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## CHEMICAL COMMUNICATION IN REPTILES: ORDER SQUAMATA

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**Abstract:** Chemoperception is the ability to detect and differentiate certain volatile chemical substances existing in the surrounding environment. The ability to detect chemical stimuli in reptiles has been studied mainly in organisms belonging to the order Squamata. Most of the work carried out has used snakes and saurians as models, where they report that chemical signals play a very important role in their ecology and behavior. They allow them to recognize the scent trails of organisms of the same species, either for the selection of a potential mate or aggregation for behaviors such as hibernation; Likewise, in the interaction with organisms of other species, chemoperception allows us to detect the smell of potential prey or that of predators; in the former, generating the display of attack behaviors when in contact with the odorous stimulus; and in the case of the latter, developing anti-predatory strategies.

**Keywords:** Squamata, chemoperception, animal communication, animal behavior.

## INTRODUCTION

Living beings obtain information from the environment through signals emitted both by organisms of the same species and by interspecific organisms. These signals can be acoustic, chemical, electrical, magnetic, tactile and visual (Lima, 2022). Chemoreception is the process by which organisms detect chemical substances in their environment. From an evolutionary perspective, it is considered the oldest sense, present among animals; It is even found in bacteria and other microorganisms (Muller-Schwarse, 2006). Chemical communication is a highly effective process used by many vertebrates due to the large number of interactions they can cause (Raya-García, 2016). Unlike visual, acoustic and tactile signals, chemical traces travel more slowly through the environment because their diffusion must be carried out from the source

of production of the odorant stimulus to the receptor's capture area. However, among its advantages are being able to be transmitted over long distances, as well as lasting longer in the medium once produced (Steinmann & Grenat, 2020).

Reptiles depend more on their chemical senses than any other class of vertebrates and various behavioral experiments suggest that chemical signals are important in their communication and reproduction, so the study of chemical communication must be an integral part of the research on the social behavior of reptiles (Muñoz, 2004). Among reptiles, the most studied order is Squamata, which includes about 11,000 species, making it the most diverse group of existing terrestrial vertebrates. In the more than 242 million years of evolutionary history they have, they have inhabited almost all terrestrial and aquatic environments. It is a monophyletic group that includes three suborders: Amphisbaenia, Lacertilia and Ophidia, which have three highly developed chemosensory systems: taste, smell and vomerolfaction that they use to detect traces of both predators and prey (Simões & Pyron, 2021).

## SUBORDER AMPHISBAENIA

Of the three suborders that make up the order Squamata, this one is definitely the least studied due to its fossorial habits, which makes it difficult to observe its behavior (Martín et al., 2021). Amphisbaenids can detect odors of conspecifics, aggregation behavior has been reported in *Trogonophis wiegmanni*, finding that adult females and males associate in pairs, as well as groups of juveniles, however, adult females and males do not associate with organisms of the same sex. Associations of females and males are more abundant in spring, so they could be associated with reproductive behavior; For its part, the absence of observations of two adult

males or two adult females together would suggest intolerance between adult organisms of the same sex (Martín et al., 2011).

Regarding the interaction with interspecific organisms, it is known that they can detect the smell of their prey, although there is little knowledge about the diet of many amphisbaenids and even less about their prey. A work carried out with *Amphisbaena heterozonata* (Figure 1) reported that they can discriminate the smell of potential prey such as tenebrio larvae (*Tenebrio* sp.), termites (*Nasutitermes* sp.) and earthworms (*Eukerria* sp.). The reptiles showed a significant increase in tongue oscillation to these and a significantly lower attraction latency towards odorous stimuli compared to the control. However, there were no differences in the behaviors displayed before each of these stimuli tested, which would probably be given by the opportunistic habits of *Amphisbaena heterozonata* (Semhan et al., 2010).



**Figure 1:** Amphisbaenids use chemical traces to locate their prey (Illustration: Viviana Domínguez Ochoa).

Amphisbaenids also use soil chemical traces to select their optimal microhabitat; Under laboratory conditions, *Trogonophis wiegmanni* remained in contact with soils with a salinity level similar to those of its natural habitat for a longer time. Likewise, an increase in the frequency of tongue oscillation was observed when in contact with soils with salinity levels similar to those of their habitat than with those that had a greater amount

of salts (Martín et al., 2021). The odor of the substrate can also modify the antipredatory response of amphisbaenids; *Blanus cinereus* in laboratory conditions showed greater activity and frequency of oscillation of the tongue when it was on unfamiliar substrates, compared to when it was kept on a substrate impregnated with its odor, where its mobility and oscillation frequency of the tongue significantly decreased. language. Which suggests that these organisms are capable of recognizing their own territory through chemical signals and that they can use this information to decide where to dig to escape from possible predators (López et al., 2000).

