Chemical Compounds from Femoral Gland Secretions of Male Iberian Rock Lizards, *Lacerta monticola cyreni*

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In spite of the importance of chemoreception and chemical signals in the social organization of lizards, there are only a few studies examining the chemical composition of secretions of lizards used for scent marking. The secretion of the femoral glands of male Iberian rock lizards (*Lacerta monticola cyreni*) contains 44 lipophilic compounds, including several steroids (mainly cholesterol), and n-C_6 to n-C_{22} carboxylic acids, and minor components such as esters of carboxylic acids, alcohols, squalene, and one lactone. These compounds were identified on the basis of mass spectra, obtained by GC-MS. Most lipids were detected in all individuals, although relative proportions of each chemical show a high interindividual variability. This variability might be related to the characteristics or physical and health condition of males and might be the basis of female choice based on chemical cues observed in this lizard species.

Key words: Lacerta monticola, Femoral Glands, Fatty Acids, Steroids

Introduction

Chemical cues play an important role in the intraspecific communication of lizards (Mason, 1992; Halpern, 1992). Several studies have shown pheromonal detection in different species, which is often based on precloacal and femoral gland secretions (*e.g.*, Cooper and Vitt, 1984; Alberts, 1993; Aragón *et al.*, 2001b). The femoral pores are epidermal structures on the ventral surface of the thigh of many squamates that have been extensively used in taxonomy, but relatively little is known of its functional significance (Alberts, 1993). They are connected to glands that are formed by an invagination of the stratum germinativum, which forms a follicular unit, and produce copious amounts of holocrine secretion (Mason, 1992). The secretory activity of the femoral glands is greatest in the breeding season, males produce more secretion than females and androgens can influence their development and maintain their activity (Cole, 1966; Van Wyk, 1990; Alberts, 1993). Moreover, the presence and relative concentration of pheromone components seem to vary not only between sexes but also consistently among individuals, which may convey information on the individual identity and serve a variety of functions (Alberts, 1993).

The ventral location of the femoral pores suggests that secretions are passively deposited on the substrate as lizards move through their home ranges, and, therefore, they could advertise residence in a home range, and/or could convey information about social status and competitive ability of the sender (Aragón *et al.*, 2001a, 2003; López and Martín, 2002). Also, femoral pores secretion might transmit chemical information about a male's quality, which may be used by female lizards in their mate choice process (Martín and López, 2000; López *et al.*, 2002, 2003).

In spite of the potential importance of chemical signals in lizard intraspecific relationships, only a few studies have analyzed the chemical composition of these secretions in a few species of lizards mainly Iguanids (Chauhan, 1986; Alberts, 1990; Weldon *et al.*, 1990; Alberts *et al.*, 1992; Escobar *et al.*, 2001, 2003). Chemical data for most groups of lizards are not available, but only for skin semiochemicals of a few species (Weldon and Bangall, 1987; Mason and Gutzke, 1990). 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 increase the potential information content of a pheromone, and are thought to be the main compounds involved in communication.

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The Iberian rock lizard (*Lacerta monticola*) is a small diurnal lacertid lizard found mainly in rocky habitats of some high mountains of the Iberian Peninsula (Pérez-Mellado, 1998). The subspecies *L. m. cyreni* occurs in the Central System (Guadarrama and Gredos Mountains, Central Spain). Male *L. monticola* deposit faecal pellets and femoral secretions on specific sites (López *et al.*, 1998; Aragón *et al.*, 2000, 2001a). Differential tongue flick rates to chemicals presented on cotton swabs and on substrate deposits demonstrated that male lizards can detect and discriminate between self-produced scents from the femoral pores and those of other conspecific males, and between odours of familiar and unfamiliar males (Aragón *et al.*, 2000, 2001a, b). Thus, scent marked substrates may probably function in home range advertisement, both for other males (López *et al.*, 1998; Aragón *et al.*, 2001a) and for females (Martín and López, 2000; López *et al.*, 2002, 2003). As a preliminary step to understand the function of chemical compounds in intraspecific communication of this lizard, we report here the results of an analysis by gas chromatography – mass spectrometry (GC-MS) of the lipidic fraction of femoral secretions of male lizards *L. monticola.*

Materials and Methods

We captured by noosing 15 adult male *L. monticola* during May 2003, which coincided with the mating season of lizards, in different places over a large area ("Puerto de Navacerrada", Guadarrama Mountains, Central Spain). Lizards were weighed (body mass: 7.6 ± 0.2 g, range: 6.7 – 8.3 g) and their snout-vent length (SVL) was measured (mean ± SE: 74.1 ± 0.3 mm, range: 73 – 76 mm). Lizards had an average (± SE) of 19.3 ± 0.3 (range: 17 – 22) 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. 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 lizard samples. Lizards were released to their initial sighting location prior to the capture. Before the analyses we added 250 μl of *n*-hexane (Sigma, capillary GC grade) to each vial.

