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Kin recognition and sibling association among wood frog (*Rana sylvatica*) tadpoles

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Summary. 1. Wood frog (*Rana sylvatica*) tadpoles, hatched from egg masses collected in the field, were reared either with siblings only or in mixed groups of eight sibships. Sibships were marked with vital stains, released in a laboratory test pool, and their spatial distributions recorded over a 4-day period.

2. An analysis of nearest-neighbor distances suggests that tadpoles preferentially associated with familiar siblings over both familiar and unfamiliar non-siblings. Kinship preferences of *R. sylvatica* tadpoles, unlike those of other anurans that have been tested, thus appear largely unaffected by prior social experience.

3. Tadpoles reared in mixed sibship groups preferentially associated with the siblings with which they were reared over unfamiliar siblings exposed to a different set of sibships. In contrast, tadpoles did not discriminate either between siblings reared in different baskets within the same mixed group tank, or between siblings housed in different single-sibship containers.

4. Because *R. sylvatica* egg masses are usually deposited in communal clumps, an ability to recognize relatives not dependent on post-embryonic experience may have been selected. The possible adaptive significance of kin association among wood frog tadpoles is unknown; their schools probably do not represent kin groups, but larval distributions in natural ponds might reflect sibling association tendencies.

5. Kin recognition "labels" of non-sibling *R. sylvatica* tadpoles reared together, like those of *R. cascadae* tadpoles similarly reared, may converge as a result of social interactions. *R. sylvatica* tadpoles, unlike *R. cascadae* tadpoles, retain an ability to discriminate between familiar siblings and familiar non-siblings; hence label transference may be incomplete.

Introduction

The abilities of organisms to recognize individuals and to behaviorally discriminate among classes of conspecifics have traditionally been of great interest to ethologists (see review in Colgan 1983). Until recently, however, few studies have focused on the question of whether individuals recognize their collateral kin (reviewed in Holmes and Sherman 1983; Waldman 1983). In his original formulation of genetical kinship theory, Hamilton (1964) observed that differential behavior toward kin need not depend on the evolution of specialized kin recognition mechanisms; in highly structured ("viscous") populations, neighbors would most often be relatives, and thus individuals that favored (i.e., acted altruistically or less agonistically toward) these familiar individuals would be effectively increasing their own inclusive fitness. While familiarity may be a major component of many kin recognition systems (see Porter et al. 1981; Buckle and Greenberg 1981; Holmes and Sherman 1982; Kareem and Barnard 1982), in some situations (e.g., when pairs mate polygynously or nest communally) familiarity or spatial proximity poorly correlate with genetic relatedness. Under these circumstances, recognition systems not dependent on prior association or spatial distribution may be selected (for possible examples, see Wu et al. 1980; Davis 1982; Grau 1982; Getz and Smith 1983).

The mechanisms underlying kin recognition abilities are amenable to experimental analysis. Environmental, genetic, or sensory factors can be selectively altered and their effects on the subsequent

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ontogeny of an organism's kin-directed behaviors recorded. Amphibian larvae may be model subjects for conducting such studies – they can be readily reared in large numbers and are easily manipulated for developmental or sensory experiments. Tadpoles of at least two toads (*Bufo americanus*: Waldman and Adler 1979; Waldman 1981, 1982b, in press; *Bufo boreas*: O'Hara and Blaustein 1982) and one ranid frog (*Rana cascadae*: Blaustein and O'Hara 1981; O'Hara and Blaustein 1981) preferentially associate with siblings in a variety of experimental tests. These sibling association patterns appear to be attributable to behavioral kin recognition mechanisms rather than differential responses to environmental factors (see Waldman 1982b).

Among anuran species, kin preferences observed (and kin recognition abilities inferred) have been found to be influenced by the social environment in which the tadpoles are reared. *B. americanus* tadpoles may form a recognition "template" based in part upon traits of conspecifics to which they are exposed during an early sensitive period and later recognize these individuals or others that share their traits (Waldman 1981). Recognition preferences of *B. boreas* tadpoles appear also to be susceptible to modification, but over a longer developmental time frame (O'Hara and Blaustein 1982). Similarly, *R. cascadae* larvae are evidently unable to discriminate between familiar kin and familiar non-kin; however, *R. cascadae* tadpoles exposed to non-siblings retain an ability to recognize unfamiliar relatives (O'Hara and Blaustein 1981). In this paper, I report the results of experiments aimed at understanding the ontogeny of kin recognition abilities in larvae of the wood frog, *Rana sylvatica*, a species taxonomically close to and in some ways ecologically similar to its allopatric congener, *R. cascadae* (see Martof 1970).

