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ORIGINAL ARTICLE

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Femoral secretions and copulatory plugs convey chemical information about male identity and dominance status in Iberian rock lizards (*Lacerta monticola*)

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Abstract Socially dominant males often signal their status to rival males and/or females. We tested the hypotheses that *Lacerta monticola* femoral gland secretions and copulatory plugs convey chemical information about male identity and dominance status. We estimated male dominance status by staging male–male agonistic encounters in a neutral arena. We then conducted two experiments to compare male tongue-flick behavior toward chemical stimuli consisting of cotton swabs bearing (1) deionized water (control), the lizard's own femoral secretions, and the femoral secretions of another male and (2) phosphate-buffered saline solution (control), the lizard's own plug products, and the plug products of another male. Results indicate that males discriminated their own femoral secretions and plugs from those of other males. They also discriminated morphological attributes of other males that were associated with dominance status based on chemical cues arising from femoral secretions and discriminated the dominance status of other males based on chemical cues arising from the plugs. Femoral secretions that convey information about male identity and dominance status may be hypothesized to function in the establishment of *L. monticola* dominance hierarchies through scent-marking of territories. We suggest that copulatory plugs and femoral secretions may allow males to scent-mark the female body and postulate that this behavior may influence male and female reproductive decisions under selective pressures of sperm competition.

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Introduction

Male competition over females often favors male attributes conferring fighting ability and correlated status signaling traits, resulting in the evolution of conspicuous male secondary sex traits that, in some cases, may also be favored by female mate choice (Andersson 1994; Berglund et al. 1996). When females are sexually promiscuous, sperm competition and cryptic female choice may occur after insemination, further increasing the role of sexual selection in shaping the physiology, morphology, and behavior of sexually reproducing organisms (Birkhead and Møller 1998). Male fighting ability and correlated status signaling traits may therefore be intra- and intersexually selected at both pre- and postcopulatory levels. For instance, in the red jungle fowl (*Gallus gallus*), males with higher fighting ability carry larger and brighter combs, assume socially dominant status, and enjoy increased mating success through male–male competition (Parker and Ligon 2002). They are also favored by female mate choice as females show preference for their status-signaling trait (Parker and Ligon 2003) and promote insemination by dominant males through pre- and postcopulatory mechanisms (Pizzari et al. 2002). In this species, females obtain increased material (Pizzari 2003) and genetic (Parker 2003) benefits by mating with dominant males. However, that is not the case in many organisms, leading to sexual conflict and to antagonistic coevolution of male and female reproductive traits (Qvarnström and Forsgren 1998; Moore et al. 2001, 2003; López et al. 2002).

Studies on reptilian status signaling traits have focused mainly on conspicuous visual traits (see Olsson and Madsen 1998 for review). In the sand lizard (*Lacerta agilis*), visual skin traits contribute to male individual recognition (Olsson 1994b), and the extension of male nuptial coloration correlates with fighting ability (Olsson

1994a) and mating success (Anderholm et al. 2004). The role of female mate choice in selection of visual traits that are known to mediate male–male competition is unclear because it was rarely demonstrated in reptiles (Olsson and Madsen 1995; Tokarz 1995). Chemical signals may offer an alternative model system to address the roles of intra- and intersexual selection in the evolution of reptilian sexually dimorphic traits and male social dominance (Martín and López 2000; López et al. 2002). In reptiles, intraspecific chemosensory communication is widespread and plays an important role in social organization; moreover, reptilian nasal chemical systems and organs for production of pheromones are often highly specialized (Halpern 1992; Mason 1992).

Tongue-flick (TF) behavior (extrusion of the tongue is associated with the vomeronasal organ) reflects chemical investigation of a stimulus (Halpern 1992) and is often analyzed in experiments addressing chemosensory discrimination in lizards (Cooper 1994). Many lizards possess holocrine glands that deliver secretions through open pores located on the femoral region (Mason 1992). Males often have more and/or larger femoral glands than females and produce secretions more abundantly with peak production during the breeding season (Alberts et al. 1992; Alberts 1993). Self-recognition and discrimination between familiar vs unfamiliar males based on femoral secretions was shown for several species (e.g., Alberts 1992b; Alberts and Werner 1993; Aragón et al. 2001b). Both the protein (Alberts et al. 1993) and lipid (Alberts 1990; López and Martín 2005) fractions can provide the basis for chemical communication.

