

This item is the archived peer-reviewed author-version of:

Environmental conditions shape the chemical signal design of lizards

Reference:

Baeckens Simon, Martín José, García-Roa Roberto, Pafilis Panayiotis, Huyghe Katleen, Van Damme Raoul.- Environmental conditions shape the chemical signal design of lizards

Functional ecology / British Ecological Society - ISSN 0269-8463 - Hoboken, Wiley, 32:2(2018), p. 566-580

Full text (Publisher's DOI): <https://doi.org/10.1111/1365-2435.12984>

To cite this reference: <https://hdl.handle.net/10067/1475540151162165141>

How environmental conditions shape the chemical signal design of lizards

Simon Baeckens^{1,2, *}, José Martín³, Roberto García-Roa³, Panayiotis Pafilis⁴, Katleen Huyghe¹ & Raoul Van Damme¹

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

² Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, USA

³ Department of Evolutionary Ecology, Museo Nacional de Ciencias Naturales, CSIC, José Gutiérrez Abascal 2, 28006 Madrid, Spain

⁴ School of Biology, Department of Zoology and Marine Biology, National and Kapodistrian University of Athens, 157-84 Panepistimioupolis, Ilissia, Greece

* Corresponding author. E-mail address: simon.baeckens@uantwerp.be. Phone: +3232652259. Fax: +32(0)32652271

Short running title: Ecomorphology of lizard chemical signals

Key words: Adaptive evolution, Chemical communication, Epidermal gland secretions, Lacertidae, Macroevolution, Pheromones, Phylogenetic comparative methods, Signal efficacy

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 the chemical signalling system.
2. Here, we take 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 by using gas chromatography-mass spectrometry (GC-MS). 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. While neither insularity nor substrate use affected chemical signal composition, 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 chemical signal design results from differential natural selection, optimizing signal efficacy under contrasting environmental conditions.

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. Laidre & Johnstone 2013; Lichtenberg et al. 2014; Haven Wiley 2015; Tibbets et al. 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 influences 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 et al. (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 (Ng et al. 2013; Driessens et al. 2017).

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 (Stevens 2013; Searcy & Nowicki 2005; but see e.g. Steiger et al. 2011; delBaro-Trillo & Drea 2014; Weber et al. 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 & Silverstein 1980; Müller-Schwarze 2006; 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. Vinson 1972; Durak & Kalender 2009; Lacey et al. 2009), decreases two- to fourfold when air temperature rises from 20 to 30°C (McDonough et al. 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 et al. 2015). To illustrate, high temperatures accelerate scent-mark decay in ants, and hence limit the

trail-following behaviour of these insects (Van Oudenhove et al. 2011, 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 (Regnier & Goodwin 1977; Alberts 1992a; Müller-Schwarze 2012; Apps et al. 2015). 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 two hours (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 et al. 2005). Thirdly, high levels of solar – and UV radiation, which is closely linked with altitude (Blumthaler et al. 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 (Bossert & Wilson 1963; Elkington & Cardé 1984; Alberts 1992a). 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 et

al 1997; Rantala et al. 2003; Rundle et al. 2005; Kopena et al. 2011; García-Roa et al. 2017a), animals are unlikely to invest in costly signalling compounds when the benefit is economically low.

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. Mason & Parker 2010; Martín & López 2014; Pruett et al. 2016; Mangiacotti et al. 2016; Baeckens et al. 2017a; García-Roa et al. 2017b,c). 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 (Fig. 1), or ‘epidermal (femoral) pores’ (Mayerl et al. 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 et al. 1993), such as territory demarcation and assessment (Aragón et al. 2001; Martins et al. 2006; Font et al. 2012; Martín & López 2012; Leu et al. 2016), male rival assessment (López & Martín 2002; Carazo et al. 2007; Hews et al. 2011; Khannoon et al. 2011), female choice (Carazo et al. 2011; Kopena et al. 2011, 2014; Gabirot et al. 2013; Martín & López 2013a, 2015), assessment of female reproductive status (Cooper & Pérez-Mellado 2002; Thomas 2011), individual recognition (Alberts 1992b; Alberts & Werner 1993; Gabirot et al. 2010a,b), sex identification (Cooper & Trauth 1992; Cooper & Steele 1997; Khannoon et al. 2010)

and species recognition (Aragón 2001; Barbosa et al. 2006; Gabirot 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 (Mason & Parker 2010; de Villiers et al. 2015). 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 et al. 2012). Since the longevity and detectibility 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 et al. 2013a). 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 (Gabirot 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 et al. 2001, 2003; Martín & López 2014).

Most studies on the ecological factors influencing chemical signal evolution concentrate on one or two species (e.g. Rouault et al. 2004; Rundle et al. 2005; Martín & López 2014; Martín et al. 2015), and cannot provide the broad-scale evolutionary insight that can be gained from multi-species comparative studies (see Garamszegi et

al. 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 signalling signature’) 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 (Fig. 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 et al. 2015). All species (except one) carry epidermal glands (although in different number, Baeckens et al. 2015), and many are known to use chemical signalling in several contexts (Martín & López 2014; Mayerl et al. 2015).

Material and methods

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 approximately 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 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 (Fig. 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 (Smith 1946; Cole 1966), we exclusively sampled adult lizards during the mating season (i.e. spring – early summer; Arnold & Ovenden 2007; Carretero 2007; Pianka et al. 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 approximately two weeks prior to purchase, and their epidermal gland secretions were collected in the lab 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).

Collection and extraction of glandular 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 et al. 2017b). 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 five months. The identification of each chemical compound and estimation of its relative abundance (as percentage) was estimated using 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 et al. 2007, 2013a). 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 et al. 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.

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 was downloaded from WorldClim (Hijmans et al. 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 ± 1 km², we extracted data on mean air temperature (taken ± 2 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 (Tieleman et al. 2003; Oufiero et al. 2011). 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-hourly; 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 approximately 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 (12:00h), and from March until September for European/Mediterranean lacertid species (Perry et al. 1990; Carretero 2006; Arnold & Ovendon 2007), 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 *Holaspis 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, and generalist.

