

Sexual selection and the chemical signal design of lacertid lizards

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Received 14 May 2017; revised 29 August 2017; accepted for publication 9 September 2017

Animal signals can differ considerably in complexity and composition, even among closely related species. Work on vocal and visual signals has revealed how sexual selection can elaborate signals relevant in mate choice or rival assessment, but few studies have investigated this process in chemical signals. In this study, we correlated chemical signalling diversity and richness with degree of sexual dimorphism in a data set of 60 species of the lizard family Lacertidae. The femoral glands of male lacertid lizards exude waxy secretions, of which the lipophilic fraction is known to function in chemical communication. We determined the composition of the gland secretions using gas chromatography–mass spectrometry and calculated the chemical richness (total number of compounds) and the chemical diversity (Shannon–Wiener H') for each species. We used sexual dimorphism in size, in shape, and a combination of both, as proxies for the intensity of sexual selection acting on each species. Although our data revealed considerable interspecific variation in the composition and complexity of the chemical signals, as well as in sexual dimorphism, we found no evidence for the idea that more elaborate signals arise through intensified sexual selection. We offer a number of conceptual and methodological explanations for this unexpected finding.

ADDITIONAL KEYWORDS: animal communication – femoral gland secretions – Lacertidae – lizards – phylogenetic comparative methods – sexual size dimorphism.

INTRODUCTION

From the chaffinch's single-noted 'rain call' to the intricate vocal and visual displays of the lyrebird, animal signals differ widely in complexity (Rogers & Kaplan, 2002; Smith & Harper, 2003). There are many good ideas on why the complexity of a signal would benefit the signaller and/or the receiver. More complex signals may carry more content or may be transmitted, received and processed more effectively (Candolin, 2000; Hebets & Papaj, 2005). On the other hand,

evolution towards ever more elaborate signals is likely to be tempered by constraints and costs involved in developing broadcasting and sensory machinery and by the increased exposure to eavesdropping (Endler, 2000). In the end, the intricacy of signals produced by members of a species must represent a local balance between these two opposed sets of evolutionary forces.

Although animals use signals in virtually all interactions with other living creatures, mate choice and male–male competition for access to females are generally thought to have shaped animal signalling systems more than any other form of selection (Steiger, Schmitt & Schaefer, 2011). By consequence, it seems logical to assume that interspecific variation in signal complexity

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to a large extent reflects differences in the magnitude of sexual selection among species. This especially holds true for signals that exhibit significant sexual dimorphism. Comparative studies on visual cues have corroborated this idea. In agamid and iguanid lizards, species with high sexual size dimorphism (characteristic of high levels of male–male competition) have more complex displaying behaviours (Ord, Blumstein & Evans, 2001). In Australian dragon lizards (Agamidae), males of species with high levels of head size dimorphism and sexual dichromatism also have more complex colour patterns (Chen *et al.*, 2012). Among lacertid lizards (Lacertidae), the intensity of intrasexual selection predicts the complexity of male-biased sexually dichromatic colour patterns (Pérez i de Lanuza, Font & Monterde, 2013). Similar results have been described for the auditory channel in avian reptiles (e.g. Cardoso, Hu & Gama Moto, 2012; Hamao, 2013). The effect of sexual selection on the elaborateness of chemical signals has received much less attention (Steiger & Stökl, 2014).

There is compelling evidence that chemical signals can be under sexual selection. Research on a wide variety of species has established that the production of chemical cues typically exhibits male-biased sexual dimorphism and is most prominent in adults and during the breeding season – elements characteristic of secondary sexual traits (Müller-Schwarze, 2006; Wyatt, 2014). A multitude of behavioural tests has shown that mate choice and rival assessment often involve olfactory cues and that chemical signals can be condition dependent (e.g. López, Amo & Martín, 2006; Martín & López, 2010, 2015). Whether among-species differences in the intensity, form or direction of sexual selection also contribute to macro-evolutionary patterns in the compositional complexity of chemical signals remains uncertain. Comparative analyses of chemical signal variability are still scarce (Symonds & Elgar, 2008; Weber *et al.*, 2016), and although the currently available data suggest that chemical signals can evolve and elaborate just as fast as visual or auditory displays (e.g. Mullen *et al.*, 2007; Zimmermann, Ramírez & Eltz, 2009), the forces driving these changes have hardly been explored (Steiger & Stökl, 2014).

In this study, we explore whether variation in the compositional complexity of femoral gland secretions among species of lacertid lizards correlates with sexual size and shape dimorphism (i.e. a putative proxy for the intensity of sexual selection). Lacertid lizards possess a series of holocrine glands, positioned on their inner thighs, that produce a waxy substance containing a mix of lipophilic and proteic compounds (Baeckens *et al.*, 2015; Mayerl, Baeckens & Van Damme, 2015; Mangiacotti *et al.*, 2016). In contrast to the proteinaceous fraction, the lipophilic fraction of the glandular secretion of lacertids is well studied and is thought to be the leading source of compounds