Femoral secretions were hypothesized to signal male dominance status through productivity rates and/or the quality of the secretions (Alberts et al. 1992; López et al. 2002). In green iguanas (*Iguana iguana*), productivity of femoral secretions during the breeding season is higher for dominant adult males than for subordinate adult males and both the productivity of and percentage of lipids in secretions correlate positively with plasma testosterone levels among dominant males (Alberts et al. 1992). In Iberian rock lizards belonging to *Lacerta cyreni*, males tested in cages scent-marked by other males responded differently toward the scents of familiar vs unfamiliar males, and responses toward scents of unfamiliar males depended on the difference in body size and in fluctuating asymmetry (FA) of the femoral pores (i.e., potential dominance status) between test and scent donor males (Aragón et al. 2001a, 2001c, 2003). Although self-recognition and discrimination of familiar vs unfamiliar males can be based on femoral secretions (Aragón et al. 2001b), the specific source of chemicals that potentially signaled male dominance status could not be ascertained because cages were scent-marked by femoral secretions and fecal pellets (Aragón et al. 2001a, 2001c, 2003).

Copulatory plugs may play a role in intraspecific communication (Volsøe 1944 cited in Fox 1977). We hypothesized that plugs signal male identity and dominance status. Reptilian renal sex segments (RSS), found only in males, produce secretions that are delivered along with sperm during copulation, and that form a plug in some snakes and lizards (Devine 1984; In den Bosch 1994). Plugs potentially serve diverse roles (Fox 1977; Moreira and Birkhead 2003) but their actual function was not demonstrated. Plugs did not prevent insemination by rival males ("chastity belt" hypothesis in Devine 1975, 1977; Shine et al. 2000) in Iberian rock lizards belonging to *Lacerta monticola* (Moreira and Birkhead 2003). Plugs were not the source of pheromones ("anti-aphrodisiac" hypothesis in Ross and Crews 1977, 1978) that render garter snake (*Thamnophis* sp.) females unreceptive and/or unattractive to males after copulation (Shine et al. 2000). However, in sand lizards, Olsson et al. (2004) found that copulation duration by second males differed according to the major histocompatibility complex-type of the male that copulated previously with the female; they suggested that second males gathered such information from the plug in the female cloaca. The production of RSS secretions is androgen-dependent in many reptiles (Krohmer 2004). RSS secretions contain a large array of chemical components (Fox 1977; Sever et al. 2002) and the lipid fraction was reported to increase during the breeding season in northern water snakes (*Nerodia sipedon*; Weil and Aldridge 1984).

We tested the hypotheses that *L. monticola* femoral secretions and copulatory plugs convey chemical information about male identity and dominance status. We estimated male dominance status by staging male–male agonistic encounters in a neutral arena and examined which morphological attributes [among snout-to-vent length (SVL), tail length, head length, head width, head height, body mass, and number and FA of the femoral pores] might be associated with male dominance status. Thereafter, we studied TF behavior to examine whether males discriminated their own femoral secretions and plugs from those of other males. Moreover, we examined whether males discriminated the dominance status of other males (or the morphological attributes associated with dominance status) based on chemical cues arising from femoral secretions and plugs.

Materials and methods

Study population

Iberian rock lizards are small insectivorous lacertids of highland rocky habitats. Recent taxonomic revision suggests that Iberian rock lizards comprise eight closely related species and subspecies (Mayer and Arribas 2003). In Portugal, *L. monticola* is restricted to a single population located at Serra da Estrela mountain. Lizards are active from March–May to October–November. Adult males emerge from winter dormancy 1–2 weeks before adult females and the mating season begins in April–June, once males have shed the skin, and lasts for 2–4 weeks (Moreira 2002). As in *L. cyreni*, home ranges of neighboring males overlap considerably and males engage in frequent agonistic encounters during the mating season (Aragón et al. 2001d, 2004; P. Moreira, personal observation for *L. monticola*). Male and female Iberian rock lizards are sexually promiscuous (Martín and Salvador 1993; Moreira 2002). At Serra da Estrela, females copulate 4–8 times per mating season, frequently with several males, and produce a single clutch per year with 2–12 eggs between June and July (Moreira 2002).