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 (Fu 2000; Arnold et al. 2007; Mayer & Pavlicev 2007; Pavlicev & Mayer 2009; Kapli et al. 2011; Pyron et al. 2013; Baeckens et al. 2015; Mendes et al. 2016). Hence, this phylogenetic tree will be used in all further phylogenetic comparative tests.

Statistical analyses

We performed all analyses using the ‘ape’, ‘geiger’, ‘phylocurve’ and ‘phytools’ packages in R, version 3.3.1 (Paradis et al. 2004; Harmon et al. 2008; Revell 2012, 2013; Adams 2014; Goolsby 2016; R Core Team 2013). Probabilities (P) lower than 0.05 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 (Pagel 1999; Blomberg et al. 2003). 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 et al. (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.

Results

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 Fig. 4.

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

Phylogenetic signal

The overall composition of the epidermal gland secretion in lacertid lizards exhibited a relatively weak, albeit significant, phylogenetic signal (Blomberg's multivariate $K = 0.45$, $P < 0.001$). 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, $P < 0.001$), but intermediate K values ($K < 0.6$, $P > 0.05$). As the sole exception, alcohols exposed a high λ value ($\lambda = 0.99$, $P < 0.001$) and a K value over one ($K = 1.33$, $P = 0.001$), 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.

Environmental effects

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; $R = 0.86$, $\chi^2 = 233.58$, $P = 0.002$). Only the first canonical axis between environment and chemicals proved significant; the second axis was not (canonical axis 2; $R = 0.76$, $\chi^2 = 167.36$, $P = 0.157$). We therefore focused on canonical variable 1 (CV1). The canonical loadings (on CV1) for the environmental and chemical variables are given in Table 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. Fig. 6 visualises 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 ($F_{16,47} = 0.160$, $P = 0.904$) or substrate use ($F_{64,174.53} < 0.001$, $P = 0.321$) on species' secretion composition.

Discussion

Natural selection favours signals that work effectively (Endler 1992; Boughman 2002). 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 (Morton 1975; Fleishman 1988, 1992; Alberts 1992a; Nicholls & Goldizen 2006; Ng et al. 2013). 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. However, insularity or substrate use did not affect chemical signal composition. This study provides the first evidence of a strong relationship between chemical signal design and prevailing environmental conditions across different animal species, which may result from selection on signal efficacy.

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 signalling to phylogenetic relatedness; the phylogenetic signals 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 et al. 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 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 (*Acanthodactylus erythrurus* and *A. 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 chemical signal candidate involving interspecific communication. Evidently, more research is necessary to fully understand the chemical signalling function of alcohols.

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 Regnier & Goodwin 1977; Alberts 1992a; Martín & López 2013c) suggests that the observed co-variation 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 (Regnier & Goodwin 1977; Alberts 1992a; Müller-Schwarze 2012). 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 et al. 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 probably 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 et al. 2017c), 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 (Fable et al. 2000; Kohlpeinter et al. 2013; Apps et al. 2015). 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 will 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 possible 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; Kohlpeinter et al. 2013; Indradas et al. 2014). 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 and 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 showed that lizard species from mesic environments produce 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 *Iberolacerta cyreni* lizards, López & Martín 2005a; Martín & López 2006; (Z)-7-dodecen-1-yl acetate in *Elephas maximus* elephants, Rasmussen et al. 1997), or a combination of two specific compounds (e.g. oleic acid and ergosterol in *Iberolacerta 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 et al. 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 (Møller & Pomiankowski 1993; Hebets & Papaj 2005; Symonds & Elgar 2008; Steiger et al. 2011). The functional significance of the difference in chemical richness between lizards from mesic or xeric environments may 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 et al. 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 signalling 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 et al. 2017b). Specific site selection for secretion deposit may be another way of increasing signalling efficacy. Lizards of the species *Iberolacerta cyreni* deposit their excrements at non-random sites (preferring high positioned rocks) to facilitate chemical or even visual detection by others (López et al. 1998). Lizards might also select sites of more suitable micro-climatic conditions (under vegetation, in the shade, etc.) dissimilar to that of the suboptimal macro-

climate. 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 et al. 2004).

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 et al. (2013b) 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 et al. 2013b). 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 et al. 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 (9 out of 64 species; 14%).

In summary, by taking a multi-species 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 macro-evolutionary 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.

Acknowledgments

We thank ‘El Ventorillo’ MNCN Field Station for use of their facilities, Josie Meaney for linguistic advice, Shai Meiri and his wonderful team for assistance and guidance in the field, and four anonymous reviewers for significantly improving drafts of this manuscript. 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), South Africa (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). 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. RGR is indebted to the Spanish Ministerio de Economía y Competitividad (FPI predoctoral grant number BES-2012-054387), This work was part of SB's doctoral thesis at the University of Antwerp, and made possible through financial support from the University of Antwerp. The authors declare that they have no conflict of interest.

Author 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.

References

- Adamopoulou, C. (1999). *Structure and function of epigeic animal communities with emphasis in the lizard Podarcis milensis (Sauria: Lacertidae), in insular ecosystems of the Aegean*. PhD dissertation, University of Athens, pp. 268.
- Adams, D.C. (2014). A generalized K statistic for estimating phylogenetic signal from shape and other high-dimensional multivariate data. *Syst. Biol.*, 63, 685–697.
- Alberts, A. (1989) Ultraviolet visual sensitivity in desert iguanas: implications for pheromone detection. *Anim. Behav.*, **38**, 129–137.
- Alberts, A.C. (1992a). Constraints on the design of chemical communication systems in terrestrial vertebrates. *Am. Nat.*, 139, 62–89.
- Alberts, A.C. (1992b). Pheromonal Self-Recognition in Desert Iguanas. *Copeia*, 1, 229–232.
- Alberts, A.C. & Werner, D. (1993). Chemical recognition of unfamiliar conspecifics by green iguanas: functional significance of different signal components. *Anim. Behav.*, 46, 197–199.
- Alberts, A.C., Phillips, J. & Werner, D. (1993) Sources of Intraspecific Variability in the Protein-Composition of Lizard Femoral Gland Secretions. *Copeia*, **1993**, 775–781.
- Apps, P.J., Weldon, P.J. & Kramer, M. (2015). Chemical signals in terrestrial vertebrates: search for design features. *Nat. Prod. Rep.*, 32, 1131–1153.
- Aragón, P., López, P. & Martín, J. (2001). Effects of Conspecific Chemical Cues on Settlement and Retreat-Site Selection of Male Lizards *Lacerta monticola*. *J. Herpetol.*, 35, 684–686.
- Arnold, S. (1983). Morphology, performance and fitness. *Am. Zool.*, 23, 347–361.