Social familiarity or spatial proximity may be unreliable indicators of kinship for *R. sylvatica* tadpoles. Wood frogs breed synchronously in early spring, depositing their globular egg masses directly proximate to one another, generally within one small area of a pond (Seale 1982; Waldman 1982a; and references therein). Egg masses from a few to several hundred pairs may thus be present within a single communal clump; moreover, egg masses within a clump, and the egg jelly surrounding them, tend to coalesce during advanced embryonic stages. At hatching, when embryos emerge from their egg mass, they are thus likely to be immediately surrounded by siblings and non-siblings. In contrast, *B. americanus* egg masses are deposited as gelatinous strings; pairs either oviposit in separate areas of a pond or (especially in dense breeding aggregations) several pairs may lay eggs in one area of the pond, intertwining them with vegetation and sometimes with egg strings of other pairs (personal observations). Still, individual clutches remain distinct and separate until after hatching. Following hatching and when they begin free-swimming, toad larvae may be more likely than wood frog larvae to be surrounded by siblings. Thus while a recognition system based on early experience may suffice for toad larvae if identifiable traits are retained during development (Waldman 1981, in press), such a system would appear to be unlikely to enable wood frog larvae to identify their siblings unless recognition specificity is determined prior to hatching.

Materials and methods

Study animals. I collected 46 *R. sylvatica* egg masses from four ponds in Tompkins County, New York, during April of 1981 and 1982 and March of 1983. Two ponds (here denoted YB and LP) are permanent woodland pools, 0.6 km apart, located in Yellow Barn State Forest near Dryden, New York (elevation 567 m). Pond SW is a temporary woodland pool located in the Sapsucker Woods Preserve near Ithaca, New York (elevation 329 m), approximately 9.7 km from the Yellow Barn ponds. Pond BP, a semi-permanent pool (that dried infrequently, during extremely dry summers), is located at the edge of the Cornell University golf course near the Ithaca campus (elevation 297 m), 1.9 km from the Sapsucker Woods pond and 10.4 km from the ponds in the Yellow Barn forest.

At breeding ponds, egg masses were collected immediately after spawning occurred, before the egg jelly surrounding each clutch imbibed water. Thus egg mass boundaries were well-defined and clutches could be separated without ambiguity. Because each spawning pair was observed during its entire oviposition period, the probability that more than one male fertilized any given clutch is small. To further check the possibility that clutches could be fertilized by multiple males (i.e., by sperm shed by other males into the water), I "stripped" egg masses from two unpaired females (by applying pressure to their sides), and placed the egg masses in pond water collected near the communal oviposition site. These eggs failed to develop.

Rearing procedures. Egg masses were each placed in separate containers filled with pond water, and were transported to the laboratory within 4 h of being laid. While the embryos were still in an early stage of development (before gastrulation, $<$stage 9, Gosner 1960), most clutches were divided into two sections of approximately equal numbers of eggs. The sections of each clutch were then immediately assigned randomly to one of two rearing regimes: exclusive sibling groups or mixed sibship groups.

Sibling groups were housed in 10-l plastic buckets; water was continuously aerated, and was replaced with dechlorinated tap water twice weekly. Tadpoles reared in mixed sibship groups were segregated by sibship in separate fiberglass screen baskets (38 × 38 × 38 cm, 1.4 mm mesh) lined with nylon mosquito netting (0.5 mm mesh) within one of three 425-l plywood and glass tanks (115 × 120 × 30 cm). Each rearing tank was designed to hold eight baskets containing different sibships. Water was continuously collected from an intake pipe on one side of the tank, aerated, thoroughly mixed, and pumped (20 l/min in tanks A and B, 40 l/min in tank C) to an outlet pipe on the opposite side of the tank. Because water was thus circulated

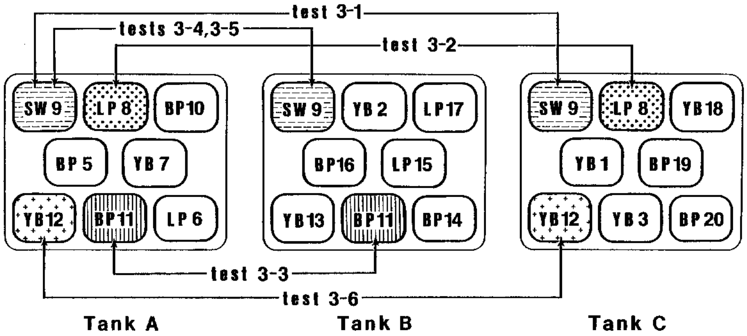

Fig. 1. Arrangement of sibships within three mixed group rearing tanks. Eight different sibships (denoted by a two letter pond designation and a clutch number) were housed in separate screen baskets within each tank, but members of four sibships (in shaded baskets) were split between more than one of the rearing tanks. Water was pumped uniformly throughout each tank. In one test series, siblings reared in different mixed group tanks were tested to determine whether their recognition labels diverged as a result of differential rearing treatment (the sibships tested and their test numbers are indicated). For further details see Table 3 and text

completely throughout each rearing tank, sibships reared in baskets within the same tank were exposed to waterborne cues emanating from non-siblings as well as from their siblings (see Waldman 1981). Water in mixed group tanks was partially changed with dechlorinated tap water once a week.

