

Chemical Exploratory Behavior in the Lizard *Liolaemus bellii*

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ABSTRACT.—An experimental study was carried out to determine whether self and conspecific chemical recognition occurs in *Liolaemus bellii*, a Tropicidurid lizard from Central Chile. Experiments were performed during the autumn and the spring. Using the number of tongue flicks as an indicator of discrimination, it was found that *L. bellii* showed both self and conspecific chemical recognition. Lizards recognized their own territories, and conspecific chemical recognition showed seasonal changes. During autumn, lizards showed higher exploratory behavior (higher numbers of tongue flicks and motion time) than in spring, and female enclosures elicited in males higher numbers of tongue flicks. Similar results were previously found in other *Liolaemus* species from a different habitat. The information available at present for *Liolaemus* suggests that recognition of own territory is more important than recognition of conspecifics, and the latter seems to be associated mainly to the reproductive season. Therefore, conspecific and self-chemical recognition seem to be independent of the habitat used by the species, although habitat could modulate the use of chemical signals.

Lizards have been described as primarily visually oriented organisms, exhibiting a variety of visual signals and behaviors during social interactions (e.g., Stamps, 1977; Martins and Lamont, 1998; Martins et al., 1998; Pough et al., 1998). However, lizard social interactions may also be mediated by pheromones (Cooper, 1996; Steele and Cooper, 1997, and references therein; for reviews see: Mason, 1992; Cooper, 1994; Font, 1996). Recently we reported that *Liolaemus tenuis*, a tree-dwelling species of the Tropiciduridae family, uses chemical signals in conspecific and self-recognition (Labra and Niemeyer, 1999). Considering that *Liolaemus* is a genus with more than 160 species (Schulte et al., 2000), generalizations about chemical recognition in the genus based on information of one species may be risky, particularly since the effectiveness, and hence the use of chemical communication can be affected by the characteristics of the environment as well as by other variables (Alberts, 1993). The use of chemical signals by *L. tenuis* may have been influenced by the presence of trees in its habitat. Trees and foliage in general can interfere with visual communication thereby favoring chemical communication. To increase our understanding of conspecific chemical communication in *Liolaemus*, we conducted a study on *L. bellii*, a mountain-hibernating and viviparous species from central Chile that lives in open rocky areas (Donoso-Barros, 1966). Individuals of *L. bellii* have been observed licking the substrate (AL, pers. obs.), suggesting che-

mosensory capabilities, although differences in the habitats of *L. bellii* and *L. tenuis* may suggest that *L. bellii* uses chemical recognition (either in intraspecific and self-recognition) comparatively less than *L. tenuis*.

Studies of communication mediated by pheromones are usually carried out only during natural or induced reproductive condition (e.g., Cooper et al., 1986; Pedersen, 1992; Steele and Cooper, 1997). The study of *L. tenuis* was conducted in two different seasons (Labra and Niemeyer, 1999); therefore, for comparative purposes, this study also investigates the effect of season on chemical communication of *L. bellii*.

Using tongue flicking as a measure of chemical discrimination (Cooper, 1998), the following questions were addressed. Does *L. bellii* show conspecific and self-chemical recognition? Does *L. bellii* exhibit seasonal changes in chemical recognition? Does *L. bellii* use chemical communication to a lesser extent than *L. tenuis*?

MATERIALS AND METHODS

Liolaemus bellii was collected at Farellones (33°20'S; 70°19'W; 2300 m) east of Santiago, Chile. Twenty-one lizards (11 males, 10 females) were obtained during the autumn (April 1998) and 23 lizards (14 males, 9 females) during the spring (November 1998). Animals were transported to the laboratory and placed in an indoor vivarium. They were housed individually in sand-filled plastic enclosures (37 × 30 × 15 cm), which were covered with thin plastic mesh. A small dish for water and a rock for shelter were placed in each enclosure. Lizards were fed three times per week with mealworms. Once a week, food was dusted with a reptile vitamin and mineral supplement.

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TABLE 1. Repeated-measures analysis of variance of the effects of sex (male vs. female), season (autumn vs. spring), experimental treatment (different enclosures) and their interactions on tongue-flick.

Source of variation	F	df	P
Between subjects			
Season	212.31	1, 40	<0.001
Sex	2.85	1, 40	0.09
Season \times sex	1.23	1, 40	0.27
Within subjects			
Treatment	31.49	3, 120	<0.001
Treatment \times season	21.91	3, 120	<0.001
Treatment \times sex	3.21	3, 120	0.026
Treatment \times season \times sex	3.29	3, 120	0.023

Lizards were maintained with a photoperiod of 10:14 L:D during the autumn and 13:11 L:D during the spring. The minimal and maximal temperatures maintained in the vivarium corresponded to those registered in the hottest day of each season (11–31 C and 13–35 C, during autumn and spring, respectively). These temperatures are within the range of the selected body temperature of the species (Labra, 1998). Halogen lights were used to maintain the thermal and photoperiod conditions.

