Kin Recognition in Tadpoles of Rana luteiventris reared in different densities

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Kin Recognition in Tadpoles of *Rana luteiventris* reared in different densities

Submitted in Partial Fulfillment of the Requirements for Graduation with Honors to the Department of Natural Sciences at Carroll College, Helena, Montana.

Douglas Barber
April 10, 2000
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Abstract

After rearing *Rana luteiventris* tadpoles at high (80-100/L) and low (2/L) densities, I tested tadpoles for kin discriminatory behavior in a standardized choice tank. To test whether density manipulations influenced growth, I measured tadpole mass at metamorphosis. Results suggest that density manipulations were not effective, as tadpole mass at metamorphosis was not different between treatments. However, tadpoles exhibited kin discriminatory behavior irrespective of density treatments. My results suggest spotted frog tadpoles exhibit kin discriminatory behavior, although it is still unknown whether rearing conditions may influence such behavior.
Introduction

The study of kin recognition systems has become increasingly important to the study of behavioral ecology (Hepper 1991). Kin recognition was first explained by the concept of inclusive fitness, proposed by Hamilton (1964). Essentially he argued that since related individuals have a number of genes in common that are identical by descent, an individual may pass on its genes to the next generation via its relations (Hepper 1991). We may then draw from this, that the inclusive fitness of an individual includes not only the reproductive success of the individual, but also the reproductive success of related conspecifics. An individual then might be able to gain fitness by recognition of related individuals. It can be widely observed throughout nature that kin recognition is operative in many animal societies on the individual level. For example, kin recognition behavior has been observed among ground squirrels, honey bees, primates, and amphibians (Porter & Blaustein 1989). Kin recognition lays the foundation of behavioral ecology. From studies of kinship, theories on altruism, cooperation, and reciprocity can be founded.

Kin recognition may be divided into two broad areas, the exhibition of differential behavior toward kin and non-kin, referred to as kin discrimination, and the mechanisms used to identify individuals as kin, non-kin, or a particular degree of relatedness, referred to as kin recognition (Hepper 1991). Several studies have shown that kin recognition can be dependent on ecological contexts including but not confined to: rearing conditions, exposure to stimulus, predatory defense mechanisms, and abundance of food (Blaustein & O’Hara 1983; Hokit & Blaustein 1995; Hokit et al. 1996). Animals that are capable of discrimination between kin and non-kin can effectively behave to the exclusive advantage of their close relatives (Porter & Blaustein 1989). By putting themselves at risk they help
their related conspecifics, which enhances conspecific reproductive success, increasing
the inclusive fitness of the original individual. Individuals, when faced with an altruistic
dilemma, should only help conspecifics if the ratio of benefit to cost is greater than the
inverse of relatedness: this ratio is known as Hamilton's rule. For example, if genes are
considered evolutionary currency, then a person should gladly give up their life for two
siblings or eight cousins. As previously stated, kin discrimination often enhances an
individual's inclusive fitness. Individuals can use kin recognition to both act
altruistically (help relatives at a cost to the individual) and avoid incest with possible
relatives.

Although kinship systems exist in many animal societies (Hepper 1991), it is
often most practical to study the physiology and ontogeny of these systems in the lab
(Blaustein 1988). Warm-blooded vertebrates can be unwieldy as subjects for ontogenetic
studies; however, kinship in amphibians is highly tractable in the lab (Blaustein 1988).
Because so little is known about the social behavior of amphibians, they are perfect
subjects for experimentation.

Kin recognition can be described as the communication of genetic relatedness
among conspecifics (Waldman 1991). For kinship identity to be communicated,
individuals (senders) must express "labels" (possibly including behaviors, morphological
traits, or signals they actively produce) that provide information concerning their
genotype. These cues are perceived and analyzed by the receivers, which may compare
perceived labels with those stored in a model template (Waldman 1991). It is thought
that anuran larvae generally rely on chemoreceptors to determine relatedness, although
other phenotypic cues might play a role in behavior (Porter & Blaustein 1989). Several
studies have been done to determine the contexts in which tadpoles display kinship behavior, which include but are not limited to: parental care, schooling behavior, territoriality, and mating (Waldman 1991). Waldman and Adler showed that American toad (*Bufo americanus*) tadpoles both recognize and preferentially associate with their sibling in the lab and field (Waldman & Adler 1979). In the early 1980s, Blaustein and O’Hara performed kin discrimination experiments on a number of anuran larvae including: *Rana cascadae, Rana aurora, Rana sylvatica,* and *Bufo boreas* (Table 1 Blaustein 1988; Table 7.1 Waldman 1991).

In a series of laboratory experiments, I tested whether *Rana luteiventris* tadpoles exhibited kin discriminatory behavior. Moreover, I reared tadpoles at different densities to determine if any observed kin discrimination was context dependent. Finally, I measured tadpole mass at metamorphosis to determine if density influences growth.
Materials and Methods

I collected four newly oviposited egg clutches of *Rana lutreiventrıs* from the Helena National Forest on May 25, 1999. Each clutch was placed in a plastic container, then in a cooler for transport back to the laboratory in Helena.

