

Variability in the Assessment of Snake Predation Risk by *Liolaemus* Lizards

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Abstract

The ability to assess and respond to predation risk is a strong selective force. Detection of predators is carried out by one or more sensory modalities, but the use of chemoreception has significant advantages. This study examines the chemosensorial assessment of snake predation risk and corresponding behaviours in different species and populations of *Liolaemus* lizards naturally exposed to different levels of snake predation pressure. The species studied were sympatric (*Liolaemus lemniscatus*), parapatric (*L. nigroviridis*) and allopatric (*L. fitzgeraldi*) to the saurophagous snake, *Philodryas chamissonis*. Additionally, two populations of *L. lemniscatus* from areas differing in snake densities were compared. Chemo-assessment of predation risk was determined by comparing the number of tongue-flicks (TF) and the behavioural responses of lizards submitted to three treatments (with semiochemicals of snake, conspecifics, and without semiochemicals – control). The results suggest that *Liolaemus* lizards can chemo-assess snake predation risk, but this was modulated by the predation pressure experienced by lizards in their natural habitats. When exposed to snake semiochemicals, the sympatric prey showed less chemical exploratory behaviour (i.e. lower number of TF), a higher frequency of antipredator behaviours that would reduce its detection by a predator, and did not show the behaviour triggered by conspecific semiochemicals. The parapatric prey showed similar number of TF across different treatments, suggesting an absence of recognition of snake semiochemicals; however, it did show antipredatory behaviours when confronted with snake semiochemicals. The allopatric prey showed similar behaviour in all treatments. Both populations of the sympatric species, *L. lemniscatus*, showed a similar ability to detect predation risk when confronted with snake semiochemicals (i.e. similar number of TF), but antipredatory behaviours were diminished, and marking behaviours were present in the population subject to lower predation pressure. Relaxed predation pressure from a predator that releases and detects semiochemicals had similar consequences at species and population levels.

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Introduction

Predation is a key selective force that governs the evolution of an array of traits that facilitate prey survival, by interrupting the predatory sequence at any of its five stages: detection, identification, approach, subjugation, and prey consumption (Endler 1986; Greene 1988; Lima & Dill 1990). Organisms that are able to assess predation risk, by identifying and/or detecting the presence of a predator before being detected by it have a higher probability of survival (Kats & Dill 1998), and many studies demonstrate a positive relationship between predation pressure and the expression of characters such as the ability to assess predation risk and exhibition of defences (e.g. Endler 1986; Kats & Dill 1998; Magurran 1999; Dicke & Grostal 2001).

Predators may be detected by one or more sensory modalities, but chemoreception has significant advantages as a predator's semiochemicals (sensu Law & Regnier 1971) can even be assessed in its absence (Weldon 1990; Kats & Dill 1998), thus strongly reducing the chance of confrontation; this chemodetection can be either innate (e.g. Van Damme et al. 1995), or learned (e.g. Chivers & Smith 1998; Dicke & Grostal 2001).

Several lizard species have an innate ability to recognize snake semiochemicals (e.g. Van Damme et al. 1995; Mori & Hasegawa 1999; Downes & Adams 2001; Stapley 2003), which determines behavioural changes that reduce predation risk including modification of the retreat sites (Van Damme & Quick 2001; Stapley 2003), and behavioural thermoregulation (Downes & Shine 1998). However, the response to snake semiochemicals is modulated by the relative predation risk given, for example, by whether a snake is saurophagous or non-saurophagous (e.g. Van Damme & Quick 2001; Bealor & Krekorian 2002), by the degree of threat imposed by snakes (Stapley 2003), or by the degree of sympatry between lizards and snakes (Mori & Hasegawa 1999; Downes & Adams 2001).

Lizards of the genus *Liolaemus* can discriminate semiochemicals of self, conspecifics and congeners (Labra & Niemeyer 1999; Labra et al. 2001a,b, 2002, 2003), although whether they can discriminate predator semiochemicals is not known. The aims of the present study were to determine the ability of *Liolaemus* to assess snake predation risk chemically, and to determine if this ability is modulated by the degree of predation pressure to which lizards are submitted under natural conditions. We examined whether different species and populations of *Liolaemus* from central Chile, naturally subject to different levels of predation pressure by the snake *Philodryas chamissonis*, would differ in their chemosensorial assessment of snake predation risk and corresponding behaviours. We compared the behaviour of species that were sympatric (*L. lemniscatus*), parapatric (*L. nigroviridis*) and allopatric (*L. fitzgeraldi*) to *P. chamissonis*. Considering that comparative studies between populations provide insight into the strength of

selection by predator pressure of a given trait, two populations of *L. lemniscatus* from places with different snake densities were compared.

