


Environmental conditions shape the chemical signal design of lizards

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Abstract

1. The signals that animals use to communicate often differ considerably among species. Part of this variation in signal design may derive from differential natural selection on signal efficacy; the ability of the signal to travel efficiently through the environment and attract the receiver's attention. For the visual and acoustic modalities, the effect of the physical environment on signal efficacy is a well-studied selective force. Still, very little is known on its impact on chemical signals.
2. Here, we took a broad, phylogenetic comparative approach to test for a relationship between animals' signal chemistry and properties of their natural environment. Our study focused on lizards from the Lacertidae family.
3. We sampled 64 species across three continents and determined the lipophilic composition of their glandular signalling secretions using gas chromatography-mass spectrometry. For each species, an array of environmental variables of high temporal and spatial resolution was obtained from climate databases.
4. Species varied considerably in the overall richness (number of constituents) of their secretions, as well as in the relative contribution of the major chemical compound classes. Signal richness and the relative contribution of the respective compounds exhibited little evidence of phylogenetic relatedness, suggesting that chemical signals may change very rapidly. Neither insularity nor substrate use affected chemical signal composition, however, we found a strong statistical relationship between the chemistry of the lizards' secretions and aspects of the thermal and hydric environment they inhabit.
5. Species from 'xeric' milieus contained high proportions of stable fatty acid esters and high molecular weight alcohols in their glandular secretions, which likely increase the persistence of secretion scent-marks. In contrast, species inhabiting 'mesic' environments produced secretions of a high chemical richness comprising high levels of aldehydes and low molecular weight alcohols. This chemical mix probably creates a volatile-rich signal that can be used for long-distance airborne communication.
6. We argue that the observed variation in signal design results from differential natural selection, optimizing signal efficacy under contrasting environmental conditions.

KEYWORDS

adaptive evolution, chemical communication, epidermal gland secretions, Lacertidae, macroevolution, pheromones, phylogenetic comparative methods, signal efficacy

1 | INTRODUCTION

The vast array of signals used in animal communication is a continuous source of awe and a hot topic in behavioural research (e.g. Haven Wiley, 2015; Laidre & Johnstone, 2013; Lichtenberg, Zivin, Hrnčir, & Nieh, 2014; Tibbetts, Mullen, & Dale, 2017; to say a few). While signal diversity can arise through genetic drift (e.g. Campbell et al., 2010; Picq et al. 2016), natural and sexual selection seem more likely causes of differentiation. One feature contributing to a signal's survival or reproductive value is its efficacy: its ability to travel through the medium and to attract the receiver's attention. Natural selection is expected to mould signal design in such a way as to maximize the efficacy of information transmission and detectability (Endler, 1992; Wyatt, 2010). Since efficacy depends on the physical properties of the environment, which may vary greatly in space and time, differential selection can be expected to result in considerable variation in signal design. This link between the design (or 'morphology') of a signal and its efficacy (or 'performance') could also be dubbed as the 'ecomorphology' of a signal (cf. Arnold, 1983; Endler, 2000).

For the auditory and visual signalling system, there is growing empirical evidence that signal efficacy largely depends on the climatic conditions and habitat characteristics under which they operate, leading to differential selection along environmental gradients. For instance, habitat density is known to influence the call of bowerbirds (*Ptilonorhynchus violaceus*), with birds singing at lower frequencies in dense habitats and at higher frequencies in open habitats (Nicholls & Goldizen, 2006). High vocalization frequencies are easily absorbed in dense habitat, so the use of low frequency calls in such an environment enables the birds to maximize the distance their songs are carried (Nicholls & Goldizen, 2006). Ng, Landeen, Logsdon, and Glor (2013) reported a strong relationship between the phenotype of an anole lizard's dewlap (i.e. sexual signalling ornament) and its environment. *Anolis distichus* populations in humid habitats have large, orange dewlaps, while populations in dryer habitats have smaller, yellow dewlaps. It is generally thought that the different dewlap designs increase visual detectability in the respective xeric and mesic habitats in which they operate (Driessens et al., 2017; Ng et al., 2013).

The majority of research on the evolution of animal signals has focused on visual and auditory communication, while the chemical communication system has received far less attention (Searcy & Nowicki, 2005; Stevens, 2013; but see e.g. delBarco-Trillo & Drea, 2014; Steiger, Schmitt, & Schaefer, 2011; Weber, Mitko, Eltz, & Ramirez, 2016). This is rather surprising since chemical signals form a substantial component of communication across many taxa, and can differ substantially, even among closely related species (Müller-Schwarze, 2006; Müller-Schwarze & Silverstein, 1980; Wyatt, 2014). Theoretical and (limited) empirical work suggests that environmental factors will affect the physico-chemical properties (and consequently the efficacy) of chemical signals—just as they influence the efficacy of auditory and visual signals.

Firstly, temperature and humidity may influence the persistence of chemical signals, with increasing evaporation rates of chemicals in hot and humid environments (Alberts, 1992a). For example,

the half-life of acetate, a pheromone in many insects (e.g. Durak & Kalender, 2009; Lacey, Millar, Moreira, & Hanks, 2009; Vinson, 1972), decreases two- to fourfold when air temperature rises from 20 to 30°C (McDonough, Brown, & Aller, 1989). Evidently, temperature has the largest functional impact on scent-mark signals compared to other chemical signals, as scent-marks are only effective when they are detectable for long periods of time (Alberts, 1992a; Apps, Weldon, & Kramer, 2015). To illustrate, high temperatures accelerate scent-mark decay in ants, and hence limit the trail-following behaviour of these insects (Van Oudenhove, Billoir, Boulay, Bernstein, & Cerdá, 2011; Van Oudenhove, Boulay, Lenoir, Bernstein, & Cerdá, 2012). Similarly, high temperatures have detrimental effects on the persistence and detectability of scent-marks in the lizard *Iberolacerta cyreni* (Martín & López, 2013b). Tongue-flick assays showed that the level of chemosensory investigation of lizards was lower towards scent-marks kept in warm rather than in cold conditions, probably due to the rapid signal fade-out in the warm setting (Martín & López, 2013b). High levels of humidity can increase evaporation and oxidation rates of chemical mixtures, resulting in accelerated rates of signal fade-out (Alberts, 1992a; Apps et al., 2015; Müller-Schwarze, 2006; Regnier & Goodwin, 1977). Experiments with chemical compounds found in the scent-marks of Mongolian gerbils (*Meriones unguiculatus*) demonstrate very little signal loss over several days at 0% relative humidity, but at 100% relative humidity, over 60% of the signal evaporates within 2 hr (Regnier & Goodwin, 1977). Secondly, precipitation may wash away scent-marks. The frequency of rainfall, for instance, has been argued to influence the predator-prey interactions among the wolf spiders *Pardosa milvina* and *Hogna helluo*, as water degrades many of the spiders' chemical cues deposited at night (Wilder, DeVito, Persons, & Rypstra, 2005). Thirdly, high levels of solar—and UV radiation, which is closely linked with altitude (Blumthaler, Ambach, & Ellinger, 1997), may increase chemical degradation rates. Lastly, extreme air currents can reduce the active spatial range of chemical signals because of high turbulent diffusivities, although moderate levels of wind may facilitate the transport of airborne volatile chemicals and can increase a signal's range (Alberts, 1992a; Bossert & Wilson, 1963; Elkinton & Cardé, 1984). Darwinian selection may favour those chemical compounds or mixtures of compounds in chemical signals that enable animals to cope with such harsh signalling environments. On the other hand, as many chemical compounds are energy-consuming to produce or difficult to obtain (Clark, DeBano, & Moore, 1997; García-Roa, Sáiz, Gómara, López, & Martín, 2017; Kopena, Martín, López, & Herczeg, 2011; Rantala, Kortet, Kotiaho, Vainikka, & Suhonen, 2003; Rundle, Chenoweth, Doughty, & Blows, 2005), animals are unlikely to invest in costly signalling compounds when the benefit is economically low.

