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Variations in chemical sexual signals of *Psammodromus algirus* lizards along an elevation gradient may reflect altitudinal variation in microclimatic conditions

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Abstract Chemical signals used in intraspecific communication are expected to evolve or to show phenotipic plasticity to maximize efficacy in the climatic conditions of a given environment. Elevational environmental gradients in mountains provide a good opportunity to test this hypothesis by examining variation in characteristics of signals in species found across different elevations with different climatic conditions. We analyzed by gas chromatography–mass spectrometry (GC-MS) the lipophilic fraction of the femoral gland secretions of male lizards *Psammodromus algirus* (Fam. Lacertidae) from six localities located along a 2200 m elevational gradient at Sierra Nevada Mountains (SE Spain). There was elevational clinal variation in climatic variables, number of femoral pores and in the relative proportions of some classes of compounds (i.e., ethyl esters of fatty acids, waxy esters, and aldehydes) but not others. We discuss how this variation would result in different physicochemical properties of the entire femoral secretion, which might help optimize the efficacy of chemical signals under the particular microclimatic conditions at each elevation.

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Introduction

Signals used in intraspecific communication are expected to evolve locally or to show phenotipic plasticity to maximize efficacy in a given environment (Guilford and Dawkins 1991; Endler and Basolo 1998; Bradbury and Vehrencamp 2011). Factors such as how the signal transmits through the environment, durability or persistence of the signal, or how well the signal is detected by the receivers are selective factors modeling the efficacy design of sexual signals (Guilford and Dawkins 1991; Alberts 1992; Endler and Basolo 1998; Boughman 2002). All of these variables largely depend on the environmental conditions. Therefore, signals are expected to vary among populations to remain informative despite geographical variation in environmental conditions.

Chemical signals play an important role in intraspecific communication of many animals (Wyatt 2014). The characteristics of chemical signals are not only expected to vary between species but also geographically within the same species, to ensure that signals are more efficient by being tailored or better suited to the local environmental conditions (e.g., humidity and temperature) where they are produced (i.e., affecting their volatility and, therefore, their persistence and transmission through the environment) (Regnier and Goodwin 1977; McDonough et al. 1989; Alberts 1992; Symonds and Elgar 2008; Martín and López 2013; Martín et al. 2015). Thus, it has been suggested that the observed interspecific or interpopulational differences in chemical signals might help maximize the efficacy of signals under different climatic conditions (Alberts 1992; Escobar et al. 2001, 2003; Martín and López 2006c, 2013; Gabirot et al. 2012a; Martín et al. 2015).

Elevational environmental gradients found in mountains provide a good opportunity to test this hypothesis by examining potential variation in characteristics of signals within

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species found across different elevations. This is because elevational gradients bring about gradual and predictable patterns of variation in climatic and environmental conditions on a small geographical scale. For example, ambient temperature and oxygen partial pressure tend to diminish in elevation, while humidity and ultraviolet radiation tend to increase (Blumthaler et al. 1997; Körner 2007; Graae et al. 2012). These environmental differences might, for example, promote the presence of more stable compounds in chemical signals of animals living under higher temperatures (Martín and López 2006c, 2013; Apps et al. 2015).

Many reptiles use chemical signals in many aspects of their social behavior (Mason 1992; Mason and Parker 2010; Martín and López 2011). For example, in many lizards, femoral or the similar precloacal glands produce large amounts of holocrine secretion, especially in males and during the mating season (reviews by Mason 1992; Alberts 1993; Martín and López 2011, 2014). The chemical composition of femoral gland secretions has been described in a few lizard species (reviewed in Weldon et al. 2008; Martín and López 2014), and it has been shown that lipophilic compounds in secretion may be involved in communication (Mason 1992; Martín and López 2006a,b, 2015). These compounds may be useful for advertising residence, dominance status, or competitive ability to other males (Aragón et al. 2001; Carazo et al. 2007; Martín and López 2007; Martín et al. 2007a). Also, femoral secretions of males may provide information to females on male characteristics, which can influence female choice, either concerning mates or settlement in a male's territory (López et al. 2002; Olsson et al. 2003; Martín and López 2006a, b, 2012, 2015).

