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Status-signalling chemical badges in male Iberian rock lizards

J. MARTÍN,*† P. L. MOREIRA‡ and P. LÓPEZ*

**Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales (MNCN), CSIC, José Gutiérrez Abascal 2, 28006 Madrid, Spain, and ‡Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Edifício C-2 Campo Grande, 1749-016 Lisboa, Portugal*

Summary

1. Male competition for females often results in the evolution of conspicuous male traits that signal fighting ability or dominance status. Most studies examining mechanisms allowing these traits to function as reliable status signals (or badges) have focused on conspicuous visual or acoustic traits. However, many animals communicate chemically, and chemical traits alone may also signal male dominance status.

2. We examined whether chemicals in femoral gland secretions of male Iberian rock lizards (*Lacerta monticola monticola* Boulenger 1905) may signal dominance status.

3. In staged encounters, larger males were dominant over smaller ones. While controlling for male body size, males of higher dominance status produced femoral secretions with higher proportions of hexadecanol and octadecanol.

4. Tongue-flick assays showed that males were able to (1) discriminate, by chemosensory cues alone, the different concentrations of hexadecanol from other chemicals presented in femoral secretions; (2) respond aggressively towards hexadecanol while responding neutrally towards other chemicals; and (3) show differential chemosensory and aggressive behaviours towards hexadecanol according to their own dominance status.

5. These results suggest that hexadecanol may be a reliable status badge. Moreover, because hexadecanol elicits male aggressive behaviour, subordinate males signalling high status ('cheaters') may end up paying high fighting costs. In addition, males that had higher dominance status, and that allocated higher proportions of hexadecanol to femoral secretions, had greater T-cell immune responses. This result suggests a possible link between quality of the immune system, dominance status, and chemical signals.

Key-words: chemical signals, dominance, femoral glands, immune response, lizards

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Introduction

Male competition over females often favours the evolution of male attributes that confer fighting ability and correlated conspicuous status-signalling traits (Andersson 1994). When males of different dominance status exhibit different badges, individuals may use these badges to judge relative fighting ability, and to modulate their own behaviour accordingly (Enquist & Leimar 1983). Thereby, males may avoid the costs associated with escalated aggressive interactions (Marler & Moore 1988).

Most studies examining the mechanisms that allow status badges to signal dominance status focused on conspicuous visual or acoustic traits (for reviews see Andersson 1994; Olsson & Madsen 1998; Searcy & Nowicki 2005), generally ignoring other systems of communication. However, chemoreception plays an important role in intraspecific communication and sexual selection in many animals, including invertebrates, mammals and reptiles (Mason 1992; Wyatt 2003). Chemicals may reveal and/or confer dominance status in animals such as cockroaches (Moore 1997; Moore *et al*. 1997; Roux *et al*. 2002), lobsters (Atema 1986), crayfish (Zulandt-Schneider, Huber & Moore 2001), salamanders (Mathis 1990), mice (Apps, Rasa & Viljoen 1988; Harvey, Jemiolo & Novotny 1989; Hurst 1993) and rabbits (Hayes, Richardson & Wyllie 2001, 2003).

Male lizards of many species possess holocrine epidermal glands that, during the breeding season, deliver secretions used in intraspecific communication (Mason 1992; Alberts 1993). For instance, secretions

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†Author to whom correspondence should be addressed. E-mail: Jose.Martin@mncn.csic.es

from femoral glands allow self-recognition, familiar discrimination and scent-marking of territories (Alberts 1993; Alberts & Werner 1993; Aragón, López & Martín 2001). Lizard femoral secretions might also signal a male's dominance status, through both productivity rates and/or the quality of the secretions (Alberts, Pratt & Phillipis 1992; López, Martín & Cuadrado 2003; Moreira, López & Martín 2006). During agonistic encounters, individual recognition based on chemical cues helps to reduce the intensity and costs of fighting (López & Martín 2002). Nevertheless, there is little knowledge regarding the relationships between dominance status and chemical signals.

On the other hand, if status signalling was based on features that are not directly related to fighting ability, subordinate males might benefit from 'cheating', signalling a higher status, to enjoy a preliminary advantage in intrasexual contests (Krebs & Dawkins 1983). This raises a question over the mechanisms allowing the maintenance of reliable signals of dominance. One possible explanation is that, for subordinate males, it may be too costly to signal too high a dominance status. For example, the costs of producing and maintaining the immune system might create a trade-off with sexual advertisement (Wedekind & Folstad 1994), such that only individuals in good condition could mount a strong immune defence and produce an extravagant sexual ornament (Sheldon & Verhulst 1996; Westneat & Birkhead 1998). In lizards, both femoral gland production (Alberts *et al*. 1992) and male dominance (Moore & Lindzey 1992) are often dependent on androgen levels. The immunosuppressive effect of testosterone (Folstad & Karter 1992; Wedekind & Folstad 1994; Belliure, Smith & Sorci 2004) therefore may provide a link between dominance status and chemical ornaments, such that only genuine dominant males could maintain a good immune response and produce the chemical signal of dominance. Costs may also be imposed by the targeted receivers. Thus, if the status signal is incongruent with behaviour, deception may be detected and punished by genuine dominant individuals (Rohwer & Rohwer 1982; Møller 1987; Olsson 1994a; Martín & Forsman 1999).

Recent studies indicated that male Iberian rock lizards (*Lacerta monticola monticola* Boulenger 1905) discriminate their own femoral secretions from those of other males, and also discriminate the dominance status of other males based on chemical cues arising from femoral secretions (Moreira *et al*. 2006). We examined whether femoral secretions may function as signals of males' dominance status, and explored the mechanism on which this could be based. We determined male dominance status by staging male–male encounters on a neutral arena. We also analysed the chemical composition of males' femoral gland secretions, and examined whether the relative proportions of lipophilic chemical compounds can be related to the dominance status of the signaller. Additionally, we evaluated the male's T-cell immune response, and explored the relationships between chemicals, dominance and the quality of the immune system.

Furthermore, based on the finding that hexadecanol might be a chemical signal of male dominance status (see Results), we used tongue-flick assays to test whether males are able to: (1) detect hexadecanol levels and changes in its concentration by chemosensory cues alone, and to discriminate it from other chemicals also found in femoral secretions; (2) respond aggressively towards hexadecanol, while responding neutrally towards chemicals unrelated to dominance status; and (3) behave differentially towards hexadecanol according to their own social dominance status.

