

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

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

Reference:

Baeckens Simon, Edwards Shelley, Huyghe Katleen, van Damme Raoul.- Chemical signalling in lizards : an interspecific comparison of femoral pore numbers in Lacertidae

Biological journal of the Linnean Society / Linnean Society of London - ISSN 0024-4066 - 114:1(2015), p. 44-57

Full text (Publishers DOI): <http://dx.doi.org/doi:10.1111/bij.12414>

Handle: <http://hdl.handle.net/10067/1205040151162165141>

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26

Journal: *Biological Journal of the Linnean Society* (2014)

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

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

**SIMON BAECKENS^{1*}, SHELLEY EDWARDS^{2,3}, KATLEEN HUYGHE¹ and
RAOUL VAN DAMME¹**

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

²Centre for Invasion Biology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa

³Applied Biodiversity Research Division, South African National Biodiversity Institute, Private Bag X7, Claremont 7735, Cape Town, South Africa

*Corresponding author: simon.baeckens@uantwerp.be

Short running head:

Femoral pore numbers in lacertid lizards

27 **ABSTRACT**

28 Animals communicate through a variety of sensory channels and signals. Studies on
29 acoustic and visual communication systems suggest that differences in the physical
30 environment contribute to the variety of signalling behaviour, with species investing
31 in those signals that are transmitted best under the local conditions. Whether or not
32 environmental tuning also occurs in chemical communication systems has received
33 much less attention. Here, we examine the effect of several aspects of the physical
34 environment on the chemical communication system of lacertid lizards (family
35 Lacertidae). The numbers of femoral pores are used as a proxy reflecting how much
36 a particular species invests in and relies upon chemical signalling. Femoral pores are
37 specialised epidermal structures that function as a secretion channel for the waxy
38 substance produced by glands. In some lacertid species, the secretion carries
39 infochemicals that play an important role in social communication. The number of
40 femoral pores varies considerably among species. We have compiled data on
41 femoral pore numbers for 162 species and tested for the effects of climate and
42 substrate use. After correcting for body size and taking the phylogenetic
43 relationships among the species into account, we found no effect of climate
44 conditions or latitude on species pore numbers. Substrate use did affect pore
45 numbers: shrub-climbing species tended to have fewer femoral pores than species
46 inhabiting other substrates.

47

48 *Key-words:* communication - comparative methods - climate conditions - lacertid -
49 latitude - olfaction - physical environment – secretion - semiochemical - substrate
50 use.

51 INTRODUCTION

52 Animals communicate with conspecifics and other species through a wide variety of
53 signals and displays. Which selection pressures and developmental constraints have
54 driven the evolution of the diversity in sending and receiving systems is the subject
55 of continued research (Bradbury & Vehrencamp, 1998; Espmark, Amundsen &
56 Rosenqvist, 2000; Greenfield, 2002; Rogers & Kaplan, 2002; Smith & Harper, 2003;
57 Searcy & Nowicki, 2005). The effects of the physical environment on the evolution of
58 particular sensory channels and signals within sensory channels have been studied
59 extensively for acoustic and visual communication systems (Morton, 1975;
60 Kroodsma & Miller, 1982; Endler, 1990; Gerhardt & Huber, 2002; Barnard, 2004;
61 Kekäläinen *et al.*, 2010). Far less is known on how the physical environment affects
62 the evolution of chemical communication, despite the fact that 'infochemicals'
63 (Dicke & Sabelis, 1988) are used by a vast number of species in a variety of aquatic
64 and terrestrial habitats (Müller-Schwarze & Silverstein, 1980; Müller-Schwarze,
65 2006; Starnberger *et al.*, 2013). Theoretical work suggests that the efficacy of
66 particular chemical signals will depend strongly on environmental conditions (i.e.
67 temperature, humidity, barometric pressure, air currents; Bossert & Wilson, 1963;
68 Moore & Crimaldi, 2004) and therefore it can be expected that varying selection
69 along an environmental gradient (Darwin, 1859), acting to maximize transmission
70 efficiency, will contribute to chemical signalling diversity (Alberts, 1992). Surprisingly
71 few studies have sought empirical support for this idea (except e.g. Escobar *et al.*,
72 2003; Pincheira-Donoso, Hodgson & Tregenza, 2008).

73 Squamate reptiles (lizards and snakes) utilize a variety of sensory systems
74 including visual, acoustic, tactile and gustatory systems (Vitt & Caldwell, 2014). They

75 also rely strongly on their ability to perceive chemicals from the environment for a
76 variety of daily activities including prey detection (e.g. Amo, López & Martín, 2004),
77 predator avoidance (e.g. Van Damme *et al.*, 1995; Aragon, López & Martín, 2008),
78 mate recognition (e.g. Cooper & Perez-Mellado, 2002; Moreira, López & Martín,
79 2006) and overall social behaviour (reviewed by Mason & Parker, 2010). Squamates
80 are equipped with a variety of glands (Quay, 1972) with which they produce and
81 send out diverse chemical messages (Martín & López, 2011). The femoral glands
82 situated in the epidermis of the inner thighs of many lizards are probably the best
83 studied (e.g. Alberts, 1990; Weldon *et al.*, 1990; Aragon, López & Martín, 2001; Louw
84 *et al.*, 2007; López & Martín, 2009). Those glands produce a holocrine secretion that
85 finds its way to the external world through epidermal structures: the 'femoral pores'
86 (Figure 1) (Mason, 1992). Pores are often larger in males than in females (Arnold &
87 Oviden, 2004) and the amount of pores present on each thigh, and the general
88 scale morphology of the pores, varies among species (Mason, 1992; Van Wyk,
89 Mouton & le, 1992). Consequently, femoral pores have been used extensively in the
90 past in identification keys and taxonomic descriptions in lizard species.

