Experience may allow increasing accuracy of the innate chemosensory recognition of snake predators by Iberian wall lizards

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Abstract Many animals can recognize chemical cues of predators and show defensive responses, but antipredatory behavior can be costly and should be modulated depending on the level of risk posed by each predatory species. Recognition ability may be innate, but it is not clear whether there might also be local adaptation to predation pressure levels or some learning component. We examined the chemosensory ability of naïve and adult Iberian wall lizards, *Podarcis hispanicus*, to detect chemical cues of two closely related *Coronella* snake species in two localities within the same continuous population. Lizards in each locality are sympatric with only one of the two snake species. Our results showed an innate chemosensory discrimination of predatory snake cues, as both adult lizards and captive reared naïve hatchlings, which had not had any previous experience with the snake cues, had short latencies to the first tongue-flick, increased tongue-flick responses, and active escape behavior from the scent of either of the two snake species. Moreover, adult lizards, but not naïve hatchlings, showed differential responses to the two different snake species, with higher responses to the snake species that is sympatric in each locality. This strongly suggests that there is a learning component acquired through previous lifetime experiences that may increase accuracy of the discrimination of the sympatric snake. Therefore, through learning of local conditions, lizards may modulate their innate antipredatory responses to the risk level posed by each snake species.

Keywords predation risk · chemical recognition · innate responses · learning · lizards · snakes

Introduction

Detection and recognition of predators before an attack occurs is an essential task for prey animals (Lima and Dill 1990). Early recognition of the predator or its cues allows prey to modify their behavior to avoid risky encounters with the predator. However, while not responding to a predatory species is costly in terms of survival, responding to a non-predatory species may be energetically costly and result in a loss of time for other activities (Ydenberg and Dill 1986; Stapley 2003). Thus, prey animals should ideally be able to accurately identify predators, assess the level of risk that each particular predator poses, and adjust the intensity of their antipredatory behavior accordingly. For this, prey should base their antipredatory decisions on cues that provide accurate information of the predator's presence and identity. However, after identification, prey should also consider the predator's relative threat and the overall predation pressure in a population. This may require previous experiences with each particular type of predator.

Many animals use chemical cues of predators as a reliable way to detect their presence (Katts and Dill 1998). In many cases, after detection and identification of the predator's scents alone, prey modify their antipredatory behavior, decrease activity or shift to secure habitats as a preventive strategy (e.g., Kiesecker et al. 1996; López and Martín 2001; Cabido et al. 2004; Apfelbach et al. 2005). Nevertheless, the combined use of multiples types of cues (e.g., chemical and visual) is sometimes needed to elicit an optimal response, avoiding overestimating risk (Chivers et al. 2001; Amo et al. 2004a, 2006).

Many species of snakes feed on lizards and many lizards are able to use their chemosensory senses to detect and recognize chemical cues of these saurophagous snakes (e.g., Thoen et al. 1986; Cooper 1990; Van Damme and Quick 2001; Bealor and Krekorian 2002; Amo et al. 2004b, 2005). According to the threat-sensitive hypothesis (Helfman 1989), natural selection should favor individuals that take action appropriate to the magnitude of threat rather than respond to chemical cues of all predators in a similar way. Thus, the defensive responses of prey animals to chemical cues of different snakes should depend on the level of risk posed by each snake species (Stapley 2003; Amo et al. 2004b). Accordingly, many lizards are able to discriminate between scents of different snake species that pose different predation threat (Thoen et al. 1986; Dial and Schwenk 1996; Downes and Shine 1998a,b; Van Damme and Quick 2001; Bealor and Krekorian 2002; Cabido et al. 2004; Labra and Niemeyer 2004). In contrast, other lizard species display generalized responses to any snake irrespective of their threat (Stapley 2003; Amo et al.

2004b, 2006; Webb et al. 2009). These differences in responses probably depend on the balance between the costs of the antipredatory response and the cost of non-responding adequately to a predator (Lima and Dill 1990).

Moreover, responses of lizards to snake scents may be often found in naïve juvenile individuals (Van Damme et al. 1995; Mori and Hasegawa 1999; Downes and Adam 2001). Also, responses may persist in a lizard population even many years after the snakes have disappeared from the population (Van Damme and Castilla 1996), although responses may be finally lost after a long enough period of predator relaxation (Durand et al. 2012). This strongly suggests that chemosensory responses of lizards to scent of predatory snakes may be innate after natural selection of those individuals that had this ability. However, responses to the scents of the same predatory snake vary geographically within lizard species depending on whether they are sympatric or not, suggesting that there may be local adaptation to predation pressure levels (Downes and Adam 2001) or that some learning component occurs in the responses (Mori and Hasegawa 1999).

