Chemical self-recognition in the lizard *Liolaemus fitzgeraldi*

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Abstract Social–chemical recognition is exhibited by all the *Liolaemus* lizards tested to date, except *Liolaemus fitzgeraldi*, which during post-hibernation did not discriminate chemosignals of same-sex individuals from a control. To clarify if *L. fitzgeraldi* is unique among the studied *Liolaemus* in lacking social–chemical recognition or if this was previously undetected, we recorded behavior during pre- and post-hibernation when confronted with chemosignals of conspecifics and from themselves. *L fitzgeraldi* showed self-recognition and seasonal changes in two exploratory behaviors. Potentially, conspecific recognition in *L fitzgeraldi* was undetected due to seasonality, but this species may rely comparatively less on chemical communication than congeners.

Introduction

Conspecific recognition has a central role in social behavior, and squamate reptiles are highly dependent on the chemosensory modality to achieve this recognition. They sample and sense chemosignals through the tongue–vomeronasal system (Font 1996), which allows them, for

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example, to recognize their partners (Bull 2000), and determine from a potential partner its body size (Lemaster and Mason 2002), body condition (Shine et al. 2003), and mating status (Cooper and Pérez-Mellado 2002; O'Donnell et al. 2004). In other social contexts, chemosignals can give detailed information about the fighting abilities of the owner (Labra 2006) and allow for the identification of the sex of the signalers (Cooper and Pérez-Mellado 2002), discrimination of familiar from unfamiliar individuals (Font and Desfilis 2002), mother–offspring recognition (Bull and Baghurst 1998), and self-recognition (Alberts 1992).

Liolaemus lizards, a highly diverse genus (>170 spp) from South America, are highly dependant on the chemosensory modality (see Labra 2008). Presently, we know that seven of eight *Liolaemus* show some types of social–chemical recognition. On one hand, *L. bellii*, *L. constanzae*, *L. eleodori*, *L. jamesi*, *L. lemniscatus*, and *L. tenuis* show clear evidence of self-recognition, as focal individuals explore less their own secretions than those of conspecifics (Labra and Niemeyer 1999, 2004; Labra et al. 2001a, b, 2003; Labra 2008). On the other hand, *L. jamesi*, *L. monticola*, and *L. tenuis* show other types of conspecific recognition, including sex-recognition (Labra and Niemeyer 1999; Labra et al. 2001b; Labra 2006). *Liolaemus fitzgeraldi*, however, was the only species that did not show evidence of social–chemical recognition, as it did not discriminate between conspecific secretion and a control (environment free of secretions), at least during post-hibernation (Labra and Niemeyer 2004).

Previous studies indicate that different social–chemical recognition in *Liolaemus* (e.g., sex and self-recognition) can be modulated by factors such as seasonality (Labra and Niemeyer 1999; Labra et al. 2001a). Since conspecific recognition in *L. fitzgeraldi* was tested during only one

season (Labra and Niemeyer 2004), it is still possible that this species exhibits conspecific chemical recognition during other seasons, or that it shows other types of social recognition, such as self-recognition. To assess the later possibility, we studied the behavior of *L. fitzgeraldi* when confronted with different types of conspecific chemosignals and with those of the focal individual in two seasons (pre- and post-hibernation).

Materials and methods

Liolaemus fitzgeraldi is a species that lives at high altitude in the Andes mountain range (Donoso-Barros 1966). We collected, in central Chile (around 32°50′S, 2,900 m asl), 22 individuals (9 ♂, 13 ♀) during the pre-hibernation season (March), and 17 individuals (11 ♂, 6 ♀) during the post-hibernation season (January). Lizards were transported to the laboratory and placed in an indoor vivarium. They were housed individually in plastic enclosures (37 × 30 × 15 cm) with plastic mesh tops. The front of each enclosure had a small window (10 × 5 cm) that was covered with plastic mesh. The floor of the enclosures had a 3-cm-thick layer of sand, a water container, and a rock used as a shelter and for basking. Water was supplied ad libitum, and food (mealworms) was supplied every other day. Food was dusted with vitamins once a week. The vivarium was maintained under conditions mimicking those recorded in the field. This included photoperiods of 11:13 L:D and 13:11 L:D for pre- and post-hibernation seasons, respectively, and a thermal range that covered the minimum and maximum temperatures recorded during hot days of both periods, 10–29°C and 12–34°C, during pre- and post-hibernation seasons, respectively. Light and temperature were provided by halogen lights.

