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Inter-island variation in femoral secretions of the Balearic lizard, *Podarcis lilfordi* (Lacertidae)

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ABSTRACT

Many lizards use femoral gland secretions in intraspecific communication. Although there is a consistent interspecific variation in chemical composition of secretions, considerable variation is also often found between populations, which may affect conspecifc recognition and lead to speciation processes. Balearic lizards (*Podarcis lilfordi*) are currently distributed only in several isolated islets offshore of the main islands with different environmental conditions (vegetation, diet, density of population, etc). Also, there is a high genetic variability between populations. We examined whether there was a similar variation in the composition of the femoral secretions of male lizards, and which could be the causes of such variation. By using GC–MS analyses, we found 75 lipophilic compounds in femoral gland secretions of male *P. lilfordi* from three representative island populations. Main compounds were steroids (94.4%), mainly cholesterol, but we also found alkanes, ketones, waxy esters, squalene, carboxylic acids and their ethyl esters, alcohols and other minor compounds. However, there were clear differences between populations with respect to the number and relative proportions of compounds. Using the patterns of presence and abundance of compounds in secretions it is possible to predict the population of origin of a lizard. We discuss how these differences could be explained considering genetic and environmental differences between populations.

1. Introduction

Many animals, including many vertebrates, use chemical signals (pheromones) in intraspecific communication (Wyatt, 2003; Müller-Schwarze, 2006). Chemicals secreted by the femoral or precloacal glands of lizards are recognized as having an important role in social organization and reproduction (Mason, 1992; Alberts, 1993; Mason and Parker, 2010; Martín and López, 2011). Male lizards scent-mark their territories using femoral secretions and these scent-marks may inform on the quality of the territory owner to other males (López and Martín, 2002, 2011; Labra, 2006; Carazo et al., 2007; Martín and López, 2007; Martín et al., 2007b) and to females (Martín and López, 2000, 2006a,b; Olsson et al., 2003; López and Martín, 2005a). This information is reliable because it is based on physiological-dependent relationships between the proportions of some compounds in secretions and the characteristics, diet and health state of males (Alberts et al., 1992a; López

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et al., 2006, 2009; Martín et al., 2007a; Kopena et al., 2011). Therefore, it is important to know which compounds are found in these femoral secretions and how they vary to understand their role in social and sexual behavior of lizards.

Femoral secretions of lizards are composed of proteins and many lipophilic compounds that may function as pheromones (reviewed in Weldon et al., 2008; Martín and López, 2011). However, these chemicals are only known for a few species, including lacertids (López and Martín, 2005b,c, 2006; 2009; Martín and López, 2006c,d; 2010; Gabirot el al., 2008, 2010; Kopena et al., 2009; Khannoon et al., 2011a,b), an African cordylid (Louw et al., 2007), an American teid (Martín et al., 2011), an iguanid (Weldon et al., 1990; Alberts et al., 1992a,b), several South American tropidurids (Escobar et al., 2001, 2003) and a few gekkonids (Khannoon, 2012). These studies show consistent interspecific variation in composition (Weldon et al., 2008; Martín and López, 2011), which probably reflects phylogenetic differences *per se*. However, most informative chemicals in secretions come from the diet, which can vary between populations and between individuals (Martín and López, 2006b; Kopena et al., 2011). Also, signals used in intraspecific communication are expected to evolve to maximize efficacy of the signal under a given climatic conditions (Alberts, 1992; Endler and Basolo, 1998). Therefore, considerable variation is expected in composition not only between species, but further, between populations within species. This is important because chemical sexual signals are often used in interspecifc recognition, and, if differences between populations are great, they could lead to reproductive isolation and speciation (Martín and López, 2006c; Gabirot et al., 2010, 2012a,b; Labra, 2011). However, we have no precise knowledge of the magnitude, the causes and the relevance of that variation.

