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Female Iberian wall lizards prefer male scents that signal a better cell-mediated immune response

Pilar López* **and José Martín**

Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, CSIC, José Gutiérrez Abascal 2, 28006 Madrid, Spain
**Author for correspondence* (*pilar.lopez@mncn.csic.es*)

In spite of the importance of chemoreception in sexual selection of lizards, only a few studies have examined the composition of chemical signals, and it is unknown whether and how chemicals provide honest information. Chemical signals might be honest if there were a trade-off between sexual advertisement and the immune system. Here, we show that proportions of cholesta-5,7-dien-3-ol in femoral secretions of male Iberian wall lizards (*Podarcis hispanica*) were related to their T-cell-mediated immune response. Thus, only males with a good immune system may allocate higher amounts of this chemical to signalling. Furthermore, females selected scents of males with higher proportions of cholesta-5,7-dien-3-ol and lower proportions of cholesterol. Thus, females might base their mate choice on the males' quality as indicated by the composition of their chemical signals.

Keywords: femoral glands; chemoreception; mate choice; immune response

1. INTRODUCTION

Chemical cues play an important role in the communication of many lizard species (Mason 1992). Pheromonal detection is often based on femoral gland secretions (e.g. Alberts 1993), which may inform about the genetic quality or compatibility of a male and consequently be used by females to choose mates (Martín & López 2000; López *et al.* 2002, 2003; Olsson *et al.* 2003). In spite of the potential importance of chemoreception in lizards' sexual selection (López & Martín 2004), only a few studies have analysed the composition of their gland secretions (reviewed in Mason 1992; see also Escobar *et al.* 2001). Chemical secretions are composed of lipids and proteins, but lipids seem to be the main compounds involved in communication (Mason 1992). However, the role of specific chemicals in sexual selection, and, moreover, whether and how they provide honest information, is unknown.

In other systems of sexual signals (e.g. visual signals), the energetic expenditure of producing and maintaining the immune system may create a trade-off between immune function and sexually selected characters (Wedekind & Folstad 1994). Therefore, only individuals in good condition can mount a strong immune defence and produce extravagant sexual ornaments (Sheldon & Verhulst 1996; Westneat & Birkhead 1998). Because parasites may affect the information conveyed by chemical signals (Penn & Potts 1998; Rantala *et al.* 2003; Zala *et al.* 2004), it is possible that pheromones also function as condition-dependent signals of mate quality. Here, we examine whether chemical compounds in femoral secretions of male lizards reliably inform on the quality of their immune system, and whether females select males' scents based on this relationship.

2. MATERIAL AND METHODS

(a) *Study species*

The Iberian wall lizard, *Podarcis hispanica*, is a small (50–70 mm adult snout-to-vent length) diurnal lacertid lizard common at rocky habitats of the Iberian Peninsula. This lizard can discriminate between sexes and individuals by chemical cues alone (Cooper & Pérez-Mellado 2002; López & Martín 2002). We captured adult lizards (18 males and 16 females) of similar body sizes by noosing at the beginning of April 2004, before the start of their mating season, near Cercedilla (Madrid province, Spain). We captured lizards over a large area to ensure that they had not been in previous contact. 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, rocks for cover, and water *ad libitum*. They were fed every day with mealworm larvae dusted with multivitamin powder. 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. Experiments were performed under licence from the 'Comunidad de Madrid' Environmental Agency.

(b) *Immune response*

To assess the T-cell-mediated immune (CMI) response of males we used the phytohaemagglutinin (PHA) injection test. This is considered to be a reliable method of measuring CMI (Norris & Evans 2000), which has been widely used in many taxa including lizards (Belliure *et al.* 2004). We used a pressure-sensitive spessimetre to measure the thickness of the foot pad of the right and left hindlimbs three times (to the nearest 0.01 mm). Then, we injected 0.02 mg of PHA dissolved in 0.01 ml of phosphate-buffered saline (PBS) water in the right foot pad, and the same volume of PBS in the left foot as a control. Lizards were released in their terraria, and after 24 h we measured again three times the feet pad thickness. Repeatability of these measures was high ($r > 0.94$ in all cases). We calculated CMI as the difference between pre- and post-injection measures on the PHA-injected foot minus the same difference on the control foot. The only appreciable effect of the PHA injection was a slight swelling that disappeared after 48 h.