In this study, we presented the results of an analysis by gas chromatography–mass spectrometry (GC-MS) of the lipophilic fraction of the femoral gland secretions of male lizards *Psammodromus algirus* (Fam. Lacertidae) from six sampling localities located along a 2200-m elevational gradient in the Sierra Nevada Mountains (SE Spain). Chemical compounds secreted by the femoral glands of male *P. algirus* (Martín and López 2006d) are important in rival recognition during male–male interactions (López et al. 2003). Also, differential responses of females to the scent of different individual males suggest that female mate choice decisions might be, at least partially, based on characteristics of chemical signals of males (Martín et al. 2007b). This signaling function of chemical secretions may be explained because the type and proportion of some compounds in secretions are related to, for example, the age (Martín and López 2006d) and health state of a male (Martín et al. 2007b). We examined here whether there was elevational variation in climatic conditions and whether this may result in a potential elevational variation in the number of femoral glands of male *P. algirus* lizards and in the characteristics of chemical signals produced by these glands.

Methods and materials

Study species and study area

The lizard *P. algirus* is a medium-sized (53–95 mm snout-to-vent length -SVL- in the study population), eurioic, oviparous lizard. It is abundant and widespread in Mediterranean woodland and shrub habitats of the Iberian Peninsula and North Africa, from the sea level to more than 2600 m above the sea level (asl) (Salvador 2015). There are divergent eastern and western mtDNA clades of *P. algirus* within the Iberian Peninsula (Carranza et al. 2006), but we focused here on *P. algirus* lizards from six localities that are genetically closely related among them and that are all included in the eastern clade (unpublished data).

During spring 2012–2013 (coinciding with the species mating season in the area), we captured a total of 72 sexually mature male lizards, 12 from each of six sampling localities located at 300, 700, 1200, 1700, 2200, and 2500 m asl respectively, covering a 2200-m elevational gradient, in Sierra Nevada Mountains southern face (36°56′ N, 03°23′ W; SE Spain) (for details of the study area, see Zamora-Camacho et al. 2013, 2016).

We captured lizards by hand and, immediately after capture, we measured SVL to the nearest millimeter with a ruler, measured weight to the nearest 0.1 g with a spring balance, and counted the number of femoral pores in both hindlimbs. The composition of chemical signals in reptiles has been repeatedly shown to vary with individual condition (Martín and López 2010, 2015). Thus, if lizards from the different localities varied in condition, this could affect to their chemical signals. Therefore, we calculated an index of body condition as the residuals from the regression equation of ln weight (g) on ln SVL (mm). This index may represent the relative amount of fat stored and hence an estimation of individual physical condition or nutritional status (Bonnet and Naulleau 1994). All males had complete or entirely regenerated tails.

Microclimatic variation with elevation

We chose sampling localities where vegetation structures were as similar as possible (a detailed description of vegetation structure at each sampling plot can be found in Appendix A in Zamora-Camacho et al. 2013). However, microclimatic variation with elevation is large. Thus, to estimate this variation in each sampling locality, during the lizard mating season, we first recorded operative temperatures (Te) on a clear day, halfway through the reproductive season of lizards of 2012 (July 13–17) using hollow copper models (Díaz 1997). In each sampling plot, 31 copper models were equally distributed 1 m from each other in three microhabitats (fully exposed to sunlight, partially exposed, or under shade). We measured Te once an hour throughout the morning (from 10:00 to 14:00 h)

by inserting in the model of the probe of a digital thermometer (model Hibok 18; precision 0.1 °C) (Díaz 1997; Zamora-Camacho et al. 2013, 2016).

Precipitations in the study area are scarce, particularly during summer, and from October to March take the form of snow in the two highest sampling localities, while snowfalls are occasional in the two central ones and improbable in the two lowest. During late spring and summer, coinciding with the lizard reproductive period, thaw keeps relatively high ground humidity levels in the two highest sampling localities. In turn, the mid and low elevation sampling localities are markedly dry during the lizards reproductive period. Within the elevational range studied here, precipitation increases with elevation (Domínguez-Rodríguez and Justicia-Segovia 1995). To estimate precipitation in each sampling plot, we used the equation provided by Raso-Nadal (2011), who developed equations allowing estimate precipitation in Sierra Nevada based on 30-year series of precipitation data collected in 35 spots. We used the equation for the entire spring period, coinciding with the mating season of lizards (precipitation in mm = $68.32 + 0.080735 \times$ Altitude $- 0.0000096716 \times$ Altitude^2; Raso-Nadal 2011).

In addition, we recorded ultraviolet radiation (UV-R) at each locality during 1 day at the beginning of August (the same day for all localities). Radiation was measured in the 305 wavelength channel, twice for each sampling point (once in the morning and once in the afternoon), every minute during a 10-min period, using a BIC compact four-channel spectroradiometer (Ocean Optics model USB2000 + UV-VIS). We used these data to predict UVR-B radiation along the day, by using a polynomial model (Reguera et al. 2014).