The Balearic lizard, *Podarcis lilfordi* (Günther, 1874), is a medium-sized lacertid lizard endemic to coastal islets of Mallorca and Menorca and to the Cabrera archipelago (Pérez-Mellado, 1998). Lizards are currently only present in some islets offshore but not on the main two major islands (Mallorca and Menorca) where they became extinct in historical times. In all populations, males have well developed femoral pores with abundant secretions (pers. observ.). These lizards have well developed chemosensory abilities as they can discriminate chemical cues from plant and animal foods from control substances (Cooper and Pérez-Mellado, 2001), and also can discriminate some lipids found in food (Cooper et al., 2002). These lipids are also found in femoral secretions and, in other closely related lizards, are known to elicit chemosensory responses to conspecific cues (Martín and López, 2006e; López and Martín, 2012). Therefore, chemical cues may be potentially important in intraspecific communication of Balearic lizards.

The complete isolation of the different populations of *P. lilfordi* lizards have led to clear morphological and genetic differences between populations, with 25 subspecies being currently recognized (Pérez-Mellado, 2009). Detailed phylogeographic analyses of all populations of this lizard indicated the existence of several well-supported major genetic clades that group several populations each (Terrasa et al., 2004, 2009; Brown et al., 2008). The first cladogenesis event led to the separation of Minorcan populations from the remaining lineages. The second separated the populations from the western Majorcan islets from the remaining Majorcan and Cabreran islets. A subsequent cladogenesis separated populations from Majorcan islets to the north east and south of the island and those from the northern Cabrera islets from the remaining Cabreran populations (Terrasa et al., 2009). The particular distribution pattern of the Balearic lizard and the genetic variation between populations, which in some cases also have different habitat and environmental conditions (Pérez-Mellado, 2009), different diets (Pérez-Mellado, 1989; Pérez-Mellado and Corti, 1993) and different population densities (Pérez-Mellado et al., 2008), provides an excellent opportunity to examine whether there was a similar variation in the chemical composition of the femoral secretions of male lizards, and to understand which could be the causes of such variation.

We report here the results of an analysis by gas chromatography–mass spectrometry (GC–MS) of the lipophilic fraction of femoral secretions of adult male Balearic lizards, *P. lilfordi*, from three isolated island populations. We chose these three populations because they are representative of the three major genetic clades of populations of this lizard (Brown et al., 2008; Terrasa et al., 2009). We specifically described and compared the composition and relative proportions of compounds in femoral secretions of lizards from these populations.

2. Material and methods

2.1. Study area

We captured adult male *P. lilfordi* in late June 2010, coinciding with the end of the mating season of these lizards (Pérez-Mellado, 1998) in three islets of the Balearic Islands: ‘Aire’, ‘Dragonera’ and ‘Moltona’. Aire Island is located off the south-eastern coast of Minorca and has a surface of around 34 ha. It is a fairly flat islet with an hallophyllous vegetation dominated by the sea fern, *Crithmum maritimum* L. and some species of the genus *Limonium* at shores, as well as shrubs of *Suaeda vera* Forssk, *Carlina corymbosa* L. and *Pistacia lentiscus* L. along the inner areas of the islet. Dragonera is the largest coastal island offshore of Majorca, located at its southwestern corner. It is a Natural Park of more than 280 ha. Its vegetation includes around 300 plant species, including some small forests of *Pinus halepensis* Mill. and a rich diversity of Mediterranean shrubs. Moltona is an islet off Southern coast of Majorca, with 4 ha of surface and densely covered vegetation with more than 60 plant species dominated by shrubs such as *Pistacia lentiscus* L., *Phillyrea latifolia* L. and *Ephedra fragilis* Desf.

