

***SMILISCA PHAEOTA* (COPE, 1862) (ANURA: HYLIDAE) TADPOLES DO NOT DISCRIMINATE BETWEEN SIBLINGS AND NON-SIBLINGS**

RENACUAJOS DE *SMILISCA PHAEOTA* (COPE, 1862) (ANURA: HYLIDAE)
NO DISCRIMINAN ENTRE HERMANOS Y NO-HERMANOS

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Abstract

Kin recognition behavior (based on the spatial affinity for siblings index [S]) was examined in *Smilisca phaeota* tadpoles injected with odorific substances (experimental tadpoles). According to the S index values obtained, these tadpoles did not discriminate between relatives and nonrelatives when they were presented with unfamiliar sibling and non-sibling individuals. Since these results were similar to those obtained from control tadpoles (not exposed to odorific substances), I conclude that this species does not exhibit kin recognition behavior. Ecological factors such as the predation risks to tadpoles in the puddles where they live and their behavior are discussed as possible explanations for the results presented here.

Key words: Amphibia, Colombia, kin recognition, larval ecology, *Smilisca phaeota*

Resumen

El comportamiento de reconocimiento de parientes (con base en el índice de afinidad espacial [S] por hermanos) fue examinado en renacuajos de *Smilisca phaeota* inyectados con sustancias oloríficas artificiales (renacuajos experimentales). Según los valores obtenidos del índice S, estos renacuajos no discriminaron entre parientes y no-parientes cuando estuvieron en presencia de individuos hermanos y no-hermanos desconocidos. Ya que los resultados fueron similares a los mostrados por renacuajos control (no expuestos a las sustancias oloríficas), se considera que en esta especie no se exhibe un comportamiento de reconocimiento de parientes. Factores ecológicos como el riesgo de depredación de los renacuajos de *S. phaeota* en los charcos donde ellos viven y el comportamiento de ellos en los mismos, son discutidos como posibles explicaciones a los resultados aquí mostrados.

Palabras clave: anfibios, Colombia, ecología de renacuajos, reconocimiento de parientes, *Smilisca phaeota*

INTRODUCTION

Olfactory chemical cues have been identified as being fundamental to individual interactions in amphibian social behavior (Hrbacek, 1950; Jaeger, 1984; Licht, 1967; Smith, 1986; Waldman, 1981, 1984). Waterborne cues are perceived by means of the olfactory sense and

are processed in order to facilitate kin recognition (Waldman, 1985), and in anuran tadpoles they have been shown to play an important role in gregarious behavior and in sibling recognition (Blaustein and O'Hara, 1981, 1986; Waldman, 1982, 1986, 2001). In tadpoles, the sense of smell is well developed and becomes functional before other sensorial systems (Spaeti, 1978).

Recibido: septiembre de 2005; aceptado: diciembre de 2006.

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In terms of the differential behaviors that individuals exhibit toward one another (altruistic versus selfish), Hamilton (1964) proposed that behavior is based on the genetic relatedness of the individuals involved, regardless of the mechanism used to determine the degree of relationships among individuals (Blaustein, 1983). In an aggregation of tadpoles, individuals have been shown to recognize each other as genetic relatives through chemical cues (Waldman, 1981, 1982), presumably by means of phenotypic matching (see Blaustein, 1983; Holmes and Sherman, 1983; Lacy and Sherman, 1983; Waldman, 2001).

How odor preferences develop in tadpoles is unknown, but it has been suggested that they are acquired during the early stages of development, being essential to the ontogeny of any kin recognition mechanism (Waldman, 1981). In addition, such early imprinting might provide a possible mechanism to permit homing to natal ponds as breeding adults (Grubb, 1973; Waldman *et al.*, 1992). Kin recognition abilities may be influenced during the embryonic stage through odor exposure, with learning capacities permitting the posterior development of preferences to these odors as larvae. Embryos exposed to odors that have a strong maternal component (Blaustein and O'Hara, 1986; Waldman, 1991), that are influenced by environmental factors during ontogeny, or odors artificially applied to embryos (Hepper and Waldman, 1992) may permit later association with siblings (Waldman, 2001).

By injecting fragrances into embryos, this study examined the effects of such odors on the kin recognition abilities in *Smilisca phaeota* (Cope, 1862) (Anura: Hylidae). If tadpoles use innate olfactory cues as the basis for kin recognition, rearing them with these substances should not interfere with the normal expression of sibling recognition abilities in this species (Waldman, 1984).