Iberian wall lizards (*Podarcis hispanicus*) are small lacertids widespread in different types of rocky habitats across the Iberian Peninsula and North-West Africa (Salvador and Carretero 2014), where individuals from different populations may face different predator species. In the plains and mountains of Central Spain, populations of *P. hispanicus* lizards that are closely related genetically occupy an altitudinal gradient from 500 to 1800 m of altitude (Gabirot et al. 2013). However, in the same area, two different species of saurophagous specialist snakes of the genus *Coronella* are clearly segregated in altitude (i.e., *C. girondica* in lowland areas and *C. austriaca* in the highlands) (Morales et al. 2002; Galán 2014a,b). These are ambush snakes that capture their main lizard prey (> 80 % of their prey) from within dark rock crevices where lizards typically take refuge (Amo et al. 2004c; Galán 2014a,b). This natural system provides an excellent opportunity to test whether populations of the same lizard species are able to respond differentially to different snake predator species as a function of their degree of sympatry with these predators, and whether these responses are innate in each locality or have a learning component.

In this paper, we examined the ability of naïve and adult Iberian wall lizards, *P. hispanicus*, from two localities within the same population to detect chemical cues of two closely related *Coronella* snake species. Lizards in each locality are sympatric with only one of the two snake species. We designed a laboratory experiment to specifically examine: 1) whether adult lizards, which presumably had experienced the scents of snakes

in their habitat, and naïve lizards, which had not had any previous contact with the scent of these snakes, were able to detect the scent of snakes and to discriminate between the two species, and 2) whether lizards' responses to the snake scent were more intense for the snake species that is sympatric with lizards in each locality.

We predicted that chemosensory detection of the snake scent would be innate in *P. hispanicus* lizards, as it occurs in related species (Van Damme and Castilla 1996), but that an accurate discrimination of the snake species would require some previous experience with the snake scent. Therefore, only adult lizards will be able to discriminate between snake species and respond adequately, whereas naïve lizards will only show a generalized response to both snake species. We also predicted that, according to the threat sensitivity hypothesis, within each locality the responses of lizards to the sympatric snake species will be higher than to the allopatric species. This difference may be explained because risk of the sympatric snake should be considered higher by lizards based on the likely higher frequency of previous encounters with this snake or its scent, in contrast to the lack of experience with the allopatric snake.

Materials and Methods

Study sites, lizard and snake husbandry

During April-May 2013 we captured by noosing male and female Iberian wall lizards (*P. hispanicus*) at two nearby sampling sites at different elevations in the Guadarrama Mountains (Central Spain) separated 6 km by air. The lowland locality was `La Dehesa de la Golondrina´, near Cercedilla village (40º43´N, 04º01´W; 1,250 m altitude), where lizards occupied granite rocky outcrops inside an oak forest. The highland locality was the upper part of `Fuenfría Valley´ (40º47´N, 04º03´W; 1,750 m altitude), where lizards occupied granite rock walls and rock piles at the edge of a pine forest. Microsatellite analyses show that lizards from these two localities are closely related genetically, with a high degree of gene flow, indicating that are actually two sampling sites of the same genetic population (Gabirot et al. 2013). In fact, lizards could be found continuously in appropriate microhabitats along the entire altitudinal gradient between the two sampling sites. Lizards from these two localities have been proposed to be included in the recently named new species *P. guadarramae* (Geniez et al. 2014).

Immediately after capture, lizards were transported by car in individual containers to "El Ventorrillo" field station facilities about 5 km away from the capture sites. Lizards were individually housed indoor in plastic terraria (40 x 30 and 25 cm high) filled with a moistened coconut fiber substrate and provided with a water bowl and a brick (24 x 11 x 8 cm) that allowed shelter and climbing opportunities. A 50 W halogen lamp was suspended over one end of the terrarium providing a diurnal temperature gradient (21-45 ℃) allowing thermoregulation of lizards within the preferred temperature range of this species (34.4 °C; Bauwens et al. 1995). In addition, a fluorescent bulb in each shelve provided ambient lighting mimicking the natural photoperiod, and mercury vapor bulbs (Exoterra Solar Glow 125 W) provided ultraviolet radiation during 1.5 h a day (from 14.00 to 15.30). Lizards were daily watered, and fed crickets (*Acheta domesticus*) and mealworms (*Tenebrio mollitor*) *ad libitum*, dusted with a commercial vitamin and calcium supplement. Adult lizards from the two sampling sites were maintained in captivity for a similar period of time (about 1 month) until the beginning of the chemosensory trials.

