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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, 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.

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 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

78 context, are scarce (and not only so in lizards, Symonds and Elgar 2008),
79 despite the fact that comparative analyses of visual (e.g. Ord and Martins 2006)
80 and acoustic interaction systems (e.g. Garamszegi et al. 2005) have proved how
81 valuable this approach can be for understanding the evolution of signal
82 diversity. The diversity and composition of glandular secretions varies widely,
83 but consistently, among lizard species, both in complexity and nature of
84 constituent molecules (see Weldon et al. 2008 for a review on this topic in
85 reptiles), but the origins and significance of this variation remain poorly
86 understood.
87
88 One factor that is likely to contribute to divergence in glandular secretion
89 composition of vertebrates is diet. If species, populations or even individuals
90 differ, quantitatively or qualitatively, in the acquisition of certain dietary
91 compounds, they may also differ in the chemical cues and signals that are
92 ultimately obtained or synthesised from them (Symonds and Elgar 2008).
93 Evidence for a direct effect of diet on glandular chemical profiles comes from
94 studies on conspecific recognition, mate selection and predation avoidance. In a
95 diverse array of species, individuals will preferentially associate with
96 conspecifics that are on some (usually rich) diet (e.g. Bryant and Atema 1987;
97 Conner et al. 1990). Diet-derived differences in chemical cues or signals may
98 also function in mate selection; females typically prefer partners whose
99 chemical signals contain particular compounds that are expensive to produce or

100 difficult to obtain (e.g.in lizards: Kopena et al. 2011; Martín and López 2006).
101 One study on lacertids has found evidence for a direct effect of diet on signal
102 expression at the individual level (Kopena et al. 2011); in *Lacerta schreiberi*,
103 experimental dietary supplementation with carotenoids and vitamin E affected
104 among-individual variation in glandular secretion composition (i.e.
105 supplemented individuals increases relative proportions of vitamin E in
106 secretions) In much the same way, dietary components may be echoed in visual
107 sexual signals (Blair 1957; Kopena et al. 2014; Martín and López 2010).
108 Finally, animals are known to sequester food-derived chemicals into toxins
109 (Daly et al. 2000; Dumbacher et al. 2000), or deploy them in chemical
110 camouflage (e.g. Brooker et al. 2014).
111
112 Here, we take a broad phylogenetic comparative approach, testing whether
113 among-species variation in the composition of epidermal (femoral) gland
114 secretions of the lizard family Lacertidae reflects dietary divergence. We
115 exclusively consider the lipophilic, and not the proteinaceous, fraction of the
116 glandular secretion, since the former is particularly comprised of metabolites or
117 metabolite-derived compounds, hence, expected to be more dietary-driven.
118 Although most lacertids have a predominantly arthropod-based diet, the relative
119 contribution of different types of arthropods varies considerably among species
120 (Carretero 2004; Herrel et al. 2004; Verwaijen et al. 2002) and some species,
121 especially —but not exclusively— island-dwellers consume large amounts of

122 plant material (Van Damme 1999). Although prey availability undoubtedly
123 drives much of the interspecific variation in diet in lacertids, several species
124 have been shown to prefer or avoid certain food items (see Carretero 2004 for a
125 review). In the current study, we specifically look for correlations between diet
126 diversity and chemical signal diversity. We test whether species that consume
127 significant fractions of plant material differ from species with a purely
128 arthropod-based diet in the overall-composition of their chemical signals, or the
129 abundance of certain chemical compounds of vegetal origin in secretions (i.e.
130 tocopherol, a compound involved in mate choice; Kopena et al. 2011). Finally,
131 we examine whether a myrmecophagous (i.e. ant-eating) diet affects the signal
132 chemistry of lizards due to the low nutritional value and the tough chitin
133 exoskeleton of ants.
134
135
136 METHODS AND MATERIALS
137 *Femoral gland secretions* Between 2005 and 2016, we collected femoral gland
138 secretions from 45 species of lacertid lizards at various locations in Europe,
139 Africa and Asia (Table S1). In total, we captured 527 lizards by hand or noose.
140 On average, we caught 12 individuals per species (range 1- 35). Since femoral
141 glands develop at the onset of sexual maturity, and their activity is greatest
142 during the reproductive period, we exclusively sampled adult males during
143 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.