Materials and Methods

Study Animals and their Maintenance

Only two snake species are present in central Chile, colubrid *Tachymesis chiliensis* and *P. chamissonis* (Núñez 1992). Although both prey upon *Liolaemus* lizards, *P. chamissonis* does so to a much greater extent (Greene & Jaksic 1992) and is by far the most abundant snake in Chile (Núñez 1992). We therefore chose *P. chamissonis* as the snake predator in our experiments. We also denote that other Chilean snakes belonging to the same genera are mainly found further north (see Núñez & Jaksic 1992) and are extremely rare (J. C. Ortiz, pers. comm.).

During the summer of 2000, lizards and snakes were collected at different sites in central Chile (see Fig. 1 and Table 1 for sample sizes); all lizards were males and the sex of the snakes was not determined. Snake densities are likely lower for the Las Vizcachas population of *L. lemniscatus* than the Codegua population. The former locality is close to a road (approx. 60 m) where basking snakes are killed by cars. We did not rigorously quantify snake densities, but snakes were observed on each field trip to Codegua, while in Las Vizcachas, snakes were observed on one in five field trips. Because snake density in Las Vizcachas is apparently reduced by human activity, we used only data of *L. lemniscatus* from Codegua for interspecific comparisons.

Lizards were placed in an indoor vivarium with a glass roof. This permitted exposure to sunlight in the natural photoperiod, allowing lizards to perform normal basking behaviours. The vivarium was also equipped with halogen lamps to maintain temperatures similar to a typical summer day (between 12 and 36°C). Lizards were housed individually in plastic enclosures (37 cm × 30 cm × 15 cm) covered with plastic mesh. Enclosures contained a bowl of water and a rock for shelter and basking, and their floors were covered with a layer of 3 cm of sand. Water was supplied ad libitum and food (mealworms) every alternate day (dusted with vitamins once per week). Snakes were maintained in a room separate and distant from the lizard vivarium. They were housed individually in enclosures identical to those used for lizards, with a rock, sand, and a bowl of water. Snakes were maintained on a natural diet, an individual of *Liolaemus* sp. twice per week, to avoid potential changes of the lizard antipredatory behaviours caused by changes in predator diet (e.g. Chivers & Mirza 2001).

Experimental Design

Animals remained in their enclosures for 1 wk without disturbance, allowing acclimation to conditions and release of semiochemicals. For the experiment, a lizard was removed from its enclosure and maintained in a cloth bag for 10 min; thereafter, the bag was opened allowing the animal to move freely into the

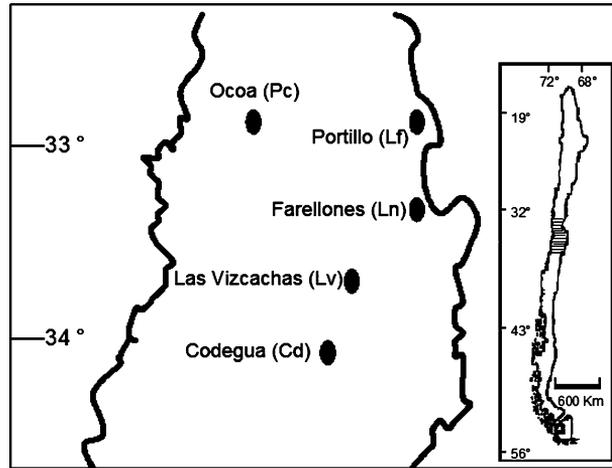


Fig. 1: Geographic location of the collecting areas of lizards and snakes. Codes for the species or population collected are given in parenthesis adjacent to the locality. Codes, nomenclature of species or populations and, in the case of prey, their geographic relation with the snake, are as follows. Pc, *P. chamissonis*; Lf, *L. fitzgeraldi* (allopatric); Ln, *L. nigroviridis* (parapatric); Cd, *L. lemmiscatus* Codegua (sympatric); Lv: *L. lemmiscatus* Las Vizcachas (sympatric)

experimental enclosure. Lizards were tested individually and randomly in different enclosures that differed in the type of semiochemicals present: (1) *conspecific*: an enclosure previously occupied by an individual of its own species; (2) *snake*: an enclosure previously occupied by *P. chamissonis*; (3) *control*: an unused and clean enclosure. Conspecific enclosure allowed the recording of behaviours in a pseudo-natural chemical social environment, and the control enclosure allowed the recording of behaviours in a completely new chemical environment. The owner of the experimental enclosure (snake or conspecific) was removed just before the trial, together with the rock and water bowl. This reduced the potential use of visual signals that may bias the results. Control enclosures were cleaned and the sand was replaced before each new trial.