involved in lacertid communication (Martín & López, 2014; Mayerl *et al.*, 2015). Lipids have the advantage of being more volatile and have a high degree of molecular diversity, which increases the potential information content of a signal (Alberts, 1992; Alberts, Phillips & Werner, 1993). Typical lipophilic compounds in glandular secretions of lacertid lizards are steroids and fatty acids, as the most dominant compounds, together with usually minor amounts of alcohols, waxy esters, squalene, tocopherol, ketones, aldehydes, furanones, alkanes or amides, and other minor and less frequent compounds (reviewed in Weldon, Flachsbarth & Schulz, 2008). Today, the lipophilic compositions of the glandular secretion of around a dozen lacertid species are known (Mayerl *et al.*, 2015). Molecules of the lipophilic fraction have been attributed a role in species recognition (Barbosa *et al.*, 2005, 2006; Gabirot, López & Martín, 2012; García-Roa *et al.*, 2016), but in several species, they also mediate mate choice in females (e.g. Martín & López, 2000, 2006a, b; Olsson *et al.*, 2003) and territory rival assessment in males (e.g. Martín & López, 2007; Font *et al.*, 2012), so therefore they may be under sexual selection. Although quantitative information on operational sex ratios, mating systems, male–male competition, territoriality, frequency of multiple mating and paternity, and sperm competition is missing except for a few species, it seems plausible that the intensity of sexual selection may vary considerably among species of lacertid lizards. For instance, while in some species males defend territories throughout the activity seasons (e.g. *Podarcis* species, Edsman, 1989; Font *et al.*, 2012), in other species, male home ranges overlap largely and males will fight over females during a narrow time window only (e.g. *Lacerta schreiberi*, Marco & Pérez-Mellado, 1999). Lacertid lizards are also known to vary considerably in sexual dichromatism (Pérez i de Lanuza *et al.*, 2013), body size and shape dimorphism (Braña, 1996) and degree of dimorphism in physiological performance measures (Van Damme *et al.*, 2008) – all of which may be targets of sexual selection. Moreover, the fact that the vast majority of female lacertids are equipped with vestigial femoral glands that do not produce any secretion (but see Khannoon *et al.*, 2011; Martín *et al.*, 2015) already reflects the presence of strong sexual selective pressure. We predict that species under high sexual selection (as indicated by high sexual dimorphism) will have evolved a more elaborate chemical signal, richer or more diverse in chemical composition.

MATERIAL AND METHODS

CHEMICAL SIGNAL COMPLEXITY

Between 2005 and 2016, we collected femoral gland secretions from 60 species of lacertid

lizards at various locations in Europe, Africa and Asia (Supporting Information, Table S1). In total, we captured 619 lizards by hand or noose. On average, we caught ten individuals per species (range 1–35). We sampled adult males only, and all data were collected during the breeding season when glandular activity is highest (Smith, 1946; Cole, 1966). Seventeen (male) *Holaspis guentheri* lizards were obtained through the pet trade (Fantasia Reptiles, Belgium, license HK51101419). Male *H. guentheri* were wild-caught in Tanzania by the trader's personnel ~2 weeks prior to purchase, and their femoral gland secretions were collected in the lab, at the University of Antwerp. Samples of the secretions were obtained by gently applying pressure around the pore-bearing scales with a forceps. The extraction procedure is harmless, and the lizards are able to produce more secretion rapidly thereafter (e.g. Baeckens *et al.*, 2017a). The samples were stored in glass vials fitted with Teflon-lined stoppers and kept at -20°C until analysis. All specimens were released immediately after sampling, at the exact site of capture.

To analyse the samples, we used a Finnigan-ThermoQuest Trace 2000 gas chromatograph (GC), fitted with a poly (5% diphenyl/95% dimethylsiloxane) column (Supelco, Equity-5, 30 m length \times 0.25 mm ID, 0.25 mm film thickness). A Finnigan-ThermoQuest Trace mass spectrometer (MS) was used as the detector. By using helium as the carrier gas, we carried out splitless sample injections (2 μL of each sample dissolved in 2 mL of GC capillary grade *n*-hexane). We maintained temperatures of injector and detector at 250 and 280 $^{\circ}\text{C}$, respectively. The oven temperature programme started at 50 $^{\circ}\text{C}$ (3 min), then increased to 300 $^{\circ}\text{C}$ (at a rate of 5 $^{\circ}\text{C}/\text{min}$), to finally stay isothermal at 300 $^{\circ}\text{C}$ (during 15 min). Mass spectral fragments below $m/z = 46$ were not recorded. Initially, we identified chemical compounds, at least to the major chemical class level, by comparing their mass spectra with those in the NIST/EPA/NIH (NIST 02) computerized mass spectral library. The identification of specific compounds was confirmed by comparing spectra and retention times with those of authentic standards when these were available (from Sigma-Aldrich Chemical Co.). Impurities in the control vial samples were not considered. Finally, we used the percent of total ion current as an estimate for the relative abundance of each chemical compound in the sample. Hence, we estimated the relative abundance of each chemical compound for every lizard individually, and averages were calculated per species (Supporting Information, Table S2). The total number of different lipophilic compounds found in the sample of a species was considered the species 'chemical richness'. To obtain another measure of the 'chemical diversity' of a species' secretion, we first determined the relative

proportions of nine major chemical compound 'classes' (alcohols, aldehydes, fatty acids, furanones, ketones, steroids, terpenoids, tocopherol and waxy esters) in the mixture and then calculated the Shannon diversity index (H_{chem} , Shannon, 1948).

