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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In species that show parental care, the ability to discriminate between offspring and unrelated young has strong and obvious implications for fitness. The success of a parent’s offspring is directly related to the quality and amount of care that the offspring receive (Hepper 1986). Also, parental care behaviours are costly to parents and their future offspring, and these costs are fruitless if care is directed at unrelated young (Trivers 1972; Queller 1997; Duckworth et al. 2003). Thus, to ensure that care is beneficial, parents from diverse taxa modulate the intensity and the target of their care according to the genetic relatedness of young. For example, in bluegill sunfish, *Lepomis macrochirus*, fathers adjust the intensity of care based on the prevalence of cuckold males and on the familiarity of offspring chemosensory cues (Neff 2003). Mother sub-Antarctic fur seals, *Arctocephalus tropicalis*, forage at sea for weeks, and return to provision their offspring after recognizing their pups’ voices among hundreds of others (Charrier et al. 2002). Mother crab spiders, *Dienea ergundros*, maintain nests with both related and unrelated young, but only catch prey and produce trophic oocytes for their own offspring (Evans 1998).

For parents to discriminate based on the degree of genetic relatedness of young, a recognition mechanism must be present (Sherman et al. 1997; Tsutsui 2004). Recognition mechanisms can indicate relatedness in one of two ways. In direct recognition mechanisms, parents use a phenotypic component of the young (Waldman 1987). The phenotypic cues that are used to recognize offspring can vary widely in modality, from chemosensory (Greenberg 1979; Main & Bull 1996; Evans 1998; Yamazaki et al. 2000; Neff & Sherman 2005) to auditory (Espmark 1971; Jouventin et al. 1999; Charrier et al. 2002; Searby & Jouventin 2003; Knörnschild & Von Helversen 2008) and less often to visual (Lahti & Lahti 2002) modes. In indirect recognition mechanisms, on the other hand, parents make use of a contextual cue such as spatial location or frequency of encounters with young (Waldman 1987). Indirect mechanisms are most commonly used when offspring are not likely to move from where parents have left them, or when offspring are not likely to be confused with unrelated young in an adjacent location (Waldman 1987; Sherman et al. 1997). In these cases, parents use spatial, chemosensory or visual cues that are derived from the nest itself (Lank et al. 1991; Bonadonna et al. 2003).
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 over siblings 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 (Pröhörl & Hödl 1999; Pröhörl & Berke 2001; Haase & Pröhörl 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 & Pröhörl 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 & Pröhörl 2002; Pröhörl 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 & Pröhörl 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. 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.
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 apospermatic 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 future source of nutritive eggs for that tadpole. On 10 occasions, mothers that were observed depositing tadpoles were marked using uniquely coloured orthodontic bracket ties (Orthodontic Supply & Equipment Co., Inc., Gaithersburg, MD, U.S.A.) on the hindlegs that fit loosely below the knee and above the calf muscle. In the days following banding, I found mothers each morning and observed them from 0700 to 1100 hours daily until eggs were deposited in the cup containing the originally deposited tadpole. Female *O. pumilio* only provision their offspring during this time period (Haase & Pröhl 2002). In addition, I considered tadpoles that were deposited more than 20 m from each other in cups to be unrelated, because mean ± SE home range size of tadpole-rearing females is 27.6 ± 11.4 m² (Haase & Pröhl 2002) and thus females are not likely to deposit tadpoles more than 20 m apart. Unrelated tadpoles used in experiments were generally found at least 100 m apart. While it would have been ideal to conduct genetic analysis and verify mother–tadpole relationships, such analysis was considered to be outside the scope of the field study.

Prior to the experiments, tadpole-rearing cups (*N = 800*) made from 15 ml clear polypropylene beakers (VWR, Batavia, IL, U.S.A.) were placed in the forest. To prevent flushing of tadpoles from the tops of cups by rain, six small holes were drilled in the sides of each cup approximately 2 cm below the top rim such that cups were generally half full. Cups were affixed to vertically aligned tongue depressors with zip ties to provide a stable base to rest against trees. After removing the natural bromeliads within reaching height, I tied cups in pairs with yarn or monofilament (>2 cm apart, 1–1.5 m high; Fig. 1a) to trees greater than 15 cm in diameter in order to mimic the natural situation of two adjacent axils of a bromeliad. Natural bromeliads at this site typically have between 2 and 7 water-filled axils per plant (Maple 2002). Mother frogs were allowed to place their tadpoles in one of these two cups and return to them repeatedly for provisioning without human interference. All cups were checked daily between 1300 and 1600 hours for the presence of new or old tadpoles and eggs from 5 June 2008 to 23 October 2008, with the exception of 7 August 2008, because a flash flood restricted researcher access to the forest. During daily cup checking, cups with low water levels were refilled with previously collected rainwater, and excess debris was removed. After a mother frog placed a tadpole in one of the two cups of a pair (Fig. 1b), that tadpole was randomly assigned to one of the seven groups described in the experiments below. Any tadpoles that I moved during manipulations were moved with the water (and possible chemical cues) in their cup. Deposited tadpoles were monitored until they were no longer in the cup. I noted whether 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 drip (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