(c) *Choice of scent experiments*

We placed in males' cages several absorbent paper strips (35×10 cm) fixed to the floor, and left them there for three weeks to obtain the scents from males. Mate choice experiments were performed at the end of April, coinciding with the mating season of lizards. Females' cages 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 (07.00 h GMT), when females were still inactive, wearing fresh gloves we fixed on one tile one paper strip from one male, and another from a different male on the other tile. The males tested and the positions of papers were randomly determined. Each female was tested once a day with papers from a pair of males over 4 days (eight different males). Each trial lasted 6 h (from 09.00 h GMT, shortly after females appeared from refuges, and until 15.00 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 details of a similar procedure see Martín & López 2000; López *et al.* 2002, 2003; Olsson *et al.* 2003). The paper on which a female spent greater than 50% of her time (excluding time in the no choice area) was designed as the preferred paper in that trial. Different papers from each male were used in eight choice tests against the papers of other eight males, with different individual females. Each individual male was assigned an 'attractiveness index', calculated as the number of females that preferred a paper with his scent.

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(d) *Chemical analyses of femoral secretions*

We extracted femoral pore secretions of males by gently pressing with forceps around the pores, depositing this directly into glass vials with Teflon-lined stoppers. Vials were stored at −20 °C until analyses. Samples were analysed by gas chromatography–mass spectrometry (Finnigan-ThermoQuest Trace 2000) equipped with a Supelco, Equity-5 column, temperature programmed from 50–280 °C at 5 °C min^{-1} and kept at 280 °C for 30 min. Identification of compounds was done by comparison of mass spectra in the NIST/EPA/NIH 1998 library, and later confirmed with authentic standards. The relative amount of each component was determined as the percentage of the total ion current (TIC). Then, we selected the peaks that represented greater than 1.0% TIC. The relative areas of the selected peaks were restandardized to 100% and 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). We used these transformed areas as independent variables in forward stepwise general regression models with CMI or attractiveness index of males as dependent variables.

3. RESULTS

Eleven major lipophilic compounds were identified in all males, although their proportions varied greatly between individuals. These were four carboxylic acids and one of their esters, 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.

Males with a greater T-cell-mediated immune response had significantly higher proportions of cholesta-5,7-dien-3-ol in their femoral secretions ($\beta=0.60$, $t=2.97$, $p=0.009$; $r^2_{\text{model}}=0.36$, $F_{1,16}=8.83$, $p=0.009$; figure 1), whereas other compounds were not related to the CMI and were not included in the model.

Femoral secretions of males more often selected by females had relatively greater proportions of cholesta-5,7-dien-3-ol ($\beta=0.53$, $t=3.08$, $p=0.008$) and relatively lower proportions of cholesterol ($\beta=-0.62$, $t=3.64$, $p=0.002$; $r^2_{\text{model}}=0.57$, $F_{2,15}=9.86$, $p=0.002$; figure 2).

Table 1. Major lipids found in femoral secretions of male Iberian wall lizards.

compound	% TIC (mean ± s.e.m.)	range
dodecanoic acid	2.3 ± 0.2	(1.3–5.8)
hexadecanoic acid	6.2 ± 0.6	(1.9–10.7)
octadecenoic acid	5.1 ± 0.6	(1.4–10.6)
octadecanoic acid	1.5 ± 0.1	(0.4–3.0)
octadecenoic acid, methyl ester	2.3 ± 0.5	(0.1–6.1)
cholesterol	56.8 ± 0.5	(40.8–68.2)
cholesta-5,7-dien-3-ol	15.4 ± 1.7	(2.8–33.3)
campesterol	2.2 ± 0.4	(0.4–6.4)
ergosta-5,8-dien-3-ol	4.2 ± 0.2	(1.8–5.5)
sitosterol	1.3 ± 0.3	(0.2–5.5)
cholesta-5,7-dien-3-ol. 4.4-dimethyl	2.7 ± 0.2	(1.1–4.6)

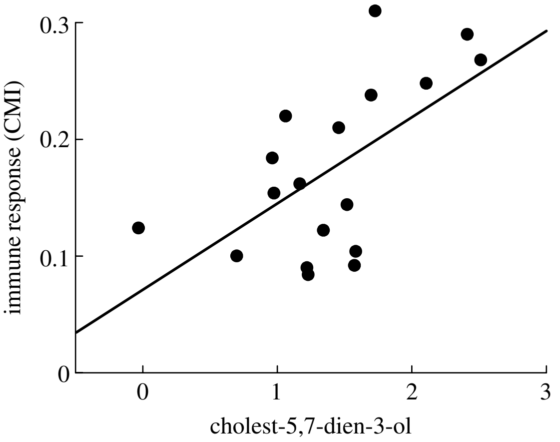

Figure 1. Relationship between relative proportions (transformed areas) of cholesta-5,7-dien-3-ol in femoral gland secretions and the T-cell-mediated immune response (CMI) of male Iberian wall lizards.