Study animals and rearing conditions

Adult lizards (56 males and 105 females) with intact or fully regenerated tails were captured at the highest elevations of Serra da Estrela (surroundings of "Torre" at 1,993 m) on emergence from winter dormancy (and before the start of the mating season) between March 16 and April 16, 2004. We recorded the lizard's color morph ("green" are more abundant than "blue," and the relative abundance of the green morph ranges between ca. 100% at lower altitudes and ca. 75% at the highest altitudes of Serra da Estrela; Moreira 2002). We measured SVL and tail length to the nearest 1 mm with a ruler; measured head length, head width, and head height to the nearest 0.05 mm with a caliper; and counted the number of femoral pores on the right and left hind legs with the aid of a magnifying glass (2–4 counts performed per animal).

Lizards were maintained under laboratory winter dormancy conditions (7°C) from the date of capture to the start of the experiments. For the present experimental study, we used 28 males and 20 females of the green morph, which ranged between 75–81 mm SVL (mean±SD=77.5±1.7 mm) and 74–83 mm SVL (78.9±2.3 mm). Based on the entire sample, the number of femoral pores (right + left) was significantly higher for males (mean±SD=36.5±2.3, n=56) than for females (33.7±2.4, n=105; t test t=7.25, P<0.001). Among males and females, right–left number of femoral pores showed the properties of FA; normal distribution (males skewness±SE=−0.06±0.32, kurtosis±SE=−0.22±0.47; females skewness=−0.02±0.24, kurtosis=0.09±0.47; t tests on skewness and kurtosis P>0.05 in all cases; methods as in Palmer 1994) around a mean of zero (one sample t test: males t_{55}=−0.76, P=0.45; females t_{104}=1.35, P=0.18). Repeatability estimates (methods as in Lessells and Boag 1987) of the number and FA of the femoral pores were very high among males (number r=0.96, $F_{55,116}$=71.9, P<0.001; FA r=0.86, $F_{55,116}$=20.3, P<0.001) and females (number r=0.97, $F_{104,183}$=95.9, P<0.001; FA r=0.90, $F_{104,183}$=26.8, P<0.001).

Experiments were conducted in indoor facilities at the "El Ventorrillo" Museo Nacional de Ciencias Naturales Field Station (Madrid, Spain). Lizards were housed in 50×25×25 cm^3 glass terraria, each provided with a cardboard substrate, cardboard rolls for refuge and a hardware cloth lid. Terraria were lighted with full spectrum fluorescent lamps and were heated by one 60 W incandescent lamp on a 10:14 light–dark cycle (light period from 9:00–19:00 h). Mealworm larvae (*Tenebrium* sp.) dusted with multivitamin, calcium/phosphorus supplements, and water were continually available. Lizards were regularly weighed to the nearest 0.01 g and maintained good condition throughout the study. They were released at their sites of capture after the studies.

Males were removed from laboratory winter dormancy conditions on May 8 and were housed initially in groups of four per terrarium. The groups were chosen randomly with the condition that each one included two animals above and two animals below the median SVL. On May 16, when males started shedding their skins (all shed between May 15 and 21), they were transferred to individual clean terraria. On May 23, females were removed from laboratory winter dormancy conditions and were housed permanently in groups of four, established in the same manner as for males. Visual communication between males (both before and after May 16) and between females in different terraria was prevented by means of cardboard screens. Males and females were housed in separate laboratory rooms.

On June 6 and 7, each of 20 males was presented with a female in the male's home terrarium and, after copulations, copulatory plugs were collected from the female cloaca with fine forceps. Plugs were preserved in Eppendorf tubes containing 0.2 ml phosphate-buffered saline (PBS) solution and were maintained at 5°C. Plugs were later divided longitudinally using surgical tweezers and half of each plug (preserved as before) was used as a sample for the chemosensory trials.

Male dominance status

We estimated male dominance status from fighting ability (López and Martín 2001) by staging 56 male–male encounters. Each male participated in four encounters against four different opponents. To avoid effects of prior residency (Olsson and Shine 2000) and familiarity (López and Martín 2001) on outcomes of encounters, trials were conducted in a neutral arena and males were paired randomly among those that were not housed together between May 6 and 16. The test arena consisted of a 50×50×25 cm^3 terrarium provided with a fresh cardboard substrate and divided into two equally sized compartments by means of a removable opaque partition. Each compartment was provided with a 60-W lamp for thermoregulation. Two sessions of 14 encounters were conducted per day (one between 11:00–13:00 h and another between 17:00–19:00 h) on two consecutive days (June 4 and 5). The order of encounters conducted per session and the 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 to adjust to the arena before the partition was removed. Encounters were filmed with an 8-mm video camera and were 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. Information on the outcome of encounters were retrieved from the films. We considered the winning male to be the one that chased its opponent, either immediately after an attack or after combat with biting. A tie was recorded 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.