Cages of females, which were already gravid when captured, were carefully checked twice daily for the presence of eggs. Females laid the eggs in the wet substrate under the brick. Eggs were individually placed in 60 mL closed plastic cups filled with 10 g of moistened perlite (10 g perlite:10 g water) and transferred to an incubator at 27.5 ℃ (IRE-160; 94 x 60 x 60 cm; Raypa, Barcelona). When incubation was about to end (beginning of June), we searched the incubator daily for newborns. Hatching rate was high (> 90%) (see Ortega et al. 2015 for more details of breeding procedures and reproductive parameters). Hatchlings lizards were kept in individual terraria in the same conditions than adults (see above), but fed with small prey, pinhead crickets (*Acheta domesticus*), fruit flies (*Drosophila hydei* and *D. melanogaster*) and small waxworms (*Galleria mellonella*). Adult lizards were returned to their capture sites in late June, and juveniles released to their mothers capture sites in October prior to winter.

During May-June 2013, we lifted stones and captured by hand three *C. girondica* snakes at the lowland locality (the same area where lizards had been captured; see above) and three *C. austriaca* snakes at the highland locality (see above). To prevent lizards from contacting the snakes' scents in the laboratory before they were tested, the snakes were housed in a different laboratory, individually in glass terraria (60 x 30 and 25 cm high) with coconut fiber substrate and a shelter. To avoid using live lizards as food (the main prey of these snakes), we used lizards found dead in the field from each locality that had been kept frozen. These lizards were unfrozen and introduced in the snakes' terraria, one

lizard every five days. Snakes readily ate them. Snakes were maintained in captivity for only a few weeks while we performed the experiments, and subsequently released at their capture sites in good condition.

Chemosensory tests

We performed two different chemosensory experiments, one at the end of May 2013 with adults ($n = 24$; six males and six females from each locality), and other at the beginning of August 2013 with hatchling naïve lizards ($n = 20$; five males and five females from each locality). Adult lizards were of similar body size within each sex and locality (as there are natural intersexual and altitudinal average body size differences in adults; Gabirot et al. 2013; Ortega et al. 2015). Hatchlings had a mean ($\pm$ SE) of 15 ± 1 days of age when they were tested. Only one hatchling from each clutch was used (i.e., they all had different mothers).

To test for differential responses to scents, we compared tongue-flick (TF) rates by lizards in response to scent stimuli arising from cotton applicators impregnated with scents of (1) *C. girondica* snake, (2) *C. austriaca* snake, or (3) deionized water (odorless control) (Cooper and Burghardt 1990; Cooper 1998). We used water to gauge baseline TF rates in the experimental situation. We did not use a pungency control (e.g., eau de cologne) or a control of odor of a non-predatory species because we already knew that this and related lizard species are able to discriminate odor of predatory snakes from control odors (Van Damme and Castilla 1996; Van Damme and Quick 2001; Amo et al. 2004b), and because we were only interested here in comparing responses to different snake species. We prepared scent stimuli by dipping the cotton tip (1 cm) of a wooden applicator attached to a long stick (50 cm) in deionized water. Snake stimuli were added by gently pressing the cloacal area of the snakes until obtaining a liquid exudate with pungent odor, and then rolling the moistened cotton over the cloaca. Different individual snakes were randomly used as donor of scents in each trial. A new swab was used in each trial.

Every responding lizard in its own terrarium was exposed to each stimulus in counterbalanced order but only one trial was conducted per day for each lizard. Trials were conducted in indoor conditions between 0900 and 1300 hours, when lizards were fully active after having been thermoregulating in their terraria for at least 2 h.

To begin a trial, one experimenter (PL) slowly approached the terrarium and slowly moved the stick with the cotton swab to a position 1 cm in front of the lizard's snout. We

recorded the number of TFs directed to the swab for 60 s, beginning with the first TF. If the lizard moved, we rapidly and gently repositioned the swab. Latency to the first TF was recorded as the time from presentation of the cotton swab to the first TF directed at the swab.

