



Antipredatory behaviour of a mountain lizard towards the chemical cues of its predatory snakes

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Abstract

The ability to early detect a potential predator is essential for survival. The potential of *Iberolacerta cyreni* lizards to discriminate between chemical cues of their two predatory snakes *Coronella austriaca* (a non-venomous active-hunter saurophagous specialist) and *Vipera latastei* (a venomous sit-and-wait generalist) was evaluated herein. A third snake species, *Natrix maura*, which does not prey on lizards, was used as a pungent control. Thus, the behaviour of *I. cyreni* was studied regarding four treatments: (1) *C. austriaca* scent, (2) *V. latastei* scent, (3) *N. maura* scent and (4) odourless control. Lizards showed antipredator behaviour (such as slow-motion and tail waving) to *C. austriaca* and *V. latastei* chemicals. The antipredatory response was similar for both predators. This ability to react with an intensive behavioural pattern to the chemical cues of their predatory snakes may prevent lizards from being detected, and, if detected, dissuade the predator from beginning a pursuit.

Keywords

lizards, chemical ecology, antipredatory responses, predators, Lacertidae.

1. Introduction

Predation risk is one of the main selective forces driving the evolution of certain morphological and behavioural traits (Endler, 1986; Sih, 1987). Potential prey should minimise the chances of being killed, while maximising the performance of other activities, such as feeding, thermoregulation or reproduction (Ydenberg & Dill, 1986; Greene, 1988; Lima & Dill, 1990; Vermeij, 1994; Lima, 1998). Failing to respond to a potential predator is costly and even deadly. However, beginning an antipredatory response, such as fleeing, when there is no real risk also entails costs, which can be energetic and/or loss of opportunities for other activities (Ydenberg & Dill, 1986; Downes & Shine, 1998; Stapley, 2003; Amo et al., 2006). Therein lies the importance of effectively identifying a potential predator (Kats & Dill, 1998; Chivers et al., 2001).

A predation sequence can be divided into six phases: (1) encounter, (2) detection, (3) identification, (4) approach, (5) subjugation and (6) consumption (Caro, 2005). Prey will maximize survival if they are capable of (1) correctly identifying and (2) successfully dissuading the predator, by different adaptations to the different phases of the predation sequence (Caro, 2005). Most animals can identify their potential predators before a visual encounter, by means of chemoreception (Schwenk, 1995; Kats & Dill, 1998). In fact, it is known that the ability to effectively identify and respond to predator scents enhances the prey survival probability (Downes, 2002). Among vertebrates, this ability has been studied in fish (e.g., Helfman, 1989; Hirvonen et al., 2000; Mitchell et al., 2015), amphibians (e.g., Semlitsch & Gavasso, 1992; Shaffery & Relyea, 2016), birds (e.g., Roth et al., 2008; Amo et al., 2017), mammals (e.g., Apfelbach et al., 2005; Garvey et al., 2016) and reptiles (e.g., Thoen et al., 1986; Dial & Schwenk, 1996; Downes, 2002; Webb et al., 2009; Ortega et al., 2017).

Lizards depend on chemoreception for different activities, such as feeding, sexual communication and detecting predators (Cooper, 1994; Martín & López, 2013; Mencía et al., 2016). Specifically, the chemical detection of predators can be achieved through gustation, olfaction and/or vomerolfaction (Cooper & Burghardt, 1990; Schwenk, 1995; Baeckens et al., 2017). Chemical discrimination confers prey the advantage of detecting predator signals when they are absent, thus avoiding the riskiest areas or moments (Cabido et al., 2004; Lloyd et al., 2009). Within European lacertid lizards, many species recognise their predator scents and react with specific antipredatory

responses (e.g., Van Damme & Quick, 2001; Mencía et al., 2017; Ortega et al., 2017).

These lacertid antipredatory behavioural patterns — vs. normal exploratory patterns — have been well-defined in experiments concerning the chemical discrimination of snake predators (Thoen et al., 1986; Mencía et al., 2016). Antipredatory responses include both slow and jerky movements, the latter including tail waving and foot shakes (Thoen et al., 1986; Van Damme et al., 1995; Mencía et al., 2016). By moving less than normal and/or moving in slow motion, an individual is less likely to be visually detected by the predator (Labra & Niemeyer, 2004). Tail waving is another clear antipredatory behaviour related to lacertid lizard caudal autotomy, confounding the predator and leading its attention to a non-vital part of the body (Arnold, 1984). On the other hand, the behaviours previously found for neutral odours (odourless controls or and non-predatory snakes) include walking normally, rubbing the snout on the walls of the terrarium, scratching the walls of the experimental terrarium and/or raising the head (Mencía et al., 2016, 2017; Ortega et al., 2017).

