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KIN PREFERENCE BEHAVIOUR IS PRESENT AFTER METAMORPHOSIS IN *RANA CASCADAE* FROGS

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Abstract. Kin recognition was investigated in newly metamorphosed *Rana cascadae* frogs. Previous work has shown that larvae of this species prefer to associate with siblings over non-siblings. Juvenile frogs from three clutches were reared with siblings and tested for sibling preference as larvae and at 4–12 days and 39–47 days after metamorphosis. Tadpoles and froglets of the three clutches displayed a significant preference to associate with siblings.

Certain species of animals can recognize their kin (see reviews by Bekoff 1981, Holmes & Sherman 1982 and references within). Studies of kin recognition have enhanced our knowledge of the ontogeny of social behaviour, animal social systems, and the sensory bases of kin-oriented behaviour, and have helped us develop ideas concerning kin selection and inclusive fitness.

Recent experimental studies of kin recognition in anuran amphibian larvae are of particular interest because the life history of these species enables the investigation of the effects of various rearing regimes on the ontogeny of recognition behaviour (Blaustein & O'Hara 1981, 1982a, b; O'Hara & Blaustein 1981, 1982; Waldman 1981). For example, tadpoles of the Cascades frog (*Rana cascadae*) can distinguish unfamiliar kin from unfamiliar non-kin after being reared in isolation from an early embryonic stage (Blaustein & O'Hara 1981, 1982a) or after being reared together with kin and non-kin (O'Hara & Blaustein 1981). These tadpoles can also distinguish between full siblings and half siblings and between half siblings and non-siblings (Blaustein & O'Hara 1982a).

Although we have learned a great deal concerning the mechanisms mediating kin recognition behaviour and have formulated hypotheses to explain its adaptive value during the larval stage (for discussions see Blaustein & O'Hara 1982a; O'Hara & Blaustein 1982; Blaustein 1983), there have been no reports on the study of kin recognition after anurans metamorphose. Generally, tadpoles are aquatic, primarily gill-breathing, swimming omnivores, which metamorphose into amphibious (or terrestrial), lung-breathing carnivores. This study investigates kin recognition behaviour after the drastic metamorphic changes have occurred in anatomy, physiology, ecology and behaviour. We also address the question of how kin recognition behaviour may be adaptive after the larval stage.

Methods

Larval Rearing Conditions and Testing

We obtained three clutches of *R. cascadae* eggs in May 1982 from one population in the Oregon Cascade Mountains (Linn County). Two clutches were obtained by capturing clasping pairs and allowing eggs to be laid and fertilized in the laboratory. A third clutch, laid by another female, was collected in the field (clutches A, B and C respectively). All clutches hatched within 4 days of one another. Each clutch was reared in a separate 38- or 98-litre aquarium. The densities of tadpoles were approximately equal in each aquarium. Tadpoles were fed daily with rabbit food pellets. All tanks were cleaned and provided with fresh water about every 5 days and were housed in the same laboratory which was kept at 20–24°C under a 14L : 10D photoperiod.

We tested tadpoles for kin recognition behaviour by assessing the amount of time a test individual spent near a sibling stimulus group and near a non-sibling stimulus group in choice experiments (see O'Hara & Blaustein 1981). Test tadpoles were used in four 5-min trials at 10-min intervals in a test aquarium. Test tadpoles were only used once and then were allowed to grow and undergo metamorphosis in their respective tanks. As we expected, given our earlier results (Blaustein & O'Hara 1981, 1982a; O'Hara & Blaustein 1981), the majority of tadpoles preferred to associate with siblings over non-siblings. The kin preference data for these experiments are presented elsewhere (Blaustein & O'Hara 1982b).

Froglet Kin Preference Tests

After metamorphosis, test froglets were maintained in groups of two to four related individuals

in 0.5-litre opaque containers. Several hundred froglets that were to be used as stimulus individuals in tests were housed separately from the test individuals in groups of one to five related individuals in 0.5-litre opaque containers. Stimulus animals were metamorphosed individuals that had not been used as test tadpoles. Each froglet was hand-fed daily with chunks of liver, live flies (*Drosophila melanogaster* and *D. gibberosa*) or a combination of flies and liver.

