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***Rana cascadae* tadpoles aggregate with siblings: an experimental field study**

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Abstract. Previous laboratory studies have shown that *Rana cascadae* larvae preferentially associate with siblings over non-siblings in choice tests. This study, conducted during three consecutive summers, tests the hypothesis that *R. cascadae* larvae aggregate nonrandomly with respect to sibship in natural ponds. Pairs of sibships were reared in separate tanks or together in the same tank in the laboratory. Each sibship within a pair was then stained with neutral red or methylene blue dye and released together in a natural pond. Over a period of several days, aggregations of tadpoles within test ponds were repeatedly captured, censused for sibship composition, and released. In control tests, two groups of tadpoles from the same sibship were dyed different colors and released together. In total, 25 different tests were conducted using tadpoles from 31 sibships and 456 aggregations were sampled. The distribution of color compositions of aggregations in control tests did not differ from an expected random distribution. Color compositions of aggregations in experimental tests differed from controls and from an expected random distribution. Aggregations in these tests tended to be dominated by one of the two colors (sibships). We conclude that *R. cascadae* tadpoles recognize and prefer to aggregate with siblings in natural field conditions. Circumstances of early rearing (i.e., whether tadpoles were reared with siblings or in mixed sibling/non-sibling groups) had no influence on preferences to associate with siblings, but there was an inverse correlation between group size and sibling association.

The concepts of inclusive fitness and kin selection (Hamilton 1964; Maynard Smith 1964) predict that genetic relatedness can be a critical variable in the evolution of social behavior. Indeed, increasing empirical evidence suggests that among group-living animals, nonrandom associations among kin are more common than previously realized (Wilson 1975; Kurland 1980). Various patterns in population structure, dispersal, mating systems, or habitat selection can act to produce nonrandom genotypic associations. Additionally, the evolutionary consequences of kin selection may influence a population's structure and life history characteristics. When kinship among social interactants affects inclusive fitness, one might expect to find patterns in gregariousness, dispersal, spacing, mating, or competitive and forag-

ing tactics to be correspondingly attuned to these relationships. For example, individuals that are cannibalistic may direct their predation towards unrelated conspecifics. Similarly, individuals competing intraspecifically for a limited resource may direct their competitive behaviors toward unrelated conspecifics. Thus, the intricacies of many social and ecological patterns may not be fully explained by models of natural selection that ignore the consequences of kinship.

It is difficult to demonstrate whether kin selection has been instrumental in shaping an organism's social behavior. The recent proliferation of studies demonstrating sibling recognition abilities among crustaceans (Linsenmair 1972), insects (e.g., Greenberg 1979; Ross and Gamboa 1981; Breed 1983; Getz and Smith 1983), fish (Quinn and Busack 1985), amphibians (O'Hara and Blaustein 1981, 1982; Waldman 1981, 1984), birds (Radesäter 1976; Bateson 1982; Beecher and Beecher 1983), small mammals (e.g., Porter and Wyrick 1979; Grau 1982; Holmes and Sherman 1982; Kareem and Barnard 1982), and non-human primates (Meikle and Vessey 1981) has provided indirect support to the postulate that kin selection is important in social behavior evolution. However, interpreting the functional significance of these recognition behaviors is often difficult.

In some studies (e.g., Greenberg 1979; O'Hara and Blaustein 1981; Beecher and Beecher 1983; Holmes and Sherman 1982), the experimental evidence of kin recognition is consistent with known social and natural history characteristics of a species. In other species, either little is known about how individuals behave under natural conditions, or the natural history evidence that is available suggests that kin associations are unlikely (e.g., O'Hara and Blaustein 1982; Waldman 1984). In the latter cases especially, it is questionable whether the recognition behavior is truly a kin selected trait, an experimental artifact of a behavior used in some different environmental context (e.g., habitat or food selection), or if kin recognition has any function in contemporary populations of that species. Unfortunately, laboratory experiments can only approximate and simplify the complex social and ecological conditions of nature. Field studies conducted in natural settings, that investigate patterns in kin associations and their adaptive significance are needed to help answer these questions.

