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Chemosensory Exploration of Male Scent by Female Rock Lizards Result from Multiple Chemical Signals of Males

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Abstract

Multiple sexual signals may convey information on males' quality. However, most research focused on visual signals, ignoring chemical signals. In vertebrates, chemical signals are probably often a multicomponent mixture of several active compounds, but they are not well known. We examined the potential additive and interactive effects of 2 compounds (oleic acid and ergosterol) naturally found in femoral gland secretions of male rock lizards on chemosensory exploration behavior of females. Tongue-flick (TF) rates of females to male secretions may result from the combination of responses to multiple compounds. There may be an additive or synergetic effect because male secretions with the highest proportions of both compounds received the highest TF rates, suggesting that their scents were more intriguing. However, there might be an interactive effect too; female TF rates were higher to males' scent with high proportions of ergosterol alone, even if proportions of oleic acid were low, than to high proportions of oleic acid but with low proportions of ergosterol. Further bioassays testing TF behavior of females to standard compounds, presented alone or combined in different concentrations confirmed these findings. Variations in female TF behavior might be explained because different compounds signal different male traits of different importance for females. Our study suggested that femoral secretion of male rock lizards may act as a multicomponent chemical signal.

Key words: chemical signals, lizards, mate choice, multiple signals, sexual selection

Introduction

Sexual selection often results in the evolution of multiple sexual signals that may convey information on the genetic and phenotypic quality of males (Iwasa and Pomiankowski 1994; Johnstone 1995). Multiple traits may either signal different characteristics of a male condition or be redundant as a way to reinforce the reliability of signals (Møller and Pomiankowski 1993; Rowe 1999). For example, in male peacocks, tail feathers and behavioral displays may signal general and current health state respectively to females (Loyau et al. 2005). Female mate attraction in ornate tree lizards, *Urosaurus ornatus*, depends on multiple male visual traits combined, such as body mass, head size, and body and tail coloration (Hamilton and Sullivan 2005).

However, most of the research on multiple sexual traits has focused on visual signals, while multiple chemical signals have been little studied (Mason 1992; Wyatt 2003). In vertebrates, chemical signals are often a multicomponent mixture of several chemical active compounds, which may have different messages or intended receivers but may also act together providing specific or individual "odor profiles," also named "gestalts," "patterns," or "mosaics" (Johnston 2005; Müller-Schwarze 2006). For example, in the badger (*Meles meles*), the subcaudal gland secretes a mixture of many carboxylic acids and other compounds, and variations in the pattern of these compounds may signal sex, group membership, or individuals (Buesching et al. 2002). Also, many lizards produce chemical secretions from the femoral glands that contain numerous compounds (Weldon et al. 2008), and multiple compounds may be used as sexual signals (Mason 1992; Alberts 1993; Mason and Parker 2010). Lizard chemical signals not only provide information on sex and age but also on morphological traits and health condition (Mason and Parker 2010; Martín and López 2011). Information from multiple chemical signals may convey multiple messages that are used in intrasexual relationships between males (Aragón et al. 2000; Carazo et al. 2007; Martín and López 2007; Martín, Moreira, and López 2007) and/or in female mate choice (Martín and López

2000, 2006a, 2006b; Olsson et al. 2003; Martín, Civantos, et al. 2007). Therefore, chemical signals of lizards may be a good example to study the evolution of multiple sexual chemical signals.

