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Section of Neurobiology and Behavior, Cornell University, Ithaca

Sibling Recognition in Toad Tadpoles: The Role of Experience

By Bruce Waldman

With 5 figures

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Abstract

To clarify the mechanism by which tadpoles of the American toad (*Bufo americanus*) recognize siblings, tadpoles were reared from eggs either (1) with siblings only, (2) in isolation from conspecifics, or (3) exposed to both siblings and non-siblings. Tadpoles reared with siblings and those reared in isolation preferentially associated with siblings in a laboratory test pool. However, tadpoles that were continuously exposed to siblings and non-siblings showed no significant sibling association unless they had been reared in sibling groups during early development. Tadpoles reared in isolation discriminated paternal — but not maternal — half-siblings from full-siblings. Although sibling preferences can develop in the absence of post-embryonic interactions with conspecifics, early experience appears to be important in the normal ontogeny of sibling recognition. Possibly the mother contributes some factor to her offspring that enables them to recognize each other as siblings.

Introduction

Kinship theory predicts that individuals should behave differently toward conspecifics according to their degree of relatedness (Hamilton 1963, 1964). Although evolutionary biologists generally agree that selection operates on individuals to maximize their inclusive fitness, mechanisms that enable organisms to act differently toward kin and non-kin have not been well studied. Some animals may behave differently toward conspecifics in response to the physical situation or social context in which they are encountered; e.g., they may act most cooperatively toward individuals at home, which frequently are kin (Hamilton 1964; Sherman 1977). In other cases, animals may learn relevant traits of kin in appropriate contexts. Thus, some birds learn vocaliza-

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tions of their offspring before they leave the nest, thereby ensuring that their later parental care is specifically directed (e.g., BEER 1970; BEECHER et al. 1981). Learning may underlie other forms of kin recognition as well, but genetic components can play a fundamental role in its ontogeny. For instance, primitively social sweat bees learn genetically determined traits (presumably odors) of their sisters, enabling them not only to discriminate kin from non-kin, but also to effectively assess their degree of relatedness to unfamiliar individuals (KUKUK et al. 1977; GREENBERG 1979; BUCKLE and GREENBERG 1981). Similarly, desert woodlice are able to recognize kin by learning family-specific chemical "badges" (LINSENMAIR 1972).

Kin recognition mechanisms in vertebrates have not been as well characterized. In spiny mice, sibling recognition may be mediated by learning during early development (PORTER et al. 1978; PORTER and WYRICK 1979). However, pigtail macaques preferentially associate with paternal half-siblings, with whom they have shared no experience, over unrelated individuals in laboratory tests (WU et al. 1980). This finding suggests that in some organisms kin recognition can develop in the absence of experience with related individuals. Both learned and genetic factors appear to be important in the ontogeny of kin recognition in ground squirrels (HOLMES and SHERMAN, in press). I report here results of experiments conducted to clarify the mechanism by which larvae of the American toad (*Bufo americanus*) recognize siblings.

Toad tadpoles are distasteful to some predators, are darkly colored, and form conspicuous schools (WASSERSUG 1973) — traits which may have evolved through kin selection (FISHER 1930; HAMILTON 1964). Aposematic individuals that recognize and preferentially associate with siblings may, by effectively deterring predation on kin, increase their inclusive fitness above that of conspecifics that associate indiscriminately with siblings and non-siblings (WALDMAN and ADLER 1979; WALDMAN, in prep.). Generally, kin recognition mechanisms should be selected for when behaviors directed toward kin augment one's inclusive fitness but individuals belonging to different kinship groups frequently come in contact. Indeed, tadpoles reared with siblings recognize and preferentially associate with these siblings when mixed with non-siblings in a laboratory pool (WALDMAN and ADLER 1979), and in natural conditions form schools consisting predominantly of siblings (WALDMAN, in prep.).

In nature, the earliest social experience of toad tadpoles is usually limited to siblings because larvae develop within a continuous jelly mass and remain aggregated together around the remnants of the mass for several days after hatching. As a basis for discriminating siblings from non-siblings, tadpoles might learn characters of individuals that surround them during these early developmental stages and later identify these individuals or others that share these traits (ALEXANDER 1979; BEKOFF 1981). Alternatively, sibling recognition might be accomplished by a process of "phenotype matching" in which individuals discriminate siblings from non-siblings by comparing other individuals' traits with their own. This assessment could be based on learning one's own phenotype (ALEXANDER 1979) or could result more directly from a "genetic recognition mechanism" (HAMILTON 1964; DAWKINS 1976), but in either case discrimination would not be dependent on prior experience with related individuals.

To investigate the roles of innate and experiential factors in the ontogeny of sibling recognition, I reared tadpoles under several different regimes in which their contact with conspecifics was controlled, and then tested their ability to discriminate full-siblings from non-siblings or half-siblings. Tadpoles were reared under three basic conditions: (1) in a social situation, exposed only to siblings, (2) in a nonsocial situation, isolated from conspecifics, and (3) in a social situation, exposed to both siblings and non-siblings.

General Methods

Animals

During April, May, and June 1978—1980, I collected pairs of toads in amplexus (before the female had begun depositing eggs) from 20 breeding ponds in Tompkins County, New York. Within 3 h after collection, pairs were transported to the laboratory where I placed each pair in a separate 10-l plastic bucket, half-filled with dechlorinated tap water. Most pairs spawned in these buckets within 24 h. Since fertilization is external in toads (as confirmed by experiments described subsequently), this procedure ensures that the parentage of each clutch is known with certainty. After oviposition was complete, I removed eggs from the spawning buckets and placed them in rearing containers that differed according to the design of each experiment. Under all regimes, tadpoles were kept on a LD 14 : 10 photoperiod, were fed spinach daily, and were reared for 4 to 8 weeks before testing.

Prior to testing, I marked 25 tadpoles from each of two different groups with dye solutions to indicate their group identities. Tadpoles from one group were placed for 12 h in a 0.00025 % aqueous solution of methylene blue, and tadpoles from a second group were placed for 12 h in a 0.00025 % aqueous solution of neutral red. These solutions were freshly prepared for each test by mixing 0.05 g stain in 20 l dechlorinated tap water. In experimental tests, the two groups consisted of different sibships; in control tests, both groups belonged to one sibship.

Data on movement patterns of breeding toads suggest that adults return to the same pond, possibly their natal pond, to breed in repeated years (OLDHAM 1966; EWERT 1969; HEUSSER 1969). If different ponds represent relatively discrete breeding units, toads found in the same pond could be more closely related than toads collected in different ponds. To control for this possibility, I conducted experimental tests both with clutches obtained from amplectant pairs collected from the same pond, and with clutches obtained from amplectant pairs collected from different ponds. Linear distances between ponds ranged from 6.0 to 13.2 km.

