Biochemical Systematics and Ecology 37 (2009) 304–307

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Lipids in femoral gland secretions of male lizards, *Psammodromus hispanicus*

Pilar López*, José Martín

Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, CSIC, José Gutiérrez Abascal 2, 28006 Madrid, Spain

ARTICLE INFO

Article history:
Received 15 January 2009
Accepted 17 May 2009

Keywords:
Psammodromus hispanicus
Femoral glands
Fatty acids
Steroids
Lizards
Lacertidae

ABSTRACT

Many lizards produce chemical secretions that may be used as pheromones in reproductive behavior, but only a few studies have identified chemical compounds in secretions. By using GC–MS, we found only 20 lipophilic compounds in femoral glands secretion of male lizards, *Psammodromus hispanicus*. Main compounds were six steroids (mainly cholesterol and campesterol) and seven n-C_9 to n-C_{18} carboxylic acids (especially dodecanoic acid), and minor components were six alcohols between C_{16} and C_{29} and squalene. We compared these chemicals with those previously found in secretions of the closely related sister species *Psammodromus algirus* and other lizard species.

1. Introduction

Chemical signals (pheromones) are important in reproductive behavior of many reptiles (Mason, 1992). Males of many lizard species produce during the reproductive season copious amounts of holocrine chemical secretion from the femoral glands (Alberts, 1993; Weldon et al., 2008). Behavioral studies indicate that femoral secretions function in intraspecific communication, signaling the characteristics and health state of males (López et al., 2006; Martín et al., 2007a), which may be useful in female mate choice (Martín and López, 2000, 2006a,d; López et al., 2002, 2003b; Olsson et al., 2003; López and Martín, 2005c) and intrasexual relationships between males (Carazo et al., 2007; Martín et al., 2007b).

However, chemicals that may function as pheromones of lizards are only known for a few species (reviewed in Weldon et al., 2008). Lipids seem to play a major role in pheromonal communication (Mason, 1992; Martín and López, 2006a). Major classes of lipophilic compounds in secretions are similar between lizard species (e.g., steroids, carboxylic acids and alcohols, among others), but the presence and abundance of specific compounds and the total number of compounds are characteristic of each species, and show a high interspecific variation (Weldon et al., 2008). Also, the average number of femoral glands is characteristic of each species and varies widely between species. The relative importance of chemical communication might affect both the amount of secretion produced, which might depend on the number of femoral glands, and the diversity of lipids. Interspecific variation may be due to phylogenetic differences, but it might also have an environmental component if the persistence of scent marks in different habitats required different chemicals with different properties (Alberts, 1992; Escobar et al., 2003; Martín and López, 2006b).

In Europe, there are two closely related species of Lacertid lizards within the Genus *Psammodromus*. The large psammodromus (*Psammodromus algirus*) is a medium sized (until 9 cm of snout-to-vent length) lizard inhabiting Mediterranean forests of the Iberian Peninsula and Northwest Africa (Böhme, 1981). Lizards become sexually mature in their second spring

* Corresponding author. Tel.: +34 91 411 1328; fax: +34 91 564 5078.
E-mail address: pilar.lopez@mncn.csic.es (P. López).

0305-1978/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bse.2009.05.006

and can reproduce during several years, showing ontogenetic changes in males' reproductive strategies (Pérez-Quintero, 1996; Salvador and Veiga, 2001; López et al., 2003a). Secretions of males contain 59 lipophilic compounds, mainly carboxylic acids between C_9 and C_{20}, and steroids (mainly campesterol, ergosterol and cholesterol), and minor components such as alcohols, aldehydes, squalene, α-tocopherol, ketones, and a furanone (Martín and López, 2006c). Chemoreception seems very important in social behavior of *P. algirus* (López et al., 2003a; Martín et al., 2007a).

In contrast, the Spanish psammodromus (*Psammodromus hispanicus*) is a very small (about 5 cm of snout-to-vent length) diurnal lizard, almost restricted to the Iberian Peninsula (Salvador, 1981). It occupies littoral sand hills or degradated garriges with patchy covering of dense humid grassland and low bushes. Lizards acquire sexual maturity at an age of 8 months and reproduce only once in their life (Pascual González and Pérez-Mellado, 1989; Pérez-Quintero, 1996). The role of chemical signals in social behavior of this species is unknown, but the low tongue-flick rates during foraging showed that lizards can recognize prey chemicals but have a low use of this ability (Verwaijen and Van Damme, 2007).

