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Differential Kin Discrimination by Red-spotted Newts (Notophthalmus viridescens) and Smooth Newts (Triturus vulgaris)

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Abstract

In organisms lacking parental care or when eggs of more than one female are laid together, parents preferentially tend to avoid eating their own offspring. In some species of newts (Caudata: Salamandridae), there is no parental care and cannibalism of eggs and larvae occurs when philopatric adults remain in the pond throughout the breeding season where eggs and larvae develop. Kin discrimination by both adult newts and larvae would be expected to occur during the breeding season as it would enable individuals to benefit from the nutrients obtained from eating eggs and larvae while they avoid eating close relatives. The effects of kinship on cannibalism of eggs and larvae were examined in two species of newts. In separate, but similar, paired behavioral trials adult female red-spotted newts, Notophthalmus viridescens, and smooth newts, Triturus vulgaris, were allowed to consume their own egg or an egg from another female. Female red-spotted newts, but not smooth newts, preferred to eat eggs of other females rather than their own, and overall smooth newts were 'reluctant' to eat conspecific eggs. To test for ontogenetic shifts in discrimination abilities, additional experiments were performed with adult female red-spotted newts paired with their own larvae and a larva from another female. In addition, the effects of kinship on the attraction or repulsion of larvae was also studied in red-spotted newts. Females showed no significant discrimination abilities during acts of cannibalism on larvae. Larvae spent similar amounts of time with siblings and non-siblings. These results suggest that the ability of adult newts to discriminate among eggs varies between species and that the elicitation of a kin-discrimination response may be context-dependent. For red-spotted newts, there was no evidence of discrimination abilities within and between other life-history stages.

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Introduction

Kin discrimination is the differential treatment of relatives and non-relatives (Waldman 1988). While kin discrimination has been well studied (review: Hepper 1991), few studies have addressed the adaptive significance of this behavior (but see

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Pfenning 1990; Pfenning et al. 1993; Walls & Blaustein 1995). When cannibalism is involved, the adaptive value of kin discrimination and the benefit to the discriminator are clearer than in some other contexts (Blaustein et al. 1987). Under certain conditions, the ability to discriminate kin, in situations where cannibalism occurs, would be expected when the benefits to fitness (in terms of the inclusive fitness) exceed the costs (Hamilton 1964; Waldman 1991; Walls & Blaustein 1995).

Amphibians have frequently been used to test aspects of kin discrimination (Walls & Roudebusch 1991; Blaustein & Waldman 1992; Pfenning et al. 1993; Pfenning et al. 1994; Walls & Blaustein 1995). The complex life cycles of many species of salamanders and anurans may facilitate intraspecific interactions, including cannibalism, among individuals within and between different life-history stages (Crump 1992). Studies of kin discrimination in anurans have dominated the literature (Pfenning 1990; Blaustein & Waldman 1992; Pfenning et al. 1993), with less known about salamanders (Walls & Roudebusch 1991; Pfenning & Collins 1993; Pfenning et al. 1994; Walls & Blaustein 1995). Adult salamanders are especially interesting because cases of filial cannibalism by egg-brooding adults of diseased or fungus-infected eggs (Forister 1979; Tilley 1972), as well as heterocannibalism (Kaplan & Sherman 1980; Morin 1983; Marshall et al. 1990; Miaud 1993; Miaud 1994), have been found.

Newts (Caudata: Salamandridae) provide an especially useful study system for kin discrimination during acts of cannibalism because both adults and larvae are likely to encounter close relatives while foraging (Waldman 1991; Crump 1992). Cannibalism of eggs and larvae by adult newts (Notophthalmus: Burton 1977; Morin 1983; Harris 1987; Taricha: Kaplan & Sherman 1980; Marshall et al. 1990; Triturus: Miaud 1993, 1994; this study) and larval cannibalism of conspecific larvae (Crump 1992; Notophthalmus: Harris 1987) are especially predominant. Adults tend to be philopatric (Notophthalmus: Gill 1978; Triturus: Joly & Miaud 1989) and remain in the pond throughout the breeding season, which may last up to three months. Therefore, kin discrimination by both adult newts and larvae would be expected to occur during the breeding season as it would enable individuals to benefit from the nutrients obtained from eating eggs and larvae while they avoid eating close relatives. However, the benefits to fitness of eating eggs and larvae over the costs of discrimination are likely to be context-dependent (Reeve 1989), with benefits being higher in less predictable environments such as ephemeral ponds (Pfenning et al. 1994; Walls & Blaustein 1995) or in ponds with limited food resources.

