Scent may signal fighting ability in male Iberian rock lizards

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Intrasexual competition favours the evolution of conspicuous fighting ability badges. However, in spite of the fact that chemoreception is important in sexual selection of many animals, such as lizards, the role of chemical signals in males’ contests is relatively unknown. Here, we show that proportions of cholesterol in femoral gland secretions of male Iberian rock lizards were related to their body size (which confers a competitive advantage in fights). Males discriminated chemically and responded aggressively to cholesterol stimuli presented on swabs. Moreover, we experimentally increased cholesterol in the scent of males, and staged encounters in neutral cages between two unfamiliar and size-matched males. Focal males lost more agonistic interactions against males manipulated with cholesterol than in control tests. We suggest that differences in scent composition may reliably signal fighting ability in many lizard species, which would help to avoid the costs of fighting.

Keywords: chemoreception; chemical signals; male–male competition; fighting badges

1. INTRODUCTION

Male competition over females or resources important to females often favours the evolution of male traits conferring fighting ability and correlated status signaling badges (Andersson 1994). During social encounters, males may use status badges to judge relative fighting ability, thus avoiding costly aggressive interactions (Maynard Smith 1982). Most studies examining status badges focused on conspicuous visual or acoustic traits (Seacry & Nowicki 2005), generally ignoring chemical signals. Chemoreception is important in intraspecific communication of many animals, including invertebrates, reptiles and mammals (Wyatt 2003), where chemical traits alone may reveal dominance status (e.g. Apps et al. 1988; Moore et al. 1997; Zulandt-Schneider et al. 2001). In lizards, femoral gland secretions might also signal dominance (Alberts et al. 1992; Moreira et al. 2006). Chemical recognition seems to reduce costs of fighting (López & Martin 2002), but no study has examined the chemical basis of this recognition, and whether lizards actually use ‘chemical badges’ during agonistic encounters.

Iberian rock lizards, Lacerta monticola, are small lacertids from the rocky mountains of the Iberian Peninsula. Fights between males are frequent and dominance hierarchies often emerge (Aragón et al. 2004). Males discriminate between self-produced scents and scents of familiar and unfamiliar males (Aragón et al. 2001). The proportion of certain chemicals (i.e. cholesterol) in males’ femoral secretions changes with body length (López et al. 2006). Because males with larger body size and larger heads have advantages in contests (López et al. 2002), we predicted that cholesterol might be a fighting ability badge in agonistic interactions.

Here, we examined whether relative proportions of cholesterol in femoral secretions of male L. monticola were related to body size. Then, we tested whether males (i) discriminated chemically cholesterol from other compounds in secretions and (ii) responded aggressively to cholesterol, while responded neutrally to other chemicals. Finally, (iii) we manipulated chemicals of size-matched males to test whether scent may signal fighting ability during agonistic contests.

2. MATERIAL AND METHODS

(a) Male body size and chemicals in femoral secretions

We captured by noosing 50 adult male lizards during the May 2005 mating season, in different places over a large area (Guadarrama Mountains, Central Spain). Lizards were individually housed at ‘El Ventorrillo’ Field Station, 5 km from capture sites, in outdoor 80×30 cm PVC terraria containing rocks for cover, and food (mosquitoes) and water ad libitum. We measured lizards’ snout-to-vent length (SVL) with a ruler (X±SE=71±1 mm; range=63–78 mm), and head depth (8.3±0.1 mm), length (15.6±0.1 mm) and width (10.7±0.1 mm) with a digital calliper. We used principal component analysis (PCA) to reduce these four variables (log-transformed). The first PC (PC-1) explained 78.8% variance and was positively correlated with all morphological measurements. We used PC-1 scores as a variable (“body size”) in subsequent analyses.

We collected femoral secretion directly in glass vials with Teflon-lined stoppers that were stored at −20°C. Samples were analysed by gas chromatography–mass spectrometry (ThermoQuest Trace 2000) with a Supelco-Equity-5 column temperature programmed (50°–280°C at 5°C min−1 and 280°C for 30 min). The compounds were identified by comparison of mass spectra in the National Institute of Standards and Technology library, and later confirmed with authentic standards. The relative amount of each component was determined as the percentage of the total ion current (TIC) area transformed following Atchison (1986; for similar analyses and details of chemicals see López et al. 2006).

(b) Chemosensory responses of males to chemical compounds

Lizards react to different chemical stimuli with increased and differential rates of tongue extrusions (Cooper & Burghardt 1990). Tongue-flick (TF) rate can, therefore, be used as a quantitative bioassay of detection of chemicals. We compared TF rate by males (n=16) in response to stimuli arising from cotton applicators bearing (i) dichloromethane (DCM; control) or DCM in which we had dissolved (ii) hexadecanoic acid, (iii) ergosterol or (iv) cholesterol. DCM was used to gauge baseline TF rates in the experimental situation. The other three chemicals were selected because they were the most abundant compounds in femoral secretions (López et al. 2006). We prepared chemical stimuli the same day of the tests by dissolving and mixing 24 mg of each compound (standards from Sigma-Aldrich Chemicals) in 1 ml DCM. In a second test, we measured TF rates to examine whether males can discriminate three different concentrations of cholesterol dissolved in DCM (8, 16 or 24 mg ml−1).

