

CHEMICAL ASSESSMENT OF PREDATION RISK IN THE WALL LIZARD, *PODARCIS MURALIS*, IS INFLUENCED BY TIME EXPOSED TO CHEMICAL CUES OF AMBUSH SNAKES

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Lizards often respond to predator presence by increasing refuge use. However, this behaviour may expose lizards to saurophagous snakes, which inhabit the same refuges to ambush their lizard prey. Snakes, which are not always visible, deposit chemical trails that can be detected by lizards. Even though there are obvious advantages of using chemical cues, chemical detection of predators might lead to very conservative estimates of risk. This is because chemical cues might indicate that an area was risky in the recent past, but not necessarily at the current time. We examined experimentally whether wall lizards (*Podarcis muralis*) avoid using refuges that contain chemical cues of smooth snakes (*Coronella austriaca*), and whether this avoidance response is maintained long term or whether it can be modified. Results suggest that wall lizards detected the chemical cues of smooth snakes inside refuges, and, in the short term, decreased the use of predator-scented refuges and increased their escape movements. However, this avoidance response seemed to decrease in the long term. By investigating the refuge again over subsequent time periods, lizards reassessed whether the snake was actually present, modified their refuge use and decreased their avoidance response. Therefore, wall lizards seem able to assess temporal variations in predation risk by snakes inside refuges and to respond accordingly.

Key words: behaviour, chemoreception, *Coronella austriaca*, predator-prey interactions

INTRODUCTION

Predation is a major selective force. However, since animals must accomplish more in their lifetime than simply avoiding predation, natural selection favours individuals that minimise their individual risk of mortality while attending to other demands (Lima & Dill, 1990). Chemosensory cues may reliably reveal the presence of predators and they may also provide information on predator activity level and diet (Kats & Dill, 1998). Snakes deposit chemical trails that can be detected by lizards with their highly developed vomeronasal system (Cooper, 1990; Van Damme *et al.*, 1995; Downes & Shine, 1998a; Van Damme & Quick, 2001; Downes & Bauwens, 2002). Because snakes are not always visible, their chemical stimuli may be particularly important for lizards that share the same refuges (Downes & Shine, 1998a). For example, some geckos used their chemosensory ability to avoid entering rock crevices with snake scent (Downes & Shine, 1998a,b).

Prey, such as lizards, often respond to predator presence by increasing refuge use (Greene, 1988; Sih *et al.*, 1992). However, refuges may have some costs that should be minimised, such as the loss of time available for other activities, or physiological costs (Dill & Fraser, 1997; Sih, 1997; Martín & López, 1999a,b). In addition, some types of refuges may only be useful against some particular type of predators, or may expose prey to other types of predators (Sih *et al.*, 1998). For example, lizards may face saurophagous, ambush-hunt-

ing snakes that share the same refuges (Downes & Shine, 1998a). The ability to detect the chemical cues of a snake may help lizards to survive an encounter with a predator (Downes, 2002). Even though there are obvious advantages of using chemical cues, especially when other cues are unavailable, chemical detection of predators may lead to very conservative estimates of risk because they indicate that a given area was risky at a certain point in time but not necessarily a current risk (Kats & Dill, 1998; Turner & Montgomery, 2003). Thus, according to the threat sensitivity hypothesis (Helfman, 1989), natural selection should favour individuals that take action appropriate to the magnitude of threat, rather than avoiding the use of refuges in response to all kinds of predator chemical cues.

The wall lizard, *Podarcis muralis*, offers an excellent opportunity to study the patterns of avoidance of hazardous refuges. Wall lizards respond to predator presence in the open by increasing refuge use (Martín & López, 1999b). However, by doing this, they may expose themselves to increased predation risk inside refuges by ambush-hunting smooth snakes (*Coronella austriaca*). This is a lizard specialist that hunts by ambush foraging, hidden in rock crevices (Rugiero *et al.*, 1995; Galán, 1998), and has a geographic distribution and habitat preferences that overlap frequently with those of wall lizards. Previous studies have shown that *P. muralis* is able to detect and discriminate the chemical cues of smooth snakes (Amo *et al.*, 2004). In this paper, we examined experimentally whether wall lizards avoid using refuges that contain chemical cues of smooth snakes, and whether this avoidance response is maintained long term or whether it can be modified.