Many lizards adapt their antipredatory responses to the hunting skills of the predator (Sherbrooke, 2008; Lloyd et al., 2009; Landová et al., 2016). Herein, the ability of the lacertid lizard *Iberolacerta cyreni* to detect the chemical cues of its two sympatric predatory snakes, *Vipera latastei* and *Coronella austriaca*, which hunt by different strategies was evaluated. *Vipera latastei* is a sit-and-wait venomous predator that consumes lizards but mainly preys on mammals (lizards represent up to 30% of its diet), while *Coronella austriaca* is an active saurophagous hunter specialist that kills by constriction (not venomous). The hypotheses of this study are that lizards would be able to (1) effectively recognize the chemical cues of their predatory snakes, discerning them from non-predatory snake chemicals (2) show different antipredatory responses to the chemical cues of different predatory snakes. To test these hypotheses, an experiment analyzing the behavioural responses of lizards exposed to four chemical treatments was carried out, namely the scent of the active saurophagous non-venomous predator (*C. austriaca*), the scent of the generalist sit-and-wait venomous predator (*V. latastei*), the scent of a non-predatory sympatric snake (*Natrix maura*), and an odourless control.

2. Material and methods

2.1. Study system

The Carpetan Rock lizard, *Iberolacerta cyreni* (Müller & Hellmich, 1937), is an endemic Spanish mountain lizard that lives in isolated populations between 1600 and 2500 m asl (Arribas, 2014). This species is active between March and October (Pérez-Mellado, 1982; Arribas, 2014) and prefers rocky and mixed shrub habitats (Martín & Salvador, 1997a; Monasterio et al., 2010a, b).

The antipredatory behaviour of the Carpetan rock lizard is probably the best studied among lacertids (see for review Arribas, 2014). Studies have mainly focused on the last phases of the predation sequence, with human-simulation attacks. Upon attack, Carpetan rock lizards flee with short sprints and hide in refuges, normally in crevices or under rocks or bushes (see for review Arribas, 2014). They adjust antipredatory behaviour, activity levels, propensity to hide and the time inside the refuge, to the intensity of risk level (Martín et al., 2009a). Particularly, fleeing behaviour has been extensively studied and depends on several factors, such as the refuge distance, body temperature, physiological costs of using a refuge, and lizard personality (Carrascal et al., 1992; Martín, 2001; Martín & López, 2004; López et al., 2005; Cabido et al., 2009). In addition, a study on simulated attacks indicates that these lizards modulate their antipredatory behaviour to the risk level, and that they respond differently when risk level time sequences are predictable compared to when randomised (Amo et al., 2007; Martín et al., 2009a). It is also known that they gather information about the predator during the attack and use this information to modulate their refuge use (Polo et al., 2011). Finally, Carpetan rock lizards use tail autotomy as a last defensive tactic, which is known to affect their mating success, activity and thermoregulation abilities (Martín & Salvador, 1993a, b, 1995). However, their antipredatory tactics in the early phases of the predator detection sequence are still less known.

The smooth snake, *Coronella austriaca* (Laurenti, 1768) (Squamata, Colubridae) is a medium-sized snake, broadly distributed throughout Europe and Asia, normally inhabiting humid mountain areas (Arnold & Oviden, 2002; Galán, 2014). In the study area, at Sierra de Gredos (Spain), this species lives above 1600 m asl (Galán, 2014). It is mainly saurophagous, and has lacertids as its main prey (Rugiero et al., 1995). In the Iberian Peninsula, reptiles represent more than the 80% of its diet (Galán, 2014). They kill by constriction

and exhibit mixed hunting strategies, depending on prey mobility. If the prey is sedentary, they will actively hunt it, while they apply an ambush strategy for active prey (Goddard, 1984; Galán, 2014).

Lataste's vipers, *Vipera latastei* (Boscá, 1878) (Squamata, Viperidae), inhabit the Iberian Peninsula (except for the Cantabrian shore), the North of Africa and the Balearic Islands (Schleich et al., 1996; Martínez-Freiría et al., 2014). Their populations are fragmented, more abundant in mountain areas (Martínez-Freiría et al., 2014). In the study area, this species is found up to 2300 m asl (García-Paris et al., 1989). *V. latastei* is a sit-and-wait generalist predator that kills its prey with poison (Bea et al., 1992; Martínez-Freiría et al., 2014). The Lataste's viper feeds on reptiles, which can represent up to a third of its diet, with the rest including small mammals, birds, and arthropods (Santos et al., 2007; Martínez-Freiría et al., 2014).

The viperine snake, *Natrix maura* (Linnaeus, 1758) (Squamata, Colubridae), inhabits Southwest Europe and Northwest Africa (Schätti, 1982; Santos, 2014). It is an aquatic snake that feeds mainly on amphibians, fish, and aquatic invertebrates (Santos, 2014). *Natrix maura* is certainly not an adult reptile predator (Santos, 2014), and has been widely used as a scent control in lizard chemical predator discrimination experiments (e.g., Thoen et al., 1986; Mencía et al., 2016, 2017).

2.2. Experimental design

During August 2012, 24 adult *I. cyreni* individuals (12 males and 12 females; mean snout-vent length, SVL, \pm SE = 70.31 \pm 2.85 mm) were captured by noosing at Lagunas del Trampal (Sierra de Gredos, Ávila, Spain), at 2200 m asl. Sex was determined based on coloration and number and size of femoral pores (see complete description in Arribas, 2014). One adult individual of each snake species was also captured: *V. latastei* (SVL = 520 mm), *C. austriaca* (SVL = 570 mm) and *N. maura* (SVL = 610 mm). Lizards and snakes were transported to the University of Salamanca where this study was conducted. The lizards were maintained in individual plastic terraria (40 \times 25 \times 30 cm) with an artificial grass substrate and daily feeding, with crickets and *Tenebrio molitor* larvae, and water ad libitum. The snakes were housed in different rooms, also in individual plastic terraria (50 \times 30 \times 30 cm) with an artificial grass substrate and water ad libitum. Since predator diets can affect prey reaction to their chemical cues (e.g., Schoeppner & Relyea, 2009), the snakes were not fed during the experiment.

