An investigation of kin recognition in Red-legged frog (*Rana aurora*) tadpoles

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(With 2 figures in the text)

The ability of Red-legged frog (*Rana aurora*) tadpoles to discriminate between siblings and non-siblings was investigated in laboratory choice experiments. Tadpoles that were reared with siblings only and tested in early developmental stages preferentially associated with siblings over non-siblings. Animals reared with siblings only and tested in later developmental stages did not preferentially associate with siblings over non-siblings. Test animals that were reared in isolation, those reared with siblings and non-siblings, or those reared with non-siblings only, displayed a random association among sibling and non-sibling stimulus choice groups. These results are compared with results obtained from testing the larvae of a closely related species, the Cascades frog (*Rana cascadae*).

Contents

Introduction

Recent studies have shown that a variety of species in different taxonomic groups have an ability to discriminate between related and unrelated individuals (reviewed by Bekoff, 1981; Holmes & Sherman, 1982; Colgan, 1983). Certain species also have the ability to discriminate between close relatives and more distantly related individuals and some species can recognize individuals with whom they have never had contact (see discussion in Blaustein, 1983). These recognition abilities may serve a variety of functions (op. cit.), one of which may be to facilitate nepotistic behaviours.

For the past several years, research investigating kin recognition in anuran amphibian larvae has revealed important differences in the ontogeny of the behaviour between species (see for examples, Blaustein & O'Hara, 1981, 1982*a*, *b*, 1983; O'Hara & Blaustein, 1981, 1982; Waldman, 1981, 1982, 1984). Our study of Cascades frog (*Rana cascadae*) larvae has revealed that this species has a well-developed ability to discriminate between kin and non-kin. *Rana cascadae* larvae can discriminate between kin and non-kin after being reared with kin only, with a mixture of kin and non-kin, with non-kin only, and after being reared in total isolation from an early embryonic stage (Blaustein & O'Hara, 1981, 1983; O'Hara & Blaustein, 1981). Furthermore, these larvae can discriminate between full siblings and half siblings (both paternal and maternal),

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and between half siblings (both paternal and maternal) and non-siblings (Blaustein & O'Hara, 1982a). Water-borne chemical cues are used for discriminating between kin and non-kin in larval *R. cascadae* (Blaustein & O'Hara, 1982b). These discriminatory abilities are retained after metamorphosis (Blaustein, O'Hara & Olson, 1984).

This paper investigates the ontogeny of kin recognition in the larvae of a species closely related to *R. cascadae*, the Red-legged frog (*R. aurora*). Similarities in morphology, life history and biochemistry (Wright & Wright, 1949; Dumas, 1966; Case, 1978) make this comparison important from a phylogenetic point of view. Furthermore, this study is the first on a comparison of kin recognition behaviour in two very closely related species. Until recently, *R. aurora* and *R. cascadae* were considered to be one species (e.g. Wright & Wright, 1949).

