The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2015, **114**, 44–57. With 2 figures

Chemical signalling in lizards: an interspecific comparison of femoral pore numbers in Lacertidae

SIMON BAECKENS[1]*, SHELLEY EDWARDS[2,3], KATLEEN HUYGHE[1] and RAOUL VAN DAMME[1]

[1]*Department of Biology, Laboratory of Functional Morphology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium*
[2]*Centre for Invasion Biology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa*
[3]*Applied Biodiversity Research Division, South African National Biodiversity Institute, Private Bag X7, Claremont 7735, Cape Town, South Africa*

Received 29 May 2014; revised 29 August 2014; accepted for publication 29 August 2014

Animals communicate via a variety of sensory channels and signals. Studies on acoustic and visual communication systems suggest that differences in the physical environment contribute to the variety of signalling behaviour, with species investing in those signals that are transmitted best under the local conditions. Whether or not environmental tuning also occurs in chemical communication systems has received much less attention. In the present study, we examined the effect of several aspects of the physical environment on the chemical communication system of lacertid lizards (family Lacertidae). The numbers of femoral pores are used as a proxy reflecting how much a particular species invests in and relies upon chemical signalling. Femoral pores are specialized epidermal structures that function as a secretion channel for the waxy substance produced by glands. In some lacertid species, the secretion carries infochemicals that play an important role in social communication. The number of femoral pores varies considerably among species. We have compiled data on femoral pore numbers for 162 species and tested for the effects of climate and substrate use. After correcting for body size and taking the phylogenetic relationships among the species into account, we found no effect of climate conditions or latitude on species pore numbers. Substrate use did affect pore numbers: shrub-climbing species tended to have fewer femoral pores than species inhabiting other substrates. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **114**, 44–57.

ADDITIONAL KEYWORDS: climate conditions – communication – comparative methods – lacertid – latitude – olfaction – physical environment – secretion – semiochemical – substrate use.

INTRODUCTION

Animals communicate with conspecifics and other species via a wide variety of signals and displays. The selection pressures and developmental constraints that have driven the evolution of the diversity in sending and receiving systems are the subject of continued research (Bradbury & Vehrencamp, 1998; Espmark, Amundsen & Rosenqvist, 2000; Greenfield, 2002; Rogers & Kaplan, 2002; Smith & Harper, 2003; Searcy & Nowicki, 2005). The effects of the physical environment on the evolution of particular sensory channels and signals within sensory channels have been studied extensively for acoustic and visual communication systems (Morton, 1975; Kroodsma & Miller, 1982; Endler, 1990; Gerhardt & Huber, 2002; Barnard, 2004; Kekäläinen *et al.*, 2010). Far less is known about how the physical environment affects the evolution of chemical communication, despite the fact that 'infochemicals' (Dicke & Sabelis, 1988) are used by a vast number of species in a variety of aquatic and terrestrial habitats (Müller-Schwarze & Silverstein, 1980; Müller-Schwarze, 2006; Starnberger *et al.*, 2013). Theoretical work suggests that the efficacy of particular chemical signals will depend strongly on environmental conditions (i.e. temperature, humidity,

---

*Corresponding author.
E-mail: simon.baeckens@uantwerp.be

barometric pressure, air currents; Bossert & Wilson, 1963; Moore & Crimaldi, 2004) and therefore it can be expected that varying selection along an environmental gradient (Darwin, 1859), acting to maximize transmission efficiency, will contribute to chemical signalling diversity (Alberts, 1992). Surprisingly few studies have sought empirical support for this idea; but see Escobar *et al*. (2003) and Pincheira-Donoso, Hodgson & Tregenza (2008).

Squamate reptiles (lizards and snakes) utilize a variety of sensory systems, including visual, acoustic, tactile, and gustatory systems (Vitt & Caldwell, 2014). They also rely strongly on their ability to perceive chemicals from the environment for a variety of daily activities including prey detection (Amo, López & Martín, 2004), predator avoidance (Van Damme *et al*., 1995; Aragon, López & Martín, 2008), mate recognition (Cooper & Pérez-Mellado; 2002; Moreira, López & Martín, 2006), and overall social behaviour (Mason & Parker, 2010). Squamates are equipped with a variety of glands (Quay, 1972) with which they produce and send out diverse chemical messages (Martín & López, 2011). The femoral glands situated in the epidermis of the inner thighs of many lizards are probably the best studied (Alberts, 1990; Weldon *et al*., 1990; Aragon, López & Martín, 2001; Louw *et al*., 2007; López & Martín, 2009). Those glands produce a holocrine secretion that finds its way to the external world through epidermal structures: the 'femoral pores' (Fig. 1) (Mason, 1992). Pores are often larger in males than in females (Arnold & Ovenden, 2004) and the amount of pores present on each thigh, as well as the general scale morphology of the pores, varies among species (Mason, 1992; Van Wyk, Mouton & Ie, 1992). Consequently, femoral pores have been used extensively in the past in identification keys and taxonomic descriptions in lizard species.

Figure 1. A row of femoral pores on the ventral surface of an adult male *Acanthodactylus schreiberi*.

The lizard family Lacertidae constitutes an excellent model for studying the effects of the physical environment on the evolution of chemical communication, with more than 300 species inhabiting a wide variety of habitats distributed over much of the Old World (Branch, 1998; Arnold & Ovenden, 2004; Spawls, Howell & Drewes, 2006; Das, 2010). Although most lacertid lizards share the same general morphology and also many aspects of their ecology (i.e. most are diurnal, heliothermic species that actively forage for invertebrate prey: Castilla, Van Damme & Bauwens, 1999; Van Damme, 1999; Vitt & Pianka, 2007), they have successfully radiated into a wide array of habitats and climate regions, from subarctic tundra over temperate heath lands and forests, alpine meadows and Mediterranean maquis, steppe and gravel semi-deserts, and monsoonal rainforest to sandy dune systems in the desert (Arnold, 1989; Harris, Arnold & Thomas, 1998; Harris *et al*., 2002). Within these habitats, they utilize a great diversity of microhabitats and substrates, ranging from herby vegetation over stony undergrounds to shifting sands (Vitt & Caldwell, 2014). Other species climb extensively in shrubs or trees (Vanhooydonck & Van Damme, 1999; Vanhooydonck *et al*., 2009). Additionally, our preliminary inquiries suggested considerable among species variation in femoral pore number. In the present study, we explored whether variation in pore number co-varies with environmental traits. In addition, the results obtained will allow us to make predictions about potentially evolutionary processes imposed by the respective physical environments.

We investigated the among-species diversity of a component of the chemical communication apparatus (i.e. the number of femoral pores). We tested the hypothesis that the number of pores varies among species living on different substrates and in dissimilar climatic conditions. The durability of an infochemical is an important aspect in chemical communication. If the signals do not last for a sufficiently long time in their environment, lizards may need to increase their effectiveness to maintain their functionality (Iraeta *et al*., 2011). Enhancing the overall production of infochemicals by increasing the number (or size) of femoral pores is, besides adjusting the composition of the secretion *per se*, a way of coping with environmental challenges that decrease the chemical signal durability (Escobar, Labra & Niemeyer, 2001; Escobar *et al*., 2003; Iraeta *et al*., 2011). We therefore assume that femoral pore number reflects an investment in and the use of chemical communication (Escobar *et al*., 2001; Pincheira-Donoso *et al*., 2008). The idea of compensating for signalling inefficiency (as a result of external factors) by increasing the investment in other communication signals or signalling apparatus within the same sensory channel is referred to in the present study as the ‘within-channel compensation hypothesis’. Chemical signalling is likely to be more challenging for species living in complex, dynamic, ephemeral habitats (e.g. flimsy, moist vegetation and shrubs), on adsorptive substrates (vegetation and sand versus rock), or in warm and humid climate conditions, as a result of the lower detectability and/or signal persistence (Alberts, 1992; Escobar *et al*., 2003; Pincheira-Donoso *et al*., 2008). We therefore predict (in line with the ‘within-channel compensation hypothesis’) that species living in such environments will tend to have more femoral pores, enhancing the overall production of secretion. The latter will consequently increase the longevity of the infochemical and thus ensuring the functionality of the signal.