- Arnold, E.N. (1987). Resource partition among lacertid lizards in southern Europe. *J. Zool.*, 1, 739–782.
- Arnold, E.N. (1989). Towards a phylogeny and biogeography of the Lacertidae: relationships within an Old-World family of lizards derived from morphology. *Bull. Br. Museum Natural Hist. Zool.*, 55, 209–257.
- Arnold, E.N., Arribas, Ó. & Carranza, S. (2007). Systematics of the Palearctic and Oriental lizard tribe Lacertini (Squamata: Lacertidae: Lacertinae), with descriptions of eight new genera. *Zootaxa*, 1430, 1–86.
- Arribas, O. & Galán, P. (2005). Reproductive characteristics of the Pyrenean high-mountain lizards: *Iberolacerta aranica* (Arribas, 1993), *I. aurelioi* (Arribas, 1994) and *I. bonnali* (Lantz, 1927). *Anim. Biol.*, 55, 163–190.
- Baeckens, S., Edwards, S., Huyghe, K. & Van Damme, R. (2015). Chemical signalling in lizards: an interspecific comparison of femoral pore numbers in Lacertidae. *Biol. J. Linn. Soc.*, 114, 44–57.
- Baeckens, S., Van Damme, R. & Cooper, W.E. (2017a) How phylogeny and foraging ecology drive the level of chemosensory exploration in lizards and snakes. *Journal of Evolutionary Biology*, **30**, 627–640.
- Baeckens, S., Huyghe, K., Palme, R. & Van Damme, R. (2017b). Chemical communication in the lacertid lizard *Podarcis muralis*: the functional significance of testosterone. *Acta Zool.*, 98: 94-103.
- Baeckens S., Herrel A., Broeckhoven C., Vasilopoulou-Kampitsi M., Huyghe K., Goyens J. & Van Damme R. (2017c) Evolutionary morphology of the lizard chemosensory system. *Sci. Rep.*
- Baha El Din, S.M. (2007). A new lizard of the *Acanthodactylus scutellatus* group (Squamata: Lacertidae) from Egypt. *Zool. Middle East*, 40, 21–32.

- Barbosa, D., Font, E., Desfilis, E. & Carretero, M.A. (2006). Chemically mediated species recognition in closely related *Podarcis* wall lizards. *J. Chem. Ecol.*, 32, 1587–1598.
- Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. & Sayers, E.W. (2009). GenBank. *Nucleic Acids Res.*, 37, D26–D31. Available at: <https://www.ncbi.nlm.nih.gov/genbank/>
- Benziane, M., Khimeche, K., Mokbel, I., Sawaya, T., Dahmani, A. & Jose, J. (2011). Experimental vapor pressures of five saturated fatty acid ethyl ester (FAEE) components of biodiesel. *J. Chem. Eng. Data*, 56, 4736–4740.
- Blomberg, S.P., Garland, T. & Ives, A.R. (2003). Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, 57, 717–45.
- Blumthaler, M., Ambach, W. & Ellinger, R. (1997). Increase in solar UV radiation with altitude. *J. Photochem. Photobiol. B Biol.*, 39, 130–134.
- Bossert, W.H. & Wilson, E.O. (1963). The analysis of olfactory communication among animals. *J. Theor. Biol.*, 5, 443–469.
- Boughman, J.W. (2002). How sensory drive can promote speciation. *TRENDS Ecol. Evol.*, 17, 571–577.
- Bradbury, J.W. & Vehrencamp, S.V. (2011). *Principles of Animal Communication*. Second edition. Sinauer Associates, Sunderland, MA.
- Braña, F. (1996). Sexual dimorphism in lacertid lizards: male head increase vs. female abdomen increase? *Oikos*, 75, 511–523.
- Braña, F., Arrayago, M. & Barahona, A. (1990). Ciclo reproductor y de cuerpos grasos de *Lacerta monticola cantabrica*. Comparación entre dos poblaciones situadas a diferente altitud. *Amphib. Reptil.*, 11, 41–52.

- Campbell, P., Pasch, B., Pino, J.L., Crino, O.L., Phillips, M. & Phelps, S.M. (2010). Geographic variation in the songs of neotropical singing mice: Testing the relative importance of drift and local adaptation. *Evolution*, 64, 1955–1972.
- Carazo, P., Font, E. & Desfilis, E. (2007). Chemosensory assessment of rival competitive ability and scent-mark function in a lizard, *Podarcis hispanica*. *Anim. Behav.*, 74, 895–902.
- Carazo, P., Font, E. & Desfilis, E. (2011). The role of scent marks in female choice of territories and refuges in a lizard (*Podarcis hispanica*). *J. Comp. Psychol.*, 125, 362–365.
- Carretero, M.A. 2006 Reproductive cycles in Mediterranean lacertids: plasticity and constraints. In: *Mainland Insular lacertid lizards: a Mediterranean Perspective* (eds. (Corti, C., Lo Cascio, P. & Biaggini, M.) Firenze University Press, Firenze, pp. 1-22.
- Clark, D., DeBano, S. & Moore, A. (1997). The influence of environmental quality on sexual selection in *Nauphoeta cinerea* (Dictyoptera: Blaberidae). *Behav Ecol.*, 8, 46-53.
- Cole, C. (1966). Femoral glands in lizards: a review. *Herpetologica*, 22, 199–205.
- Cooper, W.E. & Trauth, S.E. (1992). Discrimination of conspecific male and female cloacal chemical stimuli by males and possession of a probable pheromone gland by females in a cordylid lizard, *Gerrhosaurus nigrolineatus*. *Herpetologica*, 48, 229–236.
- Cooper, W.E. & Steele, L.J. (1997). Pheromonal discrimination of sex by male and female leopard geckos (*Eublepharis macularius*). *J. Chem. Ecol.*, 23, 2967–2977.