Currently, the literature on chemical signalling is heavily biased towards insects; other groups have received far less attention. A literature search by Symonds and Elgar in 2008 revealed that 79% of the studies on pheromone diversity have focussed on insects (38% on Lepidoptera), compared to 14% on vertebrates (1% on squamates). Within vertebrates, lizards seem to be a promising clade to study the evolution of chemical signalling (e.g. Baeckens, Van Damme,

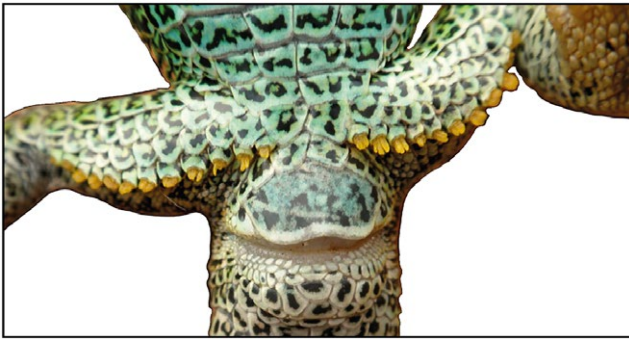


FIGURE 1 Photograph of the cloacal region of a male lacertid lizard (*Lacerta agilis*). Note the numerous epidermal pores with protruding glandular secretion. Picture from Mayerl et al. (2015) (with permission)

Cooper, 2017; García-Roa, Jara, Baeckens, et al., 2017; García-Roa, Jara, López, Martín, & Pincheira-Donoso, 2017; Mangiacotti et al., 2016; Martín & López, 2014; Mason & Parker, 2010; Pruett et al., 2016). Males of most lizard species are equipped with a series of epidermal glands located in the dermis of the inner thighs, which secrete waxy substances through pore-bearing scales (Figure 1), or 'epidermal (femoral) pores' (Mayerl, Baeckens, & Van Damme, 2015). The lipophilic compounds within the epidermal gland secretions are generally considered to be the leading source of chemical signals involved in lizard communication, and mediate behaviour in a variety of contexts (reviewed by Martín & López, 2014; Mayerl et al., 2015; but see Alberts, Phillips, & Werner, 1993), such as territory demarcation and assessment (Aragón, López, & Martín, 2001; Font, Barbosa, Sampedro, & Carazo, 2012; Leu, Jackson, Roddick, & Bull, 2016; Martín & López, 2012; Martins, Ord, Slaven, Wright, & Housworth, 2006), male rival assessment (Carazo, Font, & Desfilis, 2007; Hews, Date, Hara, & Castellano, 2011; Khannoon et al., 2011; López & Martín, 2002), female choice (Carazo, Font, & Desfilis, 2011; Gabriot, López, & Martín, 2013; Kopena, López, & Martín, 2014; Kopena et al., 2011; Martín & López, 2013a, 2015), assessment of female reproductive status (Cooper & Pérez-Mellado, 2002; Thomas, 2011), individual recognition (Alberts, 1992b; Alberts & Werner, 1993; Gabriot, Castilla, López, & Martín, 2010a, 2010b), sex identification (Cooper & Steele, 1997; Cooper & Trauth, 1992; Khannoon, Breithaupt, El-Gendy, & Hardege, 2010) and species recognition (Barbosa, Font, Desfilis, & Carretero, 2006; Gabriot et al., 2010a; Labra, 2011). Lizards passively mark or deposit these gland secretions into the environment while moving through their habitat, or they exhibit active marking behaviour to leave scent-marks on the substrate of their choice (de Villiers, Flemming, Mouton, & Le, 2015; Mason & Parker, 2010). To be effective, a scent-mark should persist for as long as possible in the absence of the signaller, and should be readily detectable to others (Alberts, 1992a; Hughes, Kelley, Banks, & Grether, 2012). Since the longevity and detectability of chemicals are strongly environment-dependent (Alberts, 1992a; Apps et al., 2015), lizard species inhabiting dissimilar environments are expected to vary in particular characteristics of their secretion (e.g. chemical

composition) in order to optimize the functionality of their signals in their environment. The glandular secretions of lizard species *Dipsosaurus dorsalis* and *Crotaphytus bicinctores*, for instance, contain large amounts of fatty acids of high molecular weight, which are thought to protect their scent-marks from rapid evaporation (Alberts, 1992b; Martín, Ortega, & López, 2013). High amounts of squalene as found in *Zootoca vivipara*, and high amounts of α -tocopherol in *Lacerta schreiberi* and *Lacerta viridis* might protect other lipophilic compounds in the secretions from oxidation in wet environments, therefore, increasing scent-mark longevity (Gabriot et al., 2008; Kopena et al., 2011, 2014). Cholesterol is usually the most abundant component in lizard gland secretions, and said to function as an unreactive apolar 'matrix' that holds and protects other lipids in the scent-marks from fading (Escobar, Escobar, Labra, & Niemeyer, 2003; Escobar, Labra, & Niemeyer, 2001; Martín & López, 2014).

