

Chemical communication in the lacertid lizard *Podarcis muralis*: the functional significance of testosterone

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Abstract

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Chemical signals are essential for intersexual communication in many animals, including lizards. While faeces have been suggested to contain socially relevant chemical stimuli, epidermal gland secretions are generally believed to be the leading source of chemosignals involved in lizard communication. Early research has shown that sex hormones affect epidermal gland activity, with androgens stimulating gland/pore size and/or gland productivity. However, the functional significance of hormone-induced glandular activity in lizard chemical communication remains unclear. In this study, we manipulated testosterone (T) concentrations in male *Podarcis muralis* lizards. While T-supplementation did not change pore size, it did increase secretion production substantially. Chemosensory tests showed that female conspecifics tongue-flick at a higher rate and more quickly towards the secretion of males with experimentally increased T levels than towards the secretion of control males, suggesting that females can discriminate between males with dissimilar T levels based on chemical cues of secretion alone. Based on the scent of faeces, however, females were unable to discriminate between males with differential T levels. Also, females reacted more quickly when offered larger amounts of secretion – irrespective of whether secretions were obtained from control or T-increased males. This result indicates that secretion quantity affects chemosignal detectability in *Podarcis muralis*.

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Introduction

Chemical signalling is the most widespread mode of communication between organisms and plays a key role in intersexual communication in many vertebrate taxa (Müller-Schwarze 2006; Wyatt 2014), including reptiles (Mason and Parker 2010). In lizards, it is generally believed that epidermal gland secretions are the leading source of chemosignals (Martín and López 2014; Mayerl *et al.* 2015), although there is some evidence that faeces, cloacal secretions and skin lipids may also contain socially relevant chemical stimuli (Cooper and Vitt 1984; Mason and Gutzke 1990; Cooper 1995; Labra 2008; Moreira *et al.* 2008). Epidermal glands of most lizards develop at the onset of sexual maturity, and their activity is often greatest in male lizards and during the reproductive

season (Smith 1946; Cole 1966). These early observations strongly suggest that the epidermal glands are controlled by androgens.

Experimental research indicates that castration of male lizards causes glandular atrophy in *Lacerta agilis*, *Podarcis muralis* and *Aspidoscelis inornatus* (Matthey 1929; Regamey 1932; Padoa 1933; Lindzey and Crews 1997). Testosterone (T) supplementation restores gland activity in castrated *Goniurosaurus lichtenfelderi* lizards (Golinski *et al.* 2015) and stimulates gland development in females of the species *Gekko gecko* and *Urosaurus ornatus* (Chiu *et al.* 1970; Hews and Moore 1995). Oestrogen supplementation in males causes glandular atrophy in *Sceloporus* sp. and *Hemidactylus bowringii*, a condition that can be reversed by exogenous androgen administration (Forbes 1941; Chiu *et al.* 1975). Gland activity also

correlates strongly with testicular activity in *Sceloporus undulatus* and *Lacerta agilis* (Regamey 1932; Altland 1941), and testis size in *Karusasaurus polyzonus* (Van Wyk 1990). Most of these studies have focused on proxies of glandular activity, such as epidermal pore size (e.g. Hews and Moore 1995; Lindzey and Crews 1997; Rhen *et al.* 2005); only a limited number of researchers scored secretion activity more directly by measuring glandular size on histological sections (e.g. Chiu *et al.* 1975; Van Wyk 1990) or by collecting and weighing the produced secretion (e.g. Fergusson *et al.* 1985; Alberts *et al.* 1992). In addition, the functional significance of increased glandular activity remains understudied. Martín *et al.* (2007b) established that T implants alter the chemical composition of the epidermal secretions of male Iberian wall lizards (*Podarcis hispanicus*) and that female conspecifics can discriminate between T-implanted and control males based on the scent of male secretions. Martins *et al.* (2006) found that seasonal increases in gland production enable male *Sceloporus graciosus* lizards to scent-mark more localities rather than to mark any single site more heavily, which may facilitate the expansion of a lizard's territory. We, however, propose an additional advantage of the production of large amounts of epidermal secretion in lizards. It is known that the absolute amount of volatile compounds determines the absolute amount of airborne chemicals in a specific environment (Apps *et al.* 2015). Based on the assumption that the proportion of volatile compounds in the epidermal gland secretion remains constant with increasing secretion quantity, we hypothesize that large amounts of secretion will increase the active space of airborne chemosignals and therefore that high secretion quantities will be detected more rapidly by lizard (vomero)lfaction.