We have investigated kin recognition in tadpoles of the Cascades frog (*Rana cascadae*) in laboratory experiments because of several key natural history characteristics displayed by this species: 1) tadpoles may not disperse far

from their hatching sites (O'Hara 1981), 2) tadpoles form relatively small, compact aggregations in nature (O'Hara 1981; Blaustein and O'Hara 1982a), and 3) the size of tadpole aggregations is much smaller than the average clutch size of a single female (O'Hara and Blaustein 1981; Blaustein and O'Hara 1982a). These characteristics led us to hypothesize that interactions among kin are likely in natural populations of *R. cascadae* larvae and that kin recognition behaviors may have evolved to facilitate cooperative behavior among kin. Our hypothesis that larval *R. cascadae* would display an ability to recognize kin has been supported by numerous laboratory experiments (e.g., Blaustein and O'Hara 1981, 1982a; O'Hara and Blaustein 1981; discussion in Blaustein 1983). *R. cascadae* larvae are capable of distinguishing between conspecifics solely on the basis of sibship specific chemical cues (Blaustein and O'Hara 1982b). The present study was designed to test the hypothesis that *R. cascadae* tadpoles aggregate nonrandomly with respect to sibship under natural field conditions.

Materials and methods

Test animals and rearing conditions. *R. cascadae* tadpoles used in field experiments were from 31 distinct clutches collected from six different populations in Deschutes, Linn, and Marion Counties, Oregon. Clutches were collected in May, June, and July of 1982, 1983, and 1984, and were returned to the laboratory for rearing.

Procedures for larval rearing were similar to those used previously (e.g., O'Hara and Blaustein 1981). Tadpoles of each sibship were either reared exclusively with siblings or as mixtures of siblings and non-siblings. In the mixed rearing treatment, equal numbers of embryos from two sibships were placed on opposite sides of a tank divided by 1.5 mm plastic mesh. The plastic mesh functioned to keep sib groups separate while allowing visual, olfactory, and some tactile exchange between groups. Air stones positioned in the center of each tank kept water constantly mixed between sides (O'Hara and Blaustein 1981). Tadpoles in both rearing treatments were kept in tanks containing 38 l or 160 l of dechlorinated tap water. The size of the tank used had no apparent effect on the outcome of experiments. Tadpoles were fed rabbit food pellets once daily and water was changed in the rearing tanks every 6–10 days. All tanks were housed in the same laboratory which was kept at 20–24°C under a 14L:10D photoperiod. All tadpole groups were reared in the laboratory for 15–40 days prior to release in natural ponds.

Experimental ponds. Field experiments were conducted in June, July, and August of 1982, 1983, and 1984. Twenty-five tests were completed at 18 different ponds, located at three different sites in Deschutes County, Oregon. The ponds selected for experiments were typical of the ephemeral montane ponds usually used by *R. cascadae* for breeding and each site supported a breeding population of *R. cascadae*. Ponds ranged in size from about 16 to 120 m² with a maximum water depth of 35 cm. *R. cascadae* also breed in some permanent lakes and large ponds and tests were attempted at two such localities. However, high dispersal and possibly predation of released larvae at these sites precluded extensive sampling. Substrates of test ponds were generally composed of compact, fine, organic particulates. The dominant macrovegetation was *Carex sp.*, which was distributed

along the pond shallows and sometimes occurred in emergent clumps throughout a pond. The water of all ponds was clear and tadpoles were easily observable.

Every test pond contained natural insect predators (dytiscid larvae and odonate naiads) of *R. cascadae* larvae, but these were generally at low densities and had little apparent impact on tadpole numbers. In only one test were predators (dytiscid larvae) in sufficient abundance to appreciably lower tadpole numbers and in that case the data were discarded from analyses.

Ponds with large resident populations of *R. cascadae* were not used in experiments. Although eight of the experimental ponds were used by *R. cascadae* for breeding, egg clutches were removed prior to the initiation of a test. Several ponds contained resident Pacific treefrog (*Hyla regilla*) tadpoles in low densities. No differences were observed in *R. cascadae* aggregation behavior in ponds with and without *H. regilla*.

Field experimental procedure. To test the hypothesis that *R. cascadae* larvae aggregate nonrandomly with respect to sibship in natural ponds, we: (1) reared sibships separately or together in mixed groups in the laboratory; (2) marked tadpoles from two sibships with different dye colors; (3) released pairs of sibships together in the same pond; and (4) repeatedly collected natural aggregations and censused their sibship composition. Ten tests were completed using 19 clutches of tadpoles reared with siblings, nine tests using 12 clutches reared in mixed sib/non-sib groups, and six control tests using six clutches. Control tests were designed to determine if tadpoles assorted randomly with respect to dye color. In these tests, two groups of tadpoles from the same sibship were dyed different colors and released together in the same pond.