The lipophilic fractions of femoral gland secretions of male lacertid lizards are mainly composed of steroids and fatty acids, with minor quantities of other compounds (Weldon et al. 2008). Chemical compositions of secretions are characteristics of each species, but within a species, proportions of some compounds show a high interindividual variability that may be related to variability in some males' traits (López et al. 2006; Martín and López 2006b; Martín, Civantos, et al. 2007). For example, in the carpetan rock lizard, *Iberolacerta cyreni* (formerly *Lacerta monticola cyreni*), the femoral glands secretion of males is a mixture of more than 40 lipophilic compounds (López and Martín 2005). Some of these male compounds have been shown to affect female responses and, thus, seem to be potentially implicated in female mate choice (Martín and López 2006b, 2010). We hypothesized that femoral secretions of male lizards may function as a multiple signal, or as multiple components of the same signal, with different compounds signaling different or related traits of a male's quality. Female *I. cyreni* show strong chemosensory responses and prefer to use areas scent marked by males with femoral secretions containing higher proportions of ergosterol (Martín and López 2006b, 2008) or oleic acid (Martín and López 2010). This has been probably favored because males with more ergosterol have a higher immune response, whereas males with more oleic acid have higher body conditions (Martín and López 2006b, 2010). Therefore, these data suggest that female rock lizards use multiple sources of information from at least these 2 compounds to choose potential mates of high quality. Because it is unlikely that all individual males with the best body condition were exactly those with the best immune system too, females might use information on the quality of both traits and decide which one, or which combination of both traits, is "more important" and favored when selecting a mate. However, it is unknown which are the additive and interactive signaling effects of multiple compounds in secretions of a male lizard, and how females could integrate and respond to information provided from different multiple chemical signals in order to assess the quality of a given male.

In this paper, we examined the potential additive and interactive effects of oleic acid and ergosterol on the chemosensory exploration behavior of females to femoral gland secretions of male rock lizards *I. cyreni*. To test this, we first analyzed the relationships between proportions of oleic acid and ergosterol in femoral secretions of several individual male lizards and the magnitude of the tongue-flick (TF) behavior of females to the scent of these individual males. Then, to test experimentally whether female chemosensory exploration was dependent on these 2 compounds, we used TF bioassays to compare the responses of females to different concentrations of oleic acid and ergosterol (commercial standards) when they were presented alone or combined with different concentrations in the same chemical stimuli.

Materials and methods

Study animals

We captured by noosing adult *I. cyreni* lizards during May 2010 and 2011 at "Alto del Telégrafo" (Guadarrama Mountains, Madrid Prov., central Spain; lat 40°47′N, long 04°01′W) at an elevation of 1900 m. The study area is characterized by a high cover of granite rock boulders and screes interspersed with shrubs (*Cytisus oromediterraneus* and *Juniperus communis*) and meadows of *Festuca* and other grasses (Martín and Salvador 1997). Activity of lizards in this area lasts from late April to early October, and the mating season occurs in May–June, producing a single clutch in July (Salvador et al. 2008). Captured lizards were transferred and 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. At the end of trials, all lizards were returned to their capture sites with a healthy state.

Chemical analyses of femoral secretions of males

Immediately after capture, we extracted femoral pores secretion of males by gently pressing with forceps around the pores. Secretion (less than 1 g per male) was collected directly in glass vials with glass inserts and Teflon-lined stoppers that were stored at –20 °C until analyses. Before the analyses, we added 50 L of *n*-hexane (Sigma-Aldrich, capillary GC grade, purity >97.0%) to each vial and mixed the solution with a vortex. Samples were analyzed by gas chromatography–mass spectrometry (Finnigan-ThermoQuest Trace 2000) equipped with a Thermo Fisher Trace TR-5 column (30 m length, 0.25 mm ID, and 0.25-mm film thickness). We used an autosampler (Finnigan-ThermoQuest AS2000) to made automatic sample injections (2 μL of each sample dissolved in *n*-hexane) using a splitless injector at 250 °C and helium as the carrier gas at 30 cm/s. The oven temperature program was as follows: 50 °C isothermal for 5 min, then increased to 270 °C at a rate of 10 °C/min, isothermal for 1 min, then increased to 300 °C at a rate of 15 °C/min, and finally isothermal (300 °C) for 10 min. Identification of compounds was done by comparison of mass spectra in the NIST/EPA/NIH 1998 library and later confirmed with authentic standards (for details of compounds in secretions, see López and Martín 2005; López et al. 2006).

