Biochemical Systematics and Ecology 64 (2016) 105–110

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

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

Interspecific differences in chemical composition of femoral gland secretions between two closely related wall lizard species, *Podarcis bocagei* and *Podarcis carbonelli*

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ARTICLE INFO

Article history:
Received 25 May 2015
Received in revised form 14 November 2015
Accepted 21 November 2015
Available online xxx

Keywords:
Chemical signals
Chemoreception
Podarcis species complex
Lizards
Steroids
Waxy esters

ABSTRACT

Chemical signals play an important role in intraspecific communication and social organization of many animals, but they also may be useful in interspecific recognition. In lizards, chemical signals are often contained in femoral gland secretions, of which composition may vary between species and populations. This may be especially important in recognition and reproductive isolation between closely related species. We analyzed by gas chromatography–mass spectrometry (GC–MS) the lipophilic fraction of femoral gland secretions of two closely related wall lizard species, *Podarcis bocagei* and *Podarcis carbonelli* to test for possible interspecific differences in chemical composition. We found 56 lipophilic compounds in femoral gland secretions of male *P. bocagei* and 60 in *P. carbonelli*. The main compounds were steroids and waxy esters, but we also found carboxylic acids and their esters, alcohols, amydes, aldehydes, squalene, ketones and furanones. There were significant differences between species with respect to the number and relative proportions of compounds. Differences in chemical composition might be a consequence of phylogenetic differences per se, but they could also be explained by ecological adaptation to different microclimatic conditions. These differences in chemical profiles may explain the known chemosensory interspecific recognition between these two lizards, contributing to their reproductive isolation.

1. Introduction

Several groups of animals have chemosensory systems that are used to detect different chemical cues involved in prey and predator detection, social organization or mate choice (Mason, 1992; Wyatt, 2014) and also in species recognition (Mas and Jaillon, 2005). Concretely, chemical ecology plays an important role in lizards because chemical signals may mediate in intra and interspecific communication (Cooper and Perez-Mellado, 2002; Weldon et al., 2008; Martín and López, 2011, 2014). Specific compounds secreted by lizards are essential in mate recognition and mate assessment and territoriality (Martín and López, 2007). In addition to this, chemoreception may be especially relevant in lizard species recognition, acting sometimes as a prezygotic barrier that may preclude reproduction between syntopic species (Gabirot et al., 2012).

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http://dx.doi.org/10.1016/j.bse.2015.11.012

In lizards, chemical cues are usually produced by males through secretions via femoral, preanal or/and postanal glands, secretion being particularly abundant during the reproductive season (Mason, 1992; Alberts et al., 1992). Proteins and lipids are the main compounds in these gland secretions and may differ thoroughly intraspecific and interspecifically (Weldon et al., 2008), probably due to environmental and phylogenetic factors (Martín and López, 2013). However, research on chemical composition of lizards' secretion and the potential information conveyed by these chemicals is relatively scarce and restricted to a few phylogentic groups (see Weldon et al., 2008; Martín and López, 2014).

In spite of recent research focused on interspecific interactions in lizards, much of the studies on this topic have only been carried out on a small number of lizard groups, for example in *Eumeces* skinks (Cooper and Vitt, 1987), tropidurid lizards of the genus *Liolaemus*, or lacertid lizards of the genus *Podarcis* (Gabirot et al., 2012). Recent molecular studies have demonstrated the complexity of the '*Podarcis hispanica*' lizard species complex (Kaliontzopoulou et al., 2012; Geniez et al., 2014), their phylogenetic relationship and underlying evolutionary processes still remaining unclear. Pinho et al. (2007) suggested that *Podarcis bocagei, Podarcis carbonelli*, *P. hispanica* and *Podarcis vaucheri* suffered a fast diversification in their evolutionary history. Concretely, the Bocage's wall lizard (*P. bocagei*) and the Carbonell's wall lizard (*P. carbonelli*) are two closely related diurnal lizard species of medium size, inhabiting western parts of the Iberian Peninsula. Some years ago, *P. carbonelli* was considered a subspecies of *P. bocagei* (Pérez-Mellado, 1981). Nevertheless, molecular studies based on morphologic and genetic data showed *P. carbonelli* as a distinct species supporting its specific status (Harris and Sá-Sousa, 2001, 2002). Recent research provides phylogenetic relationships showing both species as not being sister taxa (Kaliontzopoulou et al., 2012).

