Discrimination of offspring by indirect recognition in an egg-feeding dendrobatid frog, *Oophaga pumilio*

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Offspring discrimination, the differential treatment of offspring and unrelated young, functions in numerous animal taxa to ensure that vital and costly parental care behaviours are appropriately directed. Discrimination can be facilitated either by direct (phenotypic) recognition of offspring or by indirect (nonphenotypic) recognition of offspring location. Offspring discrimination and recognition mechanisms have not been identified in an amphibian. In the strawberry poison frog, *Oophaga pumilio*, a dendrobatid frog with obligatory maternal provisioning behaviour, I tested whether mothers discriminate between offspring and unrelated young, whether they use direct or indirect recognition cues, and whether prior parental investment plays a contextual role in the differential treatment of young. Mother frogs utilized tadpole-rearing cups attached to tree trunks in wet tropical forest. After manipulating the identity, location and/or age of tadpoles in cups, I determined whether maternal provisioning behaviour was maintained by measuring tadpole growth and development. Mothers provisioned young regardless of tadpole identity, but were sensitive to location and did not provision tadpoles that were moved 2 cm to an adjacent cup. When given a choice between related and unrelated tadpoles in originally chosen or adjacent cups, mothers discriminated by location, but not by relatedness. Maternal provisioning behaviour persisted when a tadpole provisioned for 10 days was replaced with either an age-matched or newly hatched unrelated tadpole, so direct offspring recognition does not appear to be dependent on prior parental investment. Together, these results provide strong evidence that mother *O. pumilio* use indirect recognition cues to discriminate between offspring and unrelated offspring.

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Neff 2003; Chiu & Kam 2006), or depend on temporal cues such as in several rodent species, which cease infanticidal behaviours while nursing (Elwood 1991). Both direct and indirect mechanisms can signify relatedness (Hamilton 1964), and thus both can mediate the differential treatment of young.

Kin discrimination and recognition mechanisms are known to occur in amphibian species (Blaustein & Waldman 1992), but have not been demonstrated in the context of posthatching parental care (Gibbons et al. 2003). Amphibian discrimination and recognition have been examined almost exclusively in the contexts of sibling grouping of tadpoles (Blaustein & Waldman 1992), cannibalism of larval nonsiblings and first cousins (Pfennig et al. 1993, 1994), and cannibalism of unrelated young over offspring (Forester et al. 1983; Gabor 1996; Peterson 2000; Gibbons et al. 2003). Poelman & Dicke (2007) examined but did not find evidence of an ability to discriminate offspring from unrelated young in a poison frog, *Dendrobates ventrimaculatus*. However, another poison frog, *Oophaga pumilio* (formerly *Dendrobates pumilio*; Grant et al. 2006) may be more likely than *D. ventrimaculatus* to discriminate between offspring and unrelated young because it shows more extreme parental investment that includes provisioning of offspring (Brust 1993). In addition, male *D. ventrimaculatus* defend the territories in which they rear their tadpoles (Poelman & Dicke 2007), whereas female *O. pumilio* rear tadpoles in a social environment with a greater overlap of home ranges and they compete with other females for the same tadpole-rearing sites (Prohl & Hoedl 1999; Haase & Prohl 2002). For these reasons I decided to investigate whether female *O. pumilio* are able to discriminate between offspring and unrelated young, whether they use direct or indirect recognition cues, and whether prior parental investment plays a role in differential treatment of young.

Moreover, kin recognition is often context dependent, such that its expression varies with the costs and benefits of discrimination (Sherman et al. 1997). Thus, kin recognition is often expected to vary with environmental conditions (Blaustein & Waldman 1992; Holmes & Mateo 2007). Therefore, I studied offspring recognition in a free-living population of *O. pumilio* at La Selva Biological Station in Costa Rica, where the natural environment might be more variable than a laboratory setting.

In *O. pumilio*, eggs are fertilized in leaf litter on the forest floor, and egg clutches are guarded and hydrated by fathers for 7–12 days (Weygoldt 1980; Brust 1993; Haase & Prohl 2002). Once eggs develop into tadpoles, mothers return to the clutch to transport each tadpole individually to its own water-filled axil in a bromeliad or other water-holding plant, depositing only one tadpole in each axil (Donnelly 1989; Brust 1993; Maple 2002). Then, mothers return to each tadpole every 1–8 days for approximately 6 weeks to provision them by laying unfertilized eggs into the water (Brust 1993). Even though mothers do not maintain more than one concurrent clutch of offspring (Weygoldt 1980; Brust 1993; Haase & Prohl 2002; Prohl 2005), tadpoles of vastly different sizes and stages are found in different axils of the same bromeliads (Weygoldt 1980; J.L.S., personal observation), and different mothers are seen caring for offspring in the same plants (Haase & Prohl 2002; J.L.S., personal observation). Together these observations suggest that mother *O. pumilio* need to discriminate regularly between offspring and unrelated young. Indeed, mothers spend a significant amount of time searching bromeliads, and always do some searching before depositing nutritive eggs (Brust 1990). Searching behaviour may allow mothers to distinguish between axils or between tadpoles.

