

Reliable proxies for glandular secretion production in lacertid lizards

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Abstract. The epidermal glands of lizards are considered an important source of semiochemicals involved in lizard communication. Many features of the lizard epidermal gland system vary among and within species (e.g., gland number, size, and shape), and some are believed to reflect the degree of intra- and interspecific variation in glandular secretion production, and by extension, the chemical signalling investment of lizards. Traditionally, herpetologists estimate secretion production based on the number of glands or the size of the glands, rather than quantifying the amount of secretion produced. Still, the reliability of these proxies for secretion production has never been validated. Here, I explored the relationship among secretion production (in mass), pore size (surface area, diameter), and gland number in three species of lacertid lizards (*Acanthodactylus boskianus*, *Timon lepidus*, *Holaspis guentheri*), and tested which proxies predicted secretion production variation best, and examined whether the same trend is true for all species. The findings of this study show that the total secretion production of lacertids is highly variable among and within species. Variation in secretion production among-species (but not within-species) could partly be explained by variation in body size. While both measures of pore size were positively related with secretion production, my tests revealed the model with only pore diameter as contributing variable explaining absolute secretion production variation (both within and across species) as the best one. Although gland number appeared a suboptimal estimate for secretion production in the three lacertids under study, only family-wide, multi-species comparative tests counting large within-species sample sizes can provide further insight on the matter.

Keywords. Chemical signals; epidermal gland secretions; femoral pores, Lacertidae, secretion quantity.

Chemical signals are essential for inter- and intra-sexual communication in many animals, and lizards represent no exception (Mason and Parker, 2010). Yet, the extent to which lizards utilize their chemosensory system varies greatly among species (Baeckens et al., 2017a, b). This phenomenon seems also true for the signalling system of lizards, which is illustrated by the fact that merely half of all non-ophidian squamate species are equipped with epidermal glands (lizards' leading source of socially relevant chemical signals; Mayerl et al., 2015). It is even so that the number of epidermal glands that lizards possess varies among (and sometimes even within) species (Martín and López, 2000; Pincheira-Donoso et al., 2008;

Baekens et al., 2015). In search for the constraints and selective pressures driving this variation in chemical signalling investment, researchers have focussed on various morphological characteristics of the lizard epidermal gland system to quantify chemical signalling investment, and ultimately, to compare among lizards. Based on the premise that overall secretion production (thus 'secretion quantity') reflects how much a particular lizard invests in and relies upon chemical signalling, herpetologists traditionally use gland number and/or the size of the gland opening (i.e., pore) as proxies for secretion quantity (Alberts et al., 1992; Escobar et al., 2001; Pincheira-Donoso et al., 2008; Iraeta et al., 2011; Valdecantos et al.,

2014; Baeckens et al., 2015, 2017c). However, whether these features are truly reliable measures for a lizard's total amount of secretion production (and reliable for interspecific comparisons) has never been validated.

In this study, I quantify the overall secretion production (in mass) of lizards of three different lacertid species, and test which characteristics predict secretion production best (gland number, pore diameter, and/or pore surface area).

In total, I obtained 32 adult male lizards (14 *Acanthodactylus boskianus*, 12 *Holaspis guentheri*, 6 *Timon lepidus*) from local reptile hobbyist or through the pet trade (Fantasia Reptiles, Belgium, license HK51101419). Lizards were accommodated at the University of Antwerp facility, and housed in glass terraria (100 x 40 x 50 cm). A 60-watt bulb suspended above one end of the terrarium provided light and heat so that lizards could maintain a body temperature within their preferred range. Lizards had access to freshwater at all times, and were fed up to three times a week. Snout-vent length (SVL) was measured using digital callipers (Mitutuyo, CD-15CPX, accuracy = 0.01 mm). Average pore size was estimated by digitising (ImageJ, Abramoff et al., 2004) the (1) diameter and (2) surface area of the two most proximal pores of the left femur on images obtained with a stereomicroscope (Leica M165 C), and by subsequently calculating mean pore diameter and mean pore surface area per individual (Fig. 1). Next, I collected gland secretions of all lizards, by gently pressing with forceps around the pores until each gland was completely emptied and all secretion yielded (following Baeckens et al., 2017c). Secretion collection occurred within the lizards' reproductive period in June 2014 (Castilla and Bauwens, 1989; Schleich et al.,

1996; Pianka and Vitt, 2003; Khannoon, 2009; Grimm et al., 2014), when they were active (between 10:00 and 16:00 h). Secretions extracted from all glands of the left thigh were directly weighed on a microbalance (Mettler Toledo MT5, accuracy = 1 µg). Prior to statistical analyses in SPSS v. 24 (Chicago, IL, USA), variables were log₁₀ (SVL, pore surface area, pore diameter, secretion mass) or square root (gland number) transformed to meet assumptions of normality (Shapiro-Wilks test: $W \geq 0.95$).