Under both rearing regimes, tadpoles were fed spinach daily, were maintained on a LD 14:10 photoperiod under fluorescent lamps, and were reared 24 to 58 days before testing. Approximately 2 weeks after hatching, some tadpoles were removed from buckets and baskets that were densely populated to prevent overcrowding and to establish uniform densities among all groups.

During 1982, sibships were assigned to baskets within mixed group tanks so that two different experimental questions could be asked. First, tests were planned to determine whether tadpoles associated with familiar siblings (reared in their basket) in preference to familiar non-siblings (reared in a different basket within their tank). Second, I wished to determine whether a possible decrement in sibling preference among tadpoles reared in mixed sibship groups (e.g., O'Hara and Blaustein 1981; Waldman 1981) could be attributable to convergence of recognition labels among individuals that are exposed to one another (see Waldman, in press). To accomplish both these goals, portions of four sibships were placed in baskets within two (sibships LP-8, BP-11, and YB-12) or three (sibship SW-9) mixed group tanks (see Fig. 1). Different sibships were reared in each of the remaining baskets in the three tanks.

Additionally, during 1983 tests were conducted to determine whether possible discrimination between siblings might result from simple familiarity effects (i.e., "basketmate" or "bucketmate" preferences). For one series of tests, two egg masses were each split into two portions housed in different baskets within the same mixed group tank, with the remaining four baskets housing four other sibships. This rearing procedure was duplicated in a total of three mixed group tanks. Six sibships were each split into two different 10-l buckets for a second series of tests.

Testing apparatus and procedure. Kin association was measured by sampling the positions of 50 tadpoles (25 of each of two sibships [test groups], differentially marked to indicate group identity) in a laboratory test pool. From these data, the distances of each individual to its nearest same-colored and nearest different-colored neighbors were computed and compared. In each test, all individuals in the pool were sampled repeatedly over a 4-day period during eight instantaneous sampling periods. Tadpoles were marked immediately prior to the beginning of a test by immersion either in a 0.00025% solution of neutral red for 2 to 5 h or in a 0.00025% solution of methylene blue for 5 to 8 h. In some tests, one to five tadpoles died during the 4-day test period; they were removed from the pool and were not replaced.

Test pools measured 115 × 115 cm, and were filled to a depth of 2 cm with dechlorinated tap water prior to the beginning of each test. Tadpoles of both test groups were initially placed in randomized positions within a pool, and all were simultaneously released by a raisable partitioned grid. The grid, constructed of polystyrene plastic, consisted of a series of open-ended cells, each 14 × 14 mm across and 3 cm deep (approximately the same size as a tadpole). Tadpoles were allowed to swim freely throughout the pool during a 24 h acclimation period. Data were subsequently collected by quickly lowering the grid into the bottom of the pool by means of a pulley system, thus trapping tadpoles within grid cells. Cartesian coordinates (± 7 mm) and mark identities of all individuals were recorded. The grid was then raised, and tadpoles continued to swim freely throughout the pool (for further details, see Waldman 1981).

Testing protocol and rationale. Three series of experimental tests were conducted. Tadpoles reared in sibling groups were tested for a tendency to preferentially associate with familiar siblings over unfamiliar non-siblings (13 tests). Concurrently, different sibships reared in the same mixed sibship group were tested for a tendency to preferentially associate with familiar siblings (reared in the same basket) over familiar non-siblings (reared in another basket within the same tank) (11 tests). Because portions of each clutch were initially placed in both sibling group and mixed sibship group tanks, the same sibships (but not necessarily the same sibship pairs) were used in both series of tests. As in similar tests with *B. americanus* tadpoles (Waldman 1981), different individuals (and in most cases unique sibship pairs) were used in each test.

To determine whether recognition labels of tadpoles reared together might converge, a third series of experimental tests was conducted. In each test, tadpoles of a single sibship were differentially marked by the mixed group tank in which they were reared, and their positions in the test pool subsequently recorded. A total of six tests, involving four different sibships, were conducted (see Fig. 1). As in other tests, no individual was used in more than one test. Data analysis compared mean distances to the nearest familiar (same-colored) sibling and to the nearest unfamiliar (different-colored) sibling.

The marking and testing procedures themselves might conceivably lead to differential association by treatment group. Four tests, each involving a different sibship, were conducted to control for this possibility. In each test 50 tadpoles reared in a sibling group were randomly divided into two subgroups and were differentially marked. Mean distances to the nearest same-colored sibling and to the nearest different-colored sibling were compared.

Additional series of control tests were conducted to extricate possible confounding familiarity effects from those attributable to genetic relatedness. Tadpoles reared in mixed sibship groups were tested for a preference to associate with siblings reared in the same basket as themselves over siblings from other baskets in the same mixed group tank (6 tests). Tadpoles reared in sibling groups were tested for a preference to associate with familiar siblings reared in the same bucket as themselves over unfamiliar siblings reared in another bucket (6 tests). Different sibships were used in each test.