To enable identification of different sibships, tadpoles were stained with solutions of neutral red or methylene blue vital dyes. Methods for using these dyes were similar to those used on tadpoles previously (Herreid and Kinney 1966; Guttman and Creasey 1973; O'Hara 1981; Travis 1981; Waldman 1982). Equal numbers of tadpoles from the same or different sibships were placed in solutions of each dye. After 10 to 15 h, groups were removed from the dye, mixed together, transported to a natural pond, and released. Colors remained distinguishable for 14 to 19 days. All experimental pairings were composed of sibships of the same age and developmental stage. Also, to avoid the potential confounding influence of differing population source on tadpole aggregation responses to each other (e.g., Waldman 1981, 1982), 24 of the 25 test pairings were between sibships from the same population.

R. cascadae tadpoles typically aggregate in small, stationary, dense clusters numbering less than 40 individuals (personal observations). Experimental tadpoles in this study formed aggregations indistinguishable in size and behavior from natural ones. For collection purposes, an aggregation was defined as a group of six or more tadpoles positioned in contact with one another or within approximately 2 cm of each other. Aggregation sizes of six and fewer tadpoles are common in natural populations of *R. cascadae* (personal observations).

Each pair of test groups was released in the center of an experimental pond. Censusing of aggregates commenced the following day (within 20–24 hours) and tests extended for 3 to 19 days following release. The length of the sam-

pling period depended on how long dyes were discernable, whether ponds became desiccated during sampling, and various time constraints on the observer. Ponds were sampled when tadpoles were active, between 0930 and 1800 hrs. at 1.5 to 3 h intervals. Samples were taken during sunny and overcast days, but not during rainy conditions because of the great difficulty in seeing tadpoles at these times.

At each sampling, an aggregation was located and a screened enclosure ($38 \times 38 \times 20$ cm), open at the top and bottom, was quickly dropped over it. All individuals were then transferred by dip net to a container where the numbers of tadpoles of each color were counted. Prior to capture, it was usually not possible to distinguish between red and blue tadpoles within an aggregation. If approximately 10% or more members of an aggregate escaped prior to capture, no data were taken. In no case was the sampling enclosure too small to capture all members of an aggregation. Sometimes the process of collecting one aggregation caused the scattering of individuals in other aggregations in the same pond. If this occurred, no further collections were made until the next sampling period. Otherwise, as many aggregations were collected as possible from each pond during each sampling period. After all aggregations in a pond were collected, they were mixed together and released. Although the same individuals within a pond were repeatedly sampled, the effect of each sampling was to cause a disruption and mixing of all aggregations, thus ensuring that samples taken throughout a day were independent. All collections and counts were made by one person. At the beginning and end of tests, tadpoles were developmentally staged (Gosner 1960). At the end of nine tests, all tadpoles within ponds were collected and counted to determine the extent of mortality and whether mortality was equal for the two groups.

Not all tests initiated were carried to completion and included in analyses. In three tests, excessive mortality, possibly caused by improper use of the dye, was the basis for discarding data. Although these dyes can potentially retard developmental rates (Travis 1981), when dyes are used in weak concentrations *R. cascadae* tadpoles behave normally and reach metamorphosis without any apparent adverse effects. Pond desiccation caused several tests to be terminated earlier than intended.

Statistical analyses. For each of the 25 tests, data were tabulated as the number of red and blue tadpoles in each aggregation collected. The proportion of red tadpoles (the decision to use red vs. blue tadpoles was arbitrary) comprising each aggregation was calculated and arranged in one of 20 classes, each ranging 5 percentage points, from 0 to 100% red tadpoles. We predicted that if tadpoles associate randomly with respect to sibship and color, these values should be normally distributed with a mean and median approaching 50% red tadpoles and with few aggregations falling at the extremes of the distribution (i.e., high and low percentages). If there was no sibship effect, control and experimental distributions should not differ from one another. If, however, tadpoles associate positively with sibship or color, we expected to obtain a bimodal, nonnormal distribution with most aggregations being dominated by red or blue tadpoles. The mean and median of these distributions would also be expected to approach 50% red tadpoles if there were no differential effects attributable to the dyes or sibships (e.g., differential mortality or respon-

siveness of tadpoles). Also, control and experimental distributions should differ from one another.