Materials and methods

STUDY ANIMALS

Iberian rock lizards are small, insectivorous lacertids of highland rocky habitats, which comprise several closely related species (Mayer & Arribas 2003; Carranza, Arnold & Amat 2004). In Portugal, *L. m. monticola* (= *Iberolacerta monticola*) is restricted to a single population located at Serra da Estrela mountain. Lizards are active from March to November, the mating season begins in May–June, and females produce a single clutch per year (Moreira 2002). As for the closely related *L. m. cyreni* (= *Iberolacerta cyreni*) (Martín & López 2000; Aragón, López & Martín 2004), male *L. m. monticola* aggressively defends its territories during the mating season, but as there is high home range overlap between neighbouring males, dominance hierarchies are often established (P.M., personal observation).

We captured adult male lizards at the highest elevations of Serra da Estrela (surroundings of 'Torre' at 1993 m) before the start of the mating season, during March and April. We used only lizards with intact or fully regenerated tails, because tail loss may affect dominance (Martín & Salvador 1993). Three different groups of males were used for conducting three experiments in indoor facilities at El Ventorrillo MNCN field station (Madrid Province, Spain).

We measured males' body weight with a digital balance to the nearest 0·01 g, the snout-to-vent length (SVL) with a ruler to the nearest 1 mm, and head length, width and height with a digital calliper to the nearest 0·05 mm. Given the high correlation between these morphological measurements, we used principal components analysis (PCA) to reduce these five morphological variables (log-transformed) to a smaller number of independent components. The first PC explained 67% of variance (eigenvalue = 3·35) and was positively correlated with all morphological measurements. Thus we used the resulting PC scores as a new variable (hereafter termed 'body size') in subsequent analyses, lizards with higher PC scores having relatively greater body size.

At the beginning of May, males were initially housed in groups of four per terrarium. These groups were chosen randomly, but each group included two males above and two males below the median SVL. This procedure allowed the 'stimulation' of normal social behaviour of lizards in captivity conditions after hibernation. After 10 days, when agonistic interactions became frequent, males were moved to individual terraria. We used glass terraria ($50 \times 25 \times 25$ cm^3) provided with a cardboard substrate, cardboard rolls for refuge, and a hardware cloth lid. Terraria were lighted from 9 : 00 to 19 : 00 h with full-spectrum fluorescent lamps and heated by 60-W incandescent lamps. Mealworm larvae (*Tenebrio* sp.), dusted with multivitamin powder and water, were provided *ad libitum*. Visual communication between lizards in different terraria was prevented by means of cardboard screens. Lizards maintained good conditions throughout the study and were returned to their capture sites at the end of trials.

MALE DOMINANCE STATUS

We staged encounters between pairs of males ($n = 30$ males) at the beginning of June, coinciding with the mating season, between 11 : 00 and 13 : 00 h and between 17 : 00 and 19 : 00 h. We used a neutral arena to avoid the effects of prior residency on the outcome of encounters (Olsson & Shine 2000; López & Martín 2001). The test arena was a terrarium ($50 \times 50 \times 25$ cm^3) with a fresh cardboard substrate and divided into two equally sized compartments with a removable opaque partition. Each compartment has a 60-W lamp for thermoregulation. Each male participated in four encounters against four different opponents, randomly chosen among those that had not been housed together and that had been captured in different places, so that male's familiarity did not influence the outcome of encounters (Olsson 1994b; López & Martín 2001, 2002). The order of encounters and the initial male position on the left and right sides of the arena were randomized.

Males were transferred from their home terraria to the test arena, and were given 5 min for acclimatization before the partition was removed. Encounters were filmed with a video camera and observed from a hidden point. Encounters were ended as soon as winner and loser could be established, or after 5 min when chases or combats did not occur. We considered the winning male to be the one that chased his opponent and made him flee, either immediately following an attack or after physical combat. We considered it a tie when males separated after a combat and neither chased the other, when neither retreated from a long-duration combat (>5 min; these combats were interrupted to prevent injuries), or when combats or chases did not occur. For each staged encounter, we gave scores of 1 to the winner, 0 to the loser, and 0·5 to both males for a tie. A male's dominance status score was calculated as the sum of scores obtained among his four encounters (Martín & Salvador 1993; López, Muñoz & Martín 2002a; Moreira *et al*. 2006).

ANALYSES OF CHEMICALS IN FEMORAL GLAND SECRETIONS

We collected femoral gland secretions of males the day after staged encounters finished. We pressed with forceps around the femoral pores to collect secretion directly in glass vials with Teflon-lined stoppers, which were stored at –20 °C. We analysed secretions with a ThermoQuest Trace 2000 gas chromatograph–mass spectrometer (GC–MS) fitted with a 30-m-length column (Equity-5, Supelco (Bellefonte, PA), for details see López & Martín 2005; López, Amo & Martín 2006). A total of 55 chemical lipophilic compounds were identified by comparison of their mass spectra in the National Institute of Standards and Technology's 1998 computerized mass spectral library (NIST, www.nist.gov), and later confirmed using authentic standards. We determined the relative amount of each component as the percentage of the total ion current (TIC). Then we selected the 29 peaks that represented >0·1% relative peak area (most abundant chemicals, altogether representing 98·9% of total TIC area) to reduce the number of variables to be used in multivariate statistical analysis (Dietemann *et al*. 2003). The relative areas were transformed following the formula of Aitchison (1986); and then used as variables in a PCA that produced six PCs (see Appendix S1 in Supplementary Material). To analyse relationships between male dominance status scores, proportions of chemicals in femoral secretions and T-cell-mediated immune response, we conducted forward stepwise general regression models (GRM) using the six PCs as potential predictors (López *et al*. 2006). Because body size may affect dominance status (Moreira *et al*. 2006), we also included the body size scores in the initial models as a continuous predictor.

T-CELL MEDIATED IMMUNE RESPONSE

The day after femoral gland secretions were collected, we used the phytohaemagglutinin (PHA) injection test (a delayed-type hypersensivity test) to assess the T-cell-mediated immune (CMI) response *in vivo* (Smits, Bortolotti & Tella 1999; Belliure *et al*. 2004). We measured with a pressure-sensitive spessimeter the thickness (to the nearest 0·01 mm) of the right hindlimb foot pad at a point marked with permanent ink, before and after 24 h of injecting 0·02 mg of PHA dissolved in 0·01 ml phosphate-buffered saline at the marked point. We calculated the CMI response as the difference between pre- and postinjection thickness measures (Smits *et al*. 1999). The only appreciable effect of the PHA injection was a slight swelling of the skin, which disappeared after 48 h. No lizard showed any sign of stress or pain in these tests.

