutation tests (999 tests). To test whether eggs were deposited more frequently in a specific geographic orientation of leaf axils we used a Mann–Whitney U test, because most of the eight segments received fewer than 5 ovipositions so that our data did not meet the test requirements of χ^2. We compared the scores on

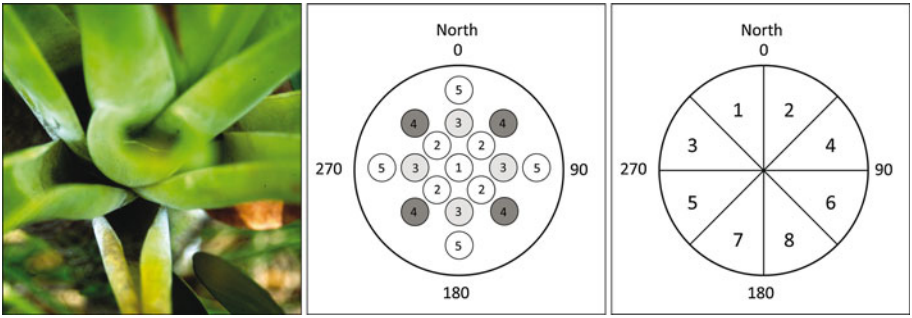

Fig. 2 *Top* view of a *C. berteroniana* bromeliad showing its leaf axils that hold phytotelmata and two panels showing the methodological numbering of the position of a leaf axil. The *middle panel* depicts the rosette numbering to indicate the position of the leaf axil towards the central rosette of the bromeliad. The *right panel* depicts the geographic orientation of the leaf axil computed from degrees into eight segments of the 360° *circle*

the geographic eight segments of the bromeliad among leaf axils that had received oviposition with those that were randomly selected and were not in use as offspring rearing site by *R. amazonica.* We used a binomial test to analyze the two-choice tests with paired plastic cups. RDA analysis was performed with CANOCO for Windows 4.5, all other tests were performed in IBM SPSS Statistics 19.

Results

In the nine study plots on Inselberg, the bromeliad *P. geykessi* was the most abundant over *C. berteroniana* and the uncommon *A. aquilega*. The leaf axils of *P. geykessi* often contained very small to no phytotelmata and we never found eggs or tadpoles deposited in *P. geykessi* leaf axils (Fig. 3). During the 4 months of study in 2002, we found 137 clutches of eggs that were almost exclusively deposited in leaf axils of *C. berteroniana* that received more oviposition events than expected by the relative abundance of these leaf axils on the study plots ($X^2 = 10425.9$, $P < 0.001$) (Fig. 3). Of the 106 tadpoles found, half of them were deposited in phytotlemata of *C. berteroinana* and the other half in *A. aquilega*. Even though *A. aquilega* was absent from many of the study plots and most male territories (Poelman and Dicke 2008), tadpoles were more frequently deposited in phytotelmata of *A. aquilega* than expected from the low availability of these tadpole deposition sites ($X^2 = 10490.4$, $P < 0.001$) (Fig. 3).

The two bromeliad species differed widely in their characteristics as identified with RDA (first PC explained 78 %, Fig. 4a). *A. aquilega* is a terrestrial species that has on average 7 leaf rosettes with over 30 leaf axils per bromeliad. These leaf axils are deeper, wider and contain eight times as much water compared to the smaller *C. berteroniana* (80 and 10 ml respectively) (Table 1; Fig. 4a). *C. berteroniana* is an epiphytic bromeliad that can be found close to the forest floor up to 2 m high in the *Clusia* trees. It has 4–5 leaf rosettes holding about 18 small phytotelmata. The water temperature and composition of K^+, NO_3^-–NO_2^- and PO_4^{3-} was similar for both species. Phytotelmata of *A. aquilega* contained more detritus, but fewer mosquito or other insect larvae than phytotelmata of *C. berteroniana*.