We determined the relative amount of each compound as the percent of the total ion current (TIC). The relative areas 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). We then used transformed TIC areas of octadecenoic (=oleic) acid and ergosterol of each individual for further analyses.

Chemosensory exploration behavior of females to scent of males

To evaluate the magnitude of the chemosensory exploration of females to chemical cues in femoral secretions of males, we used TF bioassays, which are based on the differential rates of tongue extensions of lizards to the different chemical stimuli (Cooper and Burghardt 1990; Cooper 1994, 1998). We compared TF rate by female lizards in response to chemical stimuli arising from cotton applicators impregnated with femoral gland secretions of different individual males. Based on previous studies (e.g., Martín and López 2006a), we considered that scents more intriguing or ‘‘attractive’’ to females should elicit higher TF rates by females.

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 consisted of a waxy substance, which was easily extracted by gently pressing with forceps around the femoral pores 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), thus, ensuring that any possible difference in TF rates was not due to differences in the amount of secretions presented to females but due to differences in concentration of compounds in secretions. A new swab was used in each trial. Cotton swabs with femoral secretions were used in the trials immediately after collection to avoid fading of the chemical stimuli.

Every female ($n = 16$) was exposed to scents of 2 individual males. Thus, secretions of each of 16 individual males were presented to 2 different individual females. One trial was conducted per day for each female. Trials were conducted in outdoor conditions at the end of May, coinciding with the mating season of lizards in their natural population (Salvador et al. 2008), and between 1100 and 1300 h (GMT) when lizards were fully active. Each individual was allowed to bask and attain an optimal body temperature for at least 2 h before trials (Martín and Salvador 1993). 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 during 1 min, beginning with the first TF.

To examine whether we could relate the average TF rate (log-transformed) that the femoral secretions of an individual male elicited in 2 different females with the proportions (transformed TIC areas) of oleic acid and ergosterol in his secretions, we used general regression models (GRMs) with these 2 compounds as potential predictors of the TF rate of females.

TF behavior to compounds by females

In these experiments, we used a methodology similar to the previous TF experiment (see above section, "Chemosensory exploration behavior of females to scent of males") to compare TF rate by female lizards ($n = 12$; different individuals than in the previous tests) in response to stimuli arising from cotton applicators bearing dichloromethane (DCM) alone (used as a pungency control to gauge the baseline TF rates in the experimental situation) or 2 concentrations (‘‘low’’ vs. ‘‘high’’; see following text below) of oleic acid or ergosterol. We prepared chemical stimuli the same day of the tests by dissolving either 5 mg (‘‘low’’ concentration) or 25 mg (‘‘high’’ concentration) of each compound (authentic standards, GC grade, from Sigma-Aldrich Chemicals) in 1 mL of DCM (Sigma-Aldrich Chromasol V plus for HPLC, purity >99.9%) inside glass vials closed with Teflon-lined stoppers. Then, we mixed the solution with a vortex and kept the vials in a refrigerator before trials.

We had 5 stimuli: DCM alone, low oleic (‘‘Ole–’’), high oleic (‘‘Ole+’’), low ergosterol (‘‘Erg–’’), and high ergosterol (‘‘Erg+’’). Each female was tested during 5 consecutive days with only one of these stimuli each day in a random order. Trials were conducted in outdoor conditions at the beginning of June, between 8.00 and 12.00 h (GMT) when lizards were fully active. Immediately before the trials, we prepared stimuli by dipping the cotton tip (1 cm) of a wooden applicator attached to a long stick (50 cm) for 3 s in vials containing chemical stimuli. Swabs with all stimuli were visually similar for humans. A new swab was used in each trial. One experimenter slowly moved the cotton swab to a position 2 cm anterior to the lizard’s snout. The experimenter was blind to the compounds presented. We recorded latency to the first TF and numbers of TFs directed to the swab during 1 min, beginning with the first TF.