In all experimental and control tests, groups were matched for body length and developmental stage to ensure that they were not sorting themselves based on body size differences or because of ontogenetic differences in habitat preferences (e.g., Alford and Crump 1982; Breden et al. 1982). Tadpoles were tested at all developmental stages between 26, when larvae initiate feeding, and 36, at which time toes become distinct on hind limbs (Gosner 1960). Mean tadpole body lengths ranged between 20 and 27 mm. Test plans (e.g., whether tests were experimental or control series) were not known by assistants when they were collecting data.

Statistical analyses. Mean distances between nearest (same-colored) siblings and between nearest (different-colored) non-siblings were computed for each trial (data collection period) of all tests involving two sibships. Similarly, mean nearest-neighbor distances between same-colored siblings and between different-colored siblings were computed for each trial of all tests involving subgroups of a single sibship. For each series of tests, these trial means were subjected to repeated measures analysis of variance (Keppel 1973, pp. 423–470).

In the first two series of experimental tests (different sibships reared in sibling groups or within the same mixed sibship group), the primary factor of interest in considering sources of variation is a "sibship" factor, i.e., whether the neighbors are siblings or non-siblings. In addition, because results of similar tests on *B. americanus* tadpoles suggest that kin association tendencies may vary depending on whether sibships are obtained from nearby or distant ponds (Waldman 1981), variation among tests was nested within a "pond" factor according to whether the two sibships had been obtained from the same or different ponds (for this analysis, clutches from YB and LP ponds were pooled in a single category because of the close proximity of these ponds). A term describing the interaction between sibship and pond effects provides a measure of whether kin preferences vary between these categories. The repeated measure in this three-way design, a "time" factor, is completely crossed with the sibship and test factors, as data collection periods occurred at uniform time intervals among tests.

To further examine the influence of rearing regime on sibling preferences, an additional analysis of variance was conducted on the results of six tests involving three sibship pairs that were reared under both rearing regimes (i.e., some members of each sibship pair were reared in a common mixed sibship group and others were reared in separate sibling groups). Sibships were blocked within a factorial design, considering rearing regime as a main effect.

Control tests and tests of single sibships reared in different mixed group tanks were analyzed separately. Statistical procedures similar to those applied to the experimental data were used, but variation among tests was not partitioned by a pond factor. As all individuals in these tests are siblings to one another, a "mark-color" factor (including the concomitant rearing conditions) replaces the sibship factor as the primary effect of interest.

Because repeated measures on the same groups may be correlated, the assumption of independence among measurements underlying most analyses of variance is replaced by one of homogeneity of the covariance between pairs of treatment conditions. To test this assumption, Anderson's sphericity test was applied for appropriate comparisons (see Dixon 1981, p. 379). The assumption may have been violated for some comparisons in the second and third series of experimental tests (different sibships reared within the same mixed group tank; sibships reared in different mixed group tanks) and in control tests. To compensate for failure to meet the variance-covariance assumption, Greenhouse-Geisser adjustments to probability levels were computed (see Keppel 1973); results thus affected are denoted P^*. Tadpoles move freely during the interval between data collection periods and there are certain advantages to treating trials as independent samples of tadpole positions; nonetheless, the statistical results obtained under such a model are substantially the same as those presented here (see Waldman 1983 for detailed presentation of these results).

The General Linear Models procedure of SAS (Release 79.6, Statistical Analysis System, SAS Institute, Cary, North Carolina) and various subroutines of IMSL (Edition 9, International Mathematical and Statistical Libraries, Houston) were used for analyses of variance and associated computations. Additional analyses, especially with regard to compound symmetry assumptions, were conducted with the 2V procedure of BMDP (April 1982 revision, Biomedical Computer Programs, BMDP Statistical Software, Los Angeles).

Results

Tadpoles reared in sibling groups

In 12 of 13 tests of tadpoles reared with siblings only (involving 50 tadpoles in each test, 8 samples of their positions, and 12 different sibship pairs), the mean nearest-neighbor distance to siblings was less than that to non-siblings (Table 1). Overall, this sibship effect was highly significant ($F_{1,11}=14.70$, $P=0.003$). Nearest-neighbor distances were not in general influenced by whether sibships were obtained from the same or different ponds ($F_{1,11}=0.57$, $P=0.46$), and there was no significant interaction between this pond factor and the sibship effect ($F_{1,11}=0.10$, $P=0.76$). A trend of increasing nearest-neighbor distances in trials within tests emerged ($F_{7,77}=5.91$, $P<0.0001$), perhaps related to mortality over the 4-day period in which tests were conducted or ontogenetic changes, but this factor appeared to be unrelated to sibling preference ($F_{7,77}=0.96$, $P=0.47$).

