Morphology of the Femoral Glands in the Lizard Ameiva ameiva (Teiidae) and Their Possible Role in Semiochemical Dispersion

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ABSTRACT Many lizards have epidermal glands in the cloacal or femoral region with semiochemical function related to sexual behavior and/or territorial demarcation. Externally, these glands are recognized as a row of pores, opening individually in the center of a modified scale. In many species the pores are used as systematic characters. They form a glandular cord or, in some species, a row of glandular beads below the dermis, and are connected to the exterior through the ducts, which continuously liberate a solid secretion. Dead cells, desquamated from the secretory epithelium, constitute the secretion, known as “a secretion plug.” The present work focusses on the morphology of the femoral glands of the teiid lizard Ameiva ameiva, correlating it to the way in which the secretion is deposited in the environment. The results here obtained are compared to those available for other lizards and amphisbaenians. We observed that the diameter of the glandular pores did not show significant differences between males and females. The glands comprise germinative and secretory cells, which pass through at least three stages of differentiation, during which an accumulation of cytoplasmic granules, with a glycoprotein content, occurs. The cells eventually die and desquamate from the secretory epithelium, forming a secretory plug mostly constituted by juxtaposed non-fragmented secretory cells. Because of the arrangement of the rosette-like scales surrounding the femoral pores, we suggest that when the animal is in a resting position, with its femoral regions touching the ground, these scales may be involved in the breakage of their respective plugs, depositing tiny portions on the substrate. In this manner, it seems that the method for signal dispersion in this species involves specifically adapted structures and does not simply involve the chance breakage of the plug, as the gland secretes it. Signal dispersion must also be intimately associated with the animal’s movement within its territory. J. Morphol. 00:000–000, 2007.

KEY WORDS: squamata; lizards; teiidae; femoral pores; epidermal glands; Ameiva ameiva; pheromone

Among the Squamata, semiochemicals have an important role in sexual behavior, defense, predation, territorial marking, orientation, intraspecific aggregation, and parental care (Halpern, 1992). Besides their importance in squamate biology, the morphology of the structures related to semiochemical communication are poorly known. Most morphological papers on this subject deal with European, Asian, Australian, and African species (Cole, 1966a,b; Maderson, 1968, 1970, 1985; Maderson and Chiu, 1970, 1981; Chiu and Maderson, 1975; Schaefer, 1901, 1902; Cohn, 1904; van Wyk et al., 1992; Dujsebayeva, 1998). More recently, the morphology of pre-cloacal glands in an amphisbaenian (Amphisbaena alba) was reported and their role in the natural history of this species was analyzed (Antoniazzi et al., 1993, 1994; Jared et al., 1999).

Femoral glands are the most common semiochemical sources in lizards (Jullien and Renous-Le´ curu, 1973) although some glandular scales or “generation” glands (Maderson, 1967, 1968) have been described in cordylids in other regions of the body (van Wyk et al., 1992). Femoral glands are localized in one or two rows on the ventral side of each thigh. The first morphological studies on femoral glands date from the end of XIX century and beginning of XX century (Abrahám, 1930), and concerned primarily European species. The subject was ignored by morphologists until the 1960s, when papers by Gabe and Saint-Girons (1965) and Cole (1966a,b) were published. Uva (1967), Gasc et al. (1970), Chiu and Maderson (1975) and Chauhan (1986) then reported additional data on other species.

Although semiochemical communication is very important in lizard biology, studies focusing on these aspects are rare (Mason, 1992; Cooper, 1994; Cooper et al., 1996). However, many experiments have demonstrated that several species are able to recognize chemical signals secreted by femoral glands (Cooper and Vitt, 1984; Alberts, 1989, 1993; Lopez et al., 1998, Martin and Lopez, 2000).

The localization of glands in both lizards and amphisbaenians suggests that signals are pas-
sively deposited in the environment during locomotion of the animals within their territory (Maderson, 1986; Jared et al., 1999). Among lizards possessing femoral glands, signal dispersion involves the breakage of small portions of the secretion plugs. These remain on the substrate, releasing chemical signals for a period of time that must be related to both the natural history and environment of a particular species (Alberts, 1993).