All female lizards responded to swabs by tongue flicking in all tests. To examine differences in latencies or number of directed TFs among treatments, we used one-way repeated measures analyses of variance (ANOVAs) with chemical stimuli as a within factor. Data were log-transformed to ensure normality (Shapiro–Wilk 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).

In a second experiment, we used a similar method to test the responses of females ($n = 15$; different individuals than in the previous tests) to oleic acid and ergosterol dissolved in DCM when they were presented mixed in the same scent stimuli and varying their concentrations (0, 5, or 25 mg/mL). Thus, we had a total of 9 stimuli with all possible different combinations (see Table 1). The 0/0 combination was actually DCM alone (used as a pungency control to gauge the baseline TF rates in the experimental situation), whereas the 0/5 and 0/25 (or 5/0 and 25/0) combinations were

Table 1 Mean (±standard error) latency(s) and number of TF directed to swabs by female lizards in response to cotton-tipped applicators bearing mixes of oleic acid and ergosterol (standard compounds) with different concentrations dissolved in DCM

		Ergosterol		
		0 mg/mL	5 mg/mL	25 mg/mL
Latency(s)				
	0 mg/mL	5.9 ± 0.7	4.2 ± 0.4	2.3 ± 0.4
Oleic acid	5 mg/mL	8.1 ± 0.7	3.2 ± 0.2	1.1 ± 0.1
	25 mg/mL	6.7 ± 0.4	2.3 ± 0.1	1.1 ± 0.1
			Ergosterol	
Directed TFs				
	0 mg/mL	3.0 ± 0.2	4.3 ± 0.3	8.2± 0.5
Oleic acid	5 mg/mL	4.1 ± 0.3	4.6 ± 0.3	8.5 ± 0.4
	25 mg/mL	5.9 ± 0.2	4.8 ± 0.3	10.6 ± 0.4

equivalent to the presentation of a single chemical alone. Chemical stimuli were prepared as above, and we measured the latencies and TF behavior of females to these stimuli as above. Data were log-transformed to ensure normality. To examine differences among treatments, we used a factorial 2-way repeated measures ANOVAs with the concentrations of each chemical stimuli (oleic acid or ergosterol) as factors.

Finally, to analyze the possible existence of additive effects on the TF behavior of females when ergosterol and oleic acid were found together, we designed a third experiment where we tested females (*n* = 15; different individuals than in the previous tests) with ergosterol and oleic acid presented alone or together in 4 different concentrations (0, 5, 20, and 40 mg/mL). To demonstrate the additive or synergic effects of the 2 compounds, results should show an upper-shift of the top of the dose–response curve or at least a left-shift of the dose–response curve, by the combination of the 2 compounds. To examine differences among dose–response curves, we used 2-way ANOVAs with treatment (oleic acid alone, ergosterol alone, or oleic acid and ergosterol combined) and the 4 different concentrations as factors.

Results

Chemosensory exploration behavior of females to scent of males

Males with higher proportions of oleic acid in secretions tended although nonsignificantly to have also higher proportions of ergosterol ($r = 0.41$, $F_{1,14} = 2.78$, $P = 0.12$). This lack of significance might be explained because some individuals with higher proportions of ergosterol in secretions had, however, lower proportions of oleic acid.

The scent of males with higher relative proportions of both oleic acid and ergosterol in their femoral secretions elicited significantly higher average TF rates by females (GRM model: $R^2 = 0.57$, $F_{2,13} = 8.60$, $P = 0.004$; oleic acid: $\beta = 0.43$, $F_{1,13} = 4.74$, $P = 0.048$; ergosterol: $\beta = 0.46$, $F_{1,13} = 5.47$, $P = 0.036$) (Figure 1).

Partial correlations showed that both proportions of oleic acid ($r = 0.52$, $P < 0.05$) and ergosterol ($r = 0.54$, $P < 0.05$) were significantly related to the TF rate of females when controlling for the effect of the potential correlation between the proportions of these 2 compounds.