For each of the three treatments (controls, sibships reared apart, and sibships reared together), we lumped the data from all ponds to form three frequency distributions of the percent red tadpoles composing each aggregation. Using the Kolmogorov-Smirnov goodness of fit test (Sokal and Rohlf 1981), these distributions were tested for fit to an expected normal distribution. The Kolmogorov-Smirnov two sample test (Sokal and Rohlf 1981), was used to test differences in distributions between controls and sibships reared apart, controls and sibships reared together, and between sibships reared apart and sibships reared together. In each test with ten or more observations (16 of 19 tests), observed frequency distributions were compared to an expected random distribution based on the control data. In 10 tests, it was possible to compare data collected early with data collected late in the same test using the Kolmogorov-Smirnov two sample test. This procedure would determine if there were any trends in association behavior with time (e.g., an increase or decrease in sib association behavior). To test whether group compositions significantly varied between tests within each treatment, we used the Kruskal-Wallis test (Sokal and Rohlf 1981). Finally, to determine if there was any relationship between the number of tadpoles in an aggregation and sibship composition, product-moment correlation coefficients were calculated for each of the two experimental groups (variables were aggregation size vs. percentage composition of dominant color in the aggregation). All statistical tests were two-tailed with a significance level of $P \leq 0.05$.

Results

Controls

Six control tests were conducted in which two groups of tadpoles from one sibship were dyed different colors and released together. The combined frequency distribution of these data (Fig. 1a) indicates that *R. cascadae* tadpoles responded randomly to dye color when associating with siblings in the field. The data do not differ from an expected normal distribution (Kolmogorov-Smirnov $D=0.057$, $N=103$, $P>0.05$) with most aggregations (69%) composed of 40 to 60% red tadpoles. Only 7% of all aggregations were strongly dominated (composed of 70% or greater) by red or blue tadpoles. No differential bias was apparent toward red or blue tadpoles dominating most aggregations as indicated by the centralized mean (50.9%) and median (50.0%).

A summary of results from each control test (Table 1) indicates that there was some variability between tests in number of tadpoles released, the length of a test, and the mean number of tadpoles per aggregation. No evidence was found that any of these variables or that differences between ponds, such as pond size or habitat variation, affected color compositions of aggregates. Indeed, there was no significant difference in color composition of aggregates between the six tests (Kruskal-Wallis test, $H=7.52$, d.f. = 5, $P>0.1$).

Sibships reared apart

In 10 tests, groups of tadpoles from two sibships reared apart were dyed different colors and released together. The combined frequency distribution of aggregation composi-

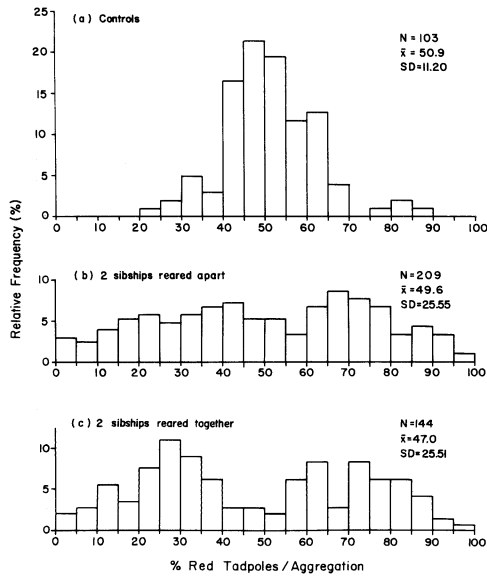


Fig. 1 a–c. Frequency distributions of the percentage of sibling tadpoles dyed red comprising each aggregation sampled in the three experimental treatments. *N* number of aggregations sampled

tions (Fig. 1 b) does not fit an expected normal distribution (Kolmogorov-Smirnov $D=0.075$, $N=209$, $P<0.01$) and significantly differs from the distribution obtained for control data ($D=0.270$, $P<0.01$). Compared with the control data, observations were skewed away from the middle and

toward each tail of the distribution; more aggregations were clearly dominated by one of the two sibships. Only 21% (vs. 69% for controls) of all aggregations were composed of 40 to 60% red tadpoles, while 51% (vs. 7% for controls) were composed of 70% or greater of either sibship. When distributions of aggregation compositions for each test were tested for goodness of fit to a hypothetical distribution based on the control data, 7 of 10 tests were significantly different (Table 1). These data demonstrate that *R. cascadae* tadpoles preferentially aggregate with siblings over non-siblings in natural ponds.