2.2. Chemical analyses of femoral gland secretions

Immediately after capture, we extracted secretions from femoral glands of male lizards by gently pressing with forceps around the femoral pores, and collected secretions directly into glass vials with glass inserts, later closed with Teflon-lined stoppers. Vials were stored at −20 °C until analyses. We also used the same procedure but without collecting secretion, to obtain blank control vials that were treated in the same procedure to compare with the samples, and be able to exclude contaminants from the handling procedure or from the environment, 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. 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 250 °C and 280 °C, respectively. The oven temperature program was as follows: 50 °C isothermal for 3 min, then increased to 300 °C at a rate of 5 °C/min, and then isothermal (300 °C) for 15 min. The carrier gas was helium at 30 cm/s. Ionization by electron impact (70 eV) was carried out at 250 °C. Mass spectral fragments below $m/z = 46$ were not recorded. Impurities identified in the solvent and/or the control vial samples are not reported. Initial identification of secretions components was done by comparison of mass spectra in the NIST/EPA/NIH (NIST 02) computerized mass spectral library. Identifications were confirmed by comparison of spectra and retention times with those of authentic standards (from Sigma–Aldrich Chemical Co) when these were available.

2.3. Statistical analyses

The relative amount of each chemical component was determined as the percent of the total ion current (TIC). We used the compositional analysis, consisting in logit transforming the proportion data by taking the natural logarithm of proportion ÷ (1 – proportion) to correct the problem of nonindependence of proportions (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 the analyses. Visual examination of the inter-individual variation of chemical profiles was made with the dendrogram plot of a cluster analysis (unweighted pair-group average) of the resemblance matrix. We used a single factor permutational multivariate analysis of variance test (PERMANOVA, Anderson, 2001; McArdle and Anderson, 2001) based on the Euclidean resemblance matrix using 9999 permutations to analyze whether the composition of the femoral secretions varied between the three islet populations. Differences between populations were investigated further using canonical analysis of principal coordinates (CAP, Anderson and Willis, 2003). The software PRIMER V6.1.13 (Clarke and Gorley, 2006) with the PERMANOVA + V1.0.3 add-on package (Anderson et al., 2008) was used to investigate differences between chemical profiles.

3. Results

We found 75 lipophilic compounds in femoral gland secretions of male *P. lilfordi* (Table 1). Considering together the three populations, the main components were 36 steroids (94.4% of TIC), but we also found four long chain alkanes between C_{28} and C_{31} (1.4%), two ketones of C_{17} and C_{19} (0.8%), eight waxy esters (0.8%), squalene (0.7%), ten carboxylic acids or their ethyl esters ranged between n-C_{14} and n-C_{22} (0.6%), six alcohols between C_{16} and C_{24} (0.5%), three furanones (0.5%), one amide (0.2%) and four aldehydes (0.1%). On average, the most abundant chemicals were cholesterol (61.0% of TIC) and 3-methoxymethoxy-cholestane (15.4%), followed by β-sitosterol methyl ether (4.2%), campesterol (4.2%), stigmastanol (1.5%) and 3-methoxy-cholestane (1.0%).

However, there were striking differences between populations. With respect to the number of compounds found, femoral secretions from lizards from Moltona had the larger number of compounds ($n = 63$), followed by lizards from Dragonera ($n = 54$) and Aire ($n = 39$) (Table 1). Only 33 of the 75 compounds (44%) were shared by the three populations but these comprised all the major compounds accounting for 97.7% of TIC on average. Moltona and Dragonera, the two Majorcan populations, shared 46 compounds, whereas the Minorcan population of Aire shared 34 compounds with Moltona and 34 slightly different compounds with Dragonera. Exclusive compounds were more frequent in Moltona ($n = 16$ exclusive compounds), than in Dragonera ($n = 7$), or Aire ($n = 4$). However, these exclusive compounds were usually less abundant (<0.6% of TIC each compound).