TF behavior to compounds by females

In the test of female TF behavior to different concentrations of oleic acid or ergosterol presented alone, the latency to the first TF differed significantly between chemical compounds stimuli (repeated measures one-way ANOVA: $F_{4,44} = 9.81$, $P < 0.0001$) (Figure 2a). Latencies to DCM were significantly longer than to Erg+ (Tukey's test: $P = 0.02$), but they did not significantly differ from latencies to other stimuli (Erg–: $P = 0.10$; Ole–: $P = 0.99$; Ole+: $P = 0.43$). Latencies to Erg+ were significantly shorter than to Erg– ($P = 0.04$) and to the 2 concentrations of oleic acid (Ole+ and Ole–; $P < 0.0003$ for both). Latencies to Ole– were significantly longer that to the 2 concentrations of ergosterol ($P = 0.00014$ for Erg+ and $P = 0.04$ for Erg–). Latencies to Ole+ and Ole– were not significantly different ($P = 0.92$) nor were latencies to Ole+ and Erg– ($P = 0.28$).

The rate of TFs directed to swabs differed significantly between chemical compounds stimuli (repeated measures one-way ANOVA: $F_{4,44} = 43.08$, $P < 0.0001$) (Figure 2b). TF rates to DCM alone were significantly lower than to any other stimuli (Tukey's tests: $P < 0.0001$ in all cases). Females made significantly more TFs directed to Erg+ than to the other chemical stimuli ($P < 0.005$ in all cases), a similar TF rate to Erg– and Ole+ ($P = 0.76$), and less TFs to Ole– than to the other stimuli (at least $P < 0.03$ in all cases).

In the second experiment to test females' TF behavior to different mixtures of oleic acid and ergosterol, latencies to the first TF differed significantly between concentrations of ergosterol (repeated measures 2-way ANOVA: $F_{2,28} = 164.84$, $P < 0.0001$) and between concentrations of oleic acid ($F_{2,28} = 3.70$, $P = 0.037$), but the interaction between the different concentrations of these 2 compounds was significant ($F_{4,56} = 11.28$, $P < 0.0001$) (Table 1). Latencies to DCM alone (i.e., concentrations of 0 mg/mL) were significantly longer than to any stimuli containing ergosterol either alone or combined (Tukey's tests: $P = 0.004$ in all cases) but significantly shorter than to any stimuli with oleic acid alone ($P < 0.04$ for all). When any of the compounds was found alone, increasing their concentration resulted in significantly shorter latencies ($P < 0.04$ for all). When the 2 compounds were mixed, latencies to the combination of the 2 compounds with low concentrations (5 mg/mL) were significantly longer than to all other combinations (Tukey's tests: $P < 0.0015$ in all cases). Also, latencies to the mix of ergosterol at low