91 The lizard family Lacertidae constitutes an excellent model to study the
92 effects of the physical environment on the evolution of chemical communication,
93 with more than 300 species inhabiting a wide variety of habitats distributed over
94 much of the Old World (Branch, 1998; Arnold & Oviden, 2004; Spawls, Howell &
95 Drewes, 2006; Das, 2010). Although most lacertid lizards share the same general
96 morphology and also many aspects of their ecology (i.e. most are diurnal,
97 heliothermic species that actively forage for invertebrate prey: Castilla, Van Damme
98 & Bauwens, 1999; Van Damme, 1999; Vitt & Pianka, 2007), they have successfully

99 radiated into a wide array of habitats and climate regions, from subarctic tundra
100 over temperate heath lands and forests, alpine meadows and Mediterranean
101 maquis, steppe and gravel semi-deserts, monsoonal rainforest to sandy dune
102 systems in the desert (Arnold, 1989; Harris, Arnold & Thomas, 1998; Harris *et al.*,
103 2002). Within these habitats, they utilize a great diversity of microhabitats and
104 substrates, ranging from herby vegetation over stony undergrounds to shifting sands
105 (Vitt & Caldwell, 2014). Other species climb extensively in shrubs or trees
106 (Vanhooydonck & Van Damme, 1999; Vanhooydonck *et al.*, 2009). Additionally, our
107 preliminary inquiries suggested considerable among species variation in femoral
108 pore number. Here, we explore whether variation in pore number co-varies with
109 environmental traits. In addition, acquired results will allow us to make predictions
110 about potentially evolutionary processes imposed by the respective physical
111 environments.

112 We investigated the among-species diversity of a component of the chemical
113 communication apparatus, i.e. the number of femoral pores. We test the hypothesis
114 that the number of pores varies among species living on different substrates and in
115 dissimilar climatic conditions. The durability of an infochemical is an important
116 aspect in chemical communication. If the signals do not last long enough in their
117 environment, lizards may need to increase their effectiveness to maintain their
118 functionality (Iraeta *et al.*, 2011). Enhancing the overall production of infochemicals
119 by increasing the number (or size) of femoral pores is, besides adjusting the
120 composition of the secretion per se, a way to cope with environmental challenges
121 that decrease the chemical signal durability (Escobar, Labra & Niemeyer, 2001;
122 Escobar *et al.*, 2003; Iraeta *et al.*, 2011). We therefore assume that femoral pore

123 number reflects investment in and use of chemical communication (Escobar *et al.*,
124 2001; Pincheira-Donoso *et al.*, 2008). The idea to compensate for signalling
125 inefficiency (due to external factors) by increasing the investment in other
126 communication signals or signalling apparatus within the same sensory channel, will
127 be referred to in this study as the ‘within-channel compensation hypothesis’.
128 Chemical signalling is likely more challenging for species living in complex, dynamic,
129 ephemeral habitats (e.g. flimsy, moist vegetation and shrubs), on adsorptive
130 substrates (vegetation and sand vs. rock), or in warm and humid climate conditions,
131 due to the lower detectability and/or signal persistence (Alberts, 1992; Escobar *et*
132 *al.*, 2003; Pincheira-Donoso *et al.*, 2008). We therefore predict – in line with the
133 ‘within-channel compensation hypothesis’ – that species living in such environments
134 will tend to have more femoral pores, enhancing the overall production of secretion.
135 The latter will consequently increase the longevity of the infochemical and thus
136 ensuring the functionality of the signal.

137

138

139 **Material and methods**

140 *Morphological analyses*

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

147

148 *Climate regions, latitude and substrate use*

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

156 Data on the species' geographical distribution was taken from herpetological
157 field guides (Schleich, Kastle & Kabisch, 1996; Branch, 1998; Disi, Neýas & Rifai,
158 2001; Arnold & Ovenden 2004; Spawls *et al.*, 2006; Das 2010), and from the website
159 of the *Arbeitsgemeinschaft Lacertiden* of the German Society of Herpetology and
160 Herpetoculture (DGHT, <http://lacerta.de>). Each species was assigned to one climate
161 region by comparing its geographical distribution to an updated digital version of the
162 Köppen-Geiger World Climates Map (Kottek *et al.*, 2006). The Köppen-Geiger system
163 (Köppen, 1900) first classifies regions according to their native vegetation into (A)
164 the equatorial zone, (B) the arid zone, (C) the warm temperate zone and (D) the
165 snow zone (Table 1). Within these four primary zones, regions are defined according
166 to precipitation levels (e.g. "Cf" for warm temperate and fully humid), and air
167 temperature (e.g. "Cfa" for warm temperate, fully humid and hot summer) (Table 2).
168 Species were assigned to the Köppen-Geiger climate region that contains the greater
169 part of their distribution range.

170 The mean latitude of the distribution area of each lacertid species was
171 estimated by taking the midpoint between the most northern and the most southern
172 distribution point. By overlapping their distribution area with a world map (using the
173 program Google Earth © 2013 Google Inc., version 7.1.1.1888;
174 <http://earth.google.com>) we obtained a rough estimate of the latitude of the centre
175 of their geographical North-South distribution. We distinguish between 'latitude' and
176 'absolute latitude'. Latitude varies from -90 to +90 degrees, whereas absolute
177 latitude varies from 0 to 90 degrees. The former variable describes a north-south
178 gradient, the latter describes seasonal day length.