The results of this study show significant intra- and interspecific variation in all aspects of the lizard epidermal gland system, including secretion production (Table 1). While interspecific variation in secretion production could be partially explained by among-species differences in body size (with the largest species in this study, *T. lepidus*, producing high amount of secretion in comparison to the other two smaller lizard species), the observed within-species variation could not (Table 1 and 2). Further, results show that none of the epidermal gland characteristics (gland number, pore area, pore diameter) of lizards belonging to *A. boskianus* and *H. guentheri* were affected by body size (all; Pearson correlation, $r < 0.45$, $P > 0.15$). The surface area and diameter of the pores of *T. lepidus*, however, were strongly linked with body size (resp., $r = 0.94$, $P = 0.006$; $r = 0.95$, $P = 0.005$). Similar to the other species, gland number did not correlate with SVL in *T. lepidus* ($r = 0.08$, $P = 0.89$). Overall, there was no significant relationship between a lizard's number of glands and its total secretion production (Table 2). It was even so that the lizard species with the least number of glands (i.e., *T. lepidus*) was equipped with the largest pores, which moreover produced the largest amount of glandular secretion (Table 1). Pore surface area and pore diameter, on the other hand, correlated strongly with secretion mass in *H. guentheri* and *T. lepidus* but not in *A. boskianus* (Table 2). In *T. lepidus*, where pore size is affected by SVL, a partial correlation test (controlling for SVL) revealed also a positive relationship between relative secretion mass and pore surface area, but not pore diameter. Multiple regression analyses (backward stepwise elimination with gland number, pore diameter, and pore surface area) indicated the model with only pore diameter as the significant contributing independent variable explaining secretion mass variation (*A. boskianus*, $R^2 = 0.31$, $F_{1,13} = 5.35$, $P = 0.039$; *H. guentheri*, $R^2 = 0.45$, $F_{1,11} = 8.27$, $P = 0.017$; *T. lepidus*, $R^2 = 0.79$, $F_{1,5} = 15.32$, $P = 0.017$). The same was true for an analogous multiple regression, but then across species encompassing all individuals ($R^2 = 0.74$, $F_{1,31} = 83.43$, $P < 0.001$, Fig. 2).

Overall, the findings of this study reveal that the total glandular secretion production of lacertid lizards is highly variable among and within species. While body size

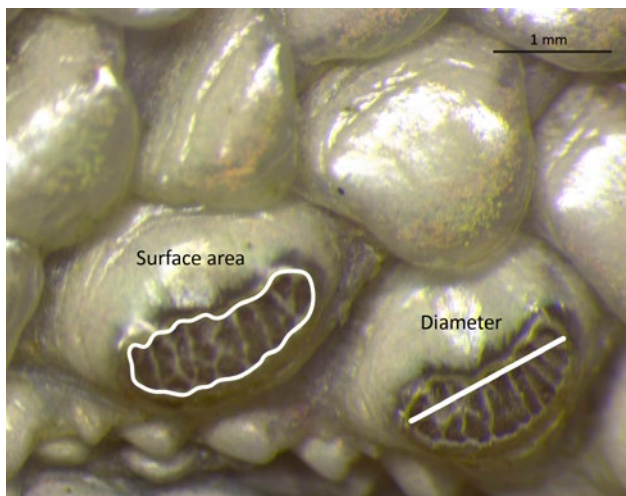


Fig. 1. Measuring pore surface area and pore diameter of the epidermal pores of a *Timon lepidus* lizard.

Table 1. Descriptive statistics for the morphological variables measured on male lacertid lizards of the three species used in this study. The table also shows the results of the analyses of variance (ANOVA) testing for absolute among-species differences (followed by Tukey post-hoc tests), and the results of the analyses of covariance (ANCOVA, with SVL as covariate) testing for relative differences.

	<i>Acanthodactylus boskianus</i> (A) n = 14				<i>Timon lepidus</i> (T) n = 6				<i>Holaspis guentheri</i> (H) n = 12				Interspecific comparison				
	Mean	SE	Min	Max	Mean	SE	Min	Max	Mean	SE	Min	Max	Absolute differences			Relative differences	
													F _{2,31}	P	Post-hoc	F _{2,31}	P
SVL (mm)	73.42	0.90	67.80	81.35	146.20	13.60	119.99	207.46	49.35	1.15	45.21	59.02	213.77	<0.001	T > A > H	—	—
Gland number	26.04	0.42	22.5	28.5	12.75	0.48	12.0	15.0	22.71	0.42	20.5	25.0	203.01	<0.001	A > H > T	0.66	0.527
Pore surface area (mm ²)	1.25	0.04	0.86	1.43	3.11	0.71	1.53	6.08	1.01	0.07	0.39	1.30	23.22	<0.001	T > A = H	1.26	0.299
Pore diameter (mm)	0.49	0.02	0.37	0.66	1.25	0.28	0.61	2.36	0.38	0.02	0.17	0.50	28.26	<0.001	T > A > H	0.55	0.586
Secretion mass (mg)	0.80	0.08	0.29	1.27	11.43	6.23	0.96	35.68	0.79	0.10	0.14	1.47	10.57	<0.001	T > A = H	2.45	0.106