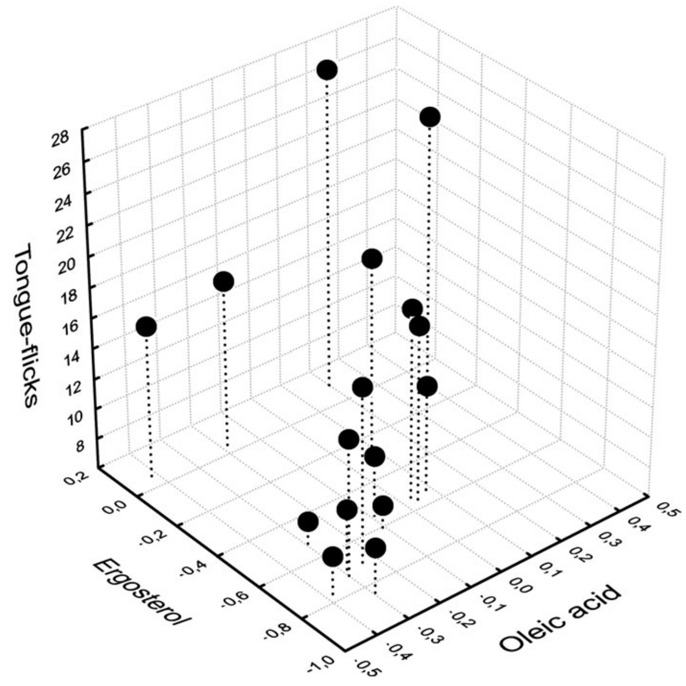

Figure 1 Relationship between relative proportions of oleic acid and ergosterol in femoral secretions of a male lizard (transformed TIC areas) with the average TF rate directed to swabs with his scent by 2 different individual female lizards.

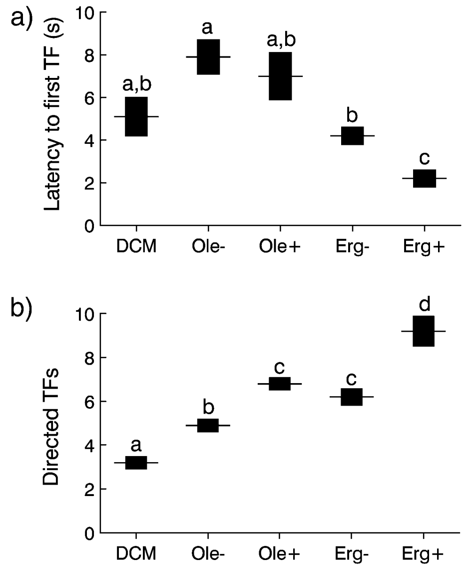

Figure 2 Mean (+standard error) **(a)** latency and **(b)** number of TFs/min directed to swabs by female lizards in response to cotton-tipped applicators bearing DCM alone or 2 different concentrations (low (−): 5 mg/mL; high (+): 25 mg/mL) of oleic acid (Ole) or ergosterol (Erg) (standard compounds), all dissolved in DCM.

concentration (5 mg/mL) with oleic acid at high concentration (25 mg/mL) were intermediate and significantly different from other combinations ($P < 0.0003$ for all), and latencies to any of the 2 combinations of ergosterol at high concentration (25 mg/mL) with oleic acid at either low or high concentration did not differ between them ($P = 0.99$) and were significantly shorter than to other stimuli ($P < 0.0003$ for all).

The rate of TFs directed to swabs differed significantly between concentrations of ergosterol (repeated measures 2-way ANOVA: $F_{2,28} = 154.65$, $P < 0.0001$) and between concentrations of oleic acid ($F_{2,28} = 16.02$, $P < 0.0001$), but the interaction between the different concentrations of these 2 compounds was significant ($F_{4,56} = 32.69$, $P < 0.0001$) (Table 1). TF rates to DCM alone (i.e., concentrations of 0 mg/mL) were significantly lower than to any other stimuli (Tukey's tests: $P = 0.00014$ in all cases). When any of the compounds was presented alone, increasing their concentration resulted in significantly higher TF rates of females ($P < 0.0015$ for all). When the 2 compounds were mixed, females made significantly more TFs directed to the combination of the 2 compounds with high concentrations (25 mg/mL each) than to other chemical stimuli ($P = 0.00015$ for all). Also, females made more TFs to the mix of ergosterol at high concentration with oleic acid at low concentration than to any of the 2 combinations of ergosterol at low concentration with oleic acid at either low or high concentration ($P < 0.00015$ for both), which were not significantly different between them ($P = 0.99$).