In the present field study, I tested whether and how mother *O. pumilio* discriminate between offspring and unrelated young when provisioning. In a series of three experiments, I determined whether mothers use either tadpole phenotypic cues or spatial location to recognize their young (recognition experiment), the relative importance of indirect and direct recognition cues in offspring discrimination during provisioning (paired discrimination experiment), and whether size and/or age contribute to offspring recognition (postparental investment recognition experiment). The postparental investment recognition experiment was necessary because offspring recognition may not occur until after parents have made some investment in their offspring (Lefevre et al. 1998; Mateo 2006).

![Figure 1](image-url) Field set-up of tadpole-rearing cups, which mimicked two adjacent natural bromeliad axils, and thus required that mothers had to make the same choices they would have made in a natural bromeliad. (a) Pair of cups tied to a tree with a bathing male *Oophaga pumilio*. (b) Mother *O. pumilio* with a recently deposited tadpole below her forelimb.
Tadpoles (N = 12) were either picked up and replaced (control), replaced with an unrelated tadpole (identity), or moved to a location 2 cm adjacent to where they were originally placed by a mother frog (location). The bottom three rows indicate results of post hoc tests between these groups.

### METHODS

This study was conducted with *O. pumilio* at La Selva Biological Station in Costa Rica. The station consists of primary and secondary lowland tropical wet forest, and receives approximately 4 m of rain annually, with slightly more precipitation occurring from May to December than in other months (McDade & Hartshorn 1994). Experiments took place in the abandoned Huertos plantations, where *O. pumilio* is very abundant and actively reproduces (Donnelly 1989), and where rows of large trees provide an organized substrate (Haggar & Ewel 1995). The environmental characteristics of the plantations mimic those of the natural habitat of *O. pumilio*, and population densities are similar to those in the adjacent secondary forests at La Selva.

*Oophaga pumilio* is a diurnal and aposematic litter frog (19–22 mm), ranging from Nicaragua to Panama, that is not harmful to humans when handled, but is often rejected by predators (Donnelly 1989; Saporito et al. 2007). Both sexes are polygamous, and males defend long-term territories and attract females by calling from perches (Limerick 1980; Pröhl & Hödl 1999). Both sexes show extensive parental care; males guard and hydrate egg clutches within their territories, whereas females transport and then provision tadpoles in water-holding plants within their home ranges (Weygoldt 1980; Brust 1993). Tadpoles are deposited and provisioned in axils of water-holding plants individually; if tadpoles are deposited in multiples, cannibalism occurs (Brust 1990).

In this study, tadpole-rearing cups were used in three experiments. During experiments, tadpoles that were deposited in these cups by a female frog were assumed to be the offspring of that female. Behavioural observations were conducted to provide evidence that the same female that transported a tadpole was the mother. Mothers were found to deposit their tadpoles in multiples, and the tadpole disappearance was due to metamorphosis, death (carcass visible in cup), predation (tadpole missing but cup water and debris were unaffected), or splash from direct raindrops or canopy drips (tadpole, debris and water in cup were missing). On the rare occasion (N = 5) that a tadpole was deposited in a cup adjacent to an existing experimental tadpole, it was removed the same day to prevent interference with provisioning of the existing tadpole.

The continuation of maternal provisioning was determined by quantifying tadpole growth and by observing changes in developmental stages (Gosner 1960) every other day for 19 days in the first two experiments and for 10 days in the third experiment. To improve precision of growth measurements in the field, I determined the total body length of tadpoles with digital photograph

![Figure 2](image-url). Change in total body length (X ± SE) of *Oophaga pumilio* tadpoles in a recognition experiment. Tadpoles in the identity group were exchanged with unrelated tadpoles. Tadpoles in the location group were moved 2 cm to an adjacent tadpole-rearing cup. Control tadpoles were picked up and replaced.
Table 2
Results of ANOVAs for effects of tadpole relatedness and location in a paired discrimination experiment