Most studies on the ecological factors influencing chemical signal evolution concentrate on one or two species (e.g. Martín & López, 2014; Martín, Ortega, & López, 2015; Rouault, Marican, Wicker-Thomas, & Jallon, 2004; Rundle et al., 2005), and cannot provide the broad-scale evolutionary insight that can be gained from multispecies comparative studies (see Garamszegi, Eens, Erritzøe, & Møller, 2005; and Ord & Martins, 2006 for excellent examples on auditory and visual signalling). Here, we examine the role of the environment on the evolution of the chemical signalling signatures of lizards. We take a broad phylogenetic comparative approach to test for co-evolution between the lipophilic chemical composition of the gland secretions (or 'chemical signal design') of lizards of the family Lacertidae, and the environment (climatic conditions and habitat characteristics) they inhabit. We hypothesize that species from areas in which the prevailing environmental conditions severely decrease signal longevity by an increase in the loss of signal compounds through evaporation (e.g. hot, humid, windy, high levels of radiation), will carry large proportions of stable and heavy weight chemicals in their glandular secretions to counter rapid signal fade-out. Lacertid lizards (Figure 2) constitute an excellent model for this particular study, because species of this clade are distributed over a wide geographical area (Arnold, 1989), and vary considerably in their micro- and macrohabitat use (Baeckens, Edwards, Huyghe, & Van Damme, 2015). All species (except one) carry epidermal glands (although they differ in the number of glands, Baeckens et al., 2015), and many are known to use chemical signalling in several contexts (Martín & López, 2014; Mayerl et al., 2015).

2 | MATERIALS AND METHODS

2.1 | Study area and species

Between 2003 and 2016, we collected epidermal gland secretions from 64 lizard (sub)species of the family Lacertidae (Squamata: Sauria) throughout Europe, Africa, and Asia. Our dataset covers half of all lacertid genera and c. 20% of all lacertid species, encompassing species belonging to both subfamilies (Gallotiinae and Lacertinae), and both major tribes within Lacertinae, i.e. Eremiadini and Lacertini (Uetz, 2017). We covered a wide array of habitats and climate regions; from



FIGURE 2 Photographs of a subset of lacertid lizard species used in this study. From the left top to the right bottom: *Acanthodactylus beershebensis*, *Lacerta bilineata**, *Dalmatolacerta oxycephala*, *Podarcis melisellensis**, *Tropidosaura gularis*, *Podarcis siculus*, *Heliobolus lugubris*, *Algroides nigropunctatus**, *Lacerta media*. With permission, pictures taken by Annelies Jacobs are marked with an asterisk (*). The others are taken by Simon Baeckens

the Mediterranean maquis over the alpine meadows in the Pyrenees Mountains, to the sandy Israeli dunes and the Kalahari Desert of South Africa. In total, we captured 627 lizards by hand or noose in 11 different countries, at 60 different locations (Figure 3). On average, we caught 10 individuals per species (range 1–35). Since epidermal glands develop at the onset of sexual maturity, and their activity is greatest during the reproductive period (Cole, 1966; Smith, 1946), we exclusively sampled adult lizards during the mating season (i.e. spring–early summer; Arnold & Ovenden, 2007; Carretero, 2007; Pianka, Huey, & Lawlor, 1979). We only collected secretion from males, since the epidermal glands of most female lacertids are vestigial and (just as juvenile males) secretion collection is simply not possible in most species (Martín & López, 2014; Mayerl et al., 2015). After secretion collection, all lizards were released at exactly the site of capture. In addition, seventeen *Holaspis guentheri* lizards were obtained through the pet trade (Fantasia Reptiles, Belgium, license HK51101419). Male *H. guentheri* were wild-caught in Tanzania by the trader's personnel c. 2 weeks prior to purchase, and their epidermal gland secretions were collected in the laboratory at the moment of arrival, at the University of Antwerp. Of the 64 species sampled, general descriptions of the chemical composition of the gland secretions of 16 species have already been published

by J. Martín's research group (Museo Nacional de Ciencias Naturales, Madrid, Spain; Table S2).

2.2 | Collection and extraction of secretions

Immediately after the lizards were captured in the field, we collected epidermal gland secretion by gently pressing around the femoral pores while wearing fresh nitrile gloves. We attempted to extract secretions from all glands from both hind limbs, providing roughly between 2 and 6 mg of secretions per individual. The extraction procedure is harmless, and the lizards are able to rapidly produce more secretion afterwards (e.g. Baeckens, Huyghe, Palme, Van Damme, 2017). We immediately placed the collected secretions in glass vials with glass inserts sealed with Teflon-lined lids. In order to obtain blank control vials (on average two per locality), we followed the same procedure without collecting secretion, in order to exclude contaminants from the handling procedure or the environment, and to examine potential impurities in the solvent or analytical procedure. Subsequently, vials were stored at -20°C until further analyses, and for not longer than 5 months. The identification of each chemical compound and estimation of its relative abundance (as percentage) was estimated using

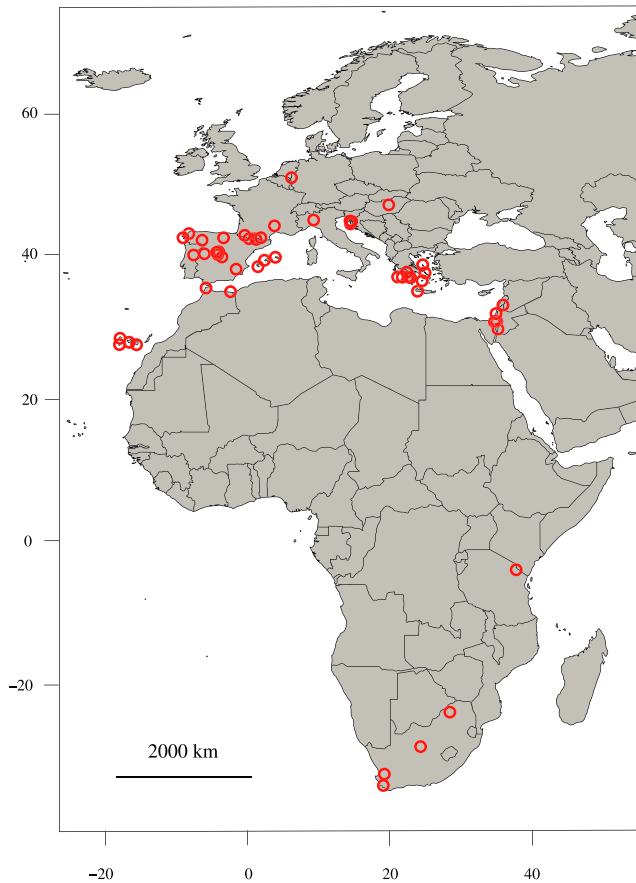


FIGURE 3 Geographical map of Europe, Africa and western Asia, showing the sample localities of the 64 lacertid lizard species under study

gas chromatography–mass spectrometry (GC–MS). Here, we used exactly the same methodology and protocol as described in earlier studies (cf. Martín & López, 2006, 2014; Martín, Moreira, & López, 2007; Martín, Ortega, et al., 2013). Details on the chemical analyses can be found in Appendix S1 in supporting information.

