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Reference:
Baeckens Simon, García-Roa Roberto, Martin José, Van Damme Raoul.- The role of diet in shaping the chemical signal design of lacertid lizards
Full text (Publisher's DOI): https://doi.org/10.1007/S10886-017-0884-2
To cite this reference: https://hdl.handle.net/10067/1460940151162165141

THE ROLE OF DIET IN SHAPING THE CHEMICAL SIGNAL DESIGN OF LACERTID LIZARDS

SIMON BAECKENS¹,* ROBERTO GARCÍA-ROA², JOSÉ MARTÍN³, RAOUl VAN DAMME¹

¹ Laboratory of Functional Morphology, Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

² Cavanilles Institute Biodiversity and Evolutionary Biology, University of Valencia, C/ Catedrático José Beltrán, nr. 2, C.P. 46980, Paterna, Valencia, Spain.

³ Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, C.S.I.C. José Gutiérrez Abascal 2, E-28006 Madrid, Spain

* Corresponding author e-mail: simon.baeckens@uantwerp.be
Abstract — Lizards communicate with others via chemical signals, whose composition varies consistently among species. Although the selective pressures and constraints affecting chemical signal diversity at the species level remain poorly understood, the possible acting role of diet has been almost fully neglected. The chemical signals of many lizards originate from the femoral glands that exude a mixture of semiochemicals, and are used in a variety of contexts. We have analysed the lipophilic fraction of the glandular secretions of 45 species of lacertid lizard species using gas chromatography-mass spectrometry (GC-MS). The proportions of nine major chemical classes of compounds (alcohols, aldehydes, fatty acids, furanones, ketones, steroids, terpenoids, tocopherols and waxy esters), the relative contribution of these different classes (‘chemical diversity’) and the total number of different lipophilic compounds in the secretions (‘chemical richness’) varied greatly among species. We examined whether interspecific differences in these chemical variables could be coupled to interspecific variation in diet. Diet data on the species in our data set were obtained from the literature. In addition, we compared chemical signal composition among species that almost never, occasionally or often eat plant material. We found very 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 allow them to include compounds of vegetal origin that are not available in animal prey. Still, overall, diet appears a relative poor predictor for interspecific differences in the broad chemical signal profiles of lacertid lizards.

Key Words — Chemical communication, Diet, Femoral gland secretions, Herbivory, Lacertidae, Lizards, Phylogenetic comparative methods.
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 for visual and auditory signals, studies of chemical signals are relatively rare, causing some authors to argue that chemical communication is ‘the last frontier in the study of animal behaviour’ (Hunt et al. 2012). With the recent improvement of analytical techniques, this is now rapidly changing, and it has become overtly clear that chemical signals are at play in multiple contexts in a wide variety of organisms (Wyatt 2014).

Lizards, for instance, are equipped with epidermal glands on their inner thighs, which produce a waxy mixture of proteins and lipids that is actively, or passively, deposited on the substrate as scent marks (Alberts 1991). Recent analyses have revealed that these glandular secretions operate as chemical signals that are 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 (reviewed by Mayerl et al. 2015).

Almost all of the studies cited in the previous paragraph have focussed 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),
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 proved 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 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 that is 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 synthesised 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. in lizards: Kopena et al. 2011; Martín and López 2006). One study on lacertids has found evidence for a direct effect of diet on signal expression at the individual level (Kopena et al. 2011); in *Lacerta schreiberi*, experimental dietary supplementation with carotenoids and vitamin E affected among-individual variation in glandular secretion composition (i.e. supplemented individuals increases relative proportions 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. 2000; Dumbacher et al. 2000), or deploy them in chemical camouflage (e.g. Brooker et al. 2014). 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 particularly comprised of metabolites or metabolite-derived compounds, hence, expected to be more dietary-driven. Although most lacertids have a predominantly arthropod-based diet, the relative contribution of different types of arthropods varies considerably among species (Carretero 2004; Herrel et al. 2004; Verwaijen et al. 2002) and some species, especially—but not exclusively— island-dwellers 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 look for correlations between diet diversity and chemical signal diversity. We test whether species that consume significant fractions of plant material differ from species with a purely arthropod-based diet in the overall-composition of their chemical signals, or the abundance of certain chemical compounds of vegetal origin in secretions (i.e. tocoferol, a compound involved in mate choice; Kopena et al. 2011). Finally, we examine whether a myrmecophagous (i.e. ant-eating) diet affects the signal chemistry of lizards due to the low nutritional value and the tough chitin exoskeleton 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 (Table S1). 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 the lizards were captured in the field, we collected femoral gland secretion 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. 2017a). The obtained secretions were instantly collected in glass vials with glass inserts closed with Teflon-lined lids. In order to obtain blank control vials, the same procedure was carried out without collecting secretion, to exclude contaminants from the handling procedure or the environment, and for examining potential impurities in the solvent. Subsequently, vials were stored at -20 °C until further analyses.

