Lipids in the Femoral Gland Secretions of Male Schreiber's Green Lizards, *Lacerta schreiberi*

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In spite of the importance of chemoreception and chemical signals in social organization of lizards, only a few studies have examined the chemical composition of secretions that lizards use for intraspecific communication. The secretion of the femoral glands of male Schreiber's green lizards (*Lacerta schreiberi*) contains 51 lipophilic compounds, including several steroids, α-tocopherol, n-C_9 to n-C_{22} carboxylic acids and their esteres, and minor components such as alcohols between C_{12} and C_{24}, two lactones, two ketones, and squalene. These compounds were identified on the basis of mass spectra, obtained by GC-MS. We compared these chemicals with those found in other lizard species, and discussed how environmental conditions could explain the differential presence of chemicals in different lizards. Particularly, the high abundance of α-tocopherol in this lizard is suggested to contribute to avoid oxidation of other lipids in secretions, increasing chemical stability of scent marks in the humid conditions of its habitat.

Key words: Lacerta schreiberi, Femoral Glands, Lipids, Tocopherol

Introduction

Many lizards use chemoreception in intraspecific communication, social organization and sexual selection (Mason, 1992). These behaviors are often based on pheromonal detection of chemicals secreted by males, *e.g.*, via the femoral glands, whose secretions are under direct androgenic control and vary seasonally with androgen production (Alberts, 1993). The presence and relative concentration of pheromone components seem to vary consistently among individuals (Alberts, 1993), which may convey information on the individual identity and on their characteristics (López *et al.*, 2006). Behavioral experiments indicated that femoral secretions of males may serve a variety of functions, such as rival recognition (Cooper and Vitt, 1987; Aragón *et al.*, 2001; López and Martín, 2002) or female mate choice (Martín and López, 2000; López *et al.*, 2002, 2003; Olsson *et al.*, 2003).

However, in spite of the potential importance of these chemical signals in lizard biology, chemical data for most groups of lizards are not available, but only for a few species of lizards mainly Iguanids (Chauhan, 1986; Alberts, 1990; Weldon *et al.*, 1990; Alberts *et al.*, 1992; Escobar *et al.*, 2001, 2003), and recently some Lacertids (López and Martín, 2005a, b; Martín and López, 2006). These studies have shown that femoral (or the analogous precloacal) gland secretions are composed of both lipids and proteins. Lipids have a high degree of molecular diversity, which increases the potential information content of a pheromone, and are thought to be the main compounds involved in communication (Mason, 1992; López and Martín, 2005c).

The information available on chemical composition of femoral secretions of several lizard species (see above) indicates that although the types of chemicals in secretions are similar (*e.g.* steroids, carboxylic acids and alcohols), the presence of specific compounds and, moreover, the relative proportions of each chemical vary widely between species. This variations may be due to phylogenetic differences between species per se, but it is also likely that, if the persistence of scent marks in the habitat is a requisite for their efficiency, there is selection for the presence of different chemicals with different properties in different habitats (Alberts, 1992; Escobar *et al.*, 2003; Martín and López, 2006). However, to test this hypothesis, we need more studies that deal with a wider range of lizard taxa and consider a larger variety of microhabitat and environmental conditions.

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"Green lizards" are a distinctive clade of Lacertid species (Harris *et al.*, 1998). They are moderate to large and very robust lizards that tend to live in dense vegetation. Adults have often green brillant dorsal colorations. The Schreiber's green lizard (*Lacerta schreiberi*) is a large (until 130 mm of snout-to-vent length) diurnal Lacertid lizard found in the northwest, west and central areas of the Iberian Peninsula (Pérez-Mellado, 1998; Marco, 2002). Adult males are predominantly green with small black spots, and show the head blue during the breeding season. This lizard occupies relatively moist, well vegetated areas, especially broad-leave, woods often close to streams (Salvador, 1988; Pérez-Mellado, 1998). Both males and females are rather sedentary and occupy optimal microhabitats with a great overlap between home ranges (Marco, 1996). Males do not defend territories, but they fight with rival males, and spent long periods of time in physical contact with the female to prevent matings from other males (Marco, 1996; Marco and Pérez-Mellado, 1999).