A cluster analysis based on the resemblance matrix of relative proportions of compounds in femoral secretions indicated greater similarities between the chemical profiles of lizards from the same population than from different populations, which is reflected in the tendency of lizards from each population to cluster closer together (Fig. 1). The PERMANOVA based on the resemblance matrix comparing the three populations was statistically highly significant (pseudo $F_{2,42} = 15.15$, $p < 0.001$). The CAP analysis classified 100% of the chemical profiles into the correct population using leave-one-out cross-validation and $m = 5$ axes ($\delta_1^2 = 0.96$, $p = 0.001$, Fig. 2). A further PERMANOVA made with compounds shared by the three populations only was not significant (pseudo $F_{2,42} = 1.43$, $p = 0.11$), but the CAP analyses still classified correctly 93.3% of samples ($\delta_1^2 = 0.99$, $p = 0.001$, $m = 29$ axes).

4. Discussion

Femoral gland secretions of the Balearic lizard *P. lilfordi* have steroids as predominant components, which is similar to that found in other lizards species (reviewed in Weldon et al., 2008). Similarly to other lacertids and other lizards from other families, cholesterol is the most abundant steroid in secretions (Weldon et al., 2008). However, in chemosensory tests, male *P. lilfordi* from the Aire population show low chemosensory responses to cholesterol, which are not different to responses to

Table 1
Lipophilic compounds found in femoral secretions of male Balearic lizards, *Podarcis lilfordi* from three different island populations (Moltona, Dragonera and Aire).

