

Effects of testosterone supplementation on chemical signals of male Iberian wall lizards: consequences for female mate choice

José Martín · Pilar López · Marianne Gabirot · Kevin M. Pilz

Received: 22 February 2006 / Revised: 22 November 2006 / Accepted: 13 January 2007 / Published online: 30 January 2007
© Springer-Verlag 2007

Abstract In some lizards, female mate choice is influenced by chemicals secreted by males, e.g., via the femoral glands. Secretions of the femoral glands are under direct androgenic control and vary seasonally with androgen production. However, whether increased testosterone (T) levels affect the concentration and chemical composition of secretions or their attractiveness to females is unknown. We manipulated T levels of male Iberian wall lizards, *Podarcis hispanica*, with silastic implants. Differential tongue-flick rates indicated that females detected and discriminated between femoral secretions of control and T-implanted males based on chemical cues alone. Females showed greater responses to secretions of T-males, which might suggest that T increased the concentration of chemical signals. Further analyses by gas chromatography–mass spectrometry indicated that T supplementation induced qualitative changes in lipid composition of femoral secretions. T-males had decreased relative proportions of cholesta-5,7-dien-3-ol in secretions, which might be related to the negative effects of T on the immune system and on lipid metabolism. However, experiments of choice of males' scent showed that females neither preferred nor avoided the scent marks of T-males but preferred males that maintained higher proportions of cholesta-5,7-dien-3-ol in secretions, independently of the experimental manipulation. Thus, because this steroid is negatively affected by T, there might be trade-offs among increasing T levels to increase

the production of chemical secretions, maintain metabolism, and attract females.

Keywords Iberian wall lizard · *Podarcis hispanica* · Femoral gland secretions · Lipids · Testosterone implants · Chemoreception · Mate choice

Introduction

Chemical cues play an important role in the intraspecific communication of lizards (Halpern 1992; Mason 1992). Several studies have shown pheromonal detection, which in some species is based on femoral gland secretions of males (e.g., Alberts 1993; Aragón et al. 2001a; reviewed in Mason 1992). The femoral pores are epidermal structures on the ventral surface of the thigh, connected to glands that produce copious amounts of holocrine secretion in males (Mason 1992; Alberts 1993). The presence and relative concentration of pheromone components seem to vary consistently among individuals, which may serve a variety of functions (Alberts 1993). For example, femoral secretions are passively deposited on the substrate as lizards move through their home ranges, and therefore, they could advertise residence and competitive ability of the signaler (Aragón et al. 2001b; López and Martín 2002). In addition, femoral pore secretions might convey information about a male's quality or genetic compatibility, which may be used by females in mate choice (Martín and López 2000, 2006; López et al. 2002b, 2003; Olsson et al. 2003; López and Martín 2005b).

The secretory activity of the femoral glands is greatest in males and in the breeding season, which indicates that femoral gland secretions are under direct androgenic control and vary seasonally with androgen production

Communicated by W. Cooper

J. Martín (✉) · P. López · M. Gabirot · K. M. Pilz
Departamento de Ecología Evolutiva,
Museo Nacional de Ciencias Naturales,
C.S.I.C., José Gutiérrez Abascal 2,
28006 Madrid, Spain
e-mail: Jose.Martin@mncn.csic.es

(Cole 1966; Van Wyk 1990; Alberts 1993). Early research indicated that castration of male lizards causes the glands to atrophy, whereas supplementation of testosterone (T) increases secretion rates (e.g., Fergusson et al. 1985; see review in Mason 1992).

The chemical composition of these secretions has been studied in a few species of lizards, mainly iguanids (Chauhan 1986; Alberts 1990; Weldon et al. 1990; Alberts et al. 1992a; Escobar et al. 2001) and lacertids (López and Martín 2005a,b). These studies have shown that femoral or the analogous precloacal gland secretions are composed of both lipids and proteins, but lipids are thought to be the main compounds involved in communication (Mason 1992). Several studies have examined seasonal variation in body fat levels and the relationships between lipid metabolism and sex steroid hormones in lizards (e.g. Ballinger et al. 1992; Sheridan 1994; Lacy et al. 2002). However, only two studies have shown that productivity and chemical composition of femoral secretions of *Iguana iguana* vary seasonally (Alberts et al. 1992a) and with plasma T levels (Alberts et al. 1992b).

It is generally assumed that success in male–male competition reflects high quality and that female preference for dominant males should be widespread (Quarnström and Forsgren 1998). Thus, traits reflecting dominance, such as large body size, are expected to be selected by females. With respect to chemical signals, T seems to stimulate the production of sex attractant pheromones in several rodent species, as females prefer males with a high concentration of T in the urine (Taylor et al. 1982; White et al. 1984; Ferkin et al. 1994; Gottreich et al. 2000). However, male dominance is not always attractive to females (Quarnström and Forsgren 1998; Moore and Moore 1999). This is because male dominance might not be a reliable indicator of better paternal care or genetic quality for females (Quarnström and Forsgren 1998; López et al. 2002b). Many lizard species exhibit social dominance systems, and T may increase aggressiveness and success in male–male competition, thus, conferring dominance (review in Moore and Lindzey 1992). Although male scent marks may play a role in female mate choice in many lizards (see above), it is unknown whether increased T levels affect the chemical composition of femoral secretions, whether females are able to detect these changes by chemoreception, and whether females select scent of males with higher levels of T.

