

The Role of Diet in Shaping the Chemical Signal Design of Lacertid Lizards

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Abstract Lizards communicate with others via chemical signals, the composition of which may vary among species. Although the selective pressures and constraints affecting chemical signal diversity at the species level remain poorly understood, the possible role of diet has been largely neglected. The chemical signals of many lizards originate from the femoral glands that exude a mixture of semiochemicals, and may be used in a variety of contexts. We analyzed the lipophilic fraction of the glandular secretions of 45 species of lacertid lizard species by gas chromatography/mass spectrometry. The proportions of nine major chemical classes (alcohols, aldehydes, fatty acids, furanones, ketones, steroids, terpenoids, tocopherols and waxy esters), the relative contributions of these different classes ('chemical diversity'), and the total number of different lipophilic compounds ('chemical richness') varied greatly among species. We examined whether interspecific differences in these chemical variables could

be coupled to interspecific variation in diet using data from the literature. In addition, we compared chemical signal composition among species that almost never, occasionally, or often eat plant material. We found little support for the hypothesis that the chemical profile of a given species' secretion depends on the type of food consumed. Diet breadth did not correlate with chemical diversity or richness. The amount of plants or ants consumed did not affect the relative contribution of any of the nine major chemical classes to the secretion. Chemical diversity did not differ among lizards with different levels of plant consumption; however, chemical richness was low in species with an exclusive arthropod diet, suggesting that incorporating plants in the diet enables lizards to increase the number of compounds allocated to secretions, likely because a (partly) herbivorous diet allows them to include compounds of plant origin that are unavailable in animal prey. Still, overall, diet appears a relatively poor predictor of interspecific differences in the broad chemical profiles of secretions of lacertid lizards.

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Introduction

Chemical communication is likely the oldest and possibly the most ubiquitous form of information exchange in the natural world (Maynard-Smith and Harper 2003). However, maybe due to our own predisposition to visual and auditory signals, studies of chemical signals in vertebrates are less common, causing some authors to argue that chemical communication is 'the last frontier in the study of animal behaviour' (Hunt et al. 2012). With the improvement in analytical techniques,

this is now changing, and it has become clear that chemical signals are at play in multiple contexts for a wide variety of organisms (Wyatt 2014).

Lizards, for instance, are equipped with epidermal glands on their inner thighs that produce a waxy mixture of proteins and lipids that is actively or passively deposited on a substrate as scent marks (Alberts 1991). Recent analyses revealed that these glandular secretions operate as chemical signals involved in a variety of contexts, such as territory demarcation and assessment, male rival assessment, female choice, assessment of female reproductive status, individual recognition, sex identification, and species recognition (recently reviewed by Mayerl et al. 2015).

Remarkable, almost all of these studies have focused on one or two study species each. Larger scale studies on chemical communication systems, comparing signals across species in a phylogenetic context, are scarce (and not only so in lizards, Symonds and Elgar 2008; but see Baeckens et al. 2017a), despite the fact that comparative analyses of visual (e.g., Ord and Martins 2006) and acoustic interaction systems (e.g., Garamszegi et al. 2005) have proven how valuable this approach can be for understanding the evolution of signal diversity. The diversity and composition of glandular secretions varies widely, but consistently, among lizard species, both in complexity and in the nature of constituent molecules (see Weldon et al. 2008 for a review on this topic in reptiles), but the origins and significance of this variation remain poorly understood.

One factor likely to contribute to divergence in glandular secretion composition of vertebrates is diet. If species, populations or even individuals differ, quantitatively or qualitatively, in the acquisition of certain dietary compounds, they may also differ in the chemical cues and signals that are ultimately obtained or synthesized from them (Symonds and Elgar 2008). Evidence for a direct effect of diet on glandular chemical profiles comes from studies on conspecific recognition, mate selection, and predation avoidance. In a diverse array of species, individuals will preferentially associate with conspecifics that are on some (usually rich) diet (e.g., Bryant and Atema 1987; Conner et al. 1990). Diet-derived differences in chemical cues or signals may also function in mate selection: females typically prefer partners whose chemical signals contain particular compounds that are expensive to produce or difficult to obtain (e.g., Kopena et al. 2011; Martín and López 2006; Rundle et al. 2005). One study on Lacertidae found evidence for a direct effect of diet on signal expression at the individual level (Kopena et al. 2014); in *Lacerta schreiberi*, dietary supplementation with carotenoids and vitamin E affected among-individual variation in glandular secretion composition (i.e., supplemented individuals increased the relative proportion of vitamin E in secretions) In much the same way, dietary components may be echoed in visual sexual signals (Blair 1957; Kopena et al. 2014; Martín and López 2010). Finally, animals are known to sequester food-derived chemicals

into toxins (Daly et al. 1994, 2000; Dumbacher et al. 1992), or deploy them for chemical camouflage (e.g., Brooker et al. 2015).