In the third experiment, comparing different concentrations of ergosterol and oleic acid presented alone or together, the TF rates of females differed significantly between treatments (2-way ANOVA: $F_{2,168} = 22.73$, $P < 0.0001$) and between concentrations ($F_{3,168} = 254.64$, $P < 0.0001$), but the interaction was significant ($F_{6,168} = 10.86$, $P < 0.0001$) (Figure 3). TF rates of females increased when concentrations increased. However, when both compounds were presented together, the TF rates of females were significantly higher than when the compounds were presented alone with the same concentrations, but only when concentrations of these compounds were of 20 mg/mL (Tukey's tests: $P < 0.01$ in both cases) or 40 mg/mL ($P < 0.0001$ in both cases), but differences were not significant when the concentration was 5 mg/mL ($P > 0.30$ in both cases), or in the control tests where concentration was 0 mg/mL ($P = 0.99$ for all).

Discussion

This study shows that the magnitude of the observed chemosensory exploration behavior of female lizards *I. cyreni* to the scent from femoral secretions of males may result from the combination of responses to the proportions of multiple compounds found in these secretions. These results suggest that femoral secretion of male lizards functions as a multicomponent chemical signal for females.

Responses to multicomponent chemical signals are probably common in vertebrates but they are not well known (Müller-Schwarze 2006). For example, the puberty-delaying pheromone from female house mice has 2 components that are active in various combinations, but one of this

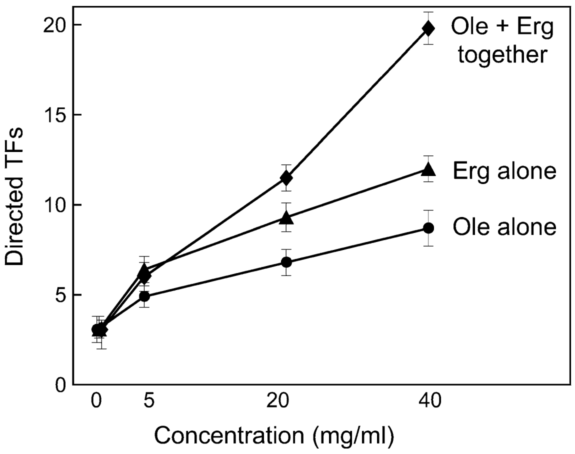

Figure 3 Mean (+standard error) number of TFs directed to swabs by female lizards in response to cotton-tipped applicators bearing different concentrations (0, 5, 20, or 40 mg/mL) of oleic acid (Ole) and ergosterol (Erg) (standard compounds) presented alone or together, all dissolved in DCM.

components is also active alone (Novotny et al. 1986). However, 2 compounds from the urine of male mice have to be present together to provoke aggressive behavior in conspecific males (Novotny et al. 1999). In the tree shrew (*Tupaia belangeri*), the chinning response is elicited by several lipophilic fractions of male urine, but the combined fractions are more active than single fractions (Stralendorff 1987). Our study suggests that also in rock lizards, there is an additive or synergetic effect of multiple compounds (oleic acid and ergosterol) such that femoral secretions of male lizards with the highest proportions of both of these 2 compounds received the highest exploratory TF rates by females, suggesting that their scents were more intriguing or attractive for females. However, not all male lizards with high proportions of oleic acid in secretions also had high proportions of ergosterol. In those cases, there might be a contrasting interactive effect of the 2 compounds. TF rates of females were higher to scent of males with high proportions of ergosterol alone, even if proportions of oleic acid were low, than to males with high proportions of oleic acid but with low proportions of ergosterol. Nevertheless, female TF rates to high proportions of a single compound were not as high as when both compounds were found together with high abundances.

Variations in the chemosensory exploration behavior of female lizards are probably explained because different compounds signal different traits of a male. Similarly, for example in birds, multiple visual ornaments may signal different aspects of male quality (Doucet and Montgomerie 2003; Loyau et al. 2005). However, some male traits might be more important for females than others when selecting a potential mate. Previous studies of rock lizards have shown a relationship between proportions of oleic acid and body condition and between proportions of ergosterol and quality of the immune system (Martín and López 2006b, 2010). Thus, females might "ideally" look for healthy males with both a high body condition and a high immune response. This would explain that in the current experiment, the scent of males with the highest proportions of these 2 compounds received the highest TF rates by females. However, the results indicated that when a male was only "good" in one of these traits, the quality of the immune system seemed to be more important than a higher body condition alone. This might be explained because the immune system quality is mainly a genotype-dependent trait that may be heritable to the offspring of the female. In contrast, although "good-quality" males also should be able to maintain a good body condition, random variations in the environment, such as limitations in prey availability or bad weather conditions, may affect negatively to the body condition of a male (e.g., Bradshaw and Dèath 1991), independently of his genotypic quality.