There are no specific studies on chemosensory detection of conspecific scents in this lizard, but, as in other Lacertid lizards, the vomeronasal organ is well developed and chemoreceptor cells are abundant (Cooper, 1996), tongue-flicking is often observed during social interactions (unpublished observations), and there is a clear sexual dimorphism in femoral pores, *i.e.*, the size of pores and the amount of secretion is higher in adult males and during the mating season; females have vestigial pores without apparent secretion. These data strongly suggests that, as in other Lacertids, *L. schreiberi* should use femoral gland secretions in some aspects of their social life. We report here the results of an analysis by gas chromatography-mass spectrometry (GC-MS) of the lipophilic fraction of femoral secretions of male *L. schreiberi.*

Materials and Methods

We captured by noosing 15 adult male *L. schreiberi* during April and May 2005, from a population occupying different bushy areas close to a mountain stream, inside a pine forest in 'Valle de la Fuenfría' (Guadarrama Mountains, Central Spain). Only adult lizards with intact or fully regenerated tails were considered. Lizards were weighed [body mass: (28 ± 1) g, range: 20–33 g] and their snout-vent length (SVL) was measured [mean ± SE = (105 ± 2) mm, range: 93–115 mm]. Lizards had an average (± SE) of 13.6 ± 0.1 (range: 13–14) femoral pores on each leg. We extracted the femoral pores' secretion by gently pressing with forceps around the femoral pores, and collected the secretion directly in glass vials with Teflon-lined stoppers. Vials were stored at −20 °C until analyses. Lizards were released to their initial sighting location prior to the capture. We also used the same procedure on each sampling occasion but without collecting secretion, to obtain blank control vials that were treated in the same manner to compare with the lizards samples and be able to exclude contaminants from the handling procedure or from the environment, where amphisbaenians were found, and for further examining impurities in the solvent.

Samples were analyzed using a Finnigan-ThermoQuest Trace 2000 gas chromatograph (GC) fitted with a poly(5% diphenyl/95% dimethylsiloxane) column (Supelco, Equity-5, 30 m length × 0.25 mm ID, 0.25-μm film thickness) and a Finnigan-ThermoQuest Trace mass spectrometer (MS) as detector. The samples, 2 ml of each sample dissolved in 250 ml of *n*-hexane (Sigma, capillary GC grade), were injected in splitless mode with an inlet temperature of 250 °C. The gas chromatograph was programmed so that the oven temperature was kept at 50 °C for 10 min, increased to a final temperature of 280 °C at a rate of 5 °C/min, and kept at this temperature for 30 min. The carrier gas was helium at 30 cm/s. Ionization by electron impact (70 eV) was carried out at 280 °C. Mass spectral fragments below m/z = 39 were not recorded. Impurities identified in the solvent and/or the control samples were not reported. Initial identification of secretion components was performed by comparison of sample mass spectra with those in the NIST/EPA/NIH 1998 computerized mass spectral library. When possible, identifications were confirmed by comparison of spectra and retention times with those of authentic standards. Authentic samples were purchased from Aldrich Chemical Co.

Results and Discussion

A total of 51 lipophilic compounds was identified in femoral gland secretions of male *L. schreiberi* (Table I). Steroids (66.7% of TIC), α-tocopherol (18.3%) and carboxylic acids ranging between n-C_9 and n-C_{22} and their esteres (10.9%) were the

Table I. Lipophilic compounds found in femoral secretions of male Schreiber's green lizards, *Lacerta schreiberi*. The relative amount of each component was determined as the percentage of the total ion current (TIC) and reported as the average (± 1 SE) for fifteen individuals. Characteristic ions (*m/z*) are reported for unidentified compounds.