Tadpoles reared in mixed sibship groups

Eleven tests involving 10 sibship pairs were conducted, and in nine of these tests, tadpoles demon-

Table 1. Experimental plan and results of tests in which individuals reared in sibling groups were marked and tested for sibling preferences. Sibships are denoted by the pond from which they were obtained (*LP*, *YB* Yellow Barn State Forest; *BP* Cornell University golf course; *SW* Sapsucker Woods Preserve) and a clutch identification number. Developmental stages (Gosner 1960) were determined at the conclusion of each test. Overall, tadpoles were significantly closer to siblings (121 ± 2 mm; $\bar{x} \pm$ SE) than to non-siblings (130 ± 2 mm)

Test no.	Mean stage	Sibship identities and mark-colors		Nearest-neighbor distance (mean ± 1 SE, mm)	
		Red	Blue	Sib	Non-sib
1-1	28	LP-15	YB-13	129 ± 4	139 ± 7
1-2	28	LP-15	YB-12	124 ± 3	135 ± 4
1-3	27	BP-11	LP-17	123 ± 3	132 ± 6
1-4	29	LP-6	BP-14	117 ± 1	145 ± 7
1-5	31	BP-11	BP-16	117 ± 6	122 ± 7
1-6	30	BP-4	LP-17	112 ± 5	131 ± 1
1-7	32	BP-16	YB-13	121 ± 6	125 ± 5
1-8	32	BP-14	YB-12	132 ± 3	128 ± 5
1-9	32	BP-4	LP-6	117 ± 4	119 ± 6
1-10	26	YB-27	YB-28	110 ± 2	112 ± 4
1-11	27	YB-25	BP-22	130 ± 5	139 ± 8
1-12	27	YB-28	YB-24	126 ± 8	132 ± 9
1-13	27	YB-28	YB-27	116 ± 5	137 ± 16

Table 2. Experimental plan and results of tests in which individuals of different sibships reared in a common mixed group tank were tested. Sibships and developmental stages are denoted as in Table 1. Overall, tadpoles were significantly closer to siblings (127 ± 2 mm; $\bar{x} \pm$ SE) than to non-siblings (138 ± 3 mm)

Test no.	Mean stage	Sibship identities and mark-colors		Nearest-neighbor distance (mean ± 1 SE, mm)	
		Red	Blue	Sib	Non-sib
2-1	28	LP-15	BP-11	139 ± 7	150 ± 7
2-2	28	YB-12	LP-6	129 ± 7	141 ± 7
2-3	27	SW-9	LP-8	129 ± 8	129 ± 6
2-4	27	BP-5	BP-10	109 ± 3	127 ± 9
2-5	32	BP-16	BP-11	116 ± 7	139 ± 14
2-6	33	YB-12	YB-7	132 ± 5	131 ± 10
2-7	32	BP-10	BP-5	114 ± 6	122 ± 6
2-8	33	BP-14	YB-12	154 ± 9	179 ± 15
2-9	26	BP-22	YB-25	123 ± 5	138 ± 14
2-10	26	YB-24	BP-22	112 ± 5	122 ± 9
2-11	26	BP-23	BP-21	134 ± 7	143 ± 8

Table 3. Experimental plan and results of tests in which members of a single sibship reared in different mixed group tanks were tested. Sibships, mark-color identities by rearing tank, and developmental stages are reported as in Table 1. Overall, tadpoles were significantly closer to familiar siblings (114 ± 3 mm; $\bar{x} \pm$ SE) than to unfamiliar siblings (126 ± 4 mm)

Test no.	Mean stage	Sibship	Mark-color identities		Nearest-neighbor distance (mean ± 1 SE, mm)	
			Red	Blue	Familiar sib	Unfamiliar sib
3-1	28	SW-9	Tank A	Tank C	123 ± 6	126 ± 7
3-2	29	LP-8	Tank C	Tank A	105 ± 6	123 ± 10
3-3	28	BP-11	Tank A	Tank B	123 ± 4	138 ± 12
3-4	31	SW-9	Tank B	Tank A	105 ± 5	109 ± 7
3-5	31	SW-9	Tank A	Tank B	88 ± 3	109 ± 10
3-6	34	YB-12	Tank C	Tank A	143 ± 6	147 ± 7

strated some preference to be closer to siblings than to non-siblings (Table 2). This sibship effect was significant among the tests ($F_{1,9}=18.77$, $P=0.002$). Again the ponds in which clutches were collected appeared neither to directly affect nearest-neighbor distances ($F_{1,9}=1.21$, $P=0.30$) nor to interact significantly with the sibship effect in influencing association tendencies ($F_{1,9}=0.02$, $P=0.89$). Variation in nearest-neighbor distance with time was also apparent ($F_{7,63}=14.56$, $P^* < 0.0001$), but this factor did not interact significantly with the sibship effect ($F_{7,63}=2.16$, $P^*=0.11$).