SIZE AND SHAPE DIMORPHISM

Information on the degree of sexual dimorphism was obtained from the literature (Carretero & Llorente, 1994; Braña, 1996; Adamopoulou, 1999; Herrel *et al.*, 1999; Moravec *et al.*, 1999; Verwajen, Van Damme & Herrel, 2002; Arribas & Carranza, 2004; Herrel, Vanhooydonck & Van Damme, 2004; Arribas, Carranza & Odierna, 2006; Ekner *et al.*, 2008; Lymberakis *et al.*, 2008; Werner & Ashkenazi, 2010; Edwards *et al.*, 2012; Kaliontzopoulou, Carretero & Llorente, 2012a; Kaliontzopoulou *et al.*, 2012b; Yalçinkaya & Göçmen, 2012; Oraie *et al.*, 2013; Sagonas *et al.*, 2014; López-Darias *et al.*, 2015; Nasri *et al.*, 2015; Runemark, Sagonas & Svensson, 2015; see also Supporting Information, Table S3) or from measurements on specimens available in the herpetology collection at the Zoological Research Museum Alexander Koenig in Bonn, Germany. In total, we gathered morphological data of 5960 lizards, with an average of 100 individuals per species (range 12–1071). We only measured those specimens of that population for which we also collected chemical data or which was geographically closest to the sampled population. We calculated sexual size dimorphism (SD_{size}) as the quotient of the average male snout–vent length (SVL_{M}) over average female SVL (SVL_{F}) if males were larger than females and as $2 - (\text{SVL}_{\text{F}}/\text{SVL}_{\text{M}})$ if females were larger (Lovich & Gibbons, 1992; Smith, 1999). In lizards, male-biased sexual size dimorphism is generally thought to arise from intrasexual selection for increased fighting capacity in males (Braña, 1996; Cox, Skelly & John-Alder, 2003). On the other hand, fecundity selection may promote relatively larger body sizes in females, leading to female-biased sexual dimorphism (Shine, 1989; Braña, 1996; Cox *et al.*, 2003). In addition, competition avoidance may select for different body sizes in males and females (Shine, 1989; Braña, 1996). Hence, sexual size dimorphism may not adequately reflect the intensity of sexual selection in lizards. In an attempt to circumvent this problem, we also calculated a measure of sexual shape dimorphism (SD_{shape}). The shape-factor considered was the quotient of head length over trunk length. Head size in male lizards is associated with bite force and fighting capacity (e.g. Huyghe *et al.*, 2005; Husak *et al.*, 2006), so dimorphism relative head size is likely to reflect the intensity of sexual selection on males of a species. Relative head size was calculated and averaged for males and females respectively, after which SD_{shape} was calculated using the same formulas used to

compute SD_{size} . Interestingly, (although somewhat complicating things), in our study system, SD_{shape} and SD_{size} tend to evolve in opposite directions, such that species showing low SD_{size} tend to exhibit high SD_{shape} and vice versa (see Results for statistics). As a consequence, neither SD_{shape} nor SD_{size} may adequately reflect the intensity of sexual selection. We therefore calculated a third measure of sexual dimorphism (SD_{comb}), by ranking species to SD_{shape} and SD_{size} , respectively, then taking the sum of both ranks per species.

PHYLOGENY AND STATISTICS

We used a tree described by Baeckens *et al.* (2017b) to analyse our data in a phylogenetic setting. The tree was constructed with information on sequences from three mitochondrial and two nuclear gene regions. We pruned the tree to include only the 60 species for which we found data. We used phylogenetic principal component analysis (pPCA) to summarize the among-species variation in chemical signal design ('*phyl.pca*' function in the '*phytools*' package in R, Revell, 2012). The input variables were signal richness (i.e. total number of lipophilic chemical compounds detected, square-root transformed), chemical diversity (H_{chem}) and the proportions of steroids, fatty acids, alcohols, aldehydes, terpenoids, ketones, furanones, waxy esters and tocopherol in the secretion (arcsin transformed). We used the '*lambda*' method in *phyl.pca* to optimize λ on the (0, 1) interval. The pPCA produced four combinatory axes. The species scores on each of these axes were then correlated to the three measures of dimorphism (SD_{size} , SD_{shape} , SD_{comb}) using phylogenetic generalized least squares regression (pGLS). Each regression was run thrice, with three different correlation structures, respectively, derived from a Brownian motion model ('*corBrownian*', Felsenstein, 1985), an Ornstein–Uhlenbeck model ('*corMartins*', Martins & Hansen, 1997) or Pagel's lambda model (which multiplies the covariances in the matrix by λ , a value between 0 and 1 that is optimized by maximum likelihood, '*corPagel*', Pagel, 1999). We ran these regressions using the '*gls*' function in the '*ape*' package in R (Paradis, Claude & Strimmer, 2004). Because the lambda model often failed to converge in *ape*, we also fitted relationships with the Pagel's lambda model using the '*pGLS*' function in the package '*caper*' (Orme *et al.*, 2013). We then compared the fit of the models using the Bayesian information criterion (BIC) and based our conclusions on the results of the model with the lowest BIC value. We calculated Blomberg's K (Blomberg, Garland & Ives, 2003) and Pagel's λ (Pagel, 1999) to index the 'phylogenetic signal' present in the three measures of sexual dimorphism and in the chemical signature. This was done using the '*phylosig*' command in the '*phytools*' package in R.