Most research on chemical signalling in lizards has focused on chemosignals originating from either epidermal gland secretions or faeces, while studies integrating chemical cues of both origins are scant (but see e.g. Labra *et al.* 2002). Incorporating multiple chemosignal sources within a study may shed light on the importance of specific chemical sources, and on the multicomponent character of a message (Bro-Jørgensen 2010).

In this study, we investigate the effect of epidermal gland secretion quantity on signal transmission in a lacertid lizard, and test whether chemosignals originating from epidermal gland secretions and faeces convey similar information. We manipulate T concentrations in male *Podarcis muralis* lizards using a non-invasive technique described by Knapp and Moore (1997). Our objectives are (i) to quantify the effect of T on the secretory activity of male epidermal glands, (ii) to test the ability of *Podarcis muralis* females to discriminate between control males and males with manipulated T levels based only on chemical cues (originating from epidermal secretion and faeces) and (iii) to determine whether the amount of epidermal secretion affects signal transmission and detectability. The potential benefits of high secretion production rates in lizards will be discussed.

Material and Methods

Animals and their maintenance

The common wall lizard *Podarcis muralis* is a diurnal saxicolous lacertid (adult snout–vent length (SVL) 51–65 mm; this study), widely distributed throughout central and southern Europe (Gassert *et al.* 2013; Michaelides *et al.* 2015). *Podarcis muralis* lizards have on average 15 pore-bearing scales on each thigh, the so-called femoral pores (Baeckens *et al.* 2015). Males of the species are highly territorial (Stamps 1977; Edsman 1986) and use scent to mark their territory (Edsman 1990). Based on chemical cues alone, female *Podarcis muralis* lizards are able to discriminate between male conspecifics of different size (Heathcote *et al.* 2014). Females of several lacertid species are known to exhibit chemically mediated mate choice (Martín and López 2015).

Early in their reproductive season (April 2015), 40 adults *Podarcis muralis* lizards (21 males; 19 females) were captured by noose at two localities in the north of Belgium ('Heverlee' and 'Muizen') and transported in cotton bags to our laboratory at the University of Antwerp. Females lacked ventral mating scars, indicating that females most probably had not mated yet. Males were individually housed in glass terraria (50 × 40 × 50 cm) to avoid physical or visual contact between them, whereas females were kept in group (maximum four females per 100 × 40 × 50 cm terrarium). Each terrarium contained a layer of sandy substrate, some vegetation and slate stones. A 60-watt bulb suspended above one end of the terrarium provided light (12L:12D) and heat that enabled the lizards to maintain a body temperature within their preferred range (i.e. between 30 °C–35 °C; Bauwens *et al.* 1995). The larger terraria were equipped with two light bulbs. Lizards had access to drinking water at all times and were fed moth larvae (*Galleria mellonella*) or crickets (*Acheta domesticus*) dusted with multivitamin powder thrice a week.

Hormonal treatments

In this study, male hormone levels were manipulated using a non-invasive technique based on the methodology described by Knapp and Moore (1997). Elevation of sustained T levels was obtained by transdermal administration of a mixture of the steroid hormone and sesame oil to the lizard. The lipid-rich character of the lizard skin enables lipophilic molecules to pass through the scales of the integument into the blood stream (Quay 1986). Unfortunately, protocolized T-administration doses for *Podarcis muralis* are not available in the literature. Therefore, we based our T dose on a study by Belliure *et al.* (2004) ensuring that a chosen T dose increases T levels – while maintaining levels within the species' estimated physiological range. In Belliure *et al.* (2004), T levels were manipulated in two lacertid species, that is *Acanthodactylus erythrurus* and *Psammodromus*

alginus, which are both comparable to the common wall lizard in body size. In accordance with their study, we created a hormone solution by diluting testosterone powder (4-androsten-17 β -ol-3-one; Sigma #86500) in commercial sesame oil (4 μ g testosterone/ μ L sesame oil). Prior to the experiments, we assigned males to one of two treatment groups: a group with experimentally manipulated T levels ('T-males', $n = 11$), or a control group ('C-males', $n = 10$). Males from the two treatment groups did not differ in SVL (ANOVA; $F_{1,20} = 0.090$; $P = 0.768$).