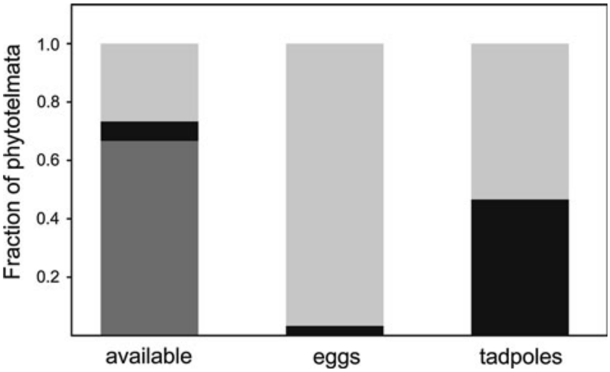

Fig. 3 Distribution of eggs and tadpoles over phytotelmata of three bromeliad species, *P. geykessi* (*dark grey*), *A. aquilega* (*black*), *C. berteroniana* (*light grey*), compared to the availability of these phytotelmata in the study area

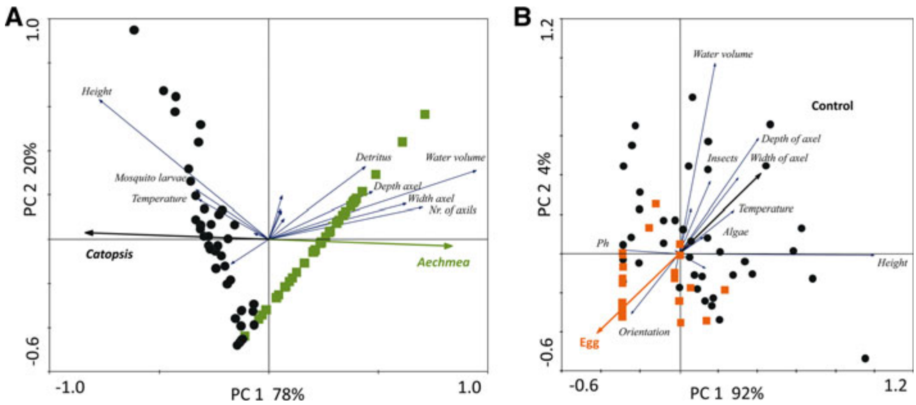

Fig. 4 RDA plots of leaf axel characteristics that differ among bromeliads (**a**) and empty control versus phytotelmata used for egg deposition in *C. berteroniana* (**b**). **a** Individual points represent single leaf axils and their phytotelm measured for 16 parameters of random axils of *Catopsis* (*black circles*) and *Aechmea* (*green squares*). **b** Individual points represent leaf axils of *Catopsis* that were randomly selected and not used for egg deposition (*black circles*) or leaf axils in which eggs were found (*orange squares*). *Blue arrows* in both plots indicate the direction of increase in value of a parameter and parameters that significantly correlated with one of the PC axis are identified with their name

In 2008, we found 24 leaf axils of *C. berteroniana* that contained eggs of *R. amazonica* and 7 that contained a freshly hatched tadpole. As identified from significant correlation of the bromeliad characteristic in RDA comparison among leaf axils that received eggs and non-selectively sampled leaf axils, leaf axils that had received oviposition were smaller and had lower water volume than the average leaf axil of a *C. berteroniana* plant (first PC explained 92 %, Fig. 4b). These leaf axils were generally in the outer rosettes of bromeliads that were growing close to the forest floor. Moreover, egg clutches were found to be deposited more frequently in leaf axils that were directed to the geographic south than our random set of leaf axils of *C. berteroniana* (Mann–Whitney U-test, $Z = -2.546$, $P = 0.011$) (Fig. 5). In RDA of a comparison among random leaf axils and those of *A. aquilega* that had received tadpole deposition none of the leaf axil characteristics correlated significantly with the Principle Components (PC).

Because *A. aquilega* was preferentially used for deposition of tadpoles and phytotelmata of this bromeliad are typically holding larger phytotelmata that are richer of detritus than leaf axils of *C. berteroniana*, we studied these two characteristics in tadpole deposition preference tests. The paired cups consisting of one holding a small water volume (10 ml) and one with a large water volume (50 ml) received only five tadpole depositions that were divided with 2 and 3 over the treatments. These observations were too few for statistical analysis, and give no indication on a preference for water volume of phytotelmata by *R. amazonica*. The pairs of cups that had similar water volume but to which detritus was added in one cup received 14 tadpoles, summed for the three study sites. *R. amazonica* males preferentially deposited their tadpoles in cups that had received detritus (Binomial test, $P = 0.013$) (Fig. 6).