RT[a] [min]	Compound	Mean ± SE	RT[a] [min]	Compound	Mean ± SE
25.3	Nonanoic acid	0.24 ± 0.06	51.8	Docosanoic acid	0.03 ± 0.02
27.9	Decanoic acid	0.28 ± 0.16	53.8	Unidentified??? (179, 291, 403)	0.02 ± 0.01
31.2	Dodecanol	0.22 ± 0.05			
32.9	Dodecanoic acid	1.34 ± 0.19	54.4	Tetracosanol	0.08 ± 0.02
35.9	Tetradecanol	1.22 ± 0.44	55.7	Squalene	0.20 ± 0.04
36.1	2-Pentadecanone	0.17 ± 0.04	56.8	Cholesta-3,5-diene	0.05 ± 0.01
37.4	Tetradecanoic acid	0.15 ± 0.03	50.2	Unidentified tocopherol derivative??? (179, 444)	0.46 ± 0.08
39.4	Pentadecanoic acid	0.06 ± 0.02			
40.1	Hexadecanol	0.08 ± 0.03	59.7	Cholesterol methyl ether	0.78 ± 0.08
40.3	2-Hexadecanone	0.05 ± 0.01	60.8	Cholesterol	6.43 ± 1.24
41.1	Hexadecenoic acid	0.48 ± 0.09	61.0	Cholestanol	12.30 ± 1.54
41.5	Hexadecanoic acid	3.43 ± 0.65	61.1	α-Tocopherol	18.26 ± 1.24
42.3	Heptadecanol	0.49 ± 0.08	61.7	Cholesta-3,5-dien-7-one	0.23 ± 0.03
43.3	Heptadecanoic acid	0.04 ± 0.01	61.9	Cholestan-3-one	0.63 ± 0.07
43.8	Octadecanol	0.34 ± 0.07	62.1	Ergost-22-en-3-ol	1.66 ± 0.09
44.2	4-Hydroxy-hexadecanoic acid γ-lactone	0.19 ± 0.03	63.2	Ergosta-7,22-dien-3-ol	0.31 ± 0.06
			63.5	Campesterol	3.90 ± 0.33
44.7	9,12-Octadecadienoic acid	0.56 ± 0.12	63.7	Ergostanol	16.54 ± 1.56
44.8	Octadecenoic acid	2.11 ± 0.29	64.2	4-Methyl-cholest-7-en-3-ol	0.22 ± 0.04
45.2	Octadecanoic acid	1.25 ± 0.23	64.9	γ-Ergostenol	1.42 ± 0.23
47.6	Eicosatetraenoic acid ethyl ester	0.07 ± 0.02	65.3	3,11-Dihydroxy-pregnan-20-one	0.15 ± 0.04
47.9	4-Hydroxy-octadecanoic acid methyl ester	0.10 ± 0.01	65.6	4,4-Dimethyl-cholest-8(14)-en-3-ol	0.10 ± 0.04
48.1	Eicosanol	0.03 ± 0.01	65.9	γ-Sitosterol	1.39 ± 0.21
48.3	Erucic acid	0.18 ± 0.06	66.3	Unidentified steroid (215, 233, 257, 383, 399, 416)	13.30 ± 2.20
48.6	Eicosanoic acid	0.87 ± 0.14			
50.3	Unidentified??? (113, 195, 307, 322)	0.09 ± 0.03	66.8	20-Methyl-pregn-20-en-3-ol	1.38 ± 0.23
			67.2	4,4-Dimethyl-cholesta-8,14-dien-3-ol	0.21 ± 0.06
51.3	4-Hydroxy-octadecanoic acid γ-lactone	0.16 ± 0.02			
			67.7	Stigmast-7-en-3-ol	5.74 ± 0.45

[a] RT, retention time.

main components, but we found also seven alcohols between C_{12} and C_{24} (2.5%), two lactones (furanones) (0.35%), two ketones (0.22%), squalene (0.20%), and three unidentified, but characteristic compounds (0.6%). Major compounds were detected in all individuals, although relative proportions of some chemicals show a high interindividual variability.

It was surprising to find such a high proportion of α-tocopherol in secretions of this lizard. In addition to α-tocopherol, two other unidentified compounds seemed to be derivatives of this compound based on their characteristic mass spectra. α-Tocopherol was also found, although in much less amounts, in secretions from the Lacertid lizards *Acanthodactylus erythrurus* (0.04%; López and Martín, 2005b) and *Podarcis muralis* (1.9%; Martín and López, 2006), in precloacal secretions of female amphisbaenians, *Blanus cinereus* (0.64%; López and Martín, 2005d), and in gular and paracloacal glands of the American alligator (Weldon *et al.*, 1987, 1988). α-Tocopherol is one of the most abundant forms of vitamin E, which is the main lipophilic antioxidant, both *in vitro* and *in vivo*, and it is involved in membrane defence (Burton and Traber, 1990; Brigelius-Flohe and Traber, 1999). The physiological relevance and the severe pathological consequences of α-tocopherol deficiency, such as neurological disorders or lung diseases, impose a major challenge to animals for sustaining an adequate supply of this vitamin to different tissues (Mardones and Rigotti, 2004), particularly those highly sensitive to α-tocopherol deficiency such as the brain and gonads.

