Chem. Senses 35: 253–262, 2010

doi:10.1093/chemse/bjq009
Advance Access publication February 22, 2010

# Condition-Dependent Pheromone Signaling by Male Rock Lizards: More Oily Scents Are More Attractive

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*Accepted January 19, 2010*

## Abstract

Pheromones of vertebrates are often a mixture of several chemicals with different properties and messages, and their production seems condition dependent. Thus, pheromones are a good, but little studied, example of multiple sexual signals. Femoral gland secretions of male rock lizards *Iberolacerta cyreni* contain steroids that may act as pheromones, but there are also many other lipids, such as oleic acid, whose allocation to secretions may be costly because it has to be diverted from body fat reserves. This suggests that oleic acid could also have some function in secretions. Chemical analyses showed that proportions of oleic acid in femoral secretions of males were positively related to body condition of males, suggesting that the oleic acid secreted may reflect the amount of body fat reserves of a male. Tongue-flick bioassays showed that females were able to detect by chemosensory cues alone differences in proportions of oleic acid in secretions of males. Scents of males with more oleic acid elicited stronger chemosensory responses by females. Further tests with chemical standards confirmed that females distinguished oleic acid, and changes in its concentration, from other chemicals that are naturally found in secretions of males. Moreover, choice trials of scent-marked substrates showed that females were more attracted to areas that were experimentally manipulated to increase the proportion of oleic acid in natural scent marks of males. We suggest that oleic acid in femoral secretions might be a reliable advertisement of a male's body condition, which females could use to select high-quality mates in conjunction with information provided by other chemicals. Alternatively, scent marks with more oleic acid might be simply more attractive to females if chemosensory responses of females to scent of males were originated by a preexisting sensory bias for food chemicals such as the oleic acid. Nevertheless, this sensory trap might have evolved into an honest signal because the elaboration of the signal seems differentially costly for males with different body conditions.

**Key words:** body condition, fatty acids, femoral glands secretion, pheromones, sexual selection

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## Introduction

The evolution of multiple sexual signals is one of the most controversial and least understood features of sexual selection. Multiple traits may either signal different characteristics of male condition or be redundant as a way to reinforce the reliability of signals (Møller and Pomiankowski 1993). In any case, a requisite for multiple signals to be stable as sexual signals may be that they are honest. Honesty can be achieved by different means, one of which is costly condition-dependent signals that act as handicaps (Johnstone 1995). Another possibility is the use of indices related to body condition (Searcy and Nowicki 2005). However, most of the research on multiple sexual traits has focused on signals that are perceived through visual sensory systems alone, despite use of other sensory systems, such as chemosensory ones by many animals (Wyatt 2003).

Chemical signals (pheromones) play an important role in intraspecific communication and sexual selection of many animals (Mason 1992; Penn and Potts 1998; Wyatt 2003). In vertebrates, pheromones are often a mixture of several chemical compounds with different properties (Müller-Schwarze 2006). Thus, a vertebrate pheromone may be defined as a group of active compounds in a secretion that supply information to, or change behavior in, another conspecific (Müller-Schwarze 2006). These pheromones may have different messages or intended receivers and different compounds or the pattern of compounds may signal sex, age, social status, group, or condition (Wyatt 2003; Müller-Schwarze 2006). For example, many lizards produce femoral chemical secretions that contain numerous chemical compounds (Weldon et al. 2008) and have pheromonal

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activity (Mason 1992; Alberts 1993). Behavioral tests have shown that lizard pheromones and chemosensory cues may give information on sex, body size, age or familiarity recognition (Mason 1992; Martín and López, forthcoming), or even more detailed characteristics, such as morphological traits and health condition (López et al. 2006; Martín, Civantos, et al. 2007). This information seems important in intrasexual relationships between males (López and Martín 2002; Carazo et al. 2007; Martín and López 2007; Martín, Moreira, and López 2007) and in female mate choice (Martín and López 2000, 2006b, 2006c; Olsson et al. 2003; Martín, Civantos, et al. 2007). These studies suggest that chemical signals of male lizards may convey multiple messages and, thus, may be a good example to study the evolution of multiple sexual traits. However, few studies have yet documented which are the chemical bases of the pheromonal differential recognition and which physiological mechanisms allow the evolutionary maintenance of pheromones as sexual signals.

Femoral gland secretions of lizards consist of both lipids and proteins (reviewed in Weldon et al. 2008). Most recent evidences point to lipids as the main compounds involved in pheromonal communication (Mason 1992; Martín and López 2006b). Lipids are regulated by the general metabolism, such that secreted lipids could be related to and, thus, signal the condition of the signaler. The lipophilic fractions of femoral secretions of male lacertid lizards are mainly composed of fatty acids and steroids, with minor quantities of other compounds (Weldon et al. 2008). Chemical compositions of secretions are characteristics of each species, but proportions of some compounds show a high interindividual variability (López et al. 2006; Martín and López 2006b; Martín, Civantos, et al. 2007). However, only a few studies have examined the function of these lipids in secretions or even whether lizards can actually discriminate by chemosensory cues alone between these compounds (Cooper and Pérez-Mellado 2001; Cooper et al. 2002a, 2002b; Martín and López 2006a, 2006b, 2008b).

