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AGE AND SEASON AFFECT CHEMICAL DISCRIMINATION OF *Liolaemus bellii* OWN SPACE

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Abstract—We explored chemical discrimination of own vs. novel space by different age classes (neonates, juveniles, and adults) of the lizard *Liolaemus bellii*, during pre- and post-hibernation seasons. We recorded the number of tongue flicks (TF) lizards produced during 10 min in their own or a novel enclosure. Age class and season affected chemical discrimination. Only adults and neonates discriminated their own space, albeit using different strategies: while adults made fewer TF in their own enclosure, neonates made more TF in their own enclosure. This difference was interpreted in terms of different requirements for discrimination of individuals during their lives. Increased chemical exploration by juveniles and adults at the onset of the post-hibernation season was associated with food-searching and reproductive behaviors.

Key Words—Semiochemicals, *Liolaemus*, lizards, tongue-flicks, chemical discrimination, age, season.

INTRODUCTION

The vomeronasal organ is one of the main chemoreceptors of squamata reptiles, allowing the reception of semiochemicals from conspecifics and heterospecifics (i.e., prey, predators, congeners). Thus, chemical discernment ultimately determines vital aspects of squamate behavior. Animals collect molecules from the environment mainly using their tongue, and discrimination of semiochemicals may be inferred from differences in the number of tongue-flicks (chemical exploratory behavior), triggered by different signals. Most studies of chemical discrimination

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have been performed with adults (Cooper, 1994), although chemoreception is functional from the first stages of development (e.g., Font and Desfilis, 2002). The number of comparative studies analyzing whether discrimination of different semiochemicals changes through the life or seasons is much lower, even though animals experience changes in ecological, physiological, and morphological features during their lives, as well as seasonally. We investigated in *Liolaemus bellii* (Tropiduridae), a viviparous lizard species inhabiting rocky mountainous areas of central Chile (Donoso-Barros, 1966), if chemical discrimination of their own space differs between age classes and seasons. This species is active from approximately October to April, and hibernates the rest of the year (Donoso-Barros, 1966). Adults show seasonal changes of home range overlap (Fox and Shipman, 2003), and of conspecific and self-chemical discrimination (Labra et al., 2001).

METHODS AND MATERIALS

Lizards were collected in Farellones (east of Santiago, Chile), during February and March 2000 (pre-hibernation) and in October 2000 (post-hibernation). Sample size is indicated in Figure 1A. In the laboratory, lizards were kept in a vivarium exposed to sunlight, and maintained at thermal and photoperiod conditions approaching values recorded in the different seasons (pre-hibernation: 11–32°C and 10:14 L:D; post-hibernation: 13–37°C and 13:11 L:D). They were assigned to different age classes based on the snout-vent length (SVL): neonates (3 to 4.8 cm), juveniles (4.4 to 6.7 cm), and adults (6 to 8.8 cm). Coloration, and thickening of the tail base (associated with the development of genitalia) were used as complementary criteria to establish age classes (Donoso-Barros, 1966).

Lizards were housed individually in sand-filled plastic enclosures (37 × 30 × 15 cm), covered with plastic mesh. Enclosures contained a bowl for water and a rock for shelter and basking. Water was supplied *ad libitum* and food (mealworms) was supplied every other day (dusted with vitamins once per week). Animals remained in their enclosures for one week without disturbance. Experiments were performed in a room without natural light, outside the vivarium. A lizard was removed from its enclosure and maintained in a cloth bag for 30 min; thereafter, the bag was opened allowing the animal to move freely into an experimental enclosure, which was selected randomly: (1) Own: the enclosure of the test animal; (2) Novel: an unused and clean enclosure, containing only sand. During trials, the stone and the bowl of the own enclosure were removed. Novel enclosures were cleaned, and the sand was replaced before each new trial. Lizards were tested only once per day, with two resting days between testing days. Behavior was video-recorded for ten min; tape playback allowed the determination of the number of tongue-flicks (TF), i.e., when the lizard protrudes and rapidly retracts the tongue, regardless of whether it touches the substrate or is waved in the air. *L. bellii* rarely performs visual displays other than tongue-flicking (Fox and Shipman, 2003); hence, no other behaviors were studied. In pilot experiments, neonates showed no differences in TF between novel, conspecific and own enclosures (one-way ANOVA $F_{(2,12)} = 0.468$; $P = 0.64$), while adults had similar TF in conspecific and novel enclosures (Labra et al., 2001). Hence, conspecific enclosures were not included in the experimental design. Once all experiments were performed, animals were released in the area where they were collected.