179

180 *Phylogenetic analyses*

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

191 Bayesian inference (BI) was performed with uniform priors for all parameters
192 (MrBayes v.3.1.2; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) at
193 the CIPRES Science Gateway (www.phylo.org/sub_sections/portal/). Each gene

194 region was partitioned separately, and the evolutionary model chosen for all gene
195 regions was GTR+I+G (jModelTest; Guindon & Gascuel, 2003; Darriba *et al.*, 2012).
196 Two parallel runs for 20×10^6 generations each were performed for the Markov chain
197 Monte Carlo (MCMC) runs, with trees sampled every 1000 generations. The number
198 of generations to discard as burn-in (4.0×10^6 generations) was determined by
199 examining the number of generations (1) at which the standard deviation of split
200 frequencies stabilized (at less than 0.001), (2) at which the log-likelihood tree scores
201 reached stationary, and (3) the effective sample sizes (ESS) of all parameters were
202 >100 (Tracer v.1.5; Rambaut & Drummond, 2007). A 50% majority rule tree was
203 constructed with the burn-in excluded using the 'sumt' command in MrBayes, and
204 nodes with ≥ 0.95 posterior probability were considered supported.

205

206 *Statistical analyses*

207 A strong phylogenetic signal was detected in most of the variables considered here
208 and therefore analysing the data in an explicit phylogenetic context seems
209 indispensable (Harvey & Pagel, 1991; Schwenk, 1993; Nunn 2011). We used both
210 traditional and phylogenetic analyses to examine how the number of femoral pores
211 varies with snout vent length and environmental conditions, although we only
212 present the result of the phylogenetic tests. All statistical analyses were conducted
213 in the program R Studio v.0.97.248 (R Core Team, 2012; R Studio, 2012) and *p*-values
214 smaller than 0.05 were considered as significant.

215 We estimated the phylogenetic signal in our data by calculating Pagel's
216 lambda for discrete variables and Blomberg's K for continuous variables. Pagel's
217 lambda was estimated by maximum likelihood optimization using the 'fitdiscrete'

218 function in the R-package 'Geiger' (Harmon *et al.*, 2008). A lambda close to zero
219 indicates no phylogenetic structure in the trait, whereas a lambda close to one
220 corresponds with the original, untransformed branch lengths (Pagel, 1999).
221 Estimates of the K-statistic were obtained with the 'Kcalc' command in the R-
222 package 'picante' (Kembel *et al.*, 2010). A $K < 1$ implies that relatives resemble each
223 other less than expected under Brownian motion evolution along the hypothesized
224 tree. A $K > 1$ implies that close relatives are more similar than expected under
225 Brownian motion evolution (stronger signal, Blomberg, Garland & Ives, 2003).

226 Absolute femoral pore number was significantly correlated with mean SVL
227 ($r=0.32$, $df=160$, $t=4.34$, $p<0.005$). Therefore, in all further analyses we used the
228 residuals extracted from the regression of pore number on mean SVL, and will be
229 referred to hereon as 'residual pore number'. Mean SVL was used as a measure of
230 body size because this was the variable most often reported in the literature. We
231 also have information on the maximal SVL reported per species, another size
232 variable commonly used in comparative studies. However, because maximal SVL is
233 probably more sensitive to sample size (Stamps & Andrews, 1992; Meiri, 2007) and
234 because our dependent variable is also an average number, we chose to use mean
235 SVL. The two body size measures are highly correlated in our dataset ($r=0.87$,
236 $df=160$, $P<0.005$). We used `phyl.resid()` function in R to achieve phylogenetic size
237 correction of femoral pore numbers (Revell, 2009). We fitted the model assuming
238 two different error structures. The first (BM) assumed simple Brownian motion
239 evolution along the hypothesised tree; the second (λ) used a 'lambda' error
240 structure, as suggested by Pagel (1999). Because the respective residuals were highly

241 correlated ($r=0.99$, $p<0.005$), we only report the results of the analyses using the
242 phylogenetic residuals obtained with the BM error structure.

243 Phylogenetic analyses of variances were used to test the differences in
244 residual pore number among species inhabiting different substrate and Köppen-
245 Geiger climate classes using the function `phylANOVA()` in the R-package `phytools`
246 (Revell, 2012). The function creates virtual data vectors by simulating evolution
247 along the hypothesized tree and performs ANOVAs on each of the data sets. The
248 resulting F-values are used to construct an empirical F-distribution, against which the
249 real F-value can be compared (Garland *et al.*, 1993). Post-hoc pair-wise differences
250 were evaluated by comparing empirical t-values to t-values obtained for each
251 simulated data vector. We used sequential-Bonferroni tests to correct for multiple
252 testing (`p.adj="holm"`, Revell, 2012).

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

257

258

259 **Results**

260 *Phylogenetic relationships*

261 We found strong branch support for the subfamilies Gallotiinae and Lacertinae
262 (Figure 2), as reported in previous studies (Mayer & Pavlicev, 2007; Kapli *et al.*, 2011;
263 Pyron *et al.*, 2013). The monophyly of most genera within the tribes Lacertini and
264 Eremiadini was also well supported, but the relationship between genera within the

265 Lacertini remained unresolved (as in previous studies: Fu, 2000; Arnold, Arribas &
266 Carranza, 2007; Mayer & Pavlicev, 2007; Pavlicev & Mayer, 2009; Kapli *et al.*, 2011;
267 Pyron *et al.*, 2013). That we were able to resolve the among-genera relationships in
268 greater detail in the Eremiadini than in the Lacertini, is most likely due to the fact
269 that nuclear gene sequences are available for Eremiadini species (Engleder *et al.*,
270 2012; Edwards *et al.*, 2012, 2013). We expect that additional information on nuclear
271 genes in Lacertini species will allow better resolution of the relationships among
272 genera in that clade.