In the laboratory, all clutches were placed in oxygenated 10-gallon aquariums filled with dechlorinated tap water. Two clutches were placed in individual aquaria and two clutches were combined in one aquarium, for a mixed group. Upon hatching, tadpoles were randomly assigned to low (2 tadpoles/L) and high (80-100 tadpoles/L) density treatments. Four treatments resulted: low-density kin group, low-density mixed group, high-density kin group, and high-density mixed group. Each high and low-density kin group treatment was represented by two sibships (A & B). Each sibship was then further divided in two and reared in separate aquaria (A1H, A2H, A1L, A2L, B1H etc.). Thus tadpoles could be tested using unfamiliar siblings raised in different tanks. A total of 10 aquaria were used, 8 for divided sibling-groups, 2 for mixed treatments. All tanks were placed on the same bench in an air-conditioned, windowless lab where water temperature was kept at 16-21°C.

Rabbit Chow was fed to the tadpoles every three days. Food quantity was adjusted throughout the experiment depending on tadpole growth, 1 to 3g for low-density treatments and 3 to 9g for high-density treatments. On any given day, all treatments of the same density received the same amount of food. Tanks were cleaned every 5-7 days and refilled with dechlorinated tap water with all tanks being cleaned on the same day.
Kin discrimination experiments

After tadpoles reached stage 29 (Gosner 1960), I tested for kin-discriminatory behavior using an apparatus following Blaustein and O'Hara (1983). The testing chamber was filled with 30L of dechlorinated tap water and refilled for each trial. For each trial a test animal was placed in a chamber (Fig 1.). Following the placement of the test animals, two opaque, mesh containers, each with 10 tadpoles, were placed at randomly determined ends of the testing chamber. One container had 10 tadpoles from the mixed treatment reared at the same density as the test subjects (high or low), and one contained 10 sibling tadpoles reared in a different aquarium. Test animals were given a 5-minute acclimation period. After acclimation, animals were tested in 5-minute trials separated by 5-minute pauses for 25 total minutes. Time spent on the kin side was recorded for each subject and summed for the 3, 5-minute tests. Twenty replicates were performed for each low and high-density sibling group for a total of 80 replicates (40 per kin group treatment). Kin discrimination was determined using a one-tailed binomial test to compare the number of trials in which tadpoles spent a greater amount of time on the kin side versus trials in which tadpoles spent a majority of time on the mixed side. The effects of density on kin discrimination behavior were examined using MANOVA, multivariate analysis of variance, which compared the time spent on each half of the chamber (kin vs. non-kin) for each treatment density.
Growth and Development

As tadpoles reached metamorphosis (stage 42, Gosner 1960), they were removed from their tanks, blotted dry and weighed. Data was recorded for each high and low-density sibling treatment, which each included four replicates, and the two mixed treatments, which only had one replicate. After weights were recorded, they were placed in a discard tank. Data was tested using MANOVA, to assess the possible influence of high and low-density rearing on mass at metamorphosis.
Results

For both replicate kin groups, a one-tailed binomial test indicated that tadpoles significantly preferred the kin side of the testing tank to the non-kin side. On average kin group A and B spent 66% and 60% respectively of testing time on the kin side (Figure 2). Binomial analysis indicated that p<0.05 for each kin group (Table 1). MANOVA tests for density effects on kin discriminatory behavior showed no significant effect of density on kin discriminatory behavior (Table 2). Furthermore, MANOVA tests for the effects of density on mass showed no significant effect (Table 3, Figure 3).

Table 1: Results of one-tailed Binomial test. All p<0.05

<table>
<thead>
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<th>Kin Group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
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</tr>
<tr>
<td>B</td>
<td>0.011</td>
</tr>
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</table>

Table 2: Results of MANOVA for effects of density rearing on kin discrimination.

<table>
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<tr>
<th>Effect</th>
<th>df effect</th>
<th>MS effect</th>
<th>df error</th>
<th>MS error</th>
<th>F</th>
<th>p-level</th>
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</thead>
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<td>4</td>
<td>7269.581</td>
<td>0.751811</td>
<td>0.434821</td>
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</table>

Table 3: Results of MANOVA for effects of density rearing on mass.

<table>
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<th>MS effect</th>
<th>Df error</th>
<th>MS error</th>
<th>F</th>
<th>p-level</th>
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</thead>
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<td>0.68367</td>
<td>0.439963</td>
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<tr>
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<td>0.894511</td>
<td>0.380772</td>
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<tr>
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<td>6</td>
<td>0.027171</td>
<td>0.408113</td>
<td>0.546526</td>
</tr>
</tbody>
</table>
Discussion

The results of my binomial test indicated that *Rana luteiventris* tadpoles exhibit kin discrimination behavior. Such a strong preference is surprising, given the work of O’Hara and Blaustein with *Rana pretiosa*, a close relative, which did not exhibit kin discrimination behavior (O’Hara & Blaustein 1988). Because there has been no study on *Rana luteiventris* with regard to kinship, more trials would reinforce my findings. A relative, *Rana cascadae*, has been studied extensively and also shows strong kin recognition behavior (Blaustein 1988). On the other hand, there are many possibilities why *R. luteiventris* tadpoles might exhibit this type of behavior.