In this paper, we report the results of an analysis by gas chromatography–mass spectrometry (GC–MS) of the lipophilic fraction of femoral secretions of male Spanish psammodromus lizards (*P. hispanicus*) from a Central Spanish population occupying humid grasslands. We compared the current data on femoral secretions of *P. hispanicus* with previous data on the related *P. algirus* (Martín and López, 2006c) and other lizard species (Weldon et al., 2008).

2. Materials and methods

We captured by hand 15 adult male *P. hispanicus* during May 2007, from a population in the region of "Campo Azálvaro", close to El Espinar (Segovia province, Central Spain; 40°40′N, 4°20′W, 1300 m a.s.l.). The habitat is extensive montane grassland dominated by large *Stipa gigantea* perennial herbs and other small annual or semiperennial herbs. Only adult males with intact or fully regenerated tails were considered. Lizards were weighed (body mass, mean $\pm$ SE $=$ 3.1 $\pm$ 0.5 g, range $=$ 2.8–4 g) and their snout-vent length (SVL) was measured (mean $\pm$ SE $=$ 50 $\pm$ 1 mm, range $=$ 50–52 mm), and the number of femoral pores of both legs counter under a magnifying glass.

Immediately after capture in the field, we extracted secretion from femoral glands of male lizards by gently pressing with forceps around the femoral pores, and collected secretion directly with glass vials, later closed 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 lizards samples, and be able to exclude contaminants from the handling procedure or from the environment where lizards were found, and for further examining impurities in the solvent. Thereafter, lizards were immediately released to their exact capture sites.

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 $\times$ 0.25 mm ID, 0.25-μm film thickness) and a Finnigan-ThermoQuest Trace mass spectrometer (MS) as detector. Sample injections (2 μl of each sample dissolved in *n*-hexane) were performed in splitless mode using helium as the carrier gas, with injector and detector temperatures at 270 °C and 250 °C, respectively. The oven temperature program was as follows: 50 °C isothermal for 10 min, then increased to 280 °C at a rate of 5 °C/min, and then isothermal (280 °C) for 30 min. Mass spectral fragments below $m/z = 39$ were not recorded. 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 in the NIST/EPA/NIH 1998 computerized mass spectral library. All identifications were confirmed by comparison of spectra and retention times with those of authentic standards. Authentic samples were purchased from Sigma–Aldrich Chemical Co.

3. Results

Male lizards *P. hispanicus* had an average ($\pm$SE) of 10 $\pm$ 1 (range $=$ 9–11) femoral pores on each leg that produce secretions. Females in the same population also had a similar number (range $=$ 9–11) of smaller vestigial femoral pores but these pores did not produce any visible secretion.

We found and identified only 20 lipophilic compounds in femoral gland secretions of male *P. hispanicus* (Table 1). The main components were six steroids (72.6% of TIC) and seven carboxylic acids ranged between n-C_9 and n-C_{18} (23.5%), but we also found six alcohols between C_{16} and C_{29} (3.8%), and squalene (0.1%). Major compounds were detected in all individuals, although relative proportions of some chemicals show interindividual variability. On average, the two most abundant chemicals were cholesterol (31.5% of TIC) and campesterol (25.6%), followed by dodecanoic acid (10.2%) and stigmast-7-en-3-ol (9.6%).

4. Discussion

The low number of compounds found in *P. hispanicus* contrasts with other lacertid lizards, including the closely related *P. algirus* (i.e., compounds ranged between 44 and 62; López and Martín, 2005a,b, 2006; Martín and López, 2006b,c). However, a similar low number (18 compounds) was found in the distantly related *Lacerta vivipara*, a species that also has few femoral pores (mean $=$ 10 pores/leg) (Gabirot et al., 2008). It is possible that scent marks are of little importance in *P. hispanicus* and *L. vivipara* because these lizards live in meadows and grasslands where persistence of scent marks would be very

Table 1
Lipophilic compounds found in femoral secretions of male lizards, *Psammodromus hispanicus*.