Lizards remained in their enclosures for 1 week prior to testing, allowing habituation to experimental conditions and deposition of chemosignals. Before each trial, the focal lizard was removed from its enclosure and held in a cloth bag for 10 min, as this procedure minimizes the stress associated with handling the lizards (A. Labra, unpublished data). The bag was then opened to allow the animal to move freely into a treatment enclosure. The top of the treatment enclosure was replaced by a glass that facilitated observation and filming. Lizards were placed randomly in the following empty enclosures (without the rock and the water container): (1) own, of the focal individual; (2) same-sex, of a conspecific of the same sex as the focal individual; and (3) opposite-sex, of a conspecific of the different sex as the focal individual. Lizards were subjected to only one trial per day, with an inter-trial interval of at least 3 days.

After placing a lizard in a treatment enclosure, the experimenter moved away and recorded the lizard's behavior using an 8 mm digital video camera installed over the enclosure and connected to a television monitor. Using a stopwatch, we recorded latency to the first tongue-flick, i.e., the time elapsed between placing the lizard in the enclosure and the occurrence of the first tongue-flick. After this, we filmed the lizard for 10 min. Later, during analysis of each video, we counted the number of tongue-flicks, an index of vomeronasal chemical exploration (Font 1996), and we recorded the time in motion, which indicates behavioral exploration (Labra 2006). The number of other behaviors that lizards displayed was also recorded, such as marking, head bobs, tail wag, and excavation (see Labra 2006). Trials in which lizards did not tongue-flick after 20 min were canceled, and repeated another day. At the conclusion of each experimental season, animals were returned in good condition to their capture site.

The lizard's sex did not affect any of the response variables; hence, data for sexes were pooled. Latency to the first tongue-flick and the number of tongue-flicks were square-root transformed to achieve normality. For each response variable, the effect of season, treatment, and their interactions, was determined by a two-way ANOVA with repeated measurements for treatments. Thereafter, LSD post hoc tests were applied. The other behaviors occurred in a low frequency that precluded any further analyses. For example, marking behavior (through cloaca and face rubbing), the dominant behavior observed, was exhibited in total by only ten lizards in the opposite-sex enclosures, eight lizards in the same-sex enclosures, and five lizards in their own enclosures.

Results and discussion

Table 1 shows the results for the three recorded response variables. The average latency to the first tongue-flick was 8.49 ± 0.33 min. This response variable was not affected by any factor, suggesting that there were no differences among treatments and/or seasons in the volatile compounds perceived by olfaction that would trigger chemical exploration by the tongue-vomeronasal system (e.g., Labra 2006), or that in this species, volatile compounds perceived by olfaction do not affect the onset of tongue-flicking behavior. The other two response variables showed seasonal changes. Lizards moved more during pre-hibernation (Fig. 1a), which can reflect an active search for hibernacula. During pre-hibernation, a posteriori tests indicated that lizards are capable of self-recognition; i.e., they moved less in their own enclosures than in any other treatment (Fig. 1a). In addition, lizards in the same-sex enclosures moved more during pre-hibernation than during post-hibernation ($P < 0.05$). Although, this might suggest sex-recognition, there were no differences in the time in motion

Table 1 Results of two-way ANOVAs, testing for effects of season (pre- and post-hibernation), treatment (own, same-sex, and opposite-sex enclosure), and their interaction on three response variables, recorded in *Liolaemus fitzgeraldi*

	df	Latency		Motion time		Number of tongue-flicks	
		F	P	F	P	F	P
Season	1,37	2.18	0.15	8.70	0.006	10.76	0.002
Treatment	2,74	2.62	0.08	1.39	0.26	4.10	0.02
Treatment × season	2,74	1.78	0.18	3.39	0.04	1.53	0.22