Chemical analyses of femoral gland secretions

Immediately after capture in the field, we extracted secretions from femoral glands of lizards by gently pressing 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. The same procedure, but without collecting secretion, was used to obtain blank control vials that were treated as the samples to allow identifying contaminants from the handling or laboratory procedures. Lizards were returned to their capture location as soon as possible. No individual suffered any damage as a consequence of this study.

Samples of femoral secretions of each individual were analyzed separately 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) coupled to a Finnigan-ThermoQuest Trace mass spectrometer (MS) as detector. Sample injections (2 μl of each sample of femoral secretions dissolved in 2 ml of GC capillary grade n-hexane), without

previous derivatization, were performed in splitless mode, with injector temperature set at 300 °C and using helium as the carrier gas at 30 cm/s. The oven temperature program was as follows: 50 °C isothermal for 3 min, then increased at a rate of 5 °C/min up to 300 °C, and then isothermal (300 °C) for 15 min. Ionization by electron impact (70 eV) was carried out at 280 °C. Mass spectral fragments below $m/z = 46$ were not recorded. Impurities identified in the control vial samples are not reported.

Initial identification of secretion 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 when these were available (from Sigma-Aldrich Chemical Co).

Statistical analyses

The relative amount of each chemical compound was determined as the percent of the total ion current (TIC). We used the compositional analysis, consisting of logit transforming the proportion data by taking the natural logarithm of proportion / (1 − proportion), thus correcting 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. To analyze whether the composition of the femoral secretions varied among localities, we used permutational multivariate analyses of variance (PERMANOVA, Anderson 2001; McArdle and Anderson 2001), followed by canonical analyses of principal coordinates (CAP, Anderson and Willis 2003), based on the Euclidean resemblance matrix using 999 permutations. Pairwise post hoc comparisons between pairs of localities were made using permutation tests. 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 perform these tests.

We used Spearman rank correlations to examine how climatic variables varied with elevation in the study area. We tested whether number of pores and proportions of type of secretion compounds differed among localities by using general linear models of ANOVA. Then, Pearson's linear regressions were used to explicitly examine whether there was a clinal variation among localities in number of pores or proportions of type of compounds due to an elevational gradient of environmental climatic conditions.

Results

Microclimatic variations with elevation

Temperature decreased significantly with elevation (Spearman's rank correlation, $r_s = -0.94$, $t_4 = -5.66$, $P = 0.0048$) (Table 1).
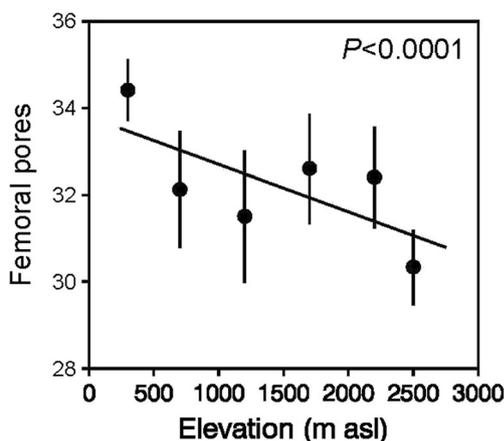
Table 1 Climatic and environmental data (mean ± SE) from six populations located along an elevational gradient in Sierra Nevada mountains (Granada, SE Spain)

Elevation (m asl)	Operative temperature (°C)	Precipitation (mm)	UV-B radiation (μW cm^{-2} nm^{-1})
300	40.0 ± 0.5	92	1.28 ± 0.03
700	46.4 ± 0.6	120	4.00 ± 0.14
1200	37.8 ± 0.5	151	4.19 ± 0.13
1700	37.6 ± 0.5	178	4.41 ± 0.15
2200	37.5 ± 0.5	199	5.15 ± 0.31
2500	31.7 ± 0.5	210	4.80 ± 0.37

Thus, operative temperatures when *P. algirus* lizards were active diminished more than 8 °C with elevation from the lowest to the highest locality. However, precipitation increased with elevation ($r_s = 0.94$, $t_4 = 5.66$, $P = 0.0048$) (Table 1), with the amount precipitation being more than the double in the highest locality in comparison with the lowest one. Similarly, UV-B radiation also increased with elevation ($r_s = 0.94$, $t_4 = 5.66$, $P = 0.0048$) (Table 1).