MATERIAL AND METHODS

Morphological analysis

We extracted data on body size and the number of femoral pores of 162 species of the family Lacertidae (Squamata: Sauria) from the literature (see Supporting information, Table S1). We recorded the mean number of femoral pores of the right thigh because this is most often reported in the literature. Our data set covers all of the genera and approximately 52% of all the lacertid species. The largest snout–vent length (SVL) recorded per species was noted, as well as the mean SVL for adult males.

Climate regions, latitude, and substrate use

Based on information available in field guides and the primary literature (see Supporting information, Table S1), each species was assigned to one of six substrate use classes: (1) sandy = species of loose sandy substrate with no or scarce vegetation; (2) rocky = species living primarily on rocky substrate; (3) low-vegetation = ground-dwelling species living on low, grassy vegetation; (4) high-vegetation = shrub-climbing species; (5) arboreal = species living mainly on tree trunks and in tree canopies, moving from tree-to-tree by gliding; or (6) generalist = species occurring on a variety of substrates.

Data on the species’ geographical distribution was taken from herpetological field guides (Schleich, Kastle & Kabisch, 1996; Branch, 1998; Disi *et al*., 2001; Arnold & Ovenden, 2004; Spawls *et al*., 2006; Das, 2010), as well as from the website of the *Arbeitsgemeinschaft Lacertiden* of the German Society of Herpetology and Herpetoculture (DGHT, http://lacerta.de). Each species was assigned to one climate region by comparing its geographical distribution to an updated digital version of the Köppen–Geiger World Climates Map (Kottek *et al*., 2006). The Köppen–Geiger system (Köppen, 1900) first classifies regions according to their native vegetation into (A) the equatorial zone, (B) the arid zone, (C) the warm temperate zone, and (D) the snow zone (Table 1). Within these four primary zones, regions are defined

Table 1. Key to climate categories, adapted from the Köppen–Geiger Climate System (Kottek *et al*., 2006): description of the first two letters of the classification system (i.e. main climate category and precipitation levels)

Type	Description	Criterion
A	Equatorial climates	$T_{\min} \geq +18$ °C
Af	Equatorial rainforest, fully humid	$P_{\min} \geq 60$ mm
Am	Equatorial monsoon	$P_{\text{ann}} \geq 25\ (100 - P_{\min})$
Aw	Equatorial savannah with dry winter	$P_{\min} < 60$ mm in winter
B	Arid climates	$P_{\text{ann}} < 10\ P_{\text{th}}$
BS	Steppe climate	$P_{\text{ann}} > 5\ P_{\text{th}}$
BW	Desert climate	$P_{\text{ann}} \leq 5\ P_{\text{th}}$
C	Warm temperate climates	-3 °C $< T_{\min} < +18$ °C
Cs	Warm temperate climate with dry summer	$P_{\text{smin}} < P_{\text{wmin}}$; $P_{\text{wmax}} > 3\ P_{\text{smin}}$ and $P_{\text{smin}} < 40$ mm
Cw	Warm temperate climate with dry winter	$P_{\text{wmin}} < P_{\text{smin}}$ and $P_{\text{smax}} > 10\ P_{\text{wmin}}$
Cf	Warm temperate, fully humid	Neither Cs, nor Cw
D	Snow climates	$T_{\min} \leq -3$ °C
Ds	Snow climate with dry summer	$P_{\text{smin}} < P_{\text{wmin}}$; $P_{\text{wmax}} > 3\ P_{\text{smin}}$ and $P_{\text{smin}} < 40$ mm
Dw	Snow climate with dry winter	$P_{\text{wmin}} < P_{\text{smin}}$ and $P_{\text{smax}} > 10\ P_{\text{wmin}}$
Df	Snow climate, fully humid	Neither Ds, nor Dw

T_{ann}, annual mean near-surface (2 m) temperature; $T_{\max}$, monthly mean temperatures of the warmest month; $T_{\min}$, monthly mean temperatures of the coldest month; P_{ann}, accumulated annual precipitation; $P_{\min}$, precipitation of the driest month; P_{smin}, lowest monthly precipitation for the summer; P_{smax}, highest monthly precipitation for the summer; P_{wmin}, lowest monthly precipitation for the winter; P_{wmax}, highest monthly precipitation for the winter. All temperatures are given in °C, monthly precipitations in mm month^{-1}, and P_{ann} in mm year^{-1}.

Table 2. Key to third letter of the climate categories, adapted from the Köppen–Geiger climate system (Kottek *et al*., 2006)

Type	Description	Criterion
H	Hot steppe/desert	$T_{\mathrm{ann}} \geq +18$ °C
K	Cold steppe/desert	$T_{\mathrm{ann}} < +18$ °C
A	Hot summer	$T_{\max} \geq +22$ °C
B	Warm summer	Not (a) and at least 4 $T_{\mathrm{mon}} \geq +10$ °C
C	Cool summer and cold winter	Not (b) and $T_{\min} > -38$ °C
d	Extremely continental	Like (c) but $T_{\min} \leq -38$ °C

Criterion abbreviations are as in Table 1.

according to precipitation levels (e.g. 'Cf' for warm temperate and fully humid) and air temperature (e.g. 'Cfa' for warm temperate, fully humid and hot summer) (Table 2). Species were assigned to the Köppen–Geiger climate region that contains the greater part of their distribution range.

The mean latitude of the distribution area of each lacertid species was estimated by taking the midpoint between the most northern and the most southern distribution point. By overlapping their distribution area with a world map (using Google Earth ©2013, version 7.1.1.1888; http://earth.google.com), we obtained an approximate estimate of the latitude of the centre of their geographical North–South distribution. We distinguish between 'latitude' and 'absolute latitude'. Latitude varies from −90° to +90°, whereas absolute latitude varies from 0° to 90°. The former variable describes a north-south gradient, the latter describes seasonal day length.

PHYLOGENETIC ANALYSIS

We used sequences obtained from GenBank (http://ncbi.nlm.nih.gov) to estimate phylogenetic relationships among the species in our femoral pore dataset. We focused on five gene regions that were effective in elucidating among-species relationships in previous analyses (Edwards *et al*., 2012, 2013; Pyron, Burbrink & Wiens, 2013): three mitochondrial regions (12S, 16S, cytochrome *b*) and two nuclear gene regions (RAG-1 and C-MOS). Species were retained in the analysis if at least two of these five regions had been sequenced. We then constructed a tree with a total of 162 tip species, representing all the genera sampled. Details of the species used in the phylogenetic analyses and the associated GenBank accession numbers for each gene region are provided in the Supporting information (Table S2).

Bayesian inference was performed with uniform priors for all parameters using MrBayes, version 3.1.2; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) at the CIPRES Science Gateway (http://www.phylo.org/sub_sections/portal/). Each gene region was partitioned separately, and the evolutionary model chosen for all gene regions was GTR + I + G (JMODELTEST; Guindon & Gascuel, 2003; Darriba *et al*., 2012). Two parallel runs for 20×10^6 generations each were performed for the Markov chain Monte Carlo runs, with trees sampled every 1000 generations. The number of generations to discard as burn-in (4.0×10^6 generations) was determined by examining the number of generations: (1) at which the standard deviation of split frequencies stabilized (at less than 0.001); (2) at which the log-likelihood tree scores reached stationary; and (3) the effective sample sizes of all parameters were > 100 (TRACER, version 1.5; Rambaut & Drummond, 2007). A 50% majority rule tree was constructed with the burn-in excluded using the 'sumt' command in MrBayes, and nodes with ≥ 0.95 posterior probability were considered supported.

STATISTICAL ANALYSIS

A strong phylogenetic signal was detected in most of the variables considered in the present study and therefore analyzing the data in an explicit phylogenetic context appears to be indispensable (Harvey & Pagel, 1991; Schwenk, 1993; Nunn, 2011). We used both traditional and phylogenetic analyses to examine how the number of femoral pores varies with SVL and environmental conditions, although we only present the result of the phylogenetic tests. All statistical analyses were conducted in R STUDIO, version 0.97.248 (R Core Team, 2012; R Studio, 2012). $P < 0.05$ was considered statistically significant.