These two wall lizard species are distributed in sympatry with other *Podarcis* spp. e.g. *P. bocagei* with *P. hispanica* type 1 (i.e. *Podarcis guadarramae*), and *P. carbonelli* with *P. hispanica* type 1 (*P. guadarramae*) and 2 (*Podarcis virescens*) (Sá-Sousa, 2001; Harris and Sá-Sousa, 2001; Geniez et al., 2014). Moreover, there exist some contact zones between *P. bocagei* and *P. carbonelli* (Carretero et al., 2002) and some cases of hybridization have been reported (Galán, 2002; Pinho et al., 2007). In relation to this, the ability to discriminate conspecific from heterospecific chemical cues may be essential for lizards with syntopic and closely related species. Cooper and Perez-Mellado (2002) analyzed the existence of interspecific recognition in *P. hispanica* (*sensu lato*), reporting the ability of males to distinguish among conspecific females and *P. carbonelli* sympatric females based on chemical cues alone. Subsequent behavioral studies demonstrated reciprocal chemosensory discrimination between *P. bocagei* and *P. carbonelli* (Barbosa et al., 2005) and between *P. bocagei* and *P. hispanica* type 1 (Barbosa et al., 2006). However, no attention has been paid to chemical composition of lizards' secretions in these studies. Nevertheless, chemical characterizations of femoral secretions of other *Podarcis* lizard species have been described in recent works (Gabirot et al., 2012) showing that steroids (mainly cholesterol) are predominant over other compounds such as fatty acids, alcohols, etc.

In the present paper, with the goal to understand the functions of chemical signals in interspecific communication and reproductive isolation in these closely related lizard species, we report the lipophilic compounds found in femoral gland secretions of male *P. bocagei* and *P. carbonelli* using gas chromatography–mass spectrometry (GC–MS). We specifically tested the existence of interspecific differences in chemical composition of secretions, which could explain the previously known chemosensory recognition between these species. We also compared composition of secretions with those of other related lizard species for which previous data is already known.

2. Material and methods

2.1. Study area and species

The Bocage's wall lizard (*P. bocagei*) and the Carbonell's wall lizard (*P. carbonelli*) are two species widespread in western Iberian Península. *P. bocagei* is distributed in Asturias, Cantabria, Galicia, north of Castilla-León (Spain) and north of River Douro and some mountains sites of Tras-os-Montes (Portugal) (Salvador et al., 2014). Populations of *P. carbonelli* inhabit mainly relict zones of Castilla León and Extremadura (Spain), and south of River Douro (Portugal) (Sá-Sousa, 2008; Galán, 2014). Both species are endemic of the Iberian Peninsula.

During field-work carried out in spring 2007 (May–June, corresponding to the species mating seasons), we captured focused on *P. bocagei* at "Louro" (Galicia, Spain; 42°44′22.30″N, 9°04′46.07″W) and *P. carbonelli* at "La Alberca" (Salamanca, Spain; 40°29′44.51″N, 6°05′17.92″W). The lizards were captured alive by noosing, and immediately after that, secretions were extracted from femoral glands by softly pressing around the femoral pores. We collected secretions into glass vials with glass inserts, and later closed them with Teflon-lined stoppers and stored at −20 °C until analyses. We also prepared blank control vials by using the same procedure, but without collecting secretion, and the same analytical methodology to compare with the secretion-samples and be able to exclude potential contaminants from the handling procedure, the solvent or laboratory equipment.