To analyse the 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 x 0.25 mm ID, 0.25 mm film thickness). A Finnigan-ThermoQuest Trace mass spectrometer (MS) was used as the detector. By using helium as the carrier gas, we carried out splitless sample injections (2 µL of each sample dissolved in 2 mL of GC capillary grade n-hexane). We maintained temperatures of injector and detector at 250 °C and 280 °C, respectively. The oven temperature program started at 50 °C (3 min), then increased to 300 °C (at a rate of 5 °C/min), to finally stay
isothermal at 300 °C (during 15 min). Mass spectral fragments below m/z = 46 were not recorded.

We first performed a preliminary tentative identification of compounds by comparison of the mass spectra in the NIST/EPA/NIH (NIST 02) computerized mass spectral library. Identifications were then confirmed, when possible, by comparison of spectra and retention times with those of authentic standards (from Sigma Aldrich Chemical Co.) when these standards were available. Impurities in the control vial samples were not considered. When compounds did not match with the available standards or we could not find a preliminary acceptable identification, we considered these compounds as "unidentified". However, the number of these unidentified compounds is relatively low (approximately between 10-20% for all vials analysed within 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, etcetera) since their mass spectra usually only differed minimally from well-known compounds. Moreover, these “unidentified” compounds could also be easily characterized across different individuals within a species by their specific retention times and characteristic mass spectra. A detailed list of all lipophilic compounds found in the glandular secretions of the lacertids under study can be found in Table S4.
Finally, we estimated the relative abundance of each chemical as the percentage of the total ion current (TIC). This was done for every lizard individual, and averages were calculated per species.

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

Diet data We searched the literature for information on the natural diet 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 that population for which we also collected chemical data, or which was geographically closest to the sampled population. The relative contribution (in terms of prey items found in the stomach, intestines or faeces) of each arthropod groups to the total diet of each species was noted. We distinguished 25 orders of Hexapoda (keeping the ants, Formicidae as a special group, separated from the rest of the 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 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. (2017b) we used a cut-off rule of 10%, were lizard from group ‘H’ are species for which plant consumption is at least 90%, and where occasional plant-eaters consume at least 10% but less than 90% plant matter. Species belonging to group ‘A’ consume less that 10% plant matter. Although arbitrary, the 10% criterion is useful because it excludes species that may incidentally ingest small amounts of plant matter (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 analyse 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 as to include only the 45 species for which we found data.
Prior to analyses, we transformed all variables to confirm to the statistics expectations of the analyses: chemical and diet diversity ($\log_{10}$), chemical richness (square-root), and all frequency data (arcsin square-root).

We used the ‘pgls’-command in the ‘caper’ package (Orme et al. 2015) to relate chemical signal diversity and richness to diet diversity, accounting for phylogenetical 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.