- Cooper, W.E. & Pèrez-Mellado, V. (2002). Pheromonal Discriminations of Sex, Reproductive Condition, and Species by the Lacertid Lizard *Podarcis hispanica*. *J. Exp. Zool.*, 292, 523–527.
- Cunningham, P.L. (2001). Notes on some aspects of the ecology of *Acanthodactylus ophiodurus* (Arnold, 1980), from the United Arab Emirates (Squamata: Sauria: Lacertidae). *Herpetozoa*, 14, 15–20.
- Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods*, 9, 772–772.
- de Villiers, A., Flemming, A. & Mouton, P.Le F.N. (2015). Generation glands of cordylid lizards: mechanism of secretion transfer to the environment. *Amphibia-Reptilia*, 36, 351–360.
- Dee, D.P., Uppala, S.M., Simmons, A.J., Berrisford, P., Poli, P., Kobayashi, S., *et al.* (2011). The ERA-Interim reanalysis: Configuration and performance of the data assimilation system. *Q. J. R. Meteorol. Soc.*, 137, 553–597. Available at: <http://www.ecmwf.int/en/research/climate-reanalysis/era-interim>
- delBarco-Trillo, J. & Drea, C.M. (2014). Socioecological and phylogenetic patterns in the chemical signals of strepsirrhine primates. *Anim. Behav.*, 97, 249–253.
- Driessens, T., Baeckens, S., Balzarolo, M., Vanhooydonck, B., Huyghe, K. & Van Damme, R. (2017) Climate-related environmental variation in a visual signalling device: the male and female dewlap in *Anolis sagrei* lizards. *J. Evol. Biol.*. doi:10.1111/jeb.13144
- Durak, D. & Kalender, Y. (2009). Fine structure and chemical analysis of the metathoracic scent gland secretion in *Graphosoma lineatum* (Linnaeus, 1758) (Heteroptera, Pentatomidae). *Comptes Rendus - Biol.*, 332, 34–42.

- Edwards, S., Vanhooydonck, B., Herrel, A., Measey, G.J. & Tolley, K.A. (2012). Convergent evolution associated with habitat decouples phenotype from phylogeny in a clade of lizards. *PLoS One*, 7, e51636.
- Edwards, S., Branch, W.R., Vanhooydonck, B., Herrel, A., Measey, G.J. & Tolley, K.A. (2013). Taxonomic adjustments in the systematics of the southern African lacertid lizards (Sauria: Lacertidae). *Zootaxa*, 3669, 101–114.
- Elkinton, J.S. & Cardé R.T. (1984). Effect of wild and laboratory-reared female gypsy moths (Lepidoptera: Lymantriidae) on the capture of males in pheromone-baited traps. *Environ. Entomol.*, 13, 1377-1385.
- Endler, J.A. (1992). Signals, signal conditions, and the direction of evolution. *Am. Nat.*, 139, 125–153.
- Endler, J.A. (1993). Some general comments on the evolution and design of animal communication systems. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 340, 215–225.
- Endler, J.A. (2000). Evolutionary implications of the interaction between animal signals and the environment. In: *Animal Signals: Signalling and Signal Design in Animal Communication* (eds. Espmark, Y., Amundsen, T. & Rosenqvist, G.). Tapir Academic Press, Norway, pp. 11-46.
- Escobar, C.A, Labra, A. & Niemeyer, H.M. (2001). Chemical composition of precloacal secretions of *Liolaemus* lizards. *J. Chem. Ecol.*, 27, 1677–1690.
- Escobar, C.M., Escobar, C.A, Labra, A. & Niemeyer, H.M. (2003). Chemical composition of precloacal secretions of two *Liolaemus fabiani* populations: are they different? *J. Chem. Ecol.*, 29, 629–368.
- Falbe, J., Bahrmann, H., Lipps, W. & Mayer, D. (2000). Alcohols, Aliphatic. In: *Ullmann's Encyclopedia of Industrial Chemistry*. pp. 255–271.

- Fleishman, L.J. (1988). Sensory and environmental influences on display form in *Anolis aeneus*, a grass anole from Panama. *Behav. Ecol. Sociobiol.*, 22, 309–316.
- Fleishman, L.J. (1992). The Influence of the Sensory System and the Environment on Motion Patterns in the Visual Displays of Anoline Lizards and Other Vertebrates. *Am. Nat.*, 139, S36–S61.
- Font, E., Barbosa, D., Sampedro, C. & Carazo, P. (2012). Social behavior, chemical communication, and adult neurogenesis: studies of scent mark function in *Podarcis wall lizards*. *Gen. Comp. Endocrinol.*, 177, 9–17.
- Fox, S.F. & Shipman, P.A. (2003). Social Behavior at high and low elevations. Environmental Release and Phylogenetic effects in *Liolaemus*. In: *Lizard Social Behavior* (eds. Fox, S., McCoy, J. & Baird, T.). John Hopkins University Press, Baltimore, MD, pp. 310–355.
- Fu, J.Z. (2000). Toward the phylogeny of the family Lacertidae - Why 4708 base pairs of mtDNA sequences cannot draw the picture. *Biological Journal of the Linnean Society*, 71, 203–217.
- Gabirot, M., López, P., Martín, J., de Fraipont, M., Heulin, B., Sinervo, B., *et al.* (2008). Chemical composition of femoral secretions of oviparous and viviparous types of male common lizards *Lacerta vivipara*. *Biochem. Syst. Ecol.*, 36, 539–544.
- Gabirot, M., Castilla, A.M., López, P. & Martín, J. (2010a). Chemosensory species recognition may reduce the frequency of hybridization between native and introduced lizards. *Can. J. Zool.*, 88, 73–80.
- Gabirot, M., Castilla, A.M., López, P. & Martín, J. (2010b). Differences in chemical signals may explain species recognition between an island lizard, *Podarcis*