Methods

Animals and rearing conditions

We collected 3 clutches of *R. aurora* eggs (A, B, and C) from 1 site in Oregon (Lincoln county, USA) in 1982. Eggs were placed in 1 of 4 rearing regimes (Fig. 1): (1) 400 tadpoles were reared exclusively with siblings in 3 130 litre aquaria; (2) tadpoles were reared with a mixture of siblings and non-siblings (mixed rearing regime) by placing an equal number (100) of tadpoles from 2 sibling groups on opposite sides of an aerated 38 litre aquarium divided by 1·5 mm plastic mesh; (3) individuals were reared in isolation by removing eggs from the egg jelly mass at the blastula stage and placing them in 0·5 litre rearing containers (details are in Blaustein & O'Hara, 1981); (4) tadpoles were reared with non-siblings only by placing one tadpole from 1 sibling group within a 9 cm diameter plastic mesh cylinder positioned in the centre of a 4 litre aquarium (28 × 17 × 12 cm) and by surrounding it with either 5 or 10 tadpoles from another sibling group (similar to methods in Blaustein & O'Hara, 1983). Partitioning the rearing aquaria in mixed rearing
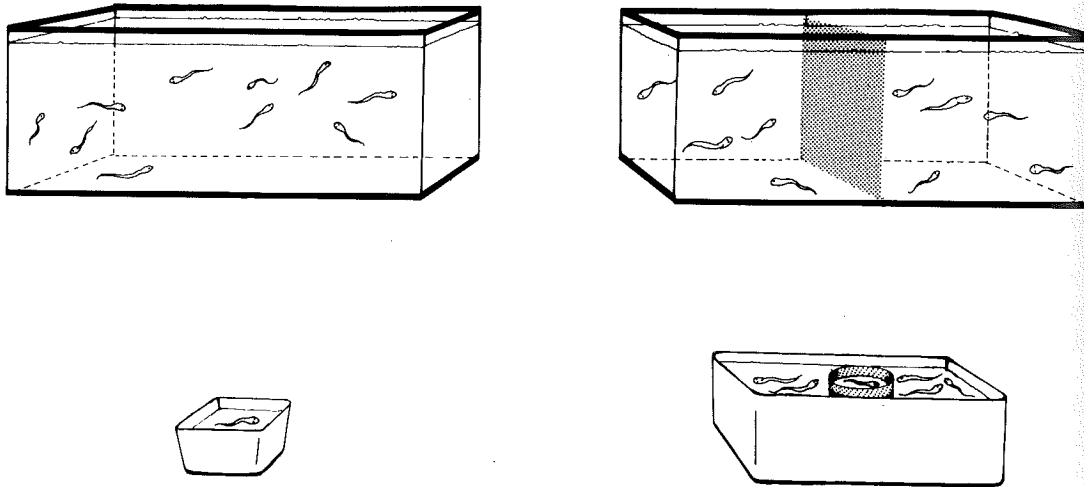

Fig. 1. Schematic diagram illustrating the four regimes under which *Rana aurora* tadpoles were reared. Tadpoles and rearing aquaria are not drawn to scale. Upper left: tadpoles reared with siblings only. Upper right: tadpoles reared within one tank in a mixed rearing regime. In this regime, tadpoles from two kin groups were separated from one another by a screen mesh. Lower left: tadpole reared in isolation. Lower right: tadpole reared with non-siblings only. In this regime, a tadpole from one kin group was separated from members of another kin group by a screen mesh cylinder.
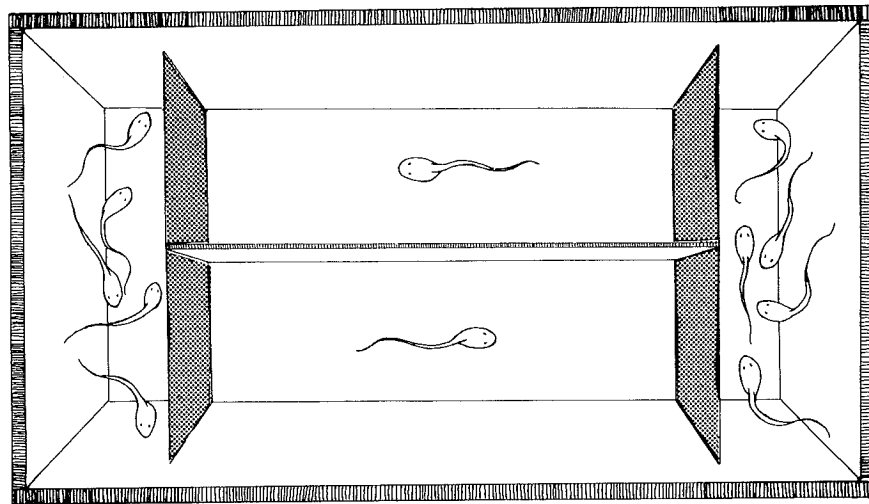
FIG. 2. Diagram showing test apparatus and placement of test individuals and stimulus tadpoles. Neither the test apparatus nor the tadpoles are drawn to scale.

regimes allowed 2 sibling groups to be reared together by permitting complete mixing of the water, and visual and some tactile contact between groups (see details of this rearing regime in O'Hara & Blaustein, 1981).

Water was changed in all rearing aquaria every 3 to 5 days. Tadpoles were fed rabbit pellets and Hartz HiProMin tropical fish food daily. All rearing aquaria, as well as the testing tank (see below), were within the same room and subjected to a 14L : 10D photoperiod at 20 to 22 °C.