For further analyses, we assigned each chemical compound to a main chemical class, and calculated the relative proportion of each class. The main chemical classes used here are alcohols, aldehydes, carboxylic or fatty acids, ethyl or methyl esters, of fatty acids, furanones, ketones, steroids, terpenoids, tocopherols and waxy esters (i.e. an ester of a fatty acid and a fatty alcohol). Additionally, we calculated the proportions of cholesterol, because this steroid is the main compound in the secretions of many lizards (Weldon, Flachsbarth, & Schulz, 2008). Since the molecular weight of alcohols and fatty acids are known to vary greatly among lizards secretions (Martín & López, 2011; Mayerl et al., 2015), we sub-categorized alcohols and fatty acids in a low molecular weight class (compounds with chains of 16 carbons or less) and a high molecular weight class (compounds with over 16 carbons). We also included the 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) established in a species' secretion, or so-called 'chemical richness', as an additional chemical variable in the analyses.

Within a lizard species, there is still measurable inter-individual variation in relative proportions of compounds in gland secretions—although, small and subtle (Martín & López, 2015). In spite of this inter-individual variation, the main chemical profile of each species (i.e. presence/absence of major compounds and relative importance of each compound) is always maintained. Essentially, and for the purpose of this study, natural variation within each species should not affect interspecific comparisons.

2.3 | Environmental data

For each species, we collated an array of environmental variables from climate databases, using the geographical coordinates of the species' catching locality (Table S1). Data were downloaded from WorldClim (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) and ERA-Interim (Dee et al., 2011). From the global WorldClim database, which provides recent long-term average climatic conditions (monthly; 1950–2000) on a spatial resolution of $\pm 1 \text{ km}^2$, we extracted data on mean air temperature (taken $\pm 2 \text{ m}$ above the surface; T_{air}), mean precipitation, and altitude. In addition, we calculated a single measure for 'aridity', the Q index:

$$Q = \frac{P}{(T_{\text{max}} + T_{\text{min}})(T_{\text{max}} - T_{\text{min}})} \times 1000,$$

where P is the average annual precipitation (mm), T_{max} is the highest monthly mean temperature, and T_{min} the lowest monthly mean temperature. Arid environments are characterized by a lower Q , whereas mesic environments have a higher Q (Oufiero, Gartner, Adolph, & Garland, 2011; Tieleman, Williams, & Bloomer, 2003). The ERA-Interim database is the latest global atmospheric reanalysis produced by the European Centre for Medium-Range Weather Forecasts (ECMWF), and provides complete datasets of multiple variables at high spatial ($\pm 17 \text{ km}^2$) and temporal resolution (6-hr; 1979–present). We extracted information on relative humidity, wind speed, proportion cloud cover, downward UV radiation at the surface level (R_{UV}), total surface solar radiation (or 'irradiance incident'; R_{solar}), soil temperature (temperature measured between 0 to 7 cm in the soil; T_{soil}), and ground surface temperature (air temperature c. 5 cm above the surface; T_{surface}). We restricted ourselves to the time and period that lizards are thought to be active (and scent-marking) and, therefore, exclusively included environmental data measured at mid-day (12:00 h), and from March until September for European/Mediterranean lacertid species (Arnold, Arribas, & Carranza, 2007; Carretero, 2006; Perry et al., 1990), October until March for South African lacertids (Huey & Pianka, 1977, 1981; Pianka et al., 1979), and annual data for species around the equator (i.e. only *H. guentheri* in our dataset). Based on daily information, we calculated species' means for every environmental variable across its activity season during the period 1979–2015.

Additionally, we (1) documented whether species were sampled on the mainland or on an island, and (2) assigned each species to one of four substrate classes based on data from Baeckens et al. (2015): sandy, rocky, vegetation or generalist.

2.4 | Phylogenetic analyses

One of the most recent (comprehensive) phylogenetic reconstruction of the family Lacertidae, which is based on both mitochondrial and nuclear gene regions, comprises 162 species and covers all lacertid genera (see Baeckens et al., 2015), but, alas, does not include all species sampled in the current study. In order to obtain a suitable phylogenetic tree for our phylogenetic comparative tests, we re-ran an identical phylogenetic analysis as Baeckens et al. (2015), but included an additional 12 (sub)species to fit our dataset. Details on the phylogenetic analyses can be found in Appendix S2. Overall, the resulted tree of the lacertid family corroborates many previously reported inter- and intrageneric relationships (Arnold et al., 2007; Baeckens et al., 2015; Edwards et al., 2013; Fu, 2000; Kapli, Poulakakis, Lymberakis, & Mylonas, 2011; Mayer & Pavlicev, 2007; Mendes, Harris, Carranza, & Salvi, 2016; Pavlicev & Mayer, 2009; Pyron, Burbrink, & Wiens, 2013). Hence, this phylogenetic tree was used in all further phylogenetic comparative tests.

2.5 | Statistical analyses

We performed all analyses using the 'ape', 'geiger', 'phylocurve' and 'phytools' packages in R, version 3.3.1 (Adams, 2014; Goolsby, 2016; Harmon, Weir, Brock, Glor, & Challenger, 2008; Paradis, Claude, & Strimmer, 2004; R Core Team, 2013; Revell, 2012, 2013). Probabilities (p) lower than .05 were considered statistically significant.

The phylogenetic signal for the proportions of each chemical class, and for the complete multivariate chemical matrix, was calculated using Pagel's λ and Blomberg's K (function 'phylosignal' and 'K.mult'). 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, Garland, & Ives, 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, therefore, indicates a substantial degree of trait conservatism (Blomberg et al., 2003). Pagel's λ is a scaling parameter that 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).

To investigate the relationship between environment 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 (Revell & Harrison, 2008). This method allows us to identify the linear functions of each set of variables that have maximum correlation with other such sets (Miles & Ricklefs, 1984). The details of this method can be found in Revell and Harrison (2008), and an easily accessible example of its application in Harrison, Revell, and Losos (2015). Prior to analyses, we transformed all variables to conform to the statistical expectations

of the analysis. Percentages were arcsine square-root transformed, and count-data (i.e. chemical richness) square-root transformed.

We used phylogenetic MANOVAs (function 'aov.phylo') to test whether substrate use and insularity affects species' secretion composition. We implemented the complete chemical matrix as multivariate character set in the MANOVA analyses.