RT (min)	Compound	Moltona	Dragonera	Aire
		Mean ± SE	Mean ± SE	Mean ± SE
13.2	Nonanal	–	–	0.02 ± 0.03
16.2	Decanal	–	–	0.02 ± 0.03
28.1	Hexadecanol	0.17 ± 0.10	0.25 ± 0.21	0.18 ± 0.11
30.8	Tetradecanal	0.08 ± 0.06	–	0.04 ± 0.07
30.9	Tetradecanoic acid, 1-methylethyl ester	0.04 ± 0.12	–	–
32.2	Heptadecanol	0.07 ± 0.05	0.16 ± 0.12	0.07 ± 0.05
32.5	2-Heptadecanone	0.31 ± 0.10	1.24 ± 1.81	0.33 ± 0.18
32.9	Hexadecanoic acid, methyl ester	0.03 ± 0.05	–	–
33.8	Hexadecanoic acid	0.54 ± 0.57	0.23 ± 0.21	0.11 ± 0.08
34.8	Pentadecanal	0.06 ± 0.08	–	–
36.0	Nonadecanol	0.07 ± 0.05	0.04 ± 0.06	–
36.3	2-Nonadecanone	0.14 ± 0.11	0.28 ± 0.17	0.20 ± 0.20
36.6	25-Dodecyldihydro-(3H)-Furanone	0.04 ± 0.03	0.04 ± 0.03	–
37.0	9,12-Octadecadienoic acid	–	0.02 ± 0.03	–
37.1	9-Octadecenoic acid	0.11 ± 0.19	0.20 ± 0.28	–
37.5	Octadecanoic acid	0.16 ± 0.23	0.23 ± 0.39	0.04 ± 0.07
37.9	Octadecanoic acid ethyl ester	–	0.01 ± 0.04	–
39.5	Eicosanol	0.09 ± 0.13	0.00 ± 0.01	–
40.2	Dihydro-5-tetradecyl 2(3H)-Furanone	0.22 ± 0.12	0.32 ± 0.19	0.20 ± 0.09
41.1	9-Octadecenamide	0.15 ± 0.19	0.29 ± 0.23	0.15 ± 0.22
41.3	Hexanedioic acid, bis(2-ethylhexyl) ester	0.02 ± 0.02	–	–
42.8	Docosanol	0.13 ± 0.16	0.05 ± 0.10	–
43.5	Unidentified furanone?	0.23 ± 0.09	0.23 ± 0.13	0.20 ± 0.07
44.2	Docosanoic acid, ethyl ester	–	0.01 ± 0.04	–
45.5	Docosyl heptanoate	0.06 ± 0.04	0.13 ± 0.10	0.08 ± 0.05
45.6	Octacosane	0.05 ± 0.04	0.12 ± 0.05	0.12 ± 0.07
45.8	Tetracosanol	0.06 ± 0.12	0.17 ± 0.30	–
46.5	13-Docosenoic acid	–	0.03 ± 0.05	–
47.0	Cholest-5-en-3-ol, tetradecanoate	–	0.02 ± 0.03	–
47.6	Squalene	0.66 ± 0.40	0.96 ± 0.67	0.63 ± 0.44
48.0	Cholesta-2,4-diene	–	0.08 ± 0.17	0.13 ± 0.28
48.3	Nonacosane	0.39 ± 0.25	0.56 ± 0.25	1.07 ± 0.87
48.5	Cholesta-4,6-dien-3-ol	0.37 ± 0.16	0.48 ± 0.26	0.31 ± 0.08
48.6	Unidentified steroid (135,143,247,366)	0.31 ± 0.21	0.21 ± 0.12	–
48.7	Triacontane	–	–	0.14 ± 0.12
48.8	Cholesta-3,5-diene	0.38 ± 0.18	0.87 ± 0.38	0.46 ± 0.24