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

285

286 *Climate regions, latitude and substrate use*

287 See table 3 for the descriptive statistics for climate regions and substrate use
288 of lacertid pore number.

289 _____ Estimates of the amount of phylogenetic signal in the variables under study
290 here are presented in table 5. The signal is very strong for all environmental
291 variables and morphological traits, pore number and SVL.

292 When accounting for phylogenetic relationships among species in our
293 dataset, we found no effect of the first main climate region (phylANOVA, $F=1.39$,
294 $df=3$, $p=0.76$), precipitation (phylANOVA, $F=5.93$, $df=4$, $p=0.42$) or temperature
295 category (phylANOVA, $F=1.23$, $df=4$, $p=0.74$) on mean residual pore number.
296 However, the overall effect of substrate class is significant (phylANOVA, $F=16.25$,
297 $df=5$, $p=0.012$, Table 4). Species from 'high-vegetation' microhabitats on average had
298 lower residual pore numbers than 'arboreal species' (post-hoc, difference=4.32,
299 $p=0.039$), species living on 'rocky' substrates (difference=7.80, $p=0.015$), species
300 from 'sandy' areas (difference=6.60, $p=0.048$) and 'generalist' species (difference
301 =6.59, $p=0.028$). Phylogenetic tests do not suggest a difference between high- and
302 low- vegetation substrates (difference=3.62, $p=0.330$). No significant correlation was
303 found between residual pore numbers and latitude (PGLS, $r=-0.26$, $df=160$, $t=0.15$,
304 $p=0.88$) and absolute latitude (PGLS, $r=-0.34$, $df=160$, $t=-0.50$, $p=0.62$), based on
305 Brownian motion model of evolution. Likewise, no noteworthy significant correlation
306 based on Ornstein-Uhlenbeck motion model of evolution.

307

308

309 **DISCUSSION**

310 In many lizards femoral pores function as an independent component of the lizards'
311 chemical signalling system (Imparato, 2007). Our analysis of the literature revealed
312 substantial among-species variation in the number of femoral pores in the family

313 Lacertidae. In this study we aimed to investigate the hypothesis that selection
314 underlying interspecific variation in pore numbers emerge from environmental
315 factors affecting chemical signalling. Subsequently, our findings allow us to make
316 inferences regarding the environmental factors that act as selective force driving this
317 among-species variation in pore number. Differential investment in particular
318 signalling systems can arise for two reasons. First, the relevance of sending out the
319 message may vary among species. For instance, we expect territorial species to
320 invest more in scent marking than non-territorial species (e.g. Becker, Petruno &
321 Marler, 2012). Second, local environmental circumstances may hamper the
322 transmission of certain signals, forcing some species to switch to a different
323 communication channel (e.g. Endler, 1993; Hews & Bernard, 2001; Stevens, 2013).

324

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

329 Generally, climate conditions could affect species' investment in chemical
330 communication (or in this specific case; femoral pore number) in different directions.
331 First and in line with the 'within-channel compensation hypothesis'; warm, windy
332 and humid conditions (which are thought to reduce signal transmittance,
333 pervasiveness and persistence) may select for stronger signalling devices and more
334 copious excretion. This would allow the lizards to bring across their message despite
335 the difficult conditions. In accordance with this idea, Escobar *et al.* (2001) found that
336 *Liolaemus* species living in warm, windy and low pressure habitats tend to have

337 more precloacal pores than species that live in less harsh habitats. However this
338 paper ignored the effects of shared ancestry. A subsequent paper by Pincheira-
339 Donoso *et al.* (2008) on the same genus, but with additional phylogenetic input,
340 failed to find any effect of environmental conditions on pore number. Pincheira-
341 Donoso *et al.* (2008) explain the assessed variation in pore numbers in *Liolaemus*
342 rather as a result of shared ancestry than a result of phylogenetic independent
343 adaptive events. These findings emphasize the major importance of the
344 incorporation of phylogenetic information in interspecific comparative studies.
345 Second, if environmental conditions become too hostile, or the costs of producing
346 chemicals too costly, lizards may trade in chemical communication for other
347 communication channels (the 'between-channel compensation hypothesis'). This
348 would result in an increase of investment in alternative signalling channels together
349 with (1) equal or (2) decreasing investment in pore number, what would either (1)
350 invalidate or (2) reverse the relationship between environmental conditions and
351 femoral pore number. An example of a possible case of 'between-channel
352 compensation' can be found in Atlantic mollies (*Poecilia mexicana*). In cave-dwelling
353 populations of Atlantic mollies male size cannot easily be determined visually, so
354 females have evolved the ability to recognize large males on the basis of chemical
355 cues (Plath *et al.*, 2004). Also the star-nosed mole (*Condylura cristata*) and the blind
356 mole rat (*Spalax ehrenbergi*), both species living underground, have reduced
357 thalamic visual system and an expanded somatosensory representation
358 (Cooper *et al.*, 1993; Catania, 2005). Environmentally induced switches between
359 communication channels may also occur on different time scales. For instance,
360 individual male newts *Mesotriton alpestris* will use comparatively more olfactory

361 than visual cues when courting in the dark than when courting in daytime (Denoël &
362 Doellen, 2010). Guppies (*Poecilia reticulata*) exhibit developmental sensory plasticity:
363 individuals reared in the dark forage more efficiently when chemical cues are
364 available, while individuals reared in full light find food faster on visual stimuli
365 (Chapman *et al.*, 2010).

