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Conspecific Chemical Cues Influence Pond Selection by Male Newts *Triturus boscai*

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In newts of the genus *Triturus*, there is behavioral and anatomical evidence for the existence of chemical communication. A laboratory study tested whether male Bosca's newts (*Triturus boscai*) preferred water in which they had been held over clean water or water in which other males or females had been held. Males preferred water that presumably contained chemical cues from themselves or other conspecifics, and males already paired with a female spent significantly more time in their home water than did single males. These observations suggest that male *T. boscai* were able to identify chemical cues from conspecifics and discriminate cues from females.

Los tritones pertenecientes al género *Triturus* presentan evidencias tanto anatómicas como comportamentales de la existencia de comunicación química. Se realizó un estudio de laboratorio para verificar si los machos de tritón ibérico (*Triturus boscai*) prefieren un estanque que contiene agua con su propio estímulo químico frente a un estanque con el estímulo químico de otros machos o hembras, o si prefieren agua que no contiene estímulos químicos de conespecíficos. Los resultados muestran que los machos prefirieron el agua que presumiblemente contenía sus propias señales químicas o las de otros conespecíficos, y que los machos que fueron emparejados con hembras pasaron significativamente más tiempo en su propio estanque que los machos no emparejados. Estos resultados sugieren que los machos de *T. boscai* son capaces de identificar en el agua señales químicas procedentes de conespecíficos y discriminar las señales químicas procedentes de las hembras.

CHEMICAL communication is widespread among aquatic organisms (Liley, 1982; Dodson et al., 1994). In newts, the chemical sense is important in the performance of sexual and feeding behavior (Verrell, 1986; Cogălniceanu, 1992).

Many species of European newts of the genus *Triturus* are terrestrial during some period of the year (mainly summer and/or winter). However, at the end of the winter and during spring, they reproduce in water, usually in shallow, temporary ponds. Males and females congregate at ponds and are ready to mate soon after they enter the water (Verrell and McCabe, 1988; Verrell, 1989; Caetano and Leclair, 1999). However, active searching for potential mates within a pond may increase risk of predation for males (Magnhagen, 1991). Efficient location of sites that contain conspecific females might also save the male time and energy.

Although there is no direct evidence that chemical cues are used by *Triturus* newts to signal boundaries of territories, chemical stimuli are used in close range interactions such as courtship (Halliday, 1977; Malacarne and Giacoma, 1986). Olfaction is particularly important in primarily nocturnal species or those living in habitats with low visibility. These observations suggest that newts might assess the quality of a pond based on chemical cues of conspecifics present in the water. Chemical cues from conspecifics could indicate absence of predators and presence of food (Woody and Mathis, 1997) but also that potential mates are present (Rowland et al., 1990; Cogălniceanu, 1992).

In this study, we examined whether pond choice by male newts is influenced by the presence of chemical cues from conspecifics in the water. We designed a laboratory experiment to test whether male Bosca's newts (*Triturus boscai*) prefer a pond with water containing their own chemical stimuli over water with chemical stimuli of other males or females, or water without any conspecific chemical stimuli. We selected this species because, among *Triturus* species, *T. boscai* is one of those in which sexual dimorphism is least developed, and this may indicate a greater importance of olfactory stimuli in the courtship of this species when compared with others (Mouta Faria, 1993). Although newts tend to be philopatric, the distribution of their breeding ponds is dynamic and changes as seasons progress. The terrestrial phase in *T. boscai* occurs quite far from the breeding ponds (Barbadillo, 1987; Caetano and Leclair, 1999), and individuals have the opportunity to choose

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among different small ponds. Thus, this species might be a good candidate to explore the use of chemoreception in pond choice. We hypothesized that, if male *T. boscai* are not territorial, they should prefer ponds with odors of conspecifics, and they should be able to detect and discriminate odors of females.

Materials and Methods

Bosca's newt is a small salamandrid (maximum total length of adults = 10 cm) inhabiting principally small ponds with abundant vegetation of the northwest and central Iberian Peninsula. In the study area, the mating season starts in November and lasts until June. Newts are terrestrial during summer (Barbadillo, 1987; Caetano and Leclair, 1999).