We also investigated whether the chemosensory examination of chemical stimuli was accompanied by a shift in the lizard's behavior. We noted whether after the stimulus presentation lizards a) stayed immobile or moved slowly, and appear to be calm (i.e., without showing signs of fear or stress such as quick respiration or "head-up" alert postures), or b) fled quickly from the swab and tried to escape from the terrarium. However, these escape behaviors of some individual lizards observed in this experiment were only mild stress responses, and because the trials were short and lizards never actually found the predators, they did not show acute stress and behaved normally within 2 min of the end of the tests.

To examine differences in number of TFs and latency to first TF among conditions, we used General Linear Models (GLMs) with scent stimuli as a within-subject factor and locality (lowland vs. highland) and sex as fixed factors, including the interactions in the models. In all cases, after logarithmic transformation, data were normally distributed (tested with Shapiro–Wilk's tests) and variances were not significantly heterogeneous (tested with Hartley's F_{max} tests). We used Tukey's honestly significant difference tests for post-hoc pairwise comparisons (Sokal and Rohlf 1995). Differences in behavioral responses to the swab presentation among treatments and populations were examined with χ^2 tests.

Results

Chemosensory responses of adult lizards

There were significant differences among chemical stimuli in latency times (GLM, $F_{2,40}=75.93$, $P<0.0001$), but not between sexes ($F_{1,20}=1.78$, $P=0.20$) nor between localities ($F_{1,20}=2.44$, $P=0.13$), and all the interactions were not significant ($P>0.25$ in all cases) (Fig. 1a). Pairwise comparisons showed that latency times to water were significantly longer than to either of the two snake stimuli (Tukey's tests, $P=0.00012$ in both cases), but latency times did not differ between the two snake species ($P=0.99$).

The rate of TFs by adult lizards directed to the swabs varied significantly among chemical stimuli (GLM, $F_{2,40}=63.30$, $P<0.0001$), there was a significant difference between localities ($F_{1,20}=6.71$, $P=0.017$) but not between sexes ($F_{1,20}=1.38$, $P=0.25$). There was a significant interaction between sex and locality ($F_{1,20}=5.45$, $P=0.03$), indicating that there were differences between localities in overall TF responses of males (i.e., the average TF rate considering all responses to the three stimuli) (Tukey's test, $P>0.012$), but not in responses of females ($P=0.99$). More importantly, there was a significant interaction between type of stimuli and locality ($F_{2,40}=3.81$, $P=0.03$) (Fig. 1b). This indicated that, in both localities, TFs to the snake chemicals were significantly higher than to water (Tukey's tests, $P<0.001$ in all cases) and there were significant differences between snake species ($P=0.005$ in both populations). However, within each locality, TF rates to the sympatric snake species were higher than to the allopatric snake species ($P<0.05$ in both cases).

Chemosensory responses of naïve hatchlings

There were significant differences among chemical stimuli in latency times (GLM, $F_{2,36}=56.80$, $P<0.0001$), but not between localities ($F_{1,18}=0.32$, $P=0.58$) and the interaction between stimuli and locality was not significant ($F_{2,36}=2.28$, $P=0.12$) (Fig. 2a). Pairwise comparisons showed that latencies to water were significantly longer than to either of the two snake stimuli (Tukey's tests, $P=0.00012$ in both cases), but latencies did not significantly differ between the two snake species ($P=0.68$).

The rate of TFs by hatchling lizards varied significantly among chemical stimuli (GLM, $F_{2,36}=61.22$, $P<0.0001$), but there were not significant differences between localities ($F_{1,18}=0.01$, $P=0.94$) and the interaction between stimuli and locality was not significant ($F_{2,36}=1.62$, $P=0.21$) (Fig. 2b). Thus, in both localities, TFs elicited by hatchling lizards to the snake chemicals were significantly higher than to water (Tukey's tests, $P=0.00012$), but there was no significant difference between the TFs responses to the two snake species ($P=0.90$).

Moreover, to compare the accuracy of the recognition of the two snake species between adult and hatchling lizards, we compared the distribution of individual TF rates to the sympatric snake minus TF rates to the allopatric snake; there were significant differences between age classes (difference in TF rates, adults: mean$\pm$SE$=7.0\pm1.3$ TFs; hatchlings: 1.2 ± 1.4 TFs; GLM, $F_{1,40}=9.79$, $P=0.003$), but not between localities ($F_{1,40}=2.23$, $P=0.14$) and the interaction was not significant ($F_{1,40}=0.18$, $P=0.67$).