185 Finally, we estimated the relative abundance of each chemical as the
186 percentage of the total ion current (TIC). This was done for every lizard
187 individual, and averages were calculated per species.
188 The total number of different lipophilic compounds (both identified and
189 'unidentified' compounds, but that could be characterized within a species by
190 their specific retention times and characteristic mass spectra) found in the
191 samples of a species (pooling data of all individuals analysed) was considered
192 the species 'chemical richness'. To obtain another measure of the 'chemical
193 diversity' of a species' secretion, we first determined the relative proportions of
194 nine chemical compound 'classes' (alcohols, aldehydes, fatty acids, furanones,
195 ketones, steroids, terpenoids, tocopherols and waxy esters) in the mixture, and
196 then calculated the Shannon diversity index (H_{chem}, Shannon 1948).
197
198 *Diet data* We searched the literature for information on the natural diet of the
199 species for which we had chemical secretion data. When we found diet
200 information on more than one population of a specific species, we only
201 included diet-data of that population for which we also collected chemical data,
202 or which was geographically closest to the sampled population. The relative
203 contribution (in terms of prey items found in the stomach, intestines or faeces)
204 of each arthropod groups to the total diet of each species was noted. We
205 distinguished 25 orders of Hexapoda (keeping the ants, Formicidae as a special
206 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.

227 Prior to analyses, we transformed all variables to confirm to the
228 statistics expectations of the analyses: chemical and diet diversity (log_{10}),
229 chemical richness (square-root), and all frequency data (arcsin square-root).
230 We used the 'pgls'-command in the 'caper' package (Orme et al. 2015)
231 to relate chemical signal diversity and richness to diet diversity, accounting for
232 phylogenetical signal by adjusting lambda by maximal likelihood
233 transformation. We used the 'phylanova'-command in the package 'phytools'
234 (Revell 2012) to test whether chemical signal diversity and richness differed
235 among species whose diet included no, little or substantial amounts of plant
236 material.
237 To investigate co-variation between diet and chemical composition, we
238 used a phylogenetic canonical correlation analysis (pCCA, function 'phyl.cca').
239 This multivariate method enables us to calculate and analyse the correlation
240 between character sets while accounting for the non-independence of species
241 due to phylogeny (Harisson et al. 2015; Revell & Harrison 2008). To maintain
242 statistical power and stable canonical variate-variable correlations, we were
243 required to reduce the number of variables in the diet dataset prior to pCC
244 analysis: we taxonomically regrouped the diet dataset from 36 variables to
245 seven (i.e. Chelicerata, Crustacea, Hexapoda, Oligochaeta, undetermined
246 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 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 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 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 know the chemical components of the males' femoral secretions (Table S2 and S3). Diet diversity (H_{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 λ = 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 (± SE) chemical richness was 50 (± 3). Richness showed a moderate but significant phylogenetic signal (λ = 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 ± 0.05. The phylogenetical signal for chemical signal diversity was not significant (λ = 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).