I examined whether two species of newts exhibit kin-discrimination abilities during parent–offspring interactions and offspring–offspring interactions to test for ontogenetic shifts in discrimination abilities. I tested both red-spotted newts, Notophthalmus viridescens, and smooth newts, Triturus vulgaris, collected from natural populations during the spring breeding season. I employed similar experimental designs for both species. To my knowledge, no amphibian study has tested hypotheses of parent–offspring discrimination in the context of cannibalistic interactions.
Methods

Experimental Animals: Collection and Housing

Notophthalmus viridescens: Female red-spotted newts occur in ponds and lakes with submerged vegetation or in calm streams, swamps, and ditches from Nova Scotia to Florida and west to southwestern Ontario and Texas (Conant & Collins 1991). In the more northern areas, adults breed in the spring. Females lay 100-300 eggs singly, each wrapped in submerged vegetation. Females mate 2–3 times in a season (Halliday & Verrell 1984; pers. obs.). Eggs hatch in late summer or early autumn, and adults begin to leave the pond in Aug. In the first two experiments, I tested only females from which I had obtained eggs to ensure that females were related to the offspring by coefficient of relatedness $\approx 0.5$. Male parentage was difficult to determine because females multiply mate and store sperm. Moreover, male salamanders infrequently eat eggs (Kaplan & Sherman 1980; Truettner 1993).

I collected 35 gravid females from Riöpel Pond from 15 to 17 Jun. 1993 at Mountain Lake Biological Station, Giles County, Virginia, USA. I placed each female in a ‘minnow trap’ (43 × 21.5 cm) which was suspended in an outdoor tank (21°C) with a natural photoperiod. The tank contained rain water, natural leaf litter, and a supply of zooplankton and damselyf larvae from Riöpel Pond. The traps had corks at both ends of the open ends to prevent the newts from escaping. The cork at the bottom had six (15 × 1.2 cm) strips of black plastic attached to it that floated vertically when placed in the trap. I began checking for deposited eggs (on the plastic strips) 24 h later and every 24 h thereafter. I placed the strips of plastic containing eggs from each female into separate containers (10.0 × 10.0 × 6 cm deep) filled with water from the outdoor tank and maintained them in the laboratory at 22–26°C. Each container had 2–16 eggs but generally 2–8 larvae emerged in each container. If more emerged, I divided the larvae between two containers. Larvae were fed daily by replacing half of the water with water containing zooplankton from the outdoor tank.

Triturus vulgaris: Smooth newts live throughout Europe and the UK in humid wooded and open areas, mountains, and plains in temporary pools or margins of lakes (Stewart 1969). Adults breed in the spring. Females lay 200–600 eggs, each separately wrapped in a leaf or a blade of grass. Females mate 2–7 times per season (Halliday & Verrell 1984; pers. obs.). Larvae take about 3–4 mo to reach full larval size, and they leave the water in late Jul. or the following spring. I obtained 35 gravid females from three ponds in Milton Keynes, England, from Feb. to Apr. 1994. Fifteen of these females were maintained in the laboratory for at least 1 mo longer than the others, as they were first used in a mate-choice experiment. This should not have affected their behaviors in this study. The rest of the females were tested within a few days of being collected. I placed each female in a separate egg-laying chamber (27 × 16 × 11.5 cm deep) with aged tap water, gravel, live zooplankton, and live plants. Females were also fed live Tubifex worms weekly. The chambers were placed at the base of a large open window (12–16°C) with a natural photoperiod. I searched for eggs every 24 h, removed pieces of plants that contained eggs, and placed the pieces into separate containers uniquely identified for each female. Each container had 2–10 eggs and larvae depending on how many a female laid in one day.