Males of the experimental T-group received 4 μ L of the hormone dilution every 2 days over a period of 4 weeks (June 2015), while C-males were administered the same amount of sesame oil as a control. Without disturbing the lizards, a droplet of the T mixture or control oil (depending on the treatment group) was deposited onto the lizard's dorsum using a volumetric pipet. To minimize stress, administration was carried out in the early evening when the lights were off and the lizards had become inactive. To validate the hormonal manipulation, we measured male testosterone levels before and after the treatment period using a non-invasive steroid analysis based on faecal droppings. Following extraction of the droppings with 60% methanol (0.5 mL per 20 mg; Palme *et al.* 2013), testosterone (metabolites) were analysed with a testosterone enzyme immunoassay (details see Palme and Möstl 1994). Dry droppings were collected twice for every individual: 2 weeks before the start of the treatment and 3 weeks after the start of the treatment. We used forceps for faecal collection, which were rinsed in 70% ethanol, to avoid cross-contamination. Any visible sand and uric acid remains on the dry droppings were manually scraped off, and individual faecal samples were placed in labelled plastic bags and stored at -20 °C.

Femoral pore size and secretion mass

Snout–vent length was measured using digital callipers (Mitutoyo, CD-15CPX, accuracy = 0.01 mm). Female and male pore size was estimated by digitizing (ImageJ, Abràmoff *et al.* 2004) the circumference of each of the three most proximal pores of the left femur on images obtained with a stereomicroscope (Leica M165 C). Only male *Podarcis muralis* lizards possess active epidermal glands, but merely for quantitative comparative purposes, we measured the pore size of individuals of both sexes.

Next, we collected epidermal gland secretions (from all the glands of the left thigh) of male lizards, by gently squeezing the femoral pores with a forceps until each gland was completely emptied and all secretion yielded. Secretions were directly weighed on a microbalance (Mettler Toledo MT5, accuracy = 1 μ g). The measurements were conducted on the day preceding the hormonal treatment and repeated 21 days after the start of the hormonal treatment. The obtained secretions (after 21 days) were also used for the consecutive chemosensory tests.

Chemosensory tests

Squamates (amphisbaenians, lizards and snakes) react to a variety of chemical stimuli with increased and differential rates of tongue extrusions. Tongue-flick (TF) rates can therefore be used as a quantitative bioassay for the degree of chemosensory excitation (Burghardt 1970, 1980; Cooper 1994, 1998). To examine whether females can distinguish between T- and C-males on the basis of their scent, we observed female TF behaviour towards different stimuli presented on cotton-tipped applicators. This technique is a widely used method in studies on chemical detection in squamates (Cooper 1994, 1998, 2007). This particular part of the study was conducted 21 days after the start of the T- or C-administration.

Cotton swabs were presented to 19 females. These swabs were impregnated with (i) a single portion of male epidermal gland secretion (i.e. all secretion obtained from all glands of one thigh), (ii) a double portion of male secretion (i.e. all secretion obtained from all glands of both thighs) and (iii) male faeces. All three treatments were carried out in twofold, that is with excretions extracted from C-males and from T-males. Also, each female was subjected to an odourless control: a cotton swab impregnated with deionized water (Cooper and Burghardt 1990). In total, all females were subjected to seven different observation trials: one odourless control and six trials with stimuli derived from six different males. We did not implement a pungency control, as it is already well established that lacertid lizards can distinguish the scent of conspecifics from ecologically irrelevant scents (e.g. Cooper and Pérez-Mellado 2002). As a default preparation, we dipped the cotton tip of the wooden applicator in deionized water before applying secretion or faeces onto the tip. Epidermal gland secretions were extracted by pressing gently with forceps around the pores and were gathered on the swab after the secretions were weighed. Pending on the trial, we collected secretion from only one (single portion) or both (double portion) thighs. For the faecal trial, we made use of the fact that lizards often defecate when handled. As such, fresh male faeces were applied onto the cotton swab.

Prior to the experiments, female lizards were placed individually in a novel cage (50 \times 25 \times 30 cm), which contained slate stones under which the lizards could seek refuge. Each female was exposed to the scent stimuli in a randomized order, and with a minimum interval of 30 min between subsequent trials. Females were solely subjected to the scent of males of their own population. All observations were performed during the lizards' peak activity hours (10:00–16:00).

After preparation of the swab, one of the researchers (SB) quietly approached the female's cage and moved the cotton swab slowly in a straight line towards the lizard until it was approximately 2 cm anterior to the snout (Fig. 1). Subsequently, two variables were scored: (i) the time period – or 'latency' – to the first elicited TF, starting from the moment the cotton swab was in position; and (ii) the total number of elicited TFs during 60 s, starting from the first TF.