366 Contrary to our predictions, phylogenetic analyses failed to find a significant
367 relationship between temperature, humidity, latitude and femoral pore number in
368 lacertids. Similar as reported by Picheira-Donoso *et al.* (2008), each record of co-
369 variation between climatologic factors and pore number vanishes when accounting
370 for shared ancestry. Based on the strong phylogenetic signal of the climatic variables
371 used in this particular study, variation in pore number could be most parsimoniously
372 explained by phylogenetic relatedness rather than the results of adaption to climatic
373 conditions. Therefore, solely based on pore number diversity, we cannot argue that
374 climatic conditions induce 'within-channel compensation' or 'between-channel
375 compensation' in this lizard family. An alternative possibility is that other features of
376 the chemical signalling system do differ among closely related species, but that this
377 occurs without significant differentiation in the morphological expression of the
378 femoral gland system; e.g. pore number (Picheira-Donoso *et al.*, 2008). Numerous
379 studies have shown that the chemical composition of femoral secretion can differ
380 significantly between lizards of different age, sex, population and species (Cole,
381 1966; Martins *et al.*, 2006; Martín & López, 2006; López & Martín, 2009; Gabirot *et*
382 *al.*, 2010; Gabirot, López & Martín, 2011; Martín *et al.*, 2013). Information on the
383 chemical structure of secreted compounds could potentially unravel differences

384 between lacertids living in regions with dissimilar climate conditions, what could
385 strengthen the 'within-channel compensation' hypothesis.

386

387 Our results suggest that substrate may be an environmental factor affecting the
388 evolution of the chemosensory system of lacertids. Femoral pore numbers differed
389 among species inhabiting different substrate classes, even after controlling for
390 phylogenetic relationships. Several other studies have shown that characteristics of
391 the substrate may affect the efficiency of signal transmission. For instance, the
392 seismic signals produced by male jumping spiders (*Habronattus dossenus*) to attract
393 females are far less effective on sandy or rocky substrates than on leaf litter (Elias,
394 Mason & Hoy, 2004). Similar results have been found in a wolf spider (*Schizocosa*
395 *occreata*), but here males that find themselves on substrates that attenuated seismic
396 signals used more visual signals (Gordon & Uetz, 2011) - a possible case of 'between-
397 channel compensation'.

398 Substrate type may also affect the efficiency of chemical signals themselves,
399 especially that of marks that are deposited directly on the surface and must
400 communicate the identity of characteristics of a territory holder for as long as
401 possible (Alberts, 1992). Regnier & Goodwin (1977) have demonstrated
402 experimentally that the fade-out time of a chemical signal depends on its affinity for
403 the substrate. For example, in their experiments, secretions applied to clay surfaces
404 evaporated more slowly than those applied to wood surfaces. The fade out-times of
405 chemical signals on sand, stones or vegetation (applied in our study) have not been
406 compared yet, but it seems plausible that differences in affinity will prevail because
407 of their distinctive physical nature. Additionally, the dynamics of the substrate could

408 affect the volatility and fade-out time of the secretion as well. A highly dynamic
409 substrate, such as sand dunes or shrubby vegetation, has a higher level of
410 disturbance and a complex airflow character, what both influences signal fade-out
411 time (Müller-Schwarze, 2006). Scent marks deposited in dunes may be covered
412 easily with sand, and infochemicals deposited on shrubby vegetation are subjected
413 to a high level of disturbance because of their 3D complexity, which act to decrease
414 signal life.

415

416 Our results suggest that shrub-climbing lacertid species invest less in femoral pores
417 than species living on any other substrate type. Studies have indicated that
418 vegetation affects airflow patterns and may adsorb and re-emit molecules (Perry &
419 Wall, 1984), and thus the scale (density, height and species-dependent
420 characteristics) of vegetation will affect communication patterns (Müller-Schwarze,
421 2006). Accordingly, when depositing chemical secretions onto a substrate at some
422 height above the ground (e.g. shrubby vegetation), the active space of the
423 infochemical increases by the addition of the vertical dimension (Alberts, 1992).
424 Because of the developed three-dimensional spherical shape of the active space of
425 the chemical signal, the longevity of the infochemical will decrease. Subsequently, an
426 individual could respond to such environmental conditions by downsizing its
427 investment in chemical communication and investing in more suitable
428 communication systems. Natural selection could therefore act in favour of species
429 with a low number of femoral pores, supporting the 'between communication
430 channel' hypothesis. Data on investments in alternative signalling channels would
431 enable us to draw more accurate conclusions.

432 Alternatively, a trade-off might exist between proficiency in chemical
433 advertising (number of femoral pores) and climbing capacity (Iraeta *et al.*, 2011). In
434 species that climb extensively in vegetation, the need for shorter limbs would tip
435 that balance towards shorter limbs, less space for glands, and fewer femoral pores.
436 Biomechanical considerations suggest that climbing species should benefit from
437 having shorter upper limbs because this would bring their centre of body mass closer
438 to the substrate (Clemente *et al.*, 2013), which would prevent them from 'toppling
439 over'. In accordance, comparative studies have reported smaller limb lengths in
440 climbing taxa (Sinervo & Losos, 1991; Vanhooydonck & Van Damme, 1999; Herrel,
441 Meyers & Vanhooydonck, 2002; Herrel *et al.*, 2008). With less space available on the
442 inner thighs, selection for shorter hind limbs would result in fewer femoral pores as
443 a by-product. We had no information on limb length for many of the species in our
444 dataset, so we cannot assess this alternative hypothesis here. However, Iraeta *et al.*
445 (2011) found no significant correlation between pore number and hind limb length
446 in *Psammodromus algirus* lizards. Interestingly though, male hind limb length did
447 correlate with mean pore size (another potentially important variable for which we
448 currently have no information). An argument against the idea that variation in pore
449 numbers is a by-product of differential selection on hind limb length, is that rock
450 climbing species tend to have relatively large numbers of pores, while biomechanics
451 and comparative studies would predict them to have short limbs for the same
452 reasons as vegetation climbers.