*Ameiva ameiva* is a teiid lizard with a broad geographic distribution in Central and South Americas, from Panamá to South Brazil and North Argentina (Vanzolini et al., 1980; Colli, 1991). The aim of the present paper was the study of the morphology and some aspects of the histochemistry of the femoral glands of the teiid *Ameiva ameiva*, and the comparison of the results with those obtained for other lizards and amphibians. We hope to contribute to the integrative knowledge of the natural history of lizards, especially in relation to chemical communication. Our data suggest that in *Ameiva ameiva* the differentiated scales containing the femoral pores and the secretion plugs are structures adapted to the dispersion of chemical signal on the substrate and seem to be associated with the locomotion of these animals throughout their territory.

**MATERIALS AND METHODS**

**Animals**

Adult individuals of *Ameiva ameiva* Linnaeus 1758 were collected at Palmas (State of Tocantins, Brazil) and maintained for 2 years in the vivarium of the Laboratory of Cellular Biology of the Instituto Butantan. They were housed in terraria containing humid earth at the bottom with dry branches above and pieces of plastic pipe for shelter. They were daily exposed to sunlight during the morning period. Food was supplied weekly consisting of live items (beetles - *Tenebrio molitor*, crickets - *Gryllus sp.*, cockroaches - *Pycnocellus surinamensis*), fruits, eggs, and minced beef.

For the morphological studies, six animals were sacrificed with an intraperitoneal overdose of sodic thiopental just after being collected from nature. Samples of the skin containing the femoral glands were removed and processed as described below.

Voucher specimens were deposited in the collection of the Museum of Zoology, University of São Paulo (MZUSP).

**Histology**

The skin containing the femoral glands was cut in strips of about 3 × 5 mm² using a razor blade and fixed in 4% paraformaldehyde in PBS 0.1 M, pH 7.2 for 24 h. The material was dehydrated and embedded either in historesin (glycol methacrylate, Leica) or paraffin (at 60 °C).

For general study of the tissues, 2 μm historesin sections were stained with toluidine-fuchsin (Junqueira, 1995) and 5–7 μm paraffin sections were stained with hematoxylin–eosin. Picro-Sirius and N.M.C. trichrome stains (Junqueira et al., 1979) were used for the identification of collagen and muscular fibers, and the Weigert’s resorcin-fuchsin stain (Bancroft and Stevens, 1996), for the identification of elastic fibers.

**Histochemistry**

Historesin and paraffin sections were subjected to the following histochemical staining procedures (according to Bancroft and Stevens, 1996): periodic acid-Schiff (PAS) and alcian blue pH 2.5, for identification of neutral and acidic mucosubstances, respectively, and bromophenol blue, for identification of proteins. Sudan black B was used for identification of lipidic substances.

**Photomicrography**

Photographs were obtained by using a Leica DMLB microscope equipped with a Leica MPS60 photographic system.

**Scanning Electron Microscopy**

A strip of skin from the femoral region containing 5–7 femoral pores was removed from the animals, fixed in Karnovsky solution, pH 7.2 (Karnovsky, 1965) for 24 h, post-fixed in 1% osmium tetroxide, incubated in 1% tannin, and postfixed once more in 1% osmium tetroxide (Mizuhira and Futasekku, 1974; Simionescu and Simionescu, 1976). The material was dried by the critical point method, coated with gold, and examined in a JEOL JSM 6100 and in a JEOL JSM 5300, operating between 15 and 25 kV.

**Transmission Electron Microscopy**

Fragments of the femoral glands (separated from the skin) of about 1 mm³ were fixed in Karnovsky solution, pH 7.2 (Karnovsky, 1965) for 24 h, post-fixed in 1% osmium tetroxide, contrasted in 2% uranyl acetate, dehydrated in ethanol and embedded in epoxy resin. Ultrathin sections (60 μm) were obtained in a Sorvall MT 6000 ultramicrotome and were examined with a LEO 906E, operating at 80 kV.