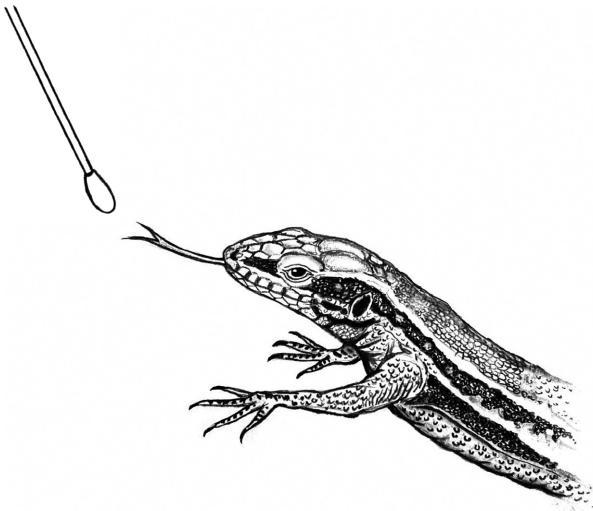


Fig. 1—An illustration of the cotton-swab technique designed to test lizard's tongue-flick behaviour towards chemical stimuli. Drawn by Detlef Tibax.

Statistics

Prior to analyses, all variables were \log_{10} -transformed to meet assumptions of normality (Shapiro–Wilks test with $W \geq 0.95$). Within each lizard, femoral pore size – determined as pore surface area – did not differ between pore number one, two and three (repeated-measure ANOVA, Wilk's $\lambda = 0.973$, $F_{2,19} = 0.234$, $P = 0.794$). Therefore, the average size of all three pores was calculated for each individual and used in further analyses. Relationships between average femoral pore size, secretion mass and SVL were explored using Pearson's correlations. To test for changes in pore size, secretion mass and testosterone levels before and after the treatment period, we used repeated-measure ANOVAs with 'time' (before versus after) as within-subject variable and 'treatment' (C versus T) as between-subject variable testing for differences in changes between C- and T-males. When necessary, an ANCOVA was used with SVL as covariate. Repeated-measures ANOVAs were used to examine differences in TF behaviour with 'scent stimuli' as within-subject factor. Pairwise comparisons were carried out using Tukey's honestly significant difference (HSD) tests. All statistical analyses were conducted in IBM SPSS Statistics for Macintosh v. 22.0 (Chicago, IL, USA), and probabilities lower than 0.05 were considered statistically significant.

Results

Validation of the hormonal treatment

Prior to the administration period, T levels did not differ between C- and T-males (average faecal steroid concentration \pm SE: 31.3 ± 10.1 ng/g for C-males versus 30.4 ± 6.0 ng/g

for T-males; ANOVA, $F_{1,18} = 0.224$, $P = 0.642$). After 3 weeks of treatment, lizards from the T-group showed a significant increase in faecal testosterone concentration, whereas males from the control group exhibited even a minor decrease in testosterone levels (repeated-measure ANOVA, 'treatment' \times 'time'; $F_{1,18} = 11.484$, $P = 0.001$), with 10.7 ± 5.6 ng/g for C-males versus 96.9 ± 23.3 ng/g for T-males (Fig. 2). The small T-drop in the control group is most probably linked with an inevitable stress increase due to captive conditions: a common phenomenon, observed in various reptile taxa (e.g. Lance and Elsey 1986; Moore *et al.* 1991).

Femoral pore size and secretion mass

Average femoral pore size differed significantly between sexes, with males having larger pores than females (average pore size \pm SE: $638 \pm 45 \mu\text{m}^2$ for males versus $364 \pm 18 \mu\text{m}^2$ for females; ANOVA, $F_{1,39} = 44.596$, $P < 0.001$). Neither male nor female pore size correlated with SVL (male, $r^2 = 0.033$, $P = 0.427$; female, $r^2 < 0.001$, $P = 0.982$). Prior to the administration period, femoral pore size did not differ between C- and T-males (ANOVA; $F_{1,20} = 0.729$, $P = 0.405$). After an administration period of 3 weeks, pore size had not changed (repeated-measure ANOVA, 'time' effect; $F_{1,20} = 1.325$, $P = 0.266$) and did not differ between treatments (repeated-measure ANOVA, 'treatment' effect; $F_{1,20} = 1.439$, $P = 0.247$).