Femoral pores, body size and body condition

Male *P. algirus* lizards in these localities had an overall average (±SE) total number of 32.5 ± 0.3 (range = 27–39) femoral pores. There were significant differences in the number of femoral pores among localities located at different elevations (GLM: $F_{5,66} = 6.20$, $P < 0.0001$) (Fig. 1), with a low but significant trend to decrease the number of femoral pores with elevations (linear regression: $r = -0.31$, $F_{1,70} = 7.36$, $P = 0.008$). Also, male lizards were significantly larger (based on SVL measurements) at higher elevations (GLM:

Fig. 1 Total number of femoral pores (mean ± 1SE) in male *Psammodromus algirus* lizards of six sampling localities located along an elevational gradient. The *line* represents the fitted lineal regression with the *P* value indicated above

$F_{5,66} = 10.91$, $P < 0.0001$; linear regression: $r = 0.54$, $F_{1,70} = 29.36$, $P < 0.0001$), but the number of femoral pores was not significantly dependent on SVL ($r = -0.16$, $F_{1,70} = 2.48$, $P = 0.12$). Moreover, the variation in the number of femoral pores with elevation remained significant after removing the potential effect of covariation with body size (GLM; elevation effect: $F_{5,65} = 5.51$, $P < 0.0003$; SVL effect: $F_{1,65} = 0.34$, $P = 0.56$). Body condition indexes of male lizards did not vary significantly with elevation (GLM: $F_{5,66} = 1.36$, $P < 0.25$; linear regression: $r = 0.10$, $F_{1,70} = 0.77$, $P = 0.38$).

Characteristics of chemical secretions

We found 57 lipophilic compounds in the femoral gland secretions of male *P. algirus* (Table 2). Considering all lizards from the six localities, the main components were 24 steroids (65.1% of TIC), nine carboxylic (=fatty) acids and their ethyl esters between C_{10} and C_{20} (9.4%), eight alcohols between C_{12} and C_{20} (7.0%), eight aldehydes (6.2%), four waxy esters (5.5%), squalene and other terpenoid (4.0%), one amide (2.4%), and one ketone (0.4%). On average, the six most abundant compounds were campesterol (17.4% of TIC), sitosterol (9.9%), ergosterol (8.6%), cholesterol (6.7%), squalene (4.0%), and hexadecanoic acid (3.8%).

Elevational variation of femoral secretions

The PERMANOVA based on the resemblance matrix comparing the presence/absence of compounds in the chemical profiles of lizards showed significant differences among localities located at different elevations (pseudo $F_{5,66} = 4.46$, $P = 0.001$). However, these differences were due to the minor compounds because when considering only the presence of the 14 major compounds (> 2% of TIC each, comprising 61.72% of the total TIC), there were no significant differences (pseudo $F_{5,66} = 1.14$, $P = 0.35$) as all of these major compounds were found in most of individuals.

There were also significant differences in the relative proportion of all compounds among localities located at different elevations (pseudo $F_{5,66} = 6.05$, $P = 0.001$). Pairwise comparisons showed significant differences in chemical profiles between almost all localities (permutation tests: $t > 1.86$, $P < 0.006$ in all cases) but there was no difference between the two highest localities (2200 vs. 2500: $t = 0.56$, $P = 0.84$). The CAP analysis assigned on average 62.5% of the chemical profiles to the correct locality using leave-one-out cross-validation and $m = 9$ axes ($\delta_1^2 = 0.88$, $P = 0.001$).

There were significant trends in changes with elevation of the proportion of some of the major classes of compounds in femoral secretions (Fig. 2). There were significant increases with elevation in relative proportion of ethyl esters of fatty acids (linear regression: $r = 0.43$, $F_{1,70} = 16.10$, $P = 0.00015$), aldehydes

Table 2　Relative proportions (mean ± SE) of lipophilic compounds found in femoral secretions of male *Psammodromus algirus* lizards (*N* = 72) from six sampling localities (12 lizards per locality) of Sierra Nevada mountains (Granada, SE Spain)