Discussion

Amazon poison frogs use different characteristics of leaf axils to assess their suitability as deposition site for eggs or tadpoles. The differences among quality characteristics of

Table 1 Measurements of leaf axil and phytotelm characteristics of control axils and those that had received egg or tadpole deposition by *R. amazonica* in two bromeliad species, *C. berteroniana* and *A. aquilega*

Leaf axil characteristic	*C. berteroniana*			*A. aquilega*[e]	
	Control (n = 40)	Egg (n = 24)	Tadpole (n = 7)	Control (n = 40)	Tadpole (n = 17)
Height above forest floor (cm)	**45.58 (36.09)**	**16.58 (20.34)**	**13.00 (23.13)**	**0 (0.00)**	**0 (0.00)**
Geographic orientation[a]	**4.25 (2.01)**	**5.71 (2.44)**	**6.14 (2.10)**	**4.45 (2.24)**	**4.41 (2.14)**
Nr. of leaf rosettes	**4.55 (0.71)**	**4.38 (0.82)**	**4.14 (0.69)**	**6.75 (1.01)**	**8.00 (1.12)**
Leaf rosette number[b]	0.66 (0.19)	0.90 (0.13)	0.84 (018)	0.65 (0.19)	0.56 (0.24)
Width (cm)	**2.83 (0.64)**	**2.50 (0.46)**	**2.66 (0.49)**	**5.29 (1.76)**	**5.39 (1.21)**
Depth (cm)	**9.52 (2.88)**	**5.95 (2.95)**	**7.19 (3.96)**	**14.03 (5.72)**	**16.52 (5.42)**
Water volume (ml)	**10.63 (6.57)**	**4.93 (3.50)**	**6.43 (3.55)**	**81.38 (35.68)**	**85.65 (40.35)**
Water temperature (°C)	26.60 (0.88)	25.74 (0.90)	25.57 (1.09)	23.99 (0.70)	24.45 (0.58)
Water Ph	5.42 (0.21)	5.48 (0.28)	5.51 (0.29)	5.41 (0.18)	5.37 (0.13)
K^+ (mol/m^3)	<0.5 (0.00)	<0.5 (0.00)	<0.5 (0.00)	<0.5 (0.00)	<0.5 (0.00)
NO_3^-–NO_2^- (mg/l)	50–10 (0.00)	50–10 (0.00)	50–10 (0.00)	50–10 (0.00)	50–10 (0.00)
PO_4^{3-} (mg/l)	2.10 (2.71)	0.67 (1.69)	0.43 (1.13)	3.83 (3.46)	3.71 (2.71)
Amount of detritus[c]	**1.20 (0.85)**	**1.54 (0.83)**	**1.29 (0.95)**	**2.13 (1.28)**	**2.94 (0.90)**
Presence of algae[d]	0.30 (0.69)	0.17 (0.48)	0.14 (0.38)	0.35 (0.77)	1.00 (0.79)
Nr. Of insect larvae	0.40 (0.55)	0.17 (0.38)	0.14 (0.38)	0.03 (0.16)	0.00 (0.00)
Nr. of other tadpoles	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.12 (0.33)	0.00 (0.00)

Bold face type indicate axil characteristics that explained variation in the RDA analyses between bromeliad species or axils used for reproduction

[a] Computed compartment number from circular degree of geographic orientation (Fig. 1)

[b] Number of rosette of measured leaf axil divided by the total number of rosettes in the bromeliad (Fig. 1)

[c] Scored visually on a scale of 0–5

[d] Scored visually on a scale of 0–3

[e] Only a single egg clutch was found in a waterless leaf axil of *A. aquilega*

deposition sites were strongest among bromeliad species largely partitioning egg and tadpole deposition over bromeliad species. However, males were also selective about the leaf axils within a bromeliad species that they used for egg or tadpole deposition. Eggs were deposited in small, resource limited water bodies that were close to the forest floor. Tadpoles were deposited in leaf axils holding phytotelmata that are resource rich and have larger water volume.

Only few poison frog species use phytotelmata for both egg and tadpole deposition. Most of the other poison frogs lay their eggs in leaf litter on the forest floor and eggs are kept moist by one of the parents (Summers et al. 1999). Terrestrial egg deposition has been hypothesized to be an escape to the many aquatic predators as compared to a lower predation risk of eggs on land (Wells 1981). The use of the smallest phytotelmata for egg deposition by *R. amazonica* in our study and avoidance of insect larvae, may suggest that also this species selects egg deposition sites that have low predation risk. Since eggs are partly submerged below the water line in phytotelmata, significant mortality may be caused by aquatic predators that include conspecific tadpoles (Summers and Amos 1997;