MATERIALS AND METHODS

During May 2001, we captured adult *P. muralis* by noosing (9 males and 10 females; snout-vent length, SVL, \pm SE = 66 ± 2 mm) at a rock wall (120 m long, 5 m high) near Cercedilla (Madrid Province, Spain). We also captured in the same wall two adult smooth snakes to be used as source of scent of potential predators. Lizards were individually housed at "El Ventorrillo" Field Station 5 km from the capture site, in outdoor 60×40 cm PVC terraria containing sand substratum and rocks for cover. Every day, they were fed mealworm larvae (*Tenebrio molitor*) dusted with multivitamin powder for reptiles, and water was provided *ad libitum*. The photoperiod and ambient temperature was that of the surrounding region. Lizards were held in captivity at least one month before testing to allow acclimation to laboratory conditions. To prevent lizards from having contact with the scent stimuli before they were tested, the snakes were housed separately in glass terraria ($60 \times 30 \times 20$ cm) with sawdust on the substrate to obtain their scent. Due to its absorbent properties, the odourless sawdust is an excellent method for obtaining snake scent without disturbing the animal. All the animals were healthy during the trials and were returned to their exact capture sites at the end of experiments. The experiment was performed under licence from the "Madrid Environmental Agency" ("Consejería del Medio Ambiente de la Comunidad de Madrid").

To compare the behaviour of lizards when they found a potentially unsafe refuge (i.e. with snake chemical cues) or an unfamiliar but predator-free refuge, we used two terraria ($60 \times 40 \times 30$ cm). Terraria were divided into two halves, and had two refuges placed symmetrically on either side, one in front of the other, with a distance of 15 cm between them. The refuges were flat rocks (10×7 cm) placed 2 cm above the substrate, allowing lizards to hide under them. In the 'predator' treatment, the terrarium had a refuge containing chemical cues of a smooth snake and an odourless refuge. In the 'control' treatment both refuges were odourless. To add the predator scent to the refuge, we used sawdust that had been in the terrarium of the snakes for at least three days, moistened with deionized water. In the odourless refuges, we applied some deionized water to a similar quantity of odourless sawdust. In both cases, sawdust was placed on the ground, inside the refuge. We did not include a pungent control (e.g. perfume) in the experimental design because results of previous experiments showed that *P. muralis* cannot distinguish it from water and from other biologically irrelevant odours, but can distinguish it from snake scent (Amo *et al.*, 2004). Moreover, *P. muralis* does not modify the use of refuges containing a pungent odour, compared to an odorless control (Amo, López & Martín, unpublished data). Every lizard was tested in each treatment once in a randomised block design, and order of trials was counterbalanced. One trial was conducted per day for each

animal. Trials were conducted under outdoor conditions during July 2001 between 1200-1700 hrs when lizards were fully active. Lizards were allowed to bask in their home terraria for at least two hours before trials. After each trial the cages and the refuges were cleaned thoroughly with water and detergent for 20 min and dried at the outdoor temperature. We used new stimuli in each trial to avoid the mixture of odours.

Experiments were recorded on videotape (Hi-8 format, 25 frames s⁻¹) using a Sony CCD-TR810E video camera aligned perpendicularly over the terrarium. Lizards were filmed as they moved spontaneously along the terrarium during 25 min. The experimenter was not present during filming to avoid disturbing lizards. After this, we noted the location of each lizard in the terrarium every 30 min over the subsequent five hours. Later, we analysed the tapes and noted lizard behaviour in the experimental half of the terrarium (i.e. the half that contained the snake-scented refuge in the 'predator' treatment, or one of the odourless refuges in the 'control' treatment). We noted the total time spent in the experimental area, time spent in movement, motionless, or standing up trying to escape (i.e. the lizard stands in a upright position against the wall of the terrarium and performs scratching movements with the forelegs), and total time spent inside each refuge. To determine possible changes through time in the responses, we divided each 25 min period into five consecutive periods of 5 min each. We chose this interval of time because previous results of Thoen *et al.* (1986) showed that the responses of common lizards, *Lacerta vivipara*, to the scent of smooth snakes was different in the first 5 min of the trial than afterwards.