RT	Compound	Mean ± SE		
19.9	Dodecanol	1.27	±	0.38
20.6	Decenoic acid	0.02	±	0.01
22.5	Tridecanol	0.05	±	0.03
24.8	Tetradecanol	0.69	±	0.13
24.9	Tridecanal	0.05	±	0.03
25.3	Tetradecanal	0.18	±	0.05
27.1	Hexadecenal	0.44	±	0.19
27.6	Pentadecanal	1.21	±	0.48
29.2	Pentadecanol	0.55	±	0.12
29.7	Hexadecanal	0.85	±	0.14
31.2	Hexadecanol	1.13	±	0.11
31.4	2-Heptadecanone	0.47	±	0.18
31.7	Heptadecanal	1.01	±	0.18
32.7	Hexadecanoic acid	3.83	±	0.56
33.2	Hexadecanoic acid, ethyl ester	0.17	±	0.05
33.7	Octadecanal	1.97	±	0.25
35.3	Octadecanol	0.52	±	0.19
35.9	9,12-Octadecadienoic acid	0.19	±	0.10
36.0	Octadecenoic acid	0.24	±	0.18
36.3	9,12-Octadecadienoic acid, ethyl ester	1.91	±	0.66
36.4	Octadecanoic acid	1.76	±	0.41
36.8	Octadecanoic acid, ethyl ester	0.21	±	0.09
37.3	Octadecenol acetate	0.25	±	0.09
38.7	Eicosanol	2.53	±	0.55
39.1	Docosanal	0.46	±	0.12
39.7	9-Octadecenamide	2.45	±	0.52
39.9	Eicosanoic acid	1.05	±	0.34
46.6	Squalene	4.03	±	0.92
47.4	Cholesta-4,6-dien-3-ol	0.11	±	0.04
47.7	Cholesta-3,5-diene	0.60	±	0.11
47.9	Unid.Steroid (197,251,349,364)	0.08	±	0.05
48.5	Unidentified terpenoid	0.01	±	0.01
48.6	Unid.Steroid(197,251,361,376)	0.64	±	0.33
48.8	Cholesta-4,6-dien-3-ol, methyl derivative?	0.11	±	0.06
49.1	Cholesta-3,5-diene, methyl derivative?	0.34	±	0.09
49.4	Unid.Steroid (197,251,363,378)	0.94	±	0.14
49.7	Unid.Steroid (195,209,249,363,378)	0.09	±	0.04
49.9	Unid.Steroid 143,158,253,366,381)	0.40	±	0.11
50.0	Unid.Steroid 141,156,380,393,396)	0.17	±	0.07
50.3	Unid.Steroid 147,159,213,255,382,397)	1.88	±	0.67
50.6	Unid.Steroid 197,251,377,392)	3.77	±	0.54
50.7	Cholesterol	6.68	±	1.20
50.9	Unid.Steroid (195,251,377,393)	0.28	±	0.11
51.1	Unid.Steroid(143,158,253,379,395)	1.88	±	0.33
51.4	Ergosta-7,22-dien-3-ol	2.32	±	0.55
51.5	Unid.Steroid (235,255,361,376)	0.86	±	0.28

Table 2　(continued)

RT	Compound	Mean ± SE		
51.8	Ergosterol	8.59	±	1.53
52.0	Campesterol	17.38	±	1.71
52.8	Ergost-7-en-3-ol	2.32	±	0.53
53.1	Stigmasterol	2.02	±	0.57
53.3	Unid.Steroid (255,271,300,351,377,410)	2.20	±	0.48
53.9	Sitosterol	9.93	±	1.09
55.4	Unid. waxy ester of hexadecanoic acid	1.00	±	0.41
59.0	Unid. waxy ester of hexadecanoic acid	1.61	±	0.53
61.2	Unid. waxy ester of octadecanoic acid	2.26	±	0.39
64.2	Unid. waxy ester of octadecanoic acid	0.62	±	0.14
66.9	Unid.Steroid (145,213,255,274,355,367,384)	1.46	±	0.66