**Morphometrical Study**

Adult male (*N* = 14) and female (*N* = 14) specimens of *Ameiva ameiva* from the zoological collection of Museu de Zoologia da Universidade de São Paulo, all from the same population, were studied. The number and the diameter of the pores in the femoral region of males and females were quantified and possible differences in both sexes were analyzed by Student test. The diameters of the pores of three regions in the pore row (the two extreme regions, named *P*₁ and *P*₃, and the middle region, named *P*₂) were measured. Because data were normally distributed and had homogeneous variances, they were analyzed by ANOVA test.

**RESULTS**

**General Anatomy and Histology of the Femoral Glands**

In *Ameiva ameiva* (Fig. 1), the ventral skin of the thighs, in both males and females, shows a single row of pores (Fig. 2), whose number varies from 17 to 23 in males and from 16 to 22 in females. Each pore opens in the center of a modified scale, resembling a rosette (Fig. 3). Because of their prominence, the row of pore-bearing scales stand out from the neighboring scales and, for this reason, are easily recognized (Figs. 2, 3). Studying the skin from the dermal side reveals that each of the pores corresponds to a gland. The row of glands, superimposed on one another, forms a deli-
cate yellowish cord (Fig. 4), which is juxtaposed to the dermis by delicate connective tissue. When this tissue is removed, it is possible to observe that each one of the glands is branched and flat (Fig. 4). In sections of this region, the glandular bodies are cut in different planes and internal ductules appear among the lobules (Fig. 5). The main ducts connecting the glandular bodies to the exterior are visible in sections cut transverse or longitudinal to the axis of the femur. The ducts are always filled with a solid plug comprising a yellowish and brittle secretion (Fig. 5).

A thin layer of connective tissue, which is supplied by many blood vessels, covers each of the femoral glands. The connective tissue forms septa dividing the gland into lobules (Fig. 6). Each lobule comprises a holocrine epithelium that converges to the ductules, which finally connect to the main duct.

The main duct is lined by a stratified epithelium, which is continuous with the skin epidermis (Fig. 5). The holocrine secretion that fills the duct comprises small units with an affinity for toluidine blue (in toluidine blue-fuchsin staining) (Fig. 6) or for eosin (in hematoxylin–eosin staining).

### Scanning Electron Microscopy of the Glandular Pore Region

The scale containing the glandular pore comprise three or four plates arranged like the petals of a flower (Fig. 7). One of the plates is always larger and more prominent when compared to the others (Fig. 7, insert). Each one of the scale plates, specially the larger one, shows a fine arched internal border, which contacts the plug (Fig. 7).

The secretion plug emerges from the pore in the form of a rod, the tip of which is usually about the same level of the skin surface. While a smooth cover (Fig. 7) envelops its lateral aspect, small units corresponding to the dead cells, which are desquamated from the holocrine epithelium, form its distal surface (Fig. 8). Most of these dead cells are intact and show no signs of abrasion from contact with the substrate (Fig. 8).

### Light Microscopy of the Secretory Epithelium

Germinative cells and secretory cells (Fig. 9A–F) comprise the glands. To facilitate the morphological description of the differentiation process, the secretory cells were divided into three subsequent stages (S1, S2, and S3), according to their structural characteristics.

The germinative cells (G) are localized at the periphery of the lobules. They are elongated and have voluminous nuclei (Fig. 9A). The cells in the first stage of differentiation (S1) have the same localization as the germinative cells but are generally more spherical. Their nuclei are also spherical and voluminous and usually have one or two conspicuous nucleoli. During the first stage, the cytoplasm is lightly stained and does not possess secretion granules (Fig. 9A).

The second stage of differentiation (S2) is characterized by cells with a larger volume and observed in different phases of the secretory process. These phases can be identified by the increase in the number of secretory granules and were named P1, P2, and P3. In P1, the cells are usually spherical and the cytoplasm has a number of discrete granules (Fig. 9B). In P2, small granules that are sometimes metachromatic (Fig. 9B) characterize the cells. In the last phase (P3), the granules occupy a large portion of the cytoplasm, are larger and spherical, but are heterogeneous in size and in their tinctorial properties (Fig. 9C). Irregular granules containing heterogeneous secretion (Fig. 9D) which usually forms a half-stained pattern within the granule distinguish late P3. In the most differentiated cells, the irregular nuclei tend to be displaced to the periphery, and are smaller in relation to the total cell volume. The cells remain spherical.