The effect of treatment, age class, and season on TF was assessed with a three-way ANOVA with repeated measures for treatments, followed by *a posteriori* Fisher LSD tests. Partial correlation was used to determine the relationship between SVL (a rough estimator of age) and the difference between TF performed in the novel and own enclosures, thus correcting for inter-individual behavioral variability.

RESULTS AND DISCUSSION

Liolaemus bellii chemically discriminated its own vs. a novel space. However, age class and season affected discrimination in an interrelated way (Table 1 and Figure 1A): while juveniles made no distinction between enclosures in either season, adults made fewer TF in their own than in a novel enclosure during pre ($P = 0.005$) and post-hibernation ($P < 0.001$), and neonates made more TF in their own than in a novel enclosure only during post-hibernation ($P = 0.006$). This suggests that own semiochemicals produce different effects on neonates and adults, as seen also from the relationship between the differences in number of TF between enclosures and SVL, during post-hibernation ($r = 0.61$, $P < 0.001$) (Figure 1B). The correlation remained significant after removing the two extreme points. A similar trend, albeit marginally non-significant, was observed during pre-hibernation ($r = 0.26$, $P = 0.057$).

How and why there is a change in the way in which age classes achieve discrimination, is unclear. Possibly, age classes have different requirements to

TABLE 1. ANOVA RESULTS OF THE EFFECTS OF SEASON (PRE-HIBERNATION VS. POST-HIBERNATION), AGE CLASS (NEONATES, JUVENILES OR ADULTS), TREATMENT (OWN VS. NOVEL ENCLOSURE), AND THEIR INTERACTIONS, ON THE NUMBER OF TONGUE FLICKS

Source of variation	*F*	*df*	*P*
Season	39.98	1,74	<0.001
Treatment	10.01	1,74	0.002
Age class	4.87	2,74	0.010
Season × Age class	4.24	2,74	0.018
Season × Treatment	0.29	1,74	0.591
Age class × Treatment	14.14	2,74	<0.001
Season × Age class × Treatment	7.38	2,74	0.001