RESULTS

Sexual size dimorphism (SD_{size}) in our data set ranged from 0.88 in *Zootoca vivipara* to 1.31 in *Podarcis pit-yusensis* (see Supporting Information, Table S3). In 14 out of 60 species, SD_{size} was female biased; in the other 40 species, SD_{size} was male biased. Sexual size dimorphism exhibited significant phylogenetic signal (Blomberg's $K = 0.41$, $P = 0.009$; Pagel's $\lambda = 0.60$, $P = 0.002$; Fig. 1). Larger species tended to be more size-dimorphic (pGLS $SD_{size} \sim SVL_M$, $r^2 = 0.17$, $P = 0.001$, coefficient \pm SE = 0.22 ± 0.06).

Sexual shape dimorphism (SD_{shape}) ranged from 0.94 in *H. guentheri* to 1.32 in *Podarcis virescens*, but only one species had a SD_{shape} below 1, indicating that males of lacertid species almost always have larger head-trunk ratios than females. SD_{shape} also showed significant phylogenetic signal ($K = 0.498$, $P = 0.001$; $\lambda = 0.74$, $P = 0.0001$; Fig. 1). Shape dimorphism did not change with body size (pGLS $SD_{shape} \sim SVL_M$, $r^2 = 0.006$, $P = 0.56$). Interestingly, SD_{size} and SD_{shape} were inversely related (pGLS $SD_{shape} \sim SD_{size}$, $r^2 = 0.23$, $P = 0.0001$, coefficient \pm SE = -0.44 ± 0.11).

In this 60 species data set, chemical signal richness varied between 14 number of compounds (for *Ophisops elegans*) and 103 (for *Gallotia galloti*). The average (\pm SE) chemical richness was $51 (\pm 3)$. Richness showed a moderate but significant phylogenetic signal ($\lambda = 0.70$, $P = 0.011$; $K = 0.38$, $P = 0.011$). Chemical signal diversity ranged from 0.19 (*Dalmatolacerta oxycephala*) to 1.56 (*Podarcis peloponnesiacus*), with a species average of 0.80 ± 0.04 (see Supporting Information, Table S2). The phylogenetic signal for chemical signal diversity was not significant ($\lambda = 0.35$, $P = 0.10$; $K = 0.33$, $P = 0.06$). Species with a high chemical signal richness also had a high chemical diversity (pGLS $R_{chem} \sim H_{chem}$, $r^2 = 0.15$, $P = 0.0025$; Fig. 2).

The phylogenetic principal component analysis combined the 11 original chemical variables into four new axes. Together, these four axes explained 69.9% of the total variation (31.6, 14.8, 12.8 and 10.7%, respectively). The factor loadings for the four principal components are shown in Table 1. The first axis, PC1, separated species that had relatively 'simple' chemical signals (i.e. small number of compounds, low diversity) from species with more elaborate signals. Simple signals tended to contain large proportions of steroids, while more complex secretions held high proportions of ketones and aldehydes. The second axis correlated negatively with the proportions of alcohols and furanones but positively with chemical richness. PC3 had high negative loadings for the proportion of ketones; PC4 correlated positively with tocopherol concentrations. The first three principal components exhibited significant phylogenetic signal; the fourth axis did not (Table 1).

