Anim. Behav., 1986, **34**, 48–53

Preference for unfamiliar siblings over familiar non-siblings in American toad (*Bufo americanus*) tadpoles

BRUCE WALDMAN *
Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853, U.S.A.

Abstract. Kin recognition may involve comparisons between conspecifics' traits and one's own or those of known genetic relatives. In American toad (*Bufo americanus*) larvae, association patterns appear to reflect this 'phenotype matching', based, in part, upon traits of familiar conspecifics. To examine whether socially-reared tadpoles might additionally use their own traits in making such discriminations, individuals were tested in a Y-maze apparatus for their tendencies to discriminate between waterborne cues released by their unfamiliar siblings and by familiar non-siblings. Six of seven test groups spent more time oriented toward their unfamiliar siblings than toward familiar non-siblings, but the difference was significant for only two of these groups. Overall, 84 subjects oriented toward siblings and 51 toward non-siblings. Thus, in some circumstances, toad tadpoles appear to recognize siblings based on shared recognition traits rather than on prior social experience with conspecifics.

To recognize its kin, an organism can compare conspecifics' traits with its own or with those of its close genetic relatives (often known through prior interactions in unambiguous social circumstances). Kinship assessments thus made may be based on comparisons of general phenotypic characters (Alexander 1979; Getz 1981; Lacy & Sherman 1983) or of especially selected marker traits, which in principle could be encoded by 'recognition alleles' (Hamilton 1964; Dawkins 1976; Ridley & Grafen 1981). The traits compared must correlate with genotypic differences among individuals, though they may consist of genetically or environmentally determined components (discussed in Waldman 1983). If characters are genetically determined, an individual's own traits represent a subset of those expressed by its sibling group as a whole. This may also be true when recognition characters are environmentally influenced. Generalized phenotypic comparisons using characters of siblings may thus provide a broader basis for identifying kin than comparisons solely with one's own traits.

In practice, which traits serve as a model for 'phenotype matching' may not be clearly delineated. American toad (*Bufo americanus*) tadpoles, reared from an early embryonic stage in social isolation, can behaviourally discriminate between siblings and non-siblings, indicating that they can use their own traits as a model for comparison with conspecifics. Moreover, differential responses of isolates to maternal and paternal half-siblings suggest that recognition traits may incorporate a substantial maternal component (Waldman 1981). Nonetheless, toad tadpoles reared in social groups associate indiscriminately with familiar individuals, regardless of whether or not they are genetic relatives. Tadpoles may construct a recognition 'template' based upon phenotypic attributes of individuals with which they come in contact. Template formation appears to occur during early development, under natural conditions presumably before larvae (siblings) disperse from oviposition sites (Waldman 1981, 1985b).

In this paper, I evaluate the extent to which socially-reared tadpoles might use their own traits, in addition to those of known conspecifics, as a basis for phenotype matching. Tadpoles were reared in a mixed group tank, continuously exposed both to their siblings and to non-siblings. These individuals were subsequently tested in a Y-maze apparatus for their tendencies to discriminate between familiar non-siblings, with which they had been reared, and unfamiliar siblings, with which they had no previous post-embryonic experience. Phenotype matching based on one's own traits should result in a preference for siblings, even if they are unfamiliar.

---

* Present address: Department of Organismic and Evolutionary Biology, Harvard University, The Biological Laboratories, Cambridge, Massachusetts 02138, U.S.A.

MATERIALS AND METHODS

Study Animals

Tests involved seven sibships of American toad (*Bufo americanus*) larvae. Amplectant pairs of toads were collected (prior to initiating oviposition) during May 1982 at breeding ponds in Tompkins County, New York, and were transported to the laboratory. Pairs spawned in 10-litre plastic buckets filled with dechlorinated tap water. Each egg mass was split into two portions within 2 h of oviposition. One portion of each clutch was transferred to a separate 75-litre glass tank, and larvae were reared in sibling groups. The other portion was transferred to one of seven fibreglass mesh baskets within a 425-litre mixed group rearing tank (each basket housed a different sibship; tank design further described in Waldman 1981). Water was actively pumped through the baskets at a rate of 40 litres/min so that tadpoles were exposed to any waterborne cues released by their siblings and by their non-siblings. Different members of each sibship were thus reared under both social regimes from early embryonic stages until the testing period (6–8 weeks).

At testing, tadpoles ranged between developmental stages 27 and 40, which span the period between hindlimb bud formation and the external appearance of forelimbs (Gosner 1960). Tadpole body lengths ranged from 14 to 26 mm. Size variation was uniform among the test groups (mean stages fell between 32 and 34, mean body lengths between 17 and 19 mm).