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Results of ANOVAs in a recognition experiment</th>
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<tbody>
<tr>
<td>Factor(s)</td>
<td>Tadpole growth</td>
</tr>
<tr>
<td>Whole model</td>
<td>F&lt;sub&gt;2,13&lt;/sub&gt; = 6.176, p = 0.005</td>
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<tr>
<td>Location</td>
<td>Control</td>
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<td>Location</td>
<td>Identity</td>
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<tr>
<td>Identity</td>
<td>Control</td>
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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.

![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(3,48) = 12.816, F(3,48) = 24.574, F(3,48) = 6.362,</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Location:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Original</td>
<td>Adjacent</td>
<td></td>
</tr>
<tr>
<td>Tadpole:</td>
<td>Related</td>
<td>Unrelated</td>
<td>related</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Related</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Unrelated</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Related,</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>unrelated</td>
<td>p = 0.797</td>
<td>p = 0.484</td>
</tr>
<tr>
<td></td>
<td>Related,</td>
<td>p = 0.294</td>
<td>p = 0.617</td>
</tr>
<tr>
<td></td>
<td>unrelated</td>
<td>p = 0.834</td>
<td></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.

RESULTS

Tadpoles in the recognition experiment that had been relocated 2 cm adjacent to where mothers had placed them into tadpole-rearing 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_{12} = 0.941, P = 0.342; stages: t_{12} = 0.861, P = 0.363; Fig. 4) or age-matched tadpoles (10-day-old; growth: t_{12} = 0.180, P = 0.675; stages: t_{12} = 0.240, P = 0.629; Fig. 4).

Figure 3. Change in total body length (X ± SE) of Oophaga pumilio tadpoles in paired choice discrimination tests. Mothers placed tadpoles into one of two tadpole-rearing 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.
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 (\( N = 196, \) 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 provisioning. During observations of marked females depositing and later provisioning tadpoles in cups (\( N = 10 \)), females deposited eggs in the same cup in which they had previously deposited a tadpole within a mean ± SE of 2.5 ± 1.3 days. In no case was an unmarked female seen in the cup with a marked female’s tadpole, and nutritive eggs were never seen in tadpole cups before observation of a marked female’s first nutritive egg deposition. In 4.5 months of cup checking, nutritive eggs were never found in cups that did not contain a tadpole. A multiple regression showed no combined correlation or individual correlation between the number of tadpoles deposited in cups on a given day and the average temperature, maximum temperature, minimum temperature or relative humidity (multiple regression: \( F_{5,94} = 1.889, R^2 = 0.091, P = 0.104 \)). However, there was a correlation between the number of tadpoles deposited and the amount of daily rainfall (linear regression: \( F_{1,98} = 5.250, R^2 = 0.225, P = 0.024 \)).

**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 discriminate 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 environment is noteworthy. The capacity of *O. pumilio* to navigate spatially is also supported by the demonstration that individuals are able to return to their territory or home range following experimental displacement (McVey et al. 1981).

Indirect rather than direct recognition is the likely mechanism for offspring discrimination in female *O. pumilio* because tadpoles are not capable of moving from one bromeliad axil to another (Waldman 1987; Sherman et al. 1997). Thus, indirect spatial cues are presumably reliable enough to ensure that mothers are provisioning their own offspring. However, both phenotypic and non-phenotypic cues appear to play a role in maternal care in this system; mothers may also use tadpole behaviour as an auxiliary measure to verify that they are provisioning in the appropriate location. In *O. pumilio*, tadpoles perform a vibration behaviour when an adult frog approaches their axil (Weygoldt 1980; J.L.S., personal observation), and this behaviour appears to stimulate egg deposition into the axil. In both the present study and in an aquarium study (Weygoldt 1980), eggs were never found in cups or axils that did not contain tadpoles or that contained dead tadpoles. Yet, the indirect recognition mechanism shown by *O. pumilio* mothers leaves open the possibility for intraspecific reproductive parasitism, in which a female might consume the existing tadpole in an axil and deposit her own in its place to be fed by the other tadpole’s mother. Adult *O. pumilio* consume unrelated young (Weygoldt 1980; Haase & Pröhl 2002), and in this study, mothers still provisioned when their 10-day-old tadpoles were replaced with 1-day-old tadpoles. Reproductive parasitism is speculated to occur in *O. pumilio* females (Haase & Pröhl 2002) and occurs in the related *D. ventrimaculatus* (Summers 1999). Nothing is yet known about the relatedness of adult and young individuals in *O. pumilio* populations, but the hypothesis on reproductive parasitism would benefit from information on genetic relatedness.

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