The Carpetan rock lizard, *Iberolacerta cyreni* (formerly *Lacerta monticola cyreni*), is a small diurnal lacertid lizard found mainly in rocky mountain habitats of the Iberian Peninsula (Martín and Salvador 1997; Pérez-Mellado 1998). In the femoral glands secretions of males there is a mixture of more than 40 lipophilic compounds, mainly fatty acids and steroids (López and Martín 2005a). Female *I. cyreni* show stronger chemosensory responses and prefer the scent of males that allocate relatively more cholesta-5,7-dien-3-ol (= provitamin $D_3$) and ergosterol to femoral secretions (Martín and López 2006b). These preferred males are those of presumably high quality (i.e., those more symmetric and with a higher immune response), which suggests that females use these chemicals to choose potential mates. Further experiments confirmed that females can discriminate these steroids, and changes in their concentration, from similar chemicals (i.e., cholesterol) that are naturally found in males' secretions (Martín and López 2006b, 2008b). On the other hand, proportions of cholesterol in femoral secretions of males increase with body size (López et al. 2006). Cholesterol is chemically discriminated by males, elicits aggressive responses and affects agonistic interactions, likely because it signals aggressiveness or dominance (Martín and López 2007, 2008b).

However, as occurs in most lizards, there are many other chemical components in femoral secretions of male *I. cyreni*, which may function as multiple signals or as multiple components of the same signal. Thus, the question that arises is whether these "other" compounds have any function in secretions or have no function at all. The latter is especially questionable because some compounds have important physiological functions in the organism but are diverted from metabolism to be secreted. Thus, the allocation of these compounds to secretions may be costly, which suggest that these "costly" chemicals should also have some important function in secretions. Some compounds might have secondary functions such as to constitute an unreactive matrix that holds and delivers the compounds that are the true semiochemicals (e.g., cholesterol; Escobar et al. 2003) or to limit oxidation of other lipids in scent marks, contributing to their persistence (e.g., squalene, tocopherol, or waxy esters; Alberts 1992; López and Martín 2006; Gabirot et al. 2008), but some compounds might also have a signaling function per se.

Oleic acid (= cis-9-octadecenoic acid) is found in appreciable quantities in femoral secretions of many lizards, including *I. cyreni* (López and Martín 2005a; Weldon et al. 2008). This unsaturated fatty acid is a major constituent of energetic fat reserves of lizards and other animals (Derickson 1976; Loumbourdis 1987; Geiser and Learmonth 1994; Simandle et al. 2001). Therefore, lizards have to divert oleic acid from fat reserves to allocate it to femoral secretions. Correlative studies found that more symmetrical male *I cyreni* have higher proportions of oleic acid in their femoral secretions (López et al. 2006), which suggests that allocation of this lipid to secretions is differentially costly for males or that it may benefits males differentially (Johnstone 1995; Rowe 1999). Thus, we hypothesized that proportions of oleic acid in secretions of lizards may signal some characteristics of a male. More specifically, the amount of oleic acid available for secretions might be directly related to the amount of body fat reserves of a male, which would be reflected in his body condition (Bonnet and Naulleau 1994; Schulte-Hostedde et al. 2005). This suggested function of oleic acid in secretions is appealing because previous studies showed that female *I cyreni* prefer scent marks of males with higher body condition (López et al. 2002), and field studies confirmed that male *I cyreni* with a higher body condition have a greater reproductive success (Salvador et al. 2008). Moreover, male *I cyreni* that experimentally have lost their tail, and thus most of their body fat reserves, have lower mating success (Martín and Salvador 1993).

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The lacertid Lilford's wall lizard (*Podarcis lilfordi*) can discriminate among different lipids such as cholesterol and oleic and hexadecanoic acids (Cooper et al. 2002a, 2002b), and this has been related to the ability to find and identify prey by chemosensory cues. It is likely that female *I. cyreni* lizards can also discriminate oleic acid in scent marks of males. Females might prefer to establish in areas scent marked by males with more oleic acid in secretions to increase the probability of mating with males with a better body condition. A similar effect has been shown for other chemicals in secretions that are related to the quality of the immune system of males (López and Martín 2005b; Martín and López 2006b). Alternatively, scent-marked areas with more oleic acid might be simply more attractive if chemosensory responses of females to the scent of males evolved from a preexisting sensory bias for food-related chemicals such as the oleic acid (Martín and López 2008a). Nevertheless, this sensory trap for essential nutrients might evolve into an honest signal if the elaboration of the signal was differentially costly or differentially beneficial for males (Macías-García and Ramirez 2005; Martín and López 2008a).

In this paper, we explored the potential functions of oleic acid in femoral gland secretions of male rock lizards *I. cyreni* and its consequences for sexual selection processes. We hypothesized that the presence of oleic acid in femoral secretions might not be a mere by-product of the presence of other lipids but that it might have a signaling or female-attracting function. To test this, we first analyzed the relationships between proportions of oleic acid in femoral secretions of male lizards and their body condition. Then, we used tongue-flick (TF) bioassays and scent-choice trials to test the predictions that, if females used the proportions of oleic acid in scent marks of males to select mates, females should be able: 1) to detect by chemosensory cues alone changes in proportions of oleic acid in secretions of males, 2) to discriminate oleic acid, and changes in its concentration, from other chemicals that are naturally found in secretions of males, and 3) to be more attracted to areas scent marked by males with more oleic acid in their secretions.