MATERIALS AND METHODS

This study was conducted in El Danubio (Alto Anchicayá region on the western slopes of the Cordillera Occidental), Dagua municipality, Valle del Cauca department, Colombia. Gutiérrez-C. (2006) and Vargas-S. and Castro-H. (1999) offer a more complete description of this locality.

Smilisca phaeota is a hylid anuran widely distributed below elevations of approximately 1000 m from lower Central America to northwestern Colombia and inland in the valleys of the Rivers Cauca and Magdalena (Duellman and Trueb, 1966; Kattan, 1984). Three clutches were collected on February 15, 1999 in different small (less than 1m diameter) roadside puddles, where this species commonly breeds. In breeding sites, *S. phaeota* females lay eggs in loose clumps amidst vegetation on the water (Duellman and Trueb, 1966). Although mean clutch size of 1850 eggs have been reported, in this study the egg masses collected contained an average of 300 eggs.

According to Duellman and Trueb (1966), tadpoles of this species are pelagic inhabitants of shallow puddles, but when disturbed, they usually dive and seek shelter amidst vegetation or in mud on the bottom. However, based on my observations during three months in El Danubio, the tadpoles were bottom-dwellers, although when they were disturbed, the behavior that they displayed was the same as reported by Duellman and Trueb (1966). This behavior was observed both day and night.

The distance between the sites where the three *S. phaeota* clutches were collected was sufficient to guarantee the lack of relatedness among clutches. In addition, this study assumes that larvae obtained from an egg mass were full siblings. The clutches were transferred to a laboratory at the Hospedaje Brisas del Danubio, located near natural habitat for

the species, thus insuring appropriate conditions of light, temperature, and humidity during the study. Temperature in the laboratory was maintained between 24-26 °C during the day and 20-23 °C at night, with a 14:10 h photoperiod. Light was supplied by a 60 W yellow light bulb. The two experimental groups and control group were comprised of tadpoles obtained from these three clutches, as described below.

The influence of larval olfactory exposure on later sibling recognition ability. *Procedure.* At the exact moment of oviposition, three groups of 20 embryos in stages 15-18 of development (Gosner, 1960) were selected from each clutch and injected with, either 10 µl of orange essence (20%), 1 µl of blackberry essence (20%), or 10 µl isotonic saline solution, respectively. The first two groups were experimental tadpoles and the latter group was the control. The injections were made directly into the eggs using a 1-ml insulin syringe (Becton-Dickinson Co, U.S.A.) and 29-gauge needle (12.7 mm) with the aid of a dissecting microscope (30 X magnification). After this process, I placed each experimental group into 1L plastic beakers with 800-ml fresh water. Twenty embryos selected as stimulus tadpoles from each clutch were reared under the same regimen, but without any injections of odors. All hatchling tadpoles were fed boiled lettuce leaves cut into small pieces.

The behavioral trials were performed in a 40 x 20 x 15 cm glass aquarium filled to a depth of 10 cm with fresh water. The floor of the test aquarium was divided longitudinally into four equal sections of 10 cm, which were demarcated with white lines using masking tape and labeled with the letters **A**, **B**, **C**, and **D**. A 4 x 4 x 4 cm box made of fine stainless-steel mesh was placed into each end of the test apparatus. The boxes placed in the A section of the aquarium housed the stimulus larvae that were either siblings or non-siblings of the experimental and control tadpoles. Behavioral tests were conducted between stages 26-36 of development (Gosner, 1960).

The sibling recognition tests were conducted by placing individually an experimental or control tadpole in the center of the aquarium, and after two minutes of acclimatization, the time that it spent in each of the four sections of the aquarium was recorded. With these records, a spatial affinity for siblings index ($S = [1(A) + Z(B) + 3(C) + 4(D)]/T$) was calculated for every tadpole, this being an estimate of the mean section position of the test tadpole. The letters **A**, **B**, **C**, and **D** represented the time (in seconds) spent in each of the four sections and **T** was the total test time (300 s).

This index ranges from 4 (maximum affinity for siblings) to 1 (maximum affinity for non-siblings). Intermediate indices ($2.5 < S < 2.5$) indicate no clear preference between kin and non-kin stimuli. This method to quantify kin recognition abilities in anuran larvae has been recommended (Fishwild et al., 1990) because it includes information from all four sections, providing a more fine-grained estimate of the mean position of the test tadpoles than do simple bipolar indices. This method also is more comparable to the nearest-neighbor analysis used by Waldman (1981). Each calculated value of the S index was compared to the null value of 2.5, using a one-tailed Wilcoxon rank-sum test, under a critical value of $\alpha = 0.05$ (Siegel, 1956). The null value is the expected index value if the tadpoles' behavior patterns were random with respect to kinship.