<table>
<thead>
<tr>
<th>Factor(s)</th>
<th>Tadpole growth</th>
<th>Developmental stage</th>
<th>Number of days with eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole model</td>
<td>F&lt;sub&gt;3,48&lt;/sub&gt; = 12.816, P &lt; 0.0001</td>
<td>F&lt;sub&gt;3,48&lt;/sub&gt; = 24.574, P &lt; 0.0001</td>
<td>F&lt;sub&gt;3,48&lt;/sub&gt; = 6.362, P &lt; 0.001</td>
</tr>
<tr>
<td>Location:</td>
<td></td>
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</tr>
<tr>
<td>Original</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Related</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.011</td>
</tr>
<tr>
<td>Unrelated</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Adjacent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Related</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.006</td>
</tr>
<tr>
<td>Unrelated</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Tadpole:</td>
<td>Related,</td>
<td>Related,</td>
<td>Unrelated,</td>
</tr>
<tr>
<td>Original</td>
<td>P = 0.779</td>
<td>P = 0.484</td>
<td>P = 0.511</td>
</tr>
<tr>
<td>Adjacent</td>
<td>P = 0.294</td>
<td>P = 0.517</td>
<td>P = 0.834</td>
</tr>
</tbody>
</table>

Tadpoles (N = 13) were either unrelated or related to mothers, and lived individually either in the original mother-chosen location or 2 cm adjacent to the original location. The bottom six rows indicate results of post hoc tests between these four groups.

In the first experiment, I manipulated tadpole position and identity to determine which recognition cues were used by mothers when making choices about provisioning. Upon deposition, tadpoles were assigned to one of three treatment groups (N = 12). In the identity group, tadpoles were replaced with an age- and size-matched tadpole from at least 20 m away. In the location group, tadpoles were moved 2 cm to the other cup in a pair of cups on a given tree. In the control group, the existing tadpole was merely picked up and replaced.

In the second experiment, I examined the relative importance of location and identity cues in discrimination between offspring and unrelated offspring. Upon deposition, tadpoles were assigned one of four treatment groups (N = 13). For half of the tadpoles, the offspring placed into a cup by a mother was moved to the adjacent cup in a pair of cups on a tree, and an unrelated tadpole of the same age was put in its place. For the other half of the tadpoles, the offspring was merely picked up and replaced, and an unrelated tadpole was placed in the adjacent cup.

In some taxa, mechanisms of offspring recognition are not used until some minimum level of parental investment occurs (Lefèvre et al. 1998; Mateo 2006); so, in the third experiment, I tested whether mother frogs can recognize their offspring after providing 10 days of care. Ten days after the initial tadpole deposition, tadpoles (N = 12) were replaced with either a 1-day-old unrelated tadpole or a 10-day-old unrelated tadpole.

I used parametric statistics because f tests for equality of variances of groups showed no significant differences (all P > 0.05). For the first two experiments, I compared tadpole growth with univariate, repeated measures ANOVAs. I used Tukey tests post hoc to determine differences between individual groups. In the third experiment, I used paired t tests to compare the growth of 1- and 10-day-old tadpoles that replaced related offspring to the growth of unmanipulated tadpoles of the same age (either from 1 to 9 days old or from 10 to 19 days old, respectively). I used one-way ANOVAs to compare changes in developmental stages, the number of days with eggs present in a cup, and the days of survival for the first two experiments, and changes in developmental stages after manipulations in the third experiment. I also used a multiple regression to determine whether the number of tadpoles deposited in cups on a given day was correlated with daily rainfall, average temperature, maximum temperature, minimum temperature or relative humidity. All data were analysed in StatView 4.0 (SAS Institute Inc., Cary, NC, U.S.A.).

RESULTS

Tadpoles in the recognition experiment that had been relocated 2 cm apart to where mothers had placed them into tadpole-bearing cups (see Fig. 1a) differed after 19 days of cup occupancy from control tadpoles in growth, developmental stage progression (Gosner 1960) and the number of days with nutritive eggs present (Table 1, Fig. 2). Unrelated tadpoles that were exchanged with a mother-deposited tadpole, but not relocated, did not differ from control tadpoles in these three characteristics (Table 1, Fig. 2).

In the paired discrimination experiment, when tadpoles differed in whether they occupied the mother's originally chosen location or the adjacent location, they also differed in growth, developmental stage progression and the number of days with nutritive eggs present (Table 2, Fig. 3). However, when tadpoles occupied the same relative location (either the mother's originally chosen location or an adjacent location), they did not differ in these characteristics (Table 2, Fig. 3). In no case were differences dependent on whether tadpoles were related to mothers (Table 2, Fig. 3).

Tadpole growth and developmental stage progression in the postparental investment recognition experiment did not differ from that of unmanipulated tadpoles when tadpoles were replaced after 10 days of cup occupancy by either non-age-matched tadpoles (paired t test: 1-day-old: growth: t<sub>12</sub> = 0.941, P = 0.342; stages: t<sub>12</sub> = 0.861, P = 0.363; Fig. 4) or age-matched tadpoles (10-day-old: growth: t<sub>12</sub> = 0.180, P = 0.675; stages: t<sub>12</sub> = 0.240, P = 0.629; Fig. 4).