Larger males produced larger quantities of epidermal gland secretions ($r^2 = 0.263$, $P = 0.009$, Fig. 3). Prior to the

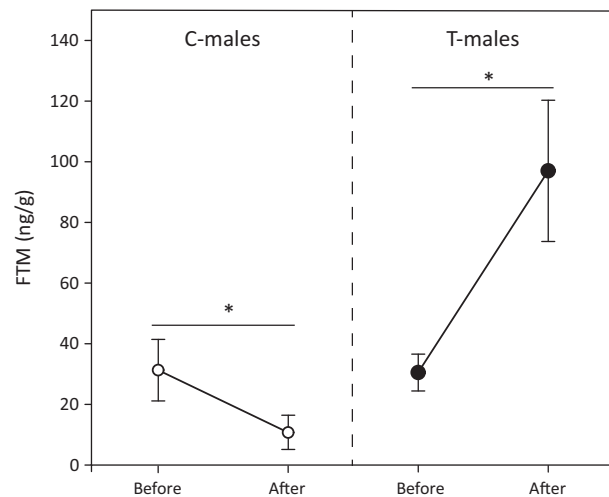


Fig. 2—Mean (\pm SE) faecal testosterone (metabolite) concentrations of *Podarcis muralis* males from the experimental group, which received a testosterone treatment (T-males) and males from the control group (C-males). Means are shown 'before' and 'after' the 3-week administration period. Steroid concentration is presented as the mass of immunoreactive testosterone metabolites per dried faecal mass. FTM = faecal testosterone metabolites. Asterisks indicate statistical differences.

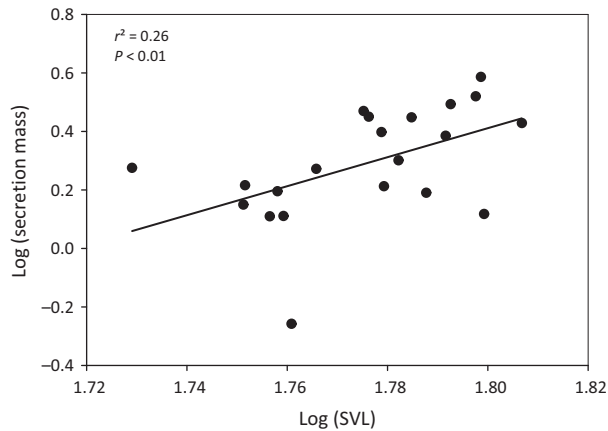


Fig. 3—Relationship between epidermal gland secretion mass and snout–vent length (SVL) in *Podarcis muralis* males.

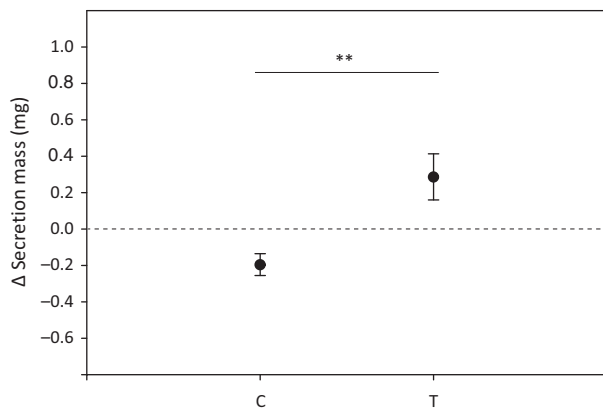


Fig. 4—Mean (\pm SE) difference in epidermal secretion mass (i.e. secretion mass after 3-week administration period subtracted by secretion mass before administration period) for control males (C) and males with experimentally increased testosterone levels (T). Asterisks indicate statistical differences.

administration period, the amount of secretion that could be obtained from the males' glands did not differ between the C- and the T-group (average secretion mass \pm SE: 2.067 ± 0.204 mg for C-males versus 2.156 ± 0.302 mg for T-males; ANCOVA; $F_{1,20} = 0.001$, $P = 0.861$). After 3 weeks of C- and T-administration, lizards from the T-group showed an increase in secretion production, whereas C-males showed a decrease (repeated-measure ANCOVA, 'treatment' \times 'time'; $F_{1,20} = 13.325$, $P = 0.002$; Fig. 4). Compared to the sampling prior to the administration period, the amount of secretion that could be obtained from the glands was lower in C-males (-9.51%) and higher in T-males ($+9.76\%$). Larger males did not show a higher increase in secretion production (correlation of 'difference in secretion mass before–after' over 'SVL', $r^2 = 0.008$, $P = 0.405$).