Apparatus and testing procedure

A rectangular tank ($122 \times 44 \times 30$ cm) was used to test 2 individuals for sibling group preferences (Fig. 2). To create end compartments for housing stimulus animals, a partition of 1·5 mm plastic mesh was placed 15 cm from each end of the tank. A longitudinal partition placed in the central compartment was opaque and water-tight and allowed us to test preferences of 2 tadpoles simultaneously, but independently.

Before each test, the tank was filled to a depth of 6 cm with 34 litres of dechlorinated tap water (at room temperature). After 2 to 4 min, 25 tadpoles of 1 sibling group and 25 from a second sibling group were placed in opposite end compartments and left undisturbed for 15 min. One test tadpole was released at tank centre on each side of the longitudinal partition and allowed to acclimate for 10 min. The time spent in seconds by each tadpole in sibling and non-sibling halves of the tank was recorded for 4 5-min trials at 10 min intervals. Observations were taken from behind a plastic blind. Following each test, the tank was drained and thoroughly rinsed. The 2 tadpoles tested simultaneously were always members of the same sibling group. No test tadpole was tested more than once and the same stimulus animals were used in no more than 6 tests. We alternated stimulus groups from one end to the other between tests. Other controls for the apparatus and testing procedures have been reported elsewhere (e.g. Blaustein & O'Hara, 1981; O'Hara & Blaustein, 1981). All stimulus tadpoles and test individuals were matched for body size and developmental stage (Gosner, 1960) within each test.

Statistical tests

We used the total amount of time spent in sibling and non-sibling halves of the test tank as a measure of a tadpole's association preference. The Wilcoxon signed-ranks test (Hollander & Wolfe, 1973) was used to

test differences in time spent on each side of the test tank. The numbers of tadpoles spending most of their time on either side of the test tank were compared using the binomial test (Siegel, 1956). Tests were 2-tailed because no predictions were made concerning sibling preferences.

Results

When reared with siblings only, tadpoles tested in early developmental stages (stages 26–28, Gosner, 1960; 9–17 days of age) showed preferences to associate in the portion of the test tank nearest siblings (Table I). Although the time spent nearest siblings in these tests was not significantly different from random when each replicate was analysed separately, when data from both replicates were combined, the time spent nearest siblings was significantly different from random. The number of tadpoles spending the majority of their time nearest siblings was significantly different from random in one of two early tests, and when data from both replicates were

TABLE I

*Results of **Rana aurora** kin recognition tests. All statistical tests were two-tailed*

Experiment	Test group (N)	Age in days (Gosner stage)	Rearing condition of test group	No. tadpoles spending majority of time nearest siblings	Binomial P	Amount of time spent on sibling side of tank; mean ±S.E.	Wilcoxon T (P)
1.		Early tests					
	A (20)	9–17 (26–28)	with siblings	15	0·042	650± 36·3	62 (>0·05)
	B (19)	9–16 (26–27)	with siblings	14	0·116	664·6± 40·9	67* (>0·05)
2.		Late tests					
	A (20)	49–71 (31–36)	with siblings	10	1·0	626·0 ±50·1	89 (>0·05)
	B (20)	50–85 (33–37)	with siblings	12	0·50	637·2 ±62·8	80 (>0·05)
3.							
	A_m (20)	30–41 (29–31)	with siblings and non-siblings	9	0·82	591·9± 55·4	100 (>0·05)
	B_m (20)	31–41 (29–32)	with siblings and non-siblings	12	0·50	645·4 ±46·6	78·5 (>0·05)
4.							
	C_I (20)	42–48 (32–35)	in isolation	9	0·82	525 ±69·3	74 (>0·05)
5.							
	C (15)	24–30 (28–31)	with 5 non-siblings	10	0·30	605·8 ±50·3	59 (>0·05)
	C (19)	17–24 (27–30)	with 10 non-siblings	8	0·64	617·8 ±50·9	93 (>0·05)

*When data from both replicates are combined, tadpoles show a significant preference to associate with siblings over non-siblings (29 of 39 tadpoles spent most time on the sibling portion of the tank, P = 0·003, binomial test; mean time spent on sibling portion of tank = 657·6±27·01 S.E., T = 250, P<0·03).