Here, we take a broad phylogenetic comparative approach, testing whether among-species variation in the composition of epidermal (femoral) gland secretions of the lizard family Lacertidae reflects dietary divergence. We exclusively consider the lipophilic, and not the proteinaceous, fraction of the glandular secretion, since the former is comprised of metabolites and hence, expected to be more dietary-driven. Although most lacertids have a predominantly arthropod-based diet, the relative contributions of different types of arthropods vary considerably among species (Carretero 2004; Herrel et al. 2004; Verwajen et al. 2002) and some species, especially island dwellers (but not exclusively), consume large amounts of plant material (Van Damme 1999). Although prey availability undoubtedly drives much of the interspecific variation in diet in lacertids, several species have been shown to prefer or avoid certain food items (see Carretero 2004 for a review). In the current study, we specifically looked for correlations between diet diversity and chemical signal diversity. We tested whether species that consume significant fractions of plant material differed from species with a purely arthropod-based diet in the overall composition of their chemical signals or in the abundance of specific chemical compounds of plant origin (e.g., tocopherol, a compound involved in mate choice; Kopena et al. 2011) in secretions. Finally, we examined whether a myrmecophagous (ant-eating) diet affected signal chemistry of lizards due to the relatively low nutritional value of ants.

Methods and Materials

Femoral Gland Secretions Between 2005 and 2016, we collected femoral gland secretions from 45 species of lacertid lizards at various locations in Europe, Africa and Asia (see Table S1 in Electronic Supplementary Materials). In total, we captured 527 lizards by hand or noose. On average, we caught 12 individuals per species (range 1–35). Since femoral glands develop at the onset of sexual maturity, and their activity is greatest during the reproductive period, we exclusively sampled adult males during mating season. After secretion collection, all lizards were released at the exact site of capture. Captures of animals were performed under licence and permission of the local, regional and/or national environmental agency (see ‘Compliance with Ethical Standards’ for more details). Immediately after lizards were captured in the field, we collected their femoral gland secretions by gently pressing around the pore-bearing scales, or ‘femoral pores’. The extraction procedure is harmless, and the lizards are able to produce more secretion rapidly thereafter (e.g., Baeckens et al. 2017b). The secretions were collected in glass vials with glass inserts, sealed with Teflon-lined caps. For blank controls, the same

procedure was carried out without collecting a secretion. Subsequently, vials were stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

To analyze samples, we used 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). A Finnigan-ThermoQuest Trace mass spectrometer (MS) was used as the detector. With helium as carrier gas, we carried out splitless injections (2 μl of each sample dissolved in 2 ml of GC capillary grade *n*-hexane). The injector and detector were set to 250 $^{\circ}\text{C}$ and 280 $^{\circ}\text{C}$, respectively. The column oven temperature program started at 50 $^{\circ}\text{C}$ (held for 3 min) and was increased to 300 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C}\cdot\text{min}^{-1}$, and held for 15 min. Mass spectral fragments below $m/z = 46$ were not recorded. Initially, we identified chemicals to (at least) a major class, by comparing their mass spectra with those in the NIST/EPA/NIH (NIST 02) mass spectral library. The identification of specific compounds was confirmed by comparing spectra and retention times with those of authentic standards when these were available (from Sigma-Aldrich Chemical Co.). Impurities in the control vial samples were not considered. When compounds did not match with available standards or we could not find a preliminary acceptable identification, compounds were considered “unidentified”. The percentage of these unidentified compounds was relatively low (ca. 10–20% for all vials analyzed for the same species) and, in practically all cases, they could be easily and reliably identified as belonging to a major class of compounds (steroids, waxy esters, etc.) since their mass spectra usually differed minimally from those of well-known compounds. Moreover, these “unidentified” compounds could also be characterized across different individuals within a species by their specific retention times and characteristic mass spectra.