In the third and last stage (S3), the secretory cells, already close to the duct, assume a flat form and the cytoplasm is full of irregular granules with heterogeneous secretion. The peripheral nuclei are pyknotic (Fig. 9E). At the end of this stage, the cells desquamate from the secretory epithelium, enter the duct, and become part of the solid plug.

During the differentiation process, cells of a different type may be seen among the secretory cells. They contain dense inclusions suggesting clusters of secretion granules lying inside a large vacuole,
and occupy a considerable part of the cell cytoplasm (Fig. 9C,F).

**Histochemical Observations of the Femoral Glands**

The secretory cells showed a strong and homogeneous PAS positivity (before and after amylase treatment) either in the secretion granules or in the plug. Similarly, granules and plug were positive to bromophenol blue, although the granules are not all positive to the same intensity.

Alcian Blue at different pHs (1.0, 2.5, and 3.1) and Sudan Black were negative for all glandular structures.

**Light Microscopy of the Rosette**

The skin of *Ameiva ameiva* comprises a cornified epidermis and a dermis essentially constituted by
dense regular connective tissue in a basket arrangement with intermingled blood vessels.

Longitudinal sections of femoral skin show the general structure of the modified scales (rosettes) containing the pores, which may also be seen in transverse section (Figs. 10–13). Picro Sirius staining showed that the dermal connective tissue is basically formed by collagen fibers, which stain bright red. N.M.C. trichrome staining revealed the characteristic arrangement of dermal collagen fibers (which appear in deep blue color) within the rosette. The dermis comprises an exceptionally well-developed deep layer and a poorly developed superficial layer, immediately below the epidermis (Figs. 10 and 11). Immediately around the duct the collagen fibers are thinner and are circularly arranged (Fig. 11). Between the deep dermis and the circular fibers, there is zone of loose and less organized connective tissue well irrigated by blood vessels (Fig. 11).

When Weigert’s Fucsin-Resorcine is applied to the sections, delicate purple elastic fibers are revealed among the collagen fibers, mainly in the well-developed deep layer in the form of a net (Fig. 12) and following a circular arrangement around the duct (Fig. 13).

**Transmission Electron Microscopy of the Femoral Glands**

At the ultrastructural level, the germinative cells are polygonal or spindle-shaped (Fig. 14A). As in other epithelial tissue, the cell membranes show many interdigitations and desmosomes. The cytoplasm of germinative cells is quite electron lucent and is rich in mitochondria, granular and agranular endoplasmic reticulum, free ribosomes, and Golgi apparatus (Fig. 14B). Bundles of intermediate filaments (tonofilaments) occur through the cytoplasm or linked to desmosomes (Fig. 14A,B). The spherical nuclei with one to three nucleoli occupy a large volume of the cell and present predominantly lose chromatin (Fig. 14A).

The secretory cells in the first stage of differentiation (S1) can be distinguished from the germinative cells by their more spherical shape, and especially by the small and scarce secretion granules in the cytoplasm (Fig. 14C). These granules are spherical and homogeneous. Similar to the germinative cells, the cytoplasm is still quite electron lucent and tonofilaments and desmosomes are abundant. An increase in the granular endoplasmic reticulum (GER) and Golgi apparatus occurs (Fig. 14D). The nuclei is spherical, with predominantly loose chromatin, and is less voluminous within the cell when compared to the situation in germinative cells (Fig. 14C).

The second stage (S2) is characterized by nuclei with irregular shapes, an increase in cytoplasmic volume and in the number of the secretion granules. It is possible to distinguish at least three phases in this stage: (P1) In the initial phase, cells have spherical, homogeneous granules of different sizes occupying part of the cytoplasm (Fig. 14E). Lamellae of the granular endoplasmic reticulum are quite developed and dilated and Golgi cisternae were often seen (Fig. 14F). (P2) The cytoplasm of the cells contains many large spherical granules, some of them with heterogeneous content (half electron dense, half electron lucent). The GER is well developed and is still dilated (Fig. 15A). Other organelles are not so visible and, as the number of granules increases, nuclei are more electron dense and are pushed towards the cell periphery (P3) In the last phase of S2, the cytoplasm is replete with secretion granules with heterogeneous content (Fig. 15B). The nuclei often appear already shrunk, are electron dense and have an irregular shape. While most organelles are barely observed among the granules, it seems that there is an increase in the number of lipidic inclusions, with medium electron density (Fig. 15B).