Testing Apparatus and Procedure

Tadpoles reared in mixed group tanks were tested for tendencies to discriminate between familiar non-siblings (with which they were reared) and unfamiliar siblings reared in sibling groups (from which test subjects were separated shortly after oviposition). Individuals were tested in a Y-maze apparatus in which they were exposed simultaneously to water flowing from a container housing familiar non-siblings and one housing unfamiliar siblings (Fig. 1). At least 2 h before each test, two 10-litre plastic buckets were filled with dechlorinated tap water, and 25 tadpoles from each stimulus group were transferred from their rearing tanks to these containers. Water flow from the source containers through connecting tubes was calibrated at a rate of 1 drop/s.

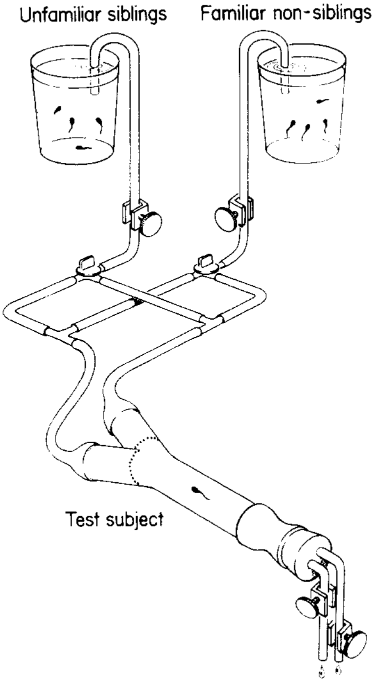

Figure 1. Testing apparatus for measuring tadpole kin preference responses (not drawn to scale). A pair of stopcocks, inserted in tubing between the stimulus containers and the testing device, facilitated the switching of stimulus directions. Water flow rates were regulated by clamps where indicated. Test individuals were considered oriented toward either of the stimulus groups if their snouts extended beyond the criterion (dotted) lines.

Test individuals were removed from the mixed group tank and each was immediately placed in the base of a Y-shaped glass tube, which served as the testing device. After a 5-min acclimation period, the subject's movements in the central, neutral part of the Y-maze and into the two stimulus arms were timed and recorded for 10 min. The stimulus directions were then reversed by adjusting stopcocks inserted in tubing between the source containers and the testing apparatus. The subject was allowed 5 min for reacclimation, and its movements were recorded for an additional 10-min period. A more detailed description of the testing apparatus and procedures is given in Waldman (1985a).

Statistical Analyses

Twenty different subjects were used in each test.

The amount of time spent by each individual in each of the arms of the Y-maze (oriented toward unfamiliar siblings or familiar non-siblings) and in the central, neutral area of the maze was computed. For every test, total times spent oriented toward the two stimulus groups were compared by a Wilcoxon matched-pairs signed-ranks test (Siegel 1956). In addition, the numbers of individuals spending most of their (non-neutral) response time oriented toward unfamiliar siblings and toward familiar non-siblings were compared with those expected under a binomial distribution (binomial test; Siegel 1956). Data from all seven tests (140 individuals) were pooled and subjected to the same analyses. Variability in responses among tests was evaluated by subjecting sibling preference scores (time oriented toward siblings minus time oriented toward non-siblings) of members of each group to a Kruskal–Wallis one-way analysis of variance (Siegel 1956). Statistical inferences were based on two-tailed probabilities.

RESULTS

Tadpoles tended to orient toward unfamiliar siblings rather than toward familiar non-siblings, but the effect lacks robustness. Significant differences were apparent in three of the seven tests (Table I). Two test groups (E,G; tests 4,7) spent significantly more time oriented toward siblings than toward non-siblings. Individual preferences of subjects in two groups (A,G; tests 1,7) indicate a significant bias toward siblings. Three of the remaining test groups (C,D,A; tests 2,3,5) showed marginal tendencies to orient toward unfamiliar siblings by these measures; the other test group (D; test 6) spent, on average, more time oriented toward familiar non-siblings than toward siblings.

Response variability is apparent even when considering test groups composed of members of the same sibship in repeated tests (e.g. tests 1,5). Although the few replicates precluded detailed analyses, variation seems not to be attributable to whether stimulus groups were obtained from the same pond (= breeding population) (tests 2,4), from nearby ponds (1 km apart; tests 1,5,7), or from more distant ponds (4 km apart; tests 3,6) (cf. Waldman 1981). Overall, sibling orientation tendencies did not significantly vary among tests (Kruskal–Wallis test, $\chi^2=9{\cdot}55$, 6 *df*, $P=0{\cdot}15$).