CHEMOSENSORY AND AGGRESSIVE RESPONSES TOWARDS CHEMICAL STIMULI

Based on the observed relationship between male dominance status and the chemical composition of femoral secretions (see Results), we conducted two chemosensory experiments using two different groups of males ($n = 16$ each). We tested whether males (1) discriminate putative chemical signals of dominance status from other chemicals present in femoral secretions; and (2) discriminate between different concentrations of putative chemical signals of dominance status. In both experiments we studied tongue-flick (TF) rates, which can be used as a bioassay for measuring discrimination of chemical cues because lizards respond to different chemical stimuli by changing the rate of tongue extrusions (Cooper & Burghardt 1990; Cooper 1994). We also investigated whether chemosensory discrimination of chemical stimuli was accompanied by a shift in male aggressive behaviour (e.g. bites to the stimuli).

In a first experiment, we compared TF rate by male lizards ($n = 16$) in response to stimuli arising from cotton applicators bearing scents of (1) cholesterol, (2) hexadecanoic acid, (3) hexadecanol, (4) octadecanol or (5) dichloromethane (DCM). The rationale for testing these chemicals was that: (1) cholesterol is the most abundant chemical in femoral secretions; (2) hexadecanoic acid is the most abundant fatty acid, is chemically similar to hexadecanol, and is negatively related to male dominance status; (3) hexadecanol and (4) octadecanol are putative chemical signals of male dominance status, as their abundances in femoral secretions correlated with dominance status; and (5) DCM was used as a control to gauge baseline TF rates under the experimental conditions.

We prepared chemical stimuli the day of the tests by dissolving each compound (authentic standards, GC grade, Sigma-Aldrich, St. Louis, MO) in DCM (30 mg ml^{-1}) in glass vials with Teflon-lined stoppers. We shook the solution for 1 min using a vortex, and kept the vials in a refrigerator.

In a second experiment, we presented a different group of males ($n = 16$) with cotton swabs bearing three different concentrations of hexadecanol dissolved in DCM: low (8 mg ml^{-1}), medium (32 mg ml^{-1}) and high (56 mg ml^{-1}), prepared as above. Although the abundances of both hexadecanol and octadecanol were correlated with male dominance status, we tested hexadecanol alone, as it is fivefold more abundant in femoral secretions than octadecanol (see Discussion).

Trials were made between 11 : 00 and 13 : 00 h (GMT). 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 DCM alone, or DCM with other chemicals dissolved in it. In each experiment, every lizard was presented with each chemical stimulus in a randomized order. Swabs with all stimuli were visually similar for humans. A new swab was used in each trial. To begin a trial, the same experimenter slowly approached the home terrarium of each male and slowly moved the cotton swab to a position 1 cm anterior to the lizard's snout. The experimenter was blind to the chemical presented. The numbers of TFs directed to the swab were recorded for 60 s, beginning with the first TF. We also recorded the number of bites directed to the swabs as a measure of a defensive or aggressive response to the chemical stimuli.

To examine differences in number of directed TFs between conditions, we used one-way repeated measures ANOVA with chemical stimuli as a within factor. Data were log-transformed to ensure normality and homogeneity of variances. Pairwise comparisons used Tukey's honestly significant difference tests (Sokal & Rohlf 1995). To analyse whether chemosensory responses could depend on the dominance status of the responding males, we used general linear modelling (GLM; Grafen & Hails 2002) to examine the influence of males' body size scores and proportions of hexadecanol in femoral secretions, which are presumably related to dominance, on chemosensory responses (log-transformed TF rates). Residuals from the final models were normally distributed.

Results

CHEMICALS IN FEMORAL SECRETIONS, DOMINANCE STATUS AND IMMUNE RESPONSE

The lipophilic fraction of femoral gland secretions of *L. m. monticola* is a mixture of steroids (85·6% of TIC), carboxylic acids ranging between C_9 and C_{22} (10·7%) and their esters (1·0%), and alcohols between C_{12} and C_{41} (2·4%). We found also two furanones (0·2%) and squalene (0·1%). On average, the 10 most abundant chemicals were cholesterol (63·7% of TIC), campesterol (8·1%), hexadecanoic acid (4·4%), sitosterol (3·1%), octadecenoic acid (2·3%), cholestanol (2·1%), hexadecanol (1·9%), octadecanoic acid (1·7%), ergosterol (1·5%) and 4,4-dimethyl-cholesta-5,7-dien-3-ol (1·4%). The PCA for relative proportions of the 29 most abundant chemicals produced six components (PCs) with eigenvalues >1, and that altogether accounted for 80·9% of the observed variance in the femoral secretions' chemical composition (Appendix S1).

Dominance status scores were significantly and positively correlated with body size scores and with PC-2 for chemicals (stepwise GRM, $R^2_{\text{model}} = 0{\cdot}38$, $F_{2,25} = 7{\cdot}76$, $P = 0{\cdot}002$; body size, $\beta = 0{\cdot}33$, $t = 2{\cdot}12$, $P = 0{\cdot}04$; PC-2, $\beta = 0{\cdot}49$, $t = 3{\cdot}07$, $P = 0{\cdot}005$). Thus larger males were dominant over smaller ones. However, while controlling for body-size effects, males with higher dominance scores had femoral secretions with higher proportions of hexadecanol and octadecanol, and lower proportions of hexadecanoic, octadecanoic, octadecenoic and octadecadienoic acids (Fig. 1).

The T-cell-mediated immune response was significantly and positively correlated with PC-2 for chemicals (stepwise GRM, $R^2_{\text{model}} = 0{\cdot}26$, $F_{1,28} = 10{\cdot}08$, $P = 0{\cdot}004$; PC-2, $\beta = 0{\cdot}52$, $t = 3{\cdot}18$, $P = 0{\cdot}004$). Thus lizards in better health, as judged from their greater CMI response, had femoral secretions with higher proportions of hexadecanol and octadecanol (Fig. 2a).