A summary of the data for these tests (Table 1) indicates comparable variability to controls in numbers of tadpoles released per test, test length, and mean number of tadpoles per aggregation. Although aggregation sizes tended to be smaller in this test series ($\bar{x}=22.0$ tadpoles) than controls ($\bar{x}=27.3$ tadpoles) the difference was not statistically significant (approximate *t*-test, $t'=1.898$, $P>0.05$). Aggregation size, however, was inversely correlated with the percentage composition of the dominant sibship within each aggregation ($r=-0.305$, $d.f.=207$, $P<0.01$). While some variability occurred between tests in the numbers of red vs. blue dominated aggregations as indicated by the mean percentage red tadpoles/aggregation/test (Table 1), no consistent bias was evident (grand mean=49.6%, median=50.0%). The mean and median percentage red tadpoles/aggregation when reds were dominant (i.e., greater than 50% reds) was 71.7%; the mean % blue tadpoles/aggregation when blues were dominant was 72.1%. There was no significant variation in group compositions by color among the 10 tests

Table 1. Summarization of relevant data for each field test. The Kolmogorov-Smirnov statistic reflects a comparison of color compositions of aggregations observed in experimental tests with an expected random distribution derived from control tests

Treatment	Test group	No. individuals released of each color	Dev. stage	Length of test (days)	No. aggregations censused	Mean no. tadpoles/aggregation	Mean % (S.D.) red tadpoles/aggregation	Kolmogorov-Smirnov statistic
I. Controls: Tadpoles from 1 sibship reared together	1	200	36–38	14	10	10.4	58.0 (15.38)	
	2	145	34–37	9	38	27.2	52.6 (10.92)	
	3	160	30–32	7	18	17.0	49.1 (9.69)	
	4	160	29–32	7	11	18.6	49.3 (14.90)	
	5	151	30–33	3	16	56.7	47.7 (8.50)	
	6	240	35–38	9	10	27.1	47.8 (6.74)	
II. Tadpoles from 2 sibships reared apart	1	150	35–37	8	37	20.1	56.1 (19.20)	0.285***
	2	200	36–38	19	29	15.1	52.7 (29.41)	0.370***
	3	200	35–38	14	32	13.4	49.1 (29.40)	0.359***
	4	170	31–34	8	11	29.3	54.9 (20.70)	0.285
	5	145	34–37	9	28	26.2	46.1 (30.41)	0.429***
	6	105	32–36	9	20	37.3	50.9 (16.92)	0.193
	7	250	34–38	9	10	28.6	47.5 (23.33)	0.419*
	8	217	35–39	9	11	25.4	50.2 (27.65)	0.396*
	9	211	35–37	3	14	24.4	50.0 (20.91)	0.224
	10	150	35–38	13	17	16.1	34.9 (25.04)	0.569***
III. Tadpoles from 2 sibships reared together	1	165	35–37	7	39	13.7	50.0 (27.25)	0.333***
	2	150	28–31	3	21	19.2	47.1 (28.77)	0.350***
	3	200	30–32	3	14	19.6	40.2 (24.70)	0.565***
	4	110	32–35	7	10	19.0	45.1 (19.62)	0.493***
	5	150	30–32	3	19	25.3	46.1 (24.92)	0.343**
	6	200	29–31	3	30	16.1	52.3 (25.93)	0.326***
	7	100	32–35	7	7	14.7	41.8 (12.53)	—
	8	222	28–30	7	2	—	—	—
	9	180	27–30	7	2	—	—	—

* $P<0.05$ ** $P<0.02$ *** $P<0.01$

(Kruskal-Wallis test, $H = 15.72$, d.f. = 9, $P > 0.05$). In six tests, a comparison of color compositions of aggregations collected on the first and last sampling days revealed no significant differences and, thus, no trends with time in sibling aggregation behavior.

Sibships reared together

In nine tests, tadpoles from two sibships were reared together and then released. The combined frequency distribution of these data (Fig. 1c) was distinctly bimodal and differed from an expected normal distribution (Kolmogorov-Smirnov $D = 0.098$, $N = 144$, $P < 0.01$) and from the distribution obtained from control tests ($D = 0.372$, $P < 0.01$). These data did not, however, differ in distribution from the data for sibships reared apart ($D = 0.111$, $P > 0.05$). Only 14% of aggregations were composed of 40 to 60% red tadpoles and 60% of all aggregations were composed of 70% or greater of one or the other sibship. The mean and median percentage red tadpoles/aggregation for the nine tests were 47.0% and 44.3% respectively, indicating a slight bias toward blue dominated aggregations. However, when blues were dominant the mean group composition was 74.2% blues and when reds were dominant was 71.4% reds.

Six tests, in which sample sizes were 10 or greater, were analyzed for goodness of fit to a hypothetical distribution based on control data and all were significantly different (Table 1). There was no significant variation in group compositions by color among tests (Kruskal-Wallis test, $H = 6.62$, d.f. = 5, $P > 0.1$). Mean aggregation sizes (Table 1) were smaller than in control tests (18.4 vs. 27.3 tadpoles; $t' = 3.19$, $P < 0.01$) but did not differ from sizes of sibships reared apart. Also, the size of an aggregation was inversely correlated with the percentage compositions of the dominant sibship within each aggregation ($r = -0.290$, d.f. = 142, $P < 0.01$). In four tests, no significant differences were found in comparison of color compositions of aggregations collected on the first and last sampling days.