2.2. Analyses of femoral gland secretions

We analyzed the samples 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 the detector. We performed splitless sample injections (2 μl of each sample dissolved in *n*-hexane) with helium as the carrier gas, and injector and detector temperatures at 250 °C and 280 °C, respectively. The oven temperature program started at 50 °C, was maintained isothermal for 3 min, then increased to 300 °C at a rate of 5 °C/min, and finally isothermal (300 °C) for 15 min. Mass spectral fragments below $m/z = 46$ were not recorded. Initial identification of secretion components was done by comparing their mass spectra with those in the NIST/EPA/NIH (NIST 02) computerized mass spectral library. We confirmed identifications by comparing spectra and retention times with those of authentic standards (from Sigma–Aldrich Chemical Co.) when these were available. Impurities identified in the control vial samples were not considered.

The relative amount of each compound was determined as the percent of the total ion current (TIC). To correct the problem of nonindependence of proportions, we used the compositional analysis, by logit transforming the proportion data taking the natural logarithm of proportion/(1−proportion) (Aebischer et al., 1993). Then, we calculated Euclidean distances between every pair of individual samples to produce a resemblance matrix that formed the basis of further analyses. We used permutational multivariate analysis of variance tests (PERMANOVA; Anderson, 2001; McArdle and Anderson, 2001) based on the Euclidean resemblance matrix using 999 permutations to analyze whether the composition of the femoral secretions varied between species. We also used a canonical analysis of principal coordinates (CAP; Anderson and Willis, 2003) to investigate differences between species. Calculations were made with the software PAST 3.05 (Hammer et al., 2001) and PRIMER V6.1.13 (Clarke and Gorley, 2006) with the PERMANOVA+ V1.0.3 add-on package (Anderson et al., 2008). We compared the relative abundance of the different types of compounds found in femoral secretions between species with one-way ANOVA tests (Sokal and Rohlf, 1995).

3. Results

We found 56 lipophilic compounds in femoral gland secretions of male *P. bocagei* (Table 1), which was a mixture of 26 steroids (61.8% of TIC), 12 waxy esters (34.3%), five carboxylic acids and their ethyl esters ranged between n-C_{16} and n-C_{18} (1.6%), two amydes (0.8%), squalene and other terpenoid (0.8%), four aldehydes (0.4%), four alcohols between C_{14} and C_{20} (0.2%) and a ketone (0.1%). On average, the five most abundant chemicals of *P. bocagei*, which together comprised nearly 75% of the TIC area, were cholesterol (42.3% of TIC), and four waxy esters: octadecyl 9-octadecenoate (9.4%), eicosyl hexadecanoate (9.2%), octadecyl hexadecanoate (8.7%), and eicosyl 9-octadecenoate (4.8%).

In addition, we found in the lipophilic fraction of femoral gland secretions of male *P. carbonelli* a mixture of 60 compounds (Table 1), including 16 waxy esters (55.5% of TIC), 27 steroids (42.6%), four alcohols between C_{14} and C_{20} (0.9%), five carboxylic acids ranged between n-C_{10} and n-C_{18} and their ethyl esters (0.3%), squalene (0.2%), four aldehydes (0.2%), a ketone (0.1%), a furanone (0.1%) and one amyde (0.1%). On average, the five most abundant chemicals, which comprised more than 60% of the TIC area, were cholesterol (20.2%), and four waxy esters: hexadecyl 9-hexadecenoate (15.4%), octadecyl hexadecanoate (9.4%), octadecyl 9-octadecenoate (8.5%) and eicosyl hexadecanoate (8.3%).

There were clear differences in the presence/absence of some lipophilic compounds between *P. bocagei* and *P. carbonelli* (Table 1). Thus, *P. bocagei* had nineteen exclusive compounds (33.9% of compounds; but only 3.1% of the TIC area) that were not found in *P. carbonelli*, which had fifteen exclusive compounds (25% of compounds; 22.4% of TIC area). The PERMANOVA analysis based on the resemblance matrix comparing individuals of each species showed that there were significant differences in the proportion of compounds between the two species (pseudo $F_{1,13} = 73.44$, $p = 0.001$). Moreover, when individuals were classified by cluster analysis based on euclidean distances among their chemical profiles (i.e. relative proportions of compounds), outstanding differences were found between the two species, showing that individuals from the same species clustered close together and separated from individuals from the other species (Fig. 1a). The CAP analysis assembled 100% of the chemical profiles into the correct species using these euclidean distances (permutational test, ${\delta_1}^2 = 0.997$, $p = 0.001$, using leave-one-out cross-validation and $m = 2$ axis). In contrast, a further PERMANOVA made with only the five major compounds shared by both species (cholesterol, octadecyl hexadecanoate, octadecyl 9-octadecenoate, eicosyl hexadecanoate, and eicosyl 9-octadecenoate) showed no significant differences between both species (pseudo $F_{1,13} = 1.08$, $p = 0.36$).