291 Chemical signal diversity appeared highest in the species that consumed
292 plants occasionally (group O, mean ± SE: 0.93 ± 0.08) and lowest in species
293 with a predominantly plant-based diet (group H, 0.68 ± 0.06); the secretion of
294 species that rarely eat plants appeared an intermediate chemical diversity (group
295 A, 0.79 ± 0.06). However, this difference is not statistically significant, thus,
296 providing no statistical evidence that the degree of plant-eating might affect
297 chemical signal diversity (traditional ANOVA: $F_{2,42}$ = 1.35, P = 0.27;
298 phylogenetic ANOVA: P = 0.25). Chemical signal richness was higher in plant-
299 consuming species (group O: 60 ± 8; group H: 58 ± 5) compared to non-plant
300 eating species (group A: 43 ± 4). Both traditional ANOVA ($F_{2,42}$ = 3.80, P =
301 0.03) and phylogenetic ANOVA (P = 0.026) indicate that this difference is
302 significant (Fig. 1). Overall, the three groups considered (A, H, O) did not
303 differ in the relative contribution of the nine major chemical compound groups
304 to the total mixture (traditional MANOVA: $F_{18,70}$ = 0.86, P = 0.63; phylogenetic
305 MANOVA: P = 0.96). Neither did they differ in the relative contribution of
306 tocopherols (traditional ANOVA: $F_{2,42}$ = 0.91, P = 0.41; phylogenetic ANOVA:
307 P = 0.43).
308 Neither chemical diversity nor chemical richness correlated significantly
309 with the proportion of ants in the diet (pgls, diversity: r^2 = 0.05, P = 0.14;
310 richness: r^2 = 0.02, P = 0.39). Species that ate larger proportions of ants tended
311 to have lower percentages of steroids in their femoral secretions, but the
312 correlation was not significant at the 0.05 level (pgls, r^2 = 0.071, slope = -0.27,

313 $P = 0.077$). No relationship whatsoever was found between the reliance on ants
314 and the relative amount of any other major component class (all $P > 0.18$).
315 A phylogenetic canonical correlation analysis revealed no significant
316 relationship between the diet and chemical matrices, providing no support that
317 diet is affecting the overall chemical composition of lizard femoral gland
318 secretion (canonical axis 1: $R = 0.72$, $\chi^2 = 79.27$, $P = 0.210$; canonical axis 2: R
319 $= 0.63$, $\chi^2 = 53.34$, $P = 0.499$).
320
321 DISCUSSION
322 Our results attest that lizard species of the family Lacertidae vary considerably,
323 albeit consistently, in the composition of their femoral gland secretions. This
324 finding is not unique. Most studies that have compared the make-up of
325 chemical signals among animal species or among populations within species
326 have documented considerable variability (Alberts 1991; Gabirot et al. 2016;
327 Pureswaran et al. 2016; Rollmann 2000).
328
329 The origin and/or functional significance of this interspecific or
330 interpopulational variation of chemical signals often remains elusive. Authors
331 that compare chemical signals between two or more closely-related species that
332 live in sympatry often interpret observed differences in the light of species
333 recognition and reproductive isolation (e.g. Escobar 2003; Gabirot et al. 2010,
334 2012; Martín et al. 2016; Martín and Lopez 2006b). Others have offered

335 adaptive explanations for the observed variability, arguing that local
336 environmental conditions (climate, substrate), through their effects on
337 transmission efficiency, may select for different chemical signal structures (e.g.
338 Baeckens et al. 2015; Escobar 2003; Martín et al. 2015). Only a few authors
339 have considered the possibility that interspecific or interpopulational variation
340 may arise from differences in diet. For instance, Gabirot et al. (2016) suggested
341 that differences in the composition of uropygial gland secretions of two
342 shearwater species (*Calonectris*) might reflect differences in the birds' feeding
343 ecologies. Diet was also mentioned as a possible cause of the differences in
344 femoral gland secretion chemistry of two closely related *Podarcis* lizard species
345 (Gabirot et al. 2012). Interestingly, Alberts (1991) found that the protein
346 mixture in femoral gland secretion of desert horned lizards *Phrynosoma*
347 *platyrhinos* differed markedly from that of other sceloporine lizards and
348 pondered whether that could be due to the species' myrmecophagous diet (the
349 other species had a much more general insectivore diet). We know of no other
350 taxon-broad studies on lizards that have explicitly linked interspecific variation
351 in chemical signal design to dietary habits.
352
353 Overall, we found no evidence that chemical signal diversity is affected by diet
354 in lacertid lizards. Lizards may prey upon a wide variety of prey items, and
355 even include plant material into their diet, and still have a low signal diversity –
356 and *vice versa*. Chemical diversity, as we calculated it here, accounts for both