We tested froglets that were fully metamorphosed (tail entirely resorbed) for kin preference behaviour by placing a single test individual into the centre of a transparent plastic tube that was 84 cm long with a 5.5-cm diameter (Fig. 1). Ten froglets from the sibling group and 10 froglets from a non-sibling group were placed in opposite end chambers. Only healthy, active froglets were used in tests. Stimulus animals and test animals were approximately 1.5–3 cm in snout–vent length. The two stimulus groups had no water-borne contact with each other or with the test animal; the tube was kept moist by initially adding a few drops of water throughout the tube. The test animal was separated from the stimulus animals by 1.5-mm plastic mesh. Thus some visual, tactile, and airborne chemical contact was possible between stimulus and test animals.

By placing marks on the tube, we divided it into thirds. The middle portion was regarded as a 'neutral' section and the two ends were designated 'sibling' and 'non-sibling' sections. We assessed sibling preference behaviour by placing a test froglet in the centre of the tube and recording where it positioned itself in 15 observation periods conducted at approximately 30-min intervals beginning 30 min after the test animal was placed in a tube. To ensure that observations were independent, a test animal that did not move at least 5 cm between observation periods was placed back into the centre of the tube for the next trial and that observation was not recorded. After each test, the tube and end compartments were thoroughly rinsed, the position of the tube was reversed, and new stimulus and test animals were used.

We obtained 66 individuals for testing. We chose 39 froglets (10 from clutch A, 15 from clutch B and 14 from clutch C) that were tested as tadpoles, and tested them 4–12 days after metamorphosis (early tests) for sibling preferences. We tested 19 of these same froglets and two additional ones in late tests (39–47 days post-metamorphosis). The 25 remaining froglets were used as controls. Control animals were subjected to the same testing procedures as experimentals except that non-sibling stimulus groups were placed in both end compartments. Control animals were tested 2–15 days after metamorphosis. Only animals from groups A and C were used in controls, owing to the low numbers of metamorphosed B individuals.

All froglets were tested once except the 19 animals used in both early and late tests. No stimulus froglet was used as a test individual and no test froglet was used as a stimulus animal. Stimulus froglets were used in no more than five

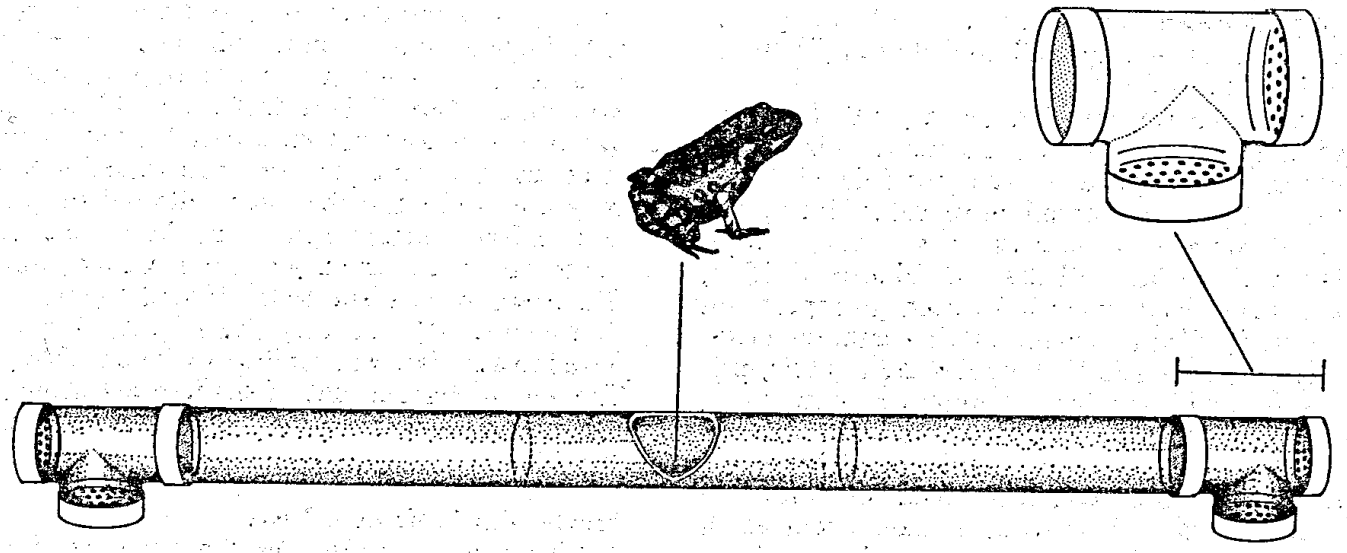

Fig. 1. Test apparatus showing placement of test individuals and stimulus animals. The cenrtal portion of the tube was 66 cm long. Each end compartment was 9 cm long.