Numerous benefits await the tadpole that can recognize its own kin. First, tadpoles with this ability are able to aggregate in groups for both protection from predators and to more easily locate food (Porter & Blaustein 1989). Tadpoles of *R. cascadae* have been shown to deliver a chemical alarm-response when injured (Hokit 1995). This chemical cue warns sibling tadpoles that newt predators are about. Tadpoles that received this cue decreased activity making them less likely to be preyed upon. Concurrently, some tadpoles might be distasteful to some predators. If one of a group of tadpoles is sampled by the predator and found inedible then the predator is less likely to feed upon tadpoles in that aggregation (Porter & Blaustein 1988). This phenomenon has been exhibited in *B. americanus* tadpoles (Waldman and Adler 1979). Kin association might also be very important for growth and development. Growth is prolonged in some tadpoles when reared with siblings versus non-kin (Porter & Blaustein 1988).

I would conclude that kin selection is a function of selective pressure. Depending on the natural environment of the tadpole, the presence of predators, limited resources, or
time period for development could all favor the tadpole that aggregates with kin groups. Moreover, kin recognition helps stop inbreeding and might enhance optimal outbreeding in adult frogs (Waldman 1991).

Rearing in low and high densities did not significantly affect kin discriminatory behavior in my trials. This could be, however, because my density manipulations were inadequate. Context-dependent studies have previously shown that both adult amphibians and tadpoles have exhibited context-dependent kin discriminatory behavior. Marbled salamanders, *Ambystoma opacum*, have been shown to express kin discrimination depending on social and environmental context (Hokit et al. 1996). If exploitative competition was allowed between salamander pairs a decrease in body size was observed. Smaller related individuals were significantly smaller than their counterpart if competition existed. Concurrently, tadpoles of *S. bombifrons* discriminate between kin and non-kin to avoid cannibalizing siblings (Pfennig et al. 1993). Therefore we can conclude that a tadpole in a highly competitive environment would be more apt to exhibit kin discrimination than a tadpole in an environment where resources are in abundance. Tadpoles that are in highly competitive situations should be forced to metamorphosis more quickly to stay competitive. Tadpoles with an abundance of resources and low competition are allowed to take more time to grow larger before metamorphosis. My density manipulations might not have been extreme enough to create environments in which tadpoles might not show kin recognition. It is possible that at densities higher than 100 tadpoles/L limited resources might counter the benefits of kin recognition. Further density manipulations are needed to indicate if *R. luteiventris* always shows kin recognition or if it is context dependent.
My experiment also indicated that there was no statistical variation in body mass dependent on high or low density rearing conditions. Body mass variance has been shown in several experiments of other species including *R. cascadae* tadpoles and *A. opacum* larvae (Hokit et al. 1994, 1995, 1997). It then stands to reason that *R. luteiventris* should exhibit this pattern. However, some studies have shown a negative correlation between kinship on growth and development. Qualitatively, I observed this pattern in my experiments. Despotic individuals could easily be observed in all high-density treatments, and tadpoles of low-density treatments were visibly much larger than tadpoles of high-density treatments. However, the data collected was dependent on time of metamorphosis, stage 42 (Gosner 1960). Because, my research was limited to a three-month time frame, I was unable to collect the largest tadpoles in low-density treatments, as they had failed to metamorphosis. If I had the time in which to measure the mass of all the individuals, I would have most likely found trends like those Blaustein and Hokit (1997) found in *R. cascadae*.

Most importantly, this study suggests that *R. luteiventris* tadpoles exhibit kin discriminatory behavior; although, it still remains unclear whether the kin discriminatory behavior is context dependent. Further studies manipulating resource availability and densities could yield a clearer picture of possible context dependence. Growth and development of the tadpoles at low and high-densities most likely would alter mass at metamorphosis; however, my experiment did not show this. This problem was largely due to time constraints. Understanding kin discriminatory behavior among species of tadpoles could provide us with a greater understanding of amphibian behavior and evolution as a whole. Hamilton’s rule suggests that kin discrimination should be context
dependent. Understanding which environments are conducive to kin discrimination and which are not may greatly augment our understanding of kin selection processes.
Figure 1. Kin discrimination testing apparatus showing placement of test subjects and stimulus groups. Neither the apparatus nor the tadpoles are drawn to scale (Blaustein & O'Hara 1983)
Figure 2. Average time spent on kin side of testing chamber.
Figure 3. Density influence on mass at metamorphosis.
Literature Cited