To eliminate the possibility that groups sorted based on differences in body size (BREDEN et al., in press), for each test the two groups of tadpoles were matched for body length and developmental stage (i. e., means and variances of these parameters did not significantly differ between groups). Overall, tadpoles used in tests ranged from stage 26, when larvae begin to feed, to stage 36, at which time toes become distinct on the hind limbs (GOSNER 1960). During this developmental period, body lengths vary from 10 to 24 mm. Preliminary tests of tadpoles reared in sibling groups indicated that sibling preferences is shown by tadpoles at all developmental stages within this range.

Testing Procedure

Tests were conducted in a 115 × 115 cm plywood test pool filled to a depth of 2 cm with dechlorinated tap water. The bottom of the pool was lined with a layer of polyurethane foam, covered with a sheet of polyethylene plastic. Between tests, the pool was emptied and the plastic sheet thoroughly cleaned or replaced prior to refilling.

To start each experimental test, I simultaneously released 50 marked tadpoles (25 from each of two sibships) at randomized locations in the test pool by means of a raisable partitioned grid (described below). After allowing tadpoles to acclimate for 24 h, the positions and clutch identities of all tadpoles in the pool were recorded twice daily for four or five consecutive days, depending on the protocol for the experiment. Thus each test consisted of either 8 or 10 recordings ("trials") of positions of a single group of 50 individuals in the pool. Using the same procedure, tests to control for the possibility that tadpole locations could be explained on the basis of their dye marks were conducted for each rearing regime with 50 tadpoles all belonging to one sibship (25 marked red and 25 marked blue).

Data were recorded by lowering a polystyrene plastic grid of open-ended partitioned cells (each 14 × 14 mm across, 3 cm depth) from approximately 3 cm above the water onto the bottom of the test pool. The grid was dropped quickly, before tadpoles could move in reaction to the disturbance, by means of a pulley system. Individuals were trapped within separate grid cells, each slightly larger than a tadpole, thus allowing accurate measurements of the two-dimensional Cartesian coordinates of all tadpoles in the pool to be made. In some trials, a few tadpoles became caught under grid partitions; they were quickly freed by depressing the foam underneath, and swam apparently unharmed into adjacent grid cells. Each tadpole was examined under a high-intensity lamp to unambiguously ascertain its dye mark. To ensure against possible observer bias, in most tests the person collecting data was unaware of the design of the test, e. g., whether it was an experimental or control test. After data were collected for each trial, the grid was raised, allowing tadpoles to move throughout the pool.

Data collection periods on any one day were at least 6 h apart, usually one in the morning and one in the afternoon. Tadpoles were not fed during the test period in either experimental or control tests.

Statistical Analysis

The coordinates and group identities of all tadpoles in the pool were entered into computer files. For each trial, the distances from each tadpole to its nearest sibling (same-colored) and to its nearest non-sibling (different-colored) neighbors were calculated. From the two nearest-neighbor distances thus obtained for each of the 50 tadpoles in the pool, mean distances to the nearest sibling (same-colored) neighbor and to the nearest non-sibling (different-colored) neighbor were calculated for each trial. Although the same individuals were sampled repeatedly within a test, replicates were considered independent samples of tadpole positions because individuals moved freely during the interval between trials. For each series of experimental tests, the trial means were compared by two-way analysis of variance (SOKAL and ROHLF 1969). By this procedure, I statistically compared sibling and non-sibling nearest-neighbor distances, factoring out variation in nearest-neighbor distances among tests not attributable to sibship, and interaction between sibship and test effects. Data obtained in control tests were similarly examined for effects resulting from the marking procedure.

I. Tadpoles Reared in Sibling Groups

I first tested whether tadpoles reared in contact only with siblings would preferentially associate with these familiar siblings over unfamiliar non-siblings. Control tests examined the role of familiarity in effecting sibling preferences by determining whether tadpoles preferentially associated with familiar siblings over unfamiliar siblings.

Methods

For experimental tests, egg masses were obtained from 41 pairs of toads. Prior to hatching, each clutch was transferred from its spawning bucket to a separate 75-l glass tank, where tadpoles were reared. Each rearing tank was aerated with an airstone, and approximately two-thirds of the tank water was replaced with dechlorinated tap water every two weeks. After marking and release in the test pool, the tadpoles' positions in the pool were recorded on four consecutive days (8 trials). To control for possible effects resulting from the marking procedure, in each of six tests, the positions of 50 siblings reared in the same tank (25 marked red and 25 marked blue) were recorded in an identical manner.

As control tests for familiarity, four egg masses were each divided into two parts of approximately equal numbers of eggs after spawning was complete. Each half was transferred to a separate 75-l glass tank, and tadpoles shared no post-embryonic experience with siblings reared in the other tank. Tadpole positions in the test pool were recorded on four consecutive days (8 trials).

Results

Tadpoles reared with siblings were significantly closer to sibling than to non-sibling nearest-neighbors (23 tests overall, $F[1,322] = 57.99$, $p < 0.0001$) regardless of whether the two sibling groups were offspring of pairs collected in the same pond (8 tests, $F[1,112] = 5.76$, $p = 0.018$) or offspring of pairs collected in different ponds (15 tests, $F[1,210] = 52.41$, $p < 0.0001$) (Fig. 1). Association with sibling nearest-neighbors was more pronounced, however, when the two sibships were from pairs collected in different ponds.

In these tests, and in subsequent tests reported here, I found no evidence that different sibships aggregated consistently in particular regions of the

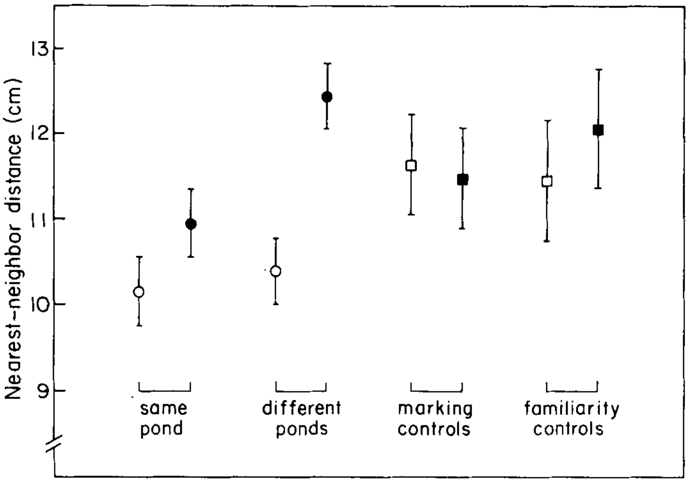

Fig. 1: Mean nearest-neighbor distances for tadpoles reared with siblings. Tadpoles were significantly closer to sibling (○) than to non-sibling (●) nearest-neighbors in both series of experimental tests. In control tests, distance to the nearest same-colored sibling (□) did not significantly differ from distance to the nearest different-colored sibling (■), regardless of whether the two groups were reared in the same or different tanks. Bars denote 95 % confidence limits

pool during repeated trials. Thus sibling association seems not to be due to differential habitat preferences of the two groups. Indeed tadpoles belonging to both sibships moved throughout the pool, and clumps of individuals belonging to either sibship were found in all areas of the pool (see WALDMAN and ADLER 1979).