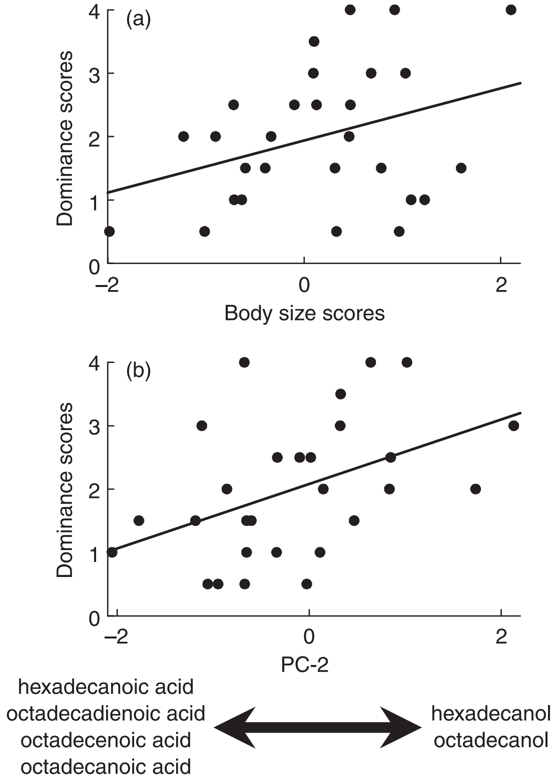

Fig. 1. Relationships between dominance status scores of male lizards *Lacerta monticola monticola* and (a) body size (PC scores describing male morphometry); (b) PC-2 (PC scores obtained from a principal components analysis on the relative proportions of chemicals in femoral gland secretions).

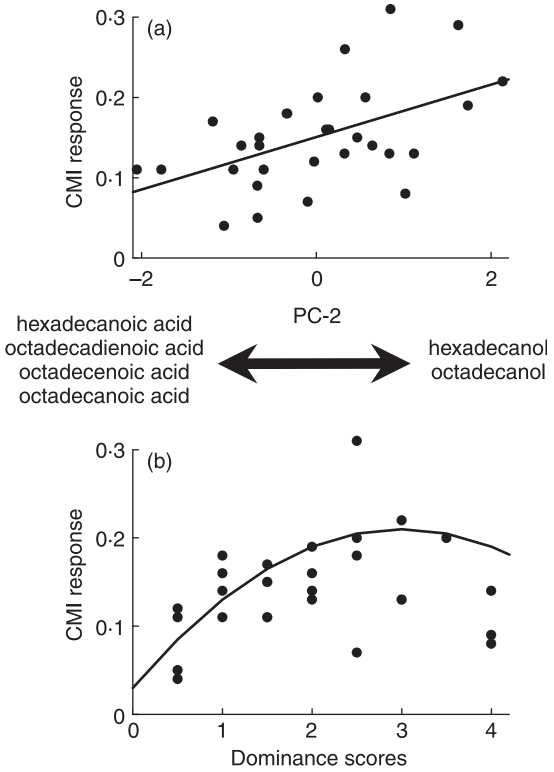

Fig. 2. Relationships between the T-cell-mediated immune (CMI) response of male lizards *Lacerta monticola monticola* and (a) PC-2 scores obtained from a principal components analysis on the relative proportions of chemicals in femoral gland secretions; (b) dominance status scores (regression line adjusted following a quadratic model).

The CMI response was not significantly dependent on body size, which was not included in the model.

There was no significant linear relationship between dominance and immune response ($R^2 = 0{\cdot}05$, $F_{1,26} = 1{\cdot}34$, $P = 0{\cdot}26$). However, dominance status scores were significantly related to immune response following a quadratic regression model ($R^2 = 0{\cdot}31$, $F_{2,25} = 5{\cdot}54$, $P = 0{\cdot}01$; intercept, $F_{1,25} = 0{\cdot}77$, $P = 0{\cdot}39$; dominance, $F_{1,25} = 10{\cdot}75$, $P = 0{\cdot}003$; dominance^2, $F_{1,25} = 9{\cdot}28$, $P = 0{\cdot}005$; Fig. 2b). The immune response increased with increasing dominance status scores, but there was a slight decrease in immune response for males with the highest dominance status scores.

CHEMOSENSORY AND AGGRESSIVE RESPONSES TOWARDS CHEMICAL STIMULI

There were significant differences in the number of TFs per minute directed towards swabs bearing different chemical stimuli (one-way repeated measures ANOVA, $F_{4,60} = 49{\cdot}30$, $P < 0{\cdot}0001$; Fig. 3a). Control DCM elicited TF rates similar to hexadecanoic acid (Tukey's tests, $P = 0{\cdot}99$), but TF rates lower than cholesterol ($P = 0{\cdot}047$) and lower than hexadecanol and octadecanol ($P = 0{\cdot}00013$ in both cases). Cholesterol and hexadecanoic acid did not elicit significantly different TF rates ($P = 0{\cdot}13$). Hexadecanol and octadecanol elicited significantly greater TF rates than all other stimuli ($P = 0{\cdot}00013$ in all cases), but TF rates did not differ significantly between these two alcohols ($P = 0{\cdot}96$).

The TF rates towards swabs bearing hexadecanol were negatively correlated with the responding male's body size (albeit short of statistical significance) and with the relative proportion of hexadecanol in his femoral secretions (GLM model, $R^2 = 0{\cdot}57$, $F_{2,13} = 8{\cdot}62$, $P = 0{\cdot}004$; body size, $\beta = -0{\cdot}38$, $F_{1,13} = 4{\cdot}10$, $P = 0{\cdot}06$; hexadecanol in secretions, $\beta = -0{\cdot}56$, $F_{1,13} = 8{\cdot}88$, $P = 0{\cdot}01$). Similar negative relationships were observed towards swabs bearing cholesterol (GLM model, $R^2 = 0{\cdot}69$, $F_{2,13} = 14{\cdot}51$, $P = 0{\cdot}0005$; body size, $\beta = -0{\cdot}50$, $F_{1,13} = 7{\cdot}74$, $P = 0{\cdot}015$; hexadecanol in secretions, $\beta = -0{\cdot}45$, $F_{1,13} = 6{\cdot}04$, $P = 0{\cdot}03$). Thus presumably more dominant males (those with larger body size and more hexadecanol in their own secretions) responded less strongly (lower TF rates) towards swabs bearing cholesterol or hexadecanol. Tongue-flick rates towards other stimuli were not significantly correlated with the male's body size or with the proportion of hexadecanol in his femoral secretions ($R^2 < 0{\cdot}17$, $P > 0{\cdot}31$ in all cases).