Antipredatory responses

Adult lizards of both localities clearly differed in their responses to the three different treatments (Fig. 3); adults always stayed immobile or moved slowly and calm after presentation of the swab with water, but lizards often showed quick escape responses after presentation of the snakes' scents (Highland: $\chi^2_2=20.28$, $P<0.0001$; Lowland: $\chi^2_2=17.14$, $P=0.0002$). However, comparing only the responses to the two snake species, adult lowland lizards were more likely to show escape responses from the scent of the sympatric snake species (*C. girondica*) than from scent of the allopatric snake species (*C. austriaca*) ($\chi^2_1=4.44$, $P=0.035$). In contrast, adult highland lizards showed similarly frequent escape responses from scent of either of the two snake species ($\chi^2_1=0.25$, $P=0.61$) (Fig. 3).

Naïve hatchlings of both localities also clearly differed in their responses to the three different treatments (Fig. 3); most of naïve lizards stayed immobile or moved slowly and calm after presentation of the swab with water, but naïve lizards often showed quick escape responses after presentation of the snakes' scents (Highland: $\chi^2_2=13.30$, $P=0.0013$; Lowland: $\chi^2_2=18.10$, $P=0.0001$). However, naïve lizards from both localities showed similar escape responses from scent of either of the two snake species (Highland: $\chi^2_1=0.01$, $P=0.99$; Lowland: $\chi^2_1=1.05$, $P=0.30$).

Discussion

Our results are consistent with the existence of an innate chemosensory recognition of predatory snake cues in *P. hispanicus* lizards, as both adult lizards and captive reared naïve hatchlings, which had not had any previous experience with the snake cues, had short latencies and increased TF responses, and showed active escape behavior from the scent of any of the two *Coronella* snake species in comparison with a blank control that did not elicit antipredatory behavior. Similar innate detection and responses have been found in other lizards (Van Damme et al. 1995; Mori and Hasegawa 1999; Downes and Adam 2001). This suggests that the ability for a quick detection of the predatory snake cues, and for eliciting an associated escape response, is very important for lizards of any age to decrease predation risk. For example, this ability would allow wall lizards to avoid entering dark rock crevices where these predatory snakes ambush for their lizard prey (Amo et al. 2004a, 2005). Innate responses could result from strong selection of those

individuals that were able to detect and respond adequately to the snake chemical cues avoiding being predated (Mery and Burns 2010). Alternatively, it could be possible that responses of naïve lizards, rather than having a genetic basis, might reflect maternal effects. Thus, in some viviparous lizards, the exposure of pregnant females to predator scents may alter behavior of their offspring to these same predator stimuli (Shine and Downes 1999; Bestion et al. 2014). However, this situation seems unlikely here because *P. hispanicus* lizards are oviparous and lack an intimate maternal-fetal connection.

However, responding in the same way to any predator cues may be costly because not all predators, nor all situations, entail the same level of risk, and prey animals should be able to balance the costs and benefits of their antipredatory responses (Lima and Dill 1990). In this way, some lizard species respond differentially to chemical cues of different snake species depending on the relative risk that each species poses (e.g., depending on whether the snake is a saurophagous specialist or a generalist that only occasionally prey on lizards) (Stapley 2003; Amo et al. 2004b, 2006; Webb et al. 2009) or of the degree of sympatry (i.e., risk of encountering it) of the prey species with the snake species (Downes and Adam 2001). More risky predatory species should require stronger and quicker, and even more specialized, but costly, antipredatory responses. In contrast, a generalist, less costly, antipredatory response may be enough to avoid a low-risk predator.

The above threat-sensitive flexible behavior requires prey to be able not only to detect the cues of any snake, but also be able to accurately identify the species. Indeed, our results indicated that adult *P. hispanicus* showed differential responses to the two different snake species (i.e., differential TF rates indicating that species recognition occurred). Chemical discrimination occurs even if these snakes are two very closely related species that, presumably, should have very similar chemical cues. In contrast, latency times were similar indicating that both snakes are detected equally fast (which will allow a quick preventive escape response). However, after discrimination occurs the subsequent response is modulated depending on how dangerous the stimulus is considered. Lizards would increase chemosensory investigation to know with more detail the intensity, age and location of the source of chemical stimuli considered more dangerous, or searching for visual cues that confirm the presence of the snake (Amo et al. 2004a, 2005).