The Iberian wall lizard, *Podarcis hispanica*, is a small (50–70 mm adult snout-to-vent length, SVL) diurnal lacertid lizard of the Iberian Peninsula, common in rocky habitats and on artificial walls (Pérez-Mellado 1998). Males are aggressive and defend some area around females (Gil et al. 1988). Overlap between male home ranges is extensive, and agonistic encounters are frequent during the mating season (López and Martín 2001a; P López and J Martín,

unpublished data). As what occurs in many other lizards (Moore and Lindzey 1992), T levels are probably linked to increased aggressiveness and male dominance. Chemosensory abilities are well-developed in *P. hispanica*. This lizard can discriminate between sexes by chemical cues alone (López and Martín 2001b; Cooper and Pérez-Mellado 2002; López et al. 2002a). Moreover, the aggressive response of males to intruding individuals depends on pheromonally mediated sex recognition (López and Martín 2001b; López et al. 2002a). Chemical cues seem involved in individual rival recognition between males (López and Martín 2002) and in female mate choice (López and Martín 2005b).

In this paper, we manipulated T levels in male *P. hispanica*, within the normal physiological range of variation, and examined whether females are able to discriminate by chemical senses alone between control males and males with increased T levels, and whether females select or avoid areas scent-marked by males with high T levels. We further analyzed by gas chromatography–mass spectrometry (GC-MS) whether increased T levels induced qualitative changes in the lipophilic fraction of male femoral gland secretions.

Materials and methods

Study area and species

We captured by noosing adult *P. hispanica* (18 males and 16 females) on rocky outcrops in an oak forest near Cercedilla (40°44'N, 4°02'W; Madrid province, Spain) at the beginning of April 2004 before the start of the mating season. We captured lizards in different places over a large area to ensure that individuals had not been in previous contact. We confirmed that females had not mated yet by their lack of characteristic mating scars on the belly (P López and J Martín, unpublished data). Lizards were weighed (body mass, males, mean±SE=3.9±0.1 g, range=2.7–5.1 g; females, 2.9±0.1 g, range=2.0–3.8 g) and their SVL was measured (males, 62.4±0.6 mm, range=58–66 mm; females, 59.1±0.8 mm, range=53–63 mm). We also took blood from the postorbital sinus by using one 9- μ l heparinized hematocrit tube for further analyses of T levels with standard enzyme-linked immunosorbent assay procedures.

All lizards were individually housed at “El Ventorrillo” field station 5 km from the capture site in outdoor 80×50 cm PVC terraria containing sand substratum and rocks for cover. Cages of males and females were in different places to ensure that there was no previous contact between them. Every day, lizards were fed with mealworm larvae (*Tenebrio molitor*) dusted with multivitamin powder for reptiles, and water was provided ad libitum. The photoper-

riod and ambient temperature were that of the surrounding region.

Testosterone implants

We administered T in silastic implants designed to elevate plasma levels by approximately 90 ng/ml (determined in preliminary trials; KM Pilz, unpublished data). This T dose should increase T level but maintain it within the normal physiological range in this lizard during the mating season (mean \pm SE=279 \pm 35 ng/ml; range=20–891 ng/ml; $N=37$; KM Pilz, unpublished data). Male lizards were cold anesthetized by placing them at 4°C for 10–20 min, inducing deep hypothermia. Silastic implants (4 mm length, 0.64 mm inner diameter, 1.19 mm outer diameter; closed with 1-mm silastic adhesive at each end) were inserted subdermally, just above the right shoulder. Implants were inserted through a small dorsolateral incision of about 3 mm, which was later closed with Vetbond brand surgical adhesive. Implants were either filled with 2 mm packed T powder (Sigma) or, for controls (C), were empty. Males were randomly assigned to each treatment, and there were no significant differences between groups in body size [analysis of variances (ANOVAs), SVL, $F_{1,16}=0.10$, $P=0.80$; body mass, $F_{1,16}=0.49$, $P=0.49$] or in initial T levels in blood (C vs T, 292 \pm 67 vs 322 \pm 73 ng/ml; $F_{1,16}=0.10$, $P=0.75$).

All animals were healthy during the trials, and all maintained or increased their original body mass. At the end of the experiment, implants were removed. After ascertaining that lizards were in good condition, we released them at their capture sites.

Discrimination of males' scent by females

We conducted this experiment during April 2004, 2 weeks after the males were implanted. To examine the hypotheses of discrimination of T levels based on chemicals from femoral secretions alone, we compared the number of tongue flicks (TFs) emitted by 16 females in their own cages in response to stimuli arising from cotton swabs impregnated with (1) femoral secretion from an unfamiliar male implanted with empty implants (C-male), (2) femoral secretion from an unfamiliar male implanted with testosterone (T-male), or (3) deionized water (odorless control; Cooper and Burghardt 1990). Water was used to gauge baseline TF rates in the experimental situation. We prepared the control stimuli by dipping the cotton tip (1 cm) of a wooden applicator (50 cm) in deionized water. Femoral secretions consisted of a waxy substance, which was easily extracted by gently pressing with forceps around the femoral pores, and collected directly on cotton swabs impregnated in distilled water. We attempted to use approximately the same amount of femoral secretion in

each stimulus (about 2 \times 1 mm of solid secretion from each of three pores), thus ensuring that any possible difference in TF rate was not due to differences in the amount of femoral secretions presented to females. Cotton swabs with scent stimuli were used in the trials immediately after collection to avoid fading of the stimuli.