49.2	Unidentified steroid (197,251,327,349,364)	0.03 ± 0.04	–	–
49.4	Unidentified steroid (195,209,251,365)	0.01 ± 0.03	–	–
49.6	Cholesta-5,7-dien-3-ol, acetate	0.21 ± 0.11	0.15 ± 0.04	–
50.1	Unidentified steroid (135,143,253,366,381)	0.15 ± 0.09	0.13 ± 0.09	–
50.6	Unidentified steroid (197,251,364,379,397)	0.44 ± 1.09	0.04 ± 0.05	0.06 ± 0.07
50.9	Hentriacontane	0.60 ± 0.37	0.70 ± 0.32	0.50 ± 0.23
51.0	Cholesterol methyl ether	0.83 ± 0.64	0.39 ± 0.25	0.10 ± 0.17
51.1	Unidentified steroid (143,158,253,366,381)	0.06 ± 0.06	0.05 ± 0.05	–
51.2	Unidentified steroid (215,281,345,364,402)	0.04 ± 0.03	–	–
51.3	Cholesta-5,22-dien-3-ol	0.16 ± 0.22	0.25 ± 0.21	–
51.6	Stigmasterol	0.77 ± 0.50	0.22 ± 0.11	0.27 ± 0.22
51.9	Cholesterol	65.20 ± 9.09	64.01 ± 5.63	53.70 ± 6.58
52.3	Sitosterol	0.55 ± 0.35	0.28 ± 0.16	0.38 ± 0.30
52.4	Ergosta-5,22-dien-3-ol	0.92 ± 0.58	0.59 ± 0.62	1.75 ± 0.79
52.5	3-Methoxy-cholestane	1.07 ± 0.53	0.64 ± 0.22	1.41 ± 0.54
52.7	Stigmasterol methyl ether	0.60 ± 0.37	0.48 ± 0.20	0.72 ± 0.41
52.9	Cholest-23-ene, unidentified derivative?	0.21 ± 0.11	0.37 ± 0.22	0.42 ± 0.27
53.0	Unidentified steroid (147,191,291,317)	0.15 ± 0.39	–	–
53.2	Campesterol	2.71 ± 1.08	2.86 ± 0.71	7.14 ± 1.71
53.4	β-Sitosterol methyl ether	3.68 ± 1.54	3.31 ± 0.78	5.62 ± 1.30
53.7	3-Methoxymethoxy-cholestane	12.22 ± 5.46	14.21 ± 3.12	19.71 ± 4.26
53.9	Unidentified steroid (215,248,283,315,330)	0.20 ± 0.08	0.32 ± 0.08	0.76 ± 0.24
54.3	Cholest-7-en-3-ol, acetate	0.23 ± 0.18	0.13 ± 0.09	0.42 ± 0.53
54.4	Sitosterol derivative?	0.55 ± 0.24	0.77 ± 0.37	1.04 ± 0.59
54.5	Stigmastanol	1.18 ± 0.42	1.95 ± 0.75	1.43 ± 0.63
54.8	Unidentified ester of hexadecanoic acid?	0.64 ± 0.55	–	–
55.0	Cholest-5-en-3-one	–	–	0.09 ± 0.10
55.2	Stigmast-7-en-3-ol	–	0.17 ± 0.18	–
56.4	Olean-12-ene	–	0.18 ± 0.14	–
56.5	Ergosta-4,6,8(14),22-tetraen-3-one	0.07 ± 0.09	–	–
56.6	Olean-18-ene	0.02 ± 0.05	0.15 ± 0.13	–