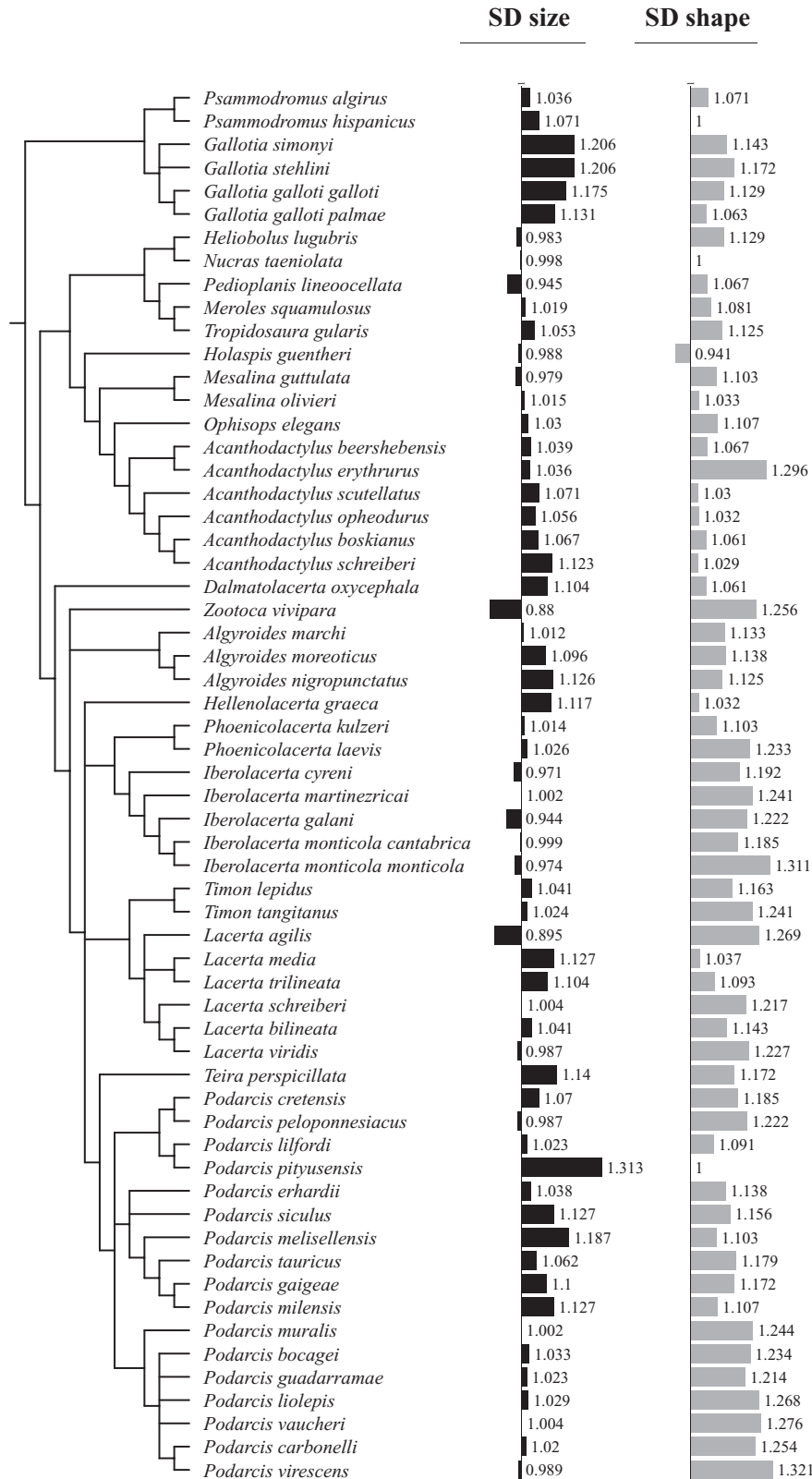


Figure 1. Visual representation of the two measures of sexual dimorphism (left: sexual size dimorphism, SD_{size} ; right: sexual shape dimorphism, SD_{shape}) along the hypothesized phylogenetic tree of lacertid lizards. Bars show the degree of sexual dimorphism for each species. The SD values are displayed alongside the bars. Bars are set zero at value 1.

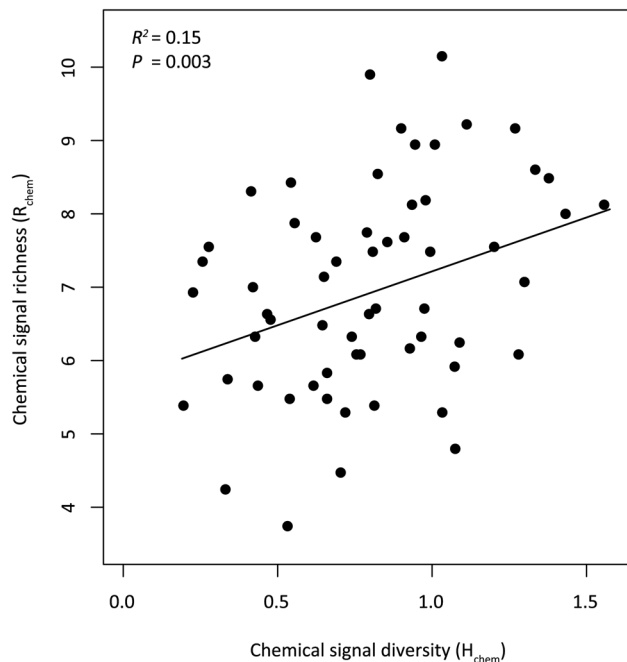


Figure 2. Scatterplot illustrating the relationship between chemical richness (R_{chem}) and chemical diversity (H_{chem}) in the femoral gland secretions of lacertid lizards. Note that R_{chem} is square-root transformed.

When analysing the relationship between the three dimorphism measures (SD_{size} , SD_{shape} , SD_{comb}) and the conjoined chemical variables (PC1–PC4), pGLS models with likelihood optimization of Pagel’s λ almost always yielded lower BIC values than Brownian motion or Ornstein–Uhlenbeck models. Only for the relationship between the SD measures and PC4, the Ornstein–Uhlenbeck model gave a slightly better fit. Overall, we found very little support for the idea that among-species variation in the elaborateness or the compositional structure of chemical signals in lacertid lizards is influenced by the intensity of sexual selection. Species scores on PC3 correlated positively with SD_{shape} ($P = 0.023$), but differences in shape dimorphism explained merely 8% of the variation in PC3, so we are reluctant to emphasize this result. For none of the other three principal component axes, we found any significant association with any of the three measures of dimorphism (Table 2).

DISCUSSION

Contrary to our expectations, we found no evidence for a relationship between the degree of sexual dimorphism in lacertid lizards and the chemical complexity or structure of their glandular secretion composition. We can think of two broad categories of explanations for this result. First, our fundamental premise was wrong, and sexual selection is simply not driving the evolution of chemical signals in these lizards. Second, more

intense sexual selection does produce more complex chemical signals, but the variables and/or methods we used were ineffective in revealing the connection.