Nine males behaved aggressively (bit the swab or pushed against the swab with the snout) when presented with swabs bearing hexadecanol, and one bit a swab bearing octadecanol. Males did not bite swabs bearing other chemical stimuli. Based on the null

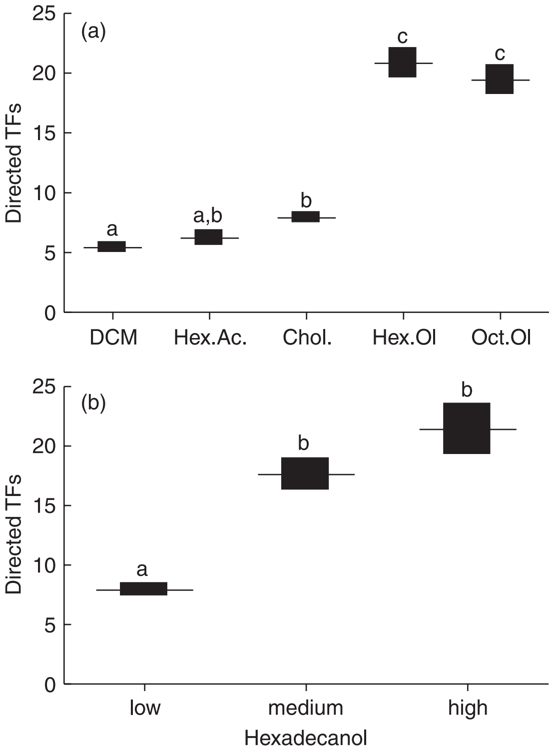

Fig. 3. Mean (± SE) directed tongue-flick (TF) rates by male lizards in response (a) towards cotton swabs bearing dichloromethane (DCM), hexadecanoic acid (Hex.Ac.), cholesterol (Chol), hexadecanol (Hex.Ol) or octadecanol (Oct.Ol); (b) towards cotton swabs bearing three different concentrations of hexadecanol. The same letter above bars denotes that means are not significantly different from each other.

hypothesis that the likelihood of biting was equal in all five conditions, the binomial probability that nine of the 10 bites would be in the same (hexadecanol) stimulus condition is <0·0001.

CHEMOSENSORY AND AGGRESSIVE RESPONSES TOWARDS DIFFERENT CONCENTRATIONS OF HEXADECANOL

There were significant differences in the number of directed TFs per minute ($F_{2,30} = 43{\cdot}06$, $P < 0{\cdot}0001$; Fig. 3b) between swabs bearing different concentrations of hexadecanol. At low concentrations of hexadecanol, TF rates were lower (Tukey's tests, $P < 0{\cdot}01$ in all cases). Differences in directed TFs between medium and high concentrations were not significantly different ($P > 0{\cdot}10$).

Tongue-flick rates towards swabs bearing high concentrations of hexadecanol were significantly and negatively correlated with the responding male's body size and with the relative proportion of hexadecanol in his femoral secretions (GLM model, $R^2 = 0{\cdot}55$, $F_{2,13} = 7{\cdot}94$, $P = 0{\cdot}006$; body size, $\beta = -0{\cdot}53$, $F_{1,13} = 7{\cdot}75$, $P = 0{\cdot}015$; hexadecanol in secretions, $\beta = -0{\cdot}44$, $F_{1,13} = 5{\cdot}40$, $P = 0{\cdot}037$). Tongue-flick rates towards swabs bearing medium concentrations of hexadecanol showed similar negative relationships with the male's body size and, although not statistically significant, with the proportion of hexadecanol in his secretions (GLM model, $R^2 = 0{\cdot}51$, $F_{2,13} = 6{\cdot}86$, $P = 0{\cdot}009$; body size, $\beta = -0{\cdot}59$, $F_{1,13} = 8{\cdot}97$, $P = 0{\cdot}01$; hexadecanol in secretions, $\beta = -0{\cdot}32$, $F_{1,13} = 2{\cdot}65$, $P = 0{\cdot}13$). In contrast, TF rates towards swabs bearing low concentrations of hexadecanol were not significantly correlated with the male's body size or with the proportion of hexadecanol in his secretions (GLM model: $R^2 = 0{\cdot}23$, $F_{2,13} = 1{\cdot}90$, $P = 0{\cdot}19$). Thus, as in the previous experiment, less-dominant males responded more strongly towards swabs bearing hexadecanol, but this relationship was statistically stronger when swabs contained higher concentrations of hexadecanol.

Three males behaved aggressively when presented with swabs bearing low concentrations of hexadecanol, eight males when presented with swabs bearing medium concentrations, and seven males when presented with swabs bearing high concentration. Based on the null hypothesis that the likelihood of biting was equal in all three conditions, the binomial probability that 15 of the 18 bites would be in the medium or high rather than in the low concentration of hexadecanol condition is 0·0075.

Discussion

This study indicates that femoral gland secretions of male *L. m. monticola* lizards contain lipophilic compounds that may function as chemical signals of male dominance status. As it occurs in most lizards (Olsson & Madsen 1998), male dominance status was largely dependent on male body size. However, while controlling for body-size effects, males with higher dominance status had femoral secretions with higher abundances of hexadecanol and octadecanol. That males can assess the dominance status of other males and change their behaviour based on these chemicals alone was supported by findings from chemosensory trials. Furthermore, our results suggest that hexadecanol may be a reliable signal of male dominance status. Hexadecanol elicits male aggressive behaviour, so that its production may be costly for subordinate males that 'cheated' on their chemical signals. Moreover, male CMI response was correlated with the abundances of hexadecanol (and octadecanol) in the femoral secretions, suggesting that both a male's dominance status and the quality of chemical signals are linked with the quality of the immune system.

Higher TF rates towards hexadecanol and octadecanol showed that males discriminated between these alcohols and other compounds present in femoral secretions, but males did not discriminate significantly between hexadecanol and octadecanol as separate alcohols. This might be explained because these alcohols are chemically very similar (hydrocarbon chains with 16 or 18 carbons), and their abundance in secretions are strongly correlated (see the PCA for chemicals). However, hexadecanol may perhaps be a more important chemical signal of male dominance than octadecanol, because hexadecanol is almost five times more abundant in femoral secretions (1·9 ± 0·7%) than octadecanol (0·4 ± 0·2%). Moreover, hexadecanol elicited stronger aggressive responses that varied according to concentrations. Therefore changes in concentration of hexadecanol in femoral secretion of males may explain the different chemosensory responses of males to secretions of other males according to their relative dominance status (Moreira *et al*. 2006).