Finally, we estimated the relative abundance of each chemical as the percentage of the total ion current (TIC). This was done for each individual lizard, with averages calculated for each species. A detailed list of all lipophilic compounds found in the glandular secretions of the lacertids under study can be found in Table S4.

The total number of different lipophilic compounds (both identified and ‘unidentified’ compounds that could be characterized within a species by specific retention times and mass spectra) found in the samples of a species (pooling data of all individuals analyzed) was considered a given species ‘chemical richness’. To obtain another measure of ‘chemical diversity’ of a species’ secretion, we first determined the relative proportions of nine compound classes (alcohols, aldehydes, fatty acids, furanones, ketones, steroids, terpenoids, tocopherols and waxy esters) in the mixture, and then calculated the Shannon diversity index (H_{chem} ; Shannon 1948).

Diet Data We searched the literature for information on the natural diets of the species for which we had chemical secretion

data. When we found diet information on more than one population of a specific species, we only included diet data of the population for which we collected chemical data, or that was geographically closest to a sampled population. The relative contribution (in terms of prey items found in the stomach, intestines or feces) of each arthropod group to the total diet of each species was noted. We distinguished 25 orders of Hexapoda (keeping formicid ants as a special group, separate from the rest of Hymenoptera), six groups of Arachnida, and five taxonomically broader groupings (Crustacea, Myriapoda, Oligochaeta, Mollusca and Vertebrata). In addition, we assigned each lizard species to one of three groups, depending on the frequency with which they are known to consume plant material. Group ‘A’ has no or very little plant material in its diet, group ‘O’ eats plants occasionally, and group ‘H’ has a diet that predominantly consists of plant material. Analogous to Cooper & Vitt (2002) and Baeckens et al. (2017c) we used a cut-off rule of 10%, in which lizards from group ‘H’ are species for which plant consumption is at least 90%, and in which occasional plant-eaters consume at least 10%, but less than 90%, plant matter. Species belonging to group ‘A’ consume less than 10% plant matter. Although arbitrary, the 10% criterion is useful because it excludes species that may incidentally ingest small amounts of plant material (Cooper & Vitt 2002).

Diet breadth was estimated by the Shannon diversity index (H_{diet} , Shannon 1948).

Phylogeny and Statistics We used the tree described by Baeckens et al. (2015) to analyze our data in a phylogenetic framework. The tree was constructed with information on sequences from three mitochondrial and two nuclear gene regions. The tree was pruned so as to include only the 45 species for which we found data.

Prior to statistical analysis, we transformed all variables to conform to statistical expectations: chemical and diet diversity (\log_{10}), chemical richness (square-root), and all frequency data (arcsin square-root).

We used the ‘ppls’-command in the ‘caper’ package (Orme et al. 2013) to relate chemical signal diversity and richness to diet diversity, accounting for phylogenetic signal by adjusting lambda by maximal likelihood transformation. We used the ‘phylanova’ command in the package ‘phytools’ (Revell 2012) to test whether chemical signal diversity and richness differed among species whose diet included no, little or substantial amounts of plant material.

We used a phylogenetic MANOVA (function ‘aov.phylo’) to test whether consuming plant material (‘H’, ‘O’, or ‘A’) affects species’ secretion composition.

The phylogenetic signal for the complete multivariate chemical matrix, chemical signal richness and chemical signal diversity, and diet-diversity was calculated using Pagel’s λ and Blomberg’s K (function ‘phylosignal’ and function ‘K.mult’ from the ‘phylocurve’ package, Goolsby 2016). Phylogenetic

signal is the tendency of related species to resemble one another due to their common ancestry, and Blomberg's K and Pagel's λ are two quantitative measures of this pattern (Blomberg et al. 2003; Pagel 1999). K values that are approximately equal to 1 match the expected trait evolution under Brownian motion (BM), and indicate an apparent phylogenetic signal; K values much less than 1 and closer to zero indicate little or no phylogenetic signal associated with random trait evolution or convergence; K values greater than 1 suggest stronger similarities among closely related species than expected under BM, and thus indicate a substantial degree of trait conservatism (Blomberg et al. 2003). Pagel's λ is a scaling parameter that typically ranges from zero to 1. Lambda values of zero indicate no phylogenetic signal, whereas values of 1 indicate a strong phylogenetic signal, matching trait evolution, expected under BM (Pagel 1999); values larger than 1 are also possible and denote a stronger phylogenetic signal than the one predicted by BM (Freckleton et al. 2002).