We estimated the phylogenetic signal in our data by calculating Pagel's λ for discrete variables and Blomberg's K for continuous variables. Pagel's λ was estimated by maximum likelihood optimization using the 'fitdiscrete' function in the R-package 'Geiger' (Harmon *et al*., 2008). A λ value close to 0 indicates no phylogenetic structure in the trait, whereas a λ value close to 1 corresponds with the original, untransformed branch lengths (Pagel, 1999). Estimates of the K-statistic were obtained with the 'Kcalc' command in the R-package 'picante' (Kembel *et al*., 2010). $K < 1$ implies that relatives resemble each other less than expected under Brownian motion evolution along the hypothesized tree. $K > 1$ implies that close relatives are more similar than expected under Brownian motion evolution (stronger signal; Blomberg, Garland & Ives, 2003).

Absolute femoral pore number was significantly correlated with mean SVL ($r = 0.32$, d.f. = 160,

$t = 4.34$, $P < 0.005$). Therefore, in all further analyses, we used the residuals extracted from the regression of pore number on mean SVL, referred to hereon as the 'residual pore number'. Mean SVL was used as a measure of body size because this was the variable most often reported in the literature. We also have information on the maximal SVL reported per species, another size variable commonly used in comparative studies. However, because maximal SVL is probably more sensitive to sample size (Stamps & Andrews, 1992; Meiri, 2007) and because our dependent variable is also an average, we chose to use mean SVL. The two body size measures are highly correlated in our dataset ($r = 0.87$, d.f. = 160, $P < 0.005$). We used the phyl.resid() function in R to achieve phylogenetic size correction of femoral pore numbers (Revell, 2009). We fitted the model assuming two different error structures. The first (BM) assumed simple Brownian motion evolution along the hypothesized tree; the second (λ) used a 'lambda' error structure, as suggested by Pagel (1999). Because the respective residuals were highly correlated ($r = 0.99$, $P < 0.005$), we only report the results of the analyses using the phylogenetic residuals obtained with the BM error structure.

Phylogenetic analyses of variances were used to test the differences in residual pore number among species inhabiting different substrate and Köppen–Geiger climate classes using the function phylANOVA() in the R-package phytools (Revell, 2012). The function creates virtual data vectors by simulating evolution along the hypothesized tree and performs an analysis of variance on each of the data sets. The resulting F-values are used to construct an empirical F-distribution, against which the real F-value can be compared (Garland *et al*., 1993). Post-hoc pairwise differences were evaluated by comparing empirical t-values with the t-values obtained for each simulated data vector. We used sequential-Bonferroni tests to correct for multiple testing (p.adj = 'holm'; Revell, 2012).

The relationship between residual pore numbers and latitude was evaluated using phylogenetic generalized least-squares regression analysis (PGLS), using a covariance matrix based on a Brownian and an Ornstein–Uhlenbeck motion model of evolution (R-package 'nlme', functions: 'gls', 'corBrownian and 'corMartin').

RESULTS

PHYLOGENETIC RELATIONSHIPS

We found strong branch support for the subfamilies Gallotiinae and Lacertinae (Fig. 2), as reported in previous studies (Mayer & Pavlicev, 2007; Kapli *et al*., 2011; Pyron *et al*., 2013). The monophyly of most genera within the tribes Lacertini and Eremiadini was also well supported, although the relationship between genera within the Lacertini remained unresolved, as in previous studies (Fu, 2000; Arnold, Arribas & Carranza, 2007; Mayer & Pavlicev, 2007; Pavlicev & Mayer, 2009; Kapli *et al*., 2011; Pyron *et al*., 2013). The reason why we were able to resolve the among-genera relationships in greater detail in the Eremiadini than in the Lacertini is most likely because nuclear gene sequences are available for Eremiadini species (Edwards *et al*., 2012, 2013; Engleder *et al*., 2012). We expect that additional information on nuclear genes in Lacertini species will allow better resolution of the relationships among genera in that clade.

Overall, our tree of the lacertid family corroborates many previously reported inter- and intrageneric relationships (Pyron *et al*., 2013), although a few details are noteworthy. The tribe *Eremiadini* consists of *Atlantolacerta andreanskyi* and two derived clades that are geographically disparate: the almost strictly Ethiopian (i.e. African south of the Saharan desert) genera and the predominantly Saharo-Eurasian genera (Mayer & Pavlicev, 2007). By contrast to earlier studies, the genus *Nucras* is now monophyletic, probably as a result of improved sampling (in contrast to Pyron *et al*., 2013). *Vhembelacerta* is recovered again as a monotypic genus, rendering *Australolacerta* as monotypic, and *Meroles squamulosus*, previously in *Ichnotropis*, is also nested in *Meroles* (*sensu* Engleder *et al*., 2012; Edwards *et al*., 2013). *Acanthodactylus* consists of three well-supported lineages, and further sampling within this genus will likely clarify the taxonomic level status of the lineages.

CLIMATE REGIONS, LATITUDE, AND SUBSTRATE USE

Descriptive statistics for climate regions and substrate use of lacertid pore number are provided in Table 3.

Estimates of the amount of phylogenetic signal in the variables under study are presented in Table 5. The signal is very strong for all environmental

Figure 2. Phylogenetic relationships for 162 species of the lizard family Lacertidae (all genera are included), estimated using a Bayesian analysis. Posterior probabilities > 0.95 are considered supported (shown at the nodes). The species' branch colour refers to the substrate usage of that species: sandy (yellow); rocky (marine blue); low-vegetation (turquoise); high-vegetation (dark green); arboreal (pink); generalist (red). (Colour version of figure available online.)