- atrata*, and related mainland lizards, *P. hispanica*. *Biochem. Syst. Ecol.*, 38, 521–528.
- Gabirot, M., López, P. & Martín, J. (2013). Female mate choice based on pheromone content may inhibit reproductive isolation between distinct populations of Iberian wall lizards. *Curr. Zool.*, 59, 210–220.
- Garamszegi, L.Z., Eens, M., Erritzøe, J. & Møller, A.P. (2005). Sexually size dimorphic brains and song complexity in passerine birds. *Behav. Ecol.*, 16, 335–345.
- García-Roa, R., Sáiz, J., Gómara, B., López, P. & Martín, J. (2017a) Dietary constraints can preclude the expression of an honest chemical sexual signal. *Sci. Rep.*, 7, 6073.
- García-Roa, R., Jara, M., López, P., Martín, J. & Pincheira-Donoso, D. (2017b) Heterogeneous tempo and mode of evolutionary diversification of compounds in lizard chemical signals. *Ecol. Evol.*, 7, 1286-1296
- García-Roa, R., Jara, M., Baeckens S., López P., Van Damme R., Martín J. & Pincheira-Donoso D. (2017c) Marcoevolutionary diversification of glands for chemical communication in squamate reptiles. *Sci. Rep.*
- Goolsby, E.W. (2016). Likelihood-Based Parameter Estimation for High-Dimensional Phylogenetic Comparative Models: Overcoming the Limitations of “Distance-Based” Methods. *Syst. Biol.*, 65, 852–870.
- Gounaris, Y. (2010). Biotechnology for the production of essential oils, flavours and volatile isolates. A review. *Flavour Fragr. J.*, 25, 367–386.
- Guindon, S. & Gascuel, O. (2003). A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. *Syst. Biol.*, 52, 696–704.

- Harmon, L.J., Weir, J.T., Brock, C.D., Glor, R.E. & Challenger, W. (2008). GEIGER: Investigating evolutionary radiations. *Bioinformatics*, 24, 129–131.
- Harrison, A.S., Revell, L.J. & Losos, J.B. (2015). Correlated evolution of microhabitat, morphology, and behavior in West Indian Anolis lizards: a test of the habitat matrix model. *Behaviour*, 152, 1187–1207.
- Haven Wiley, R. (2015) *Noise Matters: The Evolution of Communication*. Harvard University Press, Cambridge.
- Hebets, E.A. & Papaj, D.R. (2005). Complex signal function: developing a framework of testable hypothesis. *Behav. Ecol. Sociobiol.*, 57, 197-214.
- Herrel, A., Spithoven, L., Van Damme, R. & De Vree, F. (1999). Sexual dimorphism of head size in *Gallotia galloti*: testing the niche divergence hypothesis by functional analyses. *Funct. Ecol.*, 13, 289–297.
- Hews, D.K., Date, P., Hara, E. & Castellano, M.J. (2011). Field presentation of male secretions alters social display in *Sceloporus virgatus* but not *S. undulatus* lizards. *Behav. Ecol. Sociobiol.*, 65, 1403–1410.
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.*, 25, 1965–1978. Available at <http://worldclim.org/current>
- Huey, R. B. and E. R. Pianka. 1977a. Seasonal variation in thermoregulatory behavior and body temperature of diurnal Kalahari lizards. *Ecology* 58, 1066-1075.
- Huey, R. & Pianka, E.R. 1981. Ecological Consequences of Foraging Mode. *Ecology* 62, 991–999.
- Hughes, N.K., Kelley, J.L., Banks, P.B. & Grether, G. (2012). Dangerous liaisons: the predation risks of receiving social signals. *Ecol. Lett.*, 15, 1326–39.

- Indradas, B., Hansen, C., Palmer, M. & Womack, G.B. (2014). Autoxidation as a trigger for the slow release of volatile perfumery chemicals. *Flavour Fragr. J.*, 29, 313–323.
- Kaliontzopoulou, A., Adams, D.C., van der Meijden, A., Perera, A. & Carretero, M.A. (2012a). Relationships between head morphology, bite performance and ecology in two species of *Podarcis* wall lizards. *Evol. Ecol.*, 26, 825–845.
- Kaliontzopoulou, A., Carretero, M.A. & Llorente, G.A (2012b.) Morphology of the *Podarcis* wall lizards (Squamata: Lacertidae) from the Iberian Peninsula and North Africa: patterns of variation in a putative cryptic species complex. *Biol. J. Linn. Soc.*, 164, 173-193.
- Kapli, P., Poulakakis, N., Lymberakis, P. & Mylonas, M. (2011) A re-analysis of the molecular phylogeny of Lacertidae with currently available data. *Basic and Applied Herpetology*, 25, 97–104.
- Khannoon, E., Breithaupt, T., El-Gendy, A. & Hardege, J.D. (2010). Sexual differences in behavioural response to femoral gland pheromones of *Acanthodactylus boskianus*. *Herpetol. J.*, 20, 225–229.
- Khannoon, E.R., Flachsbarth, B., El-Gendy, A., Mazik, K., Hardege, J.D. & Schulz, S. (2011). New compounds, sexual differences, and age-related variations in the femoral gland secretions of the lacertid lizard *Acanthodactylus boskianus*. *Biochem. Syst. Ecol.*, 39, 95–101.
- Kohlpainter, C., Schulte, M., Falbe, J., Lappe, P., Weber, J. & Frey, G. (2013). Aldehydes, Aliphatic. In: *Ullmann's Encyclopedia of Industrial Chemistry*. pp. 647–698.

- Kopena, R., Martín, J., López, P. & Herczeg, G. (2011). Vitamin E supplementation increases the attractiveness of males' scent for female European green lizards. *PLoS One*, 6, e19410.
- Kopena, R., López, P. & Martín, J. (2014). Relative contribution of dietary carotenoids and vitamin E to visual and chemical sexual signals of male Iberian green lizards: an experimental test. *Behav. Ecol. Sociobiol.*, 68, 571–581.
- Labra, A. (2011). Chemical stimuli and species recognition in *Liolaemus* lizards. *J. Zool.*, 285, 215–221.
- Lacey, E.S., Millar, J.G., Moreira, J.A. & Hanks, L.M. (2009). Male-Produced aggregation pheromones of the cerambycid beetles *Xylotrechus colonus* and *Sarosesthes fulminans*. *J. Chem. Ecol.*, 35, 733–740.
- Laidre, M.E. & Johnstone, R.A. (2013) Animal signals. *Curr. Biol.*, **23**, R829–R833.
- Laska, M., Rother, G., Schmidt, U. & Schmid, U. (1986). Die beeinflussung des reichvermögens durch die luftfeuchte bei *Carollia perspicillata* und *Phyllostomus discolor* (Chiroptera). *Z. Säugetierkd*, 51, 129–138.
- Leu, S.T., Jackson, G., Roddick, J.F. & Bull, C.M. (2016). Lizard movement tracks : variation in path re-use behaviour is consistent with a scent-marking function. *PeerJ*, 4:e1844.
- Lichtenberg, E.M., Zivin, J.G., Hrnčir, M. & Nieh, J.C. (2014) Eavesdropping selects for conspicuous signals. *Current Biology*, **24**, R598–R599.
- López, P. & Martín, J. (2002). Chemical rival recognition decreases aggression levels in male Iberian wall lizards, *Podarcis hispanica*. *Behav. Ecol. Sociobiol.*, 51, 461–465.