We used two-way repeated measures analysis of variance (ANOVA) to test for differences between treatments ('control' vs. 'predator') and among the five time sequences of each individual (within-subjects factor). Data of total time spent in the experimental half of the terrarium were log-transformed, which successfully normalize the data. We used the time spent in movement, motionless, and in standing up acts in the experimental area, and the time spent in each refuge, in relation to the total time spent in the corresponding area. Angular transformations of all percentages were made to normalize the data.

Differences in the location of lizards during the subsequent five hours between treatments were analysed with one way ANOVA. We calculated the number of times that lizards were observed outside of refuges, and the number of times the lizards were seen hidden in the experimental refuge in relation to the number of times that the lizards were inside any refuge. Data were log- and arcsin-transformed, respectively, to normalize data. Tests of homogeneity of variances (Levene's test) showed that in all cases variances were not significantly heterogeneous after transformation. Pairwise comparisons of means were planned using the Tukey's honestly significant difference (HSD) test (Sokal & Rohlf, 1995).

RESULTS

Total time that lizards spent in the experimental area did not differ significantly between treatments (repeated measures two way ANOVA; $F_{1,18}=0.52$, $P=0.48$), although there were significant differences among sequences ($F_{4,72}=2.47$, $P<0.05$). The interaction was not significant ($F_{4,72}=1.12$, $P=0.35$). Lizards decreased the time that they spent in the experimental half of the terrarium over time, although there were only significant post-hoc differences between the first and the third sequence (Tukey's test, $P=0.04$).