453

454

455 *Sexual selection as a driving force?*

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

471

472 *Synthesis and future prospects*

473 In summary, we find large among-species variation in femoral pore numbers in
474 Lacertidae. Using phylogenetic comparative methods, this study succeeds in finding
475 a co-variation between the number of pores and species' substrate usage, whereas
476 shrub-climbing species tend to have fewer femoral pores than species inhabiting
477 other substrates. In contrast, no effect of climatic conditions (temperature and
478 precipitation) or latitude on species' pore numbers was found. These results allow us

479 to speculate about the potential role of the physical environment on the evolution of
480 the chemical communication system, in lacertid lizards.

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

489

490

491 **ACKNOWLEDGEMENT**

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

498

499

500 **REFERENCES**

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

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

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

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

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

514 **Aragon P, Massot M, Gasparini J, Clobert J. 2006.** Socially acquired information from
515 chemical cues in the common lizard, *Lacerta vivipara*. *Animal Behaviour* **72**:
516 965-974.

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

520 **Arnold EN. 1989.** Systematics and Adaptive Radiation of Equatorial African Lizards
521 Assigned to the Genera *Adolfus*, *Bedriagaia*, *Gastropholis*, *Holaspis* and
522 *Lacerta* (Reptilia, Lacertidae). *Journal of Natural History* **23**: 525-555.

523 **Arnold EN, Oviden D. 2004.** *A Field Guide to the Reptiles and Amphibians of*
524 *Britain & Europe*. London: HarperCollins Publishers.

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

528 **Barnard CJ. 2004.** *Animal Behaviour: Mechanism, Development, Function and*
529 *Evolution*. Harlow: Pearson Education Limited.

530 **Becker EA, Petruno S, Marler CA. 2012.** A comparison of scent marking between a
531 monogamous and promiscuous species of *Peromyscus*: Pair bonded males do
532 not advertise to novel females. *PloS one* **7**: e32002.

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

536 **Bossert WH, Wilson DO. 1963.** The Analysis of Olfactory Communication Among
537 Animals. *Journal of Theoretical Biology* **5**: 443-469.

538 **Bradbury JW, Vehrencamp SL. 1998.** *Principles of Animal Communication*.
539 Sunderland: Sinauer Associates.

540 **Branch B. 1998.** *Field Guide to Snakes and Other Reptiles of Southern Africa*. Cape
541 Town: Struik Publishers.

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

545 **Catania KC. 2005.** Evolution of sensory specializations in insectivores. *Anatomical*
546 *Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology*
547 **287**: 1038-1050.

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

551 **Clemente CJ, Withers PC, Thompson GG, Lloyd D. 2013.** Lizard tricks: Overcoming
552 conflicting requirements of speed versus climbing ability by altering
553 biomechanics of the lizard stride. *Journal of Experimental Biology* **216**: 3854-
554 3862.

555 **Cole CJ. 1966.** Femoral Glands in Lizards: A Review. *Herpetologica* **22**: 119-206.

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

559 **Cooper WE Jr, Pérez-Mellado V. 2002.** Pheromonal Discriminations of Sex,
560 Reproductive Condition, and Species by the Lacertid Lizard *Podarcis*
561 *hispanica*. *Journal of Experimental Zoology* **292**: 523-527.

562 **Danoël M, Doellen J. 2010.** Displaying in the dark: Light-dependent alternative
563 mating tactics in the Alpine newt. *Behavioural Ecology and Sociobiology* **64**:
564 1171-1177.

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

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

570 **Darwin C. 1859.** *On the Origin of Species by Means of Natural Selection, or the*
571 *Preservation of Favoured Races in the Struggle of Life*. London: John Murray.

572 **Das I. 2010.** *A Field Guide to the Reptiles of South-East Asia*. London: New Holland
573 Publishers.

574 **Disi AM, Modry D, Neýas P, Rifai L. 2001.** *Amphibians and Reptiles of the Hashemite*
575 *Kingdom of Jordan*. An Atlas and Field Guide. Frankfurt: Chimaira.

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

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

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

584 **Elias DO, Mason AC, Hoy RR. 2004.** The effect of substrate on the efficacy of
585 seismic courtship signal transmission in the jumping spider *Habronattus*
586 *dossenus* (Araneae: Salticidae). *Journal of Experimental Biology* **207**: 4105-
587 4110.

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

590 **Endler JA. 1993.** Some general comments on the evolution and design of animal
591 communication systems. *Philosophical Transactions of the Royal Society B:*
592 *Biological Sciences* **340**: 215-225.

593 **Engleder A, Haring E, Kirchhof S, Mayer W. 2012.** Multiple nuclear and
594 mitochondrial DNA sequences provide new insights into the phylogeny of

595 South African Lacertids (Lacertidae, Eremiadinae). *Journal of Zoological*
596 *Systematics and Evolutionary Research* **51**: 132-143.

597 **Esobar CA, Labra A, Niemeyer HM. 2001.** Chemical composition of precloacal
598 secretions of *Liolaemus* lizards. *Journal of Chemical Ecology* **27**: 1677-1690.

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

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

604 **Font E, Barbosa D, Sampedro C, Carazo P. 2012.** Social behavior, chemical
605 communication, and adult neurogenesis: Studies of scent mark function in
606 *Podarcis wall* lizards. *General and Comparative Endocrinology* **177**: 9-17.

607 **Fu J. 2000.** Toward the phylogeny of the family Lacertidae - Why 4708 base pairs of
608 mtDNA sequences cannot draw the picture. *Biological Journal of the Linnean*
609 *Society* **71**: 203-217.

610 **Gabirot M, Castilla AM, López P, Martín J. 2010.** Differences in chemical signals may
611 explain species recognition between an island lizard *Podarcis atrata*, and
612 related mainland lizards *P. hispanica*. *Biochemical Systematics and Ecology*
613 **38**: 521-528.