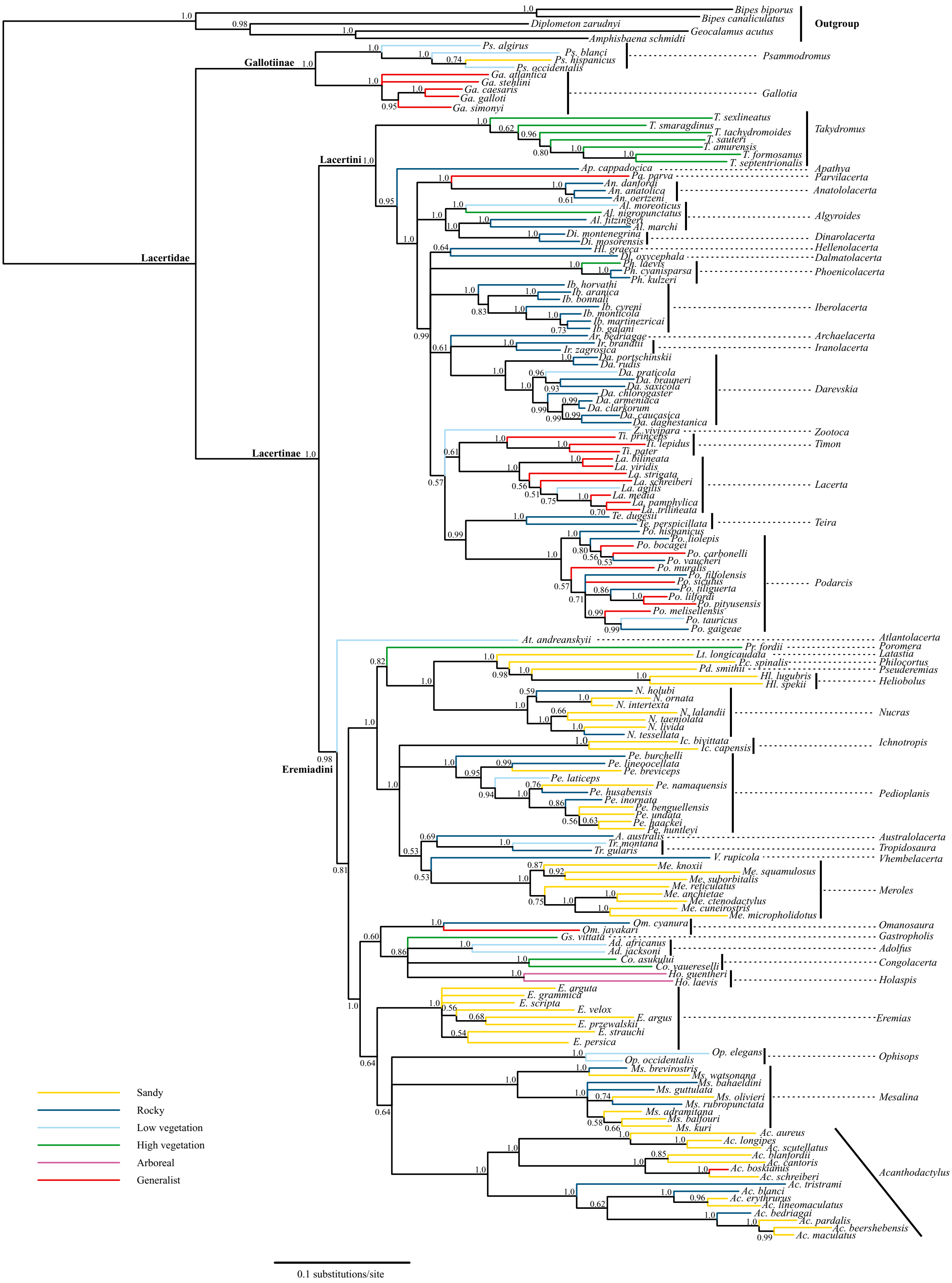
Bipes biporus
Bipes canaliculatus
Diplometon zarudnyi
Geocalamus acutus
Amphisbaena schmidti
Outgroup
Ps. algirus
Ps. blanci
Ps. hispanicus
Ps. occidentalis
Psammodromus
Gallotiinae
Ga. atlantica
Ga. stehlini
Ga. caesaris
Ga. galloti
Ga. simonyi
Gallotia
T. sexlineatus
T. smaragdinus
T. tachydromoides
T. sauteri
T. amurensis
T. formosanus
T. septentrionalis
Takydromus
Lacertini
Ap. cappadocica
Apathya
Pa. parva
Parvilacerta
An. danfordi
An. anatolica
An. oertzeni
Anatololacerta
Al. moreoticus
Al. nigropunctatus
Al. fitzingeri
Al. marchi
Algyroides
Di. montenegrina
Di. mosorensis
Dinarolacerta
Hl. graeca
Hellenolacerta
Dl. oxycephala
Dalmatolacerta
Ph. laevis
Ph. cyanisparsa
Ph. kulzeri
Phoenicolacerta
Lacertidae
Ib. horvathi
Ib. aranica
Ib. bonnali
Ib. cyreni
Ib. monticola
Ib. martinezricai
Ib. galani
Iberolacerta
Ar. bedriagae
Archaelacerta
Ir. brandtii
Ir. zagrosica
Iranolacerta
Da. portschinskii
Da. rudis
Da. praticola
Da. brauneri
Da. saxicola
Da. chlorogaster
Da. armeniaca
Da. clarkorum
Da. caucasica
Da. daghestanica
Darevskia
Z. vivipara
Zootoca
Ti. princeps
Ti. lepidus
Ti. pater
Timon
Lacertinae
La. bilineata
La. viridis
La. strigata
La. schreiberi
La. agilis
La. media
La. pamphylica
La. trilineata
Lacerta
Te. dugesii
Te. perspicillata
Teira
Po. hispanicus
Po. liolepis
Po. bocagei
Po. carbonelli
Po. vaucheri
Po. muralis
Po. filfolensis
Po. siculus
Po. tiliguerta
Po. lilfordi
Po. pityusensis
Po. melisellensis
Po. tauricus
Po. gaigeae
Podarcis
At. andreanskyii
Atlantolacerta
Pr. fordii
Poromera
Lt. longicaudata
Latastia
Pc. spinalis
Philocortus
Pd. smithii
Pseuderemias
Hl. lugubris
Hl. spekii
Heliobolus
N. holubi
N. ornata
N. intertexta
N. lalandii
N. taeniolata
N. livida
N. tessellata
Nucras
Ic. bivittata
Ic. capensis
Ichnotropis
Eremiadini
Pe. burchelli
Pe. lineoocellata
Pe. breviceps
Pe. laticeps
Pe. namaquensis
Pe. husabensis
Pe. inornata
Pe. benguellensis
Pe. undata
Pe. haackei
Pe. huntleyi
Pedioplanis
A. australis
Australolacerta
Tr. montana
Tr. gularis
Tropidosaura
V. rupicola
Vhembelacerta
Me. knoxii
Me. squamulosus
Me. suborbitalis
Me. reticulatus
Me. anchietae
Me. ctenodactylus
Me. cuneirostris
Me. microphholidotus
Meroles
Om. cyanura
Om. jayakari
Omanosaura
Gs. vittata
Gastropholis
Ad. africanus
Ad. jacksoni
Adolfus
Co. asukului
Co. vauereselli
Congolacerta
Ho. guentheri
Ho. laevis
Holaspis
E. arguta
E. grammica
E. scripta
E. velox
E. argus
E. przewalskii
E. strauchi
E. persica
Eremias
Op. elegans
Op. occidentalis
Ophisops
Ms. brevirostris
Ms. watsonana
Ms. bahaeldini
Ms. guttulata
Ms. olivieri
Ms. rubropunctata
Ms. adramitana
Ms. balfouri
Ms. kuri
Mesalina
Ac. aureus
Ac. longipes
Ac. scutellatus
Ac. blanfordi
Ac. cantoris
Ac. boskianus
Ac. schreiberi
Ac. tristrami
Ac. blanci
Ac. erythrurus
Ac. lineomaculatus
Ac. bedriagai
Ac. pardalis
Ac. beershebensis
Ac. maculatus
Acanthodactylus
Sandy
Rocky
Low vegetation
High vegetation
Arboreal
Generalist
0.1 substitutions/site

Table 3. Descriptive statistics (means, SEs, sample sizes, minima and maxima) for substrate class and climate class of lacertid absolute mean femoral pore numbers

Factor	$\bar{x}$	SE	*N*	Minimum	Maximum
Substrate class					
Arboreal	21.25	1.25	2	20.0	22.5
Generalist	20.06	1.00	25	13.5	21.0
High vegetation	7.27	1.90	13	1.0	20.0
Low vegetation	13.00	0.91	15	6.0	18.5
Rocky	17.98	0.48	55	10.5	25.0
Sandy	16.36	0.72	52	0.0	32.0
Main climate class					
Equatorial (A)	13.78	2.09	9	1.5	22.5
Arid (B)	16.77	0.70	56	0.0	32.0
Warm temperate (C)	16.76	0.62	89	1.0	31.0
Snow (D)	14.81	1.98	8	3.0	19.0
Precipitation					
Fully humid (f)	13.27	1.09	35	1.0	24.0
Summer dry (s)	18.81	0.60	53	8.0	27.5
Steppe (S)	16.70	1.09	13	10.5	21.0
Winter dry (w)	15.55	1.26	22	3.0	31.0
Desert (W)	16.73	0.90	39	0.0	32.0
Temperature					
Hot summer (a)	17.38	0.79	87	1.0	31.0
Warm summer (b)	15.21	1.00	34	1.0	27.5
Cool summer (c)	17.08	1.46	6	10.0	19.5
Hot arid (h)	16.74	0.93	34	0.0	27.5
Cold arid (k)	16.82	1.08	22	10.0	32.0

Table 4. Results of phylogenetic analyses showing the mean difference in residual pore numbers between classes of species' substrate usage

Substrate class	Generalist	High vegetation	Low vegetation	Rocky	Sandy
Arboreal	1.40	4.32	2.54	1.22	1.71
Generalist	–	**6.59**	2.70	−0.63	0.85
High vegetation	–	–	−3.62	**−7.80**	**−6.60**
Low vegetation	–	–	–	−3.55	−2.30
Rocky	–	–	–	–	1.85

Bold indicates statistical significance ($P < 0.05$).

variables, as well as morphological traits, pore number, and SVL.