### SUBORDIN LACERTILIA

The members of this suborder have the main olfactory organ and the accessory or vomeronasal organ well developed, which allows them to detect and discriminate many different odors in their environments, both from conspecifics and other species, both prey and predators (Martín & López, 2014). Chemical signals play an important role in intraspecific communication in lizards, secretions from the preloacal and femoral glands are the main source of chemical signals in this group, the femoral pores located on the ventral surface of the thigh of many scaly lizards such as Iguana, *Leposoma*, *Gecko*, *Sceloporus* (Figure 2), to name a few, are connected to glands that produce large amounts of holocrine secretion. These secretions have a variable composition of proteins and lipids between organisms and sexes, which provides an honest signal about the individual identity of the males and their genetic quality, information that allows females to make adequate mate selection (López et al, 2006).



**Figure 2:** Pheromones secreted by the femoral glands of lizards: *Sceloporus jarrovi*, they work as an honest signal that allows them to select a potential partner (Illustration: Viviana Domínguez Ochoa).

Likewise, the use of chemical signals for social recognition has been reported. Studies carried out with various species of the genus *Liolaemus* found that these organisms are capable of self-recognition, presenting a lower rate of licking when faced with their own secretions compared to licking performed before secretions of conspecifics, it has also been observed that factors such as seasonality and age of the organisms cause the response to chemical stimuli from conspecifics to vary in different species of the genus (Labra, 2008).

Research in which the relevance of chemical traces in prey foraging has been evaluated has shown that this provides preponderant information for the discrimination of a potential prey, such as that carried out by Jiang et al (2009) where the behavioral response was analyzed. of *Shinisaurus crocodilurus*, when faced with the smell of potential prey such as *Pheretima* sp. and *Tenebrio molitor* which were macerated and offered with a swab to the reptiles, reporting a significant increase in the frequency of oscillation of the tongue before both stimuli compared to the control (deionized water) and a novel stimulus (cologne water), indicating that *S. crocodilurus* is capable of discriminating food and non-food stimuli.

With respect to the detection of predators,

various works have shown that some saurians of the genera *Lacerta*, *Dipsosaurus*, *Anguis* and *Liolaemus* are capable of discriminating the smell of potential predators (sympatric, parapatric and allopatric) from those that do not represent a potential danger to them. them (Thoen et al., 1986; Bealor & Krekorian, 2002; Cabido et al., 2004; Labra & Niemeyer, 2004), and even in the absence of any previous experience (Van Damme et al., 1995) which displayed anti-predatory behaviors such as flight or immobility when coming into contact with the smell of a potential predator. However, it is also known that some saurians show a decrease in the frequency of tongue oscillation as an anti-predatory response. This is because the movement of the tongue could be detected visually by the predator, which is why reptiles reduce the number of oscillations once they have detected its odor (Labra & Niemeyer, 2004).

## SUBORDIN OPHIDIA

Various behavioral studies refer to the ability of these organisms to recognize the scent trails of conspecifics, which allows them to display behaviors such as aggregation and mate selection. The ability of snakes to produce and follow chemical traces such as pheromones has been reported by various researchers, which is why we know the importance that these traces have in many aspects of the social behavior of snakes. To secrete these pheromones, snakes have glands, which are placed in pairs and release their contents into the cloaca (Muller-Schwarse, 2006).

In snakes, conspecific tracking allows for behaviors such as aggregation, the selection of a potential partner during the reproductive season, as well as the location of hibernacula where they spend the winter (LeMaster et al., 2001). The perception of odors is predominant in a social context.

For example, in rattlesnakes such as *Crotalus horridus*, chemoperception allows them to track shelters used for aggregation, chemical traces of a potential mate during the reproductive season, as well as locate hibernacula during the winter (Brown & MacLean, 1983). At the level of agonistic behavior, it has been observed that snakes of the genus *Natrix* have paired “scent glands” that open inside the cloaca, as well as some members of the genus *Macropisthodon* that have glands on the skin of the dorsal area of the neck. In both cases, the secretions of these glands are assumed to be used for defense and/or communication with intraspecific organisms (Müller-Schwarze, 2006).