To investigate co-variation between diet and chemical composition, we used a phylogenetic canonical correlation analysis (pCCA, function ‘phyl.cca’). This multivariate method enables us to calculate and analyse the correlation between character sets while accounting for the non-independence of species due to phylogeny (Harisson et al. 2015; Revell & Harrison 2008). To maintain statistical power and stable canonical variate-variable correlations, we were required to reduce the number of variables in the diet dataset prior to pCC analysis: we taxonomically regrouped the diet dataset from 36 variables to seven (i.e. Chelicerata, Crustacea, Hexapoda, Oligochaeta, undetermined arthropods, and Vertebrata).
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 $\lambda$ 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 $\lambda$ 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 the Brownian motion (BM), and indicate an apparent phylogenetic signal; K values far under 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 indicates a substantial degree of trait conservatism (Blomberg et al. 2003). Pagel’s $\lambda$ 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 know the chemical components of the males’ femoral secretions (Table S2 and S3). Diet diversity ($H_{\text{diet}}$) varied between 0.016 (for *Meroles squamulosus*) and 2.359 (*Psammodromus hispanicus*). Twenty-six species consumed no or very little plant material (category A), fourteen 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 this 45 species dataset (Table S2 and S3), chemical signal richness varied between 14 number of compounds (for *Ophisops elegans*) and 103 (for *Gallotia galloti*). The average ($\pm$ SE) chemical richness was 50 ($\pm$ 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 a species average of 0.81 $\pm$ 0.05. The phylogenetical 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 highest in the species that consumed plants occasionally (group O, mean ± 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 appeared an intermediate chemical diversity (group A, 0.79 ± 0.06). However, this difference is not statistically significant, thus, providing no statistical evidence that the degree of plant-eating might affect chemical signal diversity (traditional ANOVA: $F_{2,42} = 1.35$, $P = 0.27$; phylogenetic ANOVA: $P = 0.25$). Chemical signal richness was higher 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 ($P = 0.026$) indicate that this difference is significant (Fig. 1). Overall, the three groups considered (A, H, O) did not differ in the 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: $P = 0.96$). Neither 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 significantly 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 at the 0.05 level (pgls, $r^2 = 0.071$, slope = -0.27,
\[ P = 0.077 \]. No relationship whatsoever was found between the reliance on ants and the relative amount of any other major component class (all \( P > 0.18 \)).

A phylogenetic canonical correlation analysis revealed no significant relationship between the diet and chemical matrices, providing no support that diet is affecting the overall chemical composition of lizard femoral gland secretion (canonical axis 1: \( R = 0.72, \chi^2 = 79.27, P = 0.210 \); canonical axis 2: \( R = 0.63, \chi^2 = 53.34, P = 0.499 \)).

**DISCUSSION**

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

The origin and/or functional significance of this interspecific or interpopulational variation of chemical signals often remains elusive. Authors that compare chemical signals between two or more closely-related species that live in sympatry often interpret observed differences in the light of species recognition and reproductive isolation (e.g. Escobar 2003; Gabirot et al. 2010, 2012; Martín et al. 2016; Martín and Lopez 2006b). 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. Baeckens et al. 2015; Escobar 2003; 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 shearwater species (Calonectris) might reflect differences in the birds’ feeding ecologies. Diet was also mentioned as a possible cause of the 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 femoral gland secretion of desert horned lizards Phrynosoma platyrhinos differed markedly from that of other sceloporine lizards and pondered whether that could be due to the species’ myrmecophagous diet (the other species had a much more general insectivore diet). We know of no other taxon-broad studies on lizards that have explicitly linked interspecific variation in chemical signal design to dietary habits.

Overall, we found no evidence that chemical signal diversity is affected by diet in lacertid lizards. Lizards may prey upon a wide variety of prey items, and even include plant material into their diet, and still have a low signal diversity – and vice versa. Chemical diversity, as we calculated it here, accounts for both
the abundance and the evenness of the major chemical classes present. Because ultimately, the elements and molecules present in the food of the lizards constitute the precursors from which signal molecules are bio-synthesised, we expected that species with a wider, more varied range of prey taxa would be able to produce more diverse signals. This proved not to be the case, which may mean several things. First, our diversity measures might be poorly chosen. 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 taxa. For similar reasons, our classification of molecules present in the secretion may be simply inappropriate or too simple. This classical classification seems logic on theoretical-chemical grounds (Apps et al. 2015; Weldon et al. 2008), but may not reflect how molecules are being acquired or produced by the emitter, or are being received by the receiver. Ordering molecules by chemical compound class makes sense if molecule shape matters, but the biophysical mechanism of (vomer)olfaction remains highly debated and some authors have argued that it is the way a molecule vibrates (not its shape) that activates the receptor (Franco et al. 2011, but see Block et al. 2015). If so, molecules with highly similar molecular structures could still ‘smell’ very 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 wish to caution the reader for the fact 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 the 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 colour 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 echo diet composition. Second, a similar caveat must be made for possible temporal variation in diet, as diet and secretion samples were not estimated at the same time. Seasonal variation in diet has also been documented repeatedly in lizards (e.g. Pérez-Mellado and Corti 1993; Pérez-Cembranos et al. 2016).

