

CHEMICAL DISCRIMINATION IN *LIOLAEMUS* LIZARDS: COMPARISON OF BEHAVIORAL AND CHEMICAL DATA

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INTRODUCTION

Chemical signals produced by lizards are involved in different aspects of their interactions at intra and interspecific levels, including sex recognition, territory marking, and individual self-recognition (Mason, 1992; Cooper, 1994; Font, 1996), and have also been proposed as pre-reproductive barriers for sympatric congeneric species (Cooper and Vitt, 1986). The range of functions of chemical recognition would suggest that lizards should be able to discriminate many different patterns of chemical signals. We postulate that it would be “more economical” for lizards to discriminate only one chemical signal pattern, the one used by individuals in their self-recognition, and that signals not matching with the basic chemical pattern will not be discriminated.

To support this proposition we studied self-recognition at individual and specific levels in different lizard species of the genus *Liolaemus* of Chile. If self-recognition predominates at individual level, lizards would show lower number of tongue flicks in their own enclosures than in other enclosures (*i.e.* Alberts, 1992; Cooper *et al.*, 1999). At specific level, it is expected that lizards behave differently in the presence of chemical signals of conspecifics than of congeneric species. Finally, we analyzed the relationship between behavior and chemical composition of secretions from precloacal pores, since these are a source of chemical signals (Labra *et al.*, 2001b).

MATERIAL AND METHODS

Lizards were carried to the laboratory, maintained individually in enclosures with sand as substrate, a bowl of water and a rock, and were tested after one week of acclimation (Labra *et al.*, 2001a). Prior to the experiments, lizards were removed from their enclosures and placed randomly in different enclosures (treatments, see below). In order to avoid the

potential use of visual signals in the discrimination, the enclosures were partially emptied before the experiments: the lizard, the rock and the bowl of water were withdrawn, and the sand remained. The number of tongue-flicks (TF) that lizards made during 10 min was recorded. Records began after lizards made their first TF. Tongue-flicks were recorded since traditionally they have been used as the way to determine if lizards are able to discriminate among different chemical signals (Cooper, 1998).

Behavior

Individual self-recognition. To test self-recognition at the individual level, we studied five *Liolaemus* species: *L. bellii*, *L. constanzae*, *L. fitzgeraldi*, *L. eleodori* and *L. tenuis*. The site of capture and the type of habitat of the species used in behavioral assays are shown in Table 1. Treatments consisted in placing each lizard in the enclosure of the tested lizard (own), that of a conspecific, and an unused enclosure (control). Unless otherwise stated, conspecific treatments referred to the use of an enclosure whose owner was of the same sex of the tested individual (*i.e.* male in a male's enclosure).

Specific self-recognition. To test self-recognition at the species level, two focal species were used, *L. jamesi* and *L. bellii*. They were tested in enclosures of conspecifics and congeneric species, both sympatric and allopatric. For *L. jamesi* its sympatric species was *L. alticolor*, and the allopatric one was *L. ornatus*. In the case of *L. bellii* the sympatric species was *L. nigroviridis* and the allopatric one was *L. fitzgeraldi* (Labra and Niemeyer, 2001). The localities where different species were collected are indicated in Table 1.

Table 1. Distribution and characteristic habitat of the *Liolaemus* species included in this study.

<i>Liolaemus</i>	Altitude (m.a.s.l.)	Latitude/Longitude (°S; °W)	Characteristic habitat
<i>alticolor</i>	4350	18°10'; 69°25'	Andean Plateau
<i>bellii</i>	2300	33°20'; 70°19'	High mountain
<i>constanzae</i>	2250	23°46'; 68°14'	Atacama Desert
<i>eleodori</i>	3670	27°04'; 69°10'	Andean Plateau
<i>fitzgeraldi</i>	3500	23°50'; 70°09'	High mountain
<i>jamesi</i>	4350	18°10'; 69°25'	Andean Plateau
<i>lemniscatus</i>	950	33°35'; 70°28'	Chaparral
<i>nigroviridis</i>	2300	33°20'; 70°19'	High mountain
<i>ornatus</i>	3710	19°15'; 68°43'	Andean Plateau
<i>tenuis</i>	1850	33°46'; 70°15'	Chaparral

Chemical data

The chemical analysis of the precloacal secretions of 20 *Liolaemus* species from different localities of Chile, was performed using GC-MS. The secretions obtained were weighed and dissolved in *n*-hexane. The secretions of three individuals of each species were pooled prior to the analysis. In addition, nine males of *L. bellii* were analyzed individually to determine the intraspecific variation in the chemical composition of the secretions. The presence or absence of a given compound in the chromatographic profile of each *Liolaemus* species and of the different individuals of *L. bellii*, were determined by comparing retention times and mass spectra with a mass spectra library and commercial standards (Escobar *et al.*, 2001).