Interestingly, adult lizards from the different localities show different responses to the two snake species, with higher TF responses directed to the species that is sympatric in each locality. Thus, highland adult lizards showed relatively higher TF responses to the sympatric highland snake *C. austriaca*, while lowland adult lizards showed higher

responses to the sympatric lowland species here, *C. girondica*. Similarly, in other lizard species, lizards sympatric with a snake predator exhibit more TFs toward the snake chemicals than those allopatric with the snake (Downes and Shine 1998a; Downes and Adams 2001). However, a scenario, as in our study system, where the same prey species may find a different, but very similar, snake predator in different localities within the same population has rarely been examined. Therefore, our results indicate the existence of a complex flexible antipredatory behavior in this lizard.

These results support the idea that adult *P. hispanicus* lizards may respond to predation pressure on a local scale and that, in addition to the innate response, there is a learning component dependent on previous experience with the odor cues of the snake species found in each locality. Depending on the relatively higher frequency of previous encounters with a snake species, it may be considered to entail a higher predation risk than the other species. Whereas the allopatric species, even if it has similar or shared chemical cues, may be considered of lower risk, probably because it has never been encountered before and might not be correctly identified in a first encounter. The existence of a high rate of gene flow between the two lizard localities (Gabirot et al. 2013) makes local adaptation by differential selection pressures less likely than a simply acquired learned experience of local conditions.

The lack of differences in the TF responses to the two snake species in naïve juveniles also strongly suggests that there is needed a learning component acquired through previous lifetime experiences with the snake cues. Similarly, many animals are born with a certain innate predisposition to perform a behavior which will be more or less modified by experience (Mery and Burns 2010). Alternatively, instead of learning, there might be a simple maturation effect that would occur regardless of any experience with snakes. Thus, lizards in the different localities might have different maturation effects of different innate responses, leading to the observed interpopulational differences in responses of adults. However, from a genetic point of view, the highland and lowland localities of *P. hispanicus* lizards form a single continuous population (Gabirot et al. 2013). This would, therefore, make very unlikely that different maturation effects, independently of the snake presence, occur in different areas of the same population. Alternatively, or in addition, even if the ability to discriminate snake species might be already innate in juveniles, it is likely that any snake species may represent a higher risk for small sized juvenile lizards in comparison with adults. Thus, juveniles could respond in a similar way to either of the two snake species even if they were able to recognize them. Only after lizards had grown

enough, and became less vulnerable, they might modulate their antipredatory behavior and start to show signs of recognition in their responses.

Although TF rates indicated that species discrimination occurred in both localities, there were differences in the escape responses. Thus, responses of lowland lizards were different depending on the snake species (i.e., a higher number of lowland lizards escaped from the sympatric snake cues). In contrast, highland lizards responded equally to both snake species. This result could be explained given the more restricted distribution of the highland snake (i.e., top of the mountain areas) (Galán 2014a), in contrast with the larger area occupied by the lowland snake (Galán 2014b), and the widespread continuous distribution of *P. hispanicus* lizards in both lowland and highland areas (Salvador and Carretero 2014). Thus, it is likely that highland lizards often moved onto the more extensive surrounding lowland areas and had some experience with the lowland snake too, and consequently showed similar antipredatory responses to both species. In contrast, just because of differences in surface between lowland and highland areas, even if lowland lizards moved, they only rarely would reach the restricted mountain area where the highland snake is found. In fact, that many lowland adult lizards did not respond adequately to the cues of the highland snake might suggest that the response to this species might be partially lost with lack of experience, in spite that there is still an innate generalized high response in juveniles.

We conclude that *P. hispanicus* lizards show an innate ability to detect chemical cues of predatory snakes and to respond with antipredatory behavior, but that lifetime experience through a learning component depending on local conditions (i.e., presence/absence of determined snake species) may allow a more accurate discrimination of the sympatric snake species. This ability would allow lizards to modulate their antipredatory behavior to the risk level estimated for each snake species. Nevertheless, to ensure that learning occurs in this lizard, further studies should consider maintaining in the laboratory or seminatural enclosures naïve lizards from the two localities under different conditions of exposure to the two snake chemical cues, examining later when lizards become adults how they respond to the two snake chemical cues.

