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### *Hyla regilla* and *Rana pretiosa* Tadpoles Fail to Display Kin Recognition Behaviour

Larval anurans have emerged as one of the most interesting and well-studied of the vertebrate groups that display kin recognition behaviour (i.e. differential association between siblings and non-siblings). Each of the five species that have been tested previously (*Bufo americanus*, *B. boreas*, *Rana cascadae*, *R. sylvatica* and *R. aurora*) displayed some ability to distinguish siblings from non-siblings (e.g. reviews in Waldman 1981, 1984; Blaustein et al. 1987; Blaustein, in press). However, each differed in how the behaviour becomes expressed ontogenetically. Interspecific comparisons of anuran kin recognition are valuable in generating and testing hypotheses concerning functional significance and in delineating relationships between patterns of kin recognition behaviour and life history and ecological variables.

This paper reports results of sibling recognition tests in the larvae of western spotted frogs, *Rana pretiosa*, and Pacific treefrogs, *Hyla regilla*. Comparisons of these species with the three others we have studied is especially meaningful because we have employed the same rearing and experimental procedures for each. Also, *R. pretiosa* is closely related phylogenetically to *R. cascadae* and *R. aurora* (Wallace et al. 1973; Case 1978; Kluge & Mickevich 1979), two species that display kin association behaviour.

Two amplexant pairs of *H. regilla* were collected from a pond in Benton County, Oregon and were housed in separate aquaria in the laboratory. On 25 February 1986, each pair laid and fertilized over 200 eggs (clutches A and B). Immediately after hatching on 1 March, larvae of each clutch were transferred to rearing regimes. They were reared either with siblings (groups A and B) or with an equal mixture of siblings and non-siblings from each of two clutches (groups Am and Bm). In the mixed-rearing treatment, tadpoles from each clutch were placed on opposite sides of an aquarium divided by 1.5-mm gauge plastic mesh.

Two clutches of *R. pretiosa* were collected from a pond in Crook County, Oregon. Each clutch was housed separately in an aquarium until hatching on 16 April 1985. At this time, tadpoles of each clutch were reared with siblings (groups A and B), in a mixed group of siblings and non-siblings (groups Am and Bm), or in isolation (groups Ai and Bi). Two additional clutches were obtained from the same population in April 1986 and tadpoles from these clutches were reared in isolation (groups Ci and Di) or in a mixed group (groups Cm and Dm). Rearing and maintenance procedures for *H. regilla* and *R. pretiosa* were identical with those used for *R.*

*cascadae*, *R. aurora* and *Bufo boreas* tadpoles (e.g. O'Hara & Blaustein 1981, 1982; Blaustein & O'Hara 1986).

Procedures for testing association preferences were the same as those we have used in choice tests with other species (e.g. O'Hara & Blaustein 1981, 1982; Blaustein & O'Hara 1986). Individual tadpoles reared previously with siblings were given a choice of spending time near a group of familiar siblings or a group of unfamiliar non-siblings (stimulus groups). Tadpoles reared previously in mixed groups or in isolation were allowed to associate with unfamiliar siblings and unfamiliar non-siblings. The testing tank (122 × 44 × 30 cm) was partitioned into four compartments. To hold stimulus groups, a partition of 1.5-mm plastic mesh was placed 15 cm from each end. The central portion of the tank was divided longitudinally by an opaque, water-tight partition. Thus, we were able to test two tadpoles simultaneously (but independently) for sibling group preferences. Elsewhere, we have reported the results of control tests for the testing procedures and apparatus (e.g. O'Hara & Blaustein 1981; Blaustein & O'Hara 1987).