Comparing between the two species the relative abundance of the different types of compounds, *P. bocagei* had significantly relatively higher proportions of amydes ($F_{1,13} = 17.88$, $p = 0.001$) and terpenoids ($F_{1,13} = 7.06$, $p = 0.019$) and lower proportions of waxy esters ($F_{1,13} = 4.68$, $p = 0.049$) and furanones ($F_{1,13} = 7.05$, $p = 0.019$) than *P. carbonelli*. Whereas there were not significant differences between species in proportions of steroids ($F_{1,13} = 4.25$, $p = 0.06$), carboxylic acids and their esters ($\mathrm{F}_{1,13} = 1.37$, $\mathrm{P} = 0.26$), alcohols ($F_{1,13} = 3.68$, $p = 0.077$), aldehydes ($F_{1,13} = 3.85$, $p = 0.071$) and ketones ($F_{1,13} = 0.005$, $p = 0.93$).

4. Discussion

The current study shows that lipophilic compounds found in femoral gland secretions of male lizards *P. bocagei* vary in composition and proportions from those of the related *P. carbonelli*. Steroids and waxy esters were the main types of compounds in both species. Cholesterol was the major compound of the secretions similarly to many other lacertid lizard species, specially other species within the genus *Podarcis* (Gabirot et al., 2012; reviewed in Weldon et al., 2008; Martín and López, 2011, 2014). However, the secretions are also clearly different from other related species within the *Podarcis hispanicus* species complex (Gabirot et al., 2012). Thus, in comparison with other closely related, but more saxicolous-dweller, species of *Podarcis* lizards, such as *Podarcis guadarrame* and *P. virescens* (Gabirot et al., 2012), the relative high proportion of waxy esters in the ground-dwelling *P. bocagei* and *P. carbonelli* is notorious. This may be an ecological adaptation to increase the

Table 1
Lipophilic compounds found in male's femoral secretions of nine Bocage's wall lizard (*Podarcis bocagei*) and six Carbonell's wall lizard (*Podarcis carbonelli*).