The experimental design was similar to that of other previous studies on lacertid chemical discrimination toward snakes (Mencía et al., 2016, 2017; Ortega et al., 2017). The lizards' behaviour was addressed when exposed to four chemical treatments: generalist predator ('Vipera'), specialist predator ('Coronella'), non-predator ('Natrix') and odorless control ('Control'). Each snake was moved from their enclosures 24 h before the beginning of the trials to an experimental terrarium (60 × 40 × 40 cm), where absorbent papers were used as a substrate and as the source of chemical cues. Only the impregnated absorbent paper was left during each trial, while snakes were returned to their previous enclosures. The occlusive plastic cover of the terrarium was closed to avoid odour loss. The snakes were removed from the terrarium 2 minutes before each experiment trial. The experimental room was dark, and only the terrarium was illuminated from above (50 cm) by a 75-W bulb, providing homogeneous lighting. A homogeneous constant temperature of 30°C (near the subjects thermal preferences, see Ortega et al., 2016) was maintained in the experimental room in order to avoid possible lizard behaviour variations due to temperature.

Each lizard was subjected once to each treatment, resulting in 96 trials (24 lizards × 4 treatments). Each lizard was tested once daily within their normal activity period (0900 to 1800 GMT; Ortega et al., 2016). The trials were carried out in a random order for each lizard, that is, lizards were randomly assigned to one of the 24 different possible sequences of trials, resulting of the permutation of the four treatments. The fact that lizards were tested in an unfamiliar experimental terrarium could affect their reactivity to predatory scents or their propensity to react to antipredatory behaviours. In addition, the use of one snake per treatment precludes results on the effect of intraspecific predator variability in lizard responses. Nonetheless, the experimental design applied herein with repeated measurements, randomising the sequences in which treatments were tested for each individual lizard allows for reliable comparisons between lizard behaviour in the different treatments.

Six equal sectors on the transparent surface of each terrarium were drawn, in order to count the number of times that lizards moved among sectors. Each trial began by introducing the lizard into the experimental terrarium, closing the terrarium with the occlusive transparent cover in order to avoid scent loss and recording behaviour with a digital recorder, for 15 min. Two observers were placed in front of the terrarium, opposite each other; while one recorded behavioural variables with binoculars, the other recorded the

number of movements and changes among the terrarium sectors. All animals remained healthy throughout the study period, and, once the experiments were finished, all lizards and snakes were released at their capture sites.

2.3. Behavioural variables

A total of 15 behavioural variables were recorded: (1) ‘walking’: the lizard moves in the same manner as it does in the wild; (2) ‘changing sectors’: the lizard moves between the six predefined sectors of the experimental terrarium; (3) ‘slow’: the lizard walks slowly, usually slower than 1 cm/s, and with stalking or scattered movements (Thoen et al., 1986; Mencía et al., 2016); (4) ‘tongue flick (TF) latency’, i.e., time until the first TF; (5) ‘TF’: number of TFs; (6) ‘snout’: the lizard taps the wall of the terrarium with its snout; (7) ‘rubbing’: the lizard rubs its head against the walls of the terrarium; (8) ‘stand and scratching’: the lizard stands up against the wall of the terrarium and scratches it with its forelegs, as if trying to escape; (9) ‘head bob’: the lizard moves its head up and down; (10) ‘head raise’: the lizard raises the head with its forelimbs straightened, adopting a resting posture, as observed in the wild; (11) ‘tail waving’: the lizard waves its tail in a horizontal plane; (12) ‘foot shake’: the lizard moves its forelimbs up and down; (13) ‘walk time’: the total amount of time that the lizard moves normally; (14) ‘slow time’: the total amount of time that the lizard moves in slow motion; and (15) ‘no movement’: total amount of time that the lizard stays immobile. The variables were quantified as frequencies, except for TF latency, walk time, slow time and no movement, which were quantified in seconds. The behaviour of each lizard was recorded 5 s after placing it in the centre of the experimental terrarium.

2.4. Data analysis

All analyses were carried out using R version 3.1.3 (R Core Team, 2015). As neither the original nor the log-transformed data met parametric statistic requirements, the data were analysed by non-parametric tests. The Mann–Whitney *U*-test was applied to assess sex differences in the behavioural variables. The Friedman test for repeated measures was applied to assess possible differences in the behavioural variables among treatments. When the result of the Friedman test was significant, multiple post hoc comparisons for the Friedman test were carried out in order to locate the differences between treatments (Giraudoux, 2012). To integrate behavioural patterns, a

Principal Components Analysis (PCA) of the 15 behavioural variables was also carried out, with the `prcomp` function (Crawley, 2012). Since the values of individuals for PCA axes met the assumptions of parametric statistics, a repeated measures ANOVA was carried out, from values of PCA axes to test for differences in the behavioural patterns between the two control treatments and the two predator treatments.