Hypothesis 1 — Cannibalistic Oophagy

Individuals that discriminate kin could profit from the nutrient- and energy-rich resource and the low handling costs that non-kin eggs provide (Kaplan & Sherman 1980; Grump 1992), while not reducing their direct fitness by eating their own eggs. Therefore, I hypothesized that females will eat: a. Significantly more eggs of other females before eating their own eggs; and b. Eggs of other females significantly sooner than their own eggs when given a choice between the two.

Notophthalmus viridescens: Within 24 h of collecting at least two eggs from a female, I removed the mother newt and placed her in a separate experimental chamber (31.1 × 18.5 × 8.5 cm deep) with deionized water (4.7 cm deep) and one small (4–5 cm) rock on each side of the chamber. These females were maintained under a natural photoperiod from windows in the testing room. I tested 25 females and each female was tested after 24 h of being placed in the chamber. Testing was performed from 20 to 28 Jun. 1993.

Triturus vulgaris: Within 24 h of collecting at least two eggs from a female, I placed the female in a separate experimental chamber (27 × 16 × 11.5 cm deep), with 5.5 cm of aged tap water, in the same room as the females were maintained. I tested 35 females 1 h after being placed in the chambers and replaced them in their own chambers each day after testing. Testing was performed from 13 Apr. to 1 May 1994.
Experimental Design

I employed two treatments. A female was placed in the test chamber and a. Her own egg and one egg from another female were carefully unwrapped and dropped simultaneously on the right and left sides randomly (all eggs sank to the bottom of the chamber); b. Two eggs originating from two other females, still attached to their plastic (red-spotted newts) or plant wrappings (smooth newts), the ends of which were secured under rocks, were floated on the right and left sides (randomly) of the chamber. I tested each female once in each treatment, in random sequence, with tests separated by 24 h. Eggs from other females were randomly chosen from eggs that had been laid in the same 24-h period. I tested each female for 1 h while measuring the latency to eat one or both eggs using a digital stopwatch. In treatment a, I focused on the first egg eaten and the latency to eat it. I examined whether females more frequently ate eggs of other females first as compared to their own eggs using a one-tailed binomial test (Siegel & Castellan 1988). I also examined the latency to eat the unrelated egg first as compared to their own egg first using a one-tailed Wilcoxon–Mann–Whitney test with all α = 0.05. In treatment b, I wanted to determine whether females detected and ate wrapped eggs. I used eggs only from other females so that I was not limited to testing females that had laid three eggs in a day. No analysis was used for this treatment because females ate few wrapped eggs.

In some organisms, especially those that exhibit some form of parental care, females may eat their own offspring (amphibians: reviewed by Crump 1992; rodents: Elwood 1992, fish: reviewed by Fitzgerald & Whisnkey 1992; birds: reviewed by Stanback & Kuenig 1992). On the other hand, in organisms lacking parental care or when eggs of more than one female are laid together, parents preferentially tend to avoid eating their own offspring (birds: Stanback & Kuenig 1992; fish: Løvik et al. 1982; Sargent 1989;...
Differential Kin Discrimination by Two Species of Newts

Fig. 2: a. Latency in s for female red-spotted newts to eat their own larvae or a larva from another female (unrelated) when given a choice (one-tailed, Wilcoxon matched-pairs signed-ranks test; \( T^+ = 22 \)); b. Time that larval red-spotted newts spent on an unfamiliar sibling’s side vs. on the same side when paired with two unfamiliar non-siblings (two-tailed, Wilcoxon matched-pairs signed-ranks test; \( T^+ = 173.5, z = -0.296 \)). Parameters as in Fig. 1a.

FITZGERALD & WHORISKEY 1992; insects: NPHILIN 1989). In red-spotted newts, there is no parental care and eggs and larvae are dispersed throughout the pond. Therefore, I utilized one-tailed tests for hypotheses 1 and 2 because my a priori predictions were based on assumptions of parents favoring their own offspring, when discrimination is possible.