RT (min)	Compound	*P. bocagei* mean ± SD	*P. carbonelli* mean ± SD
13.3	Nonanal	–	0.03 ± 0.04
18.5	Decanoic acid	–	0.01 ± 0.01
23.5	Tetradecanol	0.03 ± 0.04	0.04 ± 0.03
28.1	Hexadecenal	0.17 ± 0.10	0.09 ± 0.07
28.7	Pentadecanal	0.03 ± 0.02	0.04 ± 0.05
30.8	Hexadecanal	0.10 ± 0.08	0.01 ± 0.01
32.2	Hexadecanol	0.03 ± 0.02	0.05 ± 0.06
32.5	2-Heptadecanone	0.12 ± 0.08	0.12 ± 0.11
33.9	Hexadecanoic acid	0.82 ± 0.95	0.27 ± 0.17
34.3	Hexadecanoic acid, ethyl ester	0.02 ± 0.02	–
34.8	Octadecanal	0.06 ± 0.05	–
36.0	Octadecanol	0.07 ± 0.06	0.52 ± 0.66
36.56	5-Dodecyldihydro-2(3H)-furanone	–	0.06 ± 0.07
37.3	9,12-Octadecadienoic acid, ethyl ester	0.77 ± 2.14	–
37.4	9-Octadecenoic acid, ethyl ester	0.02 ± 0.02	–
37.7	Octadecanoic acid	0.01 ± 0.01	–
37.7	9,12-Octadecadienoic acid	–	0.01 ± 0.02
37.8	9-Octadecenoic acid	–	0.02 ± 0.03
37.9	Octadecanoic acid, ethyl ester	–	0.02 ± 0.04
39.8	Eicosanol	0.01 ± 0.01	0.31 ± 0.54
40.9	9-Octadecenamide	0.03 ± 0.02	0.05 ± 0.08
47.1	13-Docosenamide	0.82 ± 0.45	–
47.5	Squalene	0.75 ± 0.48	0.24 ± 0.23
47.7	Unid.Steroid (199,253,341,352,367)	0.03 ± 0.02	–
47.9	Unid. Terpenoid	0.05 ± 0.02	–
48.4	Cholesta-2,4-diene	0.05 ± 0.03	0.15 ± 0.16
48.7	Cholesta-4,6-dien-3-ol	0.14 ± 0.04	0.12 ± 0.10
48.6	Unid.Steroid (141,156,349,364)	1.52 ± 0.66	0.74 ± 0.43
48.9	Cholesta-3,5-diene	0.39 ± 0.24	0.67 ± 0.71
49.2	Unid.Steroid (197,251,349,365)	2.68 ± 0.63	1.67 ± 0.92
49.3	4,6,8(14)-Cholestatriene	–	0.12 ± 0.11
49.4	Unid.Steroid (195,209,251,349,365)	2.84 ± 1.12	1.63 ± 0.81
49.5	Unid.Steroid (251,349,365)	0.42 ± 0.11	0.35 ± 0.26
49.6	Cholesta-5,7-dien-3-ol, acetate	0.40 ± 0.13	0.48 ± 0.59
49.7	Unid.Steroid (210,237,350,365,389)	0.36 ± 0.11	0.26 ± 0.18
49.8	Unid.Steroid (251,347,364,377)	0.04 ± 0.02	0.16 ± 0.26
49.9	Unid. Ester of 9-Octadecenoic acid	–	0.46 ± 1.10
50.0	Unid.Steroid (141,156,209,364,379)	0.09 ± 0.04	–
50.2	Unid.Steroid (141,156,209,349,364,379,400)	0.28 ± 0.07	1.13 ± 2.17
50.4	Unid.Steroid (181,193,235,348)	–	3.06 ± 7.29
50.5	Unid.Steroid (197,251,363,379,387)	0.18 ± 0.06	–
50.6	Unid.Steroid (155,197,251,363,379)	–	0.65 ± 1.45
50.9	Unid.Steroid (195,209,363,379)	0.13 ± 0.07	–
51.0	Cholesterol methyl ether	0.38 ± 0.34	1.36 ± 1.44
51.1	Unid.Steroid (197,209,365,381)	0.08 ± 0.06	0.03 ± 0.02
51.9	Cholesterol	42.36 ± 9.80	20.20 ± 11.66
52.5	Cholesta-5,7-dien-3-ol	1.26 ± 0.93	2.27 ± 1.31
52.8	Cholesta-5,7-dien-3-ol, derivative?	–	0.02 ± 0.03
53.2	Campesterol	0.38 ± 0.42	–
53.5	Cholest-4-en-3-one	0.77 ± 0.42	2.69 ± 4.18
53.7	3-Ethoxy-methoxy-cholestane	0.42 ± 0.11	2.05 ± 1.44
54.0	Cholesta-4,6-dien-3-one	3.98 ± 2.05	1.21 ± 0.93
54.2	Hexadecyl hexadecanoate	–	0.68 ± 1.07
54.2	Octadecyl tetradecanoate	0.14 ± 0.08	–
54.3	Cholest-7-en-3-ol, acetate	–	0.46 ± 0.97
54.8	Unid.Steroid (214,267,366,381)	2.26 ± 0.98	0.90 ± 1.00
54.9	Hexadecanoic acid, ethenyl ester	–	0.88 ± 0.96
55.4	Cholest-5-en-3-one	–	0.06 ± 0.12
55.1	Unid.Steroid (253,341,353,380,414)	0.26 ± 0.47	–
55.7	Heptadecyl hexadecanoate	0.30 ± 0.12	0.25 ± 0.25
55.9	Stigmast-4-en-3-one	–	0.02 ± 0.02
56.6	Unid.Steroid (214,267,380,395)	0.10 ± 0.07	–
56.7	Tetradecyl 9-octadecenoate	0.79 ± 1.06	1.77 ± 3.39
57.1	Hexadecyl 9-octadecenoate	0.18 ± 0.16	0.94 ± 0.57
57.2	Hexadecyl 9-hexadecenoate	–	15.45 ± 34.81
57.3	Octadecyl hexadecanoate	8.67 ± 3.82	9.39 ± 9.36
58.2	Octadecanoic acid, ethenyl ester	–	0.20 ± 0.21
59.1	Heptadecyl 9-octadecenoate	0.06 ± 0.11	0.08 ± 0.07
59.3	Nonadecyl hexadecanoate	0.31 ± 0.11	0.43 ± 0.34
61.4	Octadecyl 9-octadecenoate	9.43 ± 8.64	8.53 ± 5.97