Abbreviations: A = *Acanthodactylus*; H = *Holaspis*; T = *Timon*.

Table 2. Results of Pearson correlation tests (r), testing for correlations between (a) SVL and gland traits, and (b) secretion mass and gland traits. Also shown are the results of partial correlation tests (c), which accounted for differences in SVL. Variables were transformed prior to analyses to meet the assumptions of normality. Bold values indicate statistical significance (P < 0.05).

	<i>Acanthodactylus boskianus</i> (n = 14)		<i>Timon lepidus</i> (n = 6)		<i>Holaspis guentheri</i> (n = 12)	
	r	P	r	P	r	P
(a) Correlation with SVL						
Secretion mass	0.43	0.125	0.74	0.092	-0.12	0.710
Gland number	0.15	0.959	0.76	0.886	0.43	0.163
Pore surface area	-0.01	0.985	0.94	0.006	0.39	0.211
Pore diameter	0.44	0.115	0.94	0.005	0.44	0.156
(b) Correlation with secretion mass						
Gland number	-0.12	0.679	-0.17	0.747	-0.32	0.314
Pore surface area	0.32	0.259	0.91	0.011	0.58	0.048
Pore diameter	0.56	0.039	0.89	0.017	0.67	0.017
(c) Partial correlation with secretion mass						
Gland number	-0.14	0.644	-0.34	0.577	-0.30	0.375
Pore surface area	0.36	0.226	0.92	0.026	0.69	0.020
Pore diameter	0.45	0.121	0.85	0.068	0.81	0.002

was unable to explain the observed intraspecific variation in secretion production in the species under study, a positive link between body size and secretion quantity has been observed in other lizard species, such as in the iguanid *Iguana iguana* (Alberts et al., 1992) and the lacertid *Podarcis muralis* (Baeckens et al., 2017c). Latter researchers argue that since secretion production is most probably an energetically costly affair (Martín and López, 2014; Mayerl et al., 2015), it is very likely that only individuals in a good condition (which are often the largest individuals; Jakob et al., 1996) can afford high secretory activity rates. These discordant findings among studies and species imply that the biosynthetic pathways that produce glandular secretion might be species-specific. An alterna-

tive explanation for not finding a link between body size and secretion production among individuals of the same species concerns the origin of the animals in this study. Since pet store animals generally receive plenty of nutritious food throughout their lives in the store, it is unlikely to find biological significant variation in body condition (linked with secretion production) among individuals. Notwithstanding, large-scale phylogenetical-informed comparative studies could shed light on how idiosyncratic or universal the link between body size and secretion production really is, and how they scale (allometric/isometric) among and within species.

Aside from quantifying (variation in) chemical signalling investment in lacertid lizards, the ultimate aim of this

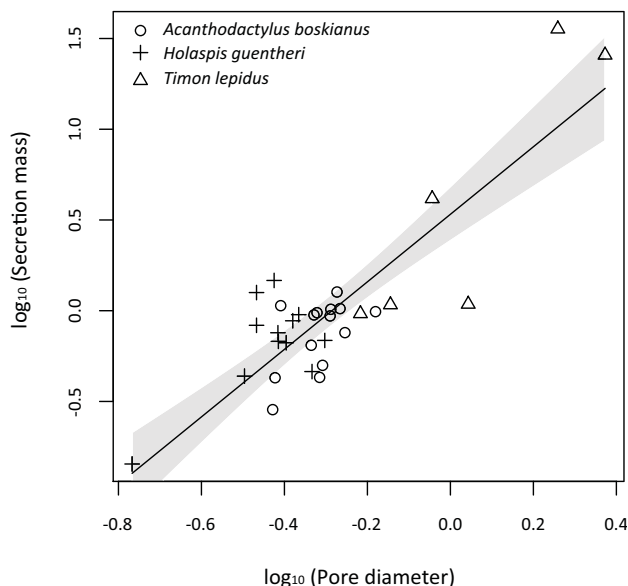


Fig. 2. A scatterplot showing the positive relationship ($R^2 = 0.74$, $F_{1,31} = 83.43$, $P < 0.001$; slope = 1.85) between pore diameter and glandular secretion production in three species of lizards. Because the slopes of the three species did not significantly differ, only one regression line (covering all lizards of the three species) is shown. Shaded area represents 95% confidence intervals.