Number in Fig. 1	RT [min]	Compound	mean ± 1SE
1	25.3	Nonanoic acid	1.15 ± 0.37
2	32.9	Dodecanoic acid	10.16 ± 1.80
3	37.4	Tetradecanoic acid	0.24 ± 0.05
4	40.1	Hexadecanol	0.12 ± 0.03
5	41.5	Hexadecanoic acid	3.23 ± 1.32
6	43.9	Octadecanol	0.82 ± 0.15
7	44.7	9,12 Octadecadienoic acid	2.90 ± 0.41
8	44.8	Octadecenoic acid	2.71 ± 0.37
9	45.2	Octadecanoic acid	3.16 ± 1.22
10	50.8	Docosanol	0.27 ± 0.11
11	53.8	Tetracosanol	1.34 ± 0.13
12	55.7	Squalene	0.09 ± 0.01
13	56.7	Hexacosanol	1.06 ± 0.45
14	60.1	Nonacosanol	0.18 ± 0.05
15	60.8	Cholesterol	31.50 ± 5.64
16	63.3	Ergosta-7,22-dien-3-ol	3.61 ± 1.20
17	63.4	Campesterol	22.94 ± 4.52
18	64.9	Ergost-7-en-3-ol	2.73 ± 0.82
19	66.0	Sitosterol	2.17 ± 1.10
20	67.8	Stigmast-7-en-3-ol	9.61 ± 3.32

The relative amount of each component was determined as the percent of the total ion current (TIC) and reported as the average (±1SE) for fifteen individuals.

low (Alberts, 1992). Nevertheless, chemical secretions may still be important in short distance interactions (López and Martín, 2002; Martín and López, 2007).

Similarly to other lizards, femoral gland secretions of *P. hispanicus* have carboxylic acids and steroids as predominant components (reviewed in Weldon et al., 2008). Among steroids, cholesterol was the main steroid, which was also found in abundance in secretions of most lizard species (Weldon et al., 2008). Campesterol, a steroid of vegetal origin that has to be obtained from the diet, is another major steroid in secretions of *P. hispanicus*. This might be characteristic of psammodromus lizards because campesterol is also the most common steroid in the related *P. algirus* (Martín and López, 2006c).

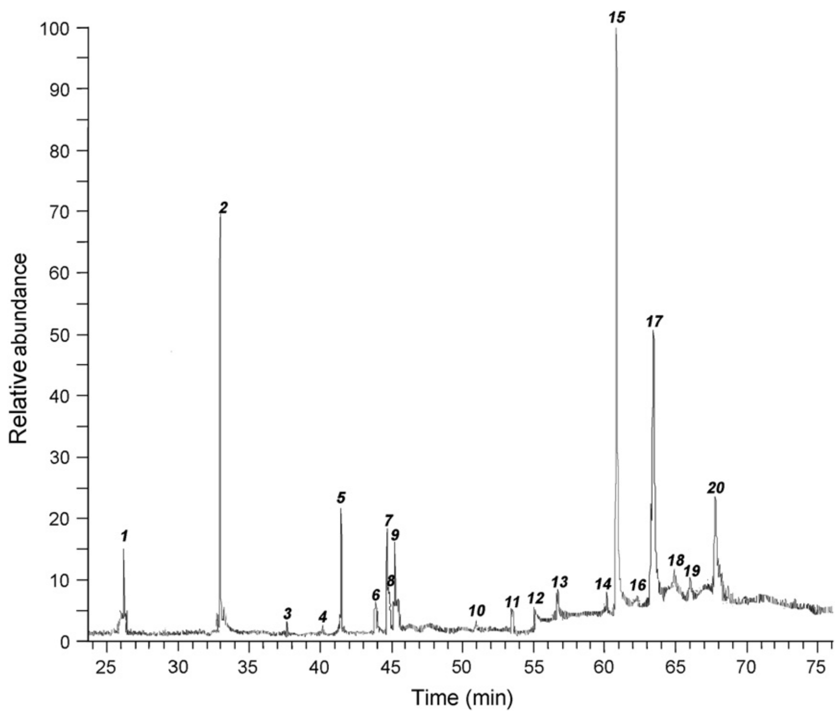

Fig. 1. Representative total ion chromatogram (GC–MS) of an extract of femoral gland secretion of a male *P. hispanicus*. Numbers on each peak indicate compounds listed in Table 1.