Tadpole mortality

In nine tests, all experimental tadpoles within a pond were collected on the final day of sampling to assess mortality. The length of the sampling period for these tests ranged from 7 to 19 days. Estimated overall mortality for each of the nine tests was 4% to 26% ($\bar{x} = 14\%$). In seven of these ponds, tadpole colors were discernible and it was possible to estimate relative mortality for red and blue-dyed tadpoles. In four tests, more blues than reds remained at the end of the experiment (percentage of all tadpoles remaining that were blue = 50%, 51%, 54%, and 56%), but the bias was not significantly different from a random expectation in any test (binomial test of numbers of blues vs. numbers of reds). In three tests, more reds remained than blues (percentage of all tadpoles remaining that were red = 51%, 55%, and 62%). One pond, the pond with 62% reds, had significantly higher mortality of blues than reds ($P < 0.001$). This is also the experimental group that sustained the highest overall mortality (26%). The specific cause of tadpole mortality in this pond is unknown, but it became manifested only on the final few days of sampling (data from these days were not used in analyses).

Discussion

The results of this study demonstrate that *R. cascadae* tadpoles are not only capable of distinguishing between siblings and non-siblings in natural field conditions, but prefer to aggregate with sibs. Whether tadpoles are reared with sibs or in mixed groups of sibs and non-sibs does not affect sib aggregation behavior. In 19 experimental tests using tadpoles from 31 sibships (from six populations), sibship compositions of 353 aggregations sampled were nonrandom when compared with control tests. Data from 13 of 16 tests significantly differed from expected random distributions. These results do not appear to be attributable to differential habitat selection among sibships. Even though aggregations were sometimes repeatedly found in the same areas of ponds, suggesting that habitat selection affected distributions, no correlation was observed between spatial positioning and color composition.

These field experiments are consistent with our previous laboratory studies in which we have shown that *R. cascadae* larvae prefer to associate with sibs over non-sibs when reared with sibs or in mixed sib/non-sib groups (O'Hara and Blaustein 1981), reared in isolation (Blaustein and O'Hara 1981, 1982a), or when individuals are reared with non-sibs only (Blaustein and O'Hara 1983). The proximate cue used to distinguish between kin and non-kin in this species is a sibship specific, waterborne chemical cue sensed through olfaction or taste (Blaustein and O'Hara 1982b).

One inconsistency exists, however, between field results and previous laboratory findings. In laboratory tests, tadpoles reared in mixed sib/non-sib groups distinguished between unfamiliar sibs and unfamiliar non-sibs, but not between familiar sibs and familiar non-sibs (i.e., tadpoles from their own rearing tank). This suggests that the chemical cues emitted by tadpoles of mixed-reared sibships are altered (perhaps through the mutual and temporary transference of odors between tadpoles) while the ability to discriminate unaltered cues remains (see discussion in O'Hara and Blaustein 1981). Strictly interpreted, these laboratory results could mean that when tadpoles encounter non-sibs during development, a condition that certainly exists in natural populations of *R. cascadae*, their sibship specific chemical cues will converge and, therefore, sibship identities will be rendered indistinguishable (Waldman 1984). This clearly did not occur, however, in our field results of both mixed-reared larvae and larvae reared with sibs; familiar sibs were distinguished from familiar non-sibs. Whether mixing with non-sibs occurred throughout embryonic and larval development (two sibships reared together) or just in later larval stages (after sibships reared apart were released together) did not seem to affect recognition behavior.

We conclude that our laboratory mixed-rearing results are probably not analogous to what takes place in natural conditions. This might be due to the rearing and testing regimes employed and because preference tests were conducted soon after tadpoles were removed from the rearing regime. One objective incorporated in the design of laboratory mixed rearing regimes was to overemphasize exposure of tadpoles to non-sibs. We confined large numbers of tadpoles from two sibships in small aquaria within which water was changed every several days. Under most natural conditions, water volume is much greater, tadpole densities are far less, and tadpoles are not forced to be in constant close association with large numbers of non-sibs or sibs. Under

laboratory conditions, it is perhaps not surprising that some temporary convergence in cues may have occurred between familiar sibships. Evidence of cue convergence among individuals has been reported for other species (e.g., Wilson 1971; Linsenmair 1972; Jutsum et al. 1979). This study suggests that if signal convergence did occur in the field it was probably weak, temporary, and not maintained. Convergence could become an important factor, however, in very dense larval populations. Although such conditions are rare in the generally small population sizes of *R. cascadae* (personal observations), they could sometimes develop during late stages of pond desiccation.