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

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

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

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

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

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

628 **Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W. 2008.** GEIGER: Investigating
629 evolutionary radiations. *Bioinformatics* **24**: 129-131

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

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

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

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

642 **Herrel A, Vanhooydonck B, Porck J, Irschick DJ. 2008.** Anatomical basis of
643 differences in locomotor behaviour in *Anolis* lizards: A comparison between
644 two ecomorphs. *Bulletin of the Museum of Comparative Zoology* **159**: 213-
645 238.

646 **Hews DK, Benard MF. 2001.** Negative association between conspicuous visual
647 display and chemosensory behavior in two phrynosomatid lizards. *Ethology*
648 **107**: 839-850.

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

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

654 **Iraeta P, Monasterio C, Salvador A, Díaz JA. 2011.** Sexual dimorphism and
655 interpopulation differences in lizard hind limb length: Locomotor
656 performance or chemical signalling? *Biological Journal of the Linnean Society*
657 **104**: 318-329.

658 **Kapli P, Poulakakis N, Lymberakis P, Mylonas M. 2011.** A re-analysis of the
659 molecular phylogeny of Lacertidae with currently available data. *Basic and*
660 *Applied Herpetology* **25**: 97-104.

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

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

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

670 **Kottek M, Grieser J, Beck C, Rudolf B, Rubel F. 2006.** World Map of the Köppen-
671 Geiger climate classification updated. *Meteorologische Zeitschrift* **15**: 259-
672 263.

673 **Kroodsma DE, Miller EH. 1982.** *Acoustic Communication in Birds*. New York:
674 Academic Press.

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

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

680 **López P, Martín J. 2009.** Lipids in femoral gland secretions of male lizards,
681 *Psammodromus hispanicus*. *Biochemical Systematics and Ecology* **37**: 304-
682 307.

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

686 **Mason RC. 1992.** *Biology of the Reptilia. Volume 18, Physiology E Hormones,*
687 *Brain, and Behavior*. Chicago: The University of Chicago Press.

688 **Mason RT, Parker MR. 2010.** Social behavior and pheromonal communication in
689 reptiles. *Journal of comparative physiology. A, Neuroethology, Sensory,*
690 *Neural, and Behavioral Physiology* **196**: 729-749.

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

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

696 **Martín J, López P. 2011.** Pheromones and reproduction in reptiles. In: Norris DO,
697 Lopez KH, eds. *Hormones and reproduction in vertebrates, Volume 3-Reptiles*.
698 New York: Academic Press, 141-167.

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

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

705

706 **Mayer W, Pavilcev M. 2007.** The phylogeny of the family Lacertidae (Reptilia) based
707 on nuclear DNA sequences: Convergent adaptations to arid habitats within
708 the subfamily Eremiainae. *Molecular Phylogenetics and Evolution* **44**: 115-
709 1163.

710 **Meiri S. 2007.** Size evolution in island lizards. *Global Ecology and Biogeography* **16**:
711 702-708.

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

714 **Moreira PL, López P, Martín J. 2006.** Femoral secretions and copulatory plugs
715 convey chemical information about male identity and dominance status in
716 Iberian rock lizards (*Lacerta monticola*). *Behavioural Ecology and Sociobiology*
717 **2**: 166-174.

718 **Morton ES. 1975.** Ecological sources of selection on avian sounds. *The American*
719 *Naturalist* **109**: 17-34.

720 **Müller-Schwarze D. 2006.** *Chemical Signals in Vertebrates*. Cambridge: Cambridge
721 University Press.

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

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

726 **Pagel M. 1999.** Inferring the historical patterns of biological evolution. *Nature* **401**:
727 877-884.

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

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

734 **Plath M, Parzefall J, Körner KE, Schlupp I. 2004.** Sexual selection in darkness?
735 Female mating preferences in surface- and cave-dwelling Atlantic mollies,

736 *Poecilia mexicana* (Poeciliidae, Teleostei). *Behavioural Ecology and*
737 *Sociobiology* **55**: 596-601.

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

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

744 **Quay WB. 1972.** Integument and the environment: Glandular composition, function
745 and evolution. *American Zoologist* **12**: 95-108.

746 **R Core Team. 2012.** *R: A language and environment for statistical computing* (R
747 Foundation for Statistical Computing, Vienna, Austria). Available at
748 <http://www.R-project.org>

749 **R Studio. 2012.** *R Studio: Integrated development environment for R* (Version
750 0.97.390) [Computer software]. Boston, MA. Accessed January 2013.
751 Available at <http://www.rstudio.org>

752 **Rambaut A, Drummond AJ. 2007.** *Tracer version 1.5*. Computer program and
753 documentation distributed by author. Accessed July 2011. Available at
754 <http://beast.bio.ed.ac.uk/Tracer>

755 **Regnier FE, Goodwin M. 1977.** On the chemical and environmental modulation of
756 pheromone release from vertebrate scent mark. In: Müller-Schwarze D,
757 Mozzel MM, eds. *Chemical Signals in Vertebrates*. New York: Plenum, 115-
758 133.

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

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

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

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

767 **Schleich HH, Kastle W, Kabisch K. 1996.** *Amphibians & Reptiles of North Africa.*
768 Germany: Koeltz Scientific Publishers.

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

772 **Searcy WA, Nowicki S. 2005.** *The Evolution of Animal Communication: Reliability and*
773 *Deception in Signalling Systems.* UK: Princeton University Press.

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

776 **Spawls S, Howell K, Drewes RC. 2006.** *Reptiles and Amphibians of East Africa.* UK:
777 Princeton University Press.