Immediately before the trials, we dipped for 3 s the cotton tip (1 cm) of a wooden applicator attached to a long stick (50 cm) in vials containing chemical stimuli. Swabs with all stimuli were visually similar for humans. A new swab was used in each trial. Every lizard was exposed to all scents (one trial per day) and order of presentation was counterbalanced. Trials were conducted outdoor in the middle of May (mating season), and between 11.00 and 13.00 h (GMT) when lizards were fully active. We slowly approached each lizard’s cage and moved the swab to a position 1 cm anterior to the lizard’s snout. We recorded numbers of TFs directed to the swab for 60 s beginning with the first TF and numbers of bites to the swabs as a measure of aggressive or defensive responses to the chemicals.

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3. RESULTS

Relative proportions of cholesterol in femoral secretions ranged between 54 and 84% (mean ± s.e. = 69 ± 2%). Larger males (those with greater body size PC scores) had relatively greater proportions of cholesterol in their femoral secretions (forward stepwise regression, $R^2 = 0.20$, $F_{1,48} = 12.04$, $p = 0.001$).

In chemosensory tests, the rate of TFs (log-transformed) directed to swabs differed between chemical stimuli (repeated measures ANOVA: $F_{3,45} = 85.26$, $p < 0.0001$; figure 1a). TF rates to cholesterol were higher than to other stimuli (Tukey’s tests: $p < 0.0002$ in all cases) and there were no differences among other chemicals ($p > 0.70$ in all cases). Six individuals bit the swabs with cholesterol vigorously and briefly, whereas no bites were recorded to other scents (binomial test, $p = 0.0015$).

In the second test, TF rates differed between concentrations of cholesterol (repeated measures ANOVA: $F_{3,30} = 167.81$, $p < 0.0001$; figure 1b), with increased TF rates to stimuli having increasing concentrations (Tukey’s tests: $p < 0.0001$ in all cases). Five lizards bit the applicators bearing ‘high’ concentrations of cholesterol, and one lizard bit the applicators bearing ‘low’ concentrations (binomial test, $p = 0.022$).

In staged encounters, there were no significant differences in the number of agonistic interactions between treatments (repeated measures two-way ANOVA, $F_{1,19} = 1.58$, $p = 0.22$) and focal males lost more interactions than manipulated males ($F_{1,19} = 6.47$, $p = 0.019$), but the interaction was significant ($F_{1,19} = 11.61$, $p = 0.003$; figure 2). Thus, in ‘cholesterol’ trials, focal males lost more interactions than they won (Tukey’s test: $p < 0.01$), but there were no differences in the ‘control’ treatment ($p = 0.62$).

4. DISCUSSION

Cholesterol proportions in femoral secretions of male *L. monticola* were related to their body size. In this lizard, cholesterol is the major lipid in secretions and its production may be size/age dependent. In lizards, social dominance, lipid metabolism, femoral gland production and percentage of lipid in secretions depend on androgen levels (Alberts et al. 1992; Sheridan 1994). Testosterone upregulates cholesterol in humans and birds (McGraw et al. 2006). Thus, it is likely that proportions of cholesterol in secretions depended on sex steroid levels, which would make it a potentially reliable signal of dominance.
Chemosensory tests clearly showed that cholesterol was readily discriminated and elicited aggressive responses. Lizards often exhibit social dominance systems and use pheromones as social signals (Mason 1992), and intrasexual aggression in many lizards is mediated by chemical recognition (Cooper & Vitt 1987; López & Martín 2002). Because a large body size is determinant in agonistic contests, it would be advantageous to assess the size of rivals in the absence of the signallers before being involved in fights. Previous experiments showed that behavioural responses of intruder male *L. monticola* to scent marks of unfamiliar males depended on the relative differences in body size (Aragón et al. 2001): the current experiment suggests that body size may be assessed from cholesterol proportions in scent.

Moreover, cholesterol may be a signal of fighting ability during agonistic contests too. Visual estimation of size should occur first, but between similarly sized males, chemoreception could allow an individual to quickly assess a rival's fighting ability, such that potentially inferior males may retreat before a costly escalated fight occurs. In fact, most interactions lost in our experimental treatment were retreats of males before manipulated males had started any aggressive approach. We suggest that differences in scent composition, based on cholesterol or other lipids, may reliably signal fighting ability in many lizard species where chemoreception is important, which would help to avoid the costs of unnecessary fighting.

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