3. Results

The values of the 15 behavioural variables were similar for adult males and females, so the data were pooled for further analyses ($p > 0.05$ for the Mann–Whitney U -test for the 15 behavioural variables, see Table A1 in the Appendix).

Statistically significant differences were observed between the treatments for 14 of the 15 behavioural variables (Friedman test, $p < 0.001$ for all comparisons; Table 1). On the other hand, TF latency was similar for all the treatments (Friedman test, $\chi^2 = 1.025$, $p = 0.795$; Table 1). Paired comparisons revealed that no significant differences for the 15 variables between the

Table 1.

Mean values (\pm SE) and results of the Friedman test ($df = 3$) of the 15 behavioural variables recorded for the Carpetan rock lizard (*Iberolacerta cyreni*) for the four treatments.

Behaviour	Control	Natrix	Coronella	Vipera	χ^2	p
Walking	71 \pm 6.27	60.79 \pm 5.37	1.83 \pm 0.52	0.92 \pm 0.48	60.04	<0.0001***
Changing sectors	32.3 \pm 3.61	27.62 \pm 2.76	0.79 \pm 0.27	0.5 \pm 0.25	62.89	<0.0001***
Slow	0	0	14.96 \pm 1.45	15.46 \pm 1.4	64.31	<0.0001***
TF	102 \pm 7.41	109.7 \pm 7.61	65.17 \pm 6.34	100.4 \pm 12.03	18.61	0.0003***
TF latency	26.46 \pm 4.29	30.5 \pm 5.63	26.33 \pm 6.04	31.08 \pm 5.53	1.03	0.7951
Snout	26.76 \pm 2.63	27.54 \pm 2.53	1.79 \pm 0.44	1.79 \pm 0.6	60.00	<0.0001***
Rubbing	79.71 \pm 7.78	66.38 \pm 7.02	3.79 \pm 1.2	2.83 \pm 0.78	59.76	<0.0001***
Stand and scratching	29.21 \pm 3.08	26.71 \pm 3.15	1.25 \pm 0.44	0.5 \pm 0.22	61.67	<0.0001***
Head bob	0	0.04 \pm 0.04	6.75 \pm 1.47	11.5 \pm 2.05	62.89	<0.0001***
Hand shake	0.37 \pm 0.23	0.12 \pm 0.09	3.87 \pm 1.21	3.29 \pm 0.85	28.71	0.0004***
Head raise	0.54 \pm 0.17	0.79 \pm 0.29	4.33 \pm 1.2	4.75 \pm 1.14	19.86	0.0002***
Tail waving	0.04 \pm 0.04	0	21.62 \pm 7.54	7.67 \pm 2.65	40.92	<0.0001***
Walk time	259.9 \pm 20.17	234.8 \pm 17.93	4.12 \pm 1.2	1.25 \pm 0.85	61.32	<0.0001***
Slow time	0	0	94.38 \pm 18.17	135.9 \pm 29.21	65.39	<0.0001***
No movement	636 \pm 20.07	652 \pm 19.53	795.7 \pm 19.41	762.5 \pm 29.03	52.25	<0.0001***

Significant results: *** $p < 0.001$. All variables are absolute frequencies (counts), except for TF latency, walk time and slow time and no movement, that are quantified in seconds.

Control and Natrix treatments, as well as between the Coronella and Vipera treatments (Table 2), while significant differences were detected between the Vipera and Natrix and the Vipera and Control treatments for 13 of the 15 behavioural variables (all variables, except for TF and Tail waving). Finally, significant differences for the 15 behavioural variables between Coronella and Control and Coronella and Natrix were also observed (Table 2).

Regarding the PCA, the sphericity Bartlett test was significant ($\chi^2 = 1769.601$, $df = 105$, $p < 0.0001$), showing that the correlation between the variables is statistically different from zero, which indicates that the data from the 15 behavioural variables were suitable for dimensionality reduction analyses. The eigenvalues indicated that retaining two dimensions is advised. The first principal component (PC1) explained 51.10% of the data variability, while the second principal component (PC2) explained 17.14%. Thus, the two retained axes explained 68.24% of the variability of the raw data. PC1 is positively related to walk time, walking, rubbing, stand and scratching, snout and changing sectors and negatively related with slow, head bob, no movement, slow time, hand shake, and head rise (Table 3). Thus, PC1 would represent exploratory (positive values) vs antipredatory (negative values) behavioural patterns. PC2 is positively related to head rise, TF, slow time and negatively related with no movement (see Table 3). Therefore, PC2 would represent the amount of movement of each individual lizard in each trial. The repeated measures ANOVA revealed significant differences between PC1 values for the two controls (treatments Control and Natrix) and the predators (treatments Coronella and Vipera; repeated measures ANOVA, $F_{1,47} = 319.084$, $p < 0.0001$; Figure 1).