In control tests for marking, sibling tadpoles reared in the same tank were not significantly closer to nearest-neighbors marked the same color as themselves than to those marked the other color (6 tests, $F[1,84] = 0.15$, $p = 0.70$). Thus the preferential sibling association demonstrated in the experimental tests is not attributable to the marking or testing procedures.

In control tests for familiarity, tadpoles from single clutches split between two different rearing tanks were not significantly closer to familiar than to unfamiliar sibling nearest-neighbors (4 tests, $F[1,56]=1.54$, $p=0.22$), suggesting that the results of the experimental tests were not simply due to tadpoles associating with familiar individuals. Nevertheless there was a trend for tadpoles to be closer to familiar than to unfamiliar siblings, raising the possibility that experience plays some role in the ontogeny of sibling recognition.

II. Tadpoles Reared in Isolation

To determine whether recognition of siblings is dependent on prior experience with them, tadpoles were reared from eggs in individual containers, with no opportunity to interact with any conspecifics. Tests were conducted to determine whether these tadpoles preferentially associated with unfamiliar siblings over unfamiliar non-siblings.

Methods

Egg masses were obtained from 17 pairs of toads. Shortly after oviposition and fertilization in the spawning buckets, 150 eggs from each clutch were separated from the egg mass, and each egg with its surrounding jelly was placed singly in an individual polystyrene Petri dish (100 × 15 mm) containing 40 ml dechlorinated tap water. Eggs were separated as soon as possible after oviposition, but in all cases before neural plate formation (stage 13, GOSNER 1960). The external surfaces of the Petri dishes were coated with paraffin wax to prevent visual interactions among tadpoles. During the 5—7 week period in which they were reared in these dishes, tadpoles were fed daily and water was changed every other day with dechlorinated tap water. Clutch identities of tadpoles were labelled on the outside of each Petri dish, and dishes containing individuals belonging to different sibships were stacked in random order to ensure uniform treatment of all groups.

For experimental tests, 25 tadpoles from each of two sibships were marked with dyes in their individual Petri dishes prior to testing, to indicate their clutch identities. They were then simultaneously released at randomized locations in the test pool — their first exposure to other tadpoles — and their positions were recorded for five consecutive days (10 trials). Control tests for dye marks were conducted in a similar manner, but all 50 tadpoles were siblings (25 marked red, 25 marked blue).

Results

In each of 7 experimental tests, isolate-reared tadpoles were closer to sibling than to non-sibling nearest-neighbors (overall, F[1,126] = 22.86, $p < 0.0001$) (Fig. 2). This difference was significant both for those tests in which the two sibships were obtained from pairs collected in the same pond (3 tests, F[1,54] = 9.26, p = 0.0036) and for those in which they were obtained from pairs collected in different ponds (4 tests, F[1,72] = 14.78, p = 0.0003). In control tests, isolate-reared tadpoles were not significantly closer to same-colored than to different-colored siblings (3 tests, F[1,54] = 0.11, p = 0.74).

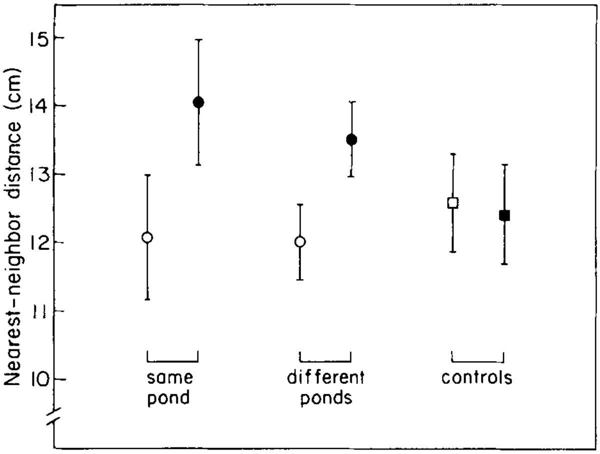

Fig. 2: Mean nearest-neighbor distances for tadpoles reared singly in Petri dishes, in isolation from conspecifics. Tadpoles were significantly closer to sibling (○) than to non-sibling (●) nearest-neighbors. In control tests, distance to nearest same-colored sibling (□) did not significantly differ from distance to nearest different-colored sibling (■). Bars denote 95% confidence limits

III. Half-siblings Reared in Isolation

Where individuals regularly interact with relatives having different degrees of relatedness, they should be selected to behaviorally discriminate among these individuals (HAMILTON 1964; SHERMAN 1980; cf: BARASH et al. 1978; SHERMAN 1979). Since male toads may sequentially amplex more than one female (pers. obs.; also: WELLS 1977; DAVIES and HALLIDAY 1979), half-sibling tadpoles could simultaneously be present in a pond. To test whether tadpoles discriminate between classes of relatives, I examined whether individuals reared in isolation associated preferentially with full-siblings over half-siblings. Tests were conducted both with half-siblings that shared the same mother, and with half-siblings that shared the same father.

Methods

I obtained half-siblings by interrupting amplectant pairs when the female had deposited about half her eggs in a spawning bucket. I removed the male amplexed to the female, rinsed the female in tap water to wash off any residual sperm, and allowed another male to clasp her. The new pair was then placed in a different bucket, and the female soon continued depositing eggs. The displaced male was also allowed to amplex another female. Thus from two males and two females, four groups of full-siblings could be obtained. The two sibling groups with the same mother but a different father are maternal half-siblings with respect to each other; the two with the same father but a different mother are paternal half-siblings with respect to each other. Six pairs each of maternal half-sibling groups and paternal half-sibling groups were tested.