Stimulus groups of 30 (*H. regilla*) or 25 (*R. pretiosa*) tadpoles, matched for size and developmental stage (Gosner 1960), were used in each test. The time in seconds spent by each test tadpole in each half of the tank was recorded for four 5-min trials, at 10-min intervals. Between tests, stimulus groups were alternated from one end to the other and the tank was rinsed thoroughly. No individual was tested more than once and the same stimulus tadpoles were used in six consecutive tests and then discarded. The Wilcoxon signed-ranks test was used to analyse the total amount of time spent by tadpoles in sibling versus non-sibling halves of the test tank. We tested differences in the amount of time spent on the sibling portion of the test tank from a hypothetical random time (600 s) expected under the null hypothesis. The binomial test was used to determine whether numbers of tadpoles that spent most of their time (more than 600 s) on the sibling side of the test tank differed from random expectation. All statistical tests were two-tailed.

Regardless of how test individuals were reared, neither *R. pretiosa* nor *H. regilla* tadpoles associated preferentially with their siblings (Table 1). In late tests, however, *R. pretiosa* tadpoles from group B exhibited a statistically significant bias toward non-siblings. We have no explanation for these latter, unexpected results.

Kin recognition has now been investigated in seven species of larval anurans. Of these, *R. pretiosa* and *H. regilla* tadpoles are the only

Table I. Sibling association preferences in *R. pretiosa* and *H. regilla* tadpoles

Rearing treatment	Sibling group	Age (days)	Developmental stage	No. spending most of time toward		Seconds out of 1200 spent on sibling side	
				Siblings	Non-siblings	$\bar{X}$	SD
<b><i>R. pretiosa</i></b>							
Siblings							
Early tests	A	15-22	26-27	12	8	621.6	115.7
	B	15-22	26-28	10	10	618.8	138.6
Late tests	A	48-53	38-40	9	11	615.2	136.5
	B	48-52	38-41	5	15	543.8	112.1*
Mixed							
Early tests	Cm	12-20	26-28	12	8	625.3	127.2
	Dm	13-20	26-27	9	11	583.8	99.4
Late tests	Am	27-32	32-34	11	9	609.5	124.6
	Bm	28-33	33-35	10	10	582.8	200.6
Isolates							
	Ai	22-27	29-31	13	7	664.6	137.8
	Bi	22-27	29-30	11	9	620.8	174.7
	Ci	20-27	30-32	11	8	587.8	136.9
	Di	21-27	29-32	9	11	609.1	149.2
<b><i>H. regilla</i></b>							
Siblings							
Early tests	A	12-20	26-28	9	11	609.2	190.4
	B	12-20	26-28	9	11	551.4	168.3
Late tests	A	37-46	37-40	10	10	595.0	155.0
	B	37-47	37-40	8	12	574.4	161.2
Mixed							
Early tests	Am	13-20	26-28	10	10	605.6	185.5
	Bm	13-21	26-28	9	11	607.8	218.5
Late tests	Am	38-47	38-40	11	9	610.2	179.2
	Bm	38-45	37-40	12	7	637.0	186.4

See text for an explanation of sibling groups.

\*  $P < 0.05$ .

anurans that show no kin association behaviour. The differences in behaviour displayed by anuran larvae may reflect differences in certain natural history traits (O'Hara & Blaustein 1985; Blaustein, in press). For example, larvae of *R. cascadae*, *B. boreas* and *B. americanus* are highly social in nature and in laboratory tests (O'Hara & Blaustein 1982, 1985; Waldman 1982). However, *H. regilla* tadpoles also exhibit a strong attraction to conspecifics (either siblings or non-siblings) in laboratory tests, whereas *R. pretiosa* tadpoles do not (O'Hara & Blaustein, unpublished data). To interpret the biological significance of kin association behaviour in anurans it will be necessary to consider other ecological and natural history traits of a species, in

addition to sociality. Among these are larval dispersal and spacing patterns, conditions surrounding early development, habitat quality and structure, food availability and distribution, population size and density, and predators. The failure of *R. pretiosa* and *H. regilla* to associate with kin may provide us with valuable comparative information for discerning patterns in kin recognition among larval anurans. In seeking causative explanations for kin recognition, it will be important to study not only those species that possess such abilities, but also those that lack the behaviour.

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