

HELLENIC ZOOLOGICAL SOCIETY

Lacertids of the Mediterranean region

A Biological Approach

R E P R I N T

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Chapter 14

Chemoreception in the Lacertidae: exploration and conspecific discrimination in the Spanish wall lizard, *Podarcis hispanica*

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Introduction

Growing evidence points to the chemical senses as playing a crucial role in lizard and snake behavior. Conspecific and sex recognition, territory marking, aggregation, foraging, exploration, and predator detection are related to some extent to chemoreception in snakes and in many lizards (see Burghardt, 1970, 1980; Simon, 1983; Mason, 1992, for reviews). Unfortunately, lizards have received far less attention than snakes on chemoreception studies. Yet, three lines of evidence suggest pheromonal communication and use of chemical information in lizards. First, many species of lizards possess secretory structures, such as femoral and urodaeal glands (Simon, 1983), that synthesize

socially relevant chemicals (Cooper *et al.*, 1986; Alberts, 1989). Second, most lizards have three chemosensory systems: vomeronasal (i.e., the tongue-Jacobson's organ system); olfactory, and gustatory. Although lizards are generally assumed to rely chiefly on vomerolfaction for their chemically guided behaviors, taste buds are present widely among lizards, including lacertids (Schwenk, 1985). However, the role of taste in chemosensory discrimination remains unclear. Olfaction, on the other hand, may be important to some squamates, but its roles appear to differ from those of vomerolfaction (Mason, 1992). In fact, Cowles and Phelan (1958) postulated that a role of the olfactory epithelium could be the activation of the vomeronasal system for the detection of low volatility chemicals. This hypothesis has received some empirical support in lizards (Duvall, 1981; Simon and Moakley, 1985). Third, a uniquely specialized behavior is associated with the chemosensory function of the vomeronasal organ (VNO): the extrusion of the tongue or tongue-flick. Many lizards regularly tongue-flick while moving about their environment, either waving the tongue in the air, or touching the substrate or an object with it (Bissinger and Simon, 1979; Gove, 1979; Duvall, 1982). During tongue-flicking the tongue picks up chemicals and transfers them from the external environment to the VNO (Graves and Halpern, 1989; 1990), though the detailed transfer mechanism has not been fully elucidated (reviewed in Young, 1990). The ease with which tongue-flicks can be observed and recorded makes tongue-flicking a reliable and widely used behavioral index of VNO stimulation.

The purpose of the present study is twofold. First, we present a brief review of the literature dealing with chemoreception in the Lacertidae. Second, we provide preliminary results on chemosensory abilities of a lacertid, the Spanish wall lizard, *Podarcis hispanica*.

Previous work on chemoreception in the Lacertidae

Lacertidae, one of the largest families of lizards, is traditionally included in the autarchoglossan division of lacertilian phylogeny (Camp, 1923; Estes *et al.*, 1988). Associated with this taxonomic view, lacertids have been implicitly assumed to rely strongly on their chemical senses. Morphological and behavioral data also support the chemosensory abilities of lacertids: their vomeronasal system is generally well developed (Parsons, 1970; Northcutt, 1978), the tongue is long and forked, and functional femoral glands are usually present as well (Arnold, 1989). Nevertheless, experimental evidence supporting a role of the chemical senses in lacertid behavior is scant (Table 1). Using the method of the odor impregnated cotton swab, Cooper (1990a; 1991) reported on the ability of some lacertids for prey odor discrimination and poststrike elevation in tongue-flick rate. A different set of studies (Thoen *et al.*, 1986; Van Damme *et al.*, 1990) indicated that *Lacerta vivipara* is capable of recognizing

the odor of its snake predator, the common viper, *Vipera berus*, although a faulty experimental design might undermine this conclusion (Cooper, 1990b).

Table 1. Previous work on chemoreception in lacertid lizards.

Reference	Species	Discrimination	Method	Result
Bauwens <i>et al.</i> (1987)	<i>L. vivipara</i>	Sex	Odorless lizards	-
Cooper (1990a)	<i>P. hispanica</i>	Prey	Swab	+
Cooper (1991)	<i>P. muralis</i>	Prey	Swab	+
Mou (1989)	<i>P. muralis</i>	Sex/species	?	-
Thoen <i>et al.</i> (1986)	<i>L. vivipara</i>	Predator	Soiled substrate	+
Vanderstighlen (1987)	<i>L. vivipara</i>	Predator	Soiled substrate	+

It is noteworthy that the few analyses performed on the potential role of lacertid chemoreception in social contexts have yielded negative results. Bauwens *et al.* (1987) reported that male *Lacerta vivipara* courted both males artificially painted so as to resemble the female color pattern, and females treated to eliminate chemical secretions. Based on this evidence, they concluded that chemical cues alone are not sufficient for sex recognition. In a different study, Mou (1989) could not find evidence for chemosensory sex or species discrimination in *Podarcis muralis*. In the present work we analyze the role of chemoreception in exploratory behavior and provide the first evidence of chemosensory conspecific discrimination in a lacertid.