Results

Hypothesis 1

Notophthalmus viridescens

Sixteen of 25 females (64%) ate eggs. The mean latency for a female to eat her own egg was significantly longer than the mean latency for a female to eat an unrelated egg (Fig. 1a). Females ate an unrelated egg first significantly more often then they ate their own egg first in treatment a (Fig. 1b). None of 25 females ate wrapped eggs in treatment b. Two females bit the eggs that were wrapped but could not obtain the eggs and did not break the jelly layer. Both females proceeded to lay their own eggs on the strips of plastic after biting the eggs of other females that were present.

Triturus vulgaris

Eight of 35 females (22%) ate eggs but only seven ate eggs in treatment a. The mean latency for a female to eat her own egg was not significantly different from the
mean latency for a female to eat an unrelated egg in treatment a (Fig. 1c), and females did not differ significantly in the frequency with which they first ate their own egg vs. an unrelated egg (Fig. 1d). Three of 35 females (six attempts) ate wrapped eggs in treatment b.

**Hypothesis 2 — Maternal Cannibalism of Larvae**

In red-spotted newts females may encounter related larvae in the natal pond. Therefore, I hypothesized that mothers of *N. viridescens* will show kin discrimination by preferentially not eating their own larvae.

I tested only red-spotted newts because smooth newts did not show evidence of parent–offspring kin discrimination. I removed females from the minnow traps within 24 h of the larvae hatching and placed the mother newts in individual chambers for 24 h prior to testing. The chambers were the same as in hypothesis 1. I tested each female once in each of two treatments, in random sequence, with tests separated by 24 h. Experiments were performed from 5 to 9 Jul. 1993.

**Experimental Design**

The two treatments, randomly assigned, were: 1. A female red-spotted newt tested with one of her own larvae; and 2. The same female tested with one larva from another female. Females were tested for 1 h. I recorded latency to eat each larva using a digital stopwatch. Females were paired with larvae that had hatched from eggs laid within the same 24-h period and had hatched =72 h prior to testing. Larvae were chosen haphazardly from those available from each female. I used a one-tailed Wilcoxon signed-ranks test to compare the latency for females to eat their own larva or an unrelated larva, with α = 0.05. A maximum time of 3600 s (1 h) was recorded to indicate that a female did not eat a larva so that the response of all females had scores that could be analyzed with paired statistics.

**Results**

Eight of 27 females (30%) ate larvae. There was no significant difference between the mean latency for females to eat their own larva or to eat an unrelated larva (Fig. 2a).

**Hypothesis 3 — Larval Association**

In some species of salamanders, larvae avoid eating kin (Pfenning et al. 1993) while in other species, larvae preferentially eat siblings over non-siblings (Walls & Blaustein 1995). In red-spotted newts, larger larval *N. viridescens dorsalis* may cannibalize smaller larvae in laboratory experiments (Harris 1987), and density-dependent mortality may regulate populations of newts in the larval stage (Morin et al. 1983; Fauth et al. 1990). While larvae of this species apparently do not aggregate in ponds (Chadwick 1950; pers. obs.), upon encountering a conspecific, larvae may benefit from either avoiding or associating with an individual, depending on kinship. Therefore, I hypothesized that larvae of *N. viridescens* will preferentially associate with or preferentially avoid siblings.

I separated eggs collected from females and put eggs laid on separate days in separate containers (unfamiliar individuals) in Jun. 1994. Each container had 2–10 eggs. Experiments were performed from 16 to 18 Jul. 1994.
Experimental Design

I tested unfamiliar siblings and unfamiliar non-siblings in two treatments: 1. The test larva in the center of a chamber with a sibling on one partitioned side and a non-sibling on the other partitioned side; and, in random sequence 2. The same test larva paired with two non-siblings (control). The test chamber was made of an 18-cm long × 1.3-cm wide piece of butylate tube with a 1.1-cm opening cut along the length of the tube. I divided the tube into four parts: two 3-cm partitions on opposite ends and two 4-cm segments on either side of the center line. I used 1.5-cm diameter pieces of mesh screen to partition off the end segments and placed two screens 2 cm apart on either side of the center line of the tube to form a habituation chamber for the test animal. The mesh screen allowed potential transmission of visual and chemical cues.