When accounting for phylogenetic relationships among species in our dataset, we found no effect of the first main climate region (phylANOVA, $F = 1.39$, d.f. = 3, $P = 0.76$), precipitation (phylANOVA, $F = 5.93$, d.f. = 4, $P = 0.42$) or temperature category (phylANOVA, $F = 1.23$, d.f. = 4, $P = 0.74$) on mean residual pore number. However, the overall effect of substrate class is significant (phylANOVA, $F = 16.25$, d.f. = 5, $P = 0.012$, Table 4). Species from 'high-vegetation' microhabitats on average had lower residual pore numbers than 'arboreal species (post-hoc, difference = 4.32, $P = 0.039$), species living on 'rocky' substrates (difference = 7.80, $P = 0.015$), species from 'sandy' areas (difference = 6.60, $P = 0.048$), and 'generalist' species (difference = 6.59, $P = 0.028$). Phylogenetic tests do not suggest a difference between high- and low-vegetation substrates (difference = 3.62, $P = 0.330$). No significant correlation was found between residual pore numbers and latitude (PGLS, $r = -0.26$, d.f. = 160, $t = 0.15$, $P = 0.88$) and absolute latitude (PGLS, $r = -0.34$, d.f. = 160, $t = -0.50$, $P = 0.62$) based on a Brownian motion model of evolution. Similarly, no noteworthy significant correlation was found based on an Ornstein–Uhlenbeck motion model of evolution.

Table 5. The degree to which the environmental factor variables (climate, temperature, precipitation, and substrate usage) and continuous variables (latitude, mean male SVL, and mean pore number) exhibits phylogenetic signals, indicated by a significant Pagel's λ value (for factor variables) and Blomberg's *K* values (for continuous variables)

	λ	*K*
Climate	0.976	_
Temperature	0.928	_
Precipitation	0.990	_
Substrate usage	0.940	_
Latitude	_	3.64
SVL	_	0.64
Mean pore number	_	0.67

SVL, snout–vent length.

DISCUSSION

In many lizards, femoral pores function as an independent component of the lizards' chemical signalling system (Imparato, 2007). Our analysis of the literature revealed substantial among-species variation in the number of femoral pores in the family Lacertidae. In the present study, we aimed to investigate the hypothesis that selection underlying interspecific variation in pore numbers emerge from environmental factors affecting chemical signalling. Subsequently, our findings allow us to make inferences regarding the environmental factors that act as selective force driving this among-species variation in pore number. Differential investment in particular signalling systems can arise for two reasons. First, the relevance of sending out the message may vary among species. For example, we expect territorial species to invest more in scent marking than nonterritorial species (Becker, Petruno & Marler, 2012). Second, local environmental circumstances may hamper the transmission of certain signals, forcing some species to switch to a different communication channel (Endler, 1993; Hews & Benard, 2001; Stevens, 2013).

We find little support for the hypothesis that climate conditions co-vary with the diversification of femoral pore number across lacertid lizards. However, differences in substrate use may explain part of the variation in femoral pore numbers in lacertids.

Generally, climate conditions could affect species' investment in chemical communication (or in this specific case; femoral pore number) in different directions. First, and in line with the 'within-channel compensation hypothesis', warm, windy and humid conditions (which are assumed to reduce signal transmittance, pervasiveness, and persistence) may select for stronger signalling devices and more copious excretion. This would allow the lizards to bring across their message despite the difficult conditions. In accordance with this idea, Escobar *et al*. (2001) found that *Liolaemus* species living in warm, windy, and low-pressure habitats tend to have more precloacal pores than species that live in less harsh habitats. However this paper ignored the effects of shared ancestry. A subsequent study by Pincheira-Donoso *et al*. (2008) on the same genus (but with additional phylogenetic input) failed to identify any effect of environmental conditions on pore number. Pincheira-Donoso *et al*. (2008) explain the assessed variation in pore numbers in *Liolaemus* rather as a result of shared ancestry than a result of phylogenetic independent adaptive events. These findings emphasize the major importance of the incorporation of phylogenetic information in interspecific comparative studies. Second, if environmental conditions become too hostile, or the costs of producing chemicals too costly, lizards may trade in chemical communication for other communication channels (the 'between-channel compensation hypothesis'). This would result in an increase of investment in alternative signalling channels together with (1) equal or (2) decreasing investment in pore number, which would either (1) invalidate or (2) reverse the relationship between environmental conditions and femoral pore number. An example of a possible case of 'between-channel compensation' can be found in Atlantic mollies (*Poecilia mexicana*). In cave-dwelling populations of Atlantic mollies, male size cannot easily be determined visually, and so females have evolved the ability to recognize large males on the basis of chemical cues (Plath *et al*., 2004). Also the star-nosed mole (*Condylura cristata*) and the blind mole rat (*Spalax ehrenbergi*), both of which are species living underground, have reduced thalmocortical visual system and an expanded somatosensory representation (Cooper, Herbin & Nevo, 1993; Catania, 2005). Environmentally-induced switches between communication channels may also occur on different time scales. For example, individual male newts *Mesotriton alpestris* will use comparatively more olfactory than visual cues when courting in the dark than when courting in daytime (Danoël & Doellen, 2010). Guppies (*Poecila reticulata*) exhibit developmental sensory plasticity: individuals reared in the dark forage more efficiently when chemical cues are available, whereas individuals reared in full light find food faster on visual stimuli (Chapman *et al*., 2010).

By contrast to our predictions, phylogenetic analyses failed to identify a significant relationship between temperature, humidity, latitude, and femoral pore number in lacertids. Similar to that the findings reported by Pincheira-Donoso *et al*. (2008), each record of co-variation between climatologic factors and pore number vanishes when accounting for shared ancestry. Based on the strong phylogenetic signal of the climatic variables used in the present study, variation in pore number could be most parsimoniously explained by phylogenetic relatedness rather than the results of adaption to climatic conditions. Therefore, solely based on pore number diversity, we cannot argue that climatic conditions induce 'within-channel compensation' or 'between-channel compensation' in this lizard family. An alternative possibility is that other features of the chemical signalling system do differ among closely-related species, although this occurs without significant differentiation in the morphological expression of the femoral gland system (e.g. pore number) (Pincheira-Donoso *et al*., 2008). Numerous studies have shown that the chemical composition of femoral secretion can differ significantly between lizards of different age, sex, population, and species (Cole, 1966; Martín & López, 2006; Martins *et al*., 2006; López & Martín, 2009; Gabirot *et al*., 2010; Gabirot, López & Martín, 2011; Martín *et al*., 2013). Information on the chemical structure of secreted compounds could potentially unravel differences between lacertids living in regions with dissimilar climate conditions, which could strengthen the 'within-channel compensation' hypothesis.

The results of the present study suggest that substrate may be an environmental factor affecting the evolution of the chemosensory system of lacertids. Femoral pore numbers differed among species inhabiting different substrate classes, even after controlling for phylogenetic relationships. Several other studies have shown that characteristics of the substrate may affect the efficiency of signal transmission. For example, the seismic signals produced by male jumping spiders (*Habronattus dossenus*) to attract females are far less effective on sandy or rocky substrates than on leaf litter (Elias, Mason & Hoy, 2004). Similar results have been found in a wolf spider (*Schizocosa ocreata*), although males finding themselves on substrates that attenuated seismic signals used more visual signals (Gordon & Uetz, 2011), which is a possible case of 'between-channel compensation'.

Substrate type may also affect the efficiency of chemical signals themselves, especially for marks that are deposited directly on the surface and must communicate the identity of characteristics of a territory holder for as long as possible (Alberts, 1992). Regnier & Goodwin (1977) have demonstrated experimentally that the fade-out time of a chemical signal depends on its affinity for the substrate. For example, in their experiments, secretions applied to clay surfaces evaporated more slowly than those applied to wood surfaces. The fade out-times of chemical signals on sand, stones or vegetation (applied in the present study) have not been compared yet, although it is plausible that differences in affinity will prevail because of their distinctive physical nature. Additionally, the dynamics of the substrate could affect the volatility and fade-out time of the secretion as well. A highly dynamic substrate, such as sand dunes or shrubby vegetation, has a higher level of disturbance and a complex airflow character, both of which influence signal fade-out time (Müller-Schwarze, 2006). Scent marks deposited in dunes may be covered easily with sand, and infochemicals deposited on shrubby vegetation are subjected to a high level of disturbance because of their three-dimensional complexity, acting to decrease signal life.