Individuals remained in their enclosures for one week prior to experimentation, allowing them to habituate to experimental conditions. This period would also allow the potential liberation of chemical cues, which probably are associated with the cloaca. Both sexes have been observed dragging this area of the body (SB, pers. obs.), a behavior normally associated with pheromone release (Mason, 1992). After the habituation period, lizards were maintained individually in fabric bags for 45 min and thereafter placed individually and randomly in one of four treatments: (1) the empty enclosure of the tested individual; (2) the empty enclosure of an unfamiliar male; (3) the empty enclosure of an unfamiliar female; or (4) an untreated control enclosure. When the enclosure of the conspecific was used, the owner was previously removed. Lizards were subjected to only one trial per day, and they had one day to rest before being tested in another treatment. At the end of each trial, we recorded the lizard's cloacal body temperature.

Data were collected using the Observer software (Noldus, 1995) on a personal computer. Three variables were determined. First, latency to first tongue flick equals the time (min) elapsed from placing the lizard in the enclosure to the onset of the first tongue flick. After the first tongue flick, the following behaviors were recorded for 10 min: number of tongue flicks (TF) equals the number of times the lizard protrudes and rapidly retracts its tongue, regardless of whether the tongue touches the substrate

or is waved in the air; and motion time equals the time the lizard remains in motion (min), which includes adjustments in body posture, head movements, and displacements of the body's center of gravity through the enclosure.

Of the 176 trials (44 individuals each subjected to four treatments), 16 trials were repeated, six because lizards finished with a body temperature at least three degrees lower than their seasonal selected body temperatures (Labra, 1998), a fact that could have biased the behavioral patterns (Cooper and Vitt, 1986), and 10 because lizards retreated under the rock or failed to protrude the tongue within 20 min. There was no relationship between treatment type and the trials that were repeated. After the experiments, lizards were returned to the field.

Pearson product-moment correlations (Zar, 1984) were used to test relationships between variables. TF and motion time were not normally distributed; therefore they were transformed using square-root and log-transformations, respectively (Zar, 1984). Because there was no correlation between any variable and body temperatures ($P > 0.05$), the effect of season, sex, treatment, and their interactions on the number of TF, motion time, and latency to first TF, was tested by an ANOVA with a three-factor design and repeated measurements for treatment (Edwards, 1968). After the ANOVA, Tukey's tests were used for multiple comparisons.

RESULTS

Season and treatment significantly affected TF (Table 1). During autumn, lizards showed higher number of TF than during spring, and lizards exhibited a lower mean TF in their home enclosure than in any other enclosure (Fig. 1). The interaction between season and treatment was significant, because lizards showed lower TF during spring, in all the enclosures, whereas during the autumn, lizards had lower number of TF in their home enclosure (Fig. 1). Sex did not affect TF, although its interaction with treat-

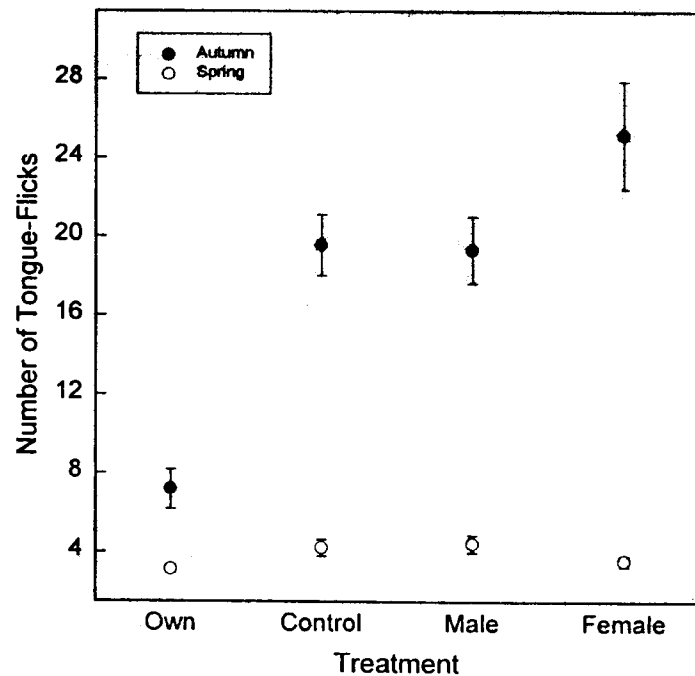


FIG. 1. Mean number of tongue flicks (\pm SE) exhibited by *Liolaemus bellii* individuals in two seasons, when exposed for 10 min to enclosures of the tested individual (own), a male, a female, or an untreated control enclosure. Sample size: 21 lizards in autumn, and 23 in spring.