I chose 25 test larvae (focal animals) haphazardly, randomly assigned them to test treatments 1 or 2, and randomly assigned treatment larvae (non-focal animals) to one partitioned side or the other. I recorded the side that the test larva was on for 15 min after an initial 15-min habituation period. I considered the larva to be on a particular side if its snout crossed the center line. After the first test, the test larva was placed in a small cup (3.0 × 3.4 cm with 2.5 cm of water) for 1 h before being re-tested. Testing was performed using spring water, and chambers were rinsed between trials. The same test animal was re-tested in the same test chamber. I used a two-tailed Wilcoxon signed-ranks test to compare the time spent on the sibling side by the test animal in treatment 1 with time spent on the same side with the non-sibling in the control treatment 2, with α = 0.05. I employed a two-tailed test because I made no prior assumption of kin discrimination. Results in either direction were potentially interesting for this hypothesis.

Results

There was no significant difference between the mean amount of time spent on the sibling (vs. non-sibling) side of the chamber and the mean time spent on the same side when paired with two non-siblings (Fig. 2b).

Discussion

Hypotheses 1–3

Females of N. viridescens discriminated against eating their own eggs vs. eating eggs of different females. The latency to eat eggs of other females was shorter than the latency to eat their own eggs. Thus, females were able to obtain nutrients and energy from eating eggs of other females while reducing predation on their own eggs. However, female red-spotted newts did not appear to have the capacity to eat wrapped eggs (in plastic strips). This suggests that egg wrapping (attaching eggs to plants) may be a form of protection from egg predation, as has been demonstrated for two species of Triturus (Miaud 1993, 1994). No evidence of kin discrimination abilities during acts of cannibalism was found beyond the mother-egg stage. Female red-spotted newts did not demonstrate a significant discrimination response between eating their own larvae and larvae of other females in the second experiment. In experiment 3, there was no
evidence of kin communication in larval newts as they did not show a preference to associate with siblings or non-siblings.

Whereas red-spotted newts demonstrated kin discrimination toward eggs, there was no evidence that smooth newts had this ability. Moreover, females of *T. vulgaris* infrequently ate eggs. Considering the apparent lack of ability of these females to discriminate between their own eggs and another female’s eggs, not eating eggs may be the best way to avoid cannibalizing one’s kin in this species.

Wood & Goodwin (1954) suggested that distributing eggs around the pond and egg wrapping may decrease the risk of cannibalism for *N. viridescens*. My data support this hypothesis. However, in my experiment with red-spotted newts, the eggs were wrapped in plastic strips and may have been more difficult to eat by predatory females than if they were wrapped in live plants. Moreover, a few smooth newts did eat wrapped eggs and these were wrapped in live plants. These results suggest that while egg wrapping does not completely prevent predation, wrapped eggs may reduce the probability of successful egg predation.

**Cannibalistic Oophagy**

Riebe (1989) suggested that eliciting a kin-discrimination response may be context-dependent or environmentally induced, with the threshold for a discrimination response varying according to the fitness consequences in different ecological contexts (see also Blaustein & Waldman 1992). The apparent variance ability of *N. viridescens* and *T. vulgaris* to discriminate among conspecific eggs may be context-dependent or environmentally induced. That is, female newts of these two species may have similar acceptance thresholds that enable them to discriminate between their own eggs and eggs of other females (Riebe 1989) but the conditions (i.e. ecological context) of the test may not have been sufficient to elicit a discrimination response from *T. vulgaris*. Based on two studies by Miaud (1993, 1994), egg eating in *Triturus* appears to be more prevalent in other populations than in the populations of *T. vulgaris* that I studied. This apparent contradiction may result from variance in the abundance of food available in the different ponds from which the newts originated. One hypothesis, then, is that newts from ponds where food resources are less abundant have reached the threshold for eliciting a kin-discrimination response while those with abundant food resources have not.