tests. Chi-square tests were used to determine if the observations of froglets in the three portions of the test apparatus, or in the two ends only, were distributed differently from a random expectation. We used the binomial test to determine if the number of individuals associating most often on either end of the apparatus differed from a random expectation.

Results

The distribution of observations in early and late tests departed from random expectation in two ways (Table I). First, the relatively small number of observations in the neutral portion of the tube suggests that froglets preferred to associate with conspecifics (or preferred tank ends, possibly because of edge effects) rather than to remain in the middle portion of the test apparatus. Second, excluding observations from the middle portion of the tube, results of the analysis of observations in the end portions of the tube revealed that froglets preferred to associate with siblings than with non-siblings. The number of froglets preferring to associate on the sibling portion of the tube also indicated a preference for siblings (Table I). Control animals did not prefer one non-sibling stimulus group to another (Table II). However, there was a significant departure from random expectation in the observations from all three portions of the tube, with few observations in the neutral portion of the tube. Control animals, like experimental animals, chose to associate with conspecifics rather than remain in the neutral portion of the tube. Furthermore, there was no bias in the testing procedures or in the apparatus, since froglets did not prefer one particular end of the apparatus to another (Table III). Froglets were generally active in tests. For example, 37 of the 39 animals tested in early tests sampled both stimulus portions of the tube. Animals in control tests also moved readily from one stimulus end to another.

Since test and control froglets used in this study were taken from a group of 120 tadpoles that were tested for sibling association preferences (see Blaustein & O'Hara 1982b), we tested if the proportion of froglets (using the 39 froglets from experimental tests) that were observed most often near siblings was significantly different from the proportion of tadpoles that spent

Table I. Summary of the Number of Observations of Subjects in the Three Portions of the Test Apparatus, and the Number of Froglets Associating Most Often in the Sibling Portion of the Test Apparatus, in Experimental Groups

Group	N	Number of observations in: Sibling area	Neutral area	Non-sibling area	Number of froglets spending most of time in sibling area	Binomial $P <$ (two-tailed)
Early tests (4–12 days after metamorphosis)						
A	10	79	21	50	7	
B	15	149	19	57	14	
C	14	108	34	68	10	
Total	39	336	74	175	31	0.0003
Comparison of the distribution of all observations combined: $\chi^2 = 192.0$, $df = 4$, $P < 0.001$. Comparison of the distribution of observations in the sibling and non-sibling portions for all groups combined: $\chi^2 = 56.7$, $df = 2$, $P < 0.001$.						
Late tests (39–47 days after metamorphosis)						
A	6	53	11	26	4	
B	3	28	9	8	3	
C	12*	93	32	55	10	
Total	21	174	52	89	17	0.007
Comparison of the distribution of all observations combined: $\chi^2 = 78.8$, $df = 4$, $P < 0.05$. Comparison of the distribution of observations in the sibling and non-sibling portions for all groups combined: $\chi^2 = 30.1$, $df = 2$, $P < 0.001$.						

*Two animals were not tested as tadpoles.

Table II. Summary of the Number of Observations of Subjects in the Three Portions of the Test Apparatus in Control Tests. Stimulus Groups were Two Non-sibling Groups Composed of 10 Animals Each

Control group	N	Number of observations near:			Comparison of total distributions χ^2	df	P	Comparison of distributions in stimulus ends χ^2	df	P
		C stimulus	Neutral	B stimulus						
A	11	77	18	70	37.8	2	<0.005	0.33	1	NS
		A stimulus	Neutral	B stimulus						
C	14	73	57	80	3.95	2	<0.005	0.32	1	NS

NS=not significant.