Lizards were subjected to only one trial per day, with an inter-trial period of 2 d. The cloacal temperature of the individual was recorded at the end of each trial to ensure that, during experiments, lizards had temperatures of approx. 35°C, the selected body temperature of *Liolaemus* (Labra 1998). If the temperature was not near this value ($\pm 2^\circ\text{C}$), the trial was cancelled and repeated later. After each trial, the lizard was removed from the experimental enclosure and placed back in its own enclosure. Snakes remained undisturbed in their enclosure for at least 5 d to ensure the release of enough semiochemicals, before their enclosures were re-used; hence, the possibility that the tested lizard would perceive semiochemicals previously released by a conspecific was minimized. Additionally, there are no data concerning lizards suggesting the production of alarm pheromones, and the

Table 1: Mean (\pm SE) duration (seconds) of the behaviours recorded for three species of *Liolaemus*, and two populations of *L. lemniscatus* in different treatments (conspecific, snake and control enclosure)

Variable	<i>L. fitzingeri</i> (Lf; n = 11; 51.7 \pm 1.5 mm)			<i>L. nigroviridis</i> (Ln; n = 11; 73.3 \pm 0.7 mm)		
	Conspecific	Snake	Control	Conspecific	Snake	Control
Latency to TF	70.36 \pm 18.07	57.55 \pm 15.51	80.18 \pm 21.38	183.32 \pm 37.50	233.00 \pm 51.67	196.33 \pm 5 6.23
Motion time	161.46 \pm 20.91	132.68 \pm 18.98	197.39 \pm 21.72	91.90 \pm 37.60	99.71 \pm 26.74	125.52 \pm 21.26
Slow motion	-	1.27 (1)	-	-	10.63 \pm 4.33 (5)	-
Tail waving	-	0.25 \pm 0.17 (2)	-	15.74 \pm 11.63 (4)	53.42 \pm 25.03 (9)	27.73 \pm 14.8 (7)
Visual displays	-	0.19 \pm 0.15 (2)	-	0.06 (1)	-	-
Marking behaviours	-	-	-	7.64 \pm 3.98 (3)	3.68 \pm 2.58 (4)	2.08 \pm 1.80 (3)
	<i>L. lemniscatus</i> (Codegua, Cd; n = 9; 47.6 \pm 0.8 mm)					
	<i>L. lemniscatus</i> (Las Vizzachas, Lv; n = 8; 49.9 \pm 0.6 mm)					
Latency to TF	213.69 \pm 26.50	118.76 \pm 18.79	145.10 \pm 21.10	199.11 \pm 32.10	112.94 \pm 20.53	139.12 \pm 33.30
Motion time	128.26 \pm 16.57	95.54 \pm 13.42	192.97 \pm 39.02	157.74 \pm 18.50	154.05 \pm 21.77	105.88 \pm 14.50
Slow motion	3.2 \pm 2.1 (2)	25.50 \pm 7.0 (8)	-	-	3.55 \pm 3.13 (2)	-
Tail waving	11.3 \pm 9.2 (3)	19.60 \pm 16.4 (5)	44.35 \pm 40.21 (4)	0.38 (1)	3.97 (1)	1.55 (1)
Visual displays	6.4 \pm 3.2 (8)	-	-	1.35 \pm 0.44 (5)	-	-
Marking behaviours	-	-	-	15.15 \pm 8.14 (3)	1.14 (1)	5.23 \pm 4.89 (3)

In parenthesis following the species names are the codes used for each species and population (see Fig. 1), the sample size (n), and the mean snout-vent length (\pm SE). The number in parentheses following the SE of each variable corresponds to the number of individuals that performed the behaviour, but mean values were obtained on the basis of all individuals studied, i.e. those that did and those that did not perform a given behaviour; in the latter case, the value was zero. Latency and motion time were recorded for all individuals. -, behaviour did not occur.

experimental period (10 min) was not sufficiently long enough to allow lizards to release a detectable quantity of semiochemicals (Labra & Niemeyer 1999). Natural light was not used in the experiments, thus minimizing the chance of semiochemicals reflecting UV light that may be used by lizards as visual signals (Alberts 1989).