Another explanation for the lack of relationship between diet and signal diversity, might be that most lipids present in the lizards’ secretions can be biosynthesised by the animal that make the secretions, starting from simpler
carbon chains readily available in most food items. Studies on insects suggest that such *de novo* synthesis of chemical signals predominates (Tillman et al. 1999), but in some species, chemical signals do arise through sequestration (e.g. Aldrich et al. 2016), or through moderate 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 now and then. This result seems to suggest that there may be individual molecules in the chemical signature of lizards that are primarily derived from plants and can only be acquired if lizards include plants in their diet. Weldon et al. (2008), in their review of squamate integumentary molecules indicate 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 increases the concentration of this molecule in the femoral gland secretions.
(Kopena et al. 2014). Because this compound 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 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 signalling role in other lacertids, but our results suggest that 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 may obtain tocopherols from other sources than plants, e.g. from the fat of herbivorous insect prey (Barbehenn 2003) or earthworms (Marconi et al. 2002), or that we have failed to detect the consumption of plant material in some species. It would be interesting for future studies to experimentally assess whether the diet of the prey (e.g. polyphagous vs. graminivorous) might influence 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 dimensions, is furthermore difficult to exploit due to the presence of a tough chitin exoskeleton (Redford and Dorea 1984). In many myrmecophagous
species, the morphological adaptations required to capture and process sufficient numbers of ants lead to a further specialisation in this prey type (Meyers et al. 2006). For these reasons, one might expect the chemical signals of ant-eating lizards to be relatively poor in compounds. On the other hand, several dendrobatid and microhyliid 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 signalling molecules. As mentioned earlier, Alberts (1991) has 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 as well. However, from our results, there is no evidence that the femoral secretions of myrmecophagous lacertid species contain 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 statistically significant, for a lower proportion of steroids in secretions of species than include more ants in the diet, which suggests that there could be some limitations for ant-eaters. 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 signal signature in the lizard family Lacertidae. Our wide-angle shot revealed considerable among-species variation in both diet and secretion chemistry. Although plant-eaters were shown to produce secretions of a higher chemical richness than species that do not eat plants, our overall findings established little co-variation between the diet and chemical signal profiles of lacertids. This may indicate that the precursors of the signal components are widely available in the prey species, or that lizards can bio-synthesize the compounds *de novo* or from simpler precursors. However, as admitted above, our data may also lack the resolution required to demonstrate any direct connections between the intake and the secretion of major types of chemicals. Because experimental studies have shown that interindividual variations in the diet may affect variation in chemical signal composition (e.g. Kopena et al. 2014; Martín and López 2006b), and because there exists interpopulational 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 contemporary and local food availability and consumption, preferably at several, contrasting
locations in the field. Also, the relationship between chemical richness and plant diet should be examined in more detail.

Acknowledgments. — We thank Josie Meaney for linguistic advice, and three anonymous reviewers for significantly improving drafts of this manuscript. Financial support to JM and RGR was provided by the Spanish’s Ministerio de Economía y Competitividad projects MICIIN-CGL2011-24150/BOS and MINECO CGL2014-53523-P.

Compliance with Ethical Standards. — This work was conducted under permits for Croatia (UP/I-612-07/14-48/111 & UP/I-612-07/14-48/33); The Netherlands (FF/74A/2015/009); Israel (2014/40323), SA Free State Province (S54C-515022511060), SA Eastern Cape Province (CRO 45/15CR & 46/15CR), SA Western Cape Province (0056-AAA041-00093), SA Northern Cape Province (FAUNA 229/2015 & 230/2015) and SA Limpopo Province (0092-MKT001-00004), and in accordance with University of Antwerp (Belgium) animal welfare standards and protocols (ECD 2014-32). Captures of lizards and sampling procedures were performed under different licenses for the 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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Fig. 1 — Ancestral character estimation of chemical signal richness along the branches and nodes of the 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 red bar below the cricket = predominantly arthropod diet; one green bar below the plant = predominantly herbivorous diet.