Ethical standards The captures and experiments enforced all the present Spanish laws and ethical principles and were performed under license (permit number: 10/024398.9/13) from the Environmental Organism of Madrid Community ("Area de Conservación de Flora y Fauna. Dirección General del Medio Ambiente. Consejería de Medio Ambiente y Ordenación del Territorio") where they were carried out.

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Fig. 1 Mean (+ SE) (a) latency (s) and (b) number of tongue-flicks (TF) directed to swabs by adult *P. hispanicus* lizards from two localities (highland vs. lowland) within the same population in response to swabs bearing odorless water (white) or scent from two snake species: *C. austriaca* (black) or *C. girondica* (gray)
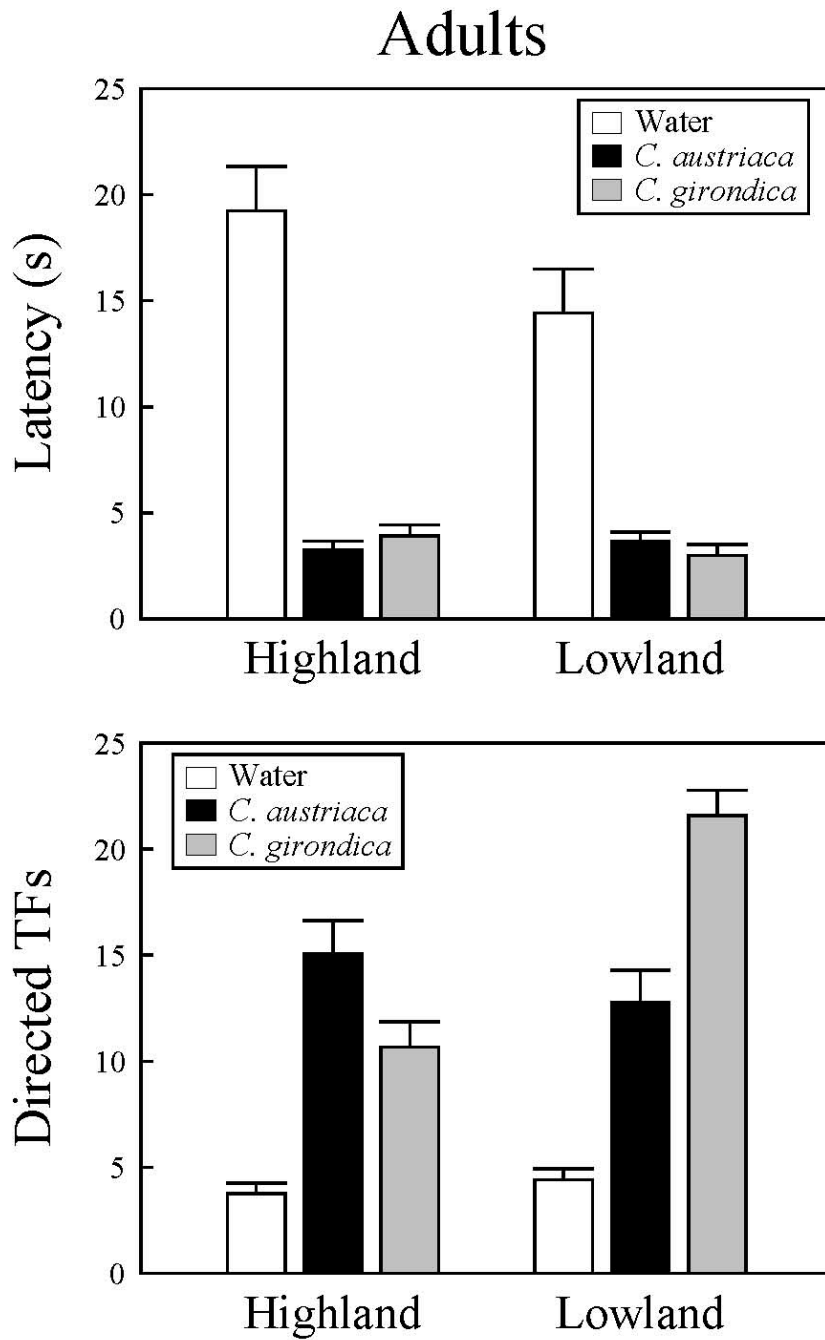
Fig. 2 Mean (+ SE) (a) latency (s) and (b) number of tongue-flicks (TF) directed to swabs by naïve hatchling *P. hispanicus* lizards from two localities (highland vs. lowland) within the same population in response to swabs bearing odorless water (white) or scent from two snake species: *C. austriaca* (black) or *C. girondica* (gray)
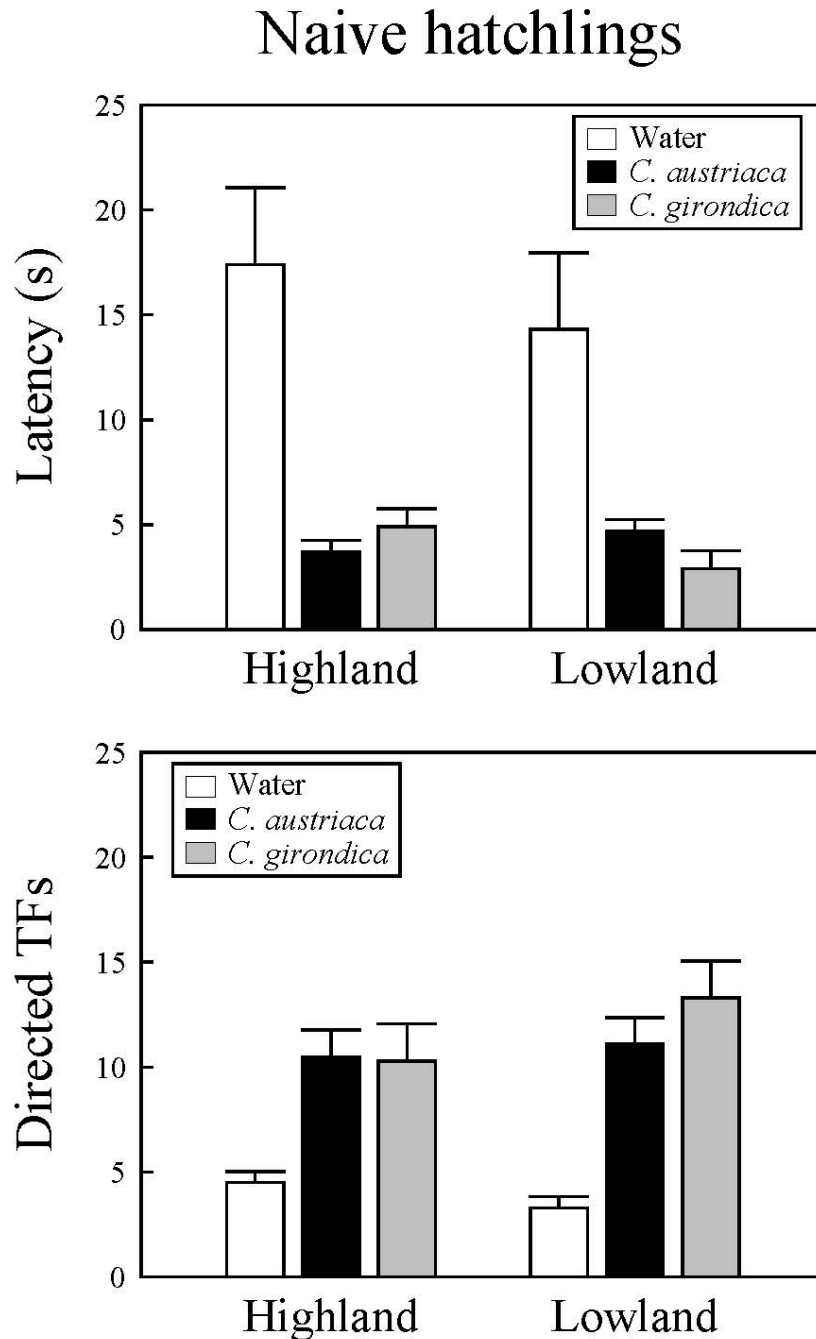
Fig. 3 Numbers of individual (a) adult and (b) naïve hatchling *P. hispanicus* lizards from two localities (highland vs. lowland) within the same population that stayed calm and/or did not move (black) vs. those that showed escape responses (gray) in response to the presentation of the swab with water (W) or scent from one of two snake species, *C. austriaca* (CA) or *C. girondica* (CG)