Chemosensory tests

All female lizards responded to swabs by tongue flicking. Mean latency to the first TF differed significantly among conditions (repeated-measures ANOVA; Wilk's $\lambda = 0.064$, $F_{6,13} = 31.612$, $P < 0.001$; Fig. 5A). Latencies to the first TF in response to male stimuli were significantly shorter than to water (Tukey's post hoc test, all $P < 0.002$). Latency times in response to scent stimuli were similar for C- and T-males (comparing secretions from one thigh: $P = 0.222$; from both thighs: $P = 0.772$; from faeces: $P = 0.191$). However, females reacted more rapidly when offered large amounts of epidermal secretions (from both thighs) than when offered smaller quantities (from one thigh only) (comparing C-males: $P < 0.001$; comparing T-males: $P < 0.001$). TF latency times did not

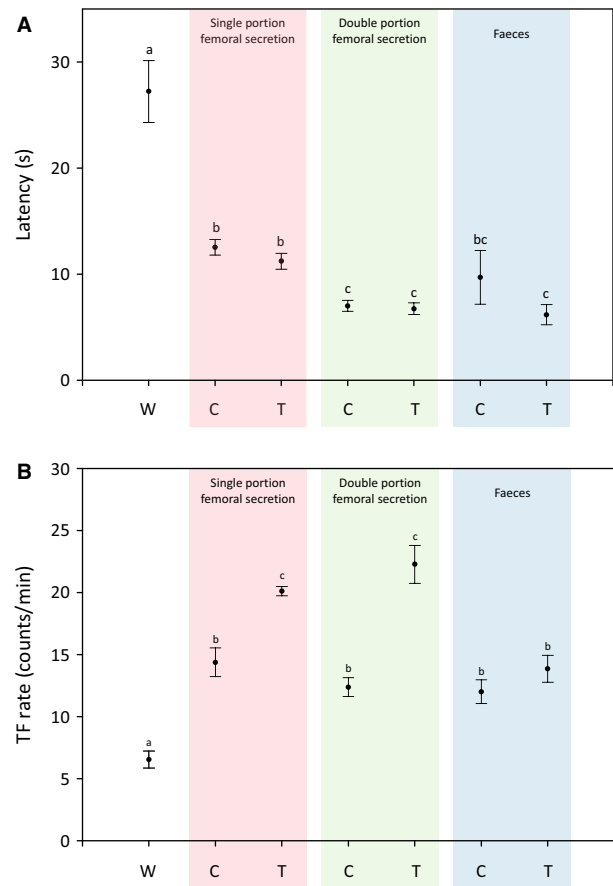


Fig. 5—(A) Mean latency (in seconds) to the first TF and—(B) mean number of tongue-flicks (TF) elicited in 1 min by female conspecifics in response to different stimuli (symbolized by different background colours) originating from control males (C) and males with experimentally increased testosterone levels (T). The stimuli are as follows: deionized water (W); a single portion of male epidermal gland secretion (red); a double portion of male epidermal gland secretion (green); faeces (blue). Error bars represent SE. The same letters above the bars denote that means are not significantly different from each other.

differ with origin (one thigh, two thighs, faeces) when stimuli were taken from C-males ($P \geq 0.191$). T-males faeces and secretions from both thighs elicited earlier reactions than T-male secretions from one thigh ($P < 0.001$).

The mean number of TFs differed among stimulus conditions (repeated-measures ANOVA, Wilk's $\lambda = 0.026$, $F_{6,13} = 81.282$, $P < 0.001$; Fig. 5B). Swabs impregnated with male epidermal gland secretions or faeces elicited significantly more TFs than control swabs with water (all $P < 0.001$). Regardless of whether secretions were obtained from a single thigh or from both, swabs with epidermal secretions from T-males elicited more TFs than secretions from C-males ($P < 0.001$). The amount of male secretion did not matter (quantity effect in C-males: $P = 0.120$; in T-males: $P = 0.164$). Females did not TF more when confronted with faeces from T-males than with C-male faeces ($P = 0.253$). Similar numbers of TFs were performed in response to C-male epidermal secretions and C-male faeces ($P = 0.120$), but epidermal secretions of T-males elicited more TFs than T-male faeces ($P < 0.001$).