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Discussion

Although *R. aurora* and *R. cascadae* are closely related, the two species do not display similar abilities to recognize kin. *Rana cascadae* test tadpoles maintain their abilities to recognize kin in laboratory tests and under field conditions after they have been reared under a variety of rearing regimes (e.g. Blaustein & O'Hara, 1981, 1982a, 1983; O'Hara & Blaustein, 1981, 1985). *Rana aurora* test tadpoles failed to recognize kin after being reared in the same rearing regimes, except when they were reared with kin only and tested early in development. We suggest that differences in kin recognition ability exhibited by *R. aurora* and *R. cascadae* are correlated with differences in key life history characteristics.

The most critical parameters associated with kin recognition in larval anurans appear to be: 1) the dispersal characteristics of the larvae, and 2) the aggregation behaviour of the larvae. Anuran species whose larvae randomly disperse from their sites of oviposition would have a relatively low probability of encountering and interacting with siblings. Therefore, we would not predict that such species would exhibit kin-selected behaviours. Likewise, in species whose larvae do not socially interact (e.g. form cohesive aggregations) with conspecifics, kin-selected behaviours would have little opportunity to evolve. If there is a net benefit of associating with kin, and kin have an opportunity to interact with one another, there would be selection pressure to maintain kin groups and an ability to discriminate between kin and non-kin could facilitate the maintenance of such groups.

Little is known concerning the larval ecology of *R. aurora*. However, there is no evidence that larval *R. aurora* form prolonged aggregations with conspecifics in nature. They do, however, seem to disperse rapidly and far from their sites of oviposition and may mix freely with members of other clutches during the initial stages of dispersal (Calef, 1973). Thus, the weak tendency to recognize kin in this species is consistent with the larval ecology which shows a low likelihood of kin group formation. Our results of kin recognition tests in *R. aurora* revealed that test individuals reared with kin displayed an ability to discriminate between kin and non-kin during early developmental stages. There may be selective pressures for having an ability to recognize kin early in development and this ability may be lost because the selective pressures for its maintenance may be diminished in later stages. For example, it is possible that *R. aurora* tadpoles may exist in aggregations in early tadpole stages and they may become less social in later stages. With our limited knowledge of *R. aurora* tadpole ecology, we are uncertain as to why these larvae only displayed an ability to discriminate between kin and non-kin in early developmental stages. It is also possible that kin selection was instrumental in the evolution of the social behaviour of *R. aurora* in the past, possibly under different environmental and social conditions. A remnant of this recognition ability may exist that enables *R. aurora* larvae to display nepotism under certain present environmental regimes.

In contrast to *R. aurora*, field observations of *R. cascadae* tadpoles and controlled field and laboratory experiments using *R. cascadae* larvae are consistent with a kin selection model

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(Hamilton, 1964). *Rana cascadae* larvae are almost never found alone in nature and exist in close, social aggregations composed generally of fewer than 100 individuals, a number much smaller than their clutch size (O'Hara, 1981; Robert M. Storm, pers. obs.). Because eggs are sometimes laid communally, embryonic and larval development may occur in proximity to both kin and non-kin (Sype, 1975; Blaustein & O'Hara, 1982*a*, *b*. Thus, there may have been selection pressure for developing a kin recognition mechanism that is resistant to experiential modification in *R. cascadae*. Moreover, field experiments conducted by O'Hara (1981) illustrated that dispersal from sites of oviposition may be low. The larval ecology of *R. cascadae* suggests that the tadpole aggregations are composed of many kin.

The ability of *R. cascadae* to distinguish between individuals of various degrees of relatedness by using either paternal or maternal cues, also suggests that sibling recognition in this species is well developed. This ability may be important in *R. cascadae* because relatedness among individuals in a population is potentially high; adults are philopatric and population sizes are small (Blaustein & O'Hara, pers. obs.; Deanna H. Olson, pers. comm.). Recognition of half siblings could further ensure against recognition errors if altruistic or cooperative acts are important in increasing an individual's inclusive fitness (Hamilton, 1964). Moreover, these tadpoles may be subjected to intense predation pressure and they elicit an alarm response from chemicals emanating from damaged conspecifics (Hews & Blaustein, 1985). The alarm response could be important in warning relatives within an aggregation. Kin recognition could enhance the ability of related individuals to reform in groups after disturbances, such as predation, cause tadpoles to disperse. Altruists in groups with many relatives could increase their inclusive fitness by aiding (e.g. warning) members of the group, compared to those individuals giving similar aid in groups with fewer or no kin.

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