3 | RESULTS

3.1 | Chemical composition and richness

The most abundant chemical class found in the glandular secretions of the 64 lacertid species under study were steroids, with a mean (\pm SE) proportion of $64.2 \pm 2.5\%$ (of TIC) ranging from 19.6% in *Acanthodactylus erythrurus* to 96.3% in *Dalmatolacerta oxycephala*. Alcohols ($8.2 \pm 1.9\%$), fatty acids ($8.1 \pm 1.3\%$), waxy esters ($7.9 \pm 1.5\%$) and tocopherols ($6.9 \pm 1.5\%$) were present in intermediate concentrations. Aldehydes ($1.6 \pm 0.3\%$), terpenoids ($1.4 \pm 0.3\%$), esters of fatty acids ($0.7 \pm 0.2\%$), furanones ($0.5 \pm 0.1\%$) and ketones ($0.3 \pm 0.1\%$) were the five chemical classes with the lowest average proportion. Descriptive statistics on the relative abundance of chemicals are shown for each species in Table S3, and visualized in Figure 4.

The chemical richness of the secretion was on average (\pm SE) 50 (± 2), and ranged from 14 different lipophilic compounds in *Ophisops elegans* and 18 in *Z. vivipara*, to 98 chemicals in *Tropidosaura gularis* and 103 in *Gallotia galloti galloti* (Figure 5).

3.2 | Phylogenetic signal

The overall composition of the epidermal gland secretion in lacertid lizards exhibited a relatively weak, albeit significant, phylogenetic signal (Blomberg's multivariate $K = 0.45$, $p < .001$). The contribution of the individual chemical classes also carried relatively weak phylogenetic signals (Table S3). The proportion of fatty acid esters, furanones and terpenoids, however, showed high Pagel's λ values (all 0.99, $p < .001$), but intermediate K values ($K < 0.6$, $p > .05$). As the sole exception, alcohols exposed a high λ value ($\lambda = 0.99$, $p < .001$) and a K value over one ($K = 1.33$, $p = .001$), which implies that neighbouring lizard species tend to resemble each other more—with regard to the proportion of alcohols in their secretion—than expected under Brownian motion of evolution.

3.3 | Environmental effects

A phylogenetic canonical correlation analysis revealed a significant relationship between the environmental and chemical matrices, indicating that climatic conditions are affecting the overall chemical composition of lizard epidermal gland secretion (canonical axis 1; $R = 0.86$, $\chi^2 = 233.58$, $p = .002$). Only the first canonical axis between environment and chemicals proved significant; the second axis was not (canonical axis 2; $R = 0.76$, $\chi^2 = 167.36$, $p = .157$). We therefore focused on canonical variable 1 (CV1). The canonical loadings (on CV1) for the environmental and chemical variables are given in Table

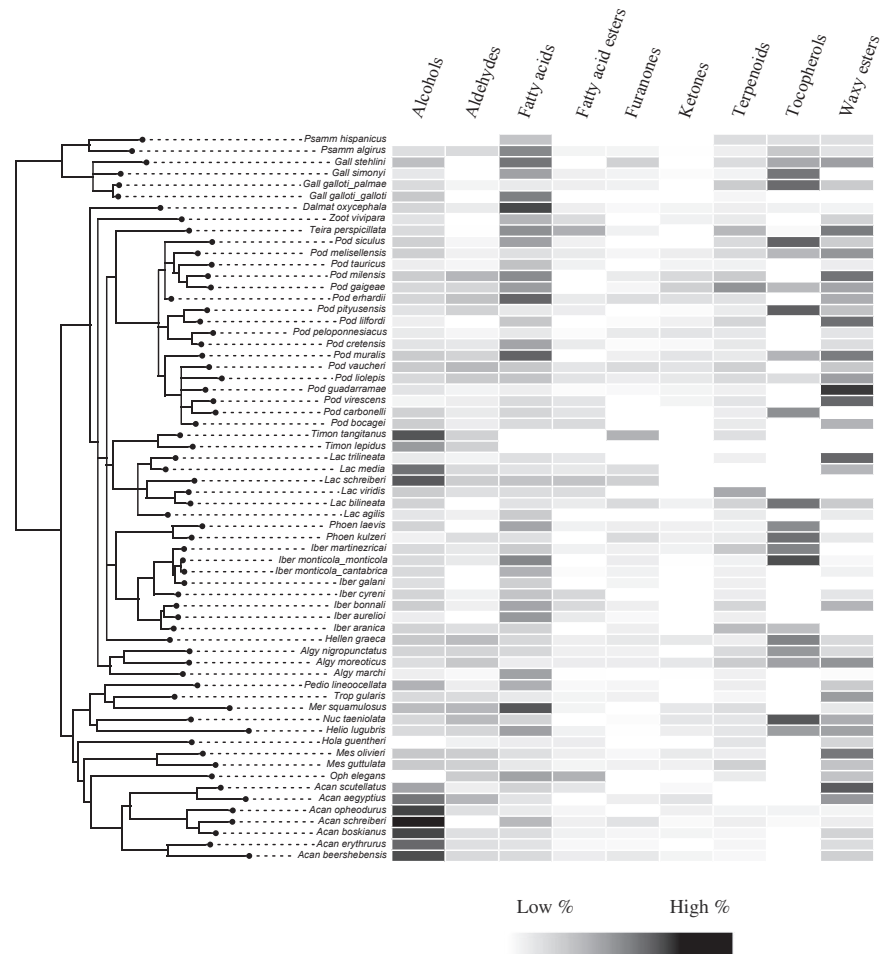


FIGURE 4 A ‘heatmap’-visualization of the variation in the relative abundance of the main chemical classes (range 0%–72%) established in the epidermal gland secretion of 64 lacertid species. Steroids are excluded, as they would skew the coloration in the representation due to their high proportion in lacertid secretion

S4. Temperature (T_{air} , T_{surface} and T_{soil}) and aridity all had high positive loadings, while altitude, precipitation, relative humidity, radiation (R_{solar} and R_{UV}) and wind speed correlated negatively with CV1. Cloud cover proved to have a low impact. Loadings on CV1 were highly positive for the relative contribution of high molecular weight alcohols and fatty acid esters, while low molecular weight alcohols, aldehydes and chemical richness showed high negative loadings. Cholesterol, fatty acids, furanones, ketones, steroids, terpenoids, tocopherol and waxy esters had a lower impact. Figure 6 visualizes the relationship between the environment and the chemical composition using the species' canonical scores (for CV1) in a scatterplot (see also Appendix S3).

Phylogenetic MANOVAs did not detect any significant effect of insularity ($F_{16,47} = 0.160$, $p = .904$) or substrate use ($F_{64,174.53} < 0.001$, $p = .321$) on species' secretion composition.