4. Discussion

The results indicate that *Iberolacerta cyreni* recognises the chemical cues of its predatory snakes, *Vipera latastei* and *Coronella austriaca*, reacting with a similar and a strong antipredatory behavioural pattern to both. This pattern mainly consisted in reducing movement activity, moving in slow motion, and exhibiting other specific behaviours, such as tail waving, forelimb shaking and head bobbing. On the other hand, the behavioural pattern of lizards in the control treatments (odourless control and scent of *Natrix maura*) was similar to the normal exploratory pattern found in close lacertids, including behaviours such as snout, rubbing, walking, and stand up and scratching

Table 2. Values of the post hoc paired comparisons for the 14 behavioural variables significant for the Friedman test.

Behaviour	A	Control–Natrix	Control–Coronella	Control–Vipera	Natrix–Vipera	Natrix–Coronella	Coronella–Vipera
Walking	0.01	6	48.5*	53.5*	47.5*	42.5*	5
Changing sectors	0.01	11	52*	55*	44*	41*	3
Slow	0.01	0	48.5*	47.5*	47.5*	48.5*	1
TF	0.01	5	30.5*	10.5	15.5	35.5*	20
Snout	0.01	4	44*	48*	52*	48*	4
Rubbing	0.01	8	50.5*	53.5*	45.5*	42.5*	3
Stand and scratching	0.01	1	45.5*	49.5*	50.5*	46.5*	4
Head bob	0.01	1	40*	53*	52*	39*	13
Hand shake	0.05	0.5	29*	27.5*	28*	29.5*	1.5
Head raise	0.05	1	25.5*	27.5*	26.5*	24.5*	2
Tail waving	0.01	1.5	38.5*	21	22.5	40*	17.5
Walk time	0.01	4	47*	53*	49*	43*	6
Slow time	0.01	0	43*	53*	53*	43*	10
No movement	0.01	3	45*	32*	29*	42*	13

Significant results are marked with asterisks. The critical value of Friedman’s post hoc comparisons is 23.60 for $\alpha = 0.05$.

Table 3.

Correlation values between each of the 15 behavioural variables studied in *Iberolacerta cyreni* in the four experimental treatments (Control, Natrix, Vipera and Coronella) and the first (PC1) and second principal component axes (PC2) of PCA.

Behaviour	PC1	PC2
Walking	0.940	0.183
Changing sectors	0.927	0.200
Slow	−0.835	0.300
TF	0.252	0.660
TF latency	−0.015	−0.034
Snout	0.869	0.133
Rubbing	0.903	0.208
Stand and scratching	0.878	0.188
Head bob	−0.670	0.469
Hand shake	−0.512	0.283
Head rise	−0.509	0.661
Tail waving	−0.446	0.426
Walk time	0.969	0.187
Slow time	−0.586	0.638
No movement	−0.607	−0.717

(Thoen et al., 1986; Mencía et al., 2016). These exploratory behaviours were almost absent in the predator treatments.

One important defensive strategy is the selection of safe microhabitats, which allow prey to optimize their requirements while avoiding predators (Amo et al., 2007). Thus, prey often assess predation risk whereas keeping a suitable distance from a safe shelter (Lima, 1993). When predation risk increases, as in the present study, with intense predatory snake scents, many prey species reduce their activity to avoid predator encounters (Lima & Dill, 1990; Constanzo-Chávez et al., 2018) or modify their locomotion pattern to reduce the chances of being captured (McAdam & Kramer, 1998). The results reported herein indicate that *I. cyreni* lizards use this strategy, in a combination of moving in slow-motion and reducing the frequency and duration of displacements. These results agree with previous studies concerning other lacertids (Thoen et al., 1986; Van Damme & Quick, 2001; Mencía et al., 2016; 2017; Ortega et al., 2017) and groups, such as liolaemid lizards (Labra & Niemeyer, 2004) and the Australian gecko *Amalasia lesueurii*

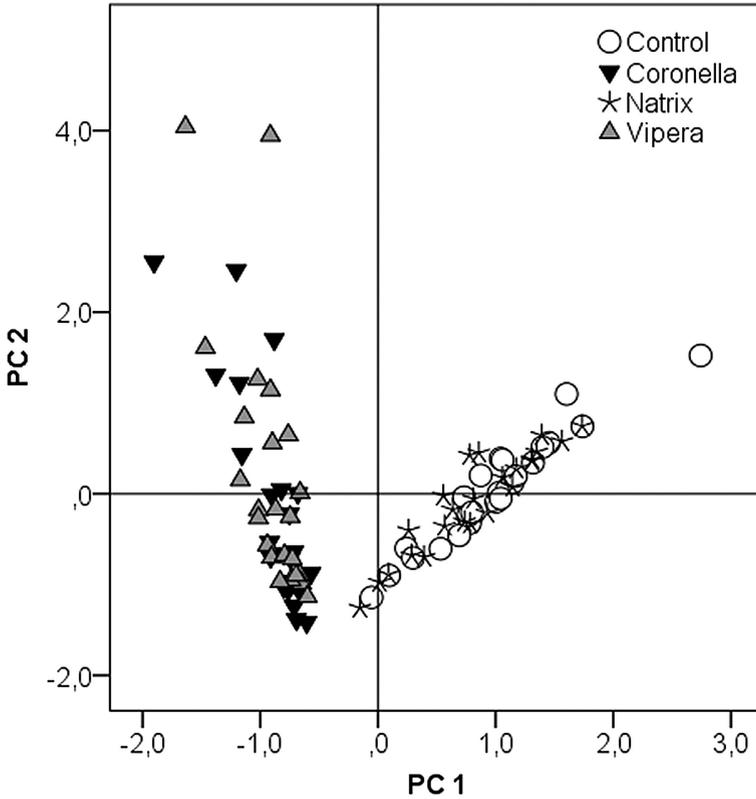


Figure 1. Scatterplot of the PCA values of each Carpetan rock lizard (*Iberolacerta cyreni*) for the four treatments. A repeated measures ANOVA demonstrated that the values of the control and Natrix treatments were significantly different than those of the Coronella and Vipera treatments values for the PC1 (see the text).