The results of the present study also suggest that shrub-climbing lacertid species invest less in femoral pores than species living on any other substrate type. Studies have indicated that vegetation affects airflow patterns and may adsorb and re-emit molecules (Perry & Wall, 1984), and thus the scale (density, height, and species-dependent characteristics) of vegetation will affect communication patterns (Müller-Schwarze, 2006). Accordingly, when depositing chemical secretions onto a substrate at some height above the ground (e.g. shrubby vegetation), the active space of the infochemical increases by the addition of the vertical dimension (Alberts, 1992). Because of the developed three-dimensional spherical shape of the active space of the chemical signal, the longevity of the infochemical will decrease. Subsequently, an individual could respond to such environmental conditions by downsizing its investment in chemical communication and investing in more suitable communication systems. Natural selection could therefore act in favour of species with a low number of femoral pores, supporting the 'between communication channel' hypothesis. Data on investments in alternative signalling channels would enable us to draw more accurate conclusions.

Alternatively, a trade-off might exist between proficiency in chemical advertising (i.e. number of femoral pores) and climbing capacity (Iraeta *et al*., 2011). In species that climb extensively in vegetation, the need for shorter limbs would tip that balance towards shorter limbs, less space for glands, and fewer femoral pores. Biomechanical considerations suggest that climbing species should benefit from having shorter upper limbs because this would bring their centre of body mass closer to the substrate (Clemente *et al*., 2013), which would prevent them from 'toppling over'. In accordance, comparative studies have reported smaller limb lengths in climbing taxa (Sinervo & Losos, 1991; Vanhooydonck & Van Damme, 1999; Herrel, Meyers & Vanhooydonck, 2002; Herrel *et al*., 2008). With less space available on the inner thighs, selection for shorter hind limbs would result in fewer femoral pores as a by-product. We had no information available on limb length for many of the species in our dataset, and so we are unable to assess this alternative hypothesis here. However, Iraeta *et al*. (2011) found no significant correlation between pore number and hind limb length in *Psammodromus algirus* lizards. Interestingly, male hind limb length did correlate with mean pore size (another potentially important variable for which we currently have no information). As an argument against the idea that variation in pore numbers is a by-product of differential selection on hind limb length, rock-climbing species tend to have relatively large numbers of pores, whereas biomechanics and comparative studies would predict them to have short limbs for the same reasons as vegetation climbers.

Sexual selection as a driving force?

There is strong evidence that the holocrine excretions of the femoral glands of lacertids function primarily in territory demarcation and/or in mate choice (Martín & López, 2000; López, Aragon & Martín, 2003; Aragon *et al*., 2006; López, Amo & Martín, 2006; Font *et al*., 2012) and therefore it is likely that their production is under sexual selection. This is further supported by the fact that males tend to have larger and more femoral glands than females and that their secretional activity increases during the breeding season in response to rising levels of circulating androgens (Díaz, Alonso-Gómez & Delgado, 1994; Iraeta *et al*., 2011). Changes in the chemical composition of the secretion during the mating season have also been observed (Alberts *et al*., 1992). We therefore hypothesize that differences in femoral pore number among species could reflect variation in the intensity of sexual selection. Unfortunately, for most species in our dataset, detailed information on territoriality, male–male combat, and female choice is simply lacking. For this reason, we are reluctant to discard sexual selection as a factor influencing the evolution of femoral pore numbers.

Synthesis and future prospects

In summary, we find large among-species variation in femoral pore numbers in Lacertidae. Using phylogenetic comparative methods, the present study succeeds in finding a co-variation between the number of pores and species' substrate usage, whereas shrub-climbing species tend to have fewer femoral pores than species inhabiting other substrates. By contrast, no effect of climatic conditions (temperature and precipitation) or latitude on species' pore numbers was found. These results allow us to speculate about the potential role of the physical environment on the evolution of the chemical communication system in lacertid lizards.

We consider that the vast among-species variation in pore numbers invites further investigation. To address these shortcomings, more research needs to be undertaken to acquire comparative data on the size of femoral pores and their distribution on the limb, their rate of secretion, the physical characteristics of the chemicals produced, the chemical composition of the secretions, and their biological meaning. Studies on variation at the receptor side of the chemical communication channel (e.g. characteristics of the lingual delivery system, Jacobson's organ, and receptive areas in the brain) should also be encouraged.

ACKNOWLEDGEMENTS

Shelley Edwards was supported by NRF (National Research Foundation) of South Africa Free-standing and SABI Grant-holder linked bursaries, and the Erasmus Mundus (EuroSA) scholarship. Katleen Huyghe is a postdoctoral fellow of the FWO-Flanders. We thank the three anonymous reviewers for their helpful comments on earlier versions of the manuscript.

REFERENCES

Alberts AC. 1990. Chemical properties of femoral gland secretions in the desert iguana, *Dipsosaurus dorsalis*. *Journal of Chemical Ecology* **16:** 13–25.

Alberts AC. 1992. Constraints of the design of chemical communication systems in terrestrial vertebrates. *American Naturalist* **139:** 62–89.

Alberts AC, Sharp TR, Werner DI, Weldon PJ. 1992. Seasonal variation of lipids in femoral gland secretions of male Green Iguanas (*Iguana iguana*). *Journal of Chemical Ecology* **18:** 703–712.

Amo L, López P, Martín J. 2004. Wall lizards combine chemical and visual cues of ambush snake predators to avoid overestimating risk inside refuges. *Animal Behaviour* **67:** 647–653.

Aragon P, López P, Martín J. 2001. Discrimination of femoral gland secretions from familiar and unfamiliar conspecifics by male Iberian rock lizards, *Lacerta monticola*. *Journal of Herpetology* **35:** 346–350.

Aragon P, López P, Martín J. 2008. Increased predation risk modifies lizards' scent-mark chemicals. *Journal of Experimental Zoology Part A – Ecological Genetics and Physiology* **309A:** 427–433.

Aragon P, Massot M, Gasparini J, Clobert J. 2006. Socially acquired information from chemical cues in the common lizard, *Lacerta vivipara*. *Animal Behaviour* **72:** 965–974.

Arnold EN. 1989. Systematics and adaptive radiation of Equatorial African lizards assigned to the genera *Adolfus*, *Bedriagaia*, *Gastropholis*, *Holaspis* and *Lacerta* (Reptilia, Lacertidae). *Journal of Natural History* **23:** 525–555.

Arnold EN, Arribas O, Carranza S. 2007. Systematics of the Palaearctic and Oriental lizard tribe Lacertini (Squamata: Lacertidae: Lacertinae), with descriptions of eight new genera. *Zootaxa* **1430:** 1–86.

Arnold EN, Ovenden D. 2004. *A field guide to the reptiles and amphibians of Britain & Europe*. London: HarperCollins Publishers.

Barnard CJ. 2004. *Animal behaviour: mechanism, development, function and evolution*. Harlow: Pearson Education Limited.

Becker EA, Petruno S, Marler CA. 2012. A comparison of scent marking between a monogamous and promiscuous species of *Peromyscus*: pair boned males do not advertise to novel females. *PLoS ONE* **7:** e32002.

Blomberg SP, Garland T, Ives AR. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution: International Journal of Organic Evolution* **57:** 717–745.

Bossert WH, Wilson DO. 1963. The analysis of olfactory communication among animals. *Journal of Theoretical Biology* **5:** 443–469.

Bradbury JW, Vehrencamp SL. 1998. *Principles of animal communication*. Sunderland, MA: Sinauer Associates.

Branch B. 1998. *Field guide to snakes and other reptiles of Southern Africa*. Cape Town: Struik Publishers.

Castilla MA, Van Damme R, Bauwens D. 1999. Field body temperatures, mechanisms of thermoregulation and evolution of thermal characteristics in lacertid lizards. *Natura Croatica* **8:** 253–257.

Catania KC. 2005. Evolution of sensory specializations in insectivores. *Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology* **287:** 1038–1050.

Chapman BB, Morell LJ, Tosh CR, Krause J. 2010. Behavioural consequences of sensory plasticity in guppies. *Proceedings of the Royal Society of London Series B, Biological Sciences* **277:** 1395–1401.

Clemente CJ, Withers PC, Thompson GG, Lloyd D. 2013. Lizard tricks: overcoming conflicting requirements of speed versus climbing ability by altering biomechanics of the lizard stride. *Journal of Experimental Biology* **216:** 3854–3862.

Cole CJ. 1966. Femoral glands in lizards: a review. *Herpetologica* **22:** 119–206.

Cooper HM, Herbin M, Nevo E. 1993. Visual system of a naturally microphthalmic mammal: the blind mole rat, *Spalax ehrenbergi*. *Journal of Comparative Neurology* **328:** 313–350.