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 μl of each sample dissolved in *n*-hexane) were injected in the splitless mode. The gas chromatograph was programmed so the oven temperature was kept at 50 °C for 10 min, then increased to a final temperature of 280 °C at a rate of 5 °C/min, and kept at this temperature for 30 min. Mass spectral fragments below $m/z = 39$ were not recorded. The relative amount of each component was determined as the percentage of the total ion current (TIC) and reported as the average for all individuals (± standard error). Impurities identified in the solvent and/or the control vial samples are not reported. Initial identification of secretion components was done by comparison of mass spectra with those from 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 44 lipophilic compounds were identified in femoral gland secretions of *L. monticola* (Table I). Steroids (84.6%) and carboxylic acids (14.1%) and their esteres (0.5%) were the main components, but we found also alcohols (0.4%), squalene (0.3%) and one lactone (0.06%). Most compounds were detected in all individuals, although relative proportions of some chemicals show a high interindividual variability.

Cholesterol was the main lipid found in all secretions (ranged between 58 and 77% of total TIC area). Cholesterol was also found in abundance in other lizards, both in the skin (Weldon and Bangall, 1987; Mason and Gutzke, 1990), and in femoral and precloacal gland secretions (Alberts *et al.*, 1992; Escobar *et al.*, 2001). The abundance and ubiquity of this component in secretions was thought to be useful to constitute an unreactive apolar matrix that delivers the compounds that are the true semiochemicals (Escobar *et al.*, 2003).

Table I. Lipophilic compounds found in femoral secretions of male Iberian rock lizards, *Lacerta monticola*. The relative amount of each component was determined as the percentage of the total ion current (TIC) and reported as the average (± SE) for fifteen individuals.

RT[a] [min]	Compound	Mean ± SE
20.4	Hexanoic acid	0.01 ± 0.01
22.2	Octanoic acid	0.01 ± 0.01
25.3	Nonanoic acid	0.10 ± 0.01
27.7	Decenoic acid methyl ester	0.02 ± 0.01
27.9	Decanoic acid	0.05 ± 0.01
32.9	Dodecanoic acid	0.48 ± 0.05
34.4	Dodecanoic acid methylethyl ester	0.02 ± 0.01
37.4	Tetradecanoic acid	0.03 ± 0.01
38.8	Tetradecanoic acid methylethyl ester	0.01 ± 0.01
39.4	Pentadecanoic acid	0.12 ± 0.10
41.1	Hexadecenoic acid	0.20 ± 0.02
41.4	Hexadecanoic acid	5.51 ± 0.84
42.1	Hexadecanoic acid ethyl ester	0.42 ± 0.38
43.3	Heptadecanoic acid	0.65 ± 0.59
43.8	Octadecanol	0.07 ± 0.01
44.2	4-Hydroxy-hexadecanoic acid γ-lactone	0.06 ± 0.01
44.7	9,12-Octadecadienoic acid	0.58 ± 0.08
44.8	Octadecenoic acid	3.98 ± 0.40
45.2	Octadecanoic acid	1.73 ± 0.17
45.5	Oleic Acid	0.29 ± 0.02
47.4	Eicosanol	0.12 ± 0.02
47.6	Eicosatetraenoic acid methyl ester	0.03 ± 0.01
48.6	Eicosanoic acid	0.28 ± 0.04
50.7	Docosanol	0.11 ± 0.02
51.8	Docosanoic acid	0.09 ± 0.01
53.8	Tetracosanol	0.07 ± 0.01
55.7	Squalene	0.30 ± 0.03
56.4	Cholesta-2,4-diene	0.07 ± 0.01
56.5	Cholesta-4,6-dien-3-ol	0.09 ± 0.01
56.7	Hexacosanol	0.06 ± 0.01
56.8	Cholesta-3,5-diene	0.17 ± 0.01
60.9	Cholesterol	67.01 ± 1.48
61.7	Cholesta-5,7-dien-3-ol	0.14 ± 0.02
61.9	Ergosta-5,22-dien-3-ol	0.15 ± 0.02
62.8	Ergosterol	1.02 ± 0.14
63.2	Stigmasta-5,24(28)-dien-3-ol	0.96 ± 0.14
63.4	Campesterol	5.33 ± 0.22
64.5	Ergosta-5,8-dien-3-ol	2.29 ± 0.24
65.2	Lanost-8-en-3-ol	0.65 ± 0.05
65.6	2,2-Dimethyl-cholest-8(14)-en-3-ol	0.08 ± 0.01
65.9	γ-Sitosterol	1.88 ± 0.08
66.5	24-Propylidene-cholest-5-en-3-ol	0.38 ± 0.04
66.8	4,4-Dimethyl-cholest-7-en-3-ol	0.13 ± 0.01
67.2	4,4-Dimethyl-cholesta-5,7-dien-3-ol	4.25 ± 0.34

[a] RT: Retention time.