Table III. Distributions of Control Froglets in the Ends of the Tube Nearest or Farthest from the Observer and in the Middle Portion of the Tube

Group	N	Number associating most often in: Near end	Far end	Tie	Number of observations Near end	Middle	Far end	Comparisons of total distributions χ^2	df	P	Comparison of distributions by stimulus ends χ^2	df	P
A	11	6	5	0	71	18	76	41.4	2	<0.001	0.33	1	>0.05
C	14	5	7	2*	74	57	79						
Total	25	11	12	2	145	75	155						

*Observations of two froglets were distributed equally between the two end stimulus groups.

most of their time near siblings. These proportions were not significantly different from one another (31/39 versus 87/120; $\chi^2=0.39$, $df=1$, $P>0.05$).

Discussion

Rana cascadae tadpoles differentiate between kin and non-kin through water-borne chemical cues which they probably perceive through olfaction or taste (Blaustein & O'Hara 1982b). We do not know what mechanism froglets use to distinguish kin, but it seems likely that airborne chemical cues would allow finer discrimination than visual cues. Chemical cues are important in various contexts of amphibian life (see discussion in Blaustein & O'Hara 1982b). For example, olfaction has been implicated as an important sense in finding food (Stoddart 1980) and in homing ability and orientation in frogs, toads and salamanders (e.g. Barthalmus & Bellis 1972; Grubb 1973a, b). Furthermore, salamanders (*Plethodon jordani*) can discriminate between the odours of neighbouring and non-neighbouring conspecifics of the same sex (Madison 1975).

Few investigators have followed the retention of larval behaviour patterns through metamorphosis in amphibians. Whether behaviour patterns are retained, modified or lost during the metamorphic transition depends on the species and behavioural trait being examined. Tadpoles of several species of anurans (McKeown 1968; Goodyear & Altig 1971) and larvae of three species of ambystomatid salamanders (Tomson & Ferguson 1972) orient away from and perpendicular to the shoreline (Y-axis orientation) prior to metamorphosis, but reverse the direction of orientation during and after metamorphosis. A similar reversal in 'habitat' choice behaviours was recorded by O'Hara (1974) in *Bufo americanus* at metamorphosis. Prior to metamorphosis tadpoles avoided an artificial striped substrate in two-choice tests, but during late metamorphosis they responded positively to the same substrate. Interestingly, in similar choice tests conducted by Wiens (1970), *R. aurora* juvenile frogs retained a preference for a striped substrate which had become established in young larvae. Wassersug & Hessler (1971) reported a loss in the tendency to aggregate with conspecifics after *Xenopus laevis* tadpoles metamorphosed.

Because froglets in our tests were reared with full siblings, they may have based their kin preferences on familiarity. However, it is not

possible to distinguish between experiential and genetic determinants of recognition at this time (see Blaustein 1983).

Although kin recognition exists in metamorphosed *R. cascadae*, it is possible that an ability to recognize kin at this stage in the life cycle confers no selective advantage. The behaviour could be retained after metamorphosis because there is no selection against retaining it, or it may be gradually lost as frogs age (i.e. subsequent to the ages we used in our tests).

If there are selective advantages to kin recognition ability after metamorphosis, what might they be? Since *R. cascadae* froglets are not known to form groups either at the time of metamorphosis or thereafter, it seems unlikely that kin recognition is used in aggregation behaviour, a function we proposed for kin recognition in the larval stage (O'Hara & Blaustein 1981). However, young frogs might develop preferences for environmental cues of which chemical cues could be one component) that could be used in habitat selection or homing behaviour. In our experiments, perhaps social cues were used because they were the only 'environmental' cues familiar to the froglets.

If *R. cascadae* individuals are site-specific (philopatric), adults could use their kin recognition ability to avoid breeding with close relatives (e.g. full siblings) and ensure breeding with more distant relatives or unrelated individuals. It is also possible that there are advantages to inbreeding and frogs may seek kin for such a purpose (see papers by Bateson 1979, 1980, 1982, and discussion in Shields 1982). Certain species of frogs and toads are known to be philopatric and have a good ability to home after being displaced. Some species will not join a different breeding chorus when released in ponds near their original pond (see Jameson 1957; Oldham 1966; discussion in Baker 1978).

We are now undertaking long-term studies concerning *R. cascadae* population structure, reproductive behaviour and adult social organization to help answer some of the questions posed above.

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