357 the abundance and the evenness of the major chemical classes present. Because
358 ultimately, the elements and molecules present in the food of the lizards
359 constitute the precursors from which signal molecules are bio-synthesised, we
360 expected that species with a wider, more varied range of prey taxa would be
361 able to produce more diverse signals. This proved not to be the case, which may
362 mean several things. First, our diversity measures might be poorly chosen. We
363 calculated dietary specialization (or diversity) from the relative abundance of
364 different taxa of invertebrates and other prey items. While this is customary in
365 studies of diet breadth (Roughgarden 1979), taxonomic prey diversity may not
366 adequately reflect the variability of chemicals ingested. Ideally, one would like
367 to have information on the chemical composition of all prey taxa. For similar
368 reasons, our classification of molecules present in the secretion may be simply
369 inappropriate or too simple. This classical classification seems logic on
370 theoretical-chemical grounds (Apps et al. 2015; Weldon et al. 2008), but may
371 not reflect how molecules are being acquired or produced by the emitter, or are
372 being received by the receiver. Ordering molecules by chemical compound
373 class makes sense if molecule shape matters, but the biophysical mechanism of
374 (vomer)olfaction remains highly debated and some authors have argued that it
375 is the way a molecule vibrates (not its shape) that activates the receptor (Franco
376 et al. 2011, but see Block et al. 2015). If so, molecules with highly similar
377 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 bio-synthesised by the animal that make the secretions, starting from simpler

400 carbon chains readily available in most food items. Studies on insects suggest
401 that such *de novo* synthesis of chemical signals predominates (Tillman et al.
402 1999), but in some species, chemical signals do arise through sequestration (e.g.
403 Aldrich et al. 2016), or through moderate modification (e.g. Eisner and
404 Meinwald 1995) of dietary compounds. Alas, very little is known on the
405 biosynthetic pathways that produce the varied molecules present in lizard
406 femoral secretions, so it is difficult to judge the relative importance of these
407 mechanisms here.
408
409 In spite of the lack of a relationship between chemical signals and other diet
410 variables, we found that chemical signal richness, which varied strongly among
411 taxa, was significantly lower in species with a strictly arthropod-based diet than
412 for species that ate plants at least now and then. This result seems to suggest
413 that there may be individual molecules in the chemical signature of lizards that
414 are primarily derived from plants and can only be acquired if lizards include
415 plants in their diet. Weldon et al. (2008), in their review of squamate
416 integumentary molecules indicate that tocopherols and many phytosterols, in
417 particular, are likely sequestered from plants. In the herbivorous green iguana
418 (*Iguana iguana*), phytosterols represent up to 10% of the lipid fraction of
419 femoral gland secretions (Alberts et al. 1992). In Iberian green lizards (*Lacerta*
420 *schreiberi*), supplementing diet with α-tocopherol (vitamin E) immediately
421 increases the concentration of this molecule in the femoral gland secretions

422 (Kopena et al. 2014). Because this compound is an important antioxidant, and
423 cannot be synthesised *de novo*, vitamin E concentration in scent marks may
424 well act as an honest signal of male quality. Similarly, females of a closely
425 related green lizard species (*L. viridis*), whose secretions are similar, are
426 attracted to the scent marks of males with high concentrations of vitamin E
427 (Kopena et al. 2011). It is not clear whether tocopherols have a similar
428 signalling role in other lacertids, but our results suggest that they are present in
429 the femoral secretions of many species. Somewhat unexpectedly, we found no
430 difference in the relative abundance of tocopherols in species of different diets
431 (herbivorous/insectivorous/omnivorous). This may suggest that some species
432 may obtain tocopherols from other sources than plants, e.g. from the fat of
433 herbivorous insect prey (Barbehenn 2003) or earthworms (Marconi et al. 2002),
434 or that we have failed to detect the consumption of plant material in some
435 species. It would be interesting for future studies to experimentally assess
436 whether the diet of the prey (e.g. polyphagous vs. graminivorous) might
437 influence the signal chemistry of lizards.
438
439 For a small number of species in our data set, ants constitute an important
440 dietary component. Myrmecophagy is often considered an evolutionary
441 challenge, because the nutritional value of an ant, limited as it is due to its small
442 dimensions, is furthermore difficult to exploit due to the presence of a tough
443 chitin exoskeleton (Redford and Dorea 1984). In many myrmecophagous