Alternatively, the differential ability to discriminate kin in these two species may result from *N. viridescens* having a genetic capacity to discriminate kin that *T. vulgaris* lacks. As Blaustein (1983) argued, ruling out or determining a genetic basis for kin recognition is difficult.

Female red-spotted newts may base their discrimination of eggs on chemical cues from a maternal label that was incorporated in the jelly mass, but this remains to be tested. In anurans, maternal cues have been found to influence recognition factors in tadpoles. Waldman (1981) found evidence that maternally derived recognition factors in American toads (*Bufo americanus*) played a role in the ontogeny of sibling recognition. Blaustein & O’Hara (1982) found that maternal cues may exert stronger influences
than paternal cues in tadpoles of *Rana catesbeiana*. Other amphibian systems have provided scant evidence of kin discrimination by adults towards eggs.

**Maternal Cannibalism of Larvae**

Female red-spotted newts did not demonstrate an ability to discriminate against eating their own larvae. While discriminatory cannibalism of larvae would provide adults with rich resources (Crump 1992), larvae disperse after hatching and may escape predation by hiding in the leaf litter or among plants in the water (Chadwick 1950; pers. obs.). Therefore, these results may be a consequence of females infrequently encountering their own larvae in the pond, suggesting that the benefits to fitness gained by eating any larvae encountered outweigh the costs of discrimination. These factors, then, suggest that selection for kin discrimination at this stage would be low. Alternatively, kin discrimination during acts of cannibalism by females may not function in relation to larvae because a maternal label from the jelly mass is no longer present.

**Larval Associations**

My data did not support the predictions of preferential association or disassociation between siblings. In other amphibian systems in which sibling discrimination has been found, larvae generally: 1. Aggregated, as with schooling tadpoles (Blaustein & O'Hara 1982; Waldman 1982); 2. Showed aggression and some cannibalism, as in the salamander *Ambystoma opacum* (Walls & Roudebush 1991; Walls & Blaustein 1995); or 3. Were highly cannibalistic, as with *A. tigrinum nebulosum* (Pfenning et al. 1994). In addition, studies with amphibians in which siblings failed to discriminate between kin and non-kin consisted of species of frogs that do not aggregate (O'Hara & Blaustein 1988; Fishwild et al. 1990). This is consistent with my results for non-aggregating newt larvae.

Pfenning et al. (1994) found that in larval Arizona tiger salamanders, *A. t. nebulosum*, cannibalistic morphs fed mostly on conspecifics and these morphs preferentially consumed less related individuals. Unlike the system studied by Pfenning et al. (1994), larval red-spotted newts apparently do not depend on eating conspecifics as a major food resource. The aggressive and cannibalistic larvae of *A. opacum* discriminated siblings from non-siblings by preferentially avoiding related individuals (Walls & Roudebush 1991) and preferentially ate siblings over non-siblings (Walls & Blaustein 1995). Walls & Blaustein (1995) suggested that this behavior was not due to size-selective predation. The larvae of *A. opacum* and *A. t. nebulosum* generally occur in ephemeral ponds, such that cannibalism enhances the probability that a larva might escape the pond (through metamorphosis) before the pond dries. However, newts breed in more permanent ponds, where early metamorphosis may not be as crucial; hence, the benefits of cannibalism for larval newts may not be as high as for larval *A. opacum* or *A. t. nebulosum*. Therefore, the infrequent levels of larval interactions and the low benefits of cannibalism for larval red-spotted newts may be insufficient to elicit a kin-discrimination response.

My results suggest that the ability of adult newts to discriminate among eggs varies between species of newts. For red-spotted newts, there appears to be no further
evidence of discrimination abilities within and between other life-history stages. Determining why one species exhibits kin discrimination while there is no evidence in another species may help understand how and when discrimination responses are elicited.

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