Every female lizard was exposed to each scent stimulus in an incompletely counterbalanced order of presentation, with a minimum intertrial interval of 30 min. Tests were made in outdoor sunny conditions between 0900–1200 hours Greenwich mean time (GMT), when lizards were fully active. Each individual was allowed to bask for at least 2 h before trials, which allowed them to attain a body temperature within the activity temperature range of the species (P López and J Martín, unpublished data). Thereafter, one of the experimenters slowly approached a lizard's cage and slowly moved the cotton swab to a position 2 cm anterior to the lizard's snout. Lizards allowed approach and testing without fleeing. We recorded TFs 'directed' to the swab and TFs directed elsewhere (non-directed) for 60 s, beginning with the first TF. We also recorded the 'latency' to the first TF computed as the period elapsed between placing the swab in the cage and the first tongue extrusion.

To examine differences in number of TFs and latency to first TF among conditions, we used repeated measures ANOVAs with scent stimuli as the within-subjects factor. Data were log-transformed to ensure normality (Shapiro–Wilk's test). Tests of homogeneity of variances (Levene's test) showed that, in all cases, variances were not significantly heterogeneous after transformation. Pairwise comparisons were planned using Tukey's honestly significant difference tests (Sokal and Rohlf 1995).

Chemical analyses of femoral gland secretions

We extracted small samples of male femoral pores secretion immediately after capture, and again 3 weeks after males were implanted, by gently pressing with forceps around the femoral pores and collected secretion directly in glass vials with Teflon-lined stoppers, which were stored at –20°C until analyses. Samples were analyzed using a Finnigan-ThermoQuest Trace 2000 GC-MS fitted with a 30-m column (Supelco, Equity-5) temperature programmed from 50–280°C at 5°C/min and kept at 280°C for 30 min (see López and Martín 2005a for details). We identified chemicals by comparison of mass spectra in the NIST 1998 library, and later confirmed with authentic standards.

The relative amount of each component was determined as the percent of the total ion current (TIC). Then, we selected the peaks that represented >1.0% relative peak area and that were present in all individuals to reduce the number of variables. The relative areas of the ten selected major peaks were restandardized to 100% and transformed

following Aitchison's formula (Aitchison 1986; Dietemann et al. 2003; López and Martín 2005b). The transformed areas were used as variables in a repeated measures multivariate ANOVA (MANOVA), testing the effects of temporal changes after 3 weeks of being implanted (within factor), treatment group (between factor, C-males vs T-males), and the interaction between time and treatment (Sokal and Rohlf 1995). This interaction effect tested for actual changes due to the treatment after controlling for natural variation between individuals and temporal changes within individuals (e.g., due to captivity conditions or other confounding effects). Because we found a significant interaction term in the multivariate analyses (see "Results"), we could perform protected repeated measures ANOVAs separately on each chemical, testing the interaction between time (within factor) and treatment, to locate which chemicals (dependent variables) changed more with T supplementation and explained the significant difference found in the previous MANOVA model (Scheiner 2001). This procedure was preferred to Bonferroni corrections to avoid increasing the probability of type I errors (Legendre and Legendre 1998; Moran 2003).

Choice of males' scent by females

After males were implanted with silastic tubes, we placed in each male's cage several absorbent paper strips (35×10 cm) fixed to the floor of the cage and left them there for 3 weeks to obtain scents from lizards. Males were often observed rubbing the femoral pores on this paper substrate. Choice experiments were carried out at the end of April, 3 weeks after the implants were made, and coinciding with the mating season of lizards in the field (P. López and J. Martín, unpublished data). Papers were removed to be placed in females' cages immediately before each experiment began. Paper strips were manipulated with fresh gloves to avoid contaminating them with human odors.

Females' cages (80×50 cm) had two basking platforms (two identical flat tiles) placed symmetrically at each end of the cage and rocks for cover in the center. At the beginning of each experiment (0700 hours GMT), when females were still inactive and hidden under rocks, we fixed one paper strip from a T-male on one tile and another strip from a C-male on the other tile. The individual male tested within each category and the positions of the papers in the cages were randomly determined. Each female was tested on 4 different days, with papers from eight different males (four pairs of a T-male and a C-male). For each female, we avoided using the same individual male whose femoral secretions were used in the previous experiment. Thus, all males tested were unfamiliar to each particular female. We used each paper strip in only one test. Each trial lasted 6 h (from 0900 hours GMT, shortly after lizards had appeared

from their refuges, and until 1500 hours GMT, when lizards hid again in the refuge). We monitored females from a hidden point, recording their locations in the cages each 15 min (25 scans) to obtain a representative sample of females' space use in the cages. If a female was located on either of the two tiles with the paper strip, she was designated as having chosen that particular paper, whereas if she was located off of the tiles, she was designated as having made no choice (see Martín and López 2000; López et al. 2002b, 2003; Olsson et al. 2003 for a similar procedure). We ensured that females were exposed to both males' tiles because at least two recordings in each male's section were recorded in all trials. At the end of the trials, the papers were removed and the cage was thoroughly rinsed with clean water. We counted the number of times that each female was observed on each particular stimulus paper in each trial and used a repeated measures two-way ANOVA to test for differences between days and between treatments (T vs C; both within-subject factors; Sokal and Rohlf 1995). In addition, because a previous study suggested that females may prefer scents of unmanipulated males with relatively higher amounts of cholesta-5,7-dien-3-ol in secretions (López and Martín 2005b), we made a similar two-way ANOVA but classifying the two males within each pair according to the relative abundance of this lipid in their secretions (higher vs lower) independently of the experimental manipulation.