## Materials and methods

### Study animals

We captured by noosing adult *I. cyreni* lizards (16 males and 16 females) of similar age/body size, at the beginning of May, before the start of their mating season, in different places over a 5 km$^2$ area ("Puerto de Navacerrada," Guadarrama Mountains, Central Spain; lat 40°47′N, long 04°01′W). Males and females were captured in different areas to avoid that females had previous experience with these particular individual males. Only adult lizards with intact or fully regenerated tails were considered. Lizards were individually housed at "El Ventorrillo" Field Station, 5 km from the capture site in outdoor 80 × 50 cm PVC terraria containing rocks for cover, and food (mealworms) and water ad libitum. Cages of males and females were in different places to avoid contact between them. All animals were healthy and were returned to their capture sites at the end of trials.

We measured males' body weight with a digital balance to the nearest 0.01 g (mean ± standard error [SE] = 7.1 ± 0.2 g, range = 6.1–8.6 g), and the snout-to-vent length (SVL) with a ruler to the nearest 1 mm (mean ± SE = 75 ± 1 mm, range = 70–78 mm). We calculated individual values of body condition as the residuals from the regression equation of ln mass (g) on ln SVL (mm), which may represent an index of the relative amount of fat stored, and hence an estimation of individual physical condition or nutritional status (Bonnet and Naulleau 1994; Naulleau and Bonnet 1996; Schulte-Hostedde et al. 2005).

### Chemical analyses of femoral secretions of males

On the same day of capture, we extracted small samples of femoral pores secretion of males by gently pressing with forceps around the pores and collected secretion directly in glass vials with Teflon-lined stoppers. Vials were stored at –20 °C until analyses were carried out. Samples were analyzed by gas chromatography–mass spectrometry (Finnigan-ThermoQuest Trace 2000) equipped with a Supelco, Equity-5 column, temperature programmed at 50 °C for 10 min, then, increased to 280 °C at 5 °C/min and kept at 280 °C for 30 min (for details see López and Martín 2005a; López et al. 2006). Identification of compounds was done by comparison of mass spectra in the National Institute of Standards and Technology/Environmental Protection Agency/National Institutes of Health 1998 library and later confirmed with authentic standards. A full description of methods of analyses and lipophilic compounds in femoral secretions of this lizard can be found in López and Martín (2005a) and López et al. (2006). The relative amount of each component was determined as the percent of the total ion current (TIC). The relative areas of the peaks were transformed following Aitchison's formula: $[Z_{ij} = \ln(Y_{ij}/g(Y_j))]$, where $Z_{ij}$ is the standardized peak area $i$ for individual $j$, $Y_{ij}$ is the peak area $i$ for individual $j$, and $g(Y_j)$ is the geometric mean of all peaks for individual $j$ (Aitchison 1986; Dietemann et al. 2003). The transformed areas of the different compounds found in each individual were used in posterior statistical analyses.

Relationships between proportions in secretions of oleic acid and proportions of other compounds or between proportion of compounds and body size and body condition of lizards were analyzed with nonparametric rank Spearman's correlations (Siegel and Castellan 1988). We estimated significance levels by using the sequential Bonferroni adjustment of Rice (1989) for multiple comparisons.

### Chemosensory responses of females to scent of males

We used TF bioassays as a test of detection of chemical cues based on the differential rates of tongue extensions of lizards to the different chemical stimuli (Cooper and Burghardt

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1990; Cooper 1994, 1998). We measured TF rate by females in response to chemical stimuli arising from cotton applicators impregnated with femoral gland secretions of different individual males. Every female (*n* = 16) was exposed to the scents of 2 different individual males, and, thus, secretions of each individual male (*n* = 16) were presented to 2 different individual females. Order of presentation was randomized.

We prepared stimuli by dipping the cotton tip (1 cm) of a wooden applicator attached to a long stick (50 cm) in deionized water. Femoral secretions of males were taken out with a pair of forceps and collected directly on cotton tips. We used approximately the same amount of femoral secretion in each stimulus (about 2 × 1 mm of solid secretion from each of 3 pores) to minimize likelihood that differences in TF rates were due to differences in the amount of secretions presented to females (Martín and López 2006b; Martín, Civantos, et al. 2007). Swabs were used in the trials immediately after collection to avoid fading of the stimuli, and a new swab was used in each trial.