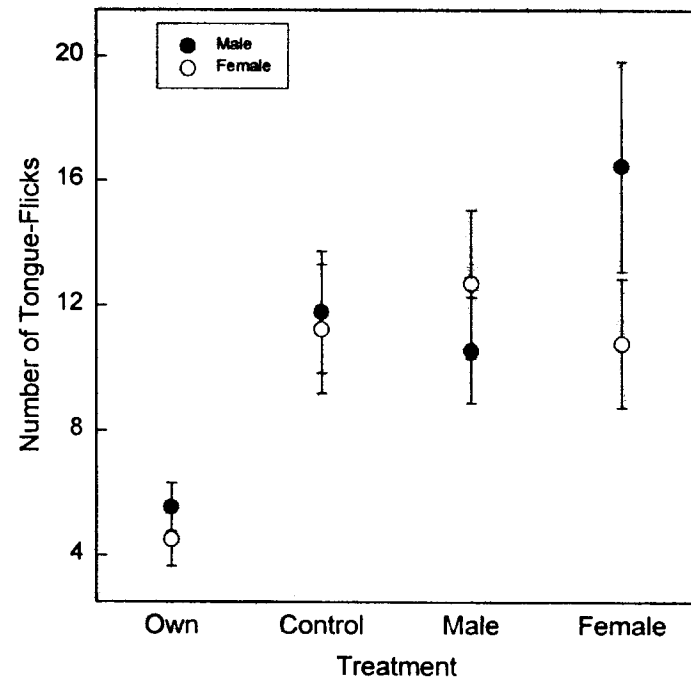


FIG. 2. Mean number of tongue flicks (\pm SE) exhibited by both sexes of *Liolaemus bellii* in the four treatments (for explanations see Fig. 1). Data for both seasons were pooled to show the interaction between sex and treatment. Sample size: 25 males and 19 females.

ment was significant. Males showed higher TF than did females in female enclosures ($P = 0.03$; Fig. 2). The interaction of the three variables (sex, season, and treatment) on TF was also significant, because males show higher TF in female enclosures during autumn ($P = 0.001$).

Season, treatment, and sex significantly affected motion time (Table 2). Lizards spent more time moving during autumn in enclosures other than their own (Fig. 3). Finally, males were more active than females. None of the interactions among variables affected motion time (Table 2). Overall motion time was positively correlated with TF ($r = 0.46$, $P < 0.001$, $N = 176$).

Latency to first tongue flicking was only affected by season ($F_{1,40} = 14.11$, $P \ll 0.001$). Shorter latencies were noted in autumn than in spring (2.07 ± 0.18 ; 3.41 ± 0.24 , respectively;

mean \pm SE). Latency was negatively correlated with the mean number of TF ($r = -0.22$, $P = 0.003$, $N = 176$) and with motion time ($r = -0.209$, $P = 0.005$, $N = 176$).

DISCUSSION

It is generally accepted that the number of tongue flicks reflects chemosensory investigation of a stimulus (Font, 1996; Cooper, 1998; but see Halpern, 1992). Under this context, *L. bellii* shows differences in chemical investigation determined by the season and the treatment. During autumn, lizards showed more TF than during spring and also more exploratory locomotion (motion time). The exact mating season of *L. bellii* is not well established. Leyton and Valencia (1992) indicated that the reproductive period (from mating to parturition) of *L. bellii* is

TABLE 2. Repeated-measures analysis of variance of the effects of sex (male vs. female), season (autumn vs. spring), experimental treatment (different enclosures) and their interactions on motion time (min).

Source of variation	F	df	P
Between subjects			
Season	36.18	1, 40	<0.001
Sex	7.05	1, 40	0.011
Season \times sex	0.04	1, 40	0.840
Within subjects			
Treatment	3.60	3, 120	0.016
Treatment \times season	1.98	3, 120	0.120
Treatment \times sex	0.70	3, 120	0.550
Treatment \times season \times sex	0.27	3, 120	0.850