- López, P. & Martín, J. (2005a). Female Iberian wall lizards prefer male scents that signal a better cell-mediated immune response. *Biol. Lett.*, 1, 404–406.
- López, P. & Martín, J. (2012) Chemosensory exploration of male scent by female rock lizards result from multiple chemical signals of males. *Chemical senses*, 37, 47–54.
- López, P., Aragón, P. & Martín, J. (1998). Iberian Rock Lizards (*Lacerta monticola cyreni*) Assess Conspecific Information Using Composite Signals from Faecal Pellets. *Ethology*, 104, 809–820.
- Losos, J.B. & Ricklefs, R.E. (2009). Adaptation and diversification on islands. *Nature*, 457, 830–836.
- Lymberakis, P., Poulakakis, N., Kaliontzopoulou, a., Valakos, E. & Mylonas, M. (2008). Two new species of Podarcis (Squamata; Lacertidae) from Greece. *Syst. Biodivers.*, 6, 307–318.
- Mangiacotti, M., Fumagalli, M., Scali, S., Zuffi, M.A.L., Cagnone, M., Salvini, R. & Sacchi, R. (2016) Inter- and intra-population variability of the protein content of femoral gland secretions from a lacertid lizard. *Current Zoology*, zow113. doi: 10.1093/cz/zow113.
- Martín, J. & López, P. (2006). Links between male quality, male chemical signals, and female mate choice in Iberian Rock Lizards. *Funct. Ecol.*, 20, 1087–1096.
- Martín, J. & López, P. (2011). Pheromones and reproduction in Reptiles. In: *Hormones and Reproduction in Vertebrates, vol. 3. Reptiles* (eds. Norris, D.O. & Lopez K.H.). Academic Press, San Diego, CA, pp. 141–167.
- Martín, J. & López, P. (2012). Supplementation of male pheromone on rock substrates attracts female rock lizards to the territories of males: a field experiment. *PLoS One*, 7, e30108.

- Martín, J. & López, P. (2013a). Responses of female rock lizards to multiple scent marks of males: effects of male age, male density and scent over-marking. *Behav. Processes*, 94, 109–114.
- Martín, J. & López, P. (2013b). Effects of global warming on sensory ecology of rock lizards: Increased temperatures alter the efficacy of sexual chemical signals. *Funct. Ecol.*, 27, 1332–1340.
- Martín, J. & López, P. (2014). Pheromones and other Chemical Communication in Animals. In: *Reproductive Biology and Phylogeny of Lizards and Tuatara* (eds. Rheubert, J.L., Siegel, D.S. & Trauth, S.E.). CRC Press, pp. 43–77.
- Martín, J. & López, P. (2015). Condition-dependent chemosignals in reproductive behavior of lizards. *Horm. Behav.*, 68, 14–24.
- Martín, J., Moreira, P.L. & López, P. (2007). Status-signalling chemical badges in male Iberian rock lizards. *Funct. Ecol.*, 21, 568–576.
- Martín, J., Ortega, J. & López, P. (2013a). Lipophilic compounds in femoral secretions of male collared lizards, *Crotaphytus bicinctores* (Iguania, Crotaphytidae). *Biochem. Syst. Ecol.*, 47, 5–10.
- Martín, J., López, P., Garrido, M., Pérez-Cembranos, A. & Pérez-Mellado, V. (2013b). Inter-island variation in femoral secretions of the Balearic lizard, *Podarcis lilfordi* (Lacertidae). *Biochem. Syst. Ecol.*, 50, 121–128.
- Martín, J., Ortega, J. & López, P. (2015). Interpopulational Variations in Sexual Chemical Signals of Iberian Wall Lizards May Allow Maximizing Signal Efficiency under Different Climatic Conditions. *PLoS One*, 10, e0131492.
- Martín, J., Castilla, A.M., López, P., Al-Jaidah, M., Mohannadi, S. & Al-Hamaidi, A. (2016). Chemical signals in desert lizards: Are femoral gland secretions of

- male and female spiny-tailed lizards, *Uromastyx aegyptia microlepis* adapted to arid conditions? *J. Arid Environ.*, 127, 192–198
- Martins, E.P. & Lamont, J. (1998). Estimating ancestral states of a communicative display: a comparative study of *Cyclura* rock iguanas. *Anim. Behav.*, 55, 1685–706.
- Martins, E.P., Ord, T.J., Slaven, J., Wright, J.L. & Housworth, E.A. (2006). Individual, sexual, seasonal, and temporal variation in the amount of sagebrush lizard scent marks. *J. Chem. Ecol.*, 32, 881–893.
- Mason, R.T. & Parker, M.R. (2010). Social behavior and pheromonal communication in reptiles. *J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol.*, 196, 729–749.
- Mateo, J. A. (1990). Taxonomy and evolution of the North African ocellated lizard, *Lacerta pater* (Lataste, 1880) (Sauria: Lacertidae). *Bonner Zool. Beiträge*, 41, 2003–2012.
- Mayer, W. & Pavlicev, M. (2007) The phylogeny of the family Lacertidae (Reptilia) based on nuclear DNA sequences: convergent adaptations to arid habitats within the subfamily Eremiainae. *Molecular phylogenetics and evolution*, 44, 1155–63.
- Mayerl, C., Baeckens, S. & Van Damme, R. (2015). Evolution and role of the follicular epidermal gland system in non-ophidian squamates. *Amphibia-Reptilia*, 36, 185–206.
- Maynard Smith, J. & Harper, D.G.C. (2003). *Animal signals*. Oxford University Press, Oxford.