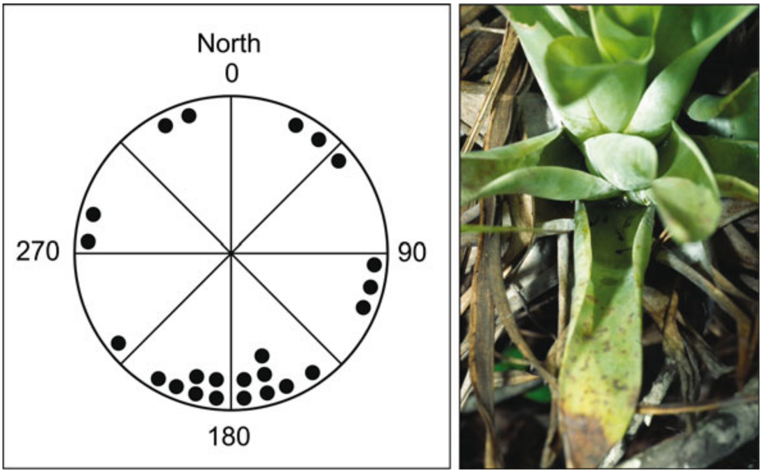

Fig. 5 Orientation distribution of *Catopsis* leaf axils that were used for egg deposition. Each *black dot* represents a single case of egg deposition and its position corresponds with the degrees derived from the north on a 360° *circle* as well as the eight segments in which the degree values were computed for statistical analysis

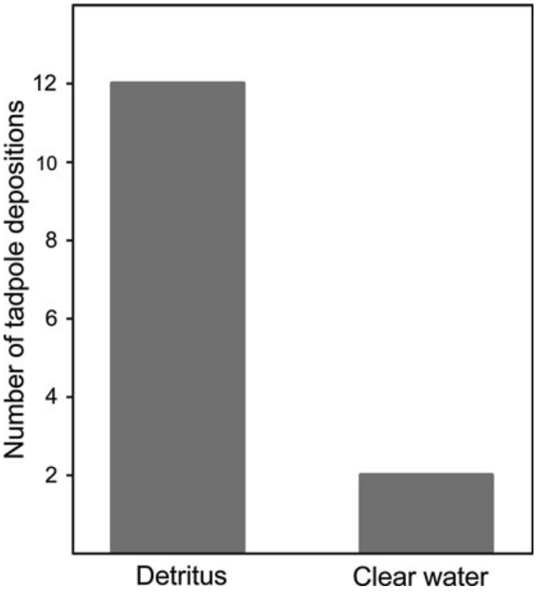

Fig. 6 Number of tadpoles deposited in plastic cups containing 50 ml of clear water or water with detritus that were offered in pair wise attached to bromeliads in the field. Cups with detritus received significantly more tadpoles than cups holding water only (Binomial test $P = 0.013$)

Poelman and Dicke 2007). The presence of conspecific tadpoles in many of the phytotelmata of the terrestrial bromeliad *A. aquilega* may for the same reason explain why these leaf axils were not used for oviposition. In contrast to a preference for egg deposition in bromeliads on the forest floor by *R. amazonica*, the sister species *Ranitomeya variabilis* was found to select phytotelmata that were located 2 m above the forest floor. Schulte et al. (2010) hypothesized that this may be a strategy to avoid the many predators on the forest floor. Since eggs of *R. variabilis* are deposited above the water line of the phytotelm

(Brown et al. 2008a, b), terrestrial predation may be selecting stronger on oviposition site selection than aquatic predators in this species. Interestingly, we found eggs to be deposited primarily in leaf axils that were directed towards the geographic south. Although these leaf axils were also containing a smaller volume of the phytotelm than the average leaf axil in *C. berteroniana*, we noted that this is confounded with a more horizontal position of the leaf axil. Most of the *C. berteroniana* bromeliads were tilted so that their southern half was more horizontal and leaves on the northern half were standing more vertically. We speculate that the choice for phytotelmata on the southern half of the bromeliad is due to the preference of horizontal surfaces for oviposition and courtship. Space requirements for egg deposition were found to explain oviposition preference in *R. sirensis* (*R. biolat* in von May et al. 2009b) that uses bamboo internodes as egg deposition site. Here eggs were deposited in internodes that provided sufficient space above the water line of the phytotelm to attach eggs to the bamboo so that they would not run the risk of sliding into the phytotelm (von May et al. 2009b).