4 | DISCUSSION

Natural selection favours signals that work effectively (Boughman, 2002; Endler, 1992,1993). For the auditory and visual communication systems, there is growing evidence that the efficacy of signals is strongly dependent on the environmental conditions under which

signals operate, leading to differential selection along environmental gradients (Alberts, 1992a; Fleishman, 1988, 1992; Morton, 1975; Ng et al., 2013; Nicholls & Goldizen, 2006). This study sought to test the idea that the physical properties of the environment drive the evolution of chemical signals by comparing the chemistry of 64 species of lizards inhabiting a wide range of habitats. Our analyses revealed substantial variation in the chemical richness and the relative proportions of the components of the glandular secretions. Shared-ancestry failed to explain among-species patterns of variation, although the relative proportion of alcohols proved to be a highly phylogenetic conservative trait within the chemical signalling signature of lacertid lizards. Most interestingly, our findings revealed a strong relationship between the environmental conditions species live in and the chemical composition of their epidermal gland secretions. On the one hand, lizards living in ‘xeric’ environments, characterized by high temperatures and arid conditions, had large proportions of high molecular weight alcohols and fatty acid esters in their gland secretions. On the other hand, in lizards inhabiting more ‘mesic’ environments with high levels of precipitation, humidity, UV- and solar-radiation, and wind, we found gland secretions of a high chemical richness, with large proportions of aldehydes and low molecular weight alcohols. Insularity or substrate use did not affect chemical signal composition. This study provides the first evidence of a strong relationship between chemical

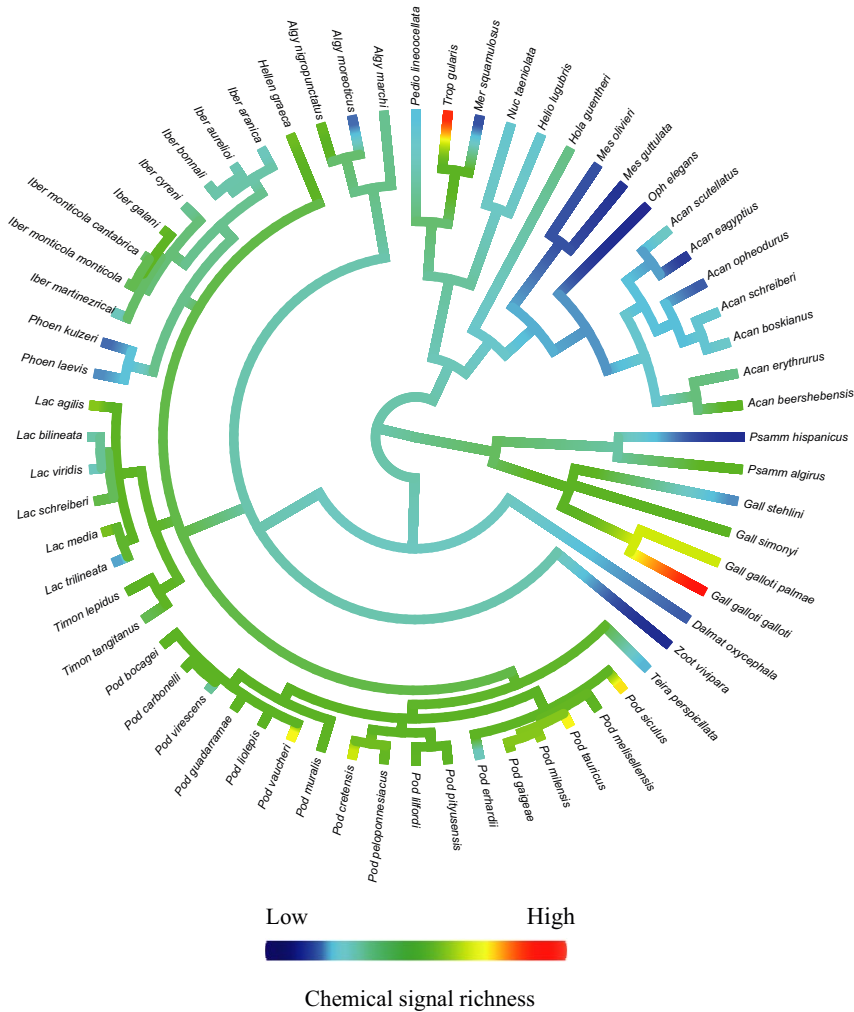


FIGURE 5 Ancestral character estimation of chemical signal richness along the branches and nodes of the tree for 64 lacertid lizard species. The illustration succeeds in visualizing the large variation in chemical richness scattered among (even closely related) species. Illustration made in R (function `contMap` using `type = 'fan'`, in package `phytools`; Revell, 2013)

signal design and prevailing environmental conditions across different animal species, which may result from selection on signal efficacy.

4.1 | Diversity in secretion composition

The lipophilic compounds of epidermal gland secretion of lacertids serve important functions in inter- and intraspecific communication (reviewed by Martín & López, 2014; Mayerl et al., 2015). In all 64 lacertid species examined, steroids were the most common compounds in the glandular secretion, but the relative proportions of all the compounds, and the chemical richness, varied dramatically even among closely related species. Our analyses were unable to describe the interspecific disparity in chemical signal design to phylogenetic relatedness; the phylogenetic signal for chemical richness, the complete chemical composition, and the major chemical classes separately (except alcohols) were all relatively low. Similar findings are reported for other signalling modalities, with low phylogenetic signal in e.g. the advertisement calls of African clawed frogs (Tobias, Evans, & Kelley, 2011) and the display behaviour of *Cyclura* rock iguanas (Martins & Lamont, 1998). Similar to many behavioural traits (Blomberg et al., 2003), the chemical signal design in lacertids seems highly evolutionary malleable and appears to have changed rapidly over a small

evolutionary time-scale. Interestingly, and in contrast to all other chemical classes, the relative proportion of alcohols appeared highly phylogenetically conservative within the Lacertidae (Blomberg's $K > 1$). While the reason or functional significance of the conservative character of alcohols in lizard secretion is uncertain, behavioural studies link the functional biology of alcohols with territoriality. For example, in rock lizards, *Iberolacerta monticola*, males respond aggressively towards cotton tips impregnated with the alcohols hexadecanol and octadecanol, and males with high levels of these alcohols in their secretion have a high dominance status (Martín et al., 2007). Male spiny-footed lizards (*A. erythrurus* and *Acanthodactylus boskianus*) respond with aggressive behaviour towards the alcohols hexacosanol and tetracosanol, both abundant in the secretion of these lizards (Khannoon et al., 2011). Secretions that mark territories may not only target conspecifics, but also members of competing species. In lacertids, pairs of closely related species often live in sympatry and feed on similar prey (Arnold, 1987, 1989), which is why lizards would benefit from keeping both hetero- and conspecifics out of their territory. The conservative character of alcohols promotes alcohols as a suitable signal candidate involving interspecific communication. Evidently, more research is necessary to fully understand the signalling function of alcohols.