Trials were conducted in outdoor conditions at the end of May, which coincided with the mating season of lizards in their original natural population (López et al. 2003), and between 1100 and 1300 h (GMT) when lizards were fully active. Females were allowed to bask and attain an optimal body temperature before trials. Only one trial was conducted per day for each animal. In each trial, one experimenter, blind to the treatment, slowly approached a lizard's home 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 during 1 min, beginning with the first TF. Based on previous studies with this and other lizard species (e.g., Martín and López 2006b; Martín, Civantos, et al. 2007), we hypothesized that scents more attractive to females should elicit higher TF rates by females. We used nonparametric rank Spearman's correlations (Siegel and Castellan 1988) to analyze the relationships between the average TF rates of 2 different females to femoral secretions of each individual male and his body condition or proportions of oleic acid in his femoral secretions.

### Detection of chemical compounds by females

In these experiments, we used a methodology similar to the previous experiment (see above) to compare TF rate by female lizards (*n* = 16) in response to stimuli arising from cotton applicators bearing chemical standards: 1) dichloromethane (DCM), 2) cholesterol, 3) hexadecanoic acid, and 4) oleic acid. The rationale for testing these chemicals was the following: 1) DCM was used as a pungency control, so as to gauge baseline TF rates under the experimental conditions, 2) cholesterol is the most abundant compound in femoral secretions of males, 3) hexadecanoic acid is the most abundant saturated fatty acid in secretions, and 4) oleic acid is the most abundant unsaturated fatty acid in secretions (see López and Martín 2005a; López et al. 2006). We prepared chemical stimuli the same day of the tests by dissolving 20 mg of each compound (authentic standards, GC grade, from Sigma-Aldrich Chemicals) in 1 mL of DCM inside glass vials closed with Teflon-lined stoppers. Then, we mixed the solution with a vortex and kept the vials in a refrigerator between trials. To human observers, the swabs prepared with all stimuli were visually similar. A new swab was used in each trial.

In a second test, we used a similar method to test whether females (*N* = 16) can discriminate differences in relative abundance of oleic acid when it was presented mixed with a majority of cholesterol. Thus, we aimed to simulate the natural occurrence of these compounds in femoral secretions of males (López and Martín 2005a). We measured the TF rate of females to cotton applicators bearing 2 different concentrations of oleic acid dissolved in DCM: "low" (5 mg/mL) and "high" (25 mg/mL), both mixed with cholesterol (100 mg/mL). We also used DCM as the pungency control.

To examine differences in latency to the first TF and number of directed TFs among conditions, we used one-way repeated measures analyses of variance (ANOVA) with scent stimuli as a within 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 used Tukey's honestly significant difference (HSD) tests (Sokal and Rohlf 1995).

### Effects of oleic acid on female choice of males' scent marks

At the beginning of the experiments, we placed in the males' cages several absorbent paper strips (35 × 10 cm) fixed to the floor and left them there for 2 weeks to allow males to scent mark them. The ventral location of the femoral pores allowed that secretions were passively deposited on the paper substrate as male lizards moved through their terraria, but active rubbing of the legs on the substrate has also been observed in this and other lizards (Martín and López 2006c; Martins et al. 2006).

Female choice of scent experiments were performed at the end of May, which coincided with the mating season of lizards in their original natural population (López et al. 2003). Females' cages had 2 basking platforms (2 identical flat tiles) placed symmetrically at each end of the cage and rocks for cover in the center. At the beginning of each test (0700 h GMT), when females were still inactive, we fixed with fresh gloves on one tile one paper strip scent marked by a male and another paper scent marked by a different male on the other tile. Then, we used glass syringes to spray over all the surface of each scent-marked paper with either 2 mL of DCM (control) or 2 mL of DCM that have dissolved oleic acid (20 mg/mL; mixed as above). DCM readily evaporated in a few minutes. With this procedure, we added supplemented oleic acid to the whole femoral secretions of males already presented on the scent marks of the paper.

Each female (*N* = 16) was tested over 2 days, once a day, with papers from 2 different pairs of males. The males tested

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and the positions of papers and treatments were randomly determined, but for each female, we excluded those males that have been used for the TF trials of that female. To control for potential differences in body condition of the pair of males tested, we calculated the average body condition of males used as control or experimental and found that they were not significantly different (one-way repeated measures ANOVA; $F_{1,31} = 0.03$, $P = 0.87$). Different papers from each male were used in each test. Each trial lasted 6 h (from 0900 h GMT, shortly after females appeared from night refuges, and until 1500 h GMT, when females hid again), Females were monitored each 15 min (25 scans) from a hidden point. If a female was located on a tile with the paper strip, she was designated as having chosen temporarily that particular paper, whereas if she was located outside of the tiles she was designated as having made no choice (for a similar procedure see Martín and López 2000; López et al. 2002, 2003; Olsson et al. 2003). To ensure that females were exposed to both males' tiles and were aware of both male's stimuli, at least 2 recordings in each male's section were considered necessary for a trial to be valid. This presumption was fulfilled in all tests. At the end of the trials the papers were removed and the cage was thoroughly rinsed with alcohol and clean water.

We compared the number of observations (squared root-transformed) of each female on each section of the terraria with a 2-way repeated measures ANOVA with treatment (control vs. oleic acid vs. nonchoice) and day of the trial, both as within factors. Pairwise comparisons used Tukey's HSD tests (Sokal and Rohlf 1995).