The Spanish wall lizard

The Spanish wall lizard, *Podarcis hispanica*, is a small diurnal warm-temperate lacertid found mainly in rocky habitats in the Iberian Peninsula (Arnold and Burton, 1982). This lizard feeds on arthropods and has been described as a food generalist (Pérez-Mellado, 1983). In the only study dealing with chemoreception in this species, Cooper (1990a) determined that *P. hispanica* is capable of discriminating prey from non-prey odor impregnated cotton swabs. Our own observations indicate that *P. hispanica* displays elevated tongue-flick rates when moved to a new environment, in feeding, and in social contexts. These behavioral correlates of a use of chemoreception prompted us to carry out the present study.

Materials and Methods

Animals and their maintenance

The lizards used in these experiments were wild-caught by noosing in Valencia in summer-fall 1989 (Experiment 1) and in Summer 1991 (Experiment 2). Experimental subjects were 15 male and 6 female *Podarcis hispanica*. Sex was determined by the presence of swellings at the base of the tail, reflecting the presence of hemipenes in males, head and body proportions, and sexually dimorphic features of body coloration and design (Arnold and Burton, 1982). Reproduction in *P. hispanica* lasts from March to July (Pérez-Mellado, 1982). Thus, all the subjects were non-reproductive when tested. Snout-vent length (mean \pm 1 SE) was 52.6 \pm 0.9 mm in males and 48.3 \pm 1.5 mm in females. All the experimental lizards were housed individually in glass or Plexiglas cages (25 x 30 x 50 cm) for at least three days prior to the beginning of testing. Each cage had a soil substrate, a rock for shelter, and a water dish. Cages were covered with softboard screens on three sides to provide visual isolation from lizards in neighboring cages. A 40-W bulb suspended ca. 20 cm above the substrate provided light and heat 14 h daily. Large windows offered a superimposed natural photoperiod. The lizards were fed mealworms (*Tenebrio molitor* larvae) several times a week. Food items were often dusted with a commercial vitamin and mineral supplement before being offered to the lizards.

General testing procedures

All testing took place between the second and fifth hour of the photophase. Room temperature at the time of testing ranged 22-29 °C, although temperature ranges were narrower for each individual lizard; individual temperature range was 1.4 \pm 0.21 °C (mean \pm 1 SE), with a maximum individual range of 2.5 °C. Preliminary observations revealed a strong observer effect (Sugerman and Hacker, 1980), consequently, we made all observations from behind a blind. All recordings were made by the same observer (AG) with the aid of a portable microcomputer equipped with event recording software (slightly modified after Unwin and Martin, 1987). After the experiments, lizards were released at their respective capture sites.

Experiment 1

The purpose of this experiment was to assess the role of chemoreception in the exploratory behavior of male *Podarcis hispanica*. A second goal was to find out if long-term habituation to a novel cage took place.

Materials and methods

Each lizard's behavior was observed in three conditions daily. First, the lizard was observed while undisturbed in its home cage for 5 min (home cage condition). The lizard was then either rapidly picked up and returned to its home cage to assess possible restraint effects (handling control), or moved to an adjacent unoccupied cage (novel cage condition) visually equivalent to the home habitat. Observations in the handling control and novel cage conditions lasted for 15 min each, and their order was alternated on successive days. The novel cage was the same for all the lizards, and no effort was made to neutralize potential chemical cues left by previously tested lizards. Nine male lizards were tested in all three conditions for 13 consecutive days. The following dependent variables were recorded:

1. POSTURE CHANGE (PC): Adjustments in body posture not associated with locomotion, such as limb or head movements.
2. SITE CHANGE (SC): Lizard walks through the cage.
3. TONGUE-FLICK (TF): The animal extrudes and quickly retracts its tongue which may or not touch the substrate (Fig. 1). Tongue-flicks displayed when the lizard was attempting to climb the cage walls were not recorded.
4. LIP-LICKING (LL): The tongue is extruded and wrapped around the snout before being retracted. Although each tongue extrusion was recorded separately, the behavior usually occurs in bouts.



Fig. 1. Male *Podarcis hispanica* tongue-flicking at the substrate.