In contrast to eggs, tadpoles were found in larger water bodies that were rich in detritus on which the tadpoles feed. Phytotelmata of *A. aquilega* were offering larger water volume and contained higher abundance of detritus than phytotelmata in *C. berteroniana* and therefore *A. aquilega* may have been preferred for tadpole deposition. Although phytotelm volume and detritus abundance were confounding factors explaining the tadpole deposition preference in *A. aquilega*, our choice tests revealed that *R. amazonica* males select phytotelmata on the abundance of detritus, i.e. select on phytotelmata that offer food to their offspring (compare von May 2009b). Nevertheless, water volume may still co-account for preference of tadpole deposition in *A. aquilega*, since our choice test for different water volumes received too few depositions to rule out this parameter. Before releasing the tadpole off their back, males were observed to repeatedly dive into the phytotelm. Potentially, males may visually explore the phytotelm for presence of predators or food, but may also use taste or smell to assess the quality of the phytotlem (Schulte et al. 2011). Water exposed to conspecific tadpoles was found to be avoided for egg and tadpole deposition by *R. variabilis*, confirming that chemical cues in water may be used to assess phytotelm quality (Schulte et al. 2011, Schulte and Lötters 2013). The use of chemical cues to assess the quality of reproductive sites is well known for insects. For example, several aquatic insects use both odours and contact with water to assess presence of conspecifics, predators or nutrients (Fincke 1994; Silberbush and Blaustein 2008). Also terrestrial insects rely on both odour profiles and taste of their food plant to assess the content of for example defensive chemistry of the plant and therefore suitability as food plant for their offspring (Mayhew 1997; Schoonhoven et al. 2005).

As in insects there is only a single phase in which females decide to which reproductive site her offspring will be confined by oviposition, parental care in poison frogs includes relocation of tadpoles after they hatch from their eggs and offers parents an opportunity to partition eggs and tadpoles over reproductive sites. Here we show that parents have distinct preferences for egg and tadpole deposition sites and can make this distinction because of tadpole transport on the back of the parent. However, preferred tadpole sites were absent from many of the male territories in our study. Males were observed to visit these preferred tadpole deposition sites in territories of other males (Summers and Amos 1997; Poelman and Dicke 2008). This behaviour has resulted in an array of reproductive strategies that infer costs to the reproductive success of other males and may result in sexual conflicts among parents of the tadpole. Tadpole carrying males that intrude other territories, may have the opportunity to deposit their tadpoles in phytotelmata containing eggs sired by an unrelated male (Brown et al. 2009). Deposition of tadpoles in egg containing phytotelmata may be beneficial for the male siring the tadpole, as the eggs provide the tadpoles with a high quality food source. Males that pirate on reproductive sites of other males, reduce the reproductive success of other breeding pairs (Summers and Amos 1997; Poelman and Dicke 2007; Brown et al. 2009). Such deposition strategies may however also result in sexual conflict when males deposit tadpoles in phytotelmata that contain eggs or tadpoles they sire, but are offspring of another female (Poelman and Dicke 2007; Schulte and Lötters 2013). These food provisioning strategies may be beneficial to females when some of her eggs or tadpoles are provided to her own offspring and enhance survival chances of the provisioned tadpole (Poelman and Dicke 2007; Schulte and Lötters 2013). To ensure provisioning only to her own offspring, females may be selected for involvement in parental care.

In addition to the ecological parameter of reproductive sites such as water volume or food availability that may directly drive transitions in parental care (Brown et al. 2010), the distribution of reproductive sites with preferred quality characteristics may indirectly contribute to selection on parental care by sexual conflicts involved in reproductive strategies to exploit reproductive sites. Such transitions may be enhanced by co-existence of several species of poison frogs that use phytotelmata for reproduction and in which at least one of the species has predatory tadpoles. To avoid predation by other poison frog tadpoles, poison frog species with less competitive larvae may colonize (smaller) phytotelmata that are not used for tadpole deposition by species with predaceous tadpoles, i.e. niche partitioning (Brown et al. 2008a, b; Ryan and Barry 2011). Selection of smaller phytotelmata to avoid predation, coinsides with reduced resource availability and potentially increased risk of desiccation (Summers and McKeon 2004). Through plastic reproductive strategies that include provisioning of offspring with either eggs (Poelman and Dicke 2007) or young tadpoles (Schulte and Lötters 2013), for example driven by seasonal changes is phytotelm stability, females may have been selected for involvement in parental care. This dynamic interplay between ecology and behavioral plasticity may have driven transitions in the mode of parental care by poison frogs and explain the association of species with female involvement in parental care with small phytotelmata as reproductive sites. Simple ecological parameters may therefore drive evolution of parental care (Brown et al. 2010) and these insights from studies on poison frogs should encourage to explore potential ecological parameters that underlie evolution of parental care in other organisms.

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