To confirm that eggs were not fertilized prior to oviposition, i.e., in the female's ovisac or cloaca (TOWNSEND et al. 1981), I interrupted four amplectant pairs midway through oviposition, removing the male clasped to the female but not replacing him with another male. As in the procurement of half-siblings, I rinsed the female thoroughly in tap water to remove sperm from her body, and then placed her alone in a newly prepared bucket. Within 30 min, each female spontaneously continued oviposition. Although in each case greater than 98 % of the eggs deposited while the female was amplexed were fertilized and developed, none of the eggs spontaneously deposited after the male was removed developed. This finding assures the identity of half-siblings.

All tadpoles were reared and marked in individual Petri dishes, in the same manner as in the previous series of tests. Half-sibling groups used in any test were obtained from pairs collected in the same pond. Tests were conducted for five consecutive days (10 trials).

Results

When maternal half-siblings were tested, individuals were not significantly closer to full-sibling than to half-sibling nearest-neighbors (6 tests, $F[1,108] = 0.66$, $p = 0.42$) (Fig. 3). However, tadpoles preferentially associated with full-siblings over paternal half-siblings (6 tests, $F[1,108] = 44.03$, $p < 0.0001$). Thus these data suggest that tadpoles are able to discriminate full-siblings from paternal half-siblings, but not from maternal half-siblings. This discrimination is independent of post-embryonic experience with other individuals, since all tadpoles were reared in isolation from conspecifics.

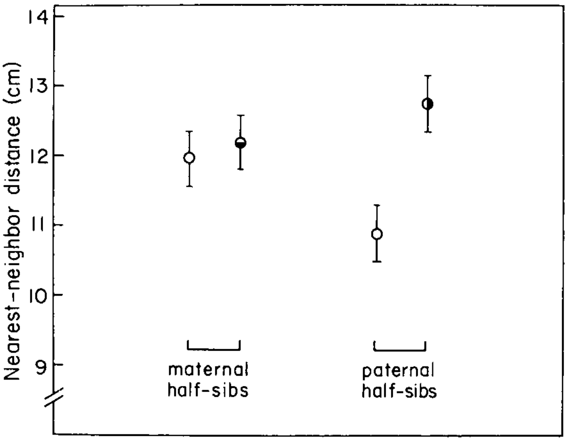

Fig. 3: Mean nearest-neighbor distances for tadpoles tested with siblings and half-siblings. Distance to nearest full-sibling (o) did not significantly differ from that to nearest maternal half-sibling (◒). However distance to nearest full-sibling (o) was significantly less than that to nearest paternal half-sibling (◑). All tadpoles tested were reared in isolation. Bars denote 95 % confidence limits

IV. Tadpoles Reared with Exposure to Siblings and Non-siblings

I examined whether experience with non-siblings affects sibling association by rearing several different sibships in a single tank. Water was actively pumped throughout the tank so that during development tadpoles were continuously exposed to waterborne cues emanating from both siblings and non-siblings. Tadpoles also had some visual interactions with non-siblings through screen partitions. Tests were conducted to determine whether tadpoles preferentially associated with familiar siblings over familiar non-siblings. In addition, to investigate whether simultaneous exposure to several sibships alters discrimination generally, I conducted tests to determine whether tadpoles reared in mixed group tanks preferentially associated with familiar siblings over unfamiliar non-siblings.

Methods

Clutches were obtained from 25 pairs of toads. Pairs spawned in 10-l buckets, and when oviposition was complete, each egg mass was moved to a separate fiberglass screen basket (38 × 38 × 38 cm, 1.4 mm mesh) within a 425-l rearing tank (115 × 120 × 30 cm). Three rearing tanks constructed of plywood and glass were used, each designed to hold up to 8 baskets containing different sibships. During early tadpole developmental stages, the baskets were lined with nylon mosquito netting (0.5 mm mesh) to prevent larvae from wriggling through the fiberglass screening. For the entire period in which eggs and tadpoles were in a tank, water was continuously collected on one side of the tank through an intake pipe, thoroughly mixed, and pumped (20 l/min) through a tube to an outlet pipe on the other side of the tank. Both intake and outlet pipes extended the entire length of a side; thus water was circulated in a unidirectional path from one side of the tank to the other, passing through all baskets contained within the rearing tank. Tests with dye solutions introduced into various regions of the tank confirmed that substances were distributed throughout the tank within 5 min of their introduction.

In the first series of tests, tadpoles belonging to two sibships reared in different baskets within the same mixed group tank were used (Fig. 4, condition A). For control tests, two egg masses were each divided into two parts of approximately equal numbers of eggs shortly after spawning was complete, and the parts were placed in separate baskets within the same rearing tank. Test groups consisted of sibling tadpoles reared in separate baskets (Fig. 4, condition C).

In addition to tests with tadpoles reared in a single mixed group tank, two tests were conducted with different sibships reared in different mixed group tanks. These tadpoles had been exposed to other sibships reared in different baskets within the same rearing tank, but they had never been exposed to the other sibship with which they were tested (Fig. 4, condition B). In these tests, sibships were obtained from pairs collected in different ponds. For all three series of tests, coordinate positions of tadpoles were recorded on four consecutive days (8 trials).

Results

Tadpoles reared in the same tank with siblings and non-siblings were not significantly closer to sibling than to non-sibling nearest-neighbors (10 tests, F[1,140]=2.36, p=0.13) (Fig. 4). Although the results were similar for those tests in which the two sibships were obtained from pairs collected in the same pond (3 tests, F[1,42] = 0.058, p = 0.81) and for those in which they were obtained from pairs collected in different ponds (7 tests, F[1,98] = 3.52, p = 0.064), there was some tendency in the latter tests for tadpoles to associate with siblings. In control tests in which all tadpoles tested were siblings, individuals were not significantly closer to same-colored than to different-colored nearest-neighbors (2 tests, F[1,28] = 0.42, p = 0.52). However, when the two sibships were reared in different mixed group tanks, tadpoles associated preferentially with familiar siblings over unfamiliar non-siblings (2 tests, F[1,28] = 6.84, p = 0.014).