Cooper WE Jr, Pérez-Mellado V. 2002. Pheromonal discriminations of sex, reproductive condition, and species by the lacertid lizard *Podarcis hispanica*. *Journal of Experimental Zoology* **292:** 523–527.

Danoël M, Doellen J. 2010. Displaying in the dark: light-dependent alternative mating tactics in the Alpine newt. *Behavioural Ecology and Sociobiology* **64:** 1171–1177.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9:** 772.

Darwin C. 1859. *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle of life*. London: John Murray.

Das I. 2010. *A field guide to the reptiles of South-East Asia*. London: New Holland Publishers.

Díaz JA, Alonso-Gómez AL, Delgado MJ. 1994. Seasonal variation of gonadal development, sexual steroids, and lipid reserves in a population of the lizard *Psammodromus algirus*. *Journal of Herpetology* **28:** 199–205.

Dicke M, Sabelis MW. 1988. Infochemical terminology: based on cost-benefit analysis rather than origin of compounds? *Functional Ecology* **2:** 131–139.

Disi AM, Modry D, Neýas P, Rifai L. 2001. *Amphibians and reptiles of the Hashemite Kingdom of Jordan. An atlas and field guide*. Frankfurt: Chimaira.

Edwards S, Branch WR, Vanhooydonck B, Herrel A, Measey GJ, Tolley KA. 2013. Taxonomic adjustments in the systematics of the southern African lacertid lizards (Sauria: Lacertidae). *Zootaxa* **3669:** 101–114.

Edwards S, Vanhooydonck B, Herrel A, Measey GJ, Tolley KA. 2012. Convergent evolution associated with habitat decouples phenotype from phylogeny in a clade of lizards. *PLoS ONE* **7:** e51636.

Elias DO, Mason AC, Hoy RR. 2004. The effect of substrate on the efficacy of seismic courtship signal transmission in the jumping spider *Habronattus dossenus* (Araneae: Salticidae). *Journal of Experimental Biology* **207:** 4105–4110.

Endler JA. 1990. On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal of the Linnean Society* **41:** 315–352.

Endler JA. 1993. Some general comments on the evolution and design of animal communication systems. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **340:** 215–225.

Engleder A, Haring E, Kirchhof S, Mayer W. 2012. Multiple nuclear and mitochondrial DNA sequences provide new insights into the phylogeny of South African lacertids (Lacertidae, Eremiadinae). *Journal of Zoological Systematics and Evolutionary Research* **51:** 132–143.

Escobar CA, Labra A, Niemeyer HM. 2001. Chemical composition of precloacal secretions of *Liolaemus* lizards. *Journal of Chemical Ecology* **27:** 1677–1690.

Escobar CM, Escobar CA, Labra A, Niemeyer HM. 2003. Chemical composition of precloacal secretions of two *Liolaemus fabiani* populations: are they different? *Journal of Chemical Ecology* **29:** 629–638.

Espmark Y, Amundsen T, Rosenqvist G. 2000. *Animal signals: signalling and signal design in animal communication*. Trondheim: Tapir Forlag.

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. *General and Comparative Endocrinology* **177:** 9–17.

Fu J. 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, Castilla AM, López P, Martín J. 2010. Differences in chemical signals may explain species recognition between an island lizard *Podarcis atrata,* and related mainland lizards *P. hispanica*. *Biochemical Systematics and Ecology* **38:** 521–528.

Gabirot M, López P, Martín J. 2011. Interpopulational variation in chemosensory responses to selected steroids from femoral secretions of male lizards, *Podarcis hispanica*, mirrors population differences in chemical signals. *Chemoecology* **22:** 65–73.

Garland T Jr, Dickerman AW, Janis CM, Jonas JA. 1993. Phylogenetic analysis of covariance by computer simulation. *Systematic Biology* **42:** 265–292.

Gerhardt HC, Huber F. 2002. *Acoustic communication in insects and anurans*. Chicago, IL: University of Chicago Press.

Gordon SD, Uetz GW. 2011. Multimodal communication of wolf spiders on different substrates: evidence for behavioural plasticity. *Animal Behaviour* **81:** 367–375.

Greenfield MD. 2002. *Signallers and receivers: mechanisms and evolution of arthropod communication*. New York, NY: Oxford University Press.

Guindon S, Gascuel O. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* **52:** 696–704.

Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W. 2008. GEIGER: investigating evolutionary radiations. *Bioinformatics* **24:** 129–131.

Harris DJ, Arnold EN, Thomas RH. 1998. Rapid speciation, morphological evolution, and adaptation to extreme environments in South African sand lizards (*Meroles*) as revealed by mitochondrial gene sequences. *Molecular Phylogenetics and Evolution* **10:** 37–48.

Harris DJ, Carranza S, Arnold EN, Pinho C, Ferrand N. 2002. Complex biogeographical distribution of genetic variation within *Podarcis* wall lizards across the Strait of Gibraltar. *Journal of Biogeography* **29:** 1257–1262.

Harvey PH, Pagel MD. 1991. *The comparative method in evolutionary biology*. New York, NY: Oxford University Press.

Herrel A, Meyers JJ, Vanhooydonck B. 2002. Relations between microhabitat use and limb shape in phrynosomatid lizards. *Biological Journal of the Linnean Society* **77:** 149–163.

Herrel A, Vanhooydonck B, Porck J, Irschick DJ. 2008. Anatomical basis of differences in locomotor behaviour in *Anolis* lizards: a comparison between two ecomorphs. *Bulletin of the Museum of Comparative Zoology* **159:** 213–238.

Hews DK, Benard MF. 2001. Negative association between conspicuous visual display and chemosensory behavior in two phrynosomatid lizards. *Ethology* **107:** 839–850.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17:** 754–755.

Imparato B. 2007. Morphology of the femoral glands in the lizard *Ameiva ameiva* (Teiidae) and their possible role in semiochemical dispersion. *Journal of Morphology* **648:** 636–648.

Iraeta P, Monasterio C, Salvador A, Díaz JA. 2011. Sexual dimorphism and interpopulation differences in lizard hind limb length: locomotor performance or chemical signalling? *Biological Journal of the Linnean Society* **104:** 318–329.

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.

Kekäläinen J, Huuskonen H, Kiviniemi V, Taskinen J. 2010. Visual conditions and habitat shape the coloration of the Eurasian perch (*Perca fluviatilis* L.): a trade-off between camouflage and communication? *Biological Journal of the Linnean Society* **99:** 47–59.

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26:** 1463–1464.

Köppen W. 1900. Versuch einer Klassifikation der Klimate, vorzugsweise nach ihren Beziehungen zur Pflanzenwelt. *Geographische Zeitschrift* **6:** 593–611.

Kottek M, Grieser J, Beck C, Rudolf B, Rubel F. 2006. World Map of the Köppen–Geiger climate classification updated. *Meteorologische Zeitschrift* **15:** 259–263.

Kroodsma DE, Miller EH. 1982. *Acoustic communication in birds*. New York, NY: Academic Press.

López P, Amo L, Martín J. 2006. Reliable signalling by chemical cues of male traits and health state in male lizards. *Journal of Chemical Ecology* **32:** 473–488.

López P, Aragon P, Martín J. 2003. Responses of female lizards, *Lacerta monticola*, to males' chemical cues reflect their mating preference for older males. *Behavioural Ecology and Sociobiology* **55:** 73–79.

López P, Martín J. 2009. Lipids in femoral gland secretions of male lizards, *Psammodromus hispanicus*. *Biochemical Systematics and Ecology* **37:** 304–307.

Louw S, Burger BV, LeRoux M, Van Wyk JH. 2007. Lizard epidermal gland secretions I: chemical characterization of the femoral gland secretion of the sungazer, *Cordylus giganteus*. *Journal of Chemical Ecology* **33:** 1806–1818.

Martín J, López P. 2000. Chemoreception, symmetry, and mate choice in lizards. *Proceedings of the Royal Society of London Series B, Biological Sciences* **267:** 1265–1269.

Martín J, López P. 2006. Age-related variation in lipophilic chemical compounds from femoral gland secretions of male lizards *Psammodromus algirus*. *Biochemical Systematics and Ecology* **34:** 691–697.