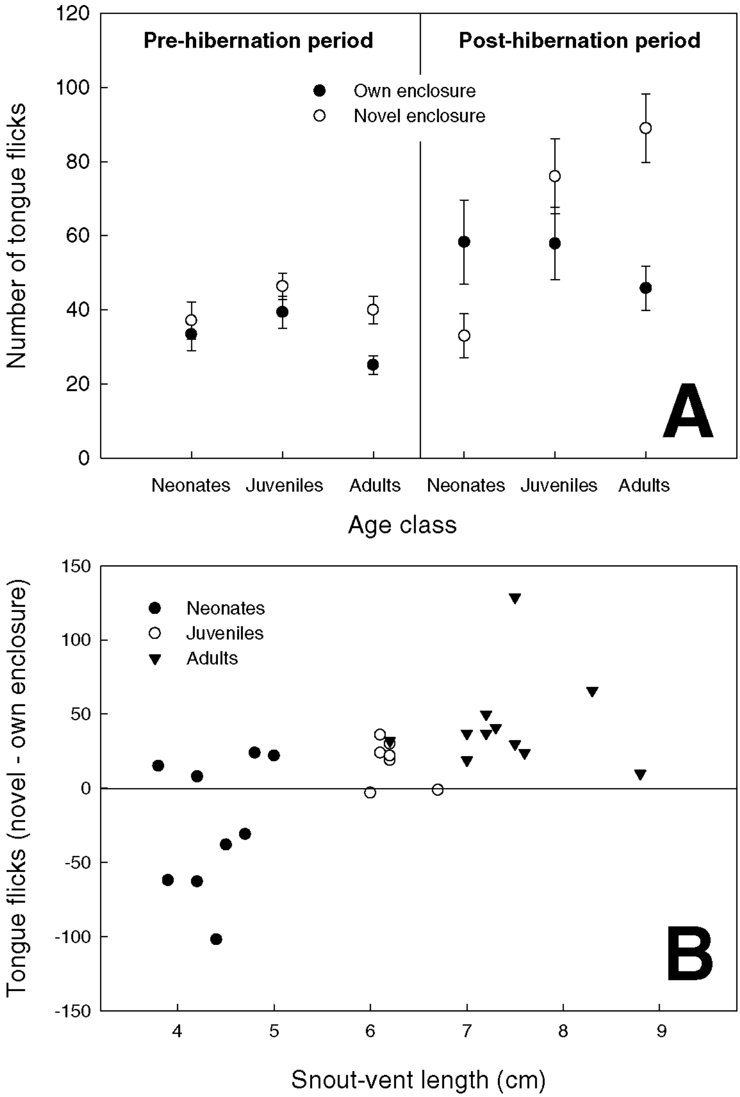

FIG. 1. A. Mean number of tongue flicks (±SE) performed by three age classes of *Liolaemus bellii*, during pre and post-hibernation periods, in own and novel enclosures. Sample sizes for pre and post-hibernation, respectively: adults (13, 9), juveniles (13, 7) and neonates (27, 11). B. Relationship between snout-vent length of *Liolaemus bellii* and the difference between the numbers of tongue flicks performed in novel minus those made in own enclosures during post-hibernation. The horizontal line indicates equal number of tongue flicks in both enclosures.

discriminate their own space. If *L. bellii* requires a familiarization period with its own semiochemicals in order to discriminate other conspecifics in the future ("self-referent matching," Wyatt, 2003), neonates would be the appropriate age for such familiarization, and, thus, their own semiochemicals would trigger higher exploration. Additionally, neonates are usually observed in association with an adult female (probably the mother), so they would not have strong requirements to explore novel semiochemicals. In fact, neonates showed fewer TF in the novel enclosure than juveniles and adults ($P < 0.001$ for both comparisons). Juveniles are frequently observed moving in the field, and being chased by adults when entering their defended areas. Hence, they probably still lack their own space and constitute the age class that disperses. Active exploration of all semiochemicals is, thus, expected from juveniles. This is supported by the higher TF shown by juveniles compared with neonates ($P = 0.01$) or adults ($P = 0.04$). Juveniles may have a low requirement, if any at all, to discriminate own from novel space. Finally, adults have preferred sites for activities; they are frequently observed using the same rocks (unpub. obs.). This necessarily requires discriminating their own semiochemicals. However, since adults are likely to be already familiarized with their own semiochemicals, exploration of new ones seems to acquire priority.

The season effect was manifested by a higher mean chemical exploration of juveniles and adults during post-hibernation ($P < 0.001$ for both comparisons). In addition, from pre to post-hibernation there was a significant increase in TF performed by neonates and adults in their own enclosure ($P = 0.009$ and 0.008, respectively), and by juveniles and adults in the novel enclosure ($P = 0.004$ and $P < 0.001$, respectively). Following hibernation, many species from temperate zones, such as *L. bellii*, search food, establish territories, and/or start their reproductive period (Gregory, 1982). Additionally, reproduction of *L. bellii* appears to occur during spring and early summer, i.e., during post-hibernation (Leyton and Valencia, 1992). All these behaviors require increased chemical exploration. In the case of neonates, familiarization with their semiochemicals would explain the increased exploration of their own space.

Results suggest that animals experience different requirements during their lives that affect the way they discriminate their own semiochemicals from those in the environment.

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