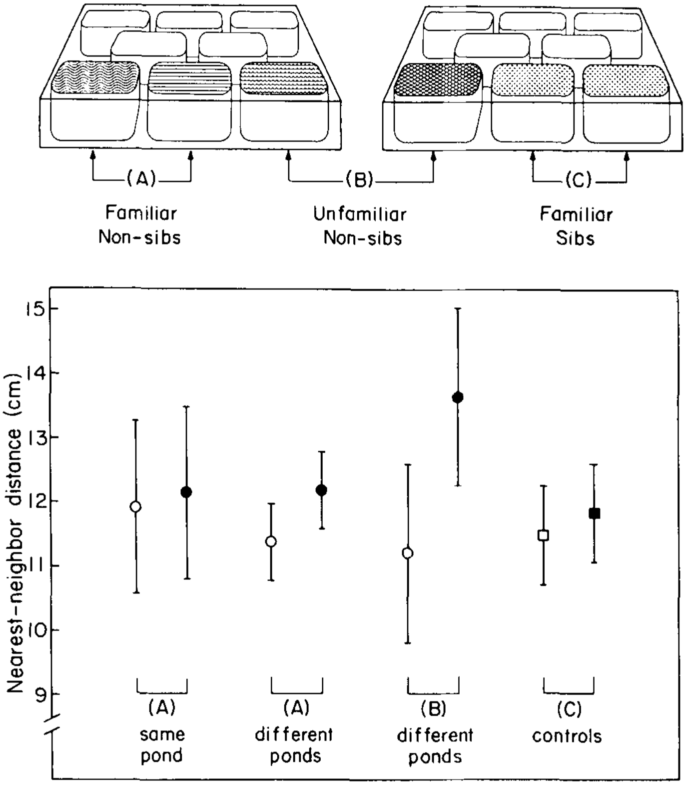

Fig. 4: Mean nearest-neighbor distances for tadpoles reared in mixed group tanks, continuously exposed to siblings and non-siblings (top). When different sibships from the same mixed group tank were tested (condition A), distance to the nearest sibling (o) did not significantly differ from that to the nearest non-sibling (●). Sibships from different mixed group tanks (condition B) were significantly closer to sibling neighbors (o) than to non-sibling neighbors (●). In control tests, all animals were siblings (condition C). Distances to neighbors marked the same color (□) and to neighbors marked the other color (■) did not significantly differ. Bars denote 95 % confidence limits

V. Tadpoles Reared with Limited Ontogenetic Exposure to Non-siblings

In the previous series of tests, exposure of tadpoles to both siblings and non-siblings from an early embryonic stage until the time of testing appeared to interfere with their ability to discriminate between these familiar groups. Early in ontogeny, before dispersal from the oviposition site, tadpoles' experience is likely to be with siblings. Thus if tadpoles learn traits of individuals around them or acquire identifiable kinship factors during this early period, they might later be able to recognize siblings on this basis. One might then hypothesize that sibling recognition would not be affected by later experience with non-siblings. I tested this possibility by exposing tadpoles only to siblings during early development, and later transferring them into tanks in which they were exposed to both siblings and non-siblings. I also conducted the reciprocal experiment, first rearing groups of tadpoles exposed both to siblings and non-siblings, and later moving each sibship into a separate tank.

Methods

Four egg masses were each initially reared in separate 75-l glass tanks for various periods ranging between 18 days and five weeks, after which the clutches were transferred into separate screen baskets within a single 425-l rearing tank (described above) in which they were exposed to each other and four other sibships. Two other clutches were placed as egg masses in separate baskets within a single tank, exposed to each other and to six other sibships, and after 18 days these sibling groups were moved into separate 75-l glass tanks. In each case, tadpoles were reared under the second set of conditions until they were tested, i. e., for periods ranging from two to six weeks.

In the first series of tests (sibships initially reared apart), tadpoles were tested to determine whether they preferentially associated with siblings with which they had been continuously reared since oviposition over non-siblings to which they had been exposed later in development. In the second series of tests (sibships initially reared together), tadpoles were tested to determine whether they preferentially associated with siblings with which they had been continuously reared over non-siblings to which they had been exposed only during the first 18 days of development. Tests were conducted for four days (8 trials). As only two groups were reared for the second series of tests, two tests were conducted with the same sibships; however different individuals were used and each sibship was marked differently in the two tests. In three of the four tests of the first series and in both tests in the second series, the sibships used in each test were obtained from pairs collected in different ponds.

Results

Tadpoles whose early ontogenetic experience was limited to siblings preferentially associated with these siblings even after up to six weeks of exposure to non-siblings (4 tests, F[1,56] = 4.99, p = 0.030) (Fig. 5). However tadpoles that had early experience with both siblings and non-siblings did not preferentially associate with familiar siblings over these non-siblings

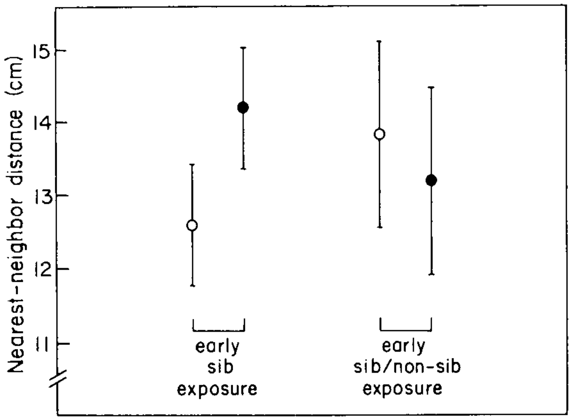

Fig. 5: Mean nearest-neighbor distances for tadpoles reared under different regimes during ontogeny. Tadpoles that had been reared with siblings prior to exposure to non-siblings were significantly closer to these familiar sibling nearest-neighbors (o) than to familiar non-sibling nearest-neighbors (●). However tadpoles reared in the reverse ontogenetic sequence were not significantly closer to familiar sibling nearest-neighbors (o) than to non-sibling nearest-neighbors (●) to which they had been exposed early in development. Bars denote 95 % confidence limits

(2 tests, F[1,28] = 0.51, p = 0.46), even though their later experience was limited to siblings. Thus early interactions seem important in the ontogeny of sibling recognition, and recognition abilities appear not to be greatly affected by later experience.

Discussion

Tadpoles reared in groups consisting exclusively of siblings could discriminate familiar siblings from unfamiliar non-siblings. This preferential sibling association is not simply the result of a preference to associate with familiar individuals since in control tests tadpoles did not show a significant preference for familiar over unfamiliar siblings. Furthermore, tadpoles lacking any contact with conspecifics from an early ontogenetic stage until the time of testing were also able to discriminate siblings from non-siblings. Thus sibling recognition in toad tadpoles can develop in the absence of post-embryonic experience with conspecifics.

Yet experience apparently plays an important role in the ontogeny of sibling recognition. Tadpoles reared from an early embryonic stage until testing in an environment in which they were continuously exposed to both siblings and non-siblings failed to show significant sibling association. The rearing tanks provide a relatively homogeneous environment in which tadpoles are exposed to cues emanating from non-siblings as well as from siblings. Tadpoles may express traits or produce signals that convey kinship identity (e.g., GREENBERG 1979); if individuals develop a basis for sibling preference as a result of learning these cues during an appropriate ontogenetic stage, they might later be unable to discriminate among individuals belonging to the groups to which they were exposed. Identifiable traits used in kin recognition might be "fixed" (i.e., non-modifiable by social interactions) or they might be labile, perhaps only during an early ontogenetic period. If an individual's kinship-identifying "label" were modifiable, labels might be transferred among proximate individuals regardless of their relatedness. Thus all tadpoles reared in a single mixed group tank might acquire the same mixture of kinship-identifying traits, as in the "Gestalt" model considered by CROZIER and DIX (1979). More generally, as a result of their social interactions, individuals belonging to different sibships might express overlapping sets of traits. If variation in kinship-identifying traits within sibships increased relative to variation among sibships, sibships reared together might be indistinguishable from one another based on these characters. The experiments reported here do not resolve the nature of the cues used.