Several different chemicals related to male dominance status have been identified in other taxa. For instance, subordinate and dominant male cockroaches, *Nauphoeta cinerea*, differ in the ratio between 2-methylthiazolidine and 3-hydroxy-2-butanone (Moore 1997; Moore *et al*. 1997), and in their cuticular hydrocarbon profile (Roux *et al*. 2002). Dominant male mice (*Mus musculus*) scent-mark their territories extensively with urine streaks, and major urinary proteins in these marks bind two semiochemically active molecules (2-s-butyl-4,5-dihydrothiazole and 2,3-dehydro-exo-brevicomin) that are associated with the males' aggressive status (Apps *et al*. 1988; Harvey *et al*. 1989; Hurst 1993; Hurst *et al*. 1998). In other lizard species, intrasexual aggression was mediated by pheromonal detection of rival males, but the specific chemicals involved were not identified (Cooper & Vitt 1987; López, Martín & Cuadrado 2002b, 2003).

Moreover, our results showed that chemosensory responses of males depended on their own body size and dominance status, further supporting the idea that hexadecanol is a chemical status badge. Larger and more dominant males responded less strongly towards hexadecanol. Considering that the presence of another male's chemical signals in a male's own home terrarium may represent a lesser threat for dominant than for subordinate males, dominant males may be expected to respond less aggressively. Surprisingly, chemosensory (but not aggressive) responses towards cholesterol were also related to body size and dominance. Cholesterol is a highly abundant steroid (64 ± 3%) in femoral gland secretions of this and other Lacertids (López & Martín 2005). Cholesterol might constitute an unreactive apolar matrix that aids in the delivery of other true semiochemicals (Escobar *et al*. 2003). Similarly, increased dominance in male rabbits is associated with increased secretion of 2-phenoxy-ethanol from the chin gland (Hayes *et al*. 2001), which is not itself detected olfactorially, but functions as a secretion fixative that lowers the release rate of other chemicals (Hayes *et al*. 2003). Nonetheless, male lizards chemically discriminated cholesterol, which suggests that this steroid might, at least, convey information regarding the presence of other males. As cholesterol is more abundant and may be 'easier to find' in scent marks, it may elicit further chemosensory investigation of other chemicals. However, cholesterol does not itself seem to function as a signal of dominance status in *L. m. monticola*, as males did not behave aggressively when presented with cholesterol. In contrast, in the related *L. monticola cyreni*, which does not secrete hexadecanol (López & Martín 2005), cholesterol alone may signal dominance (unpublished data), possibly because larger males secrete more cholesterol (López *et al*. 2006).

However, why should male *L. m. monticola* lizards rely on the concentration of hexadecanol to estimate the dominance status of other males? Evolutionary theories of honest signalling (Zahavi 1975; Pomiankowski 1988; Grafen 1990) predict that signals must be costly to produce and maintain over evolutionary time. Our results suggest that secretion of hexadecanol by *L. m. monticola* may be costly as it elicits aggressive behaviour by other males. This chemical trait might therefore be honest, as cheating by subordinate males might be checked through social control from genuine dominant males (Rohwer & Rohwer 1982; Møller 1987; Olsson 1994a; Martín & Forsman 1999). Even if subordinate males initially could allocate large amounts of hexadecanol to their secretions, the detection of this chemical would elicit an aggressive response by dominant rivals so that subordinate males, with inferior fighting ability, would end up paying high fighting costs. Instead, subordinate individuals might be better off avoiding the signalling of their inferior dominance status (that is, not allocating hexadecanol to secretions). Similarly, adult dominant male *Iguana iguana* produced larger amounts of femoral gland secretions than adult subordinates (Alberts *et al*. 1992).

We also found that male CMI response was correlated with the abundance of hexadecanol (and octadecanol) in femoral secretions. This result suggests the existence of a possible link between quality of the immune system, dominance status, and ability of a male to allocate hexadecanol to secretions. Accordingly, it might be suggested that secretion of hexadecanol may also be physiologically costly, so that only males in good physical condition and health can afford to signal high dominance status. Male birds that allocated more carotenoids to producing more elaborate visual badges were also healthier individuals with superior immunocompetence (Olson & Owens 1998; Blount *et al*. 2003). In *L. m. monticola*, it is possible that increasing the abundance of long-chain fatty alcohols in holocrine secretions may require these chemicals to be diverted from the metabolism. For example, in humans, long-chain fatty alcohols originate from both endogenous synthesis and dietary sources, and aberrant metabolism of long-chain fatty alcohols impairs a number of neurological and immunological functions (Hargrove *et al*. 2004). Then, only males with the capacity to obtain enough long-chain fatty alcohols from the diet might be able to maintain both the metabolism and the production of 'extravagant' chemical badges (see Martín & López 2006 for a similar situation with other chemicals in *L. m. cyreni*).

We conclude that the relative proportions of hexadecanol in femoral secretions of male lizards (*L. m. monticola*) may provide reliable information on the dominance status of males because it elicits aggressive behaviour by rivals, and proportions of hexadecanol are possibly linked with the immune system. These sorts of mechanism may be the basis of intrasexual selection processes based on chemical signals observed in this and other lizard species, and in many other animals that use chemoreception in the organization of their social system.

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References

Aitchison, J. (1986) *The Statistical Analysis of Compositional Data*. Chapman & Hall, London.

Alberts, A.C. (1993) Chemical and behavioral studies of femoral gland secretions in iguanid lizards. *Brain, Behavior and Evolution* **41**, 255–260.

Alberts, A.C. & Werner, D.I. (1993) Chemical recognition of unfamiliar conspecifics by green iguanas: functional significance of different signal components. *Animal Behaviour* **46**, 197–199.

Alberts, A.C., Pratt, N.C. & Phillipis, J.A. (1992) Seasonal productivity of lizard femoral glands: relationship to social dominance and androgen levels. *Physiology and Behavior* **51**, 729–733.

Andersson, M. (1994) *Sexual Selection*. Princeton University Press, Princeton, NJ, USA.