Table 1 (*continued*)

RT (min)	Compound	*P. bocagei* mean ± SD	*P. carbonelli* mean ± SD
61.8	Eicosyl hexadecanoate	9.20 ± 3.02	8.30 ± 8.61
62.9	Unid.Steroid (211,295)	–	0.15 ± 0.20
64.1	Nonadecyl 9-octadecenoate	0.21 ± 0.32	0.31 ± 0.22
64.6	Heneicosyl hexadecanoate	0.21 ± 0.10	0.17 ± 0.20
67.7	Eicosyl 9-octadecenoate	4.79 ± 3.28	7.70 ± 7.03

The relative amount of each component was determined as the percent of the total ion current (TIC) and reported as the average (±1SD). Characteristic ions (m/z) are reported for unidentified compounds. RT: Retention time.

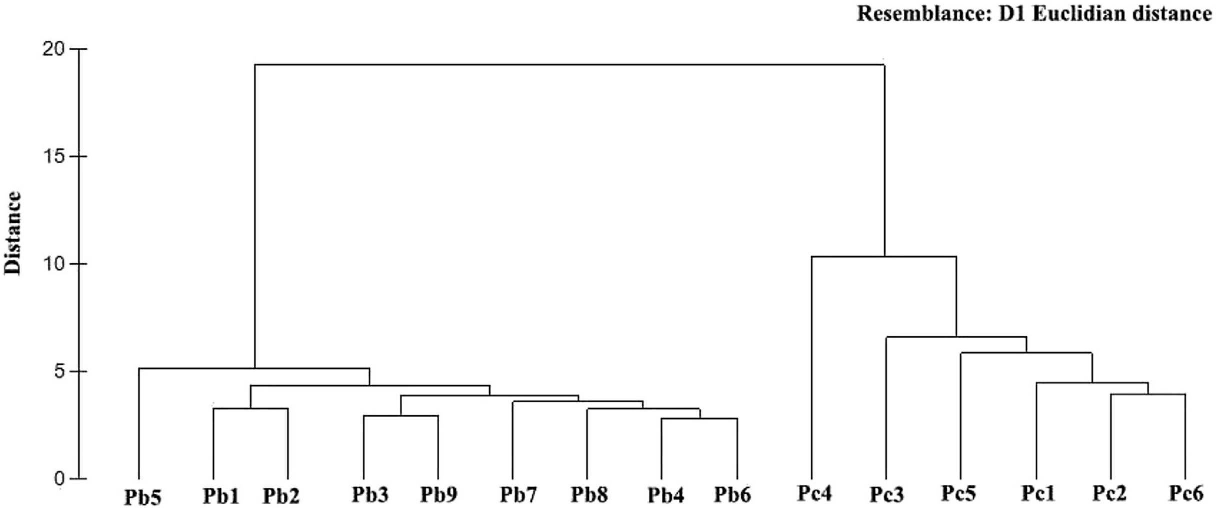

Fig. 1. Dendrogram of cluster analysis for individual samples of femoral secretions of male lizards *Podarcis bocagei* (Pb1 to Pb9) and *P. carbonelli* (Pc1 to Pc6).

persistence of scent marks under the microclimatic conditions experienced within the vegetated ground, in comparison to on exposed rocks, due to the more stable chemical characteristics of waxy esters (Alberts et al., 1992).