Carboxylic acids found in *P. hispanicus* have a low number of carbons and should be highly volatile and, thus, might be mainly used in short-distance communication. Especially, it is noteworthy the high abundance of dodecanoic acid. In contrast, hexadecanoic, octadecanoic and octadecenoic acids are the most abundant ones in most lacertids (López and Martín, 2005a,b, 2006; Martín and López, 2006b,c). Other compounds, such as alcohols and squalene, are found in very low proportions in secretions of *P. hispanicus*. In other lizards, squalene is considered to limite oxidation of other lipids (Gabirot et al., 2008). A similar function was suggested for tocopherol and waxy esters in other lizards inhabiting grassy and humid areas (López and Martín, 2006; Martín and López, 2006b; Gabirot et al., 2008). Tocopherol and waxy esters were not found in *P. hispanicus*, suggesting that avoiding lipid oxidation in scent marks may be difficult or unimportant for this lizard. Finally, it is also noteworthy the absence of other compounds that appear "regularly" in other lizards, including the related *P. algirus*, such as aldehydes, ketones, or furanones (Martín and López, 2006c; Weldon et al., 2008).

We suggest that phylogenetic affinities alone might not explain the compounds found in secretions, and that environmental and social organization factors might also influence the composition of femoral secretions. Further studies are clearly needed to understand the patterns of presence and abundance of different chemicals in femoral secretions of lizards.

Acknowledgements

We thank three anonymous reviewers for helpful comments, and "El Ventorrillo" MNCN Field Station for use of their facilities. Financial support was provided by the projects MEC-CGL2005-00391/BOS and MCI-CGL2008-02119/BOS. Captures were performed under license from the Castilla y León Environmental Agency.