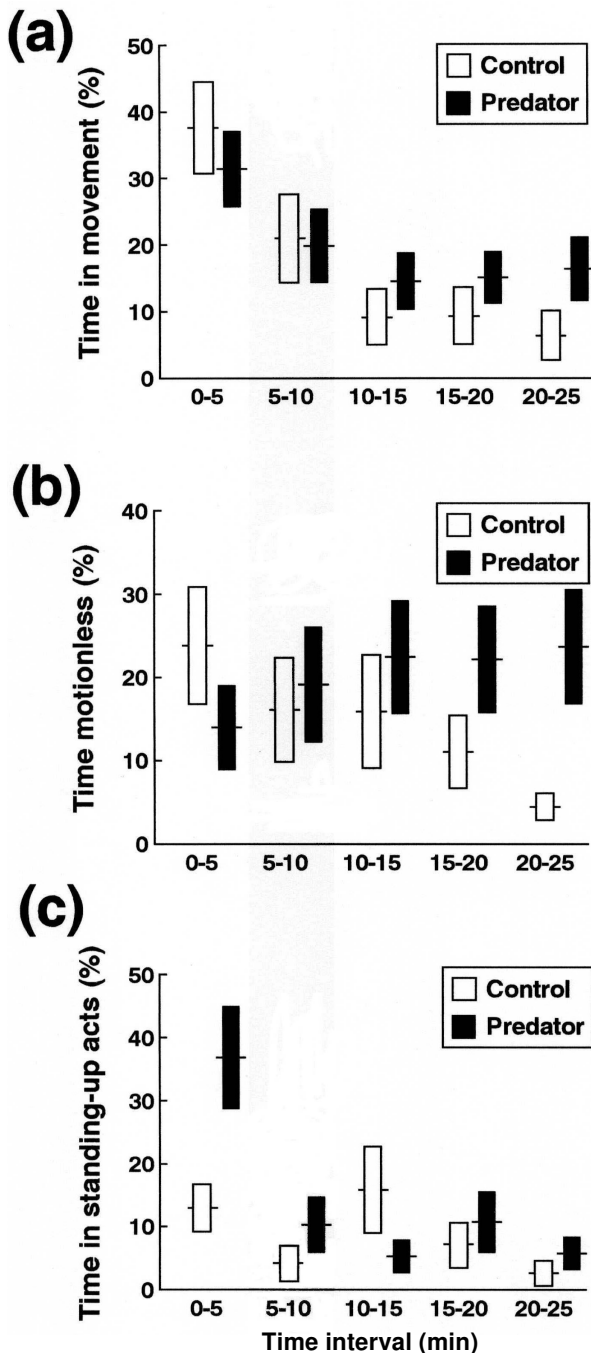


FIG. 1. Percent time (mean \pm SE) spent (a) in movement, (b) motionless, and (c) in standing up acts, in relation to the total time spent in the experimental area, in the 'control' (open boxes) and 'predator' (black boxes) treatments.

Time spent moving did not differ significantly between treatments ($F_{1,18}=0.10$, $P=0.75$), but there were significant differences among sequences ($F_{4,72}=11.92$, $P<0.0001$) (Fig. 1a). The interaction was not significant ($F_{4,72}=1.50$, $P=0.21$). Lizards decreased their movement rate across time, especially after the first 5 min. Thus, there were significant differences between the first sequence and the subsequent four ($P<0.001$ in all cases), but not between the rest of sequences ($P>0.21$ in all cases).

Time spent motionless did not differ significantly either between treatments ($F_{1,18}=0.58$, $P=0.46$), or among sequences ($F_{4,72}=0.31$, $P=0.87$), but the interaction was significant ($F_{4,72}=2.51$, $P<0.05$) (Fig. 1b). Lizards increased the time spent motionless in the 'predator' treatment whereas they decreased it in the 'control' treatment in the course of time, although post hoc comparisons did not show significant differences (Tukey's test, $P>0.10$ in all cases).

Duration of standing up acts did not differ significantly between treatments (repeated measures two way ANOVA; $F_{1,18}=2.14$, $P=0.16$), although there were significant differences among sequences ($F_{4,72}=6.07$, $P=0.0003$) and the interaction was significant ($F_{4,72}=4.34$, $P=0.003$) (Fig. 1c). During the first 5 min the time spent by lizards in standing up acts was significantly higher in the 'predator' treatment than in the 'control' one ($P=0.006$). Whereas, later, there were no significant differences either between sequences, when considering each treatment alone, or between treatments in each sequence ($P>0.57$ in all cases).

Time spent inside the refuge did not differ significantly either between treatments (repeated measures two way ANOVA; $F_{1,18}=0.001$, $P=0.98$) or among sequences ($F_{4,72}=2.06$, $P=0.09$), but the interaction was significant ($F_{4,72}=3.94$, $P=0.006$) (Fig. 2). During the first 5 min there were no significant differences between treatments in time spent in refuges ($P=0.30$). However, in the course of time lizards increased the time they

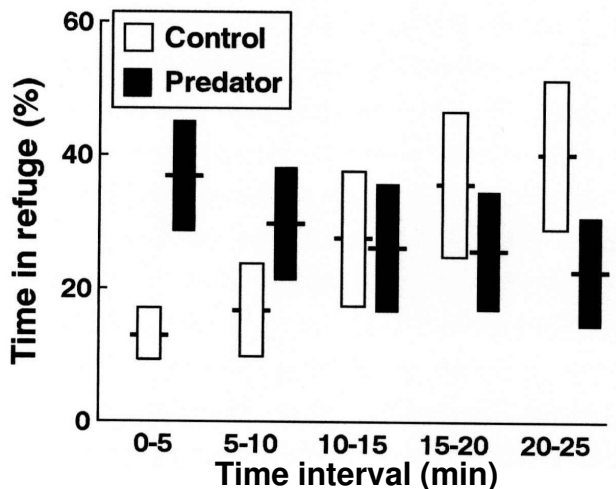


FIG. 2. Percent time (mean \pm SE) spent inside the experimental refuge in relation to the total time spent in the experimental area, in the 'control' (open boxes) and 'predator' (black boxes) treatments.