Taken together, these results suggest that tadpoles can discriminate their siblings from non-siblings even though reared with both. Statistical comparison of the association tendencies of particular sibships reared under both rearing regimes corroborates these findings. Considering just this subset of the larger data set, overall sibling preference remains highly significant ($F_{1,2}=219.48$, $P=0.004$), and the interaction between this sibling preference and rearing regime is nonsignificant ($F_{1,2}=5.69$, $P=0.14$). Whether tadpoles were reared in sibling groups or in mixed sibship groups did not affect their nearest-neighbor distances ($F_{1,2}=1.24$, $P=0.38$). Within tests, there was again variation among replicate measures ($F_{7,14}=4.58$, $P^*=0.10$), although the interaction between sibship and time effects is nonsignificant ($F_{7,14}=3.25$, $P^*=0.17$).

Siblings reared in different mixed sibship environments

In each of the six tests, involving four sibships and three rearing tanks (see Fig. 1), the mean nearest-neighbor distance between individuals marked the same color was less than that between individuals marked differently (Table 3). Thus tadpoles appeared to preferentially associate with familiar siblings, reared in the same mixed group tank as themselves, over unfamiliar siblings, reared in another mixed group tank in which they were exposed to a different set of sibships. This effect was significant among the six tests ($F_{1,5}=10.90$, $P=0.02$). No significant differences in nearest-neighbor distance in replicate measures within tests were found ($F_{7,35}=0.75$, $P=0.63$), and interaction between mark-color and time effects was negligible ($F_{7,35}=0.31$, $P=0.95$).

Control tests

Among four control tests of siblings reared together but differentially marked and tested, distances between same-colored siblings (132±4 mm, $\bar{x}\pm$ SE) and different-colored siblings (132±4 mm) did not significantly differ (overall, $F_{1,3}=0.05$, $P=0.84$). Thus, the marking and testing procedures do not appear to contribute to the observed differential association by treatment group. In addition, no trends of variation in nearest-neighbor distances attributable to time effects within tests ($F_{7,21}=2.93$, $P=0.26$) or to the interaction of time and mark-color effects ($F_{7,21}=0.73$, $P=0.65$) were noted. Although control procedures were virtually identical to those of the other tests, patterns of variation seen in experimental data are not reflected in these control data.

Results of tests of single sibships that had been split between different baskets in the same mixed group tank likewise reveal no discrimination between familiar siblings that might be attributable to the basket in which they were reared. In six tests, tadpoles were not significantly closer to siblings from their own basket (127±3 mm) than to siblings reared in another basket in the same tank (124±2 mm) (overall, $F_{1,5}=0.40$, $P=0.56$). Variation in nearest-neighbor distance with time was apparent ($F_{7,35}=4.34$, $P^*=0.05$), but did not interact with the mark-color effect ($F_{7,35}=0.34$, $P=0.93$).

Finally, when single sibships that had been split between two different buckets as eggs were marked and tested, association tendencies appeared not to be affected by differential rearing experience. Based on the results of six tests, distances between siblings reared in the same bucket (120±2 mm) were not significantly different from those between siblings reared in different buckets (118±3 mm) ($F_{1,5}=0.37$, $P=0.57$). Variation in distances attributable to time effects ($F_{7,35}=1.26$, $P=0.30$) or interaction between time and mark-color effects ($F_{7,35}=0.68$, $P=0.69$) was negligible.

Discussion

Kin recognition abilities of wood frog tadpoles appear generally more stable than those of toad tadpoles, under equivalent experimental rearing regimes. Whereas toad larvae reared in mixed sibship groups subsequently fail to behaviorally discriminate between familiar siblings and non-siblings in laboratory tests (*B. americanus*: Waldman 1981; *B. boreas*: O'Hara and Blaustein 1982), wood frog tadpoles reared in mixed sibship groups preferentially associate with familiar siblings over familiar non-siblings. Because the ontogeny of recognition specificity seems to be unaffected by exposure to non-siblings, one would predict that members of different sibships present within a communal clump (Waldman 1982a) retain the ability to discriminate between their siblings and non-siblings hatching from adjacent egg masses. Although tests under natural conditions are needed, the ontogeny of wood frog kin recognition appears to mesh well with its larval ecology.

From an evolutionary perspective, however, the finding that wood frog tadpoles associate preferentially with siblings is somewhat surprising. Toad tadpoles (genus *Bufo*) are generally characterized by highly gregarious behavior, conspicuous black body coloration, distastefulness to some predators, and alarm pheromones in their skin that may elicit a fleeing reaction among conspecifics upon a predator's attack (see reviews in Wassersug 1973; Waldman 1982b). These traits, although not necessarily conferring advantage on the individuals expressing them, may benefit conspecifics. If kin selection (Hamilton 1964) is important in the spread and maintenance of the alleles encoding these characters, tadpoles that school preferentially with kin may be favored (Waldman and Adler 1979; Waldman 1982b). In contrast, wood frog tadpoles are rather cryptically colored, for most of their larval period are apparently palatable to many vertebrate (Walters 1975) and invertebrate (Formanowicz and Brodie 1982) predators, and do not commonly aggregate in schools. Thus the selective advantages attained by associating with genetic relatives may be different than those postulated for toad larvae.