## Results

### Relationships between femoral secretions and male body condition

Proportions of oleic acid in femoral secretions of males varied between 0.7% and 3.9% (mean ± SE = 2.1 ± 0.2%) of TIC untransformed areas. Males with higher proportions of oleic acid also had significantly higher proportions of other fatty acids in secretions, such as hexadecanoic, hexadecenoic, otadecanoic, octadecadienoic (= linoleic), and eicosanoic acids (Spearman's rank-correlations with transformed TIC areas: $0.68 < r_s < 0.86$, $n = 16$, $t > 3.30$, $P < 0.004$ in all cases) but had significantly lower proportions of cholesterol ($r_s = -0.61$, $t = -2.87$, $n = 16$, $P = 0.012$). Proportions of oleic acid were not significantly related with proportions of ergosterol ($r_s = 0.29$, $t = 1.13$, $n = 16$, $P = 0.28$) or cholesta-5,7-dien-3-ol ($r_s = 0.30$, $t = 1.19$, $n = 16$, $P = 0.25$).

Males with a higher body condition had significantly higher proportions of oleic acid in their femoral secretions (Spearman's rank-correlation: $r_s = 0.69$, $t = 3.60$, $n = 16$, $P = 0.003$) (Figure 1a). Proportions of oleic acid were not significantly related to SVL ($r_s = -0.05$, $t = -0.20$, $n = 16$, $P = 0.84$) or body weight ($r_s = 0.29$, $t = 0.85$, $n = 16$, $P = 0.41$). Body condition was not significantly related to proportions of other fatty acids ($-0.11 < r_s < 0.12$, $n = 16$, $-0.42 < t < 0.47$, $P > 0.65$ in all cases).

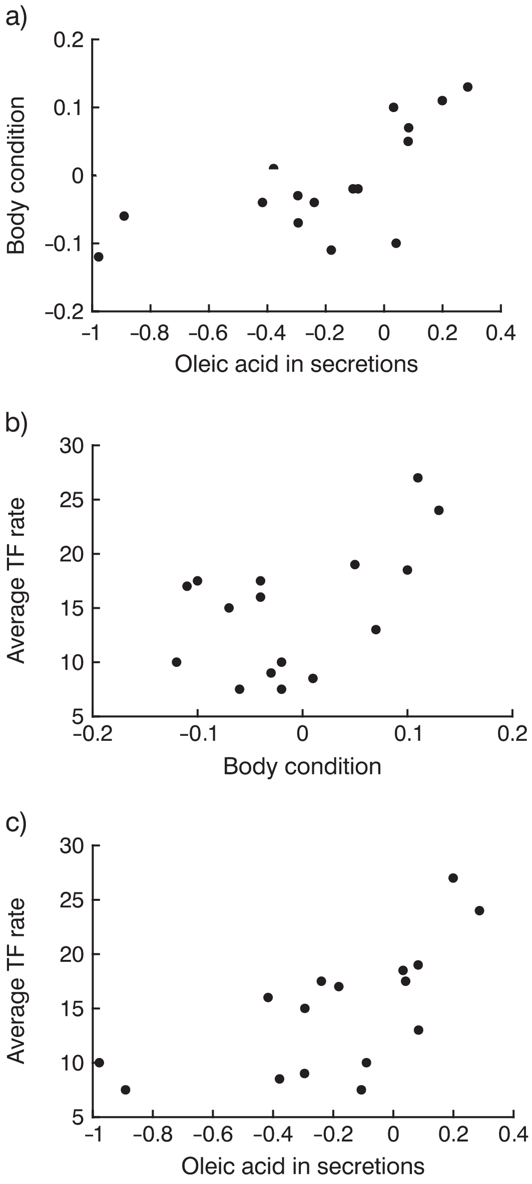

**Figure 1** **(a)** Relationships between body condition of males and proportions of oleic acid (transformed TIC areas; see Materials and methods) in femoral secretions of these individual males. **(b)** Relationship between the average TF rate per minute of 2 females to the scent of each individual male and the body condition of these males. **(c)** Relationship between average TF rate per minute of females and proportions of oleic acid in secretions of males.

### Chemosensory responses of females to scent of males

There was a trend for a positive relationship, but that did not reach statistical significance, between the average TF rate of females to the scent of each individual male and the body condition of this male (Spearman's rank-correlation: $r_s = 0.38$, $t = 1.56$, $n = 16$, $P = 0.14$) (Figure 1b), with scent from

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males with a higher body condition tending, although not significantly, to elicit higher average TF rates by females. However, there was a significant positive relationship between the average TF rate of females to the scent of each individual male and the proportions of oleic acid (transformed TIC area) in femoral secretions of this male ($r_s = 0.68$, $t = 3.44$, $n = 16$, $P = 0.004$) (Figure 1c). Thus, scent from males with higher proportions of oleic acid in their secretions elicited higher TF rates by females.

### Detection of chemical compounds by females

All female lizards responded to swabs by tongue flicking. The latency to the first TF differed significantly between chemical compounds stimuli (repeated measures one-way ANOVA: $F_{3,45} = 6.70$, $P < 0.001$) (Figure 2a). Latencies to DCM did not differ from those to cholesterol (Tukey's test: $P = 0.20$) but were significantly longer than for hexadecanoic and oleic acid ($P < 0.008$ in both cases), which did not differ between them ($P = 0.84$). Latencies to cholesterol did not differ from latencies to the 2 fatty acids ($P > 0.14$ in both cases).