Statistical analysis

A natural log (+ 1) transformation was performed on the raw data prior to analysis. Transformed scores for each of the dependent variables were analyzed with a 3 (condition) x 13 (days) within-subjects design using procedure MANOVA available in SPSS/PC+. This procedure yields both multivariate and univariate results. Whenever possible, a multivariate approach using Pillai's test statistic was adopted in order to eliminate sphericity problems associated with repeated measures ANOVA (Hand and Taylor, 1987). Probability level for rejection of the null hypothesis was set at $p < 0.05$ for all tests.

Results

MANOVA procedures revealed a significant effect of testing condition on all the behaviors (Pillai's trace statistic = 0.95, $p < 0.004$). Additionally, a multiple comparisons test showed that tongue-flick, posture change, and site change scores were significantly higher in the novel cage than in the handling control condition ($p < 0.002$; Table 2; Fig. 2). This relationship was reversed for lip-licking scores, which were higher in the handling control than in the novel cage condition ($p = 0.012$). Of all the behaviors, only TF showed a significant effect of testing day ($F = 2.55$, $p = 0.006$) and condition by day interaction ($F = 1.90$, $p = 0.01$). All other terms in the analysis were insignificant.

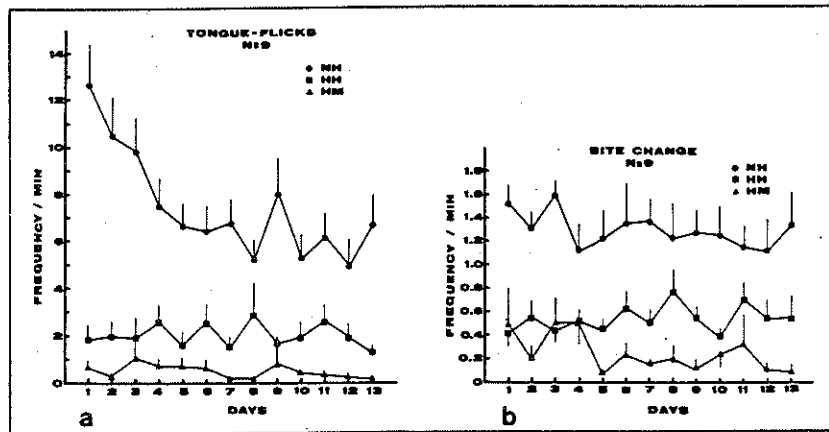


Fig. 2. a) Mean (± 1 SE) tongue-flick rate of male *Podarcis hispanica* in 13 consecutive days in the three treatment conditions. b) Mean (± 1 SE) site change rate of male *P. hispanica* in 13 consecutive days in the three treatment conditions.

Tongue-flick scores in the novel cage condition decreased over the 13 daily tests (Fig. 2a), presumably as a result of habituation. This was confirmed by Page's L test ($L = 6415$, $p < 0.001$), a non-parametric test for monotonic trends of repeated measures data (Meddis, 1984).

Table 2. Overall response of the lizards in Experiment 1. The data shown are rates (frequency/min) of behaviors for which a day effect was not significant. The values are means (± 1 SE) for nine individuals over 13 days. Abbreviations as in text.

Treatment	Behavior		
	LL	SC	PC
Home cage	0.17 \pm 0.11	0.25 \pm 0.04	0.71 \pm 0.07
Handling control	0.87 \pm 0.07	0.54 \pm 0.03	1.15 \pm 0.06
Novel cage	0.55 \pm 0.04	1.29 \pm 0.04	2.07 \pm 0.06

In the novel cage condition, lizards spent most of the time walking about the cage while tongue-flicking frequently. These "bouts of exploration" were interrupted by periods of basking under the light bulb. Occasionally, foot-shakes (see Experiment 2) were seen, but not recorded. Exploration (i.e., SC plus TF) in the handling condition was restricted to the first few minutes of observation, after which the lizards would usually stop to bask under the bulb. It was during these periods devoted to basking that LL was seen most frequently. Across days, the lizards' behavior in the novel cage condition progressively approached that in the handling control condition, the animals spending most of the trial basking under the bulb. The lizards made few movements, if any, in the control condition.

Experiment 2

In this experiment we aimed to find out whether *Podarcis hispanica* can use chemosensory information to discriminate between conspecific and heterospecific lizards. We further tested whether *P. hispanica* is capable of discriminating between sexes by chemical cues alone. One of the most widely used methods for obtaining evidence of chemical discrimination in reptiles is

the odor-impregnated cotton swab method. Using this technique, Cooper (1990a; 1991) succeeded in demonstrating chemosensory prey discrimination in our experimental species and a closely related one (*P. muralis*). However, this technique did not, in our hands, yield consistent results when applied to other aspects of chemoreception. Fortunately, alternative procedures for obtaining evidence of chemosensory discrimination are available in the literature (e.g., DeFazio *et al.*, 1977). In particular, although much more time consuming, experiments in which the lizards are tested in cages with soiled substrates seemed to us much more useful.