The relative amount of each component was determined as the percentage of the total ion current (TIC). Characteristic ions (m/z) are reported for unidentified (Unid.) steroids
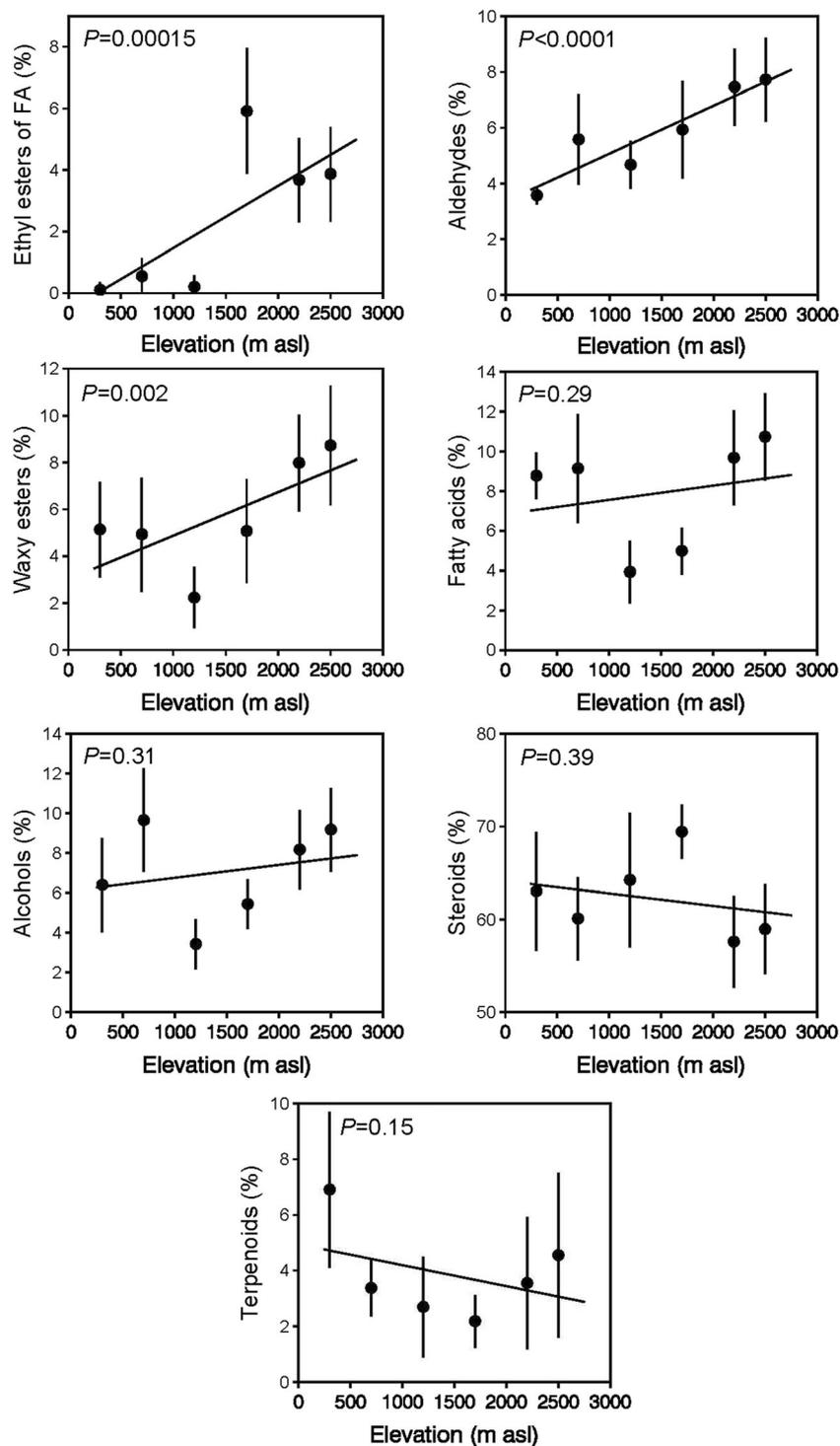
RT retention time

($r = 0.46$, $F_{1,70} = 19.11$, $P < 0.0001$) and waxy esters ($r = 0.36$, $F_{1,70} = 10.16$, $P = 0.002$), while proportions of other classes of compounds did not significantly change with elevation (fatty acids: $r = 0.13$, $F_{1,70} = 1.13$, $P = 0.29$; alcohols: $r = 0.12$, $F_{1,70} = 1.06$, $P = 0.31$; steroids: $r = -0.10$, $F_{1,70} = 0.74$, $P = 0.39$; terpenoids: $r = -0.17$, $F_{1,70} = 2.14$, $P = 0.15$).

Considering the five main major compounds, there were low but significant decreases with elevation in the relative proportion of cholesterol ($r = -0.29$, $F_{1,70} = 6.37$, $P = 0.014$) and squalene ($r = -0.29$, $F_{1,70} = 6.29$, $P = 0.014$), but proportions of the other main compounds did not significantly change with elevation (campesterol: $r = -0.15$, $F_{1,70} = 1.52$, $P = 0.22$: sitosterol: $r = -0.06$, $F_{1,70} = 0.27$, $P = 0.60$; ergosterol: $r = -0.23$, $F_{1,70} = 3.75$, $P = 0.057$; hexadecanoic acid: $r = -0.03$, $F_{1,70} = 0.06$, $P = 0.80$).