During February 1997, we captured adult *T. boscai* in a small stream, to which ponds are connected by shallow runs. The stream is located between Navia and Ortiguera (Asturias Province, northern Spain). Newts were transferred to the laboratory at "El Ventorrillo" Field Station (Madrid Province, Spain) and maintained in aquaria with water at ambient temperature of 14–16 C (coming from a nearby clean mountain stream without newts) and under a natural photoperiod. We fed newts to satiation on earthworms three times each week. Tests were made within one month from the capture date. All newts were healthy during the trials and at the end of the experiment were released at their capture sites.

Male newts were maintained under two different experimental conditions: 13 males were isolated in individual small aquaria (20 × 30 cm), whereas 10 other males were maintained in individual aquaria (20 × 30 cm) with a conspecific female. Thus, we aimed to test whether there are differences between the responses to chemical stimuli from a female that had previously shared an aquarium with the responding male and the responses to stimuli from females that had been held with another male.

Newts were tested during February to March 1997 in a rectangular aquarium (65 × 30 × 40 cm) filled to a depth of 5 cm with gravel. Two plastic circular containers (20 cm in diameter and 5 cm deep) were placed 5 cm from the aquarium walls at opposite ends of the tank, buried entirely into the gravel, and filled with water and a substratum of gravel. Thus, we simulated two different ponds separated by 15 cm of exposed gravel.

We planned a repeated measures design in which each individual male newt was tested under each of the three different treatments in randomized sequence, but participated in only one test per day. In the control treatment, water from the experimental newt's own container (here termed the home container) was placed in one pond and clean water was placed in the other. In experimental treatment 1, we used water from the home container in one pond and water from the container of another single male to test for possible avoidance of water from another male or self-recognition in male newts. In the experimental treatment 2, we used water from the home container in one pond and water from the container of a male living with a female to test for attraction to cues of conspecific males and females. We randomly chose which male was used in each trial and randomly altered the position of the ponds within a treatment. We took the water with chemical stimuli used in each test from containers in which the newts had been maintained for five days, and, when water was changed, we waited another five days before new trials were performed.

Tests were made during twilight hours, which coincide with the maximum activity period of this species (Díaz Paniagua, 1989). For each treatment, the ponds were filled with water 15 min prior to the start of each test. Individual test newts were placed in the center of the experimental aquarium, covered with a small cage, and given 15 min for habituation. Each trial began when the cage was lifted, releasing the newt. Typically, newts explored the aquarium, entered the water of one pond, and occasionally changed from one pond to the other. We noted the location of the newt each 5 min and the number of times that the newt switched between ponds. If a newt was located in either of the two ponds, it was designated as having chosen that pond, whereas if it was located out of the water, it was designated as having made no choice. We determined the newt's preferred pond by calculating in which pond the newt spent greater than 50% of its time (excluding time spent in the no-choice area). Each trial lasted 3 h, after which the aquarium was drained and thoroughly rinsed with clean water. Two new plastic ponds were used for each test to avoid odor contamination.

To assess whether newts chose one of the ponds, we calculated the number of trials out of the total in which the newts spent greater than 50% of their time on a particular stimulus pond and compared it to an expected binomial distribution assuming frequencies to be equiprobable on each side (for a similar procedure, see Horne and Jaeger, 1988; Chivers et al., 1997). To compare the time spent in their own pond across treatments, we used nonparametric

TABLE 1. SUMMARY STATISTICS OF TIME SPENT BY MALE *Triturus boscai* IN THE POND WITH WATER FROM THEIR HOME CONTAINER FOR EACH TREATMENT. The number of newts that spent greater than 50% of their time in each pond (water from their home container *vs* clean water or water from conspecifics) for each treatment, and the corresponding *P*-values from binomial tests, are indicated. Mean ± SE, median, and range of the time (min.) spent in the pond with water from their home container for each treatment are also shown.

	Own water *vs*		
	Clean water	Other male water	Other male and female water
Male alone (n = 13)			
(own/other)	11/2	5/8	7/6
P-value	0.002	0.58	0.99
$\bar{x}$ ± SE	74.5 ± 17.8	26.5 ± 9.3	61.5 ± 18.9
Median	77.5	17.5	45
Range	0–165	0–90	0–170
Male with female (n = 10)			
(own/other)	9/1	9/1	7/3
P-value	0.021	0.021	0.34
$\bar{x}$ ± SE	62.1 ± 13.7	76.4 ± 14.9	70.0 ± 16.6
Median	45	80	90
Range	10–110	15–135	10–120

Friedman two-way ANOVA by ranks. Pairwise comparisons of means were planned using nonparametric multiple comparisons procedures (Sokal and Rohlf, 1995). We compared times spent in home water by different types of males with Mann-Whitney *U*-tests (Siegel and Castellan, 1988).