In the last stage of differentiation (S3), the cells show evident signs of cytoplasmic disorganization and acquire a flatter form (Fig. 15C). The granules occupy practically the whole volume of the cells. The nuclei are pyknotic or absent. There is an increase in the number of lipidic inclusions (Fig. 15C). The cells in this stage are beginning to desquamate from the secretory epithelium, or are already desquamated and lie loose in the duct, forming the secretion plug. Ultrastructure of the plug

Fig. 9. Histological sections at high large magnification showing the internal arrangement of the glandular lobules. Gland cells are seen in different stages of differentiation. Germinative cells (G) lie at the periphery (A–C, E and F). Secretory cells in the first stage of differentiation (S1) have no granules (A, D, and F). Secretory cells in the second stage of differentiation, divided in three subsequent phases (P1, P2 e P3), in which the granules are secreted, increase in number, and undergoes maturation (B–D). Secretory cells in the third stage of differentiation (S3), in which they acquire a flat form, present evident signals of decline and finally desquamate from the secretory epithelium (E). Cells with dense inclusions (*), observed among the other cells (C, D, and F). Arrowheads point to pyknotic nuclei. Glycol methacrylate, toluidine-fuchsin stain.

Figs. 10–13. Fig. 10. Histological section through a rosette-shaped scale containing a femoral pore transversally sectioned. The arrangement of the dermis in the scale is better observed in Figure 11, which corresponds to a high magnification of the area indicated by the rectangle. The dermis comprises a thin superficial layer immediately below the epidermis (Ep), a well-developed deep layer (dt) mainly formed by collagen fibers, and a circular layer (ct), also mainly formed by collagen fibers, around the duct. Between the deep layer and the circular layer there are areas mainly composed of loose and less organized connective tissue (It) with many blood vessels (arrows). F, secretion plug; *, duct epithelium. Paraffin. N.M.C. trichrome stain. Fig. 11. Magnified image of the area indicated by the rectangle in Figure 10. Fig. 12. Details of a histological section through one rosette-shaped scale containing a femoral pore transversally sectioned. The section was stained to demonstrate the delicate elastic fibers (arrows) which form a disorganized net among the collagen fibers in the deep layer. Ep, epidermis; *, duct epithelium. Paraffin. Weigert’s resorcine-fuchsin. Fig. 13. The circular arrangement of collagen fibers around the duct.
Figure 14

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shows that it comprises electron-dense polyhedral units, measuring about 1.3 μm in the larger axis.

A few secretory cells, mainly in the first stages of differentiation, contain dense inclusions in their cytoplasm (Fig. 15D).

**Morphometrical Study**

The number of pores in males and females did not show differences (meanp1 = 20.27, meanp2 = 19.67, N = 28, t = 0.0159). The comparison of pore diameters among regions P1, P2, and P3 did not show differences in males (meanp1 = 0.51 mm, meanp2 = 0.57 mm, meanp3 = 0.49 mm, F = 3.15, N = 42) as well as in females (meanp1 = 0.34 mm, meanp2 = 0.36 mm, meanp3 = 0.35 mm, F = 0.52, N = 42). The comparison of pore diameters between males and females for regions P1, P2, and P3 also did not show differences (t=0.36±0.08, t=1.93±0.07, t=3.25E−05).