Under natural conditions, spatial relationships among larval wood frogs may be highly variable. Hassinger (1972) reported that wood frog tadpoles in New Jersey never schooled; indeed they actively avoided each other, and were found in well-defined aggregations only briefly before dispersal from the oviposition site. Wood frog tadpoles in Michigan apparently also form schools rarely if at all (DeBenedictis 1974). Dispersed rather than clumped spatial distributions typify wood frog tadpole behavior near Ithaca, as well (field observations during 1976–1981). In one pond (LP), however, wood frog tadpoles consistently formed mid-water polarized schools and feeding aggregations each year.

Unlike the others, this pond was characterized by dense tadpole populations ($>500{,}000$ individuals), little submerged vegetation, generally open water, and abundant predators, suggesting that under some conditions both anti-predator and feeding benefits may accrue to schooling wood frog tadpoles (Waldman, in preparation). Sometimes single polarized schools (sensu Shaw 1970) with densities approaching 160,000 individuals per m^3 were observed; at other times these schools would break up into several non-polarized, but well-defined, feeding groups of several hundred to several thousand individuals. Though the smaller feeding groups potentially might represent sibling groups, most schools clearly consist of individuals of many different sibships (cf. school size with mean *R. sylvatica* clutch size: 898 [Seale 1982]; 979 [Waldman 1982a]).

Nonetheless, these results, coupled with the recent discovery of kin association tendencies among *Rana cascadae* larvae (Blaustein and O'Hara 1981; O'Hara and Blaustein 1981), suggest that kin recognition abilities may be more widespread among anuran larvae than was previously thought. Perhaps kin association tendencies have not been directly selected for, but rather are expressed as secondary results of epigenetic processes associated with other aspects of the organism's life history. On the other hand, all social organisms, whether they display traits suggestive of phenotypic "altruism" or not, can potentially benefit by associating in kin groups. Amphibians, for example, often breed in transient environments in which selective pressures favoring rapid growth and early metamorphosis may be strong, and larval competition consequently intense (e.g., DeBenedictis 1974; Wilbur 1972, 1976, 1977). If food is limiting, tadpoles may expend energy to displace conspecifics feeding on an attractive food source, but they might conceivably act more tolerantly toward related individuals. Such responses may be expressed behaviorally, as forms of exploitative competition, or indirectly through interference mechanisms, for example by chemical growth regulators (see discussions in Steinwascher 1978; Waldman 1982b). At this point we can only speculate; explanations for why kin recognition abilities have evolved and under what conditions they are expressed await studies of the adaptive value of kin-directed behaviors.

Are recognition labels transferred among conspecifics?

Any trait an individual expresses that is correlated with its genetic identity can potentially serve as a kin recognition "label" for its conspecifics. Variation in these labels may result from, and hence directly reflect, genetic differences (e.g., Greenberg 1979), but label components might also be acquired from localized environmental sources, such as food or nesting materials (for examples, see reviews in Wilson 1971; Hölldobler and Michener 1980). As long as kin groups differentially incorporate environmental factors into recognition labels, these cues incidentally provide a means for detecting kinship. When different kin groups utilize the same materials, however, they may develop convergent labels (e.g., Jutsum et al. 1979). Genetically encoded traits might also be labile, and converge as the result of social interactions among non-kin (e.g., Linsenmair 1972). Yet in many natural circumstances, the recognition system may remain intact. The form of the recognition label used by wood frog tadpoles is not revealed by the present experiments; however, the results do bear on the efficacy of the label.

Although wood frog tadpoles were able to discriminate between their siblings and non-siblings reared in the same mixed sibship group, they also showed a significant preference to associate with familiar siblings over unfamiliar siblings reared in a different mixed sibship group. These results seem paradoxical. Non-siblings reared together appeared different to one another. But siblings thus reared apart also appeared different to one another. If recognition labels of siblings reared in different tanks diverge due to convergence in those attributes among non-siblings within tanks, how are wood frog tadpoles able to distinguish between familiar siblings and familiar non-siblings? The results suggest that association tendencies may reflect both familiarity and relatedness effects.

Different sibships reared in the same tank, although exposed both to siblings and non-siblings, are most directly in contact with their "basketmate" siblings. The apparent ability of tadpoles to discriminate between siblings from their own basket and non-siblings from a different basket, and their ability to discriminate between familiar siblings and unfamiliar siblings from a different mixed sibship group, might then both represent manifestations of a familiarity-based recognition system. The primary rule of this system would be to associate with those individuals with which one is *most* familiar. Yet the results of the control experiments defy this simple interpretation, for tadpoles did not preferentially associate with basketmate siblings over those from another basket in the same tank; indeed even when sibships were split at an early egg stage between two separate buckets, the larvae subsequently showed no preferential tendencies to associate with familiar siblings over unfamiliar siblings. Moreover, wood frog tadpoles were on average closer both to familiar siblings and unfamiliar siblings (whether reared in sibling group or mixed sibship group conditions) than to either familiar or unfamiliar non-siblings.