The mixed group rearing regime represents a situation in which water is circulated more quickly and more uniformly than in most habitats in which toad tadpoles normally develop, since toads usually breed in relatively still ponds and shallow pools. Although mixed group rearing experiments can be useful in elucidating aspects of the ontogenetic process by which kin recognition develops, the results of these studies cannot be directly extrapolated to the normal ontogeny of recognition in natural conditions, particularly if important kinship-identifying signals are waterborne. Moreover, under these experimental conditions, tadpoles have physical contact with siblings but not with non-siblings, and this differential contact might contribute to the weak tendency for tadpoles to discriminate between siblings and non-siblings, when from different ponds.

Whatever the nature of the factors that effect sibling recognition, early experience seems critical. Tadpoles whose early experience had been limited to siblings preferentially associated with these siblings over non-siblings to which they were exposed later in development. However, tadpoles exposed to both siblings and non-siblings early in development did not discriminate between these groups, even though their later experience was with siblings only. These results suggest that a critical or sensitive period exists early in development during which tadpoles learn and/or acquire appropriate identifiable traits, and that individuals form a relatively permanent recognition "template" that they later use to recognize siblings. In their natural environment, tadpoles are exposed first to their own characters and those of their siblings; thus under these conditions recognition might be limited to siblings. A mechanism by which recognition specificity is determined prior to hatching could provide a sufficient basis for later sibling recognition even when egg masses from several different pairs are contiguous to one another (in breeding aggregations of *B. americanus,* several pairs do sometimes deposit egg masses in one area of the pond). The finding that sibling preferences are essentially "imprinted" early in development (i.e., they seem not to be modifiable by later experience) is unusual, since continued association in sibling groups at ontogenetic stages after sibling recognition abilities have been expressed is apparently necessary for the maintenance of these sibling preferences in other organisms that have been studied (LINSENMAIR 1972; KUKUK et al. 1977; PORTER et al. 1978; PORTER and WYRICK 1979). Additional experiments are presently under way to further investigate possible effects of later experience on sibling preferences.

The ability of tadpoles reared in isolation to recognize siblings might be interpreted as evidence in favor of a "genetic recognition mechanism", i.e., one in which genes both impart phenotypic traits upon their bearers and endow them with the ability to recognize these traits in conspecifics (HAMILTON 1964; DAWKINS 1976). That a single gene could be capable of producing such a complex phenotypic effect seems unlikely; however these functions conceivably could be accomplished by a tightly linked group of several genes (HAMILTON 1964). Indeed, recent work on mice suggests that just such a linkage group, the H-2 histocompatibility complex, may serve both functions. In mate choice tests, mice apparently prefer individuals that differ from themselves at this particular locus (YAMAZAKI et al. 1980). Any empirical evidence demonstrating a kin recognition role for such supergenes would imply that even if they operate without regard to their effect on alleles at other loci in the genome, selection against them (ALEXANDER and BORGIA 1978; ALEXANDER 1979) might be ineffective.

More complex kin recognition mechanisms are also possible which, although genetically based, involve a more generalized ability to assess phenotypic similarity (ALEXANDER 1979) and possibly incorporate experience with proximate conspecifics in the ontogeny of recognition (ALEXANDER 1979; BEKOFF 1981). Such a mechanism seems likely in toad tadpoles, considering their apparent inability to discriminate between siblings and non-siblings when both sibships were reared together from early embryos. Although organisms with relatively small clutch sizes conceivably could identify siblings based on familiarity with their individual traits (BEKOFF 1981), given the large size of toad clutches (ranging from 2,000—13,000 eggs), the ability of tadpoles to individually recognize all members of their clutch seems highly improbable. Thus discrimination of siblings probably results from a type of group rather than individual recognition (see BREED and BEKOFF 1981) in which traits common to members of the sibship are perceived. Tadpoles reared in isolation may thus acquire some perception of their own phenotype, a "self-template", and then recognize siblings by comparing phenotypic characters of other individuals with their own. These traits could be genetically determined, in which case related individuals would be likely to possess them, or they might be acquired either from the environment (HÖLLDOBLER and MICHENER 1980) or through a non-genetic contribution of the parents.

The asymmetric results from the half-sibling experiments suggest that a sibling recognition factor may be associated with maternal contributions to the eggs. Tadpoles reared in isolation discriminated full-siblings from paternal half-siblings, but they did not discriminate full-siblings from maternal half-siblings. Most anuran amphibians whose karyotypes have been examined, including several *Bufo* species, lack dimorphic sex chromosomes and probably have few if any sexually dimorphic linkage groups (DODD 1960; OHNO 1967; BEÇAK 1968; but see SCHMID and BACHMANN 1981). Thus this effect probably cannot be attributed to recognition factors encoded on sex chromosomes; moreover, these results are not necessarily predicted by a chromosomal model of sex-linked inheritance. However, maternal half-siblings share various cytoplasmic factors (genetic and non-genetic) that paternal half-siblings do not have in common. Besides cytoplasmic contributions to the egg cells, maternal half-siblings share a common environment in the mother's body cavity and are all coated with an acellular jelly matrix as they move through her oviducts. The zygotes develop within this jelly, and the larvae aggregate around the jelly mass for several days after hatching. The chemical composition of the jelly, largely glycoproteins and hexosamines, differs among various anuran species, as do their histochemical properties (SHAVER 1966). If these traits also vary among conspecifics, a mother might "label" her offspring (GUBERNICK 1980, 1981) using properties of the jelly, possibly enabling them to recognize each other. Other maternally-derived recognition mechanisms are also possible. For instance, tadpoles might pick up an intestinal microfauna from bacteria that get into the jelly from the mother, and later use chemicals derived from these bacteria in their discrimination process.