Given all these important functions of α-tocopherol in metabolism, and given that it can only be obtained from the diet, it is likely that its actual function in femoral secretions has to be of enough importance to divert it from metabolism, and that only males with an adequate supply could do it. A possible function of α-tocopherol in secretions might be to inhibit oxidation of other glandular components. This may be explained because *L. schreiberi* is only found in places with high annual rainfall (over 800 mm), high humidity, and low sunshine levels (Marco and Pollo, 1993; Brito *et al.*, 1996). Large amounts of α-tocopherol might contribute to avoid oxidation of other lipids, thus, increase the chemical stability of scent marks in the humid conditions of their habitat (see Alberts, 1992). Similarly, those populations of the Lacertid, that occupy areas characterized by high levels of humidity *Podarcis hispanica*, have femoral secretions with less volatile compounds (*i.e.* more waxy esters and fatty acids of longer chain, which would confer more stability to secretions) than populations occupying more dry areas (Martín and López, 2006).

Ergostanol (16.5%), cholestanol (12.3%) and an unidentified steroid (13.3%), which probably was a derivative of cholestanol, were the main steroids found in femoral secretions of *L. schreiberi.* In contrast, in other rock-dwelling Lacertids, cholesterol was the main steroid, and the main lipid, and it was found in high abundance [*e.g.* 67% in *Lacerta monticola*, and between 52 and 63% in several species of *Podarcis* lizards (López and Martín, 2005a; Martín and López, 2006)]. Cholesterol was also found in *L. schreiberi*, but in considerably lower portions (6.4%). Cholesterol was thought to be useful to constitute an unreactive apolar matrix that delivers the compounds that are the true semiochemicals in some Iguanid lizards (Escobar *et al.*, 2003). However, if this was the function of cholesterol, we could expect that some other compounds might have a similar function in *L. schreiberi*; α-tocopherol might form a matrix that contain other lipids, and protect them from oxidation. Similarly, in *A. erythrurus*, a ground-dwelling species that inhabits xeric sandy habitats, long-chain waxy alcohols found in abundance could give consistence to femoral secretions (López and Martín, 2005b). Nevertheless, the function of α-tocopherol and other compounds in secretions remains to be analyzed properly.

Among the carboxylic acids, hexadecanoic (3.4%), octadecenoic (2.1%), dodecanoic (1.3%) and octadecanoic acids (1.2%) were the most abundant. This is similar to that found in femoral or precloacal glands of other Lacertid (López and Martín, 2005a, b; Martín and López, 2006) and Iguanid lizards (Weldon *et al.*, 1990; Alberts *et al.*, 1992; Escobar *et al.*, 2001). In contrast, similar free alcohols were found in the femoral secretions of *L. schreiberi* and in other Lacertid lizard species (López and Martín, 2005a, b; Martín and López, 2006), but not in femoral secretions of Iguanid lizards (*e.g.* Alberts, 1990; Escobar *et al.*, 2001), although they were reported in extracts from the skin of *I. iguana* (Roberts and Lillywhite, 1980) and of several snakes (see review in Mason 1992). Similarly, other minor componenst, such as squalene, ketones and lactones have been found in Lacertids but not in Iguanids. However, several long-chain saturated and unsaturated methyl-ketones were identified in the skin of female garter snakes acting as sex pheromones (Mason *et al.*, 1989). Thus, it seems that alcohols and other compounds might be characteristic of femoral secretions of Lacertids lizards (Scleroglossa clade), but be absent in other groups (the Iguania clade).

Further studies are clearly needed to reveal the patterns of presence and abundance of different chemicals in femoral secretions of different lizard taxa, to understand how environmental conditions can explain the characteristics of these secretions, and to clarify the possible role of the chemicals found in femoral secretions in social organization and sexual selection processes of lizards.

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