4. DISCUSSION

The relationship between proportions of cholesta-5,7-dien-3-ol in femoral secretions and CMI suggests that only males with a high-quality immune system may allocate high amounts of this compound to secretions. The chemical signal would, therefore, be costly and condition dependent, and may provide honest information on the male's condition. This would be consistent with recent findings in pheromones of beetles (Rantala *et al.* 2003) and house mice (Zala *et al.* 2004). Furthermore, our results indicate that females selected scents of males with higher proportions of cholesta-5,7-dien-3-ol. Thus, at least partially, females might base their mate choice on the quality of the males' immune system, as indicated by their chemical signals. As in other lizard species (Martín & López 2000; López *et al.* 2002, 2003), females might decide where to establish their home ranges based on scent marks left by territorial males, thus increasing the probability of mating with males of high quality.

The reason why only males with a better immune system can allocate high proportions of cholesta-5,7-dien-3-ol in secretions is not clear. However, one possible explanation is that this steroid is a precursor of vitamin D$_3$. It is found in the skin, where it will transform into vitamin D$_3$ after exposition to sun UVB irradiation (Carman *et al.* 2000). Vitamin D$_3$ is essential in calcium metabolism of lizards and other vertebrates (Fraser 1995). Therefore, by allocating this compound to femoral secretions, males are diverting it from metabolism. This should be costly for low-quality individuals if they did not produce nor had enough body reserves of this steroid. If this is the mechanism that confers honesty to chemical signals, females that preferred scents with cholesta-5,7-dien-3-ol might increase their probability of mating with high-quality males.

Another possible scenario is that some or all of these correlations were causally related to variations in testosterone or corticosterone levels, which, in other taxa, has been implicated in modulating immunity, in steroid biochemistry, and in mate choice (e.g. Wedekind & Folstad 1994; Sheldon & Verhulst 1996). In fact, seasonal changes in chemical composition of femoral gland secretion probably depends on variations in testosterone levels (Alberts 1993).

Females also selected scents of males with lower proportions of cholesterol, and, although we did not find a relationship with the immune system, it might be

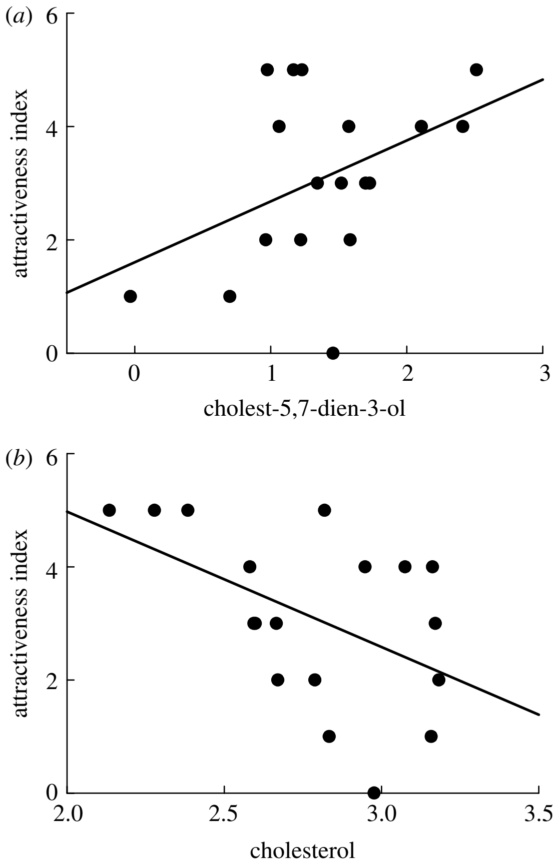

Figure 2. Relationship between relative proportions (transformed areas) of (*a*) cholesta-5,7-dien-3-ol and (*b*) cholesterol in femoral gland secretions and the attractiveness index scores of male Iberian wall lizards.

related to other male characteristics probably selected by females, such as body condition, age or testosterone levels (López *et al.* 2002, 2003). In other lizard species, genetic compatibility (based on MHC dissimilarity) was the main characteristic that females selected from the scent of a male (Olsson *et al.* 2003). However, in the large and widespread populations of Iberian wall lizards, the probability of finding genetically incompatible males may be low, and, then, it might be more important for females to select males' quality *per se*.

We suggest that chemical signals of lizards may honestly inform on the quality of a male, and that females may use this information to select mates.

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