**DISCUSSION**

Epidermal glands in pre-anal or femoral regions are well-known in several lizard families (Jullien and Renous-Lecuru, 1973; Rastogi, 1975) although they can be found in other body regions (Simon, 1983; Dujsebayeva, 1998). Many behavioral and biochemical studies conducted in recent decades have demonstrated the role of these glands in lizard chemical communication (Pough et al., 2001). There is a considerable external variation of epidermal glands in relation to their distribution in the body (Klug, 1967a, b; Dujsebayeva, 1998), the shape of the scales containing the pores (Blasco, 1975; van Wyk et al., 1992), and the coloration of the secretion plug (Cole, 1966b; Blasco, 1975). Internally these glands can also present anatomical and color variations (Gasc et al., 1970; Rastogi, 1975). Some of these differences may be related to the way epidermal glands are used for chemical communication within each group.

Rosette-like differentiated scales are not exclusive of teiids but can also be found in other lizard families such as iguanids and lacertids (Cole, 1966a; Blasco, 1975). Our histological observations on Ameiva ameiva indicate that this structure possibly derives from the fragmentation of one single scale (Fig. 5).

Femoral pores have been used as systematic characters for a long time (Linnaeus, 1758; Duméril and Bibron, 1834). They are also used to define sexual dimorphism (Cole, 1966a). In the case of captive Ameiva ameiva we have not observed any significant circannual modification in the external morphology of femoral glands although the animals remained sexually active, with copulations being frequently observed. Our morphometrical results indicated that in Ameiva ameiva no sexual dimorphism can be observed in relation to the number or to the diameter of pores. This apparent similarity in the glands of both sexes could indicate that they may be used by all individuals for territorial demarcation. However, differences in the biochemical composition of gland secretion between males and females cannot be discarded.

In relation to their internal anatomy, femoral glands of Ameiva ameiva correspond to the model proposed by Gabe and Saint Girons (1965) for lizards in general. In this model, the glands are flat and branched, with an internal ramification of ductules connected to a main duct, which leads to the secretion to the exterior. The solid secretion plug is generally the same color of the gland in which it is produced (Cole, 1966b). In Ameiva ameiva, femoral glands and secretion plugs all have the same yellowish coloration.

Femoral glands in Ameiva ameiva, as in other squamates, are holocrine. The extrusion of the solid secretion plug through the pore is due to the pressure exerted by the distal proliferation and differentiation of the secretory cells within tubules (Antoniazzi et al., 1993). This process, however, does not explain the lateral aspect of the plug, visible by scanning electron microscopy (Figs. 7, 8). This seems to be a structure formed by the accumulation of desquamated epithelial layers since, according to Maderson (1972), the gland duct epithelia do not show cyclic changes of the type that are associated with periodic shedding.

The ultrastructure of the germinative cells is typical of epidermal cells, showing large numbers of tonofilaments and desmosomes. These cells reveal an intense metabolic activity, which is morphologically evidenced by the cytoplasm rich in different organelles. The observation of histological and ultrastructural differences among the secre-
tory cells made it possible to distinguish at least three different stages of differentiation. In the third stage, small inclusions with medium electron density seem to be lipidic in nature. Furthermore, we often observed in all differentiation stages the presence of large inclusions that are apparently the accumulation of secretory material in the form of vesicles whose destiny in the cells we have not being able yet to determine. All these differentiation processes resemble what has been observed in

Journal of Morphology DOI 10.1002/jmor
the epidermal glands of other lizards (Cole, 1966a; Gasc et al., 1970; Maderson, 1972) and amphisbaenians (Antoniazzi et al., 1993, 1994; Jared et al., 1999).

In relation to the chemical nature of Ameiva ameiva femoral gland secretion, our histochemical and ultrastructural data indicate that it consists of neutral mucosubstances and proteins, probably forming glycoproteins. The same results were obtained for other lizards (Gabe and Saint Girons, 1965; Cole, 1966b; Uva, 1967; Gasc et al., 1970; Rastogi, 1975; Alberts, 1993) and amphisbaenians (Antoniazzi et al., 1993, 1994). Despite the negative result obtained with the use of Sudan Black B for detection of lipids, at the ultrastructural level, we have observed small inclusions in the secretory cell cytoplasm that seem to be lipidic in nature. Lipids are common among substances involved in chemical communication. (Alberts, 1990; Alberts et al., 1992; Halpern, 1992). Alberts (1993), working with the chemistry of pheromones in Dipsosaurus dorsalis, discussed environmental influence on the composition of the femoral gland secretion of this iguanid. In Iguana iguana and Dipsosaurus dorsalis, the low concentration of lipids in their glandular secretion could only be detected by gas chromatography (Alberts, 1990; Weldon et al., 1990). Thus, although undetectable by histochemical techniques, small amounts of lipid might be present in teiid femoral gland secretions.