Results

We found data on diet for 45 species for which we also knew the chemical components of males femoral secretions (Tables S2 and S3). Diet diversity (H_{diet}) varied between 0.016 (for *Meroles squamulosus*) and 2.359 (*Psammotromus hispanicus*). Twenty-six species consumed no or very little plant material (category A), 14 species ate plants occasionally (O) and for five species (H), plants constituted an important part of the diet. Diet diversity exhibited a low but significant phylogenetic signal (Blomberg's $K = 0.37$, $P = 0.017$; Pagel's $\lambda = 0.77$, $P = 0.0006$).

In the 45 species dataset (Table S2 and S3), chemical signal richness varied between 14 (for *Ophisops elegans*) and 103 (for *Gallotia galloti*) compounds. The average (\pm SE) chemical richness was 50 (± 3). Richness showed a moderate but significant phylogenetic signal ($\lambda = 0.78$, $P = 0.001$; $K = 0.37$, $P = 0.015$). Chemical signal diversity ranged from 0.19 (*Dalmatolacerta oxycephala*) to 1.56 (*Podarcis peloponnesiacus*), with an overall species average of 0.81 ± 0.05 . The phylogenetic signal for chemical signal diversity was not significant ($\lambda = 0.62$, $P = 0.104$; $K = 0.22$, $P = 0.140$). The overall composition of the femoral gland secretion in lacertid lizards exhibited a relatively weak phylogenetic signal (Blomberg's multivariate $K = 0.47$, $P < 0.001$).

Diet diversity did not predict chemical signal diversity (pgls, $r^2 = 0.005$, $F_{1,43} = 0.22$, $P = 0.64$) or richness (pgls, $r^2 = 0.006$, $F_{1,43} = 0.27$, $P = 0.60$).

Chemical signal diversity appeared greatest in species that consumed plants occasionally (group O, mean \pm SE: 0.93 ± 0.08) and lowest in species with a predominantly plant-based diet (group H, 0.68 ± 0.06); the secretion of species that rarely eat plants had an intermediate chemical

diversity (group A, 0.79 ± 0.06). These differences were not significant (traditional ANOVA: $F_{2,42} = 1.35$, $P = 0.27$; phylogenetic ANOVA: $P = 0.25$), and thus provide no evidence that the degree of plant-eating affects chemical signal diversity. Chemical signal richness was greater in plant-consuming species (group O: 60 ± 8 ; group H: 58 ± 5) compared to non-plant eating species (group A: 43 ± 4). Both traditional ANOVA ($F_{2,42} = 3.80$, $P = 0.03$) and phylogenetic ANOVA ($P = 0.026$) indicated that this difference was significant (Fig. 1). Overall, the three groups (A, H, O) did not differ in their relative contribution of the nine major chemical compound groups to the total mixture (traditional MANOVA: $F_{18,70} = 0.86$, $P = 0.63$; phylogenetic MANOVA: $P = 0.96$), and nor did they differ in the relative contribution of tocopherols (traditional ANOVA: $F_{2,42} = 0.91$, $P = 0.41$; phylogenetic ANOVA: $P = 0.43$).