Martín J, López P. 2011. Pheromones and reproduction in reptiles. In: Norris DO, Lopez KH, eds. *Hormones and reproduction in vertebrates, Vol. 3, reptiles*. New York, NY: Academic Press, 141–167.

Martín J, López P, Garrido M, Pérez-Cembranos A, Pérez-Mellado V. 2013. Inter-island variation in femoral secretions of the Balearic lizard, *Podarcis lilfordi* (Lacertidae). *Biochemical Systematics and Ecology* **50:** 121–128.

Martins EP, Ord TJ, Slaven J, Wright JL, Housworth EA. 2006. Individual, sexual, seasonal, and temporal variation in the amount of Sagebrush lizard scent marks. *Journal of Chemical Ecology* **32:** 881–893.

Mason RC. 1992. *Biology of the reptilia, Vol. 18, physiology E hormones, brain, and behavior*. Chicago, IL: The University of Chicago Press.

Mason RT, Parker MR. 2010. Social behavior and pheromonal communication in reptiles. *Journal of Comparative Physiology A, Neuroethology, Sensory, Neural, and Behavioral Physiology* **196:** 729–749.

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 Phyologenetics and Evolution* **44:** 115–1163.

Meiri S. 2007. Size evolution in island lizards. *Global Ecology and Biogeography* **16:** 702–708.

Moore PA, Crimaldi JP. 2004. Odor landscapes and animal behavior: tracking odor plumes in different physical worlds. *Journal of Marine Systems* **46:** 55–64.

Moreira PL, López P, Martín J. 2006. Femoral secretions and copulatory plugs convey chemical information about male identity and dominance status in Iberian rock lizards (*Lacerta monticola*). *Behavioural Ecology and Sociobiology* **2:** 166–174.

Morton ES. 1975. Ecological sources of selection on avian sounds. *American Naturalist* **109:** 17–34.

Müller-Schwarze D. 2006. *Chemical signals in vertebrates*. Cambridge: Cambridge University Press.

Müller-Schwarze D, Silverstein RM. 1980. *Chemical signals: vertebrates and aquatic invertebrates*. New York, NY: Plenum Press.

Nunn CL. 2011. *The comparative method in evolutionary anthropology and biology*. Chicago, IL: Chicago University Press.

Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* **401:** 877–884.

Pavlicev M, Mayer W. 2009. Fast radiation of the subfamily Lacertinae (Reptilia: Lacertidae): history or methodical artefact? *Molecular Phylogenetics and Evolution* **52:** 727–734.

Perry JN, Wall C. 1984. A mathematical-model for the flight of pea moth to pheromone traps through a crop. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **306:** 19–48.

Pincheira-Donoso D, Hodgson DJ, Tregenza T. 2008. Comparative evidence for strong phylogenetic inertia in precloacal signalling glands in a species-rich lizard clade. *Evolutionary Ecology Research* **10:** 11–28.

Plath M, Parzefall J, Körner KE, Schlupp I. 2004. Sexual selection in darkness? Female mating preferences in surface- and cave-dwelling Atlantic mollies, *Poecilia mexicana* (Poeciliidae, Teleostei). *Behavioural Ecology and Sociobiology* **55:** 596–601.

Pyron RA, Burbrink FT, Wiens JJ. 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology* **13:** 1–53.

Quay WB. 1972. Integument and the environment: glandular composition, function and evolution. *American Zoologist* **12:** 95–108.

R Core Team. 2012. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: http://www.R-project.org.

R Studio. 2012. *R Studio: integrated development environment for R*, Version 0.97.390. Boston, MA: R Studio. Available at: http://www.rstudio.org.

Rambaut A, Drummond AJ. 2007. Tracer version 1.5. Computer program and documentation distributed by author. Available at: http://beast.bio.ed.ac.uk/Tracer

Regnier FE, Goodwin M. 1977. On the chemical and environmental modulation of pheromone release from vertebrate scent mark. In: Müller-Schwarze D, Mozzel MM, eds. *Chemical signals in vertebrates*. New York, NY: Plenum, 115–133.

Revell LJ. 2009. Size-correction and principal components for interspecific comparative studies. *Evolution* **63:** 3258–3268.

Revell LJ. 2012. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3:** 217–223.

Rogers LJ, Kaplan G. 2002. *Songs, roars and rituals: communication in birds, mammals and other animals*. Cambridge, MA: Harvard University Press.

Ronquist F, Huelsenbeck JP. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Schleich HH, Kastle W, Kabisch K. 1996. *Amphibians & reptiles of North Africa*. Koenigstein: Koeltz Scientific Publishers.

Schwenk K. 1993. Comparative biology and the importance of cladistic classification: a case study from the sensory biology of squamate reptiles. *Biological Journal of the Linnean Society* **52:** 69–82.

Searcy WA, Nowicki S. 2005. *The evolution of animal communication: reliability and deception in signalling systems*. Princeton, NJ: Princeton University Press.

Sinervo B, Losos JB. 1991. Walking the tight rope – Arboreal sprint performance among *Sceloporus occidentalis* lizard populations. *Ecology* **72:** 1225–1233.

Smith JM, Harper D. 2003. *Animal signals*. Oxford: Oxford University Press.

Spawls S, Howell K, Drewes RC. 2006. *Reptiles and amphibians of East Africa*. Princeton, NJ: Princeton University Press.

Stamps JA, Andrews RM. 1992. Estimating asymptotic size using the largest individuals per sample. *Oecologia* **92:** 503–512.

Starnberger I, Poth D, Peram PS, Schulz S, Vences M, Knudsen J, Barej MF, Rödel MO, Walzl M, Hödl W. 2013. Take time to smell the frogs: vocal sac glands of reed frogs (Anura: Hyperollidae) contain species-specific chemical cocktails. *Biological Journal of the Linnean Society* **110:** 828–838.

Stevens M. 2013. *Sensory ecology, behaviour, and evolution,* 1st edn. Oxford: Oxford University Press.

Van Damme R. 1999. Evolution of herbivory in lacertid lizards – effects of insularity and body size. *Journal of Herpetology* **33:** 663–674.

Van Damme R, Bauwens D, Thoen C, Vanderstighelen D, Verheyen RF. 1995. Responses of naive lizards to predator chemical cues. *Journal of Herpetology* **29:** 38–43.

Van Wyk JH, Mouton P, Ie FN. 1992. Glandular epidermal structures in cordylid lizards. *Amphibia-Reptilia* **13:** 1–12.

Vanhooydonck B, Herrel A, Meyers JJ, Irschick DJ. 2009. What determines dewlap diversity in *Anolis* lizards? An among-island comparison. *Journal of Evolutionary Biology* **22:** 293–305.

Vanhooydonck B, Van Damme R. 1999. Evolutionary relationships between body shape and habitat use in lacertid lizards. *Evolutionary Ecology Research* **1:** 785–805.

Vitt LJ, Caldwell JP. 2014. *Herpetology – an introductory biology of amphibians and reptiles,* 4th edn. London: Academic Press.

Vitt LJ, Pianka ER. 2007. Feeding ecology in the natural world. In: Reilly SM, McBrayer LD, Miles DB, eds. *Lizard ecology: the evolutionary consequences of foraging mode*. Cambridge: Cambridge University Press, 141–172.

Weldon PJ, Dunn BS Jr, McDaniel CA, Werner DI. 1990. Lipids in the femoral gland secretions of the green iguana (*Iguana iguana*). *Comparative Biochemistry and Physiology* **95:** 541–543.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Morphologic, ecological, and geographical data for 162 lacertid lizards, assembled from available literature. SVL, snout–vent length; ♂, male; FP, mean femoral pore number; Subs., substrate class; Cli., Köppen–Geiger main climate class; Pre., Köppen–Geiger precipitation class; Temp., Köppen–Geiger temperature class; Lat., mean latitude op distribution, Trad. res., traditional residual values calculated from the linear regression of mean femoral pore number on male SVL; Phyl. res., phylogenetic residual values calculated from the linear regression of mean femoral pore number on male SVL; GEN., generalist class; LOW VEG., low vegetation class; HIGH VEG., high vegetation class.

Table S2. List of species used in the phylogenetic analyses for the four gene regions, including the accession numbers of sequences obtained from GenBank. NS, no sequence.