RESULTS

In their first choice, newts did not significantly select any pond in any condition (two-tailed Binomial test, $P > 0.30$ in all cases). However, when we computed the proportion of time spent in each pond, male newts in the control test clearly selected ponds containing water from their home container over ponds with clean water (Table 1). Single males did not significantly select between their own or other single male ponds. In contrast, males maintained with a female preferred the pond with water from their home container that presumably contained cues from that female. However, neither single nor paired males distinguished between ponds with their own water or that of other male and female stimuli.

For males maintained alone, there were significant differences among treatments in time spent in the pond containing water from their home container (Friedman two-way ANOVA; $\chi^2 = 6.20$, $P = 0.04$; Table 1). Single males spent significantly more time in ponds with water from their home container in the control test than in the other male treatment (experimental treatment 1) or the male and female treatment (experimental treatment 2; $P < 0.05$ in both cases). However, the responses of single males did not differ significantly between the other male and female treatment (experimental treatment 2) and the other male treatment (experimental treatment 1; $P > 0.05$). In contrast, males held with a female did not differ significantly in the time spent in their home pond across treatments (Friedman two-way ANOVA; $\chi^2 = 0.66$, $P = 0.71$; Table 1). In addition, in the other male treatment, time spent in the pond with water from the home container was significantly higher in males that previously lived with a female than in single males (Mann-Whitney *U*-test, $Z = 2.29$, $P = 0.021$), whereas there were no significant differences in the other treatments ($P > 0.05$ in both cases).

DISCUSSION

Male *T. boscai* preferred water that presumably contained chemical cues from themselves or other conspecifics. Chemical cues from conspecifics are used by other newts and salamander taxa to assess whether predators are absent (Woody and Mathis, 1997) or potential mates are present (Dawley, 1984; Rowland et al., 1990; Cogălniceanu, 1992).

The mating system of some *Triturus* closely resembles a lek where many males compete intensely for females during courtship (Verrell

and McCabe, 1988; Mouta Faria, 1993). Territoriality is probably absent in these systems, because males do not interact with one another in the absence of females, and there is no evidence of site tenacity (Verrell and McCabe, 1988). Therefore, the presence of chemical cues of a male in a pond should not cause avoidance by another male.

In accordance with this prediction, our experiment showed that male *T. boscai* used ponds with water from other males and their own water in similar proportions. This suggests that they were unable to distinguish between their own odors and those of other males or that this discrimination did not influence pond selection. This capability may not be necessary for a nonterritorial species. In contrast, some terrestrial American plethodontid salamanders are territorial and do distinguish between their own odors and those of conspecifics (Anthony, 1993), and the presence of chemical cues of one male may deter an intruding male from entering that territory (Jaeger et al., 1986; Ovaska and Davis, 1992).

Males that lived with a female selected the water from their home container over that of single males, and paired males spent significantly more time in their home pond than did single males. These observations suggest that males of *T. boscai* were able to identify chemical cues from females. In other *Triturus* species, female odors attract males (Verrell, 1986; Cogălniceanu, 1992, 1994). Male newts probably use female odors to detect and locate prospective mates by chemotaxis when vision is limited (Rowland et al., 1990). In European newt species, although male approaches are usually elicited by female movements (Halliday, 1974), olfactory investigation of females by males is particularly common during courtship (Halliday, 1977) and is particularly common in the case of *T. boscai* (Mouta Faria, 1993).

Males did not always select the pond with water from the container of other males who had lived with a female. Thus, they are probably also able to detect the presence of chemicals from another male together with chemicals from the female. Males might detect the courtship pheromones of other males, which would indicate that initial courtship interactions have commenced (Houck, 1986; Malacarne and Vellano, 1987). The detection of chemicals from potential mates and competitors may help males to decrease energetic costs and exposure to predators during their mate-searching movements.

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