Discussion

Testosterone and the epidermal gland system

Firstly, the results of this study show that artificial T-administration does not cause any changes in the size of male femoral pores in *Podarcis muralis*. Pore size also did not correlate with body size or secretion production. Interestingly, while the results of this study does not support the idea that femoral pore size can be used as a proxy for the intensity of secretion production in male wall lizards, studies on other lizard species found opposite results. Femoral pore size in male green iguanas correlates strongly with secretion mass, indicating that pore size probably provides a good estimate of gland productivity in *Iguana iguana* (Alberts *et al.* 1992). More in line with our results, Van Wyk (1990) did not find a relationship between testis volume and femoral pore size in the cordylid lizard *Karusasaurus polyzonus*. Although, based on histological section of the glands, the researcher did find a correlation between testis volume and the depth of the epidermal glands. While we are not certain on the true origin of these disparate findings, it is possible that they derive from interspecific differences in gland morphology (e.g. tubular, tubulo-acinar, tubulo-alveolar), where the form of the glands might determine whether high secretory activity affects the expansion of the pore-bearing scales. Detailed histological studies on this topic should be encouraged. Nevertheless, pore size can easily be used as a tool for sex discrimination in *Podarcis muralis*, for male femoral pores were almost twice the size of female pores. Male pores are known to be notably larger than those of females in most lizard taxa (Valdecantos *et al.* 2014; Baeckens *et al.* 2015; Mayerl *et al.* 2015). This sexual difference may be due to increased plasma oestrogen levels associated with female reproductive maturity, as administration of exogenous

oestrogen inhibits glandular activity in other lizard species (Forbes 1941; Chiu *et al.* 1975).

Secondly, our results show that T-supplementation provokes substantial changes in the secretory production of epidermal glands in male *Podarcis muralis* lizards: males with artificially increased T concentrations produced on average 19.3% more secretion than control males. In castrated *Ctenophorus ornatus*, daily T injections incite a 23.4% increase in secretion production (Fergusson *et al.* 1985). Research on seasonal T fluctuations and peak secretory activity in dominant *Iguana iguana* males showed that during the month in which the highest plasma T concentrations were observed (January), epidermal secretion production was approximately 150% higher than during the period with the lowest T concentration (May) (Alberts *et al.* 1992). Martins *et al.* (2006) have argued that an increased secretion production during the mating season may help male *Sceloporus graciosus* lizards to mark larger territories. As T levels are highest at the start of the reproductive season (Manzo *et al.* 1994; Oppliger *et al.* 2004), they may stimulate secretion production and, in some cases, lizard mobility (e.g. *Lacerta agilis*, Olsson *et al.* 2000). Then, it could be assumed that as lizards move around more often, they may release more glandular material over a larger area – which is made possible by the high (T-induced) secretion production rate – and therefore expand their scent marking space substantially. However, more research is necessary to confirm this.

In this study, large *Podarcis muralis* males produced larger amounts of secretion than small males. Similarly, dominant *Iguana iguana* males (equipped with large heads) produce gland secretions at a higher rate than subordinates with small heads (Alberts *et al.* 1992). Because secretion production is most probably an energetically costly affair (Martín and López 2015), it is very likely that only individuals in a good condition can afford high secretory activity rates. Therefore, large males with high T levels – which are often considered as most dominant (Sinervo *et al.* 2000; Husak *et al.* 2007; Huyghe *et al.* 2007, 2009) and in best condition (Tokarz 1985; Jakob *et al.* 1996), may produce high amounts of secretion, and are thus able to mark more sites and create a more widespread territory than small lizards. This is the case with sagebrush lizards (*Sceloporus graciosus*): territorially aggressive males deposit high amounts of epidermal secretion, and this over a large area (Martins *et al.* 2006).

Testosterone and signal detectability

Squamate chemoreception is said to work in a hierarchical fashion, with chemicals being received initially through the nares, processed by the nasal organs and finally triggering tongue-flick mediated vomerolfaction (Halpern 1992; Cooper 1994; Schwenk 1995). As such, different latencies to the first TF or the number of TFs elicited towards stimuli indicate distinct chemosensory activities and, thus, stimuli discrimination (Cooper 1994). Our chemosensory experiments suggest that

female *Podarcis muralis* can detect and distinguish between epidermal gland secretion of C- and T-males, as they react more quickly and more strongly with TF behaviour to secretion of T-males than to secretion of C-males. This result corroborates the findings by Martín *et al.* (2007b) in the closely related *Podarcis hispanicus*. Females may exploit T-induced differences in the chemical composition of the epidermal gland secretion or changes in the concentrations of relevant chemicals in the secretion. The ability to discriminate between differential T levels may be an important tool in lizard mate assessment, as male T concentration is commonly known to correlate strongly with various lizard performance traits, such as sprint speed (Klukowski *et al.* 1998) and bite force (Husak *et al.* 2007; Huyghe *et al.* 2009, 2010), and with overall dominant behaviour (Greenberg and Crews 1990; Pratt *et al.* 1992).