Fig. 1 Behaviors displayed by *Liolaemus fitzgeraldi* in three treatments, during pre- and post-hibernation. **a** Mean time in motion (+1 SE). **b** Mean number of tongue-flicks (+1 SE). The small histogram inside (**b**) corresponds to the total mean number of tongue-flicks in the same three treatments
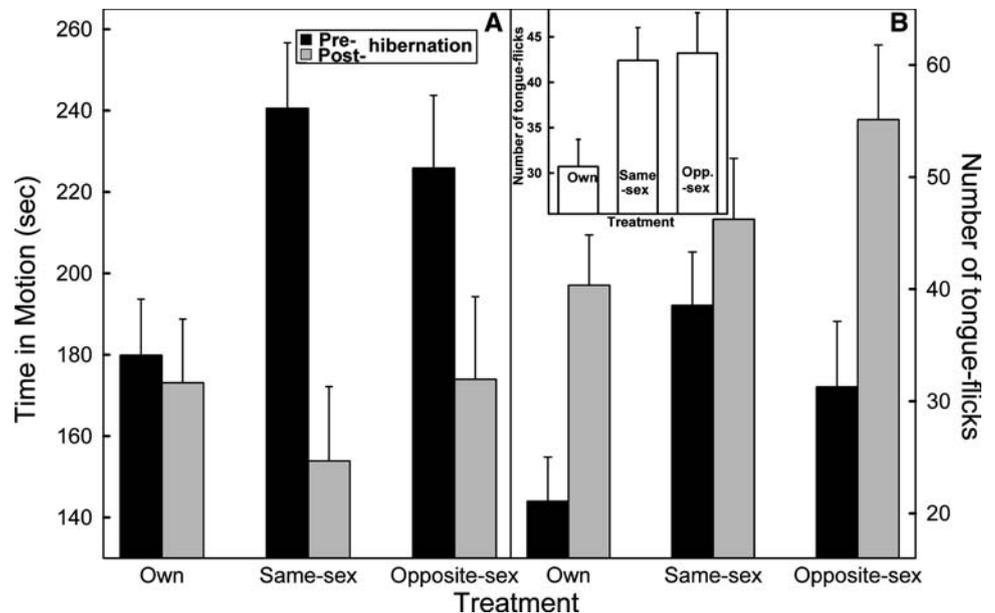
between the two types of conspecific enclosures, in any seasons (Fig. 1a).

Behavioral exploration usually correlates positively with the number of tongue-flicks or chemical exploration (e.g., Labra 2006). However, *L. fitzgeraldi* chemically explored more in the season when it moved less (Fig. 1a, b). The basis for this negative relationship is unclear, but it may be related to our proposal that during pre-hibernation lizards need to search for hibernacula, and other sensory modalities, such as vision, would be more relevant in that search. In contrast, during post-hibernation, lizards may remain in their territory and would sample their surroundings mainly using the tongue-vomeronasal system. On the other hand, the number of tongue-flicks differed among treatments (Table 1; Fig. 1b), and a posteriori tests indicated that lizards made more tongue-flicks in conspecific enclosures (same- or opposite-sex) than in their own enclosures ($P < 0.05$). This is again clear evidence of self-recognition (Labra and Niemeyer 1999; Labra 2006).

The seasonal variation in the two exploratory behaviors (time in motion and number of tongue-flicks) suggests that maybe conspecific chemical recognition in *L. fitzgeraldi* was undetected previously due to seasonality. However, a line of evidence indicates that *L. fitzgeraldi* relies comparatively less on chemical communication than the other *Liolaemus* species that show evidence of social–chemical recognition (Labra 2008). What determines this difference? Signals used for conspecific recognition evolve faster when different congeneric species live in sympatry and use the same channel of communication (e.g., Butlin and Ritchie 1994). *Liolaemus fitzgeraldi*, in contrast to all the *Liolaemus* species studied to date for social–chemical recognition, does not cohabit with congeneric species (Donoso-Barros 1966). This suggests that *L. fitzgeraldi* is not under a strong selective pressure for social–chemical discrimination, and the most significant communication possibly takes place through the visual channel. This peculiarity of *L. fitzgeraldi* among *Liolaemus* deserves further exploration. Comparative studies of the social–chemical recognition in *Liolaemus* with inclusion of more species that do not cohabit with congeners can give important insights about the evolution of chemical communication in lizards and other taxa. Moreover, if these

studies incorporate measurements of visual communication, we can have significant insights about a potential trade-off between visual and chemical communication (e.g., Martins et al. 2004).

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