Because operative temperature decreased with elevation, while precipitation and UV radiation increased with elevation (Table 1), similar results in the variation in classes of compounds in femoral secretion with respect to climatic variables were found. Thus, the proportion of ethyl esters of fatty acids decreased with temperature (linear regression: $r = -0.30$, $F_{1,70} = 7.12$, $P < 0.001$) and increased with precipitation ($r = 0.46$, $F_{1,70} = 18.34$, $P < 0.0001$) and UV radiation ($r = 0.36$, $F_{1,70} = 10.57$, $P < 0.002$). Also, the proportion of aldehydes decreased with temperature ($r = -0.55$, $F_{1,70} = 30.47$, $P < 0.0001$) and increased with precipitation ($r = 0.46$, $F_{1,70} = 18.57$, $P < 0.0001$) and UV radiation ($r = 0.44$, $F_{1,70} = 16.51$, $P = 0.00012$). Similarly, the proportion of waxy esters decreased with temperature ($r = -0.25$, $F_{1,70} = 4.79$, $P = 0.03$) and increased with precipitation ($r = 0.33$, $F_{1,70} = 8.31$, $P = 0.005$) and tended, although no significantly, to increase with UV radiation ($r = 0.21$, $F_{1,70} = 3.24$, $P = 0.076$).

Fig. 2 Major classes of lipophilic compounds found in femoral secretions of male *Psammodromus algirus* lizards of six sampling localities located along an elevational gradient. The relative amount of each component, determined as percentage of the total ion current (TIC), is reported as mean ± 1SE. The *lines* represent the fitted lineal regressions with the *P* values indicated above

Discussion

In this study, we showed clear elevational variation in characteristics of chemical signals of a lizard species occupying different elevations with different microclimatic characteristics. Although there was qualitative variation among localities in the presence/absence of minor compounds in chemical signals, the presence of the main major compounds was similar in all samples, which was expected given that lizards from all localities are connected and have minimum genetic differences (unpubl. data). However, there was elevational variation in the relative proportion of some classes of compounds (i.e., ethyl esters of fatty acids, waxy esters and aldehydes) but not others. This variation in types of compounds in secretions

would result in different physicochemical properties of the entire femoral secretion, which might help optimize the efficiency of these chemical signals under each particular local microclimatic condition. Therefore, although our results are correlational, we suggest that environmental elevational variation may drive the characteristics of chemical signals of lizards in each locality (=elevation).

Moreover, *P. algirus* lizards from the localities studied herein also differ in the number of femoral pores, males from the lowest localities having more femoral pores than males from the highest localities, independently of body size differences. A similar elevational variation in number of femoral pores was observed in two central Spain populations of *P. algirus* of the western clade (Iraeta et al. 2011) and in *Podarcis hispanicus* lizards (Gabirot et al. 2012b). A higher number of femoral pores may be related to a higher production of chemical secretions (Alberts et al. 1992; Escobar et al. 2003; Iraeta et al. 2011; Baeckens et al. 2015). This may suggest that chemical communication is more important, or more effective, for lizards in the lowest localities. Interestingly, visual signals (=nuptial bright coloration) are more developed in *P. algirus* males of the highest localities studied here (Reguera 2015), suggesting that the relative importance of chemical and visual signals depend on the effectiveness of each type of communication in different environments. A similar elevational variation in the relative importance of alternative communication systems was suggested for *Liolaemus* lizards from different elevational populations (Fox and Shipman 2003).

With respect to the elevational variations in chemical composition, we have found that high elevation male *P. algirus* have greater relative proportions of waxy esters and ethyl esters of fatty acids than males from lower elevations. These types of compounds are less volatile and less water soluble and would confer more stability to secretions and scent marks of high elevation males under conditions where high levels of humidity may increase oxidation (Alberts 1992). Similarly, in two mountain populations of *Podarcis hispanicus* (=*P. guadarramae*) lizards of Central Spain, high elevation males have higher abundances of waxy esters and of the longest chain fatty acids than low elevation males (Martín and López 2006c; Gabirot et al. 2012b). Further experimental work with these populations of *P. hispanicus* showed that femoral secretions have a lower efficacy (i.e., a lower chemosensory detectability by conspecifics and persistence) when temperature and dryness increase, but that these effects are more detrimental in the highest elevation population, which would be more adapted to the naturally colder and more humid environments that they occupy (Martín et al. 2015). Also, waxy esters and fatty acids esters might protect other compounds in secretions from higher levels of UV radiation at higher elevations (Blumthaler et al. 1997; Reguera et al. 2014), which may quickly degrade some compounds. Finally, the possible variation in microhabitats or types of substrates selected by lizards may be important; for example, in two related sympatric lizard species, the ground dwelling *Podarcis*

carbonelli have more waxy esters in secretions than the saxicolous *P. bocagei* (García-Roa et al. 2016). This may increase the persistence of scent marks under the microclimatic more humid conditions experienced in the vegetated ground in comparison to open rocks. However, the six localities of *P. algirus* studied here have similar patterns of microhabitat and substrate use (unpubl. Data), although conditions of temperature and humidity of the same type of substrate vary among elevations.