Some snakes display aggregation behaviors not only during the reproductive season or during the hibernation season, but under a wide variety of conditions this behavior has been observed both in the field (*Storeira dekayi* and *Diadophis punctatus*) and in captivity (*S. dekayi* and *Thamnophis butleri*). This behavior occurs under certain specific conditions of humidity and temperature; This type of aggregation seems to be an adaptation to reduce water loss in snakes (Burghardt, 1983).

In snakes, the chemical information provided by the lipid composition of the skin of females is very important for reproduction, since, through this, the female provides information to males about her state of receptivity and her size, which allows them to select the females most suitable for reproduction (Gabinot, 2010). Research carried out in the field showed that adults of *T. sirtalis parietalis* are capable of detecting and responding to traces of conspecifics, finding variability in the snakes’ responses both in sex and season. Males exhibited tracking behavior towards female signals during the mating season; although the females did not present any response to the pheromones of males and/

or females. Furthermore, neither sex displayed conspecific odor-tracking behavior during the autumn migration period when they returned to the hibernaculum (LeMaster et al., 2001).

Pheromone trails involved in sexual behavior have been examined in different species of the genus *Thamnophis*. A study carried out by Ford and Schofield (1984) where males of different species of the genus *Thamnophis* were subjected to traces of females of different species, yielding very particular results. Males of *T. proximus* and *T. butleri* with sympatric distribution showed that these snakes can discriminate the trail left by females of their own species. Likewise, males of *T. sirtalis* and *T. butleri* with a sympatric distribution can discriminate the tracks of females of the same species. However, when comparing organisms with allopatric distribution such as *T. radix* and *T. butleri*, it was reported that the traces left by the females of both species are indistinguishable for the males of *T. butleri*.

When they compared two subspecies they found that the males: *T. radix radix* y *T. r. haydeni*, they were able to distinguish the females of their own subspecies. Likewise, both subspecies were able to discriminate the odor of conspecific females from that of *T. marcianus* females. However, both subspecies followed the trail of *T. marcianus* females when it was presented alone, suggesting that the trail used by organisms of the genus *Thamnophis* is species-specific, as well as that the sexual pheromones of *T. radix* and *T. marcianus* are chemically similar.

Interactions with organisms of other species in snakes are basically the location of potential prey, and the detection of predators. These interactions are important for all areas of ecology, since predators guide the evolution of prey defense mechanisms, which emphasize both evasive mechanisms that will reduce the probability of detection by the predator,

as well as antipredatory mechanisms that reduce predator success after detection occurs (Madison et al., 1999).

Work carried out with ophidians has exposed their ability to detect prey odors, even without having prior experience. An investigation carried out with *T. sirtalis* neonates found that inherited chemosensory responses to the odor of specific prey can vary as the snake matures, however, the experience acquired with said prey will also influence (Burghardt, 1967).

Another work carried out with the genus *Regina* showed that neonates of *R. grahamii* and *R. septemvittata* responded significantly more to crab chemicals than any other prey stimulus (fish, odonate larvae, crayfish, crayfish in intermolt and with recent ecdysis). This corresponded well with dietary studies reporting that both species feed exclusively on soft crabs (recent ecdysis) throughout their lives.

This discriminatory ability in these species would be strongly favored by natural selection, since neither *R. grahamii* nor *R. septemvittata* present the behavioral adaptations that seem to be necessary to successfully manipulate a hard-shell crab, which could cause head damage to the snake or even death. The specific chemical basis for the discrimination of hard-shell and soft-shell crabs is unknown, however, crabs differ in the protein composition of their pre- and post-molt epidermal tissue (Waters & Burghardt, 2005).

In the case of foraging in adults, some work carried out with snakes of the genera *Natrix*, *Regina* and *Thamnophis* demonstrated that these snakes are capable of detecting the smell of potential prey and presenting predatory responses (increased frequency of tongue oscillation and emission of attacks on the source of the odor) upon coming into contact with the odorous stimulus. It is also

known that snakes can discriminate between the smell of different types of prey, showing a preference for those that are part of their feeding habits, as well as vary this response towards odorous stimuli according to their experience. Snakes that have already been in contact with prey are capable of recognizing their scent and displaying foraging behaviors (Müller-Schwarze, 2006).