Results

Discrimination of males' scent by females

All female lizards responded to swabs by tongue flicking. There were significant differences among stimulus condition in the number of TFs directed to swabs (repeated measures one-way ANOVA, $F_{2,28}=67.20$, $P<0.0001$; Fig. 1a). Chemicals from T- and C-males elicited significantly more directed TFs than water (Tukey's test, $P<0.0001$ in all cases). Chemicals from T-males elicited significantly more directed TFs than those of C-males ($P=0.012$).

The number of TFs not directed to the swab differed significantly among treatments (repeated measures one-way ANOVA, $F_{2,28}=4.17$, $P=0.003$, Fig. 1b). The number of non-directed TFs was significantly greater for the scent of T-males than to water (Tukey's test, $P<0.05$) and C-males ($P=0.027$). Responses not directed to the scent of C-males and water did not differ significantly ($P=0.80$).

Mean latency to first TF differed significantly among conditions (repeated measures one-way ANOVA, $F_{2,28}=4.82$, $P=0.016$, Fig. 1c). Latencies to the first TF in response to scents of T- and C-males were significantly shorter than to water (Tukey's test, $P<0.05$ in both cases),

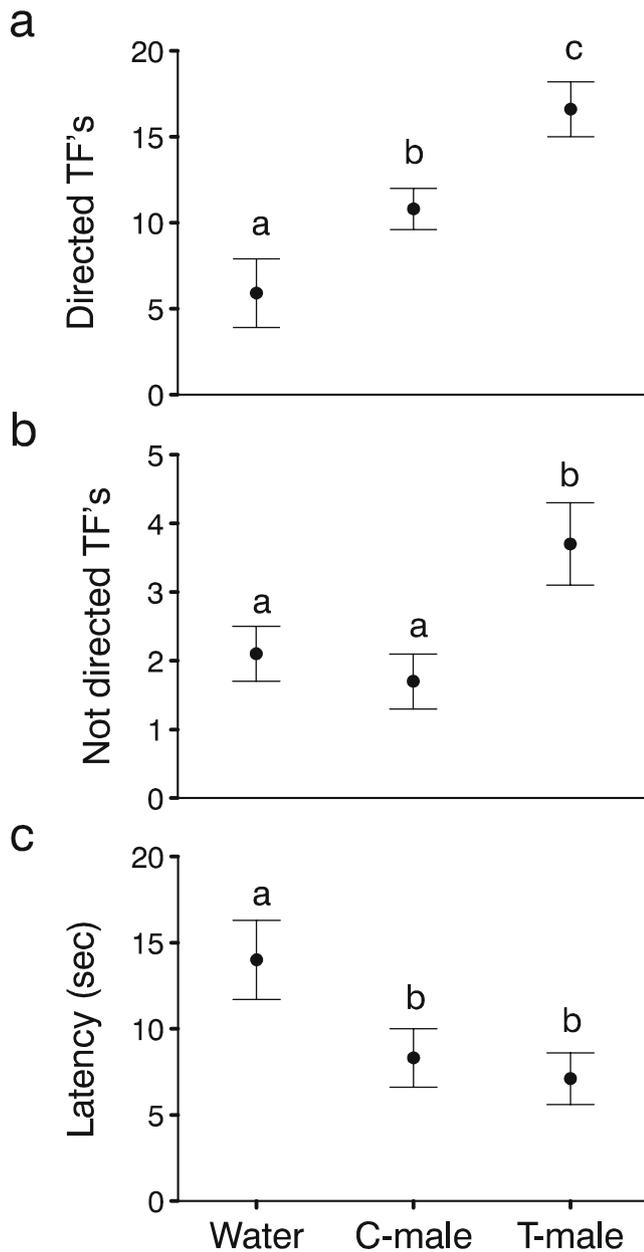


Fig. 1 Mean (\pm SE) **a** number of TFs directed to swabs, **b** non-directed TFs, and **c** latency(s) to the first TF by female lizards in response to cotton-tipped applicators bearing deionized water or scent from femoral secretions of conspecific males after 3 weeks of being implanted with empty control (C-males) or testosterone filled (T-males) silastic tubes. The same letter above the bars denotes that means are not significantly different from each other

but latencies to both types of males did not differ significantly ($P=0.76$).