(Webb et al., 2009). The strategy of reducing activity, aiding in avoiding detection, can be especially beneficial in the early stages of the predatory sequence, when a lizard identifies the chemical cues of a nearby potential predator. However, *I. cyreni* may flee to a refuge or even use tail autotomy in the last predatory sequence stages, following encounters with snakes and/or upon an attack, as they do under attacks simulated by humans (e.g., Martín & Salvador, 1992; Martín et al., 2009b).

Nonetheless, when a prey detects a predator, instead of fleeing immediately, it can sometimes display a pursuit-deterrent signal (Hasson, 1991). With this signal, the prey informs the potential predator that it has been detected (perception advertisement; Woodland et al., 1980) or that is in good

body condition to escape if attacked (quality advertisement; Zahavi, 1977), preventing a pursuit from starting. Thus, the benefits of signalling may overcome the costs when the predator is at a distance where the probability of reaching the prey in an attack is low (Ydenberg & Dill, 1986). In these cases, pursuit-deterrent signals would benefit both prey and predator, saving the energy of a pursuit that will most probably end with the prey hidden in a refuge (Murphy, 2006). Although handshakes could also have other functions, such as intraspecific communication or substrate heat avoidance (Magnusson, 1996; Cooper et al., 2004), some would fulfil a pursuit-deterrent function. This is the case of type III handshakes performed by the lacertid lizard *Podarcis muralis* (Font et al., 2012), which are similar to those performed by *I. cyreni*: rotating the whole forelimb, similarly displayed by males and females. The fact that *I. cyreni*, as reported for other lacertids (Thoen et al., 1986; Cooper et al., 2004; Mencía et al., 2016), only performed handshakes in the presence of predator chemicals reinforces the idea that they probably act as pursuit-deterrent behaviours. It is not clear whether these handshakes would signal to the predator that it is being detected or if it would also inform about the good condition of the lizard to escape, but its function as pursuit-deterrent behaviours seems quite plausible. Similar results have been reported for other lizards (Thoen et al., 1986; Cooper et al., 2004; Font et al., 2012; Mencía et al., 2016).

Another behaviour displayed by *I. cyreni* in response to predatory snake chemicals is tail waving. Tail waving is also applied as a pursuit-deterrent signal in other lizards, mainly when they are subjected to an intermediate risk level (e.g., Bohórquez-Alonso et al., 2010; Cooper, 2010). The displays performed by *I. cyreni* are similar to those described for the gecko *Gonatotodes albogularis* as a pursuit-deterrent signal, also performed similarly by both sexes (Bohórquez-Alonso et al., 2010). Thus, the lizard could communicate to the potential predator that chasing is probably a worthless energetic cost (Caro, 2005; Cooper, 2010). If the predation risk were high, then the lizard could autotomize its tail to distract the attack to a non-lethal part of its body (Bateman & Fleming, 2009). Nonetheless, *I. cyreni* lizards would only opt for tail autotomy under a high risk of an imminent attack, in the last phases of the predatory sequence, since autotomy costs are also high, affecting, among others, lizard thermoregulation, mating success, movement patterns and activity (e.g., Martín & Salvador, 1993a, b, 1995, 1997b; Martín, 2001). As with handshakes, the fact that lizards only waved their tails in the

presence of predator chemicals reinforces the idea of this behaviour as a pursuit-deterrent or deflective behaviour, and so would be the case for other similarly studied lacertids (e.g., Thoen et al., 1986; Van Damme & Quick, 2001; Mencía et al., 2016).

Iberolacerta cyreni lizards also performed head bob displays when detecting predatory snake chemicals. As with handshakes, these displays often have a communication function between conspecific lizards (e.g., Martins, 1993; Martins et al., 2004; Vicente, 2018). However, the fact that *I. cyreni* lizards were isolated in our experiment and performed head bobs from the first trial, and that the frequency of head bobs was similar between sexes, indicates another explanation. Paying attention to its head would not be advantageous for the lizard, unless it would serve, as handshakes and tail waves, to avoid a pursuit from starting. One type of head bob displayed by the lizard *Liolaemus pacha* is thought to have a pursuit-deterrent function (Vicente, 2018). Thus, it is possible that *I. cyreni* also uses this defensive tactic when suddenly confronting the predatory snake chemical scents.