Statistics

Behavioral data were analyzed using ANOVA for repeated measurements and Tukey *a posteriori* tests. The similitude in the chemical composition of the secretions, among species and among individuals of *L. bellii*, was analyzed with unweighted pair-group average (Zar, 1984).

RESULTS

Behavior

Individual self-recognition. For *L. temuis* (Labra and Niemeyer, 1999) and *L. bellii* (Labra *et al.*, 2001a) the analysis of the effects of season and sex of the enclosure owner, showed a lower number of TF in the own enclosures in both seasons in *L. temuis*, and only in the autumn in *L. bellii*. In both species, female enclosures elicited a higher number of TF during the reproductive season. However, the reanalysis of results for the reproductive season using only data of conspecifics of the same sex, showed no differences in TF between conspecific and control enclosures, and a lower number of TF in the own enclosures.

The other three species showed the same trends, i.e. lower number of tongue-flicks in their own enclosures. For *L. eleodori* differences were found among treatments, but TF was similar in conspecific and control enclosures (Fig. 1A). *L. lemniscatus* showed a lower number of TF in the own enclosure in comparison with the control condition, and *L. constanzae* also showed lower number of TF in the own enclosure, in comparison with enclosures of conspecifics (Labra and Niemeyer, 2001).

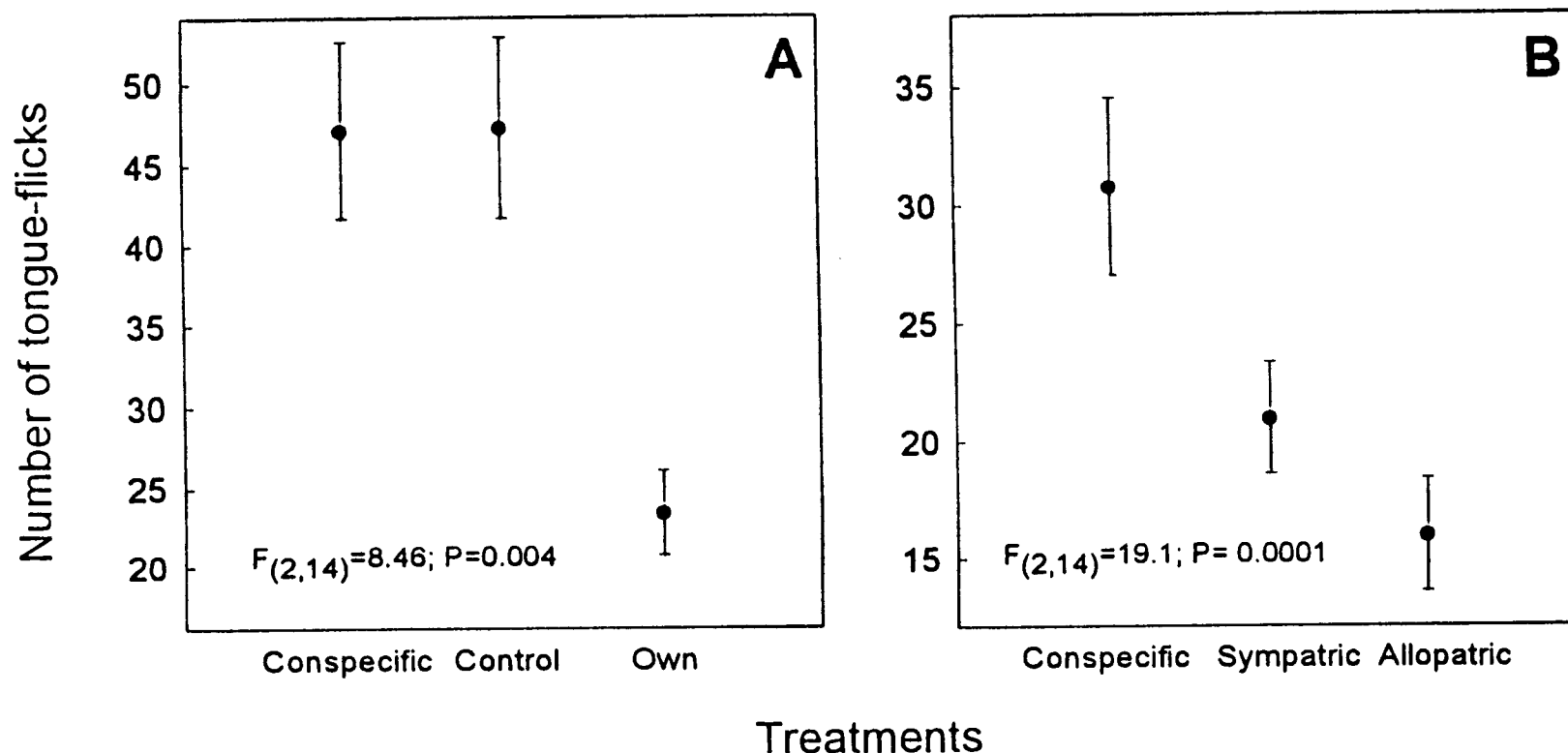


Figure 1. Self recognition: A. At individual level for *L. eleodori*, B: At specific level in *L. jamesi*. Treatments are explained in the text. Points represent mean values; bars represent one standard error. The values from the statistical tests are given in the figures.

Specific self-recognition. *Liolaemus jamesi* and *L. bellii* (Labra and Niemeyer, 2001) showed similar patterns of chemical exploratory behavior. The individuals of the focal species showed a higher number of TF in enclosures of conspecifics than in those of any of the congeneric species. There were no differences in the behaviors recorded in the enclosures of sympatric and allopatric species. Fig. 1B shows the results for *L. jamesi*.

Chemical data

The interspecific chemical analysis of male precloacal secretions of 20 *Liolaemus* species showed the presence of 50 different compounds distributed among all the species. The compounds belonged to three main categories: *n*-alkanes, long chain carboxylic acids, and steroids. Only six of these 50 compounds were present in all the species analyzed: cholesterol and five carboxylic acids (Escobar *et al.*, 2001). Using the information of presence or absence of the different compounds, preliminary analyses of similitude were performed among the species, and also among the individuals of *L. bellii*. The individuals of *L. bellii* and the pooled sample of this species were grouped together, the 20 species were well differentiated among them, and the distance indices among species were higher than those obtained between individuals of *L. bellii* (Fig. 2).