The latency to the first tongue-flick (TF) was recorded, i.e. the period (seconds) elapsed between placing the lizard in the enclosure and the occurrence of the first TF (see below). Lizards were then filmed with an 8-mm digital video camera for 10 min. We recorded six behavioural variables from videos:

(1) *Tongue-flicks (TF)*. The number of times that the lizard protruded and rapidly retracted the tongue, regardless of whether it touched the substrate or was waved in the air;

(2) *Motion time*. Total time that the lizard moved, including adjustments of body posture, head movements (scanning), and displacements of the body's centre of gravity, excluding any motion arising from the behaviours listed below;

(3) *Slow ('robotic') motion*. Very slow, stalking movements, which in the extreme appear as if lizards were lit by a stroboscopic light;

(4) *Tail waving*. The entire tail or its posterior portion moved rapidly from side to side;

(5) *Head-Bobs*. Up and down movements of the head;

(6) *Marking behaviours*. This included face and cloaca rubbing against the substrate or the walls of enclosure.

As faeces have pheromonal properties (Labra et al. 2002), defaecation was also included as marking behaviour. TFs were recorded as counts, while variables two to six were timed with a stopwatch. Animals were maintained in good condition during the whole experimental period and returned to their capture sites after the study.

Statistics

The first and second 5-min periods of recording were analysed separately. No variable showed differences between these periods; hence, data were pooled. Data for latency to the first TF and the number of TF were log-transformed, and motion time was square-root-transformed to achieve normality. Then, ANOVAS with a two-factor design (species and treatment) with repeated measurements for treatment were used, followed by Tukey tests for individual comparisons. Variables three to six did not meet the parametric assumptions even after transformation; hence non-parametric statistics were used for analysis. Kruskal–Wallis tests followed by multiple comparisons and Mann–Whitney U-tests were performed to determine major interspecific and interpopulation differences, respectively, for each variable. For these analyses data of the three treatments by species or populations were pooled. Friedman's ANOVAS with multiple comparisons were performed for intraspecific and intrapopulation comparisons of each

variable among the three treatments. Statistical analyses were performed with Statistica 6.0 (Statsoft 2001).

Results

Comparisons among Species

The mean values of the time performing different behaviours are presented in Table 1. The most relevant behaviours that suggest chemical discrimination of the different semiochemicals were TFs, slow motion, tail waving, visual displays, and marking behaviour. The other behaviours (latency of first TF and motion time) mainly reflected major interspecific differences, but not different reactions to semiochemicals. Statistics of latency of first TF, motion time and number of TF are shown in Table 2. Different species had different latency periods of first TF as *L. fitzgeraldi* had a shorter latency than either *L. nigroviridis* or *L. lemniscatus* ($p < 0.001$, in both cases). However, there was no effect of the treatment or of the interaction between species and treatment in latency of first TF. Motion time differed among species as *L. fitzgeraldi* moved more than *L. nigroviridis* ($p = 0.016$). Treatment also affected this variable; lizards spent more time moving in the control than in conspecific ($p = 0.04$) or snake enclosures ($p = 0.003$), but there was no interaction between species and treatment on motion time. TF was affected by both variables and by their interactions (Fig. 2); *L. lemniscatus* made fewer TF than *L. fitzgeraldi* ($p < 0.0001$) and *L. nigroviridis* ($p = 0.029$); lizards made fewer TF in the snake enclosure than in the conspecific ($p = 0.002$) or control enclosures ($p = 0.001$). This interaction was determined by *L. lemniscatus*, showing the lowest TF values in the snake enclosure (Fig. 2).

Table 2: Results of repeated-measures ANOVA testing for effects of group (for interspecific comparisons: *L. lemniscatus*, *L. nigroviridis* and *L. fitzgeraldi*; for intraspecific comparisons of *L. lemniscatus*: Codegua and Las Vizcachas populations), treatment (snake, conspecific, and control), and their interaction. F and p indicate the value of the ANOVA result and the probability, respectively. * $p < 0.05$.