Based on data pooled from all groups, tadpoles spent more time oriented toward unfamiliar siblings (330 ± 169 s, $\bar{X}\pm$SD; 318 s, median) than toward familiar non-siblings (277 ± 149 s; 281 s). Though the difference is significant ($T=3426$, $z=2{\cdot}56$, $P=0{\cdot}01$), tadpoles spent much more time in the neutral area (594 ± 208 s; 574 s) than in either of the stimulus arms. Excluding the amount of time spent in the neutral area, 84 individuals spent over half of their response time oriented toward unfamiliar siblings and 51 spent over half of their response time oriented toward familiar non-siblings ($P=0{\cdot}006$); five individuals spent equal amounts of time in each of the stimulus arms.

DISCUSSION

In the experiments reported here, socially reared *B. americanus* tadpoles preferentially oriented toward a group of their siblings that they had not previously encountered rather than toward the non-siblings with which they had been reared. Prior social experience with individual siblings is thus not a prerequisite for the elicitation of a sibling recognition response. Conversely, experience with individual non-siblings does not lead to the expression of a familiarity response toward them.

The magnitude of the overall response toward unfamiliar siblings is less, however, than the preference displayed for familiar siblings over unfamiliar non-siblings under otherwise identical testing conditions (see Waldman 1985a). Moreover, in the tests reported here, subjects spent somewhat more time in the neutral section of the apparatus (50% of the test period) than they did in previous tests (40% of the test period) (Waldman 1985a). This may indicate that tadpoles were less decisive in expressing a preference when familiarity and genetic relatedness were opposed rather than concomitant stimulus factors. Variability in responses among test groups was also more pronounced in the present experiment although preferences, when displayed, were consistent; significant orientation tendencies toward familiar non-siblings were never observed.

When released in a laboratory test pool, different sibling groups reared together under the same mixed group conditions used here (except with less rapid water circulation among tank compartments) associate indiscriminately with one another. Non-siblings appear to be recognized, like siblings, but only if the sibships interact during early

Table I. Summary of test results

	Sibships			Time (s)						Number of individuals oriented toward		
		Stimulus groups		Sibling	Neutral	Non-sibling	Sibling non-sibling					
Test	Test group	Unfamiliar siblings	Familiar non-siblings	mean (SD) median	mean (SD) median	mean (SD) median	T[†]	N[‡]	P[*]	Unfamiliar siblings	Familiar non-siblings	P[§*]
1	A	A	B	370 (180) 360	574 (180) 590	256 (133) 252	55	20	NS	16	4	0·01
2	C	C	A	324 (208) 264	618 (209) 572	258 (142) 270	85	20	NS	11	9	NS
3	D	D	A	306 (212) 256	602 (235) 580	291 (180) 267	102	20	NS	10	10	NS
4	E	E	F	370 (112) 355	556 (128) 560	274 (111) 292	44	19	<0·05	13	6	NS
5	A	A	E	361 (129) 393	498 (210) 490	341 (159) 347	81	19	NS	10	9	NS
6	D	D	C	253 (140) 250	643 (241) 590	304 (183) 286	75·5	19	NS	10	9	NS
7	G	G	B	324 (166) 300	664 (219) 663	212 (102) 219	26	18	<0·01	14	4	0·03

[*] Two-tailed; NS denotes $P > 0·05$.
[†] Wilcoxon matched-pairs signed-ranks test.
[‡] Excludes ties.
[§] Binomial test.

developmental stages (Waldman 1981). These results suggest that a recognition template based, in part, upon traits of conspecifics encountered during an early sensitive period, serves as a model for phenotype matching. Once formed, the template appears resistant to modification by further experience (Waldman 1981). Similarly, the recognition traits are not labile to post-embryonic social factors (Waldman 1985b).

If recognition occurs by comparing conspecifics' traits with those stored in a template, tadpoles should not discriminate between familiar non-siblings (whose traits they have 'learned') and unfamiliar siblings (whose traits are similar to their own and those of their familiar siblings). The observation that *B. americanus* tadpoles can make this discrimination suggests either (1) that individuals form biased recognition templates which incorporate sibling traits to a greater extent than non-sibling traits, or (2) that individuals can compare recognition traits that they themselves express with those of their conspecifics. Selective incorporation of sibling traits into a recognition template might result if template formation is controlled by some genetic programme (cf. song acquisition in some passerine birds; Marler 1976) or, in the case of possible non-chemical cues, because tadpoles are more directly in contact with siblings in their rearing compartments. If tadpoles use their own traits to recognize kin, these traits need not become irreversibly set in a template during early development. As an individual matures, its model for phenotype matching could be updated continually to reflect its own changing characters.

Tadpoles may have multiple, redundant recognition mechanisms. When singly testing individuals for orientation responses in a Y-maze apparatus, different mechanisms may operate or different motivational factors might come into play than in tests in which association patterns of groups are examined (also see discussion in Waldman 1982). In the Y-maze apparatus, unlike in test pools, individuals are exposed only to waterborne factors released by the stimulus groups, and though these chemical substances are sufficient to elicit kin recognition responses, other modalities are probably also important in the formation of sibling schools (Waldman 1985a). To assess the influence of the testing procedure on the kin discrimination response measured, additional tests using the Y-maze apparatus need to be done on the ability of individuals to distinguish between familiar siblings and familiar non-siblings.