The ventral localization of the epidermal glands on the body of lizards and amphisbaenians suggests that their secretions are passively deposited in the environment (Maderson, 1986). Recent papers have demonstrated that in fact many species of lizards respond to secretions derived from these glands (Cooper and Vitt, 1986; Cooper et al., 1994; Cooper et al., 1996). However, the method used by lizards for depositing chemical signals in the environment has not been studied so far. In amphisbaenians, (Antoniazzi et al., 1993, 1994), studying the morphology of the pre-cloacal glands of Amphisbaena alba, demonstrated that, as a result of the position and arrangement of these glands, the secretion plugs, perpendicular to the body, make direct contact with the tunnel walls where this species lives. These authors suggest that, during locomotion, the plugs are naturally abraded and secretion fragments are deposited on the substrate, forming a trail. In fact, Jared et al. (1999) experimentally demonstrated the formation of a secretion trail during locomotion of A. alba. Scanning electron microscopic examination, both of the trail and of the abraded surface of the plug, showed that the fragments of the trail correspond to the secretion granules of the secretory cells. The trail then may constitute an extensive area for volatilization of chemical signals, which may be an efficient way for intraspecific (or interspecific) communication within tunnels.

Differently from amphisbaenians, in lizards it has already been observed that distal portions of femoral gland secretion plugs are fractured and deposited on the substrate, where they remain in the form of small secretion blocks (Alberts, 1993). According to our observations, that seems also to be valid in Ameiva ameiva.

Scanning electron microscopy showed that the secretion plugs in this species are composed of dead cells that desquamate from the femoral gland secretory epithelium. The fact that most of the cells seen on the plug's surface are intact suggests that, rather unlike amphisbaenians, they are not abraded by the substrate. Rather, the microscopic appearance of the surface suggests that the frail plug is probably fractured, falling in small pieces on the substrate. On the other hand, the epithelial cover may function to maintain the cells' cohesion as a plug, favoring the prolongation of signal effect by preventing rapid secretion volatilization. Unlike, in Amphisbaena alba, the surface of the secretion plug shows a different arrangement, in the manner of a cigar, with interspersed layers of keratinocytes and secretory cells desquamated from the glandular epithelium. In this case, the arrangement of the plug possibly helps in the gradual fragmentation of the secretion during the trail formation, modulating friction through the presence of the interspersed keratinocyte layers (Jared et al., 1999). In Ameiva ameiva, on the other hand, we have observed that femoral pores only touch the substrate when the animal rests the internal side of its thighs on the ground. In a resting position, it probably presses the row of femoral pores against the substrate. The frail nature of the secretion, resulting from the juxtaposition of the dead secretion cells, without being interspersed with keratinocytes (as is the case in amphisbaenians) may favor transverse fracturing of the plug and the deposition of secretion tiny portions in the environment. This process may be facilitated by the rosette-like shape and histological arrangement of the pore-bearing scales.

Microscopic examination of the row of femoral pores in Ameiva ameiva shows that some plugs are more prominent than others relative to the skin surface. These differences in plug lengths are an indication of the size of the tiny pieces of secretion deposited on the substrate. The plug deposition system may function each time the animal rests relaxing its legs on the ground. Then, in this species, the method for dispersing the pheromone seems not to consist of the chance breakage of the plug but is much more complex. It involves both morphological characteristics of the rosettes and the structural and mechanical characteristics of the plug itself. These seem to be adaptations for making dispersion more efficient through the constant deposition of tiny portions of secretion every time the animal rests. Although
having its own peculiarities, this method of signal dispersion in A. ameiva, as occurs in amphibians, is intimately dependent on the locomotion of the animal within its territory.

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FEMORAL GLANDS IN THE LIZARD AMEIVA AMEIVA

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