In secretions of other lizard species typically linked to wet environments, there are higher abundances of the well-known fixers and antioxidants squalene and/or α-tocopherol (e.g., *Lacerta schreiberi*, López and Martín 2006; *Zootoca vivipara*, Gabirot et al. 2008; *L. viridis*, Kopena et al. 2009). However, tocopherol was undetected in secretions of *P. algirus* and we have not found elevational variation in terpenoids in secretions, with squalene even decreasing slightly in elevation. It is possible that phylogenetic constrains and/or physiological limitations might explain these interspecific differences in composition of secretions, but further studies are needed to test these hypotheses.

Also, there is a slight but significant decrease of cholesterol with elevation (but not of other major steroids, nor of steroids in general). Cholesterol was more abundant in low elevation localities where this steroid could protect secretions from higher temperatures and dryer conditions (Alberts 1992), as it was suggested to explain similar interpopulational variations in other lizard species (Escobar et al. 2003; Gabirot et al. 2012a,b).

Interestingly, we have also found a relatively higher proportion of aldehydes in the high elevation localities of *P. algirus*. Contrary to expectations and to the above arguments, aldehydes are highly volatile and relatively unstable compounds (Weldon et al. 2008) that would have a low efficiency and durability in substrate scent marks in the humid high elevation environment. However, rather than in scent marks, aldehydes could be alternatively used in direct intraspecific interactions where relatively long range and earlier detection by conspecifics of highly volatile compounds may be important (Apps et al. 2015). If scent marks are less effective (or costly) in the high elevation localities, an alternative use of odors coming directly from the body of the sender (Apps et al. 2015) could be favored. In fact, experimental manipulations of body odors of male lizards (i.e., secretions from the femoral glands and cloaca impregnated on the body) indicate that these odors are important in rival male recognition and dominance signaling during male–male interactions in this (López et al. 2003) and other lacertid lizards (Martín and López 2007; Martín et al. 2007a; López and Martín 2011). Aldehydes can have a prominent role under these circumstances and could be favored in the high elevation localities as an alternative when territorial scent marks were less effective.

On the other hand, proportions of other compounds (fatty acids, alcohols, and steroids) did not change with elevation. This finding might suggest that these compounds are important

in communication per se and, therefore, that their potential variation is not possible or that their proportions depend on other physiological factors or dietary constrains, independently of the environmental variables that change with elevation. For example, in other populations of *P. algirus*, the relative proportion in secretions of two alcohols (octadecanol and eicosanol) and two fatty acids (octadecanoic and eicosanoic acids) are related to the parasite load and immune response (i.e., health state) of the male that produce them (Martín et al. 2007b). This variation may have a signaling function as females show stronger chemosensory responses to secretions of healthier males, suggesting that the scents of these males are more attractive (Martín et al. 2007b). Therefore, it could be suggested that most of the compounds whose proportions do vary with microclimate may have a structural function in secretions that vary in order to protect other condition-dependent compounds with an actual signaling function.

In conclusion, our findings suggest that elevational variation in chemical characteristics of femoral secretions of *P. algirus* may reflect the different microclimatic conditions of each locality. This variation will help to maximize the efficiency of chemical communication. Further studies should examine experimentally the actual efficacy (i.e., durability and detectability by conspecifics) of the different types of secretions under different conditions of temperature and humidity. Moreover, the proximal causes of variation in the characteristics of secretions in each locality, such as different diets or thermoregulatory opportunities at different elevations (Zamora-Camacho et al. 2013, 2016), which are known to affect chemicals in secretions (Martín and López 2006a; Kopena et al. 2011; Heathcote et al. 2014), should be examined. Finally, the lack of genetic divergence between lizards of the different localities (unpublished data) strongly suggests that the variation in chemical signals in our study system reflects phenotypic plasticity rather than genetic local adaptation. However, further studies should be done to test whether environmental-dependent differences in chemical signals could lead to greater genetic differences and speciation processes in other species and environmental situations.

Compliance with ethical standards

Ethical note   The study was carried out in conformity with the Spanish current laws for lizard collection and detection, according to permits issued by Junta de Andalucía to the authors (references GMN/GyB/JMIF and ENSN/JSG/JEGT/MCF).

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