Chemical analyses of femoral gland secretions

Ten major lipophilic compounds (TIC area > 1%) were identified in all individual males. These were four carboxylic acids and six steroids (Table 1). Cholesterol and

cholesta-5,7-dien-3-ol among steroids and hexadecanoic and octadecenoic acids were the most abundant compounds. Other minor components (<1% TIC) such as other carboxylic acids and their esters, other steroids, alcohols, ketones, squalene, and one aldehyde were found in small quantities. They were not present in all individuals but were observed in similar frequencies of C- and T-males. They are not considered in this study.

The main effect of treatment was not significant (repeated measures MANOVA, treatment effect, Wilk's $\lambda=0.21$, $F_{10,7}=2.59$, $P=0.11$), but after 3 weeks of being implanted, males had significantly different proportions of compounds than initially (time effect, Wilk's $\lambda=0.09$, $F_{10,7}=6.61$, $P=0.01$; Table 1). The interaction between treatment and time was significant (interaction term, Wilk's $\lambda=0.90$, $F_{10,7}=7.43$, $P=0.007$), showing that the effects of manipulation on proportion of chemical compounds differed between T- and C-males. Univariate-protected repeated measures ANOVA showed that the T manipulation was associated with a decrease in the proportion of cholesta-5,7-dien-3-ol in T-males (interaction term, $F_{1,16}=6.38$, $P=0.02$). This was the only significant change noted in chemical composition of femoral gland secretions related to T supplementation (all other univariate tests had interaction terms with $P>0.30$).

Choice of males' scent by females

The number of observations of females on stimulus paper strips did not differ significantly between treatments (repeated measures two-way ANOVA, $F_{1,15}=0.63$, $P=0.44$) or between days ($F_{3,45}=0.92$, $P=0.44$), and the interaction was not significant ($F_{3,45}=0.03$, $P=0.99$; Fig. 2a).

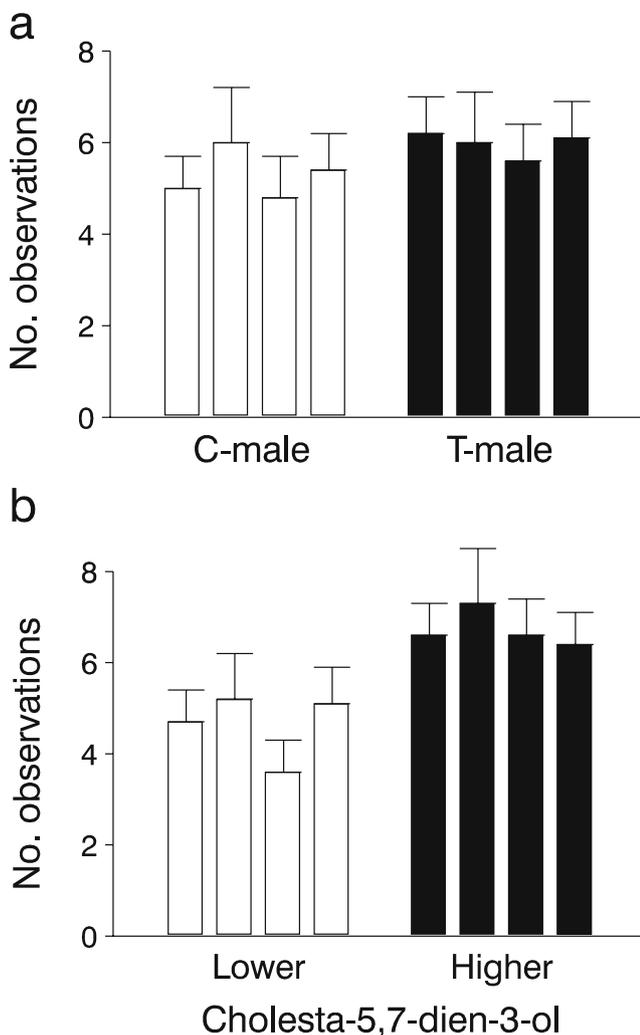
In contrast, females preferred paper strips scented by the male, within each pair, with the higher proportion of cholesta-5,7-dien-3-ol in his femoral secretions, as indicated by the significantly greater number of observations of females on paper strips labeled by males with higher cholesta-5,7-dien-3-ol (repeated measures two-way ANOVA, $F_{1,15}=11.60$, $P=0.004$). There were no significant differences between days ($F_{3,45}=0.93$, $P=0.43$), and the interaction was not significant ($F_{3,45}=0.28$, $P=0.83$; Fig. 2b).

Discussion

Differential TF rates of females indicate that females detect and discriminate between C- and T-males based on chemical cues alone. This is not surprising given the chemosensory ability of this lizard to discriminate between conspecifics, possibly even at the individual level (Cooper

Table 1 Major lipids (mean±SE of % TIC) found in femoral secretions of male Iberian wall lizards, *Podarcis hispanica*, before and after 3 weeks of being implanted with empty control (C-males) or testosterone filled (T-males) silastic tubes