Tadpoles reared in sibling groups preferentially associated with siblings regardless of whether the two sibships were obtained from pairs collected from the same or different ponds, but tadpoles generally were closer to non-siblings if they were from the same pond than if they were from different ponds. Although this trend is not apparent in the results of the experiments on tadpoles reared in isolation, these data are consistent with the hypothesis that the magnitude of sibling preference shown corresponds to possible differences in genetic relatedness. This need not imply that the recognition cue is genetically determined, however. Phenotypic differences could possibly result from influences of different environmental factors on the parents at different locations. For instance, egg-jelly or yolk composition might be influenced by diet or factors in the water at the breeding pond.

I have established that sibling recognition in toad tadpoles can develop in the absence of post-embryonic interactions with conspecifics. However, tadpoles normally do have contact with other individuals, and my results suggest that sibling preferences are affected by experience. In nature, tadpoles' early social interactions are usually with siblings, and experience during this early developmental period appears to be particularly important for the formation of sibling preferences. Unlike nestmate recognition in sweat bees, in which an individual recognizes odors of its nestmates but not its own odor (BUCKLE and GREENBERG 1981), in natural conditions tadpoles presumably utilize both their own traits and those of surrounding individuals in forming a sibling recognition "template". My results further suggest that maternally-derived recognition factors, e.g., cytoplasmic constituents or components of the egg-jelly, play a role in the ontogeny of sibling recognition.

Summary

Tadpoles of the American toad (*Bufo americanus*) were reared under various regimes and then tested for their ability to discriminate full-siblings from non-siblings or half-siblings in a laboratory pool. Tadpoles reared in sibling groups preferentially associated with familiar siblings over unfamiliar non-siblings. This sibling preference is not due to familiarity, however, since in control tests tadpoles showed no significant preferential association with familiar over unfamiliar siblings. Tadpoles reared from an early embryonic stage until testing in isolation from all conspecifics preferentially associated with unfamiliar siblings over unfamiliar non-siblings, suggesting that post-embryonic experience with conspecifics is not required for sibling preference to develop.

Isolate-reared tadpoles showed a significant preference to associate with full-siblings over paternal half-siblings, but showed no significant preference to associate with full-siblings over maternal half-siblings. Since maternal half-siblings share non-chromosomal contributions from their mother that paternal half-siblings do not share from their father, these results suggest that some factor obtained from the mother is important in the ontogeny of sibling recognition.

Tadpoles reared with continuous exposure to different sibships did not significantly associate with familiar siblings over familiar non-siblings. Tadpoles reared in sibling groups early in development but later exposed to both siblings and non-siblings preferentially associated with familiar siblings over

familiar non-siblings. However, tadpoles exposed to both siblings and non-siblings during early development did not associate with siblings, even after several weeks in which they were held in sibling groups. These results suggest that tadpoles recognize individuals that have traits similar to those of conspecifics to which they are exposed early in development. Identifiable kinship "labels" may or may not be modifiable by social interactions; if they are labile during an early developmental period, tadpoles might acquire, from proximate conspecifics, specific traits that identify them to conspecifics. Although sibling preferences are affected by experimental manipulation of rearing conditions, in natural conditions toad tadpoles' first social interactions are with siblings and in this context they may develop the ability to specifically recognize siblings.

Tadpoles reared in sibling groups preferentially associated with siblings over non-siblings regardless of whether the non-siblings were offspring of parents from the same pond as their own or from a pond some distance away. However, tadpoles associated somewhat more closely with non-siblings from the same pond than with non-siblings from a different pond. These results are consistent with the assumption that the degree of relatedness among sibships from the same pond is greater than that among sibships from different ponds, but genetic effects may be confounded by differences in environmental factors.

Zusammenfassung

Kaulquappen der amerikanischen Kröte (*Bufo americanus*) wurden unter verschiedenen Bedingungen gehalten und dann im Labor auf ihre Fähigkeit getestet, Vollgeschwister von Halbgeschwistern und Fremden zu unterscheiden. Ausschließlich mit Geschwistern aufgezogene Kaulquappen schlossen sich Geschwistern an, nicht aber fremden Nicht-Geschwistern. Das liegt aber nicht nur am Bekanntsein, denn sie machten keinen Unterschied zwischen bekannten und unbekannten Geschwistern. Auch Kaulquappen, die von frühen Embryonalstadien an isoliert aufwuchsen, verhielten sich so; Geschwisterbevorzugung bedarf also keiner nach-embryonalen Erfahrung.

Isoliert aufgewachsene Kaulquappen bevorzugten Vollgeschwister signifikant gegenüber Halbgeschwistern väterlicherseits, nicht aber gegenüber solchen mütterlicherseits. Es scheint, daß ein nicht-genetischer, von der Mutter stammender Faktor für die Geschwistererkennung maßgeblich ist.

Kaulquappen, die ständig Kontakt mit Geschwistern und Fremden hatten, zogen Geschwister nicht den bekannten Fremden vor. Diesen Unterschied machten sie aber, wenn sie zunächst nur Kontakt mit Geschwistern hatten und erst später auch Kontakt mit Fremden bekamen. Wuchsen sie in Kontakt mit Geschwistern und Nicht-Geschwistern auf, so schlossen sie sich nicht an Geschwister an, wenn sie später in reinen Geschwistergruppen gehalten wurden. Es scheint, daß Kaulquappen früh in der Ontogenese im Kontakt mit Artgenossen Merkmale von diesen erlernen und sich später an solche Individuen halten, die diese Merkmale aufweisen. (Oder sie nehmen im Kontakt dieses Merkmal selber an und weisen dann alle dasselbe Kennzeichen auf.)

Kaulquappen, die ausschließlich mit Geschwistern aufwuchsen, ziehen diese allen Fremden vor, gleichgültig ob die Fremden aus dem gleichen oder einem anderen Laichgewässer stammen. Im Wahlversuch ziehen sie aber Fremde aus dem gleichen Gewässer vor. Das deutet darauf hin, daß Kaulquappen aus dem gleichen Gewässer näher miteinander verwandt sind als solche aus verschiedenen Gewässern.

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Literature Cited

Alexander, R. D. (1979): Darwinism and Human Affairs. Univ. of Washington Press, Seattle • Alexander, R. D., and G. Borgia (1978): Group selection, altruism, and the levels of organization of life. Ann. Rev. Ecol. Syst. **9**, 449—474.