In principle, signals are likely to become more elaborate if complexity contributes to either their information content or the efficacy with which this information can be conveyed. Content-based explanations of sexual signal elaboration maintain that complex signals contain more (‘multiple message’ hypothesis) or more reliable (‘redundant message’ hypothesis) information on the sender’s quality (Møller & Pomiankowski, 1993). Efficacy-related hypotheses argue that complexity will evolve to uphold information transfer in different environments or to maximize responsiveness in different receivers (Endler, 1992, 1993; Hebets & Papaj, 2005). In a sexual selection context, the sender is most often a male, broadcasting information about aspects of its quality towards rival males, females on heat or both. Intersexual selection through female choice is generally regarded the most important driver of signal evolution (Andersson, 1994; Ord *et al.*, 2001), but, in lizards, female choice seems rare (Olsson & Madsen, 1995; Tokarz, 1995; LeBas & Marshall, 2000; Lailvaux & Irschick, 2006; Ord *et al.*, 2015). Accordingly, the evolution of elaborate coloration, ornamentation or display behaviour in lizards is usually explained in terms of intensified intrasexual selection (Ord *et al.*, 2001; Chen *et al.*, 2012; Pérez i de Lanuza *et al.*, 2013). For instance, Ord *et al.* (2001) argue that the complex behavioural displays of some agamid and iguanid lizards inform rival males on various aspects of the sender’s fighting

Table 1. Factor loadings of the original chemical variables on the first four combinatory axes produced by phylogenetic principal component axis. Also listed is the amount of total variance explained by each axis and two measures for the phylogenetic signal in the components: Blomberg's K and Pagel's λ . The P -values indicate the probability that the signal is equal to zero

	pPC1	pPC2	pPC3	pPC4
Chemical diversity	-0.940	-0.030	-0.010	-0.132
Chemical richness	-0.516	0.587	0.260	-0.069
Steroids	0.833	-0.076	-0.407	-0.121
Fatty acids	-0.409	0.195	0.268	-0.813
Alcohols	-0.300	-0.749	0.425	0.009
Aldehydes	-0.584	-0.175	-0.480	0.098
Terpenoids	-0.440	-0.019	-0.424	-0.032
Ketones	-0.638	-0.048	-0.505	0.015
Furanones	-0.454	-0.614	0.202	0.193
Waxy esters	-0.405	0.298	-0.250	0.226
Tocopherols	-0.253	0.422	0.388	0.618
% variance	31.6	14.8	12.8	10.7
K	0.375	0.664	0.449	0.319
P	0.009	0.001	0.004	0.09
λ	0.545	0.881	0.788	0.260
P	0.042	< 0.0001	0.007	0.74

Bold P -values indicate statistical significance. Factor loadings higher than $|0.500|$ are also shown in bold.

capacity, knowledge that can be used in decisions on whether or not to engage in a fight and risk injuries. [Chen *et al.* \(2012\)](#) suggest that complex coloration may promote contest success in Australian agamids. Along a very similar line, [Pérez i de Lanuza *et al.* \(2013\)](#) propose that body coloration of lacertid lizards may provide important clues to the sender's genetic quality and/or condition, thus advancing mate assessment during agonistic interactions. Could there be a reason why chemical signals are not responding in similar ways to increased sexual selection pressures? One obvious reason would be that they are not involved in mate selection or rival assessment at all, but serve some other function. Indeed, at least some lacertids can discriminate between conspecific and non-specific scent marks ([Barbosa *et al.*, 2005, 2006](#); [Gabirot *et al.*, 2010a, b](#)), suggesting that species recognition requirements may be a factor in the evolution of chemical signals. On the other hand, multiple studies have also documented that scent marks of lacertid males contain clues to the quality of the depositor, clues that can be picked up by rival males ([López & Martín, 2002, 2011](#); [Carazo, Font & Desfilis, 2007](#); [Martín & López, 2007](#)) and even by prospecting females ([Martín & López, 2000, 2006b](#); [Olsson *et al.*, 2003](#); [Martín *et al.*, 2007a, b](#); [Kopena *et al.*, 2011](#)). Although the putative importance of species recognition as an agent of chemical signal complexity remains to be tested in lacertids, it seems rash to give up sexual selection as a possible player at this moment. Perhaps

Table 2. Relationships between sexual size dimorphism (SD_{size}), sexual shape dimorphism (SD_{shape}) and the combined measure of dimorphism (SD_{comb}) as revealed by phylogenetic generalized least squares regression (pgls)

		λ	Slope	r^2	P
SD_{size}	~PC1	0.720	1.460	0.024	0.27
	~PC2	0.901	0.980	0.025	0.22
	~PC3	0.756	-0.580	0.009	0.46
	~PC4	0.000	0.070	0.000	0.92
SD_{shape}	~PC1	0.633	-1.350	0.020	0.24
	~PC2	0.880	-0.230	0.002	0.77
	~PC3	0.849	1.620	0.085	0.023
	~PC4	0.000	-0.540	0.014	0.36
SD_{comb}	~PC1	0.456	-0.005	0.015	0.34
	~PC2	0.898	0.005	0.005	0.18
	~PC3	0.858	0.006	0.038	0.13
	~PC4	0.000	-0.001	0.004	0.65