RESULTS

The experimental tadpoles (those treated with the orange and blackberry essences) and the control tadpoles showed no significant preferences for either stimulus larvae group, since the S index was greater than the null value of 2.5 (table 1). Also, there were no significant difference among S indices obtained from the two experimental groups and the control group ($F_{(2, 57, 0.05)} = 3.18$, $P > 0.25$; Dunnett's test; Zar, 1984).

Table 1. Spatial affinity index (S) based on the amount of time spent by larvae in the four sections of the test aquarium (*Wilcoxon rank-sum test, one tailed).

Experiment	n	S (Mean \pm SD)	p*
Orange injected larvae	20	2.62 \pm 0.53	0.39
Blackberry injected larvae	20	2.61 \pm 0.44	0.25
Control larvae	20	2.56 \pm 0.49	0.74

DISCUSSION

The *S. phaeota* larvae did not show a preferential association for either siblings or for non-siblings, both unfamiliar groups to the experimental individuals. The lack of preferences showed by the tadpoles injected with the odorific substances might suggest that the olfactory experience during embryogenesis influenced an “innate” kin recognition behavior. Assuming that the recognition labels for phenotype matching are genetically encoded (Blaustein and O’Hara, 1986; Waldman, 1988, 1991, 2001), and represented a subset of those expressed as a whole by their siblings (Waldman, 1986), the experimental treatments may have masked or annulled the innate abilities for recognizing and associating with relatives.

However, the mean S index of the control tadpoles (those injected with saline solution) was not different to those shown by the experimental groups. This suggests that the behavior exhibited by the tadpoles exposed to the olfactory substances was not a consequence of the modification of an innate behavior due to embryonic olfactory experiences. Apparently, the lack of kin recognition behavior or template is typical of *S. phaeota* larvae. Waldman (1986) suggested that the evolution of a kin recognition system, like any other trait or behavioral pattern, is beset with many constraints. Among the constraints in this case, I propose that the behavior and ecology of tadpoles associated

with predation risks should play an important role in whether kin recognition capabilities evolve or not. Natural selection should favor kin discrimination only when its benefits exceed its costs, measured in terms of inclusive fitness (Waldman, 1991). Thus, kin recognition is a context-dependent behavior (Reeve, 1989; Waldman, 1988).

The predation risks to a species also might significantly influence its behavior. As an anti-predator mechanism, *S. phaeota* larvae do not form aggregations in the shallow puddles where they live (Duellman and Trueb, 1966; personal observation). In fact, larval aggregations in these habitats actually make them more vulnerable to predators, especially birds (Crump and Vaira, 1991; Duellman and Trueb, 1986). The small body size and high palatability of *Smilisca* tadpoles make them susceptible to aquatic predators like odonata nymphs or carnivorous larvae of *Leptodactylus pentadactylus* (Heyer et al., 1975), but this susceptibility is offset by their benthic behavior (Heyer et al., 1975; personal observation), which is not pelagic as Duellman and Trueb (1966) reported. This behavior, coupled with their cryptic coloration, confers upon them protection from predators, especially those that rely on visual cues to find their prey (Fishwild et al., 1990). Other anuran species also depend on their cryptic coloration to avoid predators (*Pseudacris crucifer*, Fishwild et al., 1990; *Engystomops pustulosus*, Heyer et al., 1975).

In addition, a behavior detected frequently in the puddles visited in the study site was that the larvae moved rapidly from side to side, lifting mud clouds from the substrate with their tail movements. This clouded water allows them to distract the observer while they hide again, to rely on their camouflage on the bottom or to seek shelter amidst vegetation.

If these explanations for the absence of the kin recognition abilities in *S. phaeota* larvae are valid, it is probable that selection has not favored such abilities in this species. The common adaptive advantages conferred by sibling aggregations (Smith, 1986; Waldman, 1982, 1991) are, on the contrary, replaced by the advantages of the other life history traits mentioned, which is consistent with Waldman's

(1991) affirmation that kin recognition behavior may not be a universal trait in anuran larvae.

ACKNOWLEDGMENTS

This paper constituted part of my undergraduate thesis in the Department of Biology at the Universidad del Valle (Cali, Colombia). I am grateful to Fernando Garzón for providing logistic support at the lodging facility at the study site. Fernando Vargas kindly collaborated with the rearing of the larvae. Thanks to B. Waldman for provided useful comments and literature. I also thank R. García for company during the field work and to B. Bock for improving the grammar and redaction of the manuscript. My gratitude also goes to C. Gutiérrez and E. Saavedra for financial support for this project.

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