Barash, D. P., W. G. Holmes and P. J. Greene (1978): Exact versus probabilistic coefficients of relationship: some implications for sociobiology. Am. Nat. **112**, 353—363 • Beçak, M. L. (1968): Chromosomal analysis of eighteen species of *Anura*. Caryologia **21**, 191—208 • Beecher, M. D., I. M. Beecher and S. Hahn (1981): Parent-offspring recognition in bank swallows (*Riparia riparia*): II. Development and acoustic basis. Anim. Behav. **29**, 95—101 • Beer, C. G. (1970): Individual recognition of voice in the social behavior of birds. Adv. Study Behav. **3**, 27—74 • Bekoff, M. (1981): Mammalian sibling interactions. Genes, facilitative environments, and the coefficient of familiarity. In: Parental Care in Mammals. (Gubernick, D. J., and P. H. Klopfer, eds.) Plenum Press, New York, 307—346 • Breden, F., A. Lum and R. Wassersug (in press): Body size and orientation in aggregates of toad tadpoles (*Bufo woodhousei*). Copeia • Breed, M. D., and M. Bekoff (1981): Individual recognition and social relationships. J. Theor. Biol. **88**, 589—593 • Buckle, G. R., and L. Greenberg (1981): Nestmate recognition in sweat bees (*Lasioglossum zephyrum*): does an individual recognize its own odour or only odours of its nestmates? Anim. Behav. in press.

Crozier, R. H., and M. W. Dix (1979): Analysis of two genetic models for the innate components of colony odor in social Hymenoptera. Behav. Ecol. Sociobiol. **4**, 217—224.

Davies, N. B., and T. R. Halliday (1979): Competitive mate searching in male common toads, *Bufo bufo*. Anim. Behav. **27**, 1253—1267 • Dawkins, R. (1976): The Selfish Gene. Oxford Univ. Press, New York • Dodd, J. M. (1960): Genetic and environmental aspects of sex determination in cold-blooded vertebrates. Mem. Soc. Endocrinol. **7**, 17—44.

Ewert, M. (1969): Seasonal movements of the toads *Bufo americanus* and *B. cognatus* in northwestern Minnesota. Ph.D. Thesis, Univ. of Minnesota.

Fisher, R. A. (1930): The Genetical Theory of Natural Selection. Clarendon Press, Oxford.

GOSNER, K. L. (1960): A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica **16**, 183—190 • GREENBERG, L. (1979): Genetic component of bee odor in kin recognition. Science **206**, 1095—1097 • GUBERNICK, D. J. (1980): Maternal 'imprinting' or maternal 'labelling' in goats? Anim. Behav. **28**, 124—129 • GUBERNICK, D. J. (1981): Mechanisms of maternal 'labelling' in goats. Anim. Behav. **29**, 305—306.

HAMILTON, W. D. (1963): The evolution of altruistic behavior. Am. Nat. **97**, 354—356. • HAMILTON, W. D. (1964): The genetical evolution of social behaviour. I, II. J. Theor. Biol. **7**, 1—52 • HEUSSER, H. (1969): Die Lebensweise der Erdkröte, *Bufo bufo* (L.); Das Orientierungsproblem. Rev. Suisse Zool. **76**, 443—518 • HÖLLDOBLER, B., and C. D. MICHENER (1980): Mechanisms of identification and discrimination in social Hymenoptera. In: Evolution of Social Behavior: Hypotheses and Empirical Tests. (MARKL, H., ed.) Verlag Chemie, Weinheim, 35—58 • HOLMES, W. G., and P. W. SHERMAN (in press): The ontogeny of kin recognition in two species of ground squirrels. Am. Zool.

KUKUK, P. F., M. D. BREED, A. SOBTI and W. J. BELL (1977): The contributions of kinship and conditioning to nest recognition and colony member recognition in a primitively eusocial bee, *Lasioglossum zephyrum* (Hymenoptera: Halictidae). Behav. Ecol. Sociobiol. **2**, 319—327.

LINSENMAIR, K. E. (1972): Die Bedeutung familienspezifischer „Abzeichen" für den Familienzusammenhalt bei der sozialen Wüstenassel *Hemilepistus reaumuri* Audouin u. Savigny (Crustacea, Isopoda, Oniscoidea). Z. Tierpsychol. **31**, 131—162.

OHNO, S. (1967): Sex Chromosomes and Sex-linked Genes. Springer-Verlag, Berlin • OLDHAM, R. S. (1966): Spring movements in the American toad, *Bufo americanus*. Can. J. Zool. **44**, 63—100.

PORTER, R. H., and M. WYRICK (1979): Sibling recognition in spiny mice (*Acomys cahirinus*): influence of age and isolation. Anim. Behav. **27**, 761—766 • PORTER, R. H., M. WYRICK and J. PANKEY (1978): Sibling recognition in spiny mice (*Acomys cahirinus*). Behav. Ecol. Sociobiol. **3**, 61—68.

SCHMID, M., and K. BACHMANN (1981): A frog with highly evolved sex chromosomes. Experientia **37**, 243—245 • SHAVER, J. R. (1966): Immunobiological studies of the jelly-coats of anuran eggs. Am. Zool. **6**, 75—87 • SHERMAN, P. W. (1977): Nepotism and the evolution of alarm calls. Science **197**, 1246—1253 • SHERMAN, P. W. (1979): Insect chromosome numbers and eusociality. Am. Nat. **113**, 925—935 • SHERMAN, P. W. (1980): The limits of ground squirrel nepotism. In: Sociobiology: Beyond Nature/Nurture? (BARLOW, G. W., and J. SILVERBERG, eds.) Westview Press, Boulder, Colorado, 505—544 • SOKAL, R. R., and F. J. ROHLF (1969): Biometry. W. H. Freeman, San Francisco.

TOWNSEND, D. S., M. M. STEWART, F. H. POUGH and P. F. BRUSSARD (1981): Internal fertilization in an oviparous frog. Science **212**, 469—471.

WALDMAN, B. (in prep.): Sibling association among schooling toad tadpoles: field evidence and implications • WALDMAN, B., and K. ADLER (1979): Toad tadpoles associate preferentially with siblings. Nature **282**, 611—613 • WASSERSUG, R. J. (1973): Aspects of social behavior in anuran larvae. In: Evolutionary Biology of the Anurans. (VIAL, J. L., ed.) Univ. of Missouri Press, Columbia, 273—297 • WELLS, K. D. (1977): The social behaviour of anuran amphibians. Anim. Behav. **25**, 666—693 • WU, H. M. H., W. G. HOLMES, S. R. MEDINA and G. P. SACKETT (1980): Kin preference in infant *Macaca nemestrina*. Nature **285**, 225—227.

YAMAZAKI, K., M. YAMAGUCHI, E. A. BOYSE and L. THOMAS (1980): The major histocompatibility complex as a source of odors imparting individuality among mice. In: Chemical Signals. (MÜLLER-SCHWARZE, D., and R. M. SILVERSTEIN, eds.) Plenum Press, New York, 267—273.

Author's address: Bruce WALDMAN, Section of Neurobiology and Behavior, Cornell University, Langmuir Laboratory, Ithaca, New York 14850, USA.