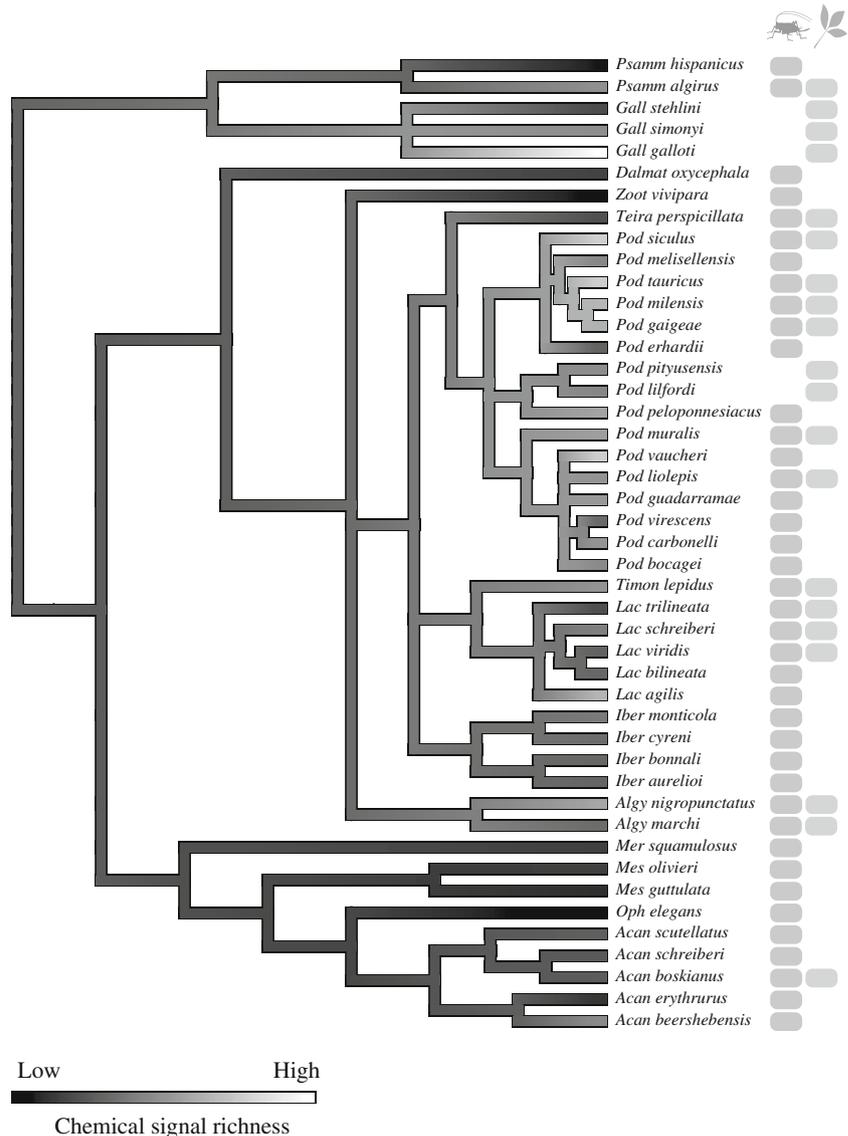
Neither chemical diversity nor chemical richness correlated with the proportion of ants in the diet (pgls, diversity: $r^2 = 0.05$, $P = 0.14$; richness: $r^2 = 0.02$, $P = 0.39$). Species that ate larger proportions of ants tended to have lower percentages of steroids in their femoral secretions, but the correlation was not significant (pgls, $r^2 = 0.071$, slope = -0.27 , $P = 0.077$). No relationship was found between the reliance on ants and the relative amount of any other major component class (all $P > 0.18$).

Discussion

Our results show that lizard species of the family Lacertidae vary considerably, but consistently, in the composition of femoral gland secretions. This finding is not unique. Most studies that have compared the composition of chemical signals among animal species or among populations within species have documented considerable variability (Alberts 1991; Rollmann et al. 2000; Gabirot et al. 2016; Pureswaran et al. 2016).

Discovering the origin and/or functional significance of this interspecific or interpopulational variation of chemical signals is elusive. Authors that compare chemical signals between two or more closely related species in sympatry often interpret differences in light of species recognition and reproductive isolation (e.g., Escobar et al. 2003; Martín and López 2006; Gabirot et al. 2010, 2012; Martín et al. 2016). Others have offered adaptive explanations for the observed variability, arguing that local environmental conditions (climate, substrate), through their effects on transmission efficiency, may select for different chemical signal structures (e.g., Escobar et al. 2003; Baeckens et al. 2015; Martín et al. 2015). Only a few authors have considered the possibility that interspecific or interpopulational variation may arise from differences in diet. For instance, Gabirot et al. (2016) suggested that differences in the composition of uropygial gland secretions of two

Fig. 1 Ancestral character estimation of chemical signal richness along the branches and nodes of a tree of 45 lacertid species with additional information on their diet (graphical method described by Revell 2013). Oval bars represent a species frequency of eating plant material; two bars = eating plants occasionally; one bar below the cricket = predominantly arthropod diet; one bar below the plant = predominantly herbivorous diet



shearwater species (*Calonectris*) might reflect differences in the birds' feeding ecologies. Diet was also mentioned as a possible cause of differences in femoral gland secretion chemistry of two closely related *Podarcis* lizard species (Gabirot et al. 2012). Interestingly, Alberts (1991) found that the protein mixture in the femoral gland secretion of desert horned lizards *Phrynosoma platyrhinos* differed markedly from that of other sceloporine lizards and suggested that this could be due to the lizard's myrmecophagous diet (the other species had a much more general insect diet). We know of no other taxon-broad study on lizards that has explicitly linked interspecific variation in chemical signal secretion to dietary habits.