Materials and methods

We tested each lizard (six males and six females) in a completely counterbalanced order in six odor treatments: (1) water as an odor control, (2) cologne (Don Algodóntm diluted 1:5 in water) as a pungency control, (3) female conspecific odor, (4) male conspecific odor, (5) female heterospecific odor, and (6) male heterospecific odor. We conducted the trials in test cages similar to the holding cages, but the floor was lined with an absorbent paper sheet that was replaced after each trial. To reduce habituation to the test procedure we tested each lizard only once a day.

Conspecific odor "donors" were several non-experimental lizards grouped by sex and maintained in conditions similar to those of the test subjects. The long-tailed lizard, *Psammodromus algirus*, was the heterospecific odor donor species. This lacertid occurs in sympatry with *Podarcis hispanica* over its whole distribution range; the two species have partially overlapping habitats and are often syntopic. The five long-tailed lizards, three males and two females, used in this study were captured in Riópar (Albacete, Spain) and Valencia in Summer 1991. Sex was determined as in *P. hispanica* (see **Animals and their maintenance**) and the lizards were kept in two large cages grouped according to capture site.

For the lizard odor trials, clean test cages were chemically marked through occupation by intact lizards prior to the introduction of the test subject. Two lizards of the same sex and species (donors) were placed in one of the test cages in the evening preceding the day of testing and were left there overnight. As the donors moved about the cage, we assume that the paper substrate was passively scent marked through skin, cloacal, or femoral gland secretions (Simon, 1983). The donor lizards were returned to their holding cages 10 min before the trial. Prior to each trial, we removed any fecal pellets and other debris to prevent discrimination by visual cues (Duvall *et al.*, 1987), and sprayed water on the marked paper substrate. In the control trials, water or cologne was sprayed on a clean paper substrate 10 min before testing.

In each test, the experimenter picked up the subject gently from its holding cage and placed it in the middle of the test cage. We observed each lizard's behavior continuously for 15 min, starting as soon as the lizard was introduced into the test cage. In addition to (1) tongue-flick and (2) lip-licking (see **Experiment 1**) we recorded the following behavior patterns:

3. FOOT-SHAKE (FS): Lizard raises and lowers one or both forelegs several times in alternate sequence.

4. WALK (W): The animal moves through the cage. We recorded presence-absence of walking at 1-min intervals during the trial (i.e. one-zero sampling).

5. ESCAPE-ATTEMPT (EA): Lizard presses its snout against the walls of the cage, making digging motions with its forelegs, or tries to climb up the walls.

6. BASKING (B): Lizard remains motionless with legs and rib cage spread laterally and head lying on the substrate.

After each trial, the test subject was returned to its home cage, the paper sheet replaced, and the floor and walls of the test cage cleaned with alcohol-impregnated paper towels.

Statistical analysis

Statistical analyses were run using SPSS/PC+. Dependent variables were analyzed by a mixed randomized block repeated measures ANOVA design, nested by sex and classified by individual, odor treatment and trial order. Possible temperature effects in tongue-flick rate (Cooper and Vitt, 1986b; Van Damme *et al.*, 1990) were taken into account statistically by using temperature during the trial as a covariate in the ANOVA design. Since sex effect was insignificant we pooled male and female results for the analysis. We tested dependent variables for normality by Kolmogorov-Smirnov's test and for homogeneity of variance using Hartley's test. When conditions for application of parametric ANOVA were not met, we analyzed data using Friedman's two-way ANOVA for a single-factor experiment with repeated measures. Non-parametric multiple comparisons were made after Zar (1984). Concordance among blocks was tested by Kendall's coefficient of concordance (Meddis, 1984). Probability level for rejection of the null hypothesis was set at $p < 0.05$ for all tests.

Results

Tongue-flick was the behavior pattern most frequently observed. Odor condition effect on TF rate was highly significant (ANOVA, $p < 0.001$). Mean number of tongue-flicks was greatest in response to the two conspecific stimuli and lowest for the water control (Fig. 3a, see Table 3 for detailed multiple comparisons results). Foot-shake scores were significantly different among treatments in males (Friedman test, $\chi^2 = 14.55$, d.f. = 5, $p = 0.062$) but not in