study was to document reliable proxies, if any, for secretion production. While both measures for pore size were positively correlated with secretion mass, the findings here suggest that pore diameter was the best predictor of secretion production quantity, hence chemical signalling investment. This was true both within species and across species, advocating pore diameter as the most adequate estimate of gland productivity in interspecific comparisons. Alberts and colleagues (1992) established a similar relationship in *I. iguana* males (with $n = 10$, $r = 0.81$, $P = 0.002$), although Baeckens et al. (2017c) did not so in the species *P. muralis*. However, the latter investigators merely measured pore surface area, not pore diameter. Why diameter turned out to be a better predictor of secretion quantity than surface area might be partly ascribed to the shape of the pores. The lacertids under study bared pores of a long-stretched oval form (unlike, for example, those of the teiid *Tupinambis merianae*; Chamut et al. 2009), which varied among individuals largely in length (diameter) and less in area. The lengthy oval shape of the pores might allow lizards to maximize their scent-mark area by increasing the contact zone between pores and substrate (along the proximal-distal limb axis of the limb). More research on lizard scent-marking behaviour and the functional significance of pore shape is necessary to make well-founded predictions on the matter.

Surprisingly, gland number came out as a poor predictor of secretion production in the three lacertids under study here. Yet, several intra- and interspecific comparative studies have assumed that gland number reflects species' investment in and use of chemical communication (e.g., Escobar et al., 2001; Pincheira-Donoso et al., 2008; Iraeta et al., 2011; Baeckens et al., 2015). Notwithstanding, their theory cannot be considered as illogically, for a lizard with x number of glands will produce a lower amount of secretion than a hypothetical identical lizard, but with $x+1$ number of glands. Besides, since this study only comprises three different lizard species belonging to merely one lizard family (Lacertidae), it would be incorrect to generalize and label gland number as a poor proxy reflecting chemical signalling investment in lizards. Clearly, only a broad, multi-species study counting large within-species sample sizes can provide further insight on the matter.

While the goal of this short note was to underscore the importance of choosing the appropriate proxy for lizard secretion production, I wish to note that the findings of this study should be interpreted cautiously due to the following. Firstly, I only used secretion quantity to estimate chemical signalling investment, whilst disregarding secretion 'quality'. The chemical composition of the secretion of lizards is a mixture of proteins and lipids and is highly species-specific (Mayerl et al., 2015; Mangiacotti et al., 2017; Baeckens et al., 2017d). One can easily imagine a trade-off between secretion quantity and 'quality', with lizards producing low amounts of secretion, but investing highly in, for instance, a rich or diverse chemical design with high concentrations of certain key compounds (such as described for some invertebrates; Wyatt, 2014). Future studies on the chemical signalling investment of lizards should, ideally, integrate both the chemical architecture of the glandular secretion and the total amount of secretion produced. Secondly, this study concentrates solely on follicular epidermal gland secretions, while neglecting any other source of semiochemicals. Although it is generally believed that follicular gland secretions are the leading source of semiochemicals (Martín and López, 2014; Mayerl et al., 2015), there is plenty of evidence that generation glands, faeces, cloacal secretions, and skin lipids contain socially relevant chemical stimuli too (Cooper and Vitt, 1984; Mason and Gutzke, 1990; Cooper, 1995; Labra, 2008; Moreira et al., 2008; Mouton et al., 2010). Whether lizards that invest little in the production of gland secretions are investing more strongly in semiochemicals of other origins (and *vice versa*) is, however, uncertain, but certainly not improbable. Thirdly, the use of animals obtained through the commercial pet trade, rather than using life-caught animals from the wild,

might bring along a series of uncertainties concerning pre-purchase animal stress, transport, and housing conditions. Yet, similar to previous experiments using pet trade lizards (obtained through the same commercial dealer as used in the current study; Herrel et al., 2007, Driessens et al., 2014), animals were in good condition at the onset of the experiments. Overall, I am confident that these limitations did not compromise the main objective of this work, which was: quantifying intra- and interspecific variation in secretion production in a small subset of lacertid lizards, and exploring the best possible morphological traits to estimate secretion production.

Based on the findings of this study, I advise scholars, at times when assessing secretion mass seems unfeasible (e.g., in museum specimen), to be cautiously thorough and integrate pore area, diameter, and number in any future studies scoring secretion production quantity. Although gland number played out to be a suboptimal quantity-proxy in the three lacertid lizards under study, broad-scale comparative analyses should examine this in more detail.

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