- McDonough, L.M., Brown, D.F. & Aller, W.C. (1989). Insect sex pheromones - Effect of temperature on evaporation rates of acetates from rubber septa. *J. Chem. Ecol.*, 15, 779–790.
- Mendes, J., Harris, J.D., Carranza, S. & Salvi, D. (2016) Evaluating the phylogenetic signal limit from mitogenomes, slow evolving nuclear genes, and the concatenation approach. New insights into the Lacertini radiation using fast evolving nuclear genes and species trees. *Molecular Phylogenetics and Evolution*, 100, 254-267.
- Meiri, S. 2010. Length-weight allometries in lizards. *J. Zool.*, 281, 218–226.
- Miles, D.B. & Ricklefs, R. (1984). The correlation between ecology and morphology in deciduous forest passerine birds. *Ecology*, 65, 629-1640.
- Miller, J.R., Baker, T.C., Carde, R.T. & Roelofs, W.L. (1976). Reinvestigation of oak leaf roller sex pheromone components and the hypothesis that they vary with diet. *Science*, 192, 140–143.
- Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*. New Orleans, LA, pp. 1-8. Available at: http://www.phylo.org/sub_sections/portal/
- Møller, A.P. & Pomiankowski, A. (1993). Why have birds got multiple sexual ornaments? *Behav. Ecol. Sociobiol.*, 32, 167-176.
- Moorcroft, P. & Lewis, M. (2006). *Mechanistic Home Range Analysis*. Princeton University Press, Princeton.
- Morton, E.S. (1975). Ecological Sources of Selection on Avian Sounds. *Am. Nat.*, 109, 17–34.

- Müller-Schwarze, D. (2006). *Chemical Ecology of Vertebrates*. Cambridge University Press, Cambridge.
- Müller-Schwarze, D. & Silverstein, R.M. (1980). *Chemical signals: vertebrates and aquatic invertebrates*. Plenum Press, New York.
- Ng, J., Landeen, E.L., Logsdon, R.M. & Glor, R.E. (2013). Correlation between anolis lizard dewlap phenotype and environmental variation indicates adaptive divergence of a signal important to sexual selection and species recognition. *Evolution*, 67, 573–582.
- Nicholls, J.A. & Goldizen, A.W. (2006). Habitat type and density influence vocal signal design in satin bowerbirds. *J. Anim. Ecol.*, 75, 549–558.
- Ord, T.J. & Martins, E.P. (2006). Tracing the origins of signal diversity in anole lizards: phylogenetic approaches to inferring the evolution of complex behaviour. *Anim. Behav.*, 71, 1411–1429.
- Oufiero, C.E., Gartner, G.E.A., Adolph, S.C. & Garland, T. (2011). Latitudinal and climatic variation in body size and dorsal scale counts in Sceloporus lizards: a phylogenetic perspective. *Evolution*, 65, 3590–3607.
- Paradis, E., Claude, J. & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20, 289–290.
- Pavlicev, M. & Mayer, W. (2009) Fast radiation of the subfamily Lacertinae (Reptilia: Lacertidae): History or methodical artefact? *Mol. Phyl. Evol.*, 52, 727–734.
- Perry, G., Lampl, I., Lerner, A., Rothenstein, D., Shani, E. & Sivan, N. 1990. Foraging mode in lacertid lizards: variation and correlates. *Amphibia-Reptilia* 11, 373–384.

- Plath, M., Parzefall, J., Körner, K.E. & Schlupp, I. (2004). Sexual selection in darkness? Female mating preferences in surface- and cave-dwelling Atlantic mollies, *Poecilia mexicana* (Poeciliidae, Teleostei). *Behav. Ecol. Sociobiol.*, 55, 596–601.
- Pianka, E.R., Huey, R.B. & Lawlor, L.R. (1979). Niche segregation in desert lizards. In: *Analysis of Ecological Systems* (eds. D.J. Horn, D.J., Mitchell, R. & G.R. Stairs, G.R.) Ohio State University Press, Columbus. pp. 67-115.
- Pruett, J.A., Zúñiga-Vega, J.J., Campos, S.M., Soini, H.A., Novotny, M. V., Vital-García, C., Martins, E.P. & Hews, D.K. (2016) Evolutionary interactions between visual and chemical signals: chemosignals compensate for the loss of a visual signal in male *Sceloporus* lizards. *J. Chem. Ecol.*, 42, 1164–1174.
- Pyron, R., Burbrink, F. & Wiens, J. (2013). A phylogeny and revised classification of Squamata , including 4161 species of lizards and snakes. *BMC Evol. Biol.*, 13, 1–53.
- R Core Team. 2013. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available at: <http://www.R-project.org>
- Rambaut, A. & Drummond, A.J. (2007). Tracer version 1.5. Computer program and documentation distributed by author. Available at: <http://beast.bio.ed.ac.uk/Tracer>
- Rantala, M.J., Kortet, R., Kotiaho, J.S., Vainikka, A. & Suhonen, J. (2003). Condition dependence of pheromones and immune function in the grain beetle *Tenebrio molitor*. *Funct. Ecol.*, 17, 534-540.
- Rasmussen, L.E., Lee, T.D., Zhang, a, Roelofs, W.L. & Daves Jr., G.D. (1997). Purification, identification, concentration and bioactivity of (Z)-7-dodecen-1-