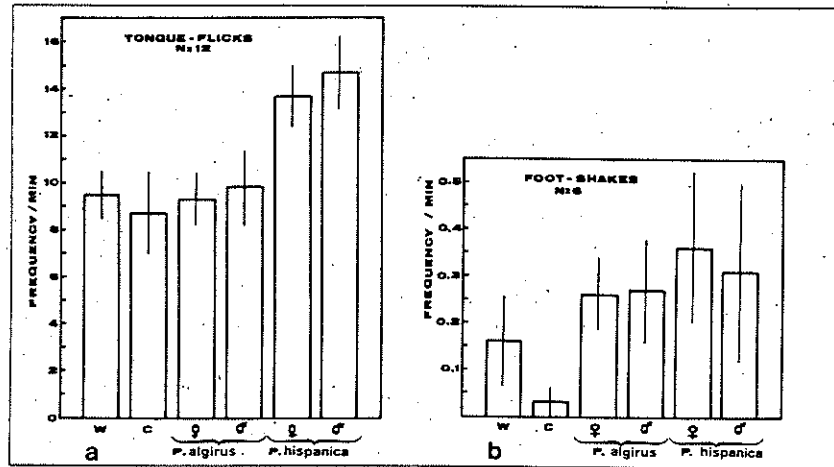


Fig. 3 a) Mean (± 1 SE) tongue-flick rate of *Podarcis hispanica* in six odor treatments.
b) Mean (± 1 SE) foot-shake rate of male *P. hispanica* in six odor treatments.

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Table 3. Overall response of the lizards in Experiment 2. Mean values (± 1 SE) after pooling male and female data. Where multiple comparisons have been conducted, columns with different superscripts are significantly different. Significance of the treatment effect is indicated in the right column (ANOVA, d.f. 5, 66). W, water; C, cologne; FN, female heterospecific; MN, male heterospecific; FC, female conspecific; MC, male conspecific. For abbreviations of behavior patterns see text.

Behavior	Treatment						Signif.
	W	C	FN	MN	FC	MC	
W	10.8 (0.8)	9.6 (1.6)	11.3 (0.8)	10.8 (1.1)	10.8 (1.2)	12.3 (0.9)	n.s.
EA	7.2 (1.7)	7.8 (2.3)	6.5 (1.6)	6.8 (1.9)	7.3 (2.1)	9.8 (2.8)	n.s.
FS	3.9 (1.1)	2.1 (1.0)	4.8 (1.0)	4.8 (0.9)	5.1 (1.1)	5.3 (1.5)	n.s.
TF	142.3 ^{ab} (15.0)	130.9 ^b (26.1)	139.6 ^b (16.6)	147.5 ^b (23.2)	205.3 ^{3c} (25.4)	220.6 ^c (29.9)	< 0.001
LL	6.0 (1.5)	5.3 (2.3)	7.1 (2.3)	3.5 (1.5)	2.1 (1.1)	2.1 (1.4)	n.s.

females ($\chi^2 = 2.67$, d.f. 5, $p = 0.75$). Males exhibited higher mean rates of FS in the presence of other lizards' chemicals than in the two controls (Fig. 3b). Odor treatment effect was not significant for the variables walk, escape-attempt and lip-licking. Order effect was insignificant for all behaviors. Temperature differences among treatments were not significant (ANOVA, $F = 0.673$, d.f. 5, 55, $p = 0.919$), but temperature effects were significant for all the behaviors excepting EA (ANOVA, $p < 0.02$).

For most of the duration of the trials, the lizards simply walked while tongue-flicking the marked substrate and the walls of the test cage. Remarkably, the TF rate in the test cage soiled by conspecifics was similar to that observed in Experiment 1 during the first few days of novel cage condition (see Figs. 2a and 3a). In most tongue-flicks the tongue actually touched the substrate or the walls, but in a few of them the tongue was simply waved in the air. In the two conspecific conditions, TF in which the snout was close to the substrate and the tongue was only slightly extruded from the mouth were very frequent, but we did not record them as different from the normal tongue-flicks. Furthermore, the subjects often stopped walking and directed multiple TF at particular spots in the paper substrate. In all conditions, the subjects also stopped occasionally to bask under the bulb. Foot-shake bouts were most often seen immediately preceding basking. As we recorded basking only in nine lizards, this behavior was not included in the statistical analyses. Lip-licking bouts took place mostly while the animals were basking.

There were considerable individual differences in all the behavioral responses. The rank orders in number of tongue-flicks contained a highly significant individual component (Kendall's coefficient of concordance, $H = 0.41$, $p = 0.0002$).

Discussion

The results of Experiment 1 demonstrate that *Podarcis hispanica* will readily explore a novel environment. Such exploratory behavior involves enhanced tongue-flick rates and movement through the environment. These results agree with those obtained in iguanians (DeFazio *et al.*, 1977; Greenberg, 1985; Burghardt *et al.*, 1986; Krekorian, 1989) and scincids (Graves and Halpern, 1990). Our results further reveal a decline in tongue-flick scores over successive tests which is presumed to result from habituation. The results are somewhat confounded due to the accumulation of chemical cues from the test subject as well as other experimental animals in the novel cage condition. However, the changing chemical environment in this experiment should, if anything, delay habituation of tongue-flicking, thus making our results even stronger.