Apps, P.J., Rasa, A. & Viljoen, H.W. (1988) Quantitative chromatographic profiling of odours associated with dominance in male laboratory mice. *Aggressive Behavior* **14**, 451–461.

Aragón, P., López, P. & Martín, J. (2001) Chemosensory discrimination of familiar and unfamiliar conspecifics by lizards: implication of field spatial relationships between males. *Behavioral Ecology and Sociobiology* **50**, 128–133.

Aragón, P., López, P. & Martín, J. (2004) The ontogeny of spatio-temporal tactics and social relationships of adult male Iberian rock lizards, *Lacerta monticola. Ethology* **110**, 1001–1019.

Atema, J. (1986) Review of sexual selection and chemical communication in the lobster, *Homarus americanus*. *Canadian Journal of Fisheries and Aquatic Sciences* **43**, 2283–2290.

Belliure, J., Smith, L. & Sorci, G. (2004) Effect of testosterone on T cell-mediated immunity in two species of Mediterranean Lacertid lizards. *Journal of Experimental Zoology A* **301**, 411–418.

Blount, J.D., Metcalfe, N.B., Birkhead, T.R. & Surai, P.F. (2003) Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science* **300**, 125–127.

Carranza, S., Arnold, E.N. & Amat, F. (2004) DNA phylogeny of *Lacerta* (*Iberolacerta*) and other lacertine lizards (Reptilia: Lacertidae): did competition cause long-term mountain restriction? *Systematics and Biodiversity* **2**, 57–77.

Cooper, W.E. (1994) Chemical discrimination by tongue-flicking in lizards: a review with hypotheses on its origin and its ecological and phylogenetic relationships. *Journal of Chemical Ecology* **20**, 439–487.

Cooper, W.E. & Burghardt, G.M. (1990) A comparative analysis of scoring methods for chemical discrimination of prey by squamate reptiles. *Journal of Chemical Ecology* **16**, 45–65.

Cooper, W.E. & Vitt, L.J. (1987) Intraspecific and interspecific aggression in lizards of the scincid genus *Eumeces*: chemical detection of conspecific sexual competitors. *Herpetologica* **43**, 7–14.

Dietemann, V., Peeters, C., Liebig, J., Thivet, V. & Hölldobler, B. (2003) Cuticular hydrocarbons mediate discrimination of reproductives and nonreproductives in the ant *Myrmecia gulosa*. *Proceedings of the National Academy of Sciences, USA* **100**, 10341–10346.

Enquist, M. & Leimar, O. (1983) Evolution of fighting behaviour: decision rules and assessment of relative strength. *Journal of Theoretical Biology* **102**, 387–410.

Escobar, C.M., Escobar, C.A., Labra, A. & Niemeyer, H.M. (2003) Chemical composition of precloacal secretions of two *Liolaemus fabiani* populations: are they different? *Journal of Chemical Ecology* **29**, 629–638.

Folstad, I. & Karter, A.K. (1992) Parasites, bright males and the immunocompetence handicap. *American Naturalist* **139**, 603–622.

Grafen, A. (1990) Biological signals as handicaps. *Journal of Theoretical Biology* **144**, 517–546.

Grafen, A. & Hails, R. (2002) *Modern Statistics for the Life Sciences*. Oxford University Press, New York.

Hargrove, J.L., Greenspan, P. & Hartle, D.K. (2004) Nutritional significance and metabolism of very long chain fatty alcohols and acids from dietary waxes. *Experimental Biological Medicine* **229**, 215–226.

Harvey, S., Jemiolo, B. & Novotny, M. (1989) Pattern of volatile compounds in dominant and subordinate male mouse urine. *Journal of Chemical Ecology* **15**, 2061–2072.

Hayes, R.A., Richardson, B.J. & Wyllie, S.G. (2001) Increased social dominance in male rabbits, *Oryctolagus cuniculus*, is associated with increased secretion of 2-phenoxy ethanol from the chin gland. *Chemical Signals in Vertebrates Vol. 9* (eds A. Marchlewska-Koj, J.J. Lepri and D. Müller-Schwarze), pp. 335–341. Plenum Press, New York.

Hayes, R.A., Richardson, B.J. & Wyllie, S.G. (2003) To fix or not to fix: the role of 2-phenoxyethanol in rabbit, *Oryctolagus cuniculus*, chin gland secretion. *Journal of Chemical Ecology* **29**, 1051–1064.

Hurst, J.L. (1993) The priming effects of urine substrate marks on interactions between male house mice *Mus musculus musculus* Schwarz and Schwarz. *Animal Behaviour* **45**, 55–81.

Hurst, J.L., Robertson, D.H.L., Tolladay, U. & Beynon, R.J. (1998) Proteins in urine scent marks of male house mice extend the longevity of olfactory signals. *Animal Behaviour* **55**, 1289–1297.

Krebs, J.R. & Dawkins, R. (1983) Animal signals: mind-reading and manipulation. *Behavioural Ecology: An Evolutionary Approach*, 2nd edn (eds J.R. Krebs and N.B. Davies), pp. 380–402. Blackwell Scientific Publications, Oxford, UK.

López, P. & Martín, J. (2001) Fighting rules and rival recognition reduce costs of aggression in male lizards, *Podarcis hispanica*. *Behavioral Ecology and Sociobiology* **49**, 111–116.

López, P. & Martín, J. (2002) Chemical rival recognition decreases aggression levels in male Iberian wall lizards, *Podarcis hispanica*. *Behavioral Ecology and Sociobiology* **51**, 461–465.

López, P. & Martín, J. (2005) Chemical compounds from femoral gland secretions of male Iberian rock lizards, *Lacerta monticola cyreni. Zeitschrift für Naturforschung C* **60**, 632–636.

López, P., Muñoz, A. & Martín, J. (2002a) Symmetry, male dominance and female mate preferences in the Iberian rock lizard, *Lacerta monticola*. *Behavioral Ecology and Sociobiology* **52**, 342–347.

López, P., Martín, J. & Cuadrado, M. (2002b) Pheromone mediated intrasexual aggression in male lizards, *Podarcis hispanicus*. *Aggressive Behavior* **28**, 154–163.

López, P., Martín, J. & Cuadrado, M. (2003) Chemosensory cues allow male lizards *Psammodromus algirus* to override visual concealment of sexual identity by satellite males. *Behavioral Ecology and Sociobiology* **54**, 218–224.