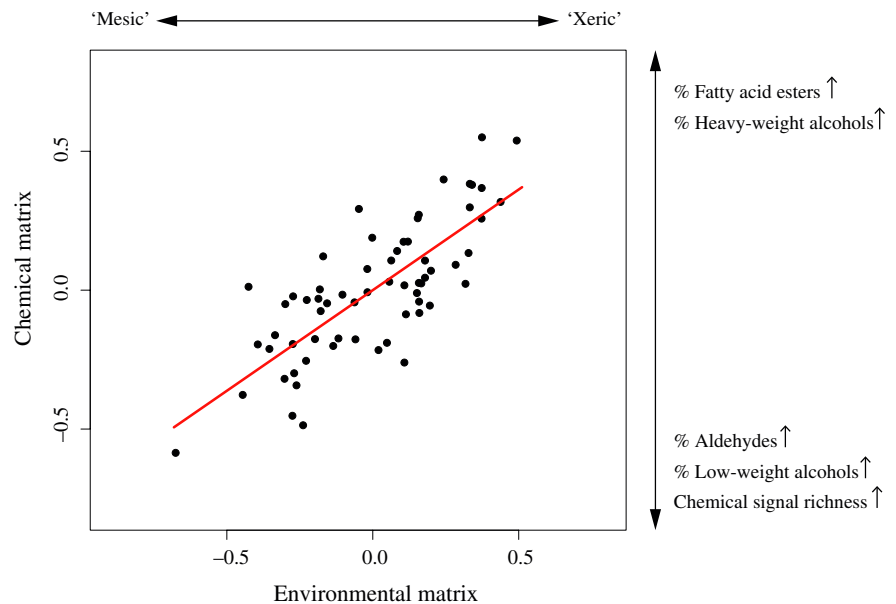


FIGURE 6 Scatterplot showing the significant relationship between the environmental and chemical matrices, indicating that climatic conditions affect the overall chemical composition of the epidermal gland secretions of lacertid lizards. Each dot represents a species' canonical score (for CV1) obtained from the phylogenetic canonical correlation analysis based on the multivariate environmental and chemical character sets

4.2 | Chemical signal efficacy

While phylogeny was unable to predict the observed patterns of variation in the chemical signalling signature of lizards, environmental factors succeeded. Earlier theoretical and experimental work (such as Alberts, 1992a; Martín et al., 2015; Regnier & Goodwin, 1977) suggests that the observed covariation may reflect differential adaptation of signal chemistry to climatic conditions, serving transmission efficacy.

'Xeric' milieus, typified by dry conditions with high temperatures, can form a harsh chemical signalling environment for animals because these high temperatures increase the evaporation and diffusion rates of chemicals, thus affecting the durability of chemical signals (Alberts, 1992a; Müller-Schwarze 2006; Regnier & Goodwin, 1977). Species inhabiting such xeric environments are therefore expected to compensate for these external factors that reduce chemical signal efficacy. One way of coping with a hostile signalling environment is by altering the chemical composition of scent-marks; investing in chemicals encompassing heavy molecules with low vapour pressures will counter rapid evaporating rates and hence increase chemical signal durability (Apps et al., 2015). For example, an interpopulational study on the lacertid lizard *Podarcis guadarramae* revealed small, but significant, differences in the chemical signal composition of two populations inhabiting environments with disparate climatic conditions (Martín et al., 2015). Moreover, chemosensory tests showed that chemical signals had a lower efficacy when temperature and dryness increases, but that these negative effects were more pronounced for signals from lizards inhabiting naturally colder and more humid environments than signals from lizards living in warm and dry conditions (Martín et al., 2015). This suggests that abiotic conditions can cause a selective pressure on the design of chemical signals in order to maximize signal efficacy. Our findings strongly support this hypothesis on a broad species-wide level, with lizard species living in xeric milieus exhibiting large

proportions of high molecular weight alcohols and fatty acid esters in their gland secretions. The heavy weight features and the low vapour pressure of long-chained $C_{>16}$ alcohols and stable long-chained fatty acid esters (Benziane et al., 2011; Saxena, Patel, & Joshipura, 2013) probably allow epidermal gland secretion to persist for longer periods of time in the environment. While scent-marks obviously benefit from these physico-chemical properties, the slow emission probably produces low gas phase concentrations, hence decreasing signal detection by airborne components (Apps et al., 2015). The glandular secretions of lizards from xeric environments, therefore, most likely serve a scent-marking function that can be detected by substrate-borne chemical sampling. Whether lizards from xeric milieus really exhibit more 'tongue-touches' than 'air-flicks' (terminology following Alberts, 1989; Carazo et al., 2007), and whether this potential shift in chemosensory behaviour has implications on the evolution of the vomeronasal-lingual system of these lizards, is uncertain. Among-species comparisons of tongue and vomeronasal organ morphology, using e.g. micro-CT imaging (as in Baeckens, Herrel, et al. 2017), would be highly informative.

'Mesic' environments, here defined by relative humid conditions with high levels of precipitation, radiation and wind, may also complicate chemical communication, although the challenges differ slightly from those faced in xeric climates. Our results showed that lizard species living in such mesic milieus exhibit large proportions of aldehydes, low molecular weight alcohols and a high chemical richness in their secretions. Low molecular weight alcohols and aldehydes evaporate rapidly, and aldehydes are also very susceptible to oxidation (Apps et al., 2015; Falbe, Bahrmann, Lipps, & Mayer, 2000; Kohlpaintner et al., 2013) While the volatile nature of these chemicals makes them, a priori, unsuitable candidates for a scent-marking function, the harsh signalling conditions of mesic environments would only accelerate the emission of the chemicals, making them even less suitable. Intuitively, these findings are not in accordance with our predictions on signalling

efficacy. However, the competing requirements for efficient scent-mark signals (persistence by low volatility) and long-distance signals (detectability by high volatility) may trigger a shift in chemical signalling tactic in mesic environments. It is plausible that lizards from mesic environments compensate the prevailing signalling challenges by utilizing their secretions for long-distance airborne communication ('high detectability-tactic'), rather than substrate-bound scent-marking ('high durability-tactic'). Indeed, a meta-analysis by Apps et al. (2015) in search of relationships between signal compound properties and signal function in terrestrial vertebrates found that aldehydes are overall less common in amniotes' scent-marks, but more common as long-distance 'odours'. Besides, aldehydes are highly odoriferous (many fragrances are aldehydes; Gounaris, 2010; Indradas, Hansen, Palmer, & Womack, 2014; Kohlpaintner et al., 2013). Their strong aromatic character, together with the moderate air currents in mesic environments, may increase the active space of the airborne chemicals and facilitate signal detection by hetero- or conspecifics from a long distance away (Martín & López, 2014). The need to shift between chemical signalling tactics ('high detectability' vs. 'high durability' tactic) could also be influenced by population density. Scent-marking in low-density populations is only effective when depositing many scent-marks over a large range, which is, by hook or by crook, very costly (Moorcroft & Lewis, 2006). Lizards from mesic environments housing low-density populations would therefore benefit from operating a long-distance signalling tactic over scent-mark signalling. Alas, we do not possess reliable data on population densities of the species sampled in this study.