The rate of TFs directed to swabs differed significantly between chemical compounds stimuli (repeated measures one-way ANOVA: $F_{3,45} = 24.66$, $P < 0.0001$) (Figure 2b). Females made significantly more TFs directed to oleic acid than to other chemical stimuli (Tukey's tests: $P < 0.01$ in all cases), a similar TF rate to hexadecanoic acid and cholesterol ($P = 0.16$), and more TFs to hexadecanoic acid and to cholesterol than to DCM ($P = 0.005$).

In the second test, latencies to the first TF differed significantly between chemical compounds stimuli (repeated measures one-way ANOVA: $F_{2,30} = 13.13$, $P < 0.0001$) (Figure 3a). Latencies to DCM were significantly longer than for the 2 concentrations of oleic acid (Tukey's tests: $P < 0.006$ in both cases). Low and high concentrations of oleic acid had not significantly different latencies ($P = 0.22$).

The rate of TFs directed to swabs differed significantly between stimuli (repeated measures one-way ANOVA: $F_{2,30} = 133.28$, $P < 0.0001$) (Figure 3b). Females made significantly more TFs directed to the higher concentration of oleic acid than to other chemical stimuli (Tukey's tests: $P = 0.00012$ in both cases), and more TFs to the low concentration of oleic acid than to DCM ($P = 0.04$).

### Effects of oleic acid on female choice of males' scent marks

In the tests with supplementation of oleic acid on papers scent marked by males, there were significant differences in the number of observations of females on a determined stimulus paper strip between treatments (repeated measures

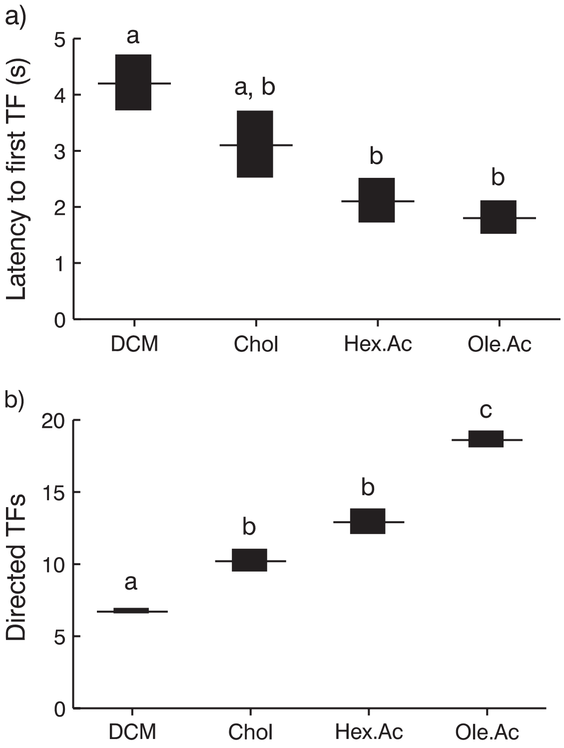

**Figure 2** Mean (±SE) **(a)** latency and **(b)** number of TFs/min directed to swabs by female lizards in response to cotton-tipped applicators bearing DCM, cholesterol (Chol), hexadecanoic acid (Hex.Ac), or oleic acid (Ole.Ac), all dissolved (20 mg/mL) in DCM.

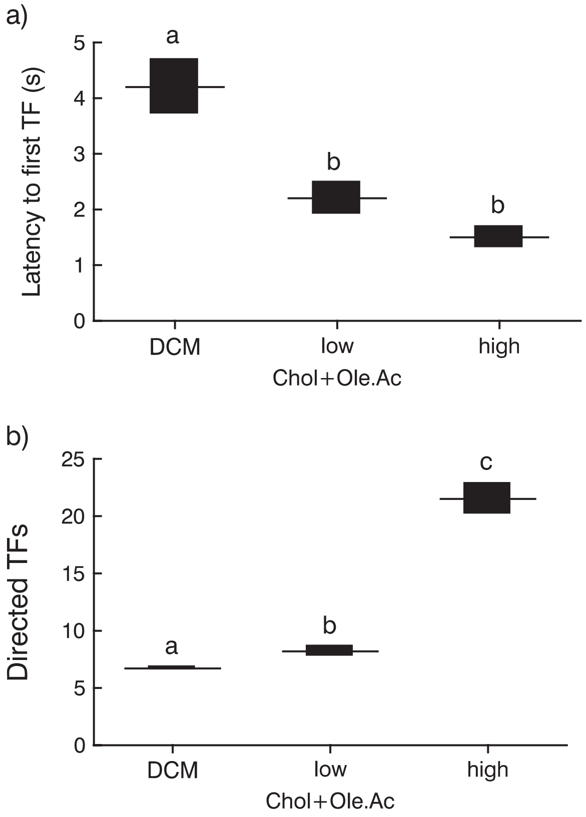

**Figure 3** Mean (±SE) **(a)** latency and **(b)** number of TFs directed to swabs by female lizards in response to cotton-tipped applicators bearing 2 different concentrations (low: 5 mg/mL; high: 25 mg/mL) of oleic acid (Ole.Ac) mixed with cholesterol (Chol; 100 mg/mL) and dissolved in DCM.