Variable	Group	Treatment	Group \times Treatment
	F (p)	F (p)	F (p)
Interspecific comparisons			
Latency to TF	13.68 (<0.001)*	1.35 (0.27)	1.37 (0.25)
Motion time	4.44 (0.02)*	6.49 (0.003)*	0.48 (0.75)
Number of tongue-flicks	9.71 (<0.001)*	3.58 (0.03)*	4.42 (0.004)*
Intraspecific comparisons			
Latency to TF	0.31 (0.59)	6.81 (0.004)*	0.23 (0.80)
Motion time	0.09 (0.77)	6.22 (0.005)*	0.95 (0.40)
Number of tongue-flicks	2.19 (0.16)	29.75 (<0.001)*	0.0 (1.00)

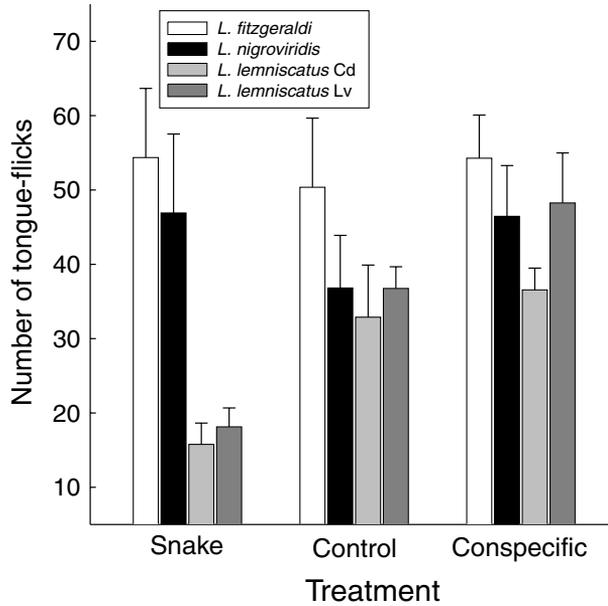


Fig. 2: Number of tongue-flicks produced by three species of *Liolaemus* under different treatments. Values are means (+ SE). For *L. lemniscatus*, Cd, Codegua; Lv, Las Vizcachas populations

Table 3: Results of Kruskal–Wallis H and Mann–Whitney U-tests, testing for differences among *Liolaemus* species and between *L. lemniscatus* populations, respectively, in the different variables recorded

Variable	Species		Populations
	H (p)		U (p)
Slow motion	12.19 (0.002)*		227.0 (0.014)*
Tail waging	24.08 (<0.0001)*		216.5 (0.012)*
Visual displays	12.35 (0.002)*		292.5 (0.44)
Marking behaviours	20.07 (<0.0001)*		229.5 (0.003)*

H and U indicate the value of the test results, and p the probability.

*p < 0.05.

Table 3 presents the results of the interspecific analyses when the data for the three treatments by species were pooled. *L. fitzgeraldi* performed less slow motion and visual displays; *L. nigroviridis* performed more tail waging and was the only species to exhibit marking behaviours. The comparisons across treatments by species are presented in Table 4. Slow motion showed significant differences among treatments in *L. lemniscatus* and *L. nigroviridis*: the former performed this motion longer in the snake enclosure; the latter only performed this behaviour in the snake enclosure. Tail waging was exhibited by *L. nigroviridis* more in the

Table 4: Results of Friedman ANOVA testing for behavioural differences among treatments in the three *Liolaemus* species and between two populations of *L. lemniscatus*

Variable	<i>L. fitzgeraldi</i> [χ^2 (p)]	<i>L. nigroviridis</i> [χ^2 (p)]	<i>L. lemniscatus</i> (Cd) [χ^2 (p)]	<i>L. lemniscatus</i> (Lv) [χ^2 (p)]
Slow motion	2.0 (0.37)	10.0 (0.007)*	12.5 (0.002)*	4.0 (0.14)
Tail waving	4.0 (0.14)	5.9 (0.05)*	0.96 (0.62)	0.0 (1)
Visual displays	4.0 (0.14)	2.0 (0.37)	16.0 (0.0003)*	10.0 (0.007)*
Marking behaviours	–	0.08 (0.96)	–	1.37 (0.50)

χ^2 and p indicate the value of the test result and its probability, respectively.