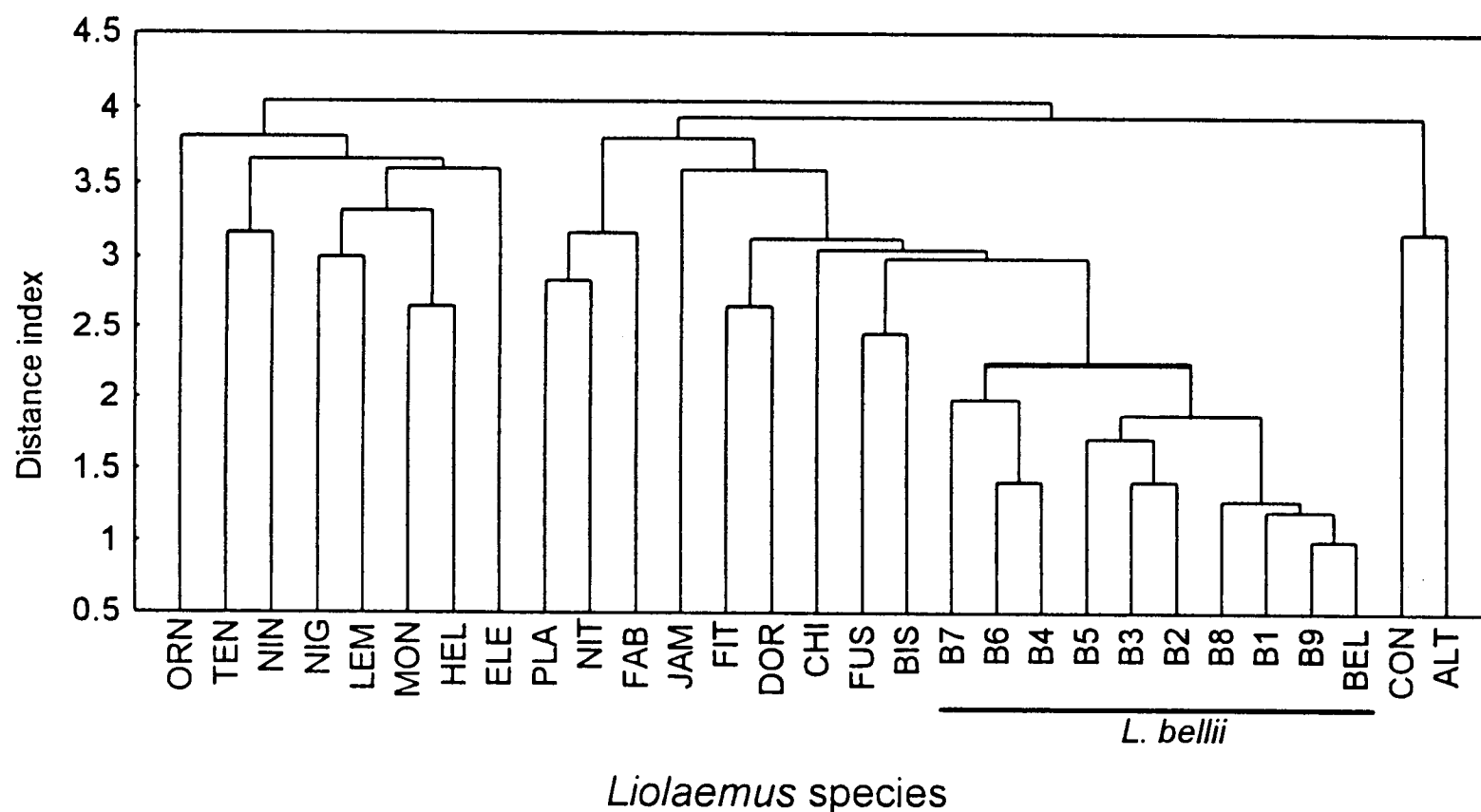


Figure 2. Phenogram showing the similitude among 20 species of *Liolaemus* and nine individuals of *L. bellii* (B1- B9). *Liolaemus* species codes are: ALT = *alticolor*; BEL = *bellii*; BIS = *bisignatus*; CHI = *chiliensis*; CON = *constanzae*; DOR = *dorbigni*; ELE = *eleodori*; FAB = *fabiani*; FIT = *fitzgeraldi*; FUS = *fuscus*; HEL = *hellmichi*; JAM = *jamesi*; LEM = *lemniscatus*; MON = *monticola*; NIG = *nigroviridis*; NIN = *nigroviridis nigroroseus*; NIT = *nitidus*; ORN = *ornatus*; PLA = *platei* and TEN = *tenuis*.

DISCUSSION

The five species of *Liolaemus* studied showed self-recognition at the individual level, as manifested by a lower number of TF in their own enclosures. The similar values of TF in control and conspecific treatments may be due to TF not being equally distributed along the duration of the experiment; this hypothesis was not tested. In addition, in *L. tenuis* and *L. bellii* female enclosures triggered higher number of TF during the reproductive season (Labra and Niemeyer, 1999; Labra *et al.*, 2001a). Self-recognition at the species level occurred, as manifested by a higher chemical exploratory behavior in enclosures of conspecific, rather than in those of congeneric species. No differences were observed between the behaviors recorded in enclosures of sympatric or allopatric species. Therefore, if chemical signals do constitute reproductive barriers, they are useful in the distinction of an individual from its conspecifics but not from individuals of any other species.

Self-recognition in *Liolaemus* at individual and specific levels seems to be quite a generalized phenomenon since the species studied came from widely different environments and belong to different clades (Etheridge, 1995). The phenogram of figure 2 shows that chemical composition of precloacal secretions from all species are different, and that there are clear differences between