- yl acetate: sex pheromone of the female Asian elephant, *Elephas maximus*. *Chem Senses*, 22, 417–437.
- Regnier, F.E. & Goodwin, M. (1977) On the chemical and environmental modulation of pheromone release from vertebrate scent marks. In: *Chemical Signals in Vertebrates* (eds Müller-Schwarze, D. & Mozell, M.M.). Plenum Press, London, pp.115-149.
- Revell, L.J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.*, 3, 217–223.
- Revell, L.J. (2013). Two new graphical methods for mapping trait evolution on phylogenies. *Methods Ecol. Evol.*, 4, 754–759.
- Revell, L.J. & Harrison, A.S. (2008). PCCA: A program for phylogenetic canonical correlation analysis. *Bioinformatics*, 24, 1018–1020.
- Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., *et al.* (2012). Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.*, 61, 539–542.
- Rouault, J.D., Marican, C., Wicker-Thomas, C. & Jallon, J.M. (2004). Relations between cuticular hydrocarbon (HC) polymorphism, resistance against desiccation and breeding temperature; a model for HC evolution in *D. melanogaster* and *D. simulans*. *Genetica*, 120, 195–212.
- Rundle, H.D., Chenoweth, S.F., Doughty, P. & Blows, M.W. (2005). Divergent selection and the evolution of signal traits and mating preferences. *PLoS Biol.*, 3, 1988–1995.
- Runemark, A., Gabirot, M. & Svensson, E.I. (2011). Population divergence in chemical signals and the potential for premating isolation between islet- and

- mainland populations of the Skyros wall lizard (*Podarcis gaigeae*). *J. Evol. Biol.*, 24, 795–809.
- Saxena, P., Patel, J.C. & Joshipura, M.H. (2013). Prediction of vapor pressure of fatty acid methyl esters. *Procedia Eng.*, 51, 403–408.
- Sayers, E.W., Barrett, T., Benson, D.A., Bryant, S.H., Canese, K., Chetvernin., V., *et al.* (2009). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res.*, 37, 5–15
- Searcy, W.A. & Nowicki, S. (2005). *The Evolution of Animal Communication: Reliability and Deception in Signalling Systems*. Princeton University Press, Princeton.
- Smith, H.M. (1946). *Handbook of Lizards: Lizards of the United States and of Canada*. Cornell University Press, New York.
- Steiger, S., Schmitt, T. & Schaefer, H.M. (2011). The origin and dynamic evolution of chemical information transfer. *Proc. R. Soc. Biol. Sci. Ser. B*, 278, 970–979.
- Stevens, M. (2013). *Sensory ecology, behaviour, and evolution*. Oxford University Press, Oxford.
- Symonds, M.R.E. & Elgar, M.A. (2008). The evolution of pheromone diversity. *Trends Ecol. Evol.*, 23, 220–228.
- Thomas, M.L. (2011). Detection of female mating status using chemical signals and cues. *Biol. Rev.*, 86, 1–13.
- Tibbetts, E.A., Mullen, S.P. & Dale, J. (2017) Signal function drives phenotypic and genetic diversity: the effects of signalling individual identity, quality or behavioural strategy. *Phil. Trans. R. Soc. B: Biol. Sci.*, **372**, 20160347.

- Tieleman, B.I., Williams, J.B. & Bloomer, P. (2003). Adaptation of metabolism and evaporative water loss along an aridity gradient. *Proc. R. Soc. B Biol. Sci.*, 270, 207–214.
- Tobias, M., Evans, B.J. & Kelley, D.B. (2011). Evolution of advertisement calls in African clawed frogs. *Behaviour*, 148, 519–549.
- Uetz, P. (editor), The Reptile Database, <http://www.reptile-database.org>, accessed May 6, 2017.
- Van Oudenhove, L., Billoir, E., Boulay, R., Bernstein, C. & Cerdá, X. (2011). Temperature limits trail following behaviour through pheromone decay in ants. *Naturwissenschaften*, 98, 1009–1017.
- Van Oudenhove, L., Boulay, R., Lenoir, A., Bernstein, C. & Cerdá, X. (2012). Substrate Temperature Constrains Recruitment and Trail Following Behavior in Ants. *J. Chem. Ecol.*, 38, 802–809.
- Vander Wall, S.B. (1998). Foraging Success of Granivorous Rodents: Effects of Variation in Seed and Soil Water on Olfaction. *Ecology*, 79, 233–241.
- Vervust, B. (2011). *Lizards on islands: a model system for studying fast phenotypic change*. Doctoral thesis, Laboratory of Functional Morphology, University of Antwerp, Belgium.
- Vinson, S.B. (1972). Courtship behavior and evidence for a sex pheromone in the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). *Environ. Entomol.*, 1, 409–414.
- Weber, M., Mitko, L., Eltz, T. & Ramirez, S. (2016). Macroevolution of perfume signalling in orchid bees. *Ecol. Lett.*, 19, 1314–1323.
- Weldon, P.J., Flachsbarth, B. & Schulz, S. (2008). Natural products from the integument of nonavian reptiles. *Nat. Prod. Rep.*, 25, 738–56

- Wilder, S.M., DeVito, J., Persons, M.H. & Rypstra, A.L. (2005). The effects of moisture and heat on the efficacy of chemical cues used in predator detection by the wolf spider *Pardosa milvina* (Araneae, Lycosidae). *J. Arachnol.*, 33, 857–861.
- Wyatt, T.D. (2010). Pheromones and signature mixtures: Defining species-wide signals and variable cues for identity in both invertebrates and vertebrates. *J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol.*, 196, 685–700.
- Wyatt, T.D. (2014). *Pheromones and Animal Behaviour. Chemical Signals and Signatures*. Cambridge University Press, Cambridge.
- Yanes-Marichal, N.R., Francisco-Sánchez, A.F. & Molina-Borja, M. (2017). Chemical discrimination of pesticide-treated grapes by lizards (*Gallotia galloti* palmae, Fam. Lacertidae). *Basic Appl. Herpetol.*, 0, 1–11.

Supporting Information

Appendix S1: Details on the GC-MS chemical analysis of the glandular secretions.

Appendix S2: Details on the reconstruction of the lacertid phylogenetic tree.

Appendix S3: Table and figure showing the species' canonical scores obtained through a phylogenetic canonical correlation analysis.

Table S1: Descriptive statistics for the environmental variables extracted from climate databases based on species' catch-locality.

Table S2: Main classes of lipophilic compounds and their relative amount found in the glandular secretions of 64 lacertid species.

Table S3: Phylogenetic signals for the chemical variables.

Table S4: Canonical loadings for the environmental and chemical variables.

Data deposited at Dryad: <https://doi.org/...>

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).

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*, *Algyroides nigropunctatus*, *Lacerta media*.

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

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 colouration in the representation due to their high proportion in lacertid secretion.

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).

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.