Overall, we found no evidence that chemical signal diversity is affected by diet in lacertid lizards. Lizards may feed

upon a wide variety of prey, and even include plant material in their diet, and still have a low signal diversity, and *vice versa*. Chemical diversity, as we calculated it here, accounts for both abundance and differences of the major chemical classes present. As the chemicals present in the food likely provide precursors from which signal molecules are biosynthesized, we expected that species with a wider, more varied range of prey should produce more diverse secretions. This proved not to be the case, which may mean several things. First, our diversity measures might be inappropriate. We calculated dietary specialization (or diversity) from the relative abundance of different taxa of invertebrates and other prey items. While this is customary in studies of diet breadth (Roughgarden 1979), taxonomic prey diversity may not adequately reflect the variability of chemicals ingested. Ideally, one would like to have

information on the chemical composition of all prey. For similar reasons, our classification of molecules present in the secretion may also be inappropriate or too simple. This classification is logical for chemical structure (Apps et al. 2015; Weldon et al. 2008), but may not reflect how molecules are acquired or produced by an emitter, or received by a receiver. Classifying chemicals by structure makes sense if molecular shape matters, but the biophysical mechanism of (vomero)lfaction remains highly debated and some authors have argued that it is the way a molecule vibrates (not its shape) that activates a receptor (Franco et al. 2011, Gane et al. 2013, Saberi and Seyed-allaei 2016, but see Block et al. 2015). If so, molecules with similar molecular structures could ‘smell’ quite differently. Also, compounds that are chemically similar could have very different origins or be more abundant in some prey types than in others.

We also caution that we used literature data to estimate dietary composition. This weakens our analysis in two ways. First, as the data on diet and the composition of femoral secretions were not always obtained for the same population, intraspecific geographical variation in dietary composition might mask any relationship between food intake and chemical signal diversity. Geographical variation in diet composition and richness has been described in several lizard species, including lacertids (e.g., Bouam et al. 2016; Scali et al. 2016). Interestingly, in the frillneck lizard, *Chlamydosaurus kingii*, among-population variation in the color of the frill seems to result from geographical differences in the availability of carotenoids and pteridines (in arthropod prey species) (Merkling et al. 2016), exemplifying how signal structure may parallel diet composition. Second, a similar caveat must be made for possible temporal variation in diet, as diet and secretion samples were not determined necessarily at the same time. Seasonal variation in diet has been documented repeatedly in lizards (e.g., Pérez-Mellado and Corti 1993; Rodríguez et al. 2008; Pérez-Cembranos et al. 2016).

Another explanation for the lack of a relationship between diet and secretion diversity, might be that most lipids present in the lizards’ secretions may be biosynthesized by the animal. Studies on insects suggest that *de novo* biosynthesis of chemical signals predominates (Tillman et al. 1999), although in some species, chemical signals arise through sequestration (e.g., Aldrich et al. 2016), or through simple chemical modification (e.g., Eisner and Meinwald 1995) of dietary compounds. Alas, very little is known on the biosynthetic pathways that produce the varied molecules present in lizard femoral secretions, so it is difficult to judge the relative importance of these mechanisms here.