However, the different proportion of some groups of lipids (e.g., waxy esters are more abundant in *P. carbonelli*), together with the presence of minor compounds exclusive of each species could explain the significant differences in the chemical profiles between *P. bocagei* and *P. carbonelli*. A possible explanation for these differences might be the different climatic conditions in each sampled area, because waxy esters could protect scent marks from rapid evaporation in the more Mediterranean-xeric habitat of *P. carbonelli*. Likewise, the observed increase of squalene in *P. bocagei* could be attributed to the wetter conditions of its habitat in comparison with that of *P. carbonelli,* due to the antioxidant function of squalene, which will protect other compounds. However, these wall lizard species also inhabit other different western areas of the Iberian Peninsula, in which climatic conditions might change slightly, potentially affecting the composition of secretions in other areas (Martín and López, 2013).

On the other hand, and according to previous research, these two lizard species may occur in sympatry with other *Podarcis* wall lizards (Sá-Sousa, 2001; Carretero et al., 2002). In this case, species-specific differentiation in sexual signals is one of the most important facts for reproductive isolation between species, especially when hybridization events have been observed (Galán, 2002). In addition to color and behavior, chemoreception may play an important role in species recognition in these lizards. In this respect, the differences observed in chemical profiles of these two species seem to be consistent with the observation of chemosensory interspecific discrimination of some wall lizard species. Barbosa et al. (2005) reported the ability of *P. bocagei* and *P. carbonelli* males to distinguish conspecific and heterospecific females. This also agrees with further research, which showed that *P. bocagei* and *P. hispanica* (type 1) (i.e. *P. guadarramae*) males discriminated females of their own species (Barbosa et al., 2006). Whereas these previous studies tended to focus on intersexual recognition based on chemical cues, they overlooked male–male discrimination. In this context, conspecific male recognition in related sympatric species may be relevant for avoiding interspecific competition due to territoriality (Labra, 2011). In other lizard species, agonistic attacks have been reported to be directed to males of the same species due to the ability to recognize conspecific even at an individual level (Cooper and Vitt, 1987; Cooper and Perez-Mellado, 2002).

Taking the above data into consideration, compounds which might facilitate long range detection by conspecifics may be aldehydes (Weldon et al., 2008; Martín and López, 2011). Curiously, in contrast to earlier research of the other sympatric wall lizard species (*P. hispanica* type 1 and 2; Gabirot et al., 2012), we found evidence of three aldehydes shared by *P. bocagei* and *P. carbonelli* (hexadecenal, pentadecenal and hexadecanal), and two more exclusive of each species (octadecanal and nonanal respectively). However, these results need to be interpreted with caution, and more research on this topic needs to be undertaken before the association between aldehydes and species recognition can be more clearly understood.

Furthermore, female choice based on chemical cues play an important role in sexual selection (Martín and López, 2011). Previous analysis observed that female wall lizards might discriminate chemical cues of males at an individual and/or conspecific level (Barbosa et al., 2005, 2006; Gabirot et al., 2012), which may affect the females' mate choice decisions. Therefore, chemical differences between males of sympatric species, may affect female mate choice and be determinant to avoid hybridization.

Future studies on chemical characterization of lizard secretions in sympatric areas or contact zones are, therefore, recommended to asses more thoroughly the role of chemical signals in reproductive isolation of these wall lizard species.

Acknowledgments

We thank Pedro Galán for indicating the location of the lizard populations, and “El Ventorrillo” MNCN Field Station for the use of their facilities. Financial support was provided by the project MICIIN-CGL2011-24150/BOS. All captures of lizards were performed under licenses from the Environmental Agencies of Galicia and Castilla y León Governments.

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