Compound	Before the implant		After the implant	
	C-males	T-males	C-males	T-males
Dodecanoic acid	2.2±0.2	2.6±0.5	1.9±0.1	2.4±0.3
Hexadecanoic acid	7.2±0.5	5.8±1.2	3.0±0.4	2.5±0.4
Octadecanoic acid	1.7±0.2	1.4±0.2	0.8±0.1	0.6±0.1
Octadecenoic acid	5.9±0.7	4.7±1.1	1.9±0.4	1.4±0.4
Cholesterol	59.3±2.3	57.2±2.7	70.9±2.7	73.0±1.4
Cholesta-5,7-dien-3-ol	13.7±1.8	16.4±1.0	13.5±1.7	9.3±0.9
Campesterol	2.0±0.5	2.6±0.6	2.2±0.5	2.9±0.3
Ergosta-5,8-dien-3-ol	4.0±0.4	4.8±0.2	2.9±0.3	3.3±0.4
Sitosterol	1.2±0.3	1.5±0.6	1.4±0.4	1.6±0.4
4,4-Dimethyl-cholesta-5,7-dien-3-ol	2.8±0.4	2.8±0.2	2.0±0.3	2.1±0.3

**Fig. 2** Number of times (mean±SE), during each of the four scent's choice trials, that female lizards were observed on paper strips scent marked by **a** C- or T-males or by **b** the male within each pair with relatively lower or higher proportions of cholesta-5,7-dien-3-ol in its femoral secretions, independently of the experimental manipulation

and Pérez-Mellado 2002; López and Martín 2002, 2005b). Discrimination between C- and T-males may be based on the amount or concentration of relevant chemicals in secretions and/or on differences in chemical composition of their femoral secretions. In other lizards, the increased T levels increased the secretion rates of femoral glands, and the natural seasonal changes in T levels increased the proportion of unsaturated lipids, which was thought to increase volatility and detectability of secretions (Alberts et al. 1992a,b; see review in Mason 1992). If a higher concentration of lipids enhances detectability of secretions, this might explain why TF rates of females to T-males were higher in our experiment.

Our analyses indicated that T supplementation induced changes in chemical composition of femoral gland secretions of males: T-males decreased the relative proportions of cholesta-5,7-dien-3-ol (=dehydrocholesterol) allocated to secretions. In a previous study, we found that proportions of this steroid in femoral gland secretions of male Iberian wall lizards were related to their cell-mediated immune response (López and Martín 2005b). Thus, changes observed in secretions of T-males might be related to changes in the immune response due to the immunosuppressive effect of T shown in similar lacertid lizard species (Belliere et al. 2004; Oppliger et al. 2004). Variations in testosterone or corticosterone levels have been implicated in lizards and other taxa in modulating immunity and lipid and steroid biochemistry (e.g., Lacy et al. 2002; Sheldon and Verhulst 1996). Other studies with lizards and tortoises have found a negative relationship between T levels and total amount of liver or plasma lipid and fat body mass (Lacy et al. 2002; Lance et al. 2002). Cholesta-5,7-dien-3-ol is a precursor for vitamin D₃ and is often found in the skin, where it is transformed into vitamin D₃ after exposure to UVB irradiation (Carman et al. 2000). Vitamin D₃ seems essential in calcium metabolism. Thus, the provitamin

excreted in the femoral secretions has to be diverted from general metabolism (Martín and López 2006). It is possible that T-males with decreased immunocompetence (Belliere et al. 2004), decreased body lipids (Lacy et al. 2002), increased metabolic rate (Oppliger et al. 2004), and possibly decreased feeding rates (Marler and Moore 1991) might not be able to allocate enough cholesta-5,7-dien-3-ol to femoral secretions without detrimental effects for metabolic functions.

The experiments of choice of males' scents indicated that females did not prefer, but neither did they avoid, the scent marks of T-males despite being able to discriminate between males' scents according to their T levels. These data suggest that the effects of increased T per se on males did not initially affect the attractiveness of their scent to females. In contrast, in mice, males with high concentrations of T in urine were preferentially selected by females (Taylor et al. 1982; White et al. 1984; Ferkin et al. 1994; Gottreich et al. 2000). In lizards, T may be needed to stimulate the secretion of femoral glands, and the rates of secretion may be also related to the levels of T (Alberts et al. 1992b; review in Mason 1992). Except in the mating season, femoral secretion of male lizards is absent or almost inexistent (Van Wyk 1990; Alberts et al. 1992b; Alberts 1993). Thus, a minimum level of T may be needed to stimulate secretion. However, because we conducted our experiment during the mating season, C-males also had femoral secretions that may have contained enough of the chemicals that can attract females to mask any effect of T on female preference.

Furthermore, female lizards might not be attracted to the presence or total amount of chemicals secreted by males per se but prefer scent of males with higher proportions of certain chemicals. Thus, in accordance with a previous study (López and Martín 2005b), we found that female Iberian wall lizards selected scents of males that maintained higher proportions of cholesta-5,7-dien-3-ol, independently of the experimental manipulation. Because we have found in this study that increased T led to decreased levels of this steroid, males might face a trade-off between increasing the amount of cholesta-5,7-dien-3-ol secreted to attract females and maintaining systemic cholesta-5,7-dien-3-ol for general metabolism (e.g., as vitamin D₃ precursor). This may represent a previously unconsidered trade-off for testosterone (Folstad and Karter 1992; Sheldon and Verhulst 1996), where only males of high quality would be able to maintain high proportions of chemicals "attractive" for females in signals despite high circulating T levels. Because, in *P. hispanica*, levels of this lipid are related to the quality of the immune system of males (López and Martín 2005b), only males in good condition could mount a strong immune defense and produce an extravagant sexual "chemical ornament" (i.e., secretions with high proportions of cho-

lesta-5,7-dien-3-ol). This trade-off between allocating lipids to secretions or to metabolism might similarly explain why dominant male *I. iguana*, of presumably higher quality, produced more secretion than subordinates and that the percentage of lipids in secretions were correlated with plasma T levels in dominant, but not in subordinate, adult males (Alberts et al. 1992b).