778 **Smith JM, Harper D. 2003.** *Animal Signals.* UK: Oxford University Press.

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

781 **Starnberger I, Poth D, Peram PS, Schulz S, Vences M, Knudsen J, Barej MF, Rödel**
782 **MO, Walzl M, Hödl W. 2013.** Take time to smell the frogs: vocal sac glands of

783 reed frogs (Anura: Hyperollidae) contain species-specific chemical cocktails.
784 *Biological Journal of the Linnean Society* **110**: 828-838.

785 **Stevens M. 2013.** *Sensory Ecology, behaviour, and evolution. First edition.* UK:
786 Oxford University Press.

787 **Van Damme R. 1999.** Evolution of herbivory in lacertid lizards - Effects of insularity
788 and body size. *Journal of Herpetology* **33**: 663-674.

789 **Van Damme R, Bauwens D, Thoen C, Vanderstighelen D, Verheyen RF. 1995.**
790 Responses of naive lizards to predator chemical cues. *Journal of Herpetology*
791 **29**: 38-43.

792 **Vanhooydonck B, Van Damme R. 1999.** Evolutionary relationships between body
793 shape and habitat use in lacertid lizards. *Evolutionary Ecology Research* **1**:
794 785-805.

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

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

800 **Vitt LJ, Caldwell JP. 2014.** *Herpetology - An introductory Biology of Amphibians and*
801 *Reptiles, Fourth Edition.* London: Academic Press.

802 **Vitt LJ, Pianka ER. 2007.** Feeding ecology in the natural world. In: Reilly SM,
803 McBrayer LD, Miles DB, eds. *Lizard Ecology: The Evolutionary Consequences*
804 *of Foraging Mode.* Cambridge: Cambridge University Press, 141-172.

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

808

809

810

811

812

813

814

815

816

817

818

819

820



822

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



824

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

829

830 **TABLES**

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

Type	Description	Criterion
A	Equatorial climates	$T_{\min} \geq + 18 \text{ }^\circ\text{C}$
Af	Equatorial rainforest, fully humid	$P_{\min} \geq 60 \text{ mm}$
Am	Equatorial monsoon	$P_{\text{ann}} \geq 25 (100 - P_{\min})$
Aw	Equatorial savannah with dry winter	$P_{\min} < 60 \text{ mm in winter}$
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	$- 3 \text{ }^\circ\text{C} < T_{\min} < + 18 \text{ }^\circ\text{C}$
Cs	Warm temperate climate with dry summer	$P_{\text{smin}} < P_{\text{wmin}}; P_{\text{wmax}} > 3 P_{\text{smin}}$ and $P_{\text{smin}} < 40 \text{ mm}$
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	$T_{\min} \leq - 3 \text{ }^\circ\text{C}$
Ds	Snow climate with dry summer	$P_{\text{smin}} < P_{\text{wmin}}; P_{\text{wmax}} > 3 P_{\text{smin}}$ and $P_{\text{smin}} < 40 \text{ mm}$
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

834 Key to Criterion abbreviations: T_{ann} = annual mean near-surface (2m) temperature; T_{max} = monthly mean
 835 temperatures of the warmest month; T_{min} = monthly mean temperatures of the coldest month; P_{ann} =
 836 accumulated annual precipitation; P_{\min} = precipitation of the driest month; P_{smin} = lowest monthly precipitation
 837 for the summer; P_{smax} highest monthly precipitation for the summer; P_{wmin} = lowest monthly precipitation for the

838 winter; P_{wmax} = highest monthly precipitation for the winter. All temperatures are given in °C, monthly
839 precipitations in mm/month and P_{ann} in mm/year.

840

841

842

843

844

845

846

847

848

849

850

851

852

853

854

855

856

857

858

859

860

861

862

863 **Table 2:** Key to third letter of the climate categories, adapted from the Köppen and Geiger
 864 climate system (Kottek *et al.*, 2006). Key to criterion abbreviations as in Table 1.

Type	Description	Criterion
h	Hot steppe / desert	$T_{\text{ann}} \geq + 18 \text{ }^\circ\text{C}$
k	Cold steppe / desert	$T_{\text{ann}} < + 18 \text{ }^\circ\text{C}$
a	Hot summer	$T_{\text{max}} \geq + 22 \text{ }^\circ\text{C}$
b	Warm summer	not (a) and at least 4 $T_{\text{mon}} \geq + 10 \text{ }^\circ\text{C}$
c	Cool summer and cold winter	not (b) and $T_{\text{min}} > - 38 \text{ }^\circ\text{C}$
d	Extremely continental	like (c) but $T_{\text{min}} \leq - 38 \text{ }^\circ\text{C}$

865

866

867

868

869

870

871

872

873

874

875

876

877

878

879

880 **Table 3:** Descriptive statistics (means, standard errors, sample sizes, minima and maxima)

881 for substrate class and climate class of lacertid absolute mean femoral pore numbers.

Factor	\bar{x}	SE	<i>n</i>	Min.	Max.
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

882 Key to abbreviations: \bar{x} = mean; SE = standard error; n = sample size.

883

884

885

886

887

888

889

890

891

892

893

894

895

896

897

898

899

900

901

902 **Table 4:** Results of phylogenetic analyses showing the mean difference in residual pore
 903 numbers between classes of species' substrate usage. Bold values indicate significant p -
 904 values ($P < 0.05$).

<i>Substrate class</i>	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

905

906

907

908

909

910

911

912

913

914

915

916

917

918

919

920 **Table 5:** The degree to which the environmental factor variables (climate, temperature,
 921 precipitation and substrate usage) and continuous variables (latitude, mean male SVL and
 922 mean pore number) exhibits phylogenetic signals, indicated by significant Pagel's λ value (for
 923 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

924