In any case, it is clear that many lacertid lizards show a consistent pattern of slow motion, handshakes, tail waving and head bobs when exposed to predatory snake chemical cues in experimental terraria (Thoen et al., 1986; Van Damme & Quick, 2001; Mencía et al., 2016, 2017; Ortega et al., 2017). Thus, it is probable that these behaviours are beneficial for lizards to prevent attacks in the early stages of the predatory sequence. In these experiments, the lizards do not see the predator, but detect its chemical cues at high intensity. Thus, the lizards seem to use a mixed strategy: reducing mobility and, thus, detectability and, if detected, signalling to the predator that it has been detected and that a pursuit will probably be unsuccessful. In addition, it is interesting that lizards use the same behavioural defences than when they detect a simulated predator (a human) under intermediate risk situations (Cooper, 2010). The next step would be to conduct experiments also focused on predator behaviour, in order to understand when lizard defensive displays effectively deter snakes in pursuing their potential prey.

Regarding tongue flicking, *I. cyreni* lizards performed less with *C. austriaca* scents compared to the other treatments. Reducing tongue flicking confronted with the saurophagous snake (*C. austriaca*) scent could indicate a faster recognition by the prey (Mori & Hasegawa, 1999; Labra & Hoare, 2015). However, differences in tongue flicking rates between both predatory snake treatments were not significant. In addition, results from previous

studies suggest that some lizards may not alter the frequency of their tongue flicks despite clearly recognizing predator chemicals and reacting with defensive patterns (Labra & Niemeyer, 2004; Mencía et al., 2016, 2017; Ortega et al., 2017).

Hence, no evidences for the hypothesis that *I. cyreni* would be able to discriminate different predator types and/or modulate its antipredatory response were obtained. Instead, lizards behaved similarly to both predatory snakes — *C. austriaca* and *V. latastei* — regardless of their different hunting strategies. Similar results have been reported for *Iberolacerta galani*, which fully recognizes the chemical cues of *C. austriaca* and *Vipera seoanei* as predatory snakes and reacts similarly to both (Mencía et al., 2016). This fact would reflect that a common response to these predators, that hunt with different strategies, is adaptative for lizards in the first stages of the predatory sequence. Analogous results were found for *Podarcis muralis*, whose tongue flick rates were similar in response to chemicals from different predatory snakes, but different for a non-predatory snake species (Amo et al., 2004). However, this seems to not be the case for *I. cyreni*. Therefore, prey that would simply adopt the strategy of avoiding all potential predators would not assume the optimal tradeoff between avoiding predation and effectively performing other activities directly related to their fitness, such as feeding, thermoregulating, or reproductive activities (Lima & Dill, 1990). Contrarily to the results reported herein, *I. cyreni* is known to adapt its use of shelter and antipredatory behaviour to the risk level (Martín, 2001; Martín & López, 2003; Polo et al., 2005; Martín et al., 2009a), so the fact that it reacts similarly to different predator snake chemical cues is surprising and deserves further consideration. One explanation is that defensive responses may differ in their specificity in the early vs last phases of the predatory sequence. The reported results suggest that reducing activity and performing pursuit-deterrent or deflective signals to a potential not-located predator is a beneficial antipredatory response when only detecting its chemical cues, regardless of whether this predatory snake is an active or ambush predator. Nonetheless, *I. cyreni* lizards could respond differently when visually detecting those predators, especially because they could identify in which direction to run if they decided to flee.

In conclusion, the experiments carried out herein proved that *I. cyreni* identifies the chemical cues of its two sympatric predatory snakes and reacts with similar and intense defensive responses, regardless of the predator

hunting strategy. Thus, lizards move less and much slower than normally, probably to go undetected, and perform visual displays that can signal the potential predator to deter a pursuit. These visual displays comprise handshakes, tail waving and head bobs, and are similar to those reported as pursuit-deterrent signals in other lizards. In addition, these displays are consistently similar to those found in analogous experiments carried out with other lacertids, so they must be an important part in avoiding attacks in the early phases of the predatory sequence. Future studies should experimentally test their biological function, as well as further investigate the factors that drive lacertid antipredatory responses to snake chemical cues.

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Table A1.

Mean values (\pm SE) of males ($n = 12$) and females ($n = 12$) on the 15 behavioural variables recorded for the Carpetan rock lizard (*Iberolacerta cyreni*) for the four treatments and their paired comparisons (Mann–Whitney U -test) between sexes.