444 species, the morphological adaptations required to capture and process
445 sufficient numbers of ants lead to a further specialisation in this prey type
446 (Meyers et al. 2006). For these reasons, one might expect the chemical signals
447 of ant-eating lizards to be relatively poor in compounds. On the other hand,
448 several dendrobatid and microhylid frog species are known to sequester certain
449 alkaloids from the ants on which they feed (Santos et al. 2003), so
450 myrmecophagy may also provide opportunities for the production of signalling
451 molecules. As mentioned earlier, Alberts (1991) has suggested that ant-eating
452 may explain the aberrant gland proteic secretion chemistry of desert horned
453 lizards (*Phryonosma platyrhinos*). Thus, we expected ant-eating lacertids to
454 have atypical femoral secretions as well. However, from our results, there is no
455 evidence that the femoral secretions of myrmecophagous lacertid species
456 contain less (or more) lipophilic compounds, or a smaller (or larger) component
457 diversity, than other species. We also did not find any consistent association
458 between ant-eating and the relative contribution of any of the major compound
459 classes. This suggests that ant-eating species can extract all necessary lipophilic
460 precursors from their prey, or that they somehow supplement their diet from
461 other sources. Nevertheless, there is a trend, although not statistically
462 significant, for a lower proportion of steroids in secretions of species than
463 include more ants in the diet, which suggests that there could be some
464 limitations for ant-eaters. Further studies that not only focus on the major
465 chemical classes in lizard secretions, but also encompass all individual

466 lipophilic compounds, might shed light on which particular steroids are affected
467 by a myrmecophagous diet. Those studies should also consider incorporating
468 true ant-specialists in their dataset, such as *Phrynosoma* (lizards of the genus
469 *Moloch* do not possess any epidermal glands; Mayerl et al. 2015).
470
471 In this study, we explored relationships between diet and chemical signal
472 signature in the lizard family Lacertidae. Our wide-angle shot revealed
473 considerable among-species variation in both diet and secretion chemistry.
474 Although plant-eaters were shown to produce secretions of a higher chemical
475 richness than species that do not eat plants, our overall findings established
476 little co-variation between the diet and chemical signal profiles of lacertids.
477 This may indicate that the precursors of the signal components are widely
478 available in the prey species, or that lizards can bio-synthesize the compounds
479 *de novo* or from simpler precursors. However, as admitted above, our data may
480 also lack the resolution required to demonstrate any direct connections between
481 the intake and the secretion of major types of chemicals. Because experimental
482 studies have shown that interindividual variations in the diet may affect
483 variation in chemical signal composition (e.g. Kopena et al. 2014; Martín and
484 López 2006b), and because there exists interpopulational variation in chemical
485 profiles within the same species (e.g. Martín et al. 2013), future studies should
486 try to associate the chemical signature of individual lizards to contemporary and
487 local food availability and consumption, preferably at several, contrasting

488 locations in the field. Also, the relationship between chemical richness and
489 plant diet should be examined in more detail.
490
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509