Furthermore, our experiments testing the exploration behavior of female lizards to standard compounds confirmed the findings from the tests with natural femoral secretions of males. Females discriminated between different concentrations of ergosterol and oleic acid presented alone and showed the highest chemosensory exploration to high concentrations of ergosterol, whereas high concentrations of oleic acid elicited TF rates of a magnitude similar to those to low concentrations of ergosterol. Nevertheless, because the compounds act as molecules in the vomeronasal organ, we should compare amounts of compounds measured in moles rather than in grams. However, because the molar mass of oleic acid (= 282.46 g/mol) is lower than that of ergosterol (= 396.65 g/mol), this results in that we actually tested a lower number of moles of ergosterol (0.0025 mol/g of ergosterol vs. 0.0035 mol/g of oleic) but we still observed higher chemosensory exploration rates to ergosterol. Therefore, if we had tested a similar number of moles of both compounds, we would have probably observed even higher differences between compounds in the exploration behavior of females.

Moreover, when standard oleic acid and ergosterol were combined in the same scent, the highest TF rates were directed to high concentrations of both compounds combined together. Also, when we tested multiple doses of oleic acid and ergosterol alone or combined, we found an upper-shift of the top of the dose–response curve by the combination of the 2 compounds when they were presented in high concentrations. These results suggested again that there are additive or synergic effects of these 2 compounds.

Similarly to chemosensory exploration of natural secretions of males, female exploration of standard compounds was intermediate when concentration of ergosterol was high but concentration of oleic acid was low. However, when concentration of ergosterol was low, female exploration was equally low, irrespective of the concentration of oleic acid. Nevertheless, latencies were shorter when concentration of oleic acid was high. These results suggested that concentration of ergosterol was more important than concentration of oleic acid to determine the magnitude of the chemosensory exploration rates of females.

Although our study suggests that chemosensory exploration behavior of females depended on ergosterol and oleic acid, there are many other compounds in femoral secretions of males (López and Martín 2005) that could also contribute to explain the variations in female exploration of males' secretion. Other compounds might be related to different male traits or be used as a way to reinforce the signal of other compounds or even be useful in different circumstances. For example, female *I. cyreni* also can detect hexadecanoic acid, a saturated fatty acid also found in secretions of males, although their TF rates to this lipid are lower than to oleic acid (Martín and López 2010). Although within a foraging context, Cooper et al. (2002) suggested that the 2 major categories of fatty acids, saturated and unsaturated, might have different effects on chemosensory responses of the lizard *Podarcis lilfordi* to prey scents 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 rock 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 (Martín and López 2010). 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 exploration of females.

In addition, some of the compounds found in femoral secretions of male lizards seem to be directed to different receivers (i.e., other males). Thus, proportions of cholesterol in femoral secretions of males increase with body size (López et al. 2006). Cholesterol elicits much higher TF rates in males than in females, and, only in males, elicits aggressive responses and affects agonistic interactions, likely because it may signal aggressiveness or dominance to other males (Martín and López 2007, 2008). In the related rock lizard *I. monticola*, proportions of hexadecanol in femoral secretions of males are related to the social status of males in intrasexual relationships, and hexadecanol elicits aggressive responses in other males (Martín, Moreira, and López 2007). These data suggest the role of femoral secretion of rock lizards as a multicomponent chemical signal, where different compounds signal different traits that are relevant for either male or female conspecifics in sexual selection processes.

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