Behaviour	Control			Natrix		
	Males	Females	Comparison	Males	Females	Comparison
Walk	65.67 \pm 7.61	76.33 \pm 10.08	$U = 56.00$ $P = 0.378$	62.00 \pm 7.42	59.58 \pm 8.06	$U = 72.00$ $P = 1.000$
Ch. among sectors	29.92 \pm 3.38	41.68 \pm 6.07	$U = 48.00$ $P = 0.178$	27.25 \pm 2.77	28.00 \pm 4.91	$U = 69.00$ $P = 0.887$
Slow	0	0	NA	0	0	NA
TF	98.42 \pm 13.13	105.67 \pm 7.39	$U = 49.50$ $P = 0.198$	103.75 \pm 8.82	115.58 \pm 12.72	$U = 61.50$ $P = 0.551$
TF latency	23.83 \pm 5.46	29.08 \pm 6.78	$U = 62.50$ $P = 0.590$	23.42 \pm 5.16	37.58 \pm 9.84	$U = 54.50$ $P = 0.319$
Snout	21.58 \pm 2.92	31.92 \pm 3.93	$U = 39.00$ $P = 0.060$	26.50 \pm 2.79	28.58 \pm 4.33	$U = 70.50$ $P = 0.932$
Rubbing	74.93 \pm 9.35	84.58 \pm 12.69	$U = 65.00$ $P = 0.713$	65.17 \pm 10.20	67.58 \pm 10.07	$U = 66.00$ $P = 0.755$
Stand and scratching	30.17 \pm 4.82	28.25 \pm 4.02	$U = 70.00$ $P = 0.932$	27.50 \pm 4.64	25.92 \pm 4.44	$U = 68.50$ $P = 0.843$
Head bob	0	0	NA	0.08 \pm 0.08	0	NA
Hand shake	0.42 \pm 0.34	0.33 \pm 0.33	$U = 66.50$ $P = 0.755$	0.08 \pm 0.09	0.17 \pm 0.17	$U = 71.50$ $P = 0.977$
Head raise	0.58 \pm 0.26	0.50 \pm 0.23	$U = 68.00$ $P = 0.843$	1.25 \pm 0.49	0.33 \pm 0.26	$U = 47.00$ $P = 0.160$
Tail waving	0	0.08 \pm 0.08	$U = 66.00$ $P = 0.755$	0	0	NA
Walk time	233.83 \pm 20.71	285.92 \pm 33.89	$U = 54.00$ $P = 0.319$	225.83 \pm 22.52	243.83 \pm 28.67	$U = 59.00$ $P = 0.478$
Slow time	0	0	NA	0	0	NA
No move	657.20 \pm 21.19	614.08 \pm 21.19	$U = 60.00$ $P = 0.514$	672.83 \pm 21.62	631.17 \pm 32.39	$U = 51.00$ $P = 0.242$

Table A1.
(Continued.)

Behaviour	Coronella			Vipera		
	Males	Females	Comparison	Males	Females	Comparison
Walk	1.82 ± 0.74	1.83 ± 0.74	$U = 68.50$ $P = 0.843$	1.33 ± 0.91	0.50 ± 0.29	$U = 65.50$ $P = 0.713$
Ch. among sectors	8.17 ± 1.70	8.25 ± 2.15	$U = 64.50$ $P = 0.671$	7.17 ± 1.36	6.75 ± 1.38	$U = 58.00$ $P = 0.443$
Slow	14.92 ± 1.21	15.00 ± 2.71	$U = 62.50$ $P = 0.590$	13.26 ± 1.74	13.83 ± 2.18	$U = 56.00$ $P = 0.378$
TF	60.33 ± 7.07	70.00 ± 10.66	$U = 64.00$ $P = 0.671$	105.00 ± 15.18	95.83 ± 19.26	$U = 56.50$ $P = 0.378$
TF latency	20.25 ± 5.22	32.42 ± 10.88	$U = 58.00$ $P = 0.443$	35.83 ± 9.23	26.33 ± 6.19	$U = 61.50$ $P = 0.551$
Snout	2.33 ± 0.70	1.25 ± 0.52	$U = 52.50$ $P = 0.266$	2.92 ± 1.08	0.67 ± 0.31	$U = 40.50$ $P = 0.068$
Rubbing	5.08 ± 2.19	2.50 ± 0.95	$U = 59.50$ $P = 0.478$	3.33 ± 0.96	2.33 ± 1.26	$U = 46.50$ $P = 0.143$
Stand and scratching	1.50 ± 0.59	1.00 ± 0.67	$U = 53.00$ $P = 0.291$	0.58 ± 0.34	0.42 ± 0.29	$U = 62.00$ $P = 0.590$
Head bob	4.92 ± 0.69	8.58 ± 2.82	$U = 62.00$ $P = 0.590$	11.33 ± 3.45	11.67 ± 2.38	$U = 61.00$ $P = 0.551$
Hand shake	1.67 ± 0.59	6.08 ± 2.21	$U = 47.00$ $P = 0.160$	3.42 ± 1.33	3.17 ± 1.11	$U = 70.50$ $P = 0.932$
Head raise	3.17 ± 0.96	5.50 ± 2.20	$U = 59.50$ $P = 0.478$	4.75 ± 1.24	4.75 ± 1.97	$U = 60.50$ $P = 0.514$
Tail waving	7.25 ± 1.77	36.00 ± 14.04	$U = 45.00$ $P = 0.128$	5.92 ± 3.14	9.42 ± 4.36	$U = 71.00$ $P = 0.977$
Walk time	3.83 ± 1.65	4.42 ± 1.83	$U = 70.00$ $P = 0.932$	2.17 ± 1.65	0.33 ± 0.33	$U = 60.50$ $P = 0.514$
Slow time	99.75 ± 29.65	89.00 ± 22.28	$U = 69.00$ $P = 0.887$	111.33 ± 23.14	160.42 ± 54.07	$U = 67.00$ $P = 0.799$
No move	787.00 ± 32.36	804.33 ± 22.67	$U = 68.50$ $P = 0.843$	786.50 ± 22.73	738.42 ± 53.88	$U = 68.00$ $P = 0.843$

All variables are absolute frequencies (counts), except for 'TF latency', 'Walk time' and 'Slow time' and 'No move', that are quantified in seconds.