López, P. & Amo, L. & Martín, J. (2006) Reliable signaling by chemical cues of male traits and health state in male lizards, *Lacerta monticola*. *Journal of Chemical Ecology* **32**, 473–488.

Marler, C.A. & Moore, M.C. (1988) Evolutionary costs of aggression revealed by testosterone manipulations in free-living male lizards. *Behavioral Ecology and Sociobiology* **23**, 21–26.

Martín, J. & Forsman, A. (1999) Social costs and development of nuptial coloration in male *Psammodromus algirus* lizards: an experiment. *Behavioral Ecology* **10**, 396–400.

Martín, J. & López, P. (2000) Social status of male Iberian rock-lizards (*Lacerta monticola*) influences their activity patterns during the mating season. *Canadian Journal of Zoology* **78**, 1105–1109.

Martín, J. & López, P. (2006) Vitamin D supplementation increases the attractiveness of males' scent for female Iberian rock lizards. *Proceedings of the Royal Society of London, Series B* **273**, 2619–2624.

Martín, J. & Salvador, A. (1993) Tail loss reduces mating success in the Iberian rock-lizard. *Behavioral Ecology and Sociobiology* **32**, 185–189.

Mason, R.T. (1992) Reptilian pheromones. *Biology of the Reptilia, Vol. 18* (eds C. Gans and D. Crews), pp. 114–228. University of Chicago Press, Chicago, IL, USA.

Mathis, A. (1990) Territorial salamanders assess sexual and competitive information using chemical signals. *Animal Behaviour* **40**, 953–962.

Mayer, W. & Arribas, O. (2003) Phylogenetic relationships of the European lacertid genera *Archaeolacerta* and *Iberolacerta* and their relationships to some other 'Archaeolacertae' (*sensu lato*) from Near East, derived from mitochondrial DNA sequences. *Journal of Zoological Systematics and Evolutionary Research* **41**, 157–161.

Møller, A.P. (1987) Social control of deception among status signalling house sparrows, *Passer domesticus*. *Behavioral Ecology and Sociobiology* **20**, 307–311.

Moore, A.J. (1997) The evolution of social signals: morphological, functional, and genetic integration of the sex pheromone in *Nauphoeta cinerea. Evolution* **51**, 1920–1928.

Moore, M.C. & Lindzey, J. (1992) The physiological basis of sexual behavior in male reptiles. *Biology of the Reptilia, Vol. 18* (eds C. Gans and D. Crews), pp. 70–113. University of Chicago Press, Chicago, IL, USA.

Moore, P.J., Reagan-Wallin, N.L., Haynes, K.F. & Moore, A.J. (1997) Odour conveys status on cockroaches. *Nature* **389**, 25.

Moreira, P.L. (2002) Sexual selection and sperm competition in the Iberian rock lizard (*Lacerta monticola*). PhD thesis, University of Sheffield, Sheffield, UK.

Moreira, P.L., López, P. & Martín, J. (2006) Femoral secretions and copulatory plugs convey chemical information about male identity and dominance status in Iberian rock lizards (*Lacerta monticola*). *Behavioral Ecology and Sociobiology* **60**, 166–174.

Olson, V.A. & Owens, I.P.F. (1998) Costly sexual signals: are carotenoids rare, risky or required? *Trends in Ecology and Evolution* **13**, 510–514.

Olsson, M. (1994a) Why are sand lizard males (*Lacerta agilis*) not equally green? *Behavioral Ecology and Sociobiology* **35**, 169–173.

Olsson, M. (1994b) Rival recognition affects male contest behavior in sand lizards (*Lacerta agilis*). *Behavioral Ecology and Sociobiology* **35**, 249–252.

Olsson, M. & Madsen, T. (1998) Sexual selection and sperm competition in reptiles. In: *Sperm Competition and Sexual Selection* (eds T.R. Birkhead and A.P. Møller), pp. 503–578. Academic Press, San Diego, CA, USA.

Olsson, M. & Shine, R. (2000) Ownership influences the outcome of male–male contests in the scincid lizard, *Niveoscincus microlepidotus*. *Behavioral Ecology* **11**, 587–590.

Pomiankowski, A.N. (1988) The evolution of female mate preferences for male genetic quality. *Oxford Surveys in Evolutionary Biology* **5**, 136–184.

Rohwer, S. & Rohwer, F.C. (1982) Status signalling in Harris sparrows: experimental deceptions achieved. *Animal Behaviour* **26**, 1012–1022.

Roux, E., Sreng, L., Provost, E., Roux, M. & Clement, J.L. (2002) Cuticular hydrocarbon profiles of dominant versus subordinate male *Nauphoeta cinerea* cockroaches. *Journal of Chemical Ecology* **28**, 1221–1235.

Searcy, W.A. & Nowicki, S. (2005) *The Evolution of Animal Communication*. Princeton University Press, Princeton, NJ, USA.

Sheldon, B.C. & Verhulst, S. (1996) Ecological immunology: costly parasite defence and trade-offs in evolutionary ecology. *Trends in Ecology and Evolution* **11**, 317–321.

Smits, J.E. & Bortolotti, G.R. & Tella, J.L. (1999) Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Functional Ecology* **13**, 567–572.

Sokal, R.R. & Rohlf, F.J. (1995) *Biometry*. Freeman, New York.

Wedekind, C. & Folstad, I. (1994) Adaptive or nonadaptive immunosuppression by sex hormones? *American Naturalist* **143**, 936–938.

Westneat, D.F. & Birkhead, T.R. (1998) Alternative hypothesis linking the immune system and mate choice for good genes. *Proceedings of the Royal Society of London, Series B* **265**, 1065–1073.

Wyatt, T.D. (2003) *Pheromones and Animal Behaviour*. Cambridge University Press, Cambridge, UK.

Zahavi, A. (1975) Mate selection – a selection for a handicap. *Journal of Theoretical Biology* **53**, 205–214.

Zulandt Schneider, R.A., Huber, R. & Moore, P.A. (2001) Individual and status recognition in the crayfish, *Orconectes rusticus*: the effects of urine release on fight dynamics. *Behaviour* **138**, 137–153.

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Supplementary material

The following supplementary material is available for this article:

Appendix S1. Principal components analysis for relative proportion of the 29 most abundant lipophilic chemicals in femoral gland secretions of male lizards *Lacerta m. monticola*.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2435.2007.01262.x