References

Alberts, A.C., 1992. Constraints on the design of chemical communication systems in terrestrial vertebrates. Am. Nat. 139, S62–S89.
Alberts, A.C., 1993. Chemical and behavioral studies of femoral gland secretions in iguanid lizards. Brain Behav. Evol. 41, 255–260.
Böhme, W., 1981. *Psammodromus algirus* (Linnaeus, 1758) – Algerischer Sandläufer. In: Böhhme, W. (Ed.), Hanbuch der Reptilien und Amphibien Europas, vol. 1/1. Akademische Verlagsgeg, Wiesbaden, Germany, pp. 479–491.
Carazo, P., Font, E., Desfilis, E., 2007. Chemosensory assessment of rival competitive ability and scent mark function in a lizard (*Podarcis hispanica*). Anim. Behav. 74, 895–902.
Escobar, C.M., Escobar, C.A., Labra, A., Niemeyer, H.M., 2003. Chemical composition of precloacal secretions of two *Liolaemus fabiani* populations: are they different? J. Chem. Ecol. 29, 629–638.
Gabirot, M., López, P., Martín, J., de Fraipont, M., Heulin, B., Sinervo, B., Clobert, J., 2008. Chemical composition of femoral secretions of oviparous and viviparous types of male common lizards *Lacerta vivipara*. Biochem. Syst. Ecol. 36, 539–544.
López, P., Martín, J., 2002. Chemical rival recognition decreases aggression levels in male Iberian wall lizards, *Podarcis hispanica*. Behav. Ecol. Sociobiol. 51, 461–465.
López, P., Martín, J., 2005a. Chemical compounds from femoral gland secretions of male Iberian rock lizards, *Lacerta monticola cyreni*. Z. Naturforsch. C 60, 632–636.
López, P., Martín, J., 2005b. Age related differences in lipophilic compounds found in femoral gland secretions of male spiny-footed lizards, *Acanthodactylus erythrurus*. Z. Naturforsch. C 60, 915–920.
López, P., Martín, J., 2005c. Female Iberian wall lizards prefer male scents that signal a better cell-mediated immune response. Biol. Lett. 1, 404–406.
López, P., Martín, J., 2006. Lipids in the femoral gland secretions of male Schreiber's green lizards, *Lacerta schreiberi*. Z. Naturforsch. C 61, 763–768.
López, P., Muñoz, A., Martín, J., 2002. Symmetry, male dominance and female mate preferences in the Iberian rock lizard, *Lacerta monticola*. Behav. Ecol. Sociobiol. 52, 342–347.
López, P., Martín, J., Cuadrado, M., 2003a. Chemosensory cues allow male lizards *Psammodromus algirus* to override visual concealment of sexual identity by satellite males. Behav. Ecol. Sociobiol. 54, 218–224.
López, P., Aragón, P., Martín, J., 2003b. Responses of female lizards, *Lacerta monticola*, to males' chemical cues reflect their mating preference for older males. Behav. Ecol. Sociobiol. 55, 73–79.
López, P., Amo, L., Martín, J., 2006. Reliable signaling by chemical cues of male traits and health state in male lizards, *Lacerta monticola*. J. Chem. Ecol. 32, 473–488.
Martín, J., López, P., 2000. Chemoreception, symmetry and mate choice in lizards. Proc. R. Soc. Lond. B 267, 1265–1269.
Martín, J., López, P., 2006a. Links between male quality, male chemical signals, and female mate choice in Iberian rock lizards. Funct. Ecol. 20, 1087–1096.
Martín, J., López, P., 2006b. Interpopulational differences in chemical composition and chemosensory recognition of femoral gland secretions of male lizards *Podarcis hispanica*: implications for sexual isolation in a species complex. Chemoecology 16, 31–38.
Martín, J., López, P., 2006c. Age-related variation in lipophilic chemical compounds from femoral gland secretions of male lizards *Psammodromus algirus*. Biochem. Syst. Ecol. 34, 691–697.
Martín, J., López, P., 2006d. Vitamin D supplementation increases the attractiveness of males' scent for female Iberian rock lizards. Proc. R. Soc. Lond. B 273, 2619–2624.
Martín, J., López, P., 2007. Scent may signal fighting ability in male Iberian rock lizards. Biol. Lett. 3, 125–127.
Martín, J., Civantos, E., Amo, L., López, P., 2007a. Chemical ornaments of male lizards *Psammodromus algirus* may reveal their parasite load and health state to females. Behav. Ecol. Sociobiol. 62, 173–179.
Martín, J., Moreira, P.L., López, P., 2007b. Status-signalling chemical badges in male Iberian rock lizards. Funct. Ecol. 21, 568–576.
Mason, R.T., 1992. Reptilian pheromones. In: Gans, C., Crews, D. (Eds.), Biology of the Reptilia, vol. 18. University of Chicago Press, Chicago, pp. 114–228.
Olsson, M., Madsen, T., Nordby, J., Wapstra, E., Ujvari, B., Wittsell, H., 2003. Major histocompatibility complex and mate choice in sand lizards. Proc. R. Soc. Lond. B 270 (Suppl.), 254–256.
Pascual González, J.A., Pérez-Mellado, V., 1989. Datos sobre la reproducción y el crecimiento de *Psammodromus hispanicus* Fitzinger, 1826 en un medio adehesado de la España Central. Doñana. Acta Vertebrata 16, 45–55.
Pérez-Quintero, J.C., 1996. Reproductive characteristics of three Mediterranean lizards: *Psammodromus algirus* (L.), *Psammodromus hispanicus* Fitzinger and *Acanthodactylus erythrurus* (Schinz). Amphib.-Rept 17, 197–208.
Salvador, A., 1981. *Psammodromus hispanicus* Fitzinger, 1826-Iberischer Sandläufer. In: Böhhme, W. (Ed.), Hanbuch der Reptilien und Amphibien Europas, vol. 1/1. Akademische Verlagsgeg, Wiesbaden, Germany, pp. 492–502.
Salvador, A., Veiga, J.P., 2001. Male traits and pairing success in the lizard *Psammodromus algirus*. Herpetologica 57, 77–86.
Verwaijen, D., Van Damme, R., 2007. Relationships between chemosensory behaviour and foraging mode within lacertid lizards. Behaviour 144, 83–99.
Weldon, P.J., Flachsbarth, B., Schulz, S., 2008. Natural products from the integument of nonavian reptiles. Nat. Prod. Rep. 25, 738–756.