spent in the control refuge (differences between the first and the other five sequences, $P=0.02$), but they did not increase it in the refuge containing chemical signals of a snake ($P>0.97$ in all cases). Nevertheless, in the long term (i.e. in the subsequent five hours), the number of times that lizards were observed out of a refuge did not significantly differ between treatments (control: 3 ± 1 times; predator: 4 ± 1 times; one way ANOVA, $F_{1,18}=1.02$, $P=0.33$). Also, there was no significant difference between treatments in the use of the experimental refuge (number of times in the experimental refuge/number of times in any of the two refuges, control: $56\pm 7\%$; predator: $51\pm 6\%$; $F_{1,18}=1.26$, $P=0.28$). Thus, in the long term lizards did not avoid to hide in the refuge soiled with snake scent.

DISCUSSION

Results of this study suggest that wall lizards were able to detect the chemical cues of smooth snakes, and to use them in the short term to assess the potential risk of predation inside a refuge, but that after some time lizards were able to reassess whether the snake was actually present and modified their response. To avoid the risk of predation by ambush snakes, lizards initially modified their behaviour and their use of potentially hazardous refuges. During the first few minutes, lizards spent the same time in both types of refuges. However, later on, lizards decreased their use of the predator-scented refuge, whereas they increased the use of the odourless refuge. This could be explained if lizards approached refuges and spent some time investigating the source of the odour, but after discriminating the snake scent, they decided to avoid using the unsafe refuge. Our results agree with previous studies that have shown that other lizard species avoid using retreats that were soiled with snake's scent (Downes & Shine, 1998a; Downes & Bauwens, 2002).

Lizards also modified their locomotor patterns in the predator treatment. Previous studies have shown that prey exposed to a potential predator odour often show behavioural changes such as reduced activity (Van Damme *et al.*, 1990), increased refuge use (Kiesecker *et al.*, 1996) or reduced use of the potential risky area (Downes & Shine, 1998a). Our results suggest that lizards increased their escape behaviour (i.e. standing up acts) when they found chemical cues of a snake inside a refuge. *Podarcis sicula* lizards also increase the time spent in standing up acts when they found chemical cues of a snake on the ground (Downes & Bauwens, 2002). Also, wall lizards showed a similar behaviour when they found chemical cues of a snake on the open ground of a terrarium (Amo, López & Martín, unpublished data). These results suggest that lizards perceived an increase in the risk of remaining near a potentially unsafe area and that they responded by trying to escape from the terrarium. A similar response to predator chemicals was observed in larval *Ambystoma* salamanders, which decreased movement only in the absence of a refuge; otherwise, increased movement in an effort to reach a

refuge (Sih & Kats, 1991). Also, increased movement in larval toads in response to an alarm substance may represent refuge-seeking behaviour (Hews, 1988). Wall lizards also tended to maintain the time spent motionless in the risky area while they decreased it in the control area across the time. By standing still, lizards may try to visually detect the snake in a potentially unsafe area (Avery, 1991, 1993; McAdam & Kramer, 1998).

However, this avoidance response seemed to decrease in the long term. Chemical detection of a snake may indicate that a refuge was risky at a certain point in time but it does not necessarily indicate a current risk. Thus, chemical assessment might lead to excessively conservative estimates of risk if prey continue avoiding the refuge despite the absence of the predator. By investigating the refuge again over the subsequent minutes, lizards may assess the absence of the snake. Thus, wall lizards responded to the temporal decrease in the risk of predation inside the refuge by decreasing their avoidance response and increasing the use of such refuge. Similarly, the avoidance response to predator chemical cues diminished with time in *Physa* snails (Turner & Montgomery, 2003) and *Plethodon cinereus* salamanders (Sullivan *et al.*, 2002). In contrast, garden skinks, *Lampropholis guichenoti*, avoided the use of predator-scented areas during six months (Downes, 2001). However, in this case, the predator odour was replaced weekly. Thus, skinks probably continued avoiding the risky area because they could perceive a fresh stimulus every week. An explanation for the lack of avoidance behaviour across the time may be that wall lizards are able to assess the age of the chemical cues they found. However, our experiment did not test this effect and, thus, further research is needed to examine whether wall lizards have this ability.

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