Although the amount of epidermal secretion did not affect female TF rates, females did respond more rapidly when offered double-loaded swabs – irrespectively of whether secretions were obtained from C- or T-males. These findings imply that the absolute amount of detectable airborne chemicals increases with increasing secretion quantity. As the absolute amount of airborne chemicals in the environment is determined by the amount of volatile chemicals in the secretion (Apps *et al.* 2015), we can infer that an increase in secretion quantity corresponds with an increase in the absolute amount of volatiles. High amounts of epidermal gland secretion may therefore increase signal detectability, with lizards responding more rapidly by vomerolfactory behaviour. These results indicate that secretion quantity – rather than T-induced changes in the physico-chemical properties of the secretion – affects chemosignal transmission and detectability in *Podarcis muralis* lizards. Accordingly, Escobar *et al.* (2001, 2003) found that *Liolaemus* lizards occurring at high altitudes and low latitudes are equipped to produce high amounts of secretion, a hypothesized compensation strategy to prevent signal loss due to the harsh signalling environment. In addition to changes in secretion quantity, changes in the chemical profile of the secretion may affect signal detectability too. For example, natural seasonal changes in T levels increase the proportion of unsaturated lipids in the secretions of male *Iguana iguana*, which are postulated to increase volatility and detectability of secretions (Alberts 1992). A recent study by Martín *et al.* (2015) established interpopulational differences in secretion composition in *Podarcis hispanicus*, enabling maximum signal detectability (and persistence) under different climatic conditions.

Our results also show that although *Podarcis muralis* females showed interest in male faeces, they could not discriminate between males with dissimilar T levels based on scent of faeces alone. Previous research has already established the ability of lacertid lizards to discriminate chemicals from faeces, and even suggested faeces as a player in intraspecific lizard communication. Tongue-flick assays

showed that Carpetan rock lizards (*Iberolacerta cyreni*) are able to distinguish between own and conspecific faeces (López *et al.* 1998) and that Iberian rock lizards (*Iberolacerta monticola*) react differently when confronted with juvenile or adult faecal deposits (Moreira *et al.* 2008). Although faecal scent elicited substantial vomerolfactory behaviour in female *Podarcis muralis* in this study, the fact that females were unable to detect T level differences in faeces (while the T enzyme immunoassay revealed clear T level variation in the faecal pellets), suggests that faeces may not only serve as a chemical signal. It has been suggested that lizards use faecal deposits to mark their territory, in which individuals exploit both the chemical and visual aspect of the mark to convey information. Male *Iberolacerta cyreni* typically deposits their excrements on rock-tops, which is thought to facilitate visual detection by conspecifics (López *et al.* 1998). After first locating and approaching the signal visually, a lizard could investigate the mark more closely through chemosensory channels (Duvall *et al.* 1987; Alberts 1989). It is generally assumed that – when the lizard moves – femoral pores scrape against the substrate and small portions of glandular material are transferred onto the substrate (Maderson 1986; Jared *et al.* 1999; Khannoon *et al.* 2013; de Villiers *et al.* 2015). Following this line of thought, it is probable that sites with faecal deposits will also be labelled with epidermal secretions. Martins *et al.* (2006) found a strong association between the amount of epidermal secretion and the amount of faecal matter on a certain marking spot in *Sceloporus graciosus*. It is therefore reasonable to hypothesize that lizards use faecal deposits as an initial long-range visual signal providing the first cue, and carrying minimal chemical information (e.g. own versus conspecific) once encountered. The neighbouring epidermal gland secretion deposits may act as chemosignal reservoirs containing more elaborate information on an individuals' condition, for example hormonal level, parasitic infection, immune response, diet quality or fighting ability (López *et al.* 2003; Labra 2006; Martín and López 2006a,b; Martín *et al.* 2007a,b, 2008; Khannoon *et al.* 2010, 2011; Kopena *et al.* 2014).

In conclusion

Our data demonstrate that testosterone affects epidermal gland activity in male *Podarcis muralis* lizards with an increased secretory production, but without any changes in the size of the pore-bearing scales. Based on the scent of male epidermal gland secretion alone, and not on the scent of faecal deposits, female conspecifics are able to discriminate between males with different testosterone levels using vomerolfaction behaviour. Moreover, females react more quickly towards higher amounts of secretion, implying that epidermal gland secretion quantity affects lizard chemosignal detectability. Still, more research on lizard scent marking behaviour is necessary to elucidate the importance of secretion quantity in a natural setting.

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