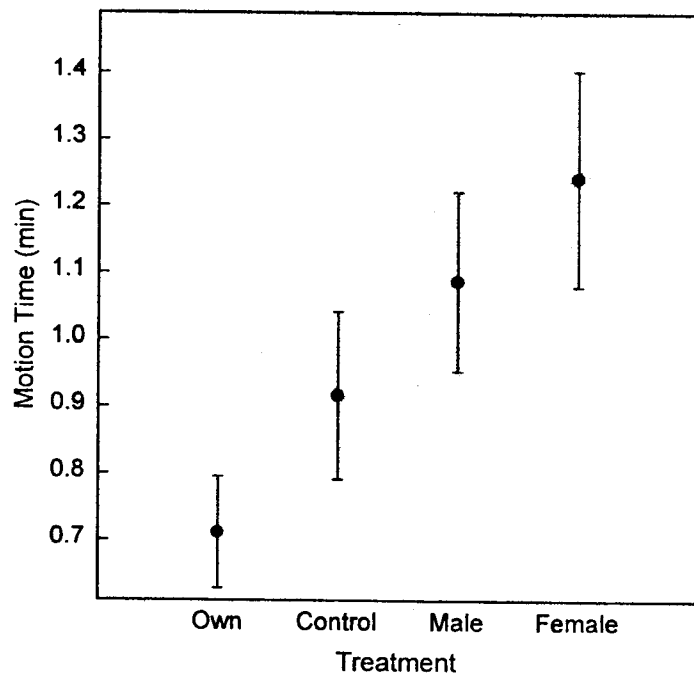


FIG. 3. Mean (\pm SE) motion time (min) exhibited by *Liolaemus bellii* in the four treatments (for explanations see Fig. 1). Data for both sexes and seasons were pooled to show the effect of treatment. Sample size: 44 lizards subjected to the different treatments.

during spring and early summer. However, pregnant females were observed during the early spring (at the end of hibernation) and juveniles at the end of the spring (AL, pers. obs.). These observations, in addition to the fact that *L. tenuis* shows higher exploratory behavior during the breeding period (Labra and Niemeyer, 1999), suggest that *L. bellii* is an autumn breeder, as are other *Liolaemus* from high elevations that gestate over winter (Leyton et al., 1982; Ramírez-Pinilla, 1991). In the future, it would be interesting to determine whether seasonal differences in TF can be used as a bioassay to determine a lizard's mating season and also whether species with continuous reproduction fail to exhibit seasonal changes in TF associated with conspecific recognition.

The number of tongue flicks varied greatly, especially in the data obtained in the female enclosures during autumn (Fig. 2). The latter probably can be explained considering that females do not reproduce synchronically (Leyton and Valencia, 1992). Therefore, the observed variability in TF in female enclosures can be ascribed to the fact that not all the females were in the same reproductive condition, and the tested lizards were hence submitted to different stimuli. However, even considering this variability during autumn, males showed highest number of TF in female enclosures, suggesting that the main conspecific chemical recognition in *L. bellii* would be restricted to the breeding season toward females, as in *L. tenuis* (Labra and Niemeyer, 1999).

During autumn, lizards showed shorter latencies than in spring. It has been proposed that differences in latency time under different experimental conditions would be a consequence of the detection by olfaction of different compounds that eventually activate tongue flicking (Cowles and Phelan, 1958; Duvall, 1981; Cooper et al., 1994). However, the seasonal differences recorded in *L. bellii* cannot be just ascribed to differences in seasonal variation in the quantity and/or quality of volatile compounds (i.e., more abundant or more active volatile pheromones during autumn), because during autumn, lizards also showed shortened latencies in control enclosures (there was no interaction between treatment and season). Probably changes in the lizards' physiology, such as those associated to the reproductive condition, determine differential exploratory "motivations" between seasons, including higher rates of movement in the autumn and, hence, differences in the onset of exploration. This is partially supported by the overall negative relationships between latency and both exploratory variables.

The treatment (i.e., type of enclosure) significantly affected exploratory chemical behavior because *L. bellii* showed lower number of TF in their own enclosures and less motion time than in any other enclosure. It is postulated that *L. bellii* produces chemical secretions used in the recognition of its territory, as has been documented for other lizard species (e.g., Graves and Halpern, 1991; Alberts, 1992; Alberts and Werner, 1993; Cooper, 1996; Cooper et al., 1999).

The prediction that *L. bellii* would show comparatively less frequent use of chemical exploratory behavior than *L. tenuis* was partially corroborated. Thus, although *L. bellii* showed a lower number of TF and shorter motion time than *L. tenuis* (Labra and Niemeyer, 1999), the overall behavioral patterns of *L. bellii* and *L. tenuis* (Labra and Niemeyer, 1999) were similar, both species showing conspecific and self-chemical recognition. These behaviors seem independent of the habitat used by the species, although habitat seems to modulate the use of chemical signals, as stated above. At present, information is available on chemical recognition in only two *Liolaemus* species, both belonging to the *Liolaemus* subgenus (Schulte et al., 2000). In the future, research will be directed to determine whether there is a phylogenetic effect in the use of chemical communication, by undertaking a phylogenetic comparative study including *Liolaemus* species distantly related to those that have been studied.

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