An unexpected finding from this study was the correlation between the number of tadpoles in an aggregation and sibship composition; sibship association (measured as the proportion of siblings comprising an aggregation) decreased with increasing aggregation size. To our knowledge, such a relationship with group size has not been reported in other kin recognition studies. These results suggest that the factors affecting sibling aggregation behavior in large groups differ from those in small groups, or that tadpoles respond differently to sibs and non-sibs in large vs. small groups. For example, large *R. cascadae* aggregations (greater than 30 tadpoles) although rare, sometimes form in response to nonsocial factors such as localized concentrations of food. Perhaps when food or other habitat requirements are limited in availability, these factors, rather than social factors such as kinship, are the primary stimuli that bring tadpoles together. In such cases, aggregations would tend to be large and composed of a random sample of the tadpole population. Also possible is that fitness costs and benefits change with group size (Pulliam and Caraco 1984) such that kinship associations are not beneficial in large groups.

Using similar dyeing techniques, Waldman (1982) has shown that *Bufo americanus* tadpoles also associate preferentially with siblings in natural ponds. In tests using sibships reared apart, 20 of 32 stationary aggregations collected were nonrandom in sibship composition. When sibships were reared together, 15 of 24 aggregations were nonrandom. Although *B. americanus* aggregation behavior differs from that of *R. cascadae* [e.g., *B. americanus* tadpoles form two types of large moving aggregations as well as stationary aggregations (Beiswenger 1975), whereas *R. cascadae* only form small stationary aggregations], the results of Waldman's field study are generally comparable to ours for *R. cascadae*. One notable difference, however, is a trend for sibling association tendencies of *B. americanus* tadpoles to diminish with time following release in some tests (Waldman 1982). We found no such trend in experiments with *R. cascadae*. Of particular interest, is Waldman's (1982) finding that mixed-reared *B. americanus* associated with familiar sibs over familiar non-sibs in field experiments but showed no discrimination in such tests conducted in the laboratory (Waldman 1981). These conflicting results are analogous to ours for *R. cascadae*. Differing rearing procedures in Waldman's laboratory and field tests of *B. americanus* could account for this inconsistency (Waldman 1982), or perhaps sibship specific cues converged between mixed reared groups in laboratory tests (see discussion above). In any case, these field studies stress the importance of conducting controlled field experiments in addition to laboratory investigations of kin recognition behavior. Waldman (1982) has concluded that field tests of *B. americanus* tad-

poles are probably more sensitive assays of sib recognition behavior than are laboratory tests. We agree. *R. cascadae* sib aggregation behavior also seems to be stronger and more definitive in the field.

Whatever the benefits might be of associating with siblings in *R. cascadae*, they are probably connected with the unusual aggregation behavior exhibited by this species. Despite one unsubstantiated implication that *R. cascadae* tadpoles generally do not aggregate (Waldman 1984), our field observations of wild and lab-reared larvae indicate that they are rarely nonaggregated (e.g., O'Hara 1981; Blaustein and O'Hara 1981, 1982a; O'Hara and Blaustein 1981, 1982). For example, our observations of *R. cascadae* tadpoles at the population localities used in this study indicate that they spend most of their active period in numerous dense (less than 2 cm between individuals) stationary aggregations. Aggregations are usually small in size (commonly range from 4 to 40 tadpoles/group) compared with other larval anurans that are known to aggregate (e.g., Wassersug 1973; Beiswenger 1975; O'Hara and Blaustein 1982). When aggregated tadpoles scatter following a disturbance individuals often rapidly reaggregate (within 30 min).