Acknowledgments We thank two anonymous reviewers for helpful comments, and "El Ventorrillo" MNCN Field Station for use of their facilities. Financial support was provided by the MEC project CGL2005-00391/BOS, and a SYNTHESYS CEE grant to M. Gabirot. The experiments were performed under license from the "Madrid Environmental Agency" (Consejería del Medio Ambiente de la Comunidad de Madrid).

References

- Aitchison J (1986) The statistical analysis of compositional data: monographs in statistics and applied probability. Chapman and Hall, London
- Alberts AC (1990) Chemical properties of femoral gland secretions in the desert iguana *Dipsosaurus dorsalis*. *J Chem Ecol* 16:13–25
- Alberts AC (1993) Chemical and behavioral studies of femoral gland secretions in iguanid lizards. *Brain Behav Evol* 41:255–260
- Alberts AC, Sharp TR, Werner DI, Weldon PJ (1992a) Seasonal variation of lipids in femoral gland secretions of male green iguanas (*Iguana iguana*). *J Chem Ecol* 18:703–712
- Alberts AC, Pratt NC, Phillips JA (1992b) Seasonal productivity of lizard femoral glands: Relationship to social dominance and androgen levels. *Physiol Behav* 51:729–733
- Aragón P, López P, Martín J (2001a) Discrimination of femoral gland secretions from familiar and unfamiliar conspecifics by male Iberian rock-lizards, *Lacerta monticola*. *J Herpetol* 35:346–350
- Aragón P, López P, Martín J (2001b) Chemosensory discrimination of familiar and unfamiliar conspecifics by lizards: implication of field spatial relationships between males. *Behav Ecol Sociobiol* 50:128–133
- Ballinger RE, Holy L, Rowe JW, Karst F, Ogg CL, Stanley-Samuelson DW (1992) Seasonal changes in lipid composition during the reproductive cycle of the red-chinned lizard, *Sceloporus undulatus erythrochelus*. *Comp Biochem Physiol B* 103:527–531
- Belliere J, Smith L, Sorci G (2004) Effect of testosterone on T cell-mediated immunity in two species of Mediterranean Lacertid lizards. *J Exp Zool* 301A:411–418
- Carman EN, Ferguson GW, Gehrman WH, Chen TC, Holick MF (2000) Photobiosynthetic opportunity and ability for UVB generated Vitamin D synthesis in free-living house geckos (*Hemidactylus turcicus*) and Texas spiny lizards (*Sceloporus olivaceous*). *Copeia* 2000:245–250
- Chauhan NB (1986) A preliminary report on the lipid components of pre-anal gland secretion of lizards *Hemidactylus flaviviridis* and *Uromastix hardwickii*. *J Anim Morphol Physiol* 33:73–76
- Cole CJ (1966) Femoral glands in lizards: a review. *Herpetologica* 22:199–206
- Cooper WE, Burghardt GM (1990) A comparative analysis of scoring methods for chemical discrimination of prey by squamate reptiles. *J Chem Ecol* 16:45–65
- Cooper WE, Pérez-Mellado V (2002) Pheromonal discrimination of sex, reproductive condition, and species by the lacertid lizard *Podarcis hispanica*. *J Exp Zool* 292:523–527