In spite of the lack of a relationship between chemical signals and other diet variables, we found that chemical signal richness, which varied strongly among taxa, was significantly lower in species with a strictly arthropod-based diet than for species that ate plants, at least occasionally. This result seems to suggest that

there may be individual molecules in the chemical signature of lizards that are primarily derived from plants and may only be acquired if lizards include plant material in their diet. Weldon et al. (2008), in their review of squamate integumentary molecules suggest that tocopherols and many phytosterols, in particular, are likely sequestered from plants. In the herbivorous green iguana, *Iguana iguana*, phytosterols represent up to 10% of the lipid fraction of femoral gland secretions (Alberts et al. 1992). In Iberian green lizards, *Lacerta schreiberi*, supplementing diet with α -tocopherol (vitamin E) immediately increased the concentration of this molecule in the femoral gland secretions (Kopena et al. 2014). Because it is an important antioxidant and cannot be synthesised *de novo*, vitamin E concentration in scent marks may well act as an honest signal of male quality. Similarly, females of a closely related green lizard species, *L. viridis*, whose femoral secretions are similar, are attracted to the scent marks of males with high concentrations of vitamin E (Kopena et al. 2011). It is not clear whether tocopherols have a similar signaling role in other lacertids, but our results suggest they are present in the femoral secretions of many species. Somewhat unexpectedly, we found no difference in the relative abundance of tocopherols in species of different diets (herbivorous/insectivorous/omnivorous). This may suggest that some species obtain tocopherols from sources other than plants, e.g., from the fat of herbivorous insects (Barbehenn 2003) or earthworms (Marconi et al. 2002), or that we failed to detect the consumption of plant material in some species. It would be interesting for future studies to assess experimentally whether the prey’s type of diet (e.g., polyphagous vs. graminivorous) influences the signal chemistry of lizards.

For a small number of species in our data set, ants constitute an important dietary component. Myrmecophagy is often considered an evolutionary challenge, because the nutritional value of an ant, limited as it is due to its small size, is difficult to exploit due to the presence of a tough chitin exoskeleton (Redford and Dorea 1984). In many myrmecophagous species, the morphological adaptations required for capturing and processing sufficient numbers of ants lead to further specialization on this prey (Meyers et al. 2006). For these reasons, one might expect the chemical signals of ant-eating lizards to contain relatively few compounds. On the other hand, several dendrobatid and microhylid frog species are known to sequester certain alkaloids from the ants on which they feed (Santos et al. 2003), so myrmecophagy may also provide opportunities for the production of signaling molecules. As mentioned earlier, Alberts (1991) suggested that ant-eating may explain the aberrant gland proteic secretion chemistry of desert horned lizards, *Phrynosoma platyrhinos*. Thus, we expected ant-eating lacertids to have atypical femoral secretions. However, from our results, there is no evidence that the femoral secretions of myrmecophagous lacertid species contained less (or more) lipophilic compounds, or a smaller (or larger) component diversity than other species. We also did not find any consistent association between ant-eating and the relative

contribution of any of the major compound classes. This suggests that ant-eating species can extract all necessary lipophilic precursors from their prey, or that they somehow supplement their diet from other sources. Nevertheless, there is a trend, although not significant, for a lower proportion of steroids in secretions of species that include more ants in the diet, which suggests that there might be some limitations for ant-eating species. Further studies that not only focus on the major chemical classes in lizard secretions, but also encompass all individual lipophilic compounds, might shed light on which particular steroids are affected by a myrmecophagous diet. Those studies should also consider incorporating true ant specialists in their dataset, such as *Phrynosoma* (lizards of the genus *Moloch* do not possess any epidermal glands; Mayerl et al. 2015).

In this study, we explored relationships between diet and chemical signatures in the lizard family Lacertidae. Our data revealed considerable among-species variation in both diet and secretion chemistry. Although plant eaters were shown to produce secretions of a greater chemical richness than species that do not eat plants, our overall findings established little covariation between diet and chemical signal profiles of lacertids. This may indicate that the precursors of the signal components are widely available in prey species, or that lizards can biosynthesize compounds *de novo* or from ubiquitous precursors. However, as discussed above, our approach may lack the requisite resolution to demonstrate any direct connections between intake and secretion of major types of chemicals. Because experimental studies have shown that inter-individual variation in diet may affect variation in chemical signal composition (e.g., Martín and López 2006b; Kopena et al. 2014), and because there exists inter-populational variation in chemical profiles within the same species (e.g., Martín et al. 2013), future studies should try to associate the chemical signature of individual lizards to local food availability and contemporary consumption, preferably at several, contrasting locations in the field. Additionally, the relationship between chemical richness and plant diet should be examined in more detail.

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Environmental Agencies of the different Regional Governments of Spain where lizards were studied. All Greek species were collected in accordance with the Hellenic National Legislation (Presidential Decree 67/81).

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