Chemoperception also provides information to snakes about predators present in the environment. Because the response to predators can involve costs (such as the loss of foraging or reproductive opportunities, for example), there must be strong selection by prey not only to identify predators, but to modify antipredatory responses according to the level of risk that the predator has (Webb et al., 2009).

Therefore, the defensive behaviors displayed by prey toward chemical traces may depend on the type of predator in question, the predator's foraging strategies, and the level of risk it represents. Thus, for example, exaggerated escape behavior in the face of a predator that has a low level of predation risk may not be appropriate because it would increase the conspicuity of the prey towards other types of predators (Amo & Bonadonna, 2018).

Predation poses one of the greatest risks for most organisms, which has given rise to the evolution of a complex formation of anti-predatory tactics that involve behavioral modifications (for example, responses to the approach of a predator), physiological (secretion of toxins), morphological (for example, defensive spines) and the deployment of their capabilities (Webb et al., 2009). Studies carried out with crotalids have shown that they present anti-predatory responses when coming into contact with predator odors, such as lifting the body in the shape of a bridge (Figure 3) in *Crotalus*

*horridus*, *C. atrox* and *C. ravus* to the smell of ophidiophagous snakes (Muller-Schwarse, 2006).



**Figure 3:** It has been reported that rattlesnakes of the genus *Crotalus* arch their bodies in the shape of a bridge as an anti-predatory response when detecting the smell of snakes with ophidiophagous habits (Illustration: Viviana Domínguez Ochoa).

The innate ability of snakes to detect and discriminate their predators has been studied in *T. elegans*; the juveniles of this ophidian were individually put in contact with three methylene chloride isotopes that were carved on the back of a male *Lampropeltis getulus californicae*, a male of *Heterodon nasicus* and a female of *Thamnophis radix* respectively. Two groups of snakes were used: 18 specimens of *T. e. vagrans* and 14 of *T. e. terrestris* both between 7 and 8 weeks old. The results showed that the snakes presented a significant increase in the frequency of tongue oscillation when faced with the odor of *Lampropeltis* (a snake with ophidiophagous habits) compared to those performed when faced with the odor of *Heterodon* and *Thamnophis*. Other works carried out with this same genus found that juveniles of *T. s. sirtalis* between 2 and 3 months of age showed a significant increase in the frequency of tongue oscillation when

faced with the odor of *Coluber c. constrictor*, a snake with ophidiophagous habits compared to the tongue oscillations performed before a non-predatory snake such as *Pituophis melanoleucos melanoleucos* (Mason, 1992).

## CONCLUSIONS

Chemical communication plays a central role in ecological, social and sexual interactions among reptiles; the diversification of signaling systems has been the result of complex evolutionary processes resulting from the interaction between natural and sexual selection. Within the Order Squamata there are animals totally dependent on their chemical senses, since, thanks to the stimuli collected from the environment, derived from prey, predators or possible partners, the members of the order Squamata display multiple well-differentiated behaviors, which indicates a specialization towards the detection and release of chemical signals.

Despite the few studies that exist on the amphisbaenia suborder, the behaviors displayed thanks to the perceived chemical traces allow us to outline their ecological interactions; Their behavior patterns are determined by the chemical stimuli present in the environment, revealing information about their ecology; Taking into account the difficulty of in vivo studies, laboratory conditions have determined the response behaviors and habits of the suborder, having important findings such as its use in habitat selection, its conspecific associations, antipredatory behaviors modified by the environment or partner selection.

Without a doubt, the discrimination and selection of prey and mates is a very important event in the life of any organism, such is the case of members of the order lacertilia that make the most of chemical communication by releasing specific chemical traces for mate selection. where females can discriminate

through vomerolfaction the honest signals emitted by the males around them; The chemical traces perceived are also used for self-discrimination of one's own secretions or the detection of prey and predators in a very accurate way.

Ophidians, for their part, present a clear specialization for the distinction of interspecific and intraspecific scent trails, allowing annual behavioral patterns, generating highly differentiated hunting and

antipredatory behaviors between families; As they are solitary organisms, aggregation behaviors are well reported, which are caused by specific chemical traces that in turn serve to identify potential mates as well as burrows used by conspecifics. The interactions and ecological plots of snakes are highly incited and determined by chemical traces that are decisive for successfully meeting the basic needs of these organisms.

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