- Dietemann V, Peeters C, Liebig J, Thivet V, Hölldobler B (2003) Cuticular hydrocarbons mediate discrimination of reproductives and nonreproductives in the ant *Myrmecia gulosa*. Proc Natl Acad Sci USA 100:10341–10346
- Escobar CA, Labra A, Niemeyer HM (2001) Chemical composition of precloacal secretions of *Liolaemus* lizards. J Chem Ecol 27:1677–1690
- Fergusson B, Bradshaw SD, Cannon JR (1985) Hormonal control of femoral gland secretion in the lizard, *Amphibolurus ornatus*. Gen Comp Endocrinol 57:371–376
- Ferkin MH, Sorokin ES, Renfro MW, Johnston RE (1994) Attractiveness of male odors to females varies directly with plasma testosterone concentration in meadow voles. Physiol Behav 55:347–353
- Folstad I, Karter AJ (1992) Parasites, bright males and the immunocompetence handicap. Am Nat 139:603–622
- Gil MJ, Pérez-Mellado V, Guerrero F (1988) Estructura del hábitat y dominios vitales en *Podarcis hispanica* (Steindachner, 1870). Misc Zool 12:273–281
- Gottreich A, Zuri I, Barel S, Hammer H, Terkel J (2000) Urinary testosterone levels in the male blind mole rat (*Spalax ehrenbergi*) affect female preference. Physiol Behav 69:309–315
- Halpern M (1992) Nasal chemical senses in reptiles: structure and function In: Gans C, Crews D (eds) Biology of the reptilia, vol 18. University of Chicago Press, Chicago, pp 423–523
- Lacy EL, Sheridan MA, Moore MC (2002) Sex differences in lipid metabolism during reproduction in free-living tree lizards (*Urosaurus ornatus*). Gen Comp Endocrinol 128:180–192
- Lance VA, Place AR, Grumbles JS, Rostal DC (2002) Variation in plasma lipids during the reproductive cycle of male and female desert tortoises, *Gopherus agassizii*. J Exp Zool 293:703–711
- Legendre P, Legendre L (1998) Numerical ecology, 2nd edn. Developments in environmental modelling 20. Elsevier, Amsterdam, The Netherlands
- López P, Martín J (2001a) Fighting rules and rival recognition reduce costs of aggression in male lizards, *Podarcis hispanica*. Behav Ecol Sociobiol 49:111–116
- López P, Martín J (2001b) Pheromonal recognition of females takes precedence over the chromatic cue in male Iberian wall lizards, *Podarcis hispanica*. Ethology 107:901–912
- López P, Martín J (2002) Chemical rival recognition decreases aggression levels in male Iberian wall lizards, *Podarcis hispanica*. Behav Ecol Sociobiol 51:461–465
- López P, Martín J (2005a) Chemical compounds from femoral gland secretions of male Iberian rock lizards, *Lacerta monticola cyreni*. Z Naturforsch C 60:632–636
- López P, Martín J (2005b) Female Iberian wall lizards prefer male scents that signal a better cell-mediated immune response. Biol Lett 1:404–406
- López P, Martín J, Cuadrado M (2002a) Pheromone mediated intrasexual aggression in male lizards, *Podarcis hispanicus*. Aggress Behav 28:154–163
- López P, Muñoz A, Martín J (2002b) Symmetry, male dominance and female mate preferences in the Iberian rock lizard, *Lacerta monticola*. Behav Ecol Sociobiol 52:342–347
- López P, Aragón P, Martín J (2003) Responses of female lizards, *Lacerta monticola*, to males' chemical cues reflect their mating preference for older males. Behav Ecol Sociobiol 55:73–79
- Marler CA, Moore MC (1991) Supplementary feeding compensates for testosterone-induced costs of aggression in male mountain spiny lizards, *Sceloporus jarrovi*. Anim Behav 42:209–219
- Martín J, López P (2000) Chemoreception, symmetry and mate choice in lizards. Proc R Soc Lond B 267:1265–1269
- Martín J, López P (2006) Vitamin D supplementation increases the attractiveness of males' scent for female Iberian rock lizards. Proc R Soc Lond B 273:2619–2624
- Mason RT (1992) Reptilian pheromones In: Gans C, Crews D (eds) Biology of the reptilia, vol 18. University of Chicago Press, Chicago, pp 114–228
- Moore MC, Lindzey J (1992) The physiological basis of sexual behavior in male reptiles In: Gans C, Crews D (eds) Biology of the reptilia, vol 18. University of Chicago Press, Chicago, pp 70–113
- Moore AJ, Moore PJ (1999) Balancing sexual selection through opposing mate choice and male competition. Proc R Soc Lond B 266:711–716
- Moran MD (2003) Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos 100:403–405
- Olsson M, Madsen T, Nordby J, Wapstra E, Ujvari B, Wittsell H (2003) Major histocompatibility complex and mate choice in sand lizards. Proc R Soc Lond B (Suppl) 270:S254–S256
- Oppliger A, Giorgi MS, Conelli A, Nembrini M, John-Alder HB (2004) Effect of testosterone on immunocompetence, parasite load, and metabolism in the common wall lizard (*Podarcis muralis*). Can J Zool 82:1713–1719
- Pérez-Mellado V (1998) *Podarcis hispanica* (Steindachner, 1870). In: Ramos MA (ed) Fauna Ibérica, vol 10. Museo Nacional de Ciencias Naturales, CSIC, Madrid, pp 258–272
- Quarnström A, Forsgren E (1998) Should females prefer dominant males? Trends Ecol Evol 13:498–501
- Scheiner MS (2001) MANOVA: multiple response variables and multispecies interactions. In: Scheiner MS, Gurevitch J (eds) Design and analysis of ecological experiments, 2nd edn. Oxford University Press, New York, pp 99–115
- Sheldon BC, Verhulst S (1996) Ecological immunology: costly parasite defence and trade-offs in evolutionary ecology. Trends Ecol Evol 11:317–321
- Sheridan MA (1994) Regulation of lipid-metabolism in poikilothermic vertebrates. Comp Biochem Physiol B 107:495–508
- Sokal RR, Rohlf FJ (1995) Biometry. Freeman, New York
- Taylor GT, Haller J, Regan D (1982) Female rats prefer an area vacated by a high testosterone male. Physiol Behav 28:953–958
- Van Wyk JH (1990) Seasonal testicular activity and morphometric variation in the femoral glands of the lizard *Cordylus polyzonus* (Sauria: Cordylidae). J Herpetol 24:405–409
- Weldon PJ, Dunn BS, McDanie CA, Werner DI (1990) Lipids in the femoral gland secretions of the green iguana (*Iguana iguana*). Comp Biochem Physiol B 95:541–543
- White PJ, Fischer RB, Meunier GF (1984) The ability of females to predict male status via urinary odors. Horm Behav 18:491–494