Until recently, support for a role of habituation in the exploratory behavior of reptiles was limited. Graves and Halpern (1990) found that the exploratory response (including tongue-flick rate) of the ocellated skink, *Chalcides ocellatus*, to a new cage habituates over a 10-min period. Similarly, Chiszar *et al.* (1976) reported short-term habituation of tongue-flicking in plains garter snakes (*Thamnophis radix*) in open field trials, but found no evidence of habituation over days (long-term habituation). Although these results may suggest that reptiles are not capable of remembering over a 24-h period, the results of Burghardt *et al.* (1986) with *Iguana iguana* as well as our own with *P. hispanica* convincingly demonstrate long-term habituation of the exploratory response to novel environments in lizards.

Results of Experiment 2 suggest that adult *Podarcis hispanica* are capable of detecting and discriminating conspecific from non-conspecific chemicals. This conclusion is supported by high rates of tongue-flicking in cages soiled by conspecifics compared to those soiled by syntopic heterospecifics. The tongue-flick response was highly variable among individuals, but consistent across experimental treatments. This variability parallels that detected by Van Damme *et al.* (1990) and can be attributed to individual differences in general reactivity, or temperature effects. Failure to find conspecific discrimination in a previous study with the congeneric *P. muralis* (Mou, 1989) may be due to inadequacies of the method used, to a faulty experimental design or, less likely, to a real inability of this species to discriminate conspecific chemicals.

Up to now, the empirical evidence showing chemosensory sex or conspecific discrimination in lizards was mostly based upon work with scincids and iguanids. Although iguanids were formerly thought to be visually guided lizards (Stamps, 1977), recent findings indicate that discrimination of sociochemicals, including kin recognition, occurs in several species of this family: *Dipsosaurus dorsalis* (Dussault and Krekorian, 1991), *Sceloporus occidentalis* (Duvall, 1979; 1981; Duvall *et al.*, 1987), *S. jarrovi* (DeFazio *et al.*, 1977; Bissinger and Simon, 1981), and *Iguana iguana* (Burghardt, 1977; Werner *et al.*, 1987). As for the scincids, chemical communication has been extensively studied in the genus *Eumeces* (reviewed in Cooper and Vitt, 1986a). Several species in this genus can discriminate conspecific from other congeners' odors.

That an animal is able to detect an odor, or distinguish between two odors, does not demonstrate its ability to recognize the odor and gather biologically relevant information from it. Although our results suggest that *Podarcis hispanica* is operationally capable of recognizing conspecifics by means of vomeronasal investigation, a tongue-flick response is of itself not valid evidence of chemosensory conspecific recognition. As lizards flick their tongues in a variety of contexts, other behaviors are often brought to the fore

in an effort to assess recognition. For example, banded geckos, *Coleonyx variegatus*, show tail-displays when exposed to the odor of a snake-predator (Dial *et al.*, 1989), and chemical recognition of snake predators in *Lacerta vivipara* is revealed by changes in locomotion and tail vibration rate (Thoen *et al.*, 1986). In both species, the behaviors elicited by chemical stimuli are antipredator behaviors displayed solely when the lizard detects a predator, and not in other contexts (Vanderstighelen, 1987). This is the kind of evidence that is required in order to show that lizards associate the detection of snake chemicals with the presence of a predator (Van Damme *et al.*, 1990). As a further example, *Dipsosaurus dorsalis* tongue-flick at their own tails after chemosensory investigation of tiles marked with secretions from their own femoral glands, suggesting that they are capable of pheromonal self-recognition (Alberts, 1992).

Foot-shakes, a putative appeasement display in lacertids (Steward, 1965), have been observed during courtship and agonistic interactions of *Podarcis hispanica* (Verbeek, 1972; our unpublished observations). This behavior is shown mainly by juveniles, females, and subordinate males, even when no other lizards are visible. Based on this evidence, we hypothesized that foot-shakes are released by the presence of sociochemicals and might possibly be used to assess chemosensory conspecific recognition. In Experiment 2, males, but not females, displayed elevated foot-shake rates in cages soiled with odors from other lizards, whether conspecific or not. Since males were introduced to unfamiliar cages marked by other lizards, it is tempting to suggest that they would behave as subordinates and exhibit this appeasement display. That males also displayed foot-shakes to heterospecifics might indicate a response to a shared sociochemical. Thus, the present data on foot-shake rates do not provide evidence for or against conspecific recognition.