Our results also show that lizard species from mesic environments produced gland secretions of a higher chemical richness (i.e. number of different lipophilic chemical compounds) than species inhabiting xeric environments. It is known that merely one individual chemical compound can hold valuable information (e.g. cholesta-5,7-dien-3-ol in *I. cyreni* lizards, López & Martín, 2005a; Martín & López, 2006; (Z)-7-dodecen-1-yl acetate in *Elephas maximus* elephants, Rasmussen, Lee, Zhang, Roelofs, & Daves, 1997), or a combination of two specific compounds (e.g. oleic acid and ergosterol in *I. cyreni* lizards; López & Martín, 2012), or a combination of compounds in a precise ratio (e.g. 67:33 blend of trans-11 and cis-11-tetradecenyl acetates in the oak leaf roller *Archips semiferanus*, Miller, Baker, Carde, & Roelofs, 1976). Therefore, animals emitting rich chemical signals probably have the potential to convey more elaborate (multiple-message hypothesis) or stronger messages (redundant message hypothesis) than animals producing signals of a low chemical richness (Hebets & Papaj, 2005; Møller & Pomiankowski, 1993; Steiger et al., 2011; Symonds & Elgar, 2008). The functional significance of the difference in chemical richness between lizards from mesic or xeric environments could be linked to the effect of humidity on the chemoreceptive organs of animals. Mammalian chemoreception, for instance, is highly modulated by relative humidity due to sensory impairment from the drying-out of nasal mucosa in low humid conditions (Laska, Rother, Schmidt, & Schmid, 1986; Vander Wall, 1998). Optimal functioning of the chemoreceptive organs in humid conditions may enable animals to detect and process

more (and) different airborne chemicals. Whether this is true for lizards, however, is still uncertain.

Although plausible, many of the above-mentioned explanations still remain speculative. While the prevailing environmental conditions strongly predict the observed variation in the chemical signal design in lizards, it still remains to be tested whether this variation is truly adaptive in terms of maximal signal efficacy.

Altering the chemical composition or the relative proportions of the compounds in the chemical signal is one way of coping with suboptimal signalling environments, but other ways are also possible. Increasing the total quantity of the deposited glandular secretions, for example, has been shown to increase the signal detectability in the lacertid lizards *Podarcis muralis* (Baeckens, Huyghe, et al., 2017). Specific site selection for secretion deposit may be another way of increasing signalling efficacy. Lizards of the species *I. cyreni* deposit their excrements at non-random sites (preferring high positioned rocks) to facilitate chemical or even visual detection by others (López, Aragón, & Martín, 1998). Lizards might also select sites of more suitable microclimatic conditions (under vegetation, in the shade, etc.) dissimilar to that of the suboptimal macroclimate. For example, the volatile-rich secretion of spiny-tailed lizards (*Uromastix aegyptia microlepis*) does not seem to be adapted to the extreme arid conditions in the desert, but they might be useful under the microclimatic conditions inside burrows where these lizards spend long periods of time (Martín et al., 2016). If environmental conditions become too hostile or the costs of producing chemicals too costly, lizards might even trade in chemical signals for other signalling modalities: the 'between-channel compensation hypothesis', as proposed by Baeckens et al. (2015) (see also Fox & Shipman, 2003; Plath, Parzefall, Körner, & Schlupp, 2004).

4.3 | Insularity and substrate use

Sixteen of the 64 species included in this study were sampled on islands. Our findings show that insularity does not affect the chemical signal design in lacertid lizards. However, lizard species on islands tend to differ notably from closely related species on the mainland in a number of morphological, behavioural, ecological, physiological and life-history characteristics (Losos & Ricklefs, 2009; Vervust, 2011). Furthermore, Martín, López, Garrido, Pérez-Cembranos, and Pérez-Mellado (2013) observed inter-island variation in the chemical composition of the secretion of the lacertid *Podarcis lilfordi* on three islands in the Balearics. However, with all islands sharing all major compounds, which accounted for 97.7% of the total chemical composition, these among-island differences turned out to be very minor (Martín, López, et al., 2013). Whilst the overall chemical profile of lacertids was unaffected by insularity in our analyses, island-life can trigger subtle changes in lacertid secretion that may be biologically significant (Runemark, Gabirot, & Svensson, 2011). Clearly, large-scale island comparisons are necessary—preferably on a populational level—in order to determine the adaptive significance of small variation in the chemical composition of lizards' secretion on islands.

A comparative study by Baeckens et al. (2015) encompassing 162 lacertid species showed that shrub-climbing species tend to have fewer secretion glands than species inhabiting other substrates, inferring that shrub-climbing species invest less in and rely less on chemical signalling. The present study was unable to find any effect of substrate use on the chemical signal composition of lacertid secretion. However, this result may be ascribed to the low number of shrub-climbing species in our dataset (nine out of 64 species; 14%).

In summary, by taking a multispecies comparative approach, we tested whether the environment is responsible for the remarkable and ubiquitous chemical signal diversity in lizards, detected in this study. Using by far the largest comparative chemical dataset amassed to-date to examine this question, our study has provided strong evidence for a significant relationship between chemical signal design and prevailing environmental conditions, which may result from differential selection on signalling efficacy. In addition, we demonstrated that lizards provide a promising model system for the study of macroevolutionary patterns of chemical signalling evolution. Future experimental studies on the biological functionality of the different chemical compounds to serve as true chemical signals or pheromones are highly encouraged.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

S.B., and R.V.D. conceived the study; all authors collected data; S.B., M.J., R.G.R., and R.V.D. analysed the data; all authors discussed the findings; S.B., M.J., and R.V.D. wrote the first draft of the manuscript; S.B. designed the figures and revised the manuscript.

DATA ACCESSIBILITY

Data deposited in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.340g8> (Baeckens, Martín, et al., 2017).

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