Results shown are for a model in which Pagel's λ was optimized by maximum likelihood. Bold indicates statistical significance.

conveying multiple or redundant messages through chemicals is problematic. Complex behavioural displays tend to consist of distinct 'building blocks' (e.g. push-ups, shudders, head bobs, dewlap displays in lizards), each of which can reflect a particular aspect of the displaying individual, or may be more effective in a particular environment (e.g. [Ord *et al.*, 2001](#); [Kelso & Martins,](#)

2008). Vocal signals can also contain different types of phrases that can easily be distinguished and have different functions (e.g. [Leboucher *et al.*, 1998](#)). Animals that use colours to communicate often have multiple badges or distinctly coloured body parts, each of which may reflect different aspects of the sender's identity or quality, may be aimed at different receivers, or may work better in particular environments (e.g. [Vergara & Fargallo, 2011](#)). Obviously, multimodal signals also have this 'modular' structure that may facilitate elaboration. It is not sure whether the same applies to lizard chemical signals. Studies on insect and mammal pheromones suggest that the respective molecules of composite chemical signals may work not only additively or synergistically ([Beynon & Hurst, 2003](#); [Greene *et al.*, 2016](#)) but also antagonistically (e.g. [Moore & Liebig, 2010](#)). Assessing whether sexual selection can act on the complexity of lizard chemical signals the way it does in visual, vocal or multimodal signals will require more detailed information on how individual molecular components, in isolation and in synergy, affect the behaviour of other individuals. Lastly, it seems important to note that although femoral gland secretions are generally believed to be the leading source of chemical signals in lizards, there is evidence that faeces, cloacal secretions and skin lipids may also contain socially relevant chemical stimuli ([Mason & Parker, 2010](#)). The ultimate challenge for chemical ecologists is to integrate information from all signalling sources in their study design.

A second series of reasons why we may have failed to establish a relationship between the intensity of sexual selection and the compositional complexity of chemical signals in our study system has to do with methodological issues. First, we have not measured the intensity of sexual selection but instead used the degree of sexual dimorphism as a proxy. Sexual dimorphism (most often, sexual size dimorphism) is customary used as an index for the intensity of sexual selection across a wide variety of taxa (e.g. [Clutton-Brock, Harvey & Rudder, 1977](#); [Price & Lanyon, 2003](#); [Lootvoet, Philippon & Bessa-Gomes, 2015](#)), including lizards (e.g. [Stuart-Fox & Ord, 2004](#); [Östman & Stuart-Fox, 2011](#); [Chen *et al.*, 2012](#)). Although comparative studies across animal taxa have usually confirmed that variation in the intensity of sexual selection to some extent contributes to sexual dimorphism (e.g. [Székely, Reynolds & Figuerola, 2000](#); [Dunn, Whittingham & Pitcher, 2001](#); [Lindenfors, Gittleman & Jones, 2007](#); [Fairbairn, 2013](#)), many of these studies have also indicated a possible role for other adaptive and non-adaptive forces. In an analysis considering 302 lizard species from 18 families, [Cox *et al.* \(2003\)](#) found that SD_{size} correlates not only with several indices of intrasexual selection (i.e. male aggressiveness, territoriality, male-to-female home range ratios) but also with measures of fecundity selection (i.e. clutch

size, reproductive frequency, reproductive mode). [Cox *et al.* \(2003\)](#) further noted that intrasexual selection and fecundity selection conjointly explained only 16% of the variation in SD_{size} and urged considering alternative routes to SD_{size} , such as intersexual trophic niche divergence or sex-specific energy allocation. In a study comparing dimorphism among eight species of lacertids, [Braña \(1996\)](#) found evidence not only for fecundity selection on SD_{size} but also for sexual selection on male body size. Because in our data set, males are larger than females in most species, and males have larger heads than females in all species, we think that the variation in sexual dimorphism is probably an effect of differential intrasexual selection. However, given the considerations above, a thorough study of the origins of sexual dimorphism in this group is needed to substantiate this assumption. Adding to complexity, the two measures of sexual dimorphism used in this study (SD_{size} and SD_{shape}) turned out to be negatively correlated, suggesting some kind of trade-off: species that have evolved size dimorphism cannot (or need not to) evolve sexual shape dimorphism and vice versa. We think that this finding is noteworthy and deserves further investigation, but in the current context, it also complicates the classification of a species on the 'scale of dimorphism'. We have tried to circumvent this problem using the combined SD_{comb} measure, but remain unsure whether any of the three measures of dimorphism adequately captures the intensity of sexual selection on males in this species. Future studies will have to look for more reliable proxies (e.g. testes size, territoriality, aggressiveness, home-range measures) or actually measure sexual selection gradients (which will be challenging, [Fitze & Le Galliard, 2011](#)).