intraspecific and interspecific variability. This suggests that: i) the six chemical compounds common to all species may be the means to chemically identify the genus *Liolaemus*; ii) that each species has its own characteristic pattern of chemical signals, as a result of qualitative and/or quantitative differences in the chemical constituents of precloacal secretions; and iii) that each species has certain variability in the basic pheromonal pattern that would enable individuals to exhibit self-recognition. In addition, lizards would also be able to discriminate (innately or through learning) other variants of the basic pattern, as it occurs in the cases of recognition of familiar individuals (Cooper, 1996) and in mother-offspring recognition (*i.e.* Bull *et al.*, 1994), and also between certain variants of pheromones from conspecifics, under particular circumstances. Thus, female enclosures elicited a higher number of TF during the breeding season (Labra and Niemeyer, 1999; Labra *et al.*, 2001a). In this case, physiological changes due to the reproductive condition would allow animals to detect (in the case of males) and/or to produce (in the case of females) the basic signal pattern with some modifications, and thus trigger an increase in chemical exploratory behavior. If the signal comes from a congeneric, so it does not match the basic chemical pattern, there is no discrimination and no particular behavior is triggered.

From the behavioral data presented we conclude that self-recognition is the basic “type of recognition” in these lizard species, at individual and specific levels. The next step will be to test self-recognition at populational level. Based on the present results, it is highly probable that self-recognition also occurs at populational level, thus constituting another mechanism that would favor the high rate of speciation found in this genus (Lambrot, 1993).

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