Kin recognition mechanisms may show substantial variability among species. For example, although *Bufo boreas* larvae, like *B. americanus* larvae, associate preferentially with familiar siblings over unfamiliar non-siblings, O'Hara & Blaustein (1982) found that *B. boreas* tadpoles exposed to non-siblings, even during late larval stages, subsequently failed to discriminate between familiar siblings and familiar non-siblings. Moreover, individual larvae reared in containers with five members of another sibship showed no significant preference either for their unfamiliar siblings or for unfamiliar members of the sibship with which they had been reared (O'Hara & Blaustein 1982). This result suggests that phenotype matching based on one's own traits can operate concurrently with matching based on traits of familiar conspecifics. In an experimental situation, the contradictory preferences thus generated may effectively cancel one another out.

While experiments such as these are useful in elucidating the mechanisms underlying kin recognition abilities, they do not necessarily reflect on the functional significance of the response. Each *B. americanus* pair generally deposits its spawn in a continuous gelatinous string within a limited area of a pond, although very occasionally (e.g., when disturbed by observers) a pair may split its clutch between two localities. In these rare cases, an ability to recognize unfamiliar siblings through comparison of common genetically determined traits or maternal labels may be adaptive. Likewise, kin recognition abilities that are not dependent on experience may be expressed in those situations in which egg strings of different pairs are extensively intermingled at the oviposition site (such oviposition strategies are apparently more common among other bufonids, e.g. *B. bufo*; personal observation). Nevertheless, the evolution of a kin recognition system, like any other trait or behavioural pattern, is beset by many constraints. Given that contacts between unfamiliar siblings in natural conditions are probably quite infrequent, an ability to recognize unfamiliar siblings may not have been specifically selected for.

ACKNOWLEDGMENTS

I thank H. Cohen for assistance conducting the experiments and F. Breden, D. K. Dawson, W. G. Holmes, G. Oettingen, and P. A. Wells for comments on the manuscript. The research was supported by grants from NSF (DEB-7909119), the Gaige Fund (American Society of Ichthyologists and Herpetologists), and Sigma Xi. Supplementary funds were provided by NSF grant BNS-7924525 (to K. Adler). While writing the manuscript, I was supported by a NATO postdoctoral fellowship at the University of Cambridge. I thank P. Bateson and R. A. Hinde for making facilities available at the Sub-department of Animal Behaviour, Madingley, and the University Computing Service for providing computer resources.

REFERENCES

Alexander, R. D. 1979. *Darwinism and Human Affairs*. Seattle: University of Washington Press.

Dawkins, R. 1976. *The Selfish Gene*. New York: Oxford University Press.

Getz, W. M. 1981. Genetically based kin recognition systems. *J. theor. Biol.*, **92,** 209–226.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16,** 183–190.

Hamilton, W. D. 1964. The genetical evolution of social behaviour. I, II. *J. theor. Biol.*, **7,** 1–52.

Lacy, R. C. & Sherman, P. W. 1983. Kin recognition by phenotype matching. *Am. Nat.*, **121,** 489–512.

Marler, P. 1976. Sensory templates in species-specific behavior. In: *Simpler Networks and Behavior* (Ed. by J. C. Fentress), pp. 314–329. Sunderland, Massachusetts: Sinauer Associates.

O'Hara, R. K. & Blaustein, A. R. 1982. Kin preference behavior in *Bufo boreas* tadpoles. *Behav. Ecol. Sociobiol.*, **11,** 43–49.

Ridley, M. & Grafen, A. 1981. Are green beard genes outlaws? *Anim. Behav.*, **29,** 954–955.

Siegel, S. 1956. *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill.

Waldman, B. 1981. Sibling recognition in toad tadpoles: the role of experience. *Z. Tierpsychol.*, **56,** 341–358.

Waldman, B. 1982. Sibling association among schooling toad tadpoles: field evidence and implications. *Anim. Behav.*, **30,** 700–713.

Waldman, B. 1983. Kin recognition and sibling association in anuran amphibian larvae. Ph.D. thesis, Cornell University.

Waldman, B. 1985a. Olfactory basis of kin recognition in toad tadpoles. *J. comp. Physiol. A*, **156,** 565–577.

Waldman, B. 1985b. Sibling recognition in toad tadpoles: are kinship labels transferred among individuals? *Z. Tierpsychol.*, **68,** 41–57.

(Received 23 July 1984; revised 23 November 1984; MS. number: A4356)