For a territorial species, the ability to recognize conspecific odors and behave accordingly has an obvious adaptive value. Although territoriality in the Lacertidae is controversial (Steward, 1965; Stamps, 1977), male *Podarcis hispanica* are very aggressive to each other (unpublished observations). Conspecific chemical detection provides them with the possibility of territory scent marking, which is energy-saving if risky conflicts with conspecifics are to be avoided.

Chemosensory sex discrimination has been clearly established in a few species of lizards. Male *Eumeces laticeps*, but not females, distinguish male from female odors and can follow scent trails (Cooper and Vitt, 1984; 1986c). Chemosensory sex discrimination has also been suggested for other lizards (Duvall, 1979; 1981; Bauwens *et al.*, 1987; Mason and Gutzke, 1990). The results of Bauwens *et al.* (1987) indicate that sex discrimination in male *Lacerta vivipara* might be accomplished through a combination of visual and chemical

stimuli. In one of the earliest experimental studies of lizard pheromones, Greenberg (1943) demonstrated that a sexual pheromone is present in the tail of the gecko, *Coleonyx variegatus*. Using a unique experimental design, Greenberg switched the tails of males and females and found that males readily courted female-tailed males. We have observed that courting male *Podarcis hispanica* tongue-flick repeatedly at the females' tails and also appear to follow females by scent trailing (unpublished observations). Furthermore, tail-biting, a behavior pattern that may be related to chemoreception (Greenberg, 1943; Gove, 1979), occurs during courtship in *P. hispanica* and some other lacertids (Kitzler, 1941; Verbeek, 1972).

Neither males nor females in our experiment exhibited a different pattern of responsiveness towards male and female odors, providing no evidence of sex discrimination. Since our experiment was conducted outside the reproductive season, the results of this study call for a replication using sexually active lizards. As in *L. vivipara*, sex discrimination might only seem apparent through a joint presentation of chemical and visual cues. Alternatively, the absence of differences in response to male and female chemical cues may indicate that these cues function not for sex recognition but, perhaps, for individual recognition (Alberts, 1989). Experimental designs with controlled interactions, as those of Greenberg (1943) or Bauwens *et al.* (1987), or simultaneous presentation of chemical stimuli (e.g. Duvall, 1979) are expected to yield better results on this particular issue.

Another point of discussion is the source of the chemical cues detected by the lizards. For males, the more likely source is the femoral glands, present in a row on the ventral surface of the hind legs. These glands are sexually dimorphic, being larger and more active in males than in females (Cole, 1966). The location of the femoral glands makes them ideal for passively marking the substrate as the lizard moves about its home range. Alberts (1989) reported that femoral gland secretions, besides being a visual signal through reflection of ultraviolet light, elicit chemosensory investigation in the iguanid *Dipsosaurus dorsalis*. In female *Podarcis hispanica*, whose femoral glands are small and probably inactive, another source of pheromone may be at work. That both male and female *P. hispanica* wipe their cloacae after defecating suggests that cloacal secretions may possibly be of importance. Another suggestive piece of evidence is the coincidence of the onset of mating activity with shedding in male *Lacerta vivipara* (Bauwens *et al.*, 1989). Although visual differences between pre-moulted and post-moulted males cannot be discarded, Bauwens *et al.* (1989) proposed that a release of pheromone following moulting might increase chemoreceptive detection of conspecific male lizards. In contrast, female moulting shows no obvious correlation with reproductive behavior.

In conclusion, our results provide evidence that *Podarcis hispanica* use chemical cues when exploring a new environment and that they can discriminate conspecific from heterospecific sociochemicals. This study also raises a number of interesting questions that can only be answered by further research.

Summary

Recent years have witnessed a remarkable surge of interest in the reptilian chemical senses. In spite of this interest, the chemosensory abilities of lacertids remain largely unexplored. The experiments described in this report were aimed at clarifying the role of the chemical senses in exploratory and social behavior of a lacertid, the Spanish wall lizard, *Podarcis hispanica*.

In the first experiment, lizards were observed in three experimental conditions: (1) home control, (2) handling control, and (3) novel cage. Each lizard was tested in all three conditions for 13 consecutive days. During each observation period we recorded several behavior patterns, including tongue-flicks. Tongue-flick rate was higher in the novel cage than in the two control conditions, suggesting a prominent role of chemoreception in the exploratory behavior of this species. Also, a decrease of tongue-flick rates over time in the novel cage condition indicates habituation of the exploratory response to novel environments.

In the second experiment we recorded the behavior of several lizards following transfer to cages soiled by conspecifics or syntopic heterospecifics (*Psammotromus algirus*) of either sex. Handling and odor controls were included for comparison. Tongue-flick rates of both males and females were significantly elevated in cages previously occupied by conspecifics. Males also exhibited high rates of foot-shake, a putative appeasement display, when transferred to cages previously occupied by any lizard. These results suggest that *Podarcis hispanica* is capable of discriminating conspecifics from heterospecifics by chemical cues alone.