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2-way ANOVA: $F_{2,30} = 69.80$, $P < 0.0001$) but not between days ($F_{1,15} = 1.00$, $P = 0.33$), and the interaction was not significant ($F_{2,30} = 0.91$, $P = 0.41$) (Figure 4). Females spent more time on paper strips scent marked by males and supplemented with oleic acid than on the nonchoice area and than on control scent marked paper strips with DCM alone (Tukey's tests: $P = 0.00012$ in both cases). Time spent on the nonchoice area did not differ from time spent on papers with DCM alone ($P = 0.66$).

## Discussion

Our results suggested that oleic acid in femoral secretions and scent marks of male *I. cyreni* lizards may have a signaling or attracting function because higher proportions of oleic acid seem to increase the attractiveness of a male scent for females. Furthermore, chemosensory tests showed that females discriminated oleic acid from other compounds and that this fatty acid elicited higher chemosensory responses. Also, the presence of oleic acid in scent marks affected females' space use. These results suggest that males that produced territorial scent marks with more oleic acid might increase their mating success because females would be more attracted and spend more time in these territories.

Oleic acid is a major constituent of animal fat (Geiser and Learmonth 1994; Simandle et al. 2001). In the organism, oleic acid has important metabolic functions as a way to store energetic reserves (Derickson 1976; Loumbourdis 1987). Therefore, secreting oleic acid for substrate scent marking should be costly for males. This is so because secreted oleic acid has to be diverted from body fat reserves and general metabolism. This cost may explain the positive relationship between oleic acid in secretions and body condition of males, suggesting that the amount of oleic acid secreted may depend on the amount of fat reserves. Thus, only males that have a good body condition (i.e., a good supply of fat body reserves) should be able to produce secretions with higher amounts of oleic acid. In this sense, the proportions of oleic acid in femoral secretions may honestly reflect the current body condition of a male, which suggests the potential role of these secretions as condition-dependent honest advertisements (Grafen 1990; Kotiaho 2001). To maintain, and signal, a good body condition would be important for male *I cyreni* because other studies have suggested that it may affect female scent preferences (López et al. 2002) and results in a greater mating (Martín and Salvador 1993) and reproductive success (Salvador et al. 2008). Similarly, in the common lizard, *Lacerta vivipara*, a species that also has oleic acid in femoral secretions (Gabirot et al. 2008), males with higher body condition have higher reproductive success (Hofmann and Henle 2006). The effect of body condition on reproductive success was explained because males with higher body condition might be more successful in male–male interactions or might be able to search more effectively for females (Hofmann and Henle 2006; Salvador et al. 2008). Additionally, our results might suggest that males with higher body condition would also produce more attractive scent marks for females. Interestingly, the nutritional condition of a male also affects the attractiveness of the male pheromones to females in cockroaches, *Nauphoeta cinerea* (Clark et al. 1997) and in mealworm beetles *Tenebrio molitor* (Rantala et al. 2003). However, to show that oleic acid is mediating female choice in rock lizards, further experiments should manipulate body condition of male lizards, through a manipulation of their diets, and then test the responses of females to their scents

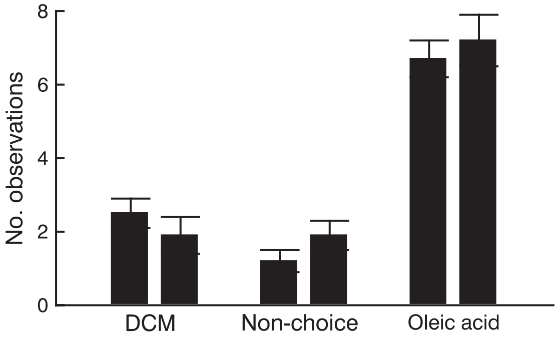

**Figure 4** Number of times (mean + SE), during each of 2 scent's choice trials, that females were observed on paper strips scent marked by males, and on which we added DCM (control) or oleic acid dissolved in DCM, or on a central nonchoice area without males' scents.

The higher and differential TF rates of female *I. cyreni* to oleic acid showed that females can detect and discriminate different concentrations of oleic acid from other chemicals naturally found in secretions of males by using chemosensory cues alone. Oleic acid elicits very strong lingual and biting responses in *P. lilfordi* lizards too (Cooper et al. 2002a), which suggests that oleic acid in particular, and probably other unsaturated fatty acids, contribute strongly to the food-related lingual and biting responses to animal fat (lipids) observed in lizards (Cooper and Pérez-Mellado 2001; Cooper et al. 2002b). Oleic and other similar fatty acids are found in many types of animal and vegetal food species, which may allow identification of a wide range of food items (Cooper et al. 2002b).