Even more than our indices of the intensity of sexual selection, our measures of signal complexity and design are open to criticism. We simply do not know, for instance, whether lizard chemical deposits with more constituents or a higher chemical diversity are more 'complex' in the sense that they carry more or more clear-cut messages, preserve better, or travel more easily through the distinctive environments. Only for a small subset of the molecules encountered in lizard femoral deposits do we have information – or hypotheses – on how they might function. For instance, high concentrations of cholesterol, campesterol and γ -sitosterol, and some free fatty acids may signal social dominance and mate attractiveness ([López *et al.*, 2006](#); [Martín & López, 2006b](#)); levels of cholesta-5-7-dien-3-ol, ergosterol and tocopherol may indicate aspects of immune capacity ([Martín & López, 2006b](#); [Kopena, López & Martín, 2014](#)); the relative proportions of some fatty acids vs. some steroids may be indicative of the depositor's age ([Martín & López, 2006a](#)); cholesterol may also serve as an inactive matrix, reducing the volatilization of other, lighter compounds ([Escobar *et al.*, 2003](#)). These studies

have typically been conducted on one or two species, and it is unclear in how far their results can be extrapolated to other species. The individual significance (if any) of the other compounds remains unknown. As indicated above, we also do not know whether the effects of chemical elements are additive or work synergistically or antagonistically. Our approach here may be overly atomistic, as if we were comparing 'Finnegans Wake' (Joyce, 1939) to 'The Very Hungry Caterpillar' (Carle, 1969) by counting the number of different letters used. Lastly, we lack information on how variation in the chemical design is linked with sexual dimorphism variation on an *intraspecific* level. Although the main chemical profile of lacertid species (i.e. presence/absence of major compounds and relative importance of each compound) is always maintained, there is still a measurable inter-individual variation in the relative proportions of compounds in gland secretions. While this intraspecific variation is much smaller than the observed variation among species, even for closely related ones (e.g. Gabirot *et al.*, 2010a, b), it is not unlikely that species, especially those inhabiting a large geographical area, might reveal larger within-species variation. For example, the lacertid *Acanthodactylus boskianus* is the most widespread species of its genus, inhabiting arid regions spanning from North Africa across to western India (Tamar *et al.*, 2016), and exhibits considerable intraspecific variability in morphology (Arnold, 1983; Harris & Arnold, 2000) and phylogenetic complexity (Tamar *et al.*, 2014). Interpopulational variation also seems apparent in the chemical signal richness of the species, as lizards of the Egyptian population carry a richer signal (Khannoon *et al.*, 2011, 2013) than the Israeli population we sampled. Further research should focus on broad-scale within-species variation in lizard's chemical signal design, and *A. boskianus* or *Z. vivipara* seem ideal candidate species due to their large geographical distribution.

In conclusion, our analyses do not support the hypothesis that the chemical signal complexity of lacertid glandular secretions has evolved in response to changes in the intensity of sexual selection. We may have mismeasured sexual selection pressure or signal complexity, or the intraspecific variation in semiochemical composition originated via other evolutionary mechanisms. Further research should focus on alternative forces driving chemical signal variation, such as the effect of variation in environmental conditions or diet.

ACKNOWLEDGEMENTS

We thank Dennis Rödder, Wolfgang Böhme, and Ursula Bott for allowing us to use the lacertid collection of the Zoological Research Museum Alexander Koenig (Bonn,

Germany) and for their hospitality; Panayiotis Pafilis, Chris Broeckhoven, and Shai Meiri for help in the field; Marta Calvo and Alberto Sánchez Vialas of the Museo Nacional de Ciencias Naturales (Madrid, Spain) for the use of the herpetological collection; Shelley Edwards for providing additional morphological data. We also thank the two anonymous reviewers, whose input significantly improved drafts of this manuscript. This work was conducted under permits for Croatia (UP/I-612-07/14-48/111 and UP/I-612-07/14-48/33), The Netherlands (FF/74A/2015/009), Israel (2014/40323), SA Free State Province (S54C-515022511060), SA Eastern Cape Province (CRO 45/15CR and 46/15CR), SA Western Cape Province (0056-AAA041-00093), SA Northern Cape Province (FAUNA 229/2015 and 230/2015) and SA Limpopo Province (0092-MKT001-00004), and in accordance with University of Antwerp (Belgium) animal welfare standards and protocols (ECD 2014-32). Captures of lizards and sampling procedures were performed under different licenses for the Environmental Agencies of the different Regional Governments of Spain where lizards were studied. All Greek species were collected in accordance with the Hellenic National Legislation (Presidential Decree 67/81). This work was part of S.B.'s doctoral thesis at the University of Antwerp.

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SUPPORTING INFORMATION

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

Table S1. Catch-locality of the sampled lacertid lizard species. Geographic coordinates are presented by latitude (Lat.) and longitude (Lon.).

Table S2. Chemical composition of the lipophilic fraction of femoral secretions of the lacertid species considered in this study. Indicated are the relative contributions (in %) of nine major chemical classes to the total content, and the chemical richness (R_{chem} , total number of compounds) and diversity (H_{chem} , the Shannon–Wiener index). Also the sample size of each species is shown (N).

Table S3. Morphometric data for the 60 species considered in this study. Indicated are male and female snout-vent length (SVL), trunk length (BL), head length (HL) and the sample size (N), alongside the calculated measures of dimorphism size (SD_{size}) and shape (SD_{shape}) and the combined index (SD_{comb}).