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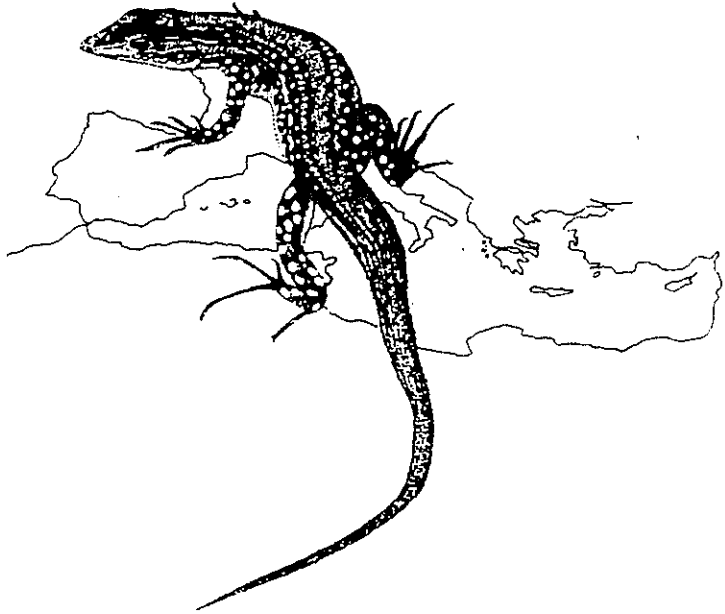
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HELLENIC ZOOLOGICAL SOCIETY

Lacertids of the Mediterranean region

A Biological approach



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Contents

Contents	I-II	Food consumption of <i>Podarcis taurica ionica</i> (Lehrs, 1902) in the Ionian islands (Greece) B. Chondropoulos, P. Maragou, E. D. Valakos	173-182
Preface	III-IV	An ethological study of feeding in the lizard, <i>Podarcis hispanica</i> E. Desfilis, E. Font, & A. Gómez	183-198
Phylogeny and the Lacertidae E.N. Arnold	1-16	Trophic ecology of <i>Acanthodactylus erythrurus</i> in central Iberian peninsula. Is there a dietary shift? M. J. Gil, V. Pérez-Mellado, F. Guerrero	199-211
Zoogeography of the lacertid lizards of the western Mediterranean basin W. Böhme & C. Corti	17-33	Chemoreception in the Lacertidae: exploration and conspecific discrimination in the Spanish wall lizard, <i>Podarcis hispanica</i> A. Gómez, E. Font, & E. Desfilis	213-230
Comparative morphology of the adrenal gland in some Mediterranean species of the family Lacertidae V. Laforgia, A. Capaldo, L. Varano, R. Putti, A. Cavagnuolo	35-42	Ecological similarity of lacertid lizards in the Mediterranean region. The case of <i>Ophisops elegans</i> and <i>Psammodromus hispanicus</i> V. Pérez-Mellado, E.D. Valakos, F. Guerrero, M.J. Gil-Costa	231-242
Clinal variation in some meristic characters of the Italian wall lizard <i>Podarcis sicula</i> (Rafinesque-Schmaltz, 1810) B. Lanza, P. Garavelli, C. Corti	43-49	Captive breeding of <i>Podarcis filfolensis</i> J. Moravec	243-248
Chromosome G-banding comparison among some mediterranean lacertid lizards G. Odierna, T. Capriglione, V. Caputo & E. Olmo	51-59	Studies of thermal biology: where should they go from here? R. A. Avery	249-252
The karyology of Mediterranean lacertid lizards E.Olmo, G.Odierna, T.Capriglione	61-84	Methods and aims in parasitology of Mediterranean reptiles, mainly lizards V. Roca	253-262
A systematic survey of the Iberian rock lizard <i>Lacerta monticola</i> Boul., 1905 V. Pérez-Mellado, L. J. Barbadillo, F. Barahona, R. P. Brown, C. Corti, F. Guerrero, B. Lanza.	85-105	The place of the herpetofauna in the design of a nature reserve system: Hypotheses, concepts and structure of data A. Y. Troumbis	263-278
An immunocytochemical study of the endocrine pancreas in three genera of lacertids from the Mediterranean basin R. Putti, M. Maglio, A. Della Rossa, V. Laforgia, L. Varano	107-119	Index of genera, species and subspecies	279-281
The many dimensions of a lizard's ecological niche E. R. Pianka	121-154		
Feeding of two sympatric lacertids in a sandy coastal area (Ebro Delta, Spain) M. A. Carretero & G. A. Llorenté	155-172		