Cd, Codegua and Lv, Las Vizcachas populations of *L. lemniscatus*.

–, not possible to perform the analyses.

*p < 0.05.

snake than in the conspecific enclosure. Visual displays were performed by *L. lemniscatus* only in the conspecific enclosure.

Comparisons between Populations

The mean values of the different behaviours of each *L. lemniscatus* populations are presented in Table 1. Both populations had similar chemical exploratory behaviours, differing in the expression of the associated behaviours. Table 2 shows that treatment was the only factor that affected latency of first TF, motion time, and number of TF: latency was longer in the conspecific enclosure than in the control (p = 0.03) and snake enclosures (p = 0.004); motion time was longer in the control than in the snake enclosure (p = 0.004), and the number of TF was lowest in the snake enclosure than in conspecific and control enclosures (both p = 0.0001). The overall comparisons of the other behaviours between populations are presented in Table 3. The Codegua population performed more tail waving, and slow-motion behaviour, and less marking behaviour than the Las Vizcachas population. The results of Friedman's ANOVAs are presented in Table 4. Both populations performed visual displays only in the conspecific enclosure.

Discussion

Liolaemus lizards chemo-assessed snake predation risk as their behaviours were affected by snake semiochemicals, although the degree of expression of the associated behaviours was modulated by the snake predation pressure experienced by the lizards under natural conditions. When confronted with snake semiochemicals, the sympatric prey showed more change in behaviours that reduce the possibility to interact with the predator (slow motion, reduced number of TF, and no visual displays). Additionally, both populations of *L. lemniscatus* showed similar exploratory chemical behaviour, but the population experiencing a relaxed predation pressure from a chemosensory predator, modified the

associated behaviours; i.e. it reduced the frequency of antipredatory behaviours and increased the use of behaviours associated with chemical communication.

Comparisons among Species

Mori & Hasegawa (1999) proposed that lizards familiar with predator semiochemicals and subject to intense predation pressure should need little chemical exploration (low number of TF) to discriminate predator stimuli. This is apparently the case of *L. lemniscatus*, the lizard sympatric with the snake predator. It was the only species that reduced the number of TF (i.e. low chemical exploratory behaviour) in the presence of snake semiochemicals. This lizard indeed suffers a high predation rate (Fox & Shipman 2003), attributed to *P. chamissonis* (Medel et al. 1990). Alternatively, the reduced number of TF of *L. lemniscatus* in the presence of snake semiochemicals may represent an antipredator mechanism, as tongue movements can be used by snakes to detect prey. Reduction of these movements decreases the probability that prey will be detected or, in case of detection, that attacks will be directed at the head (Weldon 1990; Cooper 1994). Interestingly, although *L. lemniscatus* reduced its motion time in the snake enclosure, this reduction was not significant, indicating that there is no close relationship between number of TF and motion time (see Table 2).

The relationship between number of TF and snake discrimination is at present unclear. Some have proposed that detection is associated with increased number of TF in the presence of snake semiochemicals (e.g. Van Damme & Quick 2001; Bealor & Krekorian 2002), while others suggest the opposite (e.g. Mori & Hasegawa 1999, the present study). Clearly, as pointed out by Font & Desfilis (2002), there is neither theoretical nor empirical support that allows predictions of the change of sign in TF provoked by chemical discrimination. Thus, discrimination of relevant stimuli can be manifested in higher or lower chemical exploration caused by semiochemicals, depending on the intrinsic characteristics of the species studied and/or particular selective pressures. Moreover, chemical discrimination is a complex feature as different stimuli – with probably different biological relevance – can produce similar number of TF (e.g. conspecific vs. novel semiochemicals). Therefore, behavioural studies that attempt to establish chemical discrimination should include behaviours other than number of TF (e.g. Van Damme & Quick 2001). In this context, those studies performed using cotton swabs, which clearly preclude the expression of relevant behaviours, and give sometimes peculiar results (e.g. similar number of TF for cologne and supposedly biological relevant stimuli López & Martín 2001), should be interpreted cautiously. A major reevaluation of the methodology to determine chemical discrimination seems appropriate.