Table 1 (*continued*)

RT (min)	Compound	Moltona	Dragonera	Aire
		Mean ± SE	Mean ± SE	Mean ± SE
57.2	Olean-12-en-3-yl acetate	0.02 ± 0.07	0.42 ± 0.31	–
58.2	Unidentified ester of hexadecanoic acid	0.19 ± 0.16	–	–
58.7	Unidentified steroid (147,191,291,317)	0.18 ± 0.39	–	–
62.9	Ethenyl octadecanoate	0.21 ± 0.18	–	–
63.9	2-(1-octadecenyl oxyl) hexadecanoate?	0.05 ± 0.09	0.06 ± 0.10	0.03 ± 0.05
65.2	Octadecyl 9-octadecenoate	0.48 ± 1.37	–	–
66.4	Eicosyl hexadecanoate	0.40 ± 0.83	–	–
67.5	Eicosyl 9-octadecenoate	0.05 ± 0.10	–	–

The relative amount of each component was determined as the percent of the total ion current (TIC) and reported as the average (±1SE). An asterisk denotes those compounds that were confirmed with authentic standards. Characteristic ions (*m*/*z*) are reported for unidentified compounds. RT: Retention time.

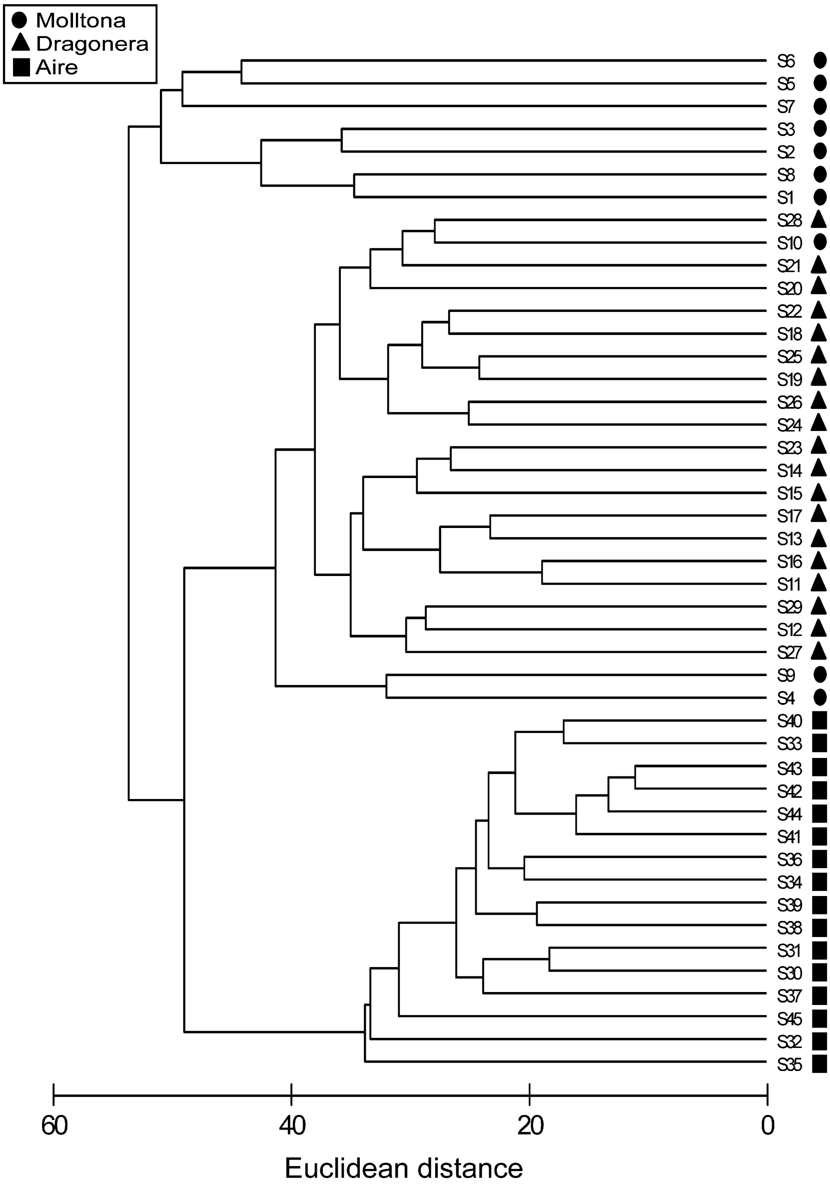

Fig. 1. Dendrogram of cluster analysis for 45 individual samples (S1 to S45) of femoral secretions of *P. lilfordi* lizards from three island populations (Moltona, Dragonera and Aire).

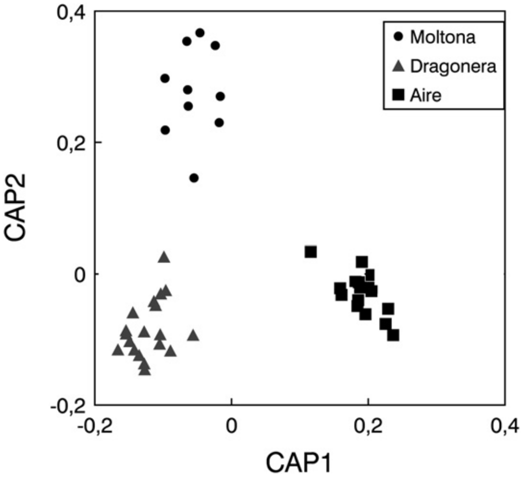

Fig. 2. Representation of the two first axes of the canonical analysis of principal coordinates (CAP) showing classification of the femoral secretions of *P. lilfordi* lizards in three island populations (Moltona, Dragonera and Aire).

control inodorous water (Cooper et al., 2002). This suggests that this steroid in femoral secretions may not be important in communication for these lizards. Similarly, in the related lizard *Podarcis hispanica*, chemosensory responses to cholesterol are lower than to other steroids (Martín and López, 2006e; Gabirot et al., 2012a). Moreover, female *P. hispanica* did not prefer to use areas scent-marked by males with higher proportions of cholesterol, but females use the proportion of other steroids to select males (López and Martín, 2005a). These results would support that, at least in *Podarcis* lizards, cholesterol in secretions might not have a signaling function, but just form an unreactive apolar matrix that could protect and deliver other compounds, which would be the true semiochemicals with a more important role in social behavior (Escobar et al., 2003). Thus, cholesterol may rather function to stabilize secretions in scent marks, especially in habitats with high evaporation rates (Alberts, 1992; Gabirot et al., 2012a).

The second most abundant steroid in femoral secretions of *P. lilfordi*, 3-methoxymethoxy-cholestane, has not been found in other lizards (Weldon et al., 2008). Nevertheless, the similar cholestanol, cholestan-3-one and some of their derivatives are found in abundance in secretions of European “green” lizards of the Gen. *Lacerta* (Kopena et al., 2009; Martín and López, 2010). The characteristic pattern of presence and abundance of this steroid, but also of others such as β-sitosterol methyl ether, campesterol or stigmastanol, in Balearic lizards might prompt future studies to analyze whether these steroids have a role in communication.