Figure 3. Change in total body length (± SE) of Oophaga pumilio tadpoles in paired choice discrimination tests. Mothers placed tadpoles into one of two tadpole-bearing cups 2 cm apart. Tadpoles were either moved to the adjacent cup and replaced with an unrelated tadpole, or picked up and replaced, and an unrelated tadpole was placed in the adjacent cup.
temperature, maximum temperature, minimum temperature or correlation or individual correlation between the number of contain a tadpole. A multiple regression showed no combined within a mean \( N \) later provisioning tadpoles in cups (\( \text{day} \)). Moreover, eggs were sometimes eaten shortly after provi-

dings did not differ between groups (ANOVA: recognition: \( F_{2,33} = 0.292, P = 0.748 \); discrimination: \( F_{3,48} = 0.987, P = 0.406 \)); this was probably due to the relatively large within-group variance cause by external factors such as predation and splash. Tadpoles measured on the day of deposition in a cup had a mean + SE total length of 9.66 + 0.78 mm (\( N = 196, \text{range 6.3–11.9} \)). Control tadpoles progressed through a mean + SE of 2 + 2.6 developmental stages (\( N = 12 \) stages) in 19 days, and in that time, eggs were found in their cups a mean + SE of 8 + 4.2 days (\( N = 12 \) days). The number of days that eggs were found in a cup differs from the number of eggs that a tadpole was fed because it was difficult to determine whether eggs were freshly deposited or remained from the previous day. Moreover, eggs were sometimes eaten shortly after provi-

Throughout the study, 196 tadpoles were deposited into tadpole-rearing cups, and of those, 51 were predated (26% as compared to 68% predation in natural bromeliads; Maple 2002), 62 were splashed out by raindrops, 62 died and 21 metamorphosed. Metamorphosis took an average of 45 days (range 41–56). The duration of survival for tadpoles in recognition and discrimination experiments did not differ between groups (ANOVA: recognition: \( F_{2,33} = 0.292, P = 0.748 \); discrimination: \( F_{3,48} = 0.987, P = 0.406 \)); this was probably due to the relatively large within-group variance cause by external factors such as predation and splash. Tadpoles measured on the day of deposition in a cup had a mean + SE total length of 9.66 + 0.78 mm (\( N = 196, \text{range 6.3–11.9} \)). Control tadpoles progressed through a mean + SE of 2 + 2.6 developmental stages (\( N = 12 \) stages) in 19 days, and in that time, eggs were found in their cups a mean + SE of 8 + 4.2 days (\( N = 12 \) days). The number of days that eggs were found in a cup differs from the number of eggs that a tadpole was fed because it was difficult to determine whether eggs were freshly deposited or remained from the previous day. Moreover, eggs were sometimes eaten shortly after provi-


days since mother’s initial tadpole deposition

Figure 4. Change in total body length (X + SE) of Oophaga pumilio tadpoles in a postparental investment recognition experiment. Mother-deposited tadpoles were replaced after 10 days of care with either 10-day-old tadpoles or 1-day-old tadpoles. Growth of unmanipulated tadpoles of the same age is also shown in grey.

DISCUSSION

During provisioning, mother \( O. \) pumilio discriminated between offspring and unrelated young using indirect spatial recognition, but not direct recognition. Provisioning of tadpoles continued both when tadpoles were picked up and replaced and when they were exchanged with an unrelated tadpole, but not when they were moved to an adjacent tadpole-rearing cup. When given a choice between provisioning unrelated and related tadpoles in either original or adjacent cups, mothers chose to provision the tadpole in the original location regardless of tadpole identity. Furthermore, if mothers’ tadpoles were replaced on day 10 with either 1-day-old or 10-day-old unrelated tadpoles, maternal provisioning was not interrupted, suggesting that mothers do not recognize offspring even after a period of parental investment has taken place. These conclusions assume that a female provisioning a tadpole was indeed its genetic mother.

These findings suggest that mother \( O. \) pumilio do not discrimi-
nate between offspring and unrelated tadpoles using direct recognition of phenotypic cues of offspring. However, previous studies have shown that the ability to recognize offspring is often context dependent (Beecher 1991; Gibbons et al. 2003), and the results of the present study of \( O. \) pumilio do not prove that mothers of this species cannot recognize offspring. None the less, because the study was conducted in the natural habitat of \( O. \) pumilio, and because offspring age, size, location and chemosensory cues were accounted for and did not appear to influence maternal behaviours, it is clear that indirect recognition based on a location cue is the primary mechanism used in offspring discrimination during provisioning in this amphibian. The spatial accuracy with which mother \( O. \) pumilio appear to navigate their tadpole-rearing envi-

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