Our experiment indicated that female *I. cyreni* also detected and discriminated different concentrations of oleic acid in femoral secretions of males. Females showed higher chemosensory responses to femoral secretions of males that had higher proportions of oleic acid, which suggest that these scents with more oleic acid were more attractive (Martín, Civantos, et al. 2007). However, we cannot discriminate whether oleic acid has a signaling function per se (i.e., signaling body condition of males) or whether the strong chemosensory responses of females to oleic acid in scent of males were originated by a preexisting sensory bias to food-related chemicals (Macías-García and Ramirez 2005; Martín and López 2008a). Nevertheless, this sensory trap might evolve into an honest signal if the elaboration of the signal was

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differentially costly or differentially beneficial for males with different body conditions.

Female *I. cyreni* also detected hexadecanoic acid, a saturated fatty acid, but their chemosensory responses to this lipid were lower. Although within a foraging context, Cooper et al. (2002a) suggested that the 2 major categories of fatty acids, saturated and unsaturated, might have different effects on chemosensory responses of lizards because at ambient temperatures unsaturated fatty acids may be accessible as liquids, whereas saturated fatty acids may be waxes. With respect to femoral secretions of male lizards, both saturated and unsaturated fatty acids are found in secretions, and proportions of different fatty acids in secretions are correlated among them such that males with higher proportions of oleic acid also have higher proportions of other fatty acids. Thus, it is likely that females actually responded to the whole mix of fatty acids or that under different temperature conditions some fatty acids were more effective than others in eliciting chemosensory responses of females.

In contrast, chemosensory responses of females to cholesterol (a major constituent of fat and femoral secretions of males) were low, although it was detected. This agrees with previous results with this and other lizards (Cooper et al. 2002a; Martín and López 2006a, 2006b, 2008b). Interestingly, proportions of cholesterol and oleic acid in secretions of males were negatively related in this study. Also, cholesterol elicits higher chemosensory responses in males than in females (Martín and López 2008b). Higher proportions of cholesterol in secretions are related to body size and might signal a greater fighting ability or aggressiveness to other males (Martín and López 2007). These results support the possibility that chemical signals of males may be advanced by different selection pressures (López et al. 2002; Martín and López 2008b). Secretion of some chemicals might have evolved to function in intrasexual relationships (Martín and López 2007), but they may be unimportant or unattractive for females, which seem to "prefer" other chemical traits in their potential mates (Martín and López 2006b, 2008b). This may be explained because, as occurs in many animals, sexual traits signaling male dominance are not always attractive to females when they are not reliable indicators of better genetic quality for females, which would lead females to base their mate choice on other male traits (Quarnström and Forsgren 1998; Andersson et al. 2002; Wong and Candolin 2005). Thus, male lizards may initially face a trade-off between allocating more cholesterol to secretions, which may benefit them in intrasexual contests (Martín and López 2007), and allocating more fatty acids and more provitamin D, which would increase the attractiveness of scent to females (Martín and López 2006b, 2006c). However, if composition of secretions depended directly on physiology and metabolism, the proportions of their lipid components might honestly reflect the actual condition of a male for intrasexual and intersexual selective processes.

Responses of females to femoral secretions of males may be more complex and be the result of additive or synergetic effects of the "cocktail" of chemicals found in natural secretions of males, which would function as multicomponent signals. For example, previous studies showed that cholesta-5,7-dien-3-ol and ergosterol, 2 steroids found in secretions of males and related to their health state, elicit strong chemosensory responses in females (Martín and López 2006b, 2006c). However, the results of the current study indicate that proportions of these steroids and those of oleic acid are not correlated, probably because they are related to different male traits. Therefore, female "preferences" for a determined scent might result from a multicomponent chemical signal. This would explain the lack of significance in the relationship between responses of females and body condition of males in our experiment. Females would base their "preferences" in a combination of characteristics of males. Similarly, in the lizard *Urosaurus ornatus*, female attraction depends on the additive and interactive effects of multiple male visual traits combined, such as body mass, head size, and body and tail coloration (Hamilton and Sullivan 2005). Multiple traits may either signal different characteristics of a male or be redundant as a way to reinforce the reliability of signals (Sullivan 1994; Rowe 1999). Further studies should examine responses to different mixtures of chemicals with different concentrations of components, examining which specific combinations of chemicals elicit stronger responses and whether there are potential trade-offs between different chemicals signaling different characteristics.

We conclude that higher amounts of oleic acid in femoral secretions of male *I. cyreni* lizards seem to increase the attractiveness of males' scent to females. Chemosensory responses of females to oleic acid may have evolved from a preexisting sensory bias for food chemicals. However, allocation of oleic acid to secretions may depend on the body condition of a male and be differentially costly for males. Therefore, it is possible that the initial sensory trap based on females' bias for oleic acid has evolved into an honest signal of the body condition of a male.

## Funding

This work was supported by Ministerio de Ciencia e Innovación (MCI-CGL2008-02119/BOS).

## Acknowledgements

We thank 2 anonymous reviewers for helpful comments and "El Ventorrillo" Museo Nacional de Ciencias Naturales Field Station for use of their facilities. Research was conducted under license from the "Comunidad de Madrid" Environmental Agency.

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