In contrast to other lizards, fatty acids were scarce in *P. lilfordi*. Although, the presence of fatty acids follows the usual pattern of other lizards, with hexadecanoic acid, followed by octadecenoic acid, being the most abundant free fatty acids in secretions (Weldon et al., 2008). Oleic acid has a signaling function in intersexual relationships in rock lizards (López and Martín, 2012). In male *P. lilfordi* from the Aire population, oleic acid elicits very strong lingual and biting responses, which suggests that this fatty acid may be used for identification of food (Cooper et al., 2002). However, oleic acid was not found in femoral secretions of lizards from this particular population. The low abundance of fatty acids in secretions of Balearic lizards, which in some populations are almost absent or appearing in very small proportions, might suggest that fatty acids are not important in communication of, at least, some populations of this lizard.

In contrast, the potential signaling function of other compounds in femoral secretions should be investigated. For example, it is noteworthy the presence in secretions of two saturated methyl ketones with odd-numbered carbon chains. A similar bishomologous series of C_{17}–C_{25} methyl ketones were found in the femoral gland secretions of the phylogenetically unrelated South African sungazer (*Cordylus giganteus*, Cordylidae) (Louw et al., 2007), and in the skin of some snakes (Mason et al., 1990; reviewed in Weldon et al., 2008). Interestingly, similar ketones from skin glands have a prominent role in social and sexual behavior of red garter snakes (Mason et al., 1990), so it would be interesting to test whether ketones might have a similar role in Balearic lizards. Also is noteworthy the presence of some long-chain alkanes, which origin and role are unknown and justify further studies.

Our results show that there were clear interpopulational differences in femoral secretions of *P. lilfordi*. Using the patterns of presence and abundance of compounds in secretions it is possible to predict the population of origin of a lizard. Thus, it is likely that lizards could also discriminate between populations based on chemical cues. Similar differences between populations in femoral secretions and chemosensory recognition have been found in populations of the related lizard *P. hispanica* (Gabirot et al., 2010, 2012a,b), and in species of *Liolaemus* lizards (Escobar et al., 2001, 2003; Labra, 2011). The long isolation history of the *P. lilfordi* islet populations might explain the clear differences in femoral secretion composition. It remains to be investigated whether these interpopulational differences may affect recognition systems, and whether this may have consequences for speciation if there were eventual future contacts between these populations, which would be likely if sea level changes only slightly, as these islets are very close to the shore and are separated by shallow waters from the main islands.

Interpopulational differences may be mainly due to the genetic differences (Terrasa et al., 2004, 2009; Brown et al., 2008). However, environmental factor, such as the clear interpopulational differences between lizards' diet (Pérez-Mellado, 1989; Pérez-Mellado and Corti, 1993) and population density (Pérez-Mellado et al., 2008) may also explain some differences. For example, *P. lilfordi* consume a wide variety of prey and plant foods that may contain a wide range of lipids (Pérez-Mellado and Corti, 1993). The presence of some compounds, such as the oleanes and other steroids of vegetal origin, in secretions of some populations but not others might derive from the inclusion of different plant species in the diet. Also, there are great differences in density of population between islets (i.e., Aire: 4099 lizards ha^{-1}; Moltona: 2515 lizards ha^{-1}; Dragonera: 729 lizards ha^{-1}; see (Pérez-Mellado et al., 2008), which could render femoral secretions more or less useful in different contexts of intraspeciifc communication. Thus, in high density populations, where many lizards are often together clustered at the same favorable sites, and home ranges overlap extensively, substrate scent-marks would not be useful. This might lead to reduce some compounds in secretions. However, other compounds used in short-range communication might be more important. For example, compounds more volatile than can be sampled directly from the lizard during direct social interactions rather than from substrate scent marks. This might explain why lizards from the highly density populated Aire islet have a low number of compounds, but have incorporated some highly volatile aldehydes, which are absent in the population with lower density of Dragonera. Further studies that include a large amount of populations of this lizard should help to test these hypotheses on the causes of variation of chemical compounds in femoral secretions.

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