A more likely hypothesis to explain these results is that recognition labels of non-siblings reared together do indeed converge, but not to the extent that they become indistinguishable. This might occur as the result of social interactions, possibly as chemical labels are transferred among conspecifics. If environmental effects are incorporated into the recognition system and differentially impinge upon mixed sibship groups, similar results might be obtained. For example, different rearing tanks may accumulate different microbial populations, have different smells, or vary in other ways that could provide a cue as to the tank in which the tadpoles were reared. Alternatively, these environmental factors might induce changes in developmental pathways that lead to the expression of genetically encoded recognition traits. Regardless of how convergence occurs, the consequent alteration of recognition labels to provide some sense of mixed group identity need not necessarily supplant coding of kinship identity; indeed these processes may result from different factors. Hence differences in kinship identity of familiar individuals and differences in familiarity of equally related individuals might both be discriminated (e.g., Holmes and Sherman 1982).

Comparison of kin recognition systems of Rana sylvatica and Rana cascadae

Wood frog (*R. sylvatica*) larvae develop specific sibling recognition abilities even when they are reared in close association with non-siblings. Their kin recognition mechanism allows them to discriminate between siblings and non-siblings, even though familiar with both. Although wood frog tadpoles probably do not form schools with siblings under field conditions, sibships might show some spatial segregation within ponds. Cascades frog (*R. cascadae*) larvae, when reared with non-siblings, subsequently fail to discriminate between familiar siblings and familiar non-siblings (O'Hara and Blaustein 1981). Yet they retain the ability to discriminate between unfamiliar siblings and unfamiliar non-siblings, and associate preferentially with unfamiliar siblings over a mixed group of familiar siblings and non-siblings (O'Hara and Blaustein 1981). Larval Cascades frogs, like wood frogs, apparently do not school frequently. In the field, O'Hara (1981) commonly observed "loose groups" of tadpoles (estimated nearest-neighbor distance of 13 cm), but rarely (in less than 10% of his observations) noted social "clusters" consisting usually of between 10 and 30 individuals within 2 cm of one another. Blaustein and O'Hara (1981) suggest that these natural groupings are likely comprised of a high proportion of siblings.

Cascades frogs, like wood frogs, are explosive breeders, depositing their egg masses directly adjacent to or on top of those of pairs that have already spawned. Hence often within a breeding pond, a single communal clump (consisting of 30–70 egg masses) forms (Sype 1975), although in some populations such clumps might be small [O'Hara and Blaustein (1981) give 4–8 as typical sizes]. Consequently, tadpoles of different broods hatch synchronously; moreover, they disperse only limited distances from the oviposition site (O'Hara 1981). On the basis of their early larval ecology, one would suspect that in natural conditions Cascades frog tadpoles would interact at least as much with non-siblings as do experimental animals segregated by plastic mesh within an aquarium (O'Hara and Blaustein 1981). Because egg masses are clumped usually at a common location, Cascades frog tadpoles are unlikely to encounter either totally unfamiliar siblings or non-siblings. Even if oviposition were not restricted to communal areas, tadpoles are unlikely to have unfamiliar siblings because each female deposits a single, compact egg mass. Although the results of their experiments are intriguing, it is not yet clear how the recognition system Blaustein and O'Hara (1981) propose could lead to the formation of sibling groups in the field.

The finding that Cascades frog larvae reared with siblings and non-siblings fail to discriminate between them, but continue to associate preferentially with unfamiliar siblings over unfamiliar non-siblings reared in sibling groups, suggests that the recognition labels of sibships reared together converge to the point that they become indistinguishable. O'Hara and Blaustein (1981, p. 1124) hypothesize that "... tadpoles reared in mixed sibling groups might assimilate and temporarily retain the 'odour' of the composite group." But tadpoles thus reared retain the ability to recognize siblings that have been reared in sibling groups, whose recognition labels have presumably remain unchanged. Thus, the tadpoles' recognition "template" (Waldman 1981) appears to be unaffected (or minimally affected) by experience, perhaps qualifying it as a component of a "genetic recognition system" (Blaustein and O'Hara 1981). Nonetheless, the template is mismatched with the labels expressed by familiar siblings, which consequently are not recognized.

Kin recognition systems in which the labels that communicate one's kinship identity to conspecifics are altered by social experience, but the recognition templates with which the labels are compared are not similarly modified, appear to be implausible in many natural situations. They may, however, evolve under certain conditions – for example, when individuals either (1) live alone or in social groups with kin only, or (2) live in social groups among non-relatives but for whom it is most important to discriminate degrees of relatedness of unfamiliar conspecifics (other advantages presumably being attained by living with a group of known individuals, perhaps through some system of reciprocity) (see Waldman 1983 for a detailed discussion of these points). Further work will be required to determine whether tadpoles of either species show sibling recognition abilities under natural conditions, and how these abilities affect the fitness of the individuals expressing them.

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