Numerous potential benefits of group living (Hamilton 1971; Alexander 1974; Bertram 1978) could be realized by tadpoles that aggregate with conspecifics. These include advantages related to predator detection and avoidance, and locating and consuming food. In theory, such benefits derived from cooperative or altruistic behavior could be augmented in sibling groups (Hamilton 1964; West Eberhard 1975) if individuals experience a net increase in inclusive fitness. We do not know what other specific costs and benefits might be involved for *R. cascadae* tadpoles that aggregate preferentially with siblings. However, *R. cascadae* tadpoles injured by predators emit a chemical substance that causes an alarm reaction in conspecifics (Hews and Blaustein 1985). Releasing an alarm substance could be a form of nepotistic behavior. Also, one potential benefit of aggregating is suggested by recent laboratory experiments (O'Hara and Blaustein, unpublished data) in which we found a negative correlation between increasing tadpole density (among siblings) and time to metamorphosis. Because of the short growing season in the Cascade Mts. where *R. cascadae* are found, and the frequent risk of pond desiccation, growth rate and time to metamorphosis can be critical variables affecting survival. We have no evidence, however, that developmental rates of tadpoles in sibling groups are faster than tadpoles in mixed sib/non-sib groups. The results of one study (Shvarts and Pyastolova 1970) actually suggest that the growth inhibitory effect among crowded *R. arvalis* tadpoles is greater in sibling groups than in mixed sibships.

In our field experiments, *R. cascadae* tadpoles rarely formed groups composed solely of siblings. The percent ratios of sibs to non-sibs/aggregation ranged from 50:50 to 100:0. The mean sibship composition of all aggregations sampled favored one of the two sibships by a ratio of 72:28%. These averages are comparable to those obtained for *B. americanus* (approximately 66:34%; Waldman 1982). Laboratory tests of *R. cascadae* (e.g. O'Hara and Blaustein 1981; Blaustein and O'Hara 1982a) also indicate substantial variability in sibling preference behavior between individuals. The potential causes of such variability in recognition systems are many (Crozier and Dix 1979; Sherman 1980; Breed and Bekoff 1981; Getz 1981; Wald-

man 1982; Colgan 1983). These may involve various experiential and genetic constraints affecting the expressions of recognition behavior, or a variable selective environment in which the costs and benefits of associating with kin fluctuate temporally and spatially. While this issue is of considerable theoretical importance, the data presently available do not allow a critical analysis of the underlying basis of variability in kin associations or what the evolutionary consequences might be of certain patterns in variability. The problem seems to be complex and will be difficult to investigate empirically.

The ability to distinguish sibs from non-sibs may be a widespread phenomenon among anuran larvae. In addition to *R. cascadae* and *B. americanus*, three species have now been tested for sib recognition behavior. All five show some recognition capabilities. The major differences between species are in the ontogenetic expression of the behavior. Tests with *B. boreas* (O'Hara and Blaustein 1982) indicate that any exposure to non-sibs, early or late in larval development, results in a loss of sib preference. Generally, larvae of both *B. boreas* and *B. americanus* interact with non-sibs throughout larval development (Beiswenger 1972; O'Hara and Blaustein 1982); eggs are laid communally, dispersal and mixing from oviposition sites is high, and aggregations mix and are composed of tadpoles from many sibships. Thus, for a sib recognition mechanism to be effective in natural populations of these species, it would need to be resistant to modification following exposure to non-sibs. At least for *B. boreas*, this does not seem to be the case.

Mixing with non-sibs is also apparently common in natural populations of the three ranids that have been investigated. Results of laboratory tests with *R. sylvatica* larvae (Waldman 1984) are similar to those for *R. cascadae*. Unlike *R. cascadae*, however, dispersal and mixing of *R. sylvatica* tadpoles from oviposition sites is high, and tadpoles seldom aggregate and may actually avoid conspecifics (Hassinger 1972; DeBenedictis 1974; Waldman 1984; personal observations) suggesting that kin associations probably do not occur in most natural populations of this species. Tadpoles of *R. aurora*, a species closely related to *R. cascadae* are also capable of distinguishing between sibs and non-sibs, but only when reared exclusively with sibs and only in early developmental stages (Blaustein and O'Hara, in preparation). However, *R. aurora* are not known to aggregate with conspecifics and mixing among sibships and dispersal is probably high (Calef 1973; personal observations).

Thus, for at least three of the five anurans tested (*B. boreas*, *R. sylvatica*, and *R. aurora*) we question whether the demonstrated kin recognition abilities are kin selected traits that function in the formation of kin groups in natural populations. This reinforces the tenet that a demonstration of kin recognition is not, by itself, evidence of kin selection; just as kin selection does not require a kin recognition mechanism to operate. Interspecific comparisons such as these, suggest that alternative explanations for kin recognition behaviors deserve consideration. For instance, perhaps recognition behaviors (1) are remnants of traits that evolved under different, past selective regimes, (2) are secondary expressions of choice behaviors selected for in different social or ecological contexts (e.g. habitat selection or food selection), or (3) conditions favoring kin associations really do exist in these species, but such conditions are rare, intermittent, and vary between populations. Studies that explore

the adaptive significance of kin associations in each species and carefully designed field experiments will help to resolve these uncertainties.

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