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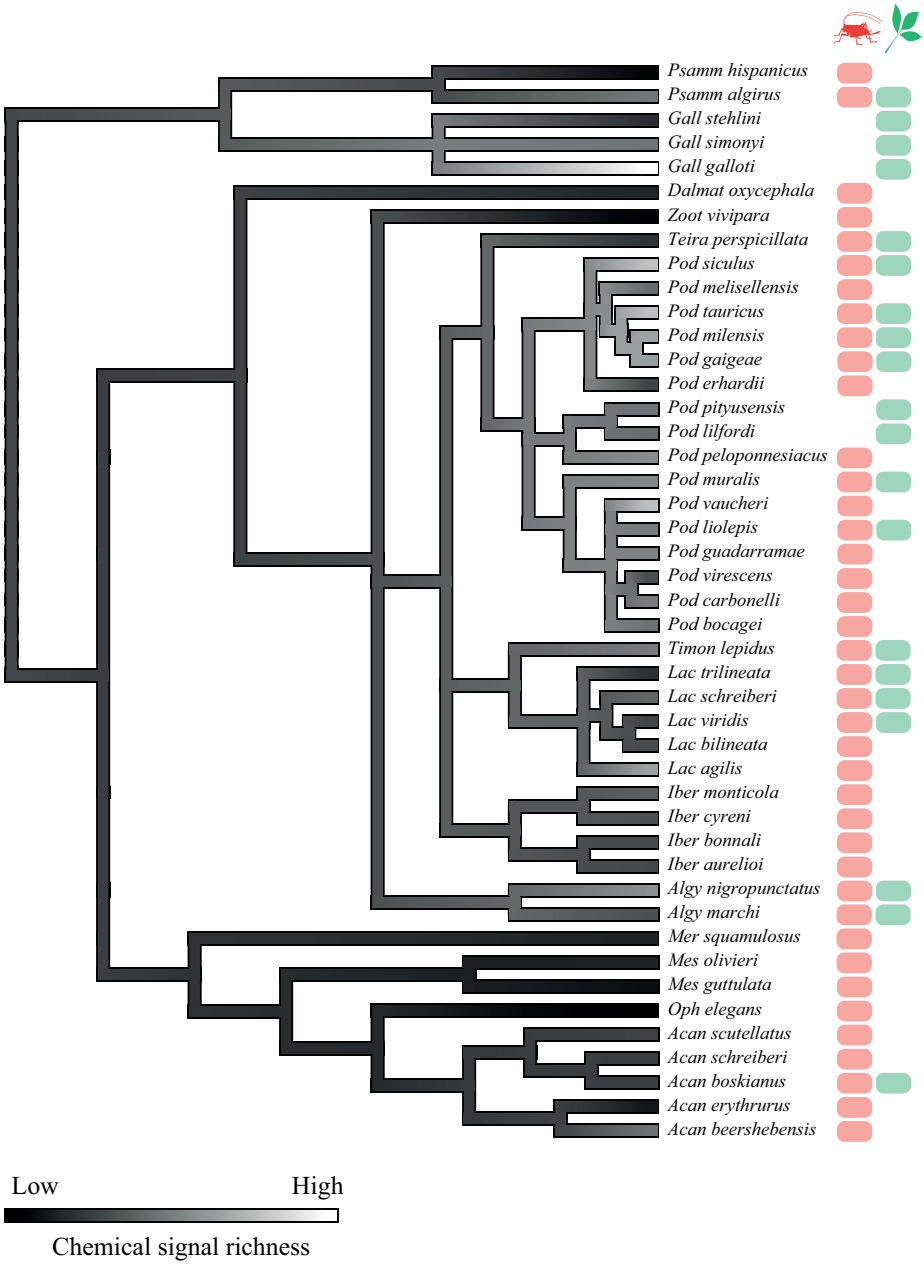
675

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.


Psamm hispanicus
Psamm algirus
Gall stehlini
Gall simonyi
Gall galloti
Dalmat oxycephala
Zoot vivipara
Teira perspicillata
Pod siculus
Pod melisellensis
Pod tauricus
Pod milensis
Pod gaigeae
Pod erhardii
Pod pityusensis
Pod lilfordi
Pod peloponnesiacus
Pod muralis
Pod vaucheri
Pod liolepis
Pod guadarramae
Pod virescens
Pod carbonelli
Pod bocagei
Timon lepidus
Lac trilineata
Lac schreiberi
Lac viridis
Lac bilineata
Lac agilis
Iber monticola
Iber cyreni
Iber bonnali
Iber aurelioi
Algy nigropunctatus
Algy marchi
Mer squamulosus
Mes olivieri
Mes guttulata
Oph elegans
Acan scutellatus
Acan schreiberi
Acan boskianus
Acan erythrurus
Acan beershebensis
Low
High
Chemical signal richness

698