We also found other steroids in significant quantities, such as campesterol, ergosta-dien-ol, γ-sitosterol, and 4,4-dimethyl-cholesta-5,7-dien-3-ol. It is interesting the presence of cholesta-5,7-dien-3-ol (= dehydrocholesterol) in femoral secretions of *L. monticola*. This is a precursor of vitamin D_3, and is often found in the skin, where it will transform into vitamin D_3 after exposition to sun UVB irradiation (Carman *et al.*, 2000). Vitamin D_3 seems essential in calcium metabolism. Thus, the provitamin excreted in the femoral secretions has to be deviated from general metabolism. All male *L. monticola* examined presented this steroid, but proportions varied greatly between individuals (range: 0.05–0.31%; CV: 53.3%). It is likely that the relative abundance of this provitamin that a male could allocate in femoral secretions was related to his physical or health condition. Thus, the amount of cholesta-5,7-dien-3-ol in secretions might indicate "quality" to conspecifics, if these could actually detect it in the scent marks. This might be the basis of female choice of scent of males of "high quality" observed in this lizard species (Martín and López, 2000; López *et al.*, 2002, 2003), but further experiments are clearly needed to test this hypothesis.

Carboxylic acids found in femoral secretions of *L. monticola* ranged between n-C_6 and n-C_{22}, with hexadecanoic, octadecenoic and octadecanoic being the most abundant. Most carboxylic acids, except hexanoic, octanoic and pentadecanoic acids, were detected in all individuals, although relative proportions varied between individuals. Similar long chain carboxylic acids have also been found in other lizards as common and major constituents of skin and femoral and precloacal glands (Mason and Gutzke, 1990; Weldon *et al.*, 1990; Alberts *et al.*, 1992; Escobar *et al.*, 2001). In these studies the range in the number of carbon atoms in carboxylic acids varied between species (*e.g.* n-C_{14} to n-C_{24} in *I. iguana*, or n-C_3 to n-C_{26} in *Liolaemus* spp.). Presumably this would reflect the environment where each lizard species lives (Alberts, 1992; Escobar *et al.*, 2001, 2003). Thus, it might be expected that under higher environmental temperatures, lizards had a larger proportion of compounds of lower volatility and higher chemical stability (Alberts, 1992; Escobar *et al.*, 2001, 2003). Low temperatures in the high altitude habitat of *L. monticola* would allow them to use more volatile carboxylic acids than, for example, *I. iguana* do in the tropics.

We also observed free alcohols in the femoral secretions of *L. monticola* ranging from 18 to 26 carbon atoms. Alcohols have not been reported previously from secretions of any lizard, although they were reported in extracts from the skin of *I. iguana* (Roberts and Lillywhite, 1980) and in the skin of several snakes (see review in Mason, 1992). Alcohols are, however, abundant in paracloacal gland secretions of crocodiles (*e.g.*, Dunn *et al.*, 1993; García-Rubio *et al.*, 2002). Finally, one terpenoid, squalene, was found in secretions of *L. monticola*, and also in the skin of male and female leopard geckos (Mason and Gutzke, 1990), skin of male garter snakes (Mason *et al.*, 1989), and paracloacal glands of crocodiles (García-Rubio *et al.*, 2002), but not in gland secretions of Iguanid lizards (Weldon *et al.*, 1990; Escobar *et al.*, 2001). Thus, it remains possible that squalene and alcohols might be characteristic of some families of lizards (those in the Scleroglossa clade), snakes and crocodiles, but be absent in others groups (the Iguania clade), but more studies with more reptile species are needed prior to any comparative study.

Alkanes have been detected in skin and precloacal secretions of other lizards (Mason and Gutzke, 1990; Escobar *et al.*, 2001). We also detected some *n*-alkanes in our lizard samples, but they were also present in blank control vials, and, thus, we could not ensure that they were not contaminants, and were excluded from the analyses. Nevertheless, some alkanes found in significant quantities in lizard samples (*e.g.*, docosane and nonacosane) might come also from the femoral secretions or the skin surrounding the femoral pores of *L. monticola.*

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