In this study, comparisons of the behaviour other than number of TF (i.e. visual displays, slow motion, tail waving and marking behaviours), were relevant to establish chemical discrimination by the *Liolaemus* species. Visual displays, a conspicuous behaviour involved in intraspecific communication (Martins et al. in press), were performed by *L. lemniscatus* only when they were confronted with conspecific semiochemicals, and not under conditions that are risky or involve

higher probability of predation (control and snake enclosures). Additionally, the three *Liolaemus* species showed slow motion in the snake enclosure – although this was not significant in *L. fitzgeraldi* – suggesting snake discrimination (e.g. Thoen et al. 1986; Downes & Adams 2001; Van Damme & Quick 2001). Slow motion enables lizards to minimize the possibility that predator and/or conspecifics pay attention to or detect them. In fact, *L. lemniscatus* also exhibited this behaviour in the conspecific enclosure. Conversely, in contrast to the other species, *L. nigroviridis* performed marking behaviours. This constitutes an increased use of chemical communication in a species subject to relaxed predation pressure by a chemo-sensing predator. Finally, tail waving may guide the attack of a detected or undetected predator to the least vital part of lizard's body, the tail, allowing it to escape via autotomy (e.g. Thoen et al. 1986; Cooper 2001). Moreover, a colourful blue tail, characteristic of *L. nigroviridis* males, is expected to evolve under high risk of predation and/or contest (Cooper & Vitt 1985). In this context, it is understandable that *L. nigroviridis* performed this behaviour for longer in the snake enclosure.

The three *Liolaemus* species differed in their expression of antipredatory responses. The sympatric species *L. lemniscatus* showed antipredatory behaviours mainly at the detection stage (reduction of different types of movement, such as TF) of the predatory sequence (Endler 1986), while the parapatric species *L. nigroviridis* showed antipredatory behaviours involved in the detection (slow motion) and subjugation (tail waving) stages of the predatory sequence. These interspecific differences are consistent with a higher predation pressure of *L. lemniscatus* than *L. nigroviridis* (Fox & Shipman 2003) and hence stronger selection for the evolution of earlier antipredator mechanisms. However, it is unclear why *L. nigroviridis* showed antipredatory behaviour. Individuals studied were from the upper part of the altitudinal distribution of the species, and given that *Liolaemus*, in general, show low vagility (Fox & Shipman 2003), the probability that individuals included in the study had real contact with *P. chamissonis*, was very low. Two not mutually exclusive hypotheses can be proposed. (1) The contiguous distributional areas of *L. nigroviridis* and *P. chamissonis* can give enough opportunities of predator–prey interactions to maintain the ability to assess snake predation risk in the whole population. (2) Fuentes & Jaksic (1979) proposed that during the last glacial period, a temporal migration of high-mountain living species, such as *L. nigroviridis*, occurred towards the lower valleys where *P. chamissonis* thrives. Thus, possibly in the past *L. nigroviridis* coexisted with *P. chamissonis* and now retains the capacity to detect the predator as a vestigial behaviour (Van Damme et al. 1995).

The allopatric species, *L. fitzgeraldi*, showed the shortest latency to the first TF, thus initiating earlier its exploratory behaviour, independent of the treatment. Whether these results merely indicate that *L. fitzgeraldi* was less stressed by the experimental conditions than the other *Liolaemus* is unclear. However, the behavioural similarities among treatments in the different variables, lead us to hypothesize that chemical discrimination may not be relevant for *L. fitzgeraldi*, or

that the experimental set-up inhibited expression of relevant behaviours in this species.

Comparisons between Populations

Comparisons across populations can yield insight into the strength of selection by predator pressure of a given trait. Data from populations of *L. lemniscatus* indicate that relaxation of the predation pressure exerted by a predator that detects and releases semiochemicals, involve at least two major changes. First, a reduction of antipredator behaviours, i.e. slow motion and tail waving; and second, an increased use of chemical communication, i.e. marking behaviours. These results are highly concordant with those obtained in the interspecific comparisons. Nevertheless, *L. nigroviridis* (parapatric prey), in contrast to the Las Vizcachas population of *L. lemniscatus* did not show differences in chemical exploratory behaviour (TF) between snake and other semiochemicals. Given that the relaxed predation pressure in Las Vizcachas is a relatively recent occurrence (approx. 30 yr) compared with the case of *L. nigroviridis*, this suggests that modification of chemical discrimination by TF evolves at a slower rate than the other behaviours recorded in this study.

Finally, the similar trends found at species and population levels refute the idea that potential interspecific differences in habitat structure, social behaviour, or other factors are the main determinants of the interspecific differences in the assessment of predation risk reported here.

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