Discrimination of femoral secretions

We studied TF behavior toward chemical stimuli using a repeated measures design (Cooper 1994). Each of the 28 males was presented with three chemical stimuli in its home terrarium in a randomized order. The stimuli consisted of cotton swabs bearing (1) deionized water (control), (2) the lizard's own femoral secretions, and (3) femoral secretions of another (donor) male. Each male was used once as test male and once as donor of femoral secretions for testing in the conditions 2 and 3. Test-secretion donor pairs were chosen randomly among males that were not housed together between May 6 and 16. They were also randomized with regard to male pairs that participated in previous staged encounters. Ten of the 28 males were presented with femoral secretions of donor males that they had previously contacted (for up to 5 min) in staged encounters. This variable was included in the analyses (see below).

TF behavior was quantified between June 14 and 17. Control stimuli were prepared by dipping cotton swabs in deionized water. Femoral secretions were collected by pressing approximately half of the number of femoral pores in the left hind leg of donor males. We attempted to express similar amounts of secretions each time and prepared stimuli consisting of femoral secretions by rubbing the expressed secretions on a moist (deionized water) cotton swab. In the chemosensory trials, we used groups of five (or four) males per session with each male in the group serving as test male, as donor of secretions to be presented to another of the five (or four) males, and as provider of his own secretions. In this manner, we controlled for manipulation and collection of femoral secretions from test males before their use in the trials and were able to present stimuli to test males shortly after their preparation (thereby avoiding fading of stimuli). During the trials, male home terraria were devoid of refuges, water, and food bowls. We also removed lighting provided by full spectrum lamps to prevent UV light from influencing stimulus recognition (Alberts 1989).

To conduct a chemosensory trial, one experimenter positioned the swab attached to a 50-cm wood applicator 2 cm anterior to the test male's snout, repositioning it every time the lizard moved away. This experimenter dictated observations to a second one who recorded them and timed events with a chronometer. We recorded the time elapsed between presentation of the swab to a lizard and the first TF directed at the swab (latency period), and the numbers of TFs directed at the swab (directed TFs) and directed elsewhere (nondirected TFs) during 60 s counted from the first directed TF. We also recorded the duration of time that the male moved away from the swab.

Discrimination of copulatory plugs

The study of TF behavior toward chemical stimuli consisting of plug products followed the same experimental design and methodological procedures as for the experiment on discrimination of femoral secretions. Each of the 20 males for which plugs were collected was presented with three stimuli in a randomized order and consisting of cotton swabs bearing (1) PBS buffer solution (control), (2) the lizard's own plug products, and (3) plug products of another male. Test-plug donor pairs were randomly chosen among males that were not housed together between May 6 and 16, and with respect to male pairs that participated in previous staged encounters or as test-secretion donor pairs in the previous experiment on discrimination of femoral secretions. Eleven of the 20 males were presented with plug products of a male with which they had previous contact (for up 5 min) in staged encounters or that served as donor of femoral secretions in the previous experiment. This variable was included in the analyses (see below). TF behavior was quantified on June 22 and 23. Control stimuli were prepared by dipping cotton swabs in PBS solution. Stimuli consisting of plug products were prepared by dipping a cotton swab in the Eppendorf tubes containing each of the plug samples and by pressing the swabs against (and macerating) the plug.

Data analyses

For each staged male–male encounter, we attributed values of 1 to the winner, zero to the loser, and 0.5 to both males for a tie. A male's dominance status was scored by the sum of values obtained among its four encounters (previous studies on *L. cyreni* showed that males establish linear dominance hierarchies; López et al. 2002). To study the morphological correlates of male dominance status, we developed generalized linear models (GLMs) of a male's dominance score based on the average difference between the morphological attributes (SVL, tail length, head length, head width, head height, body mass, and number and FA of the femoral pores) of the male and of those of its four opponents. GLMs were developed in a backward stepwise manner (Statistica 6.0) by removing nonsignificant terms at $P>0.10$ from final models. Residuals from the final model were normally distributed (Grafen and Hails 2002).

To examine whether males discriminated their own femoral secretions and plugs from those of other males, we compared TF behavior toward different chemical stimuli. We analyzed latency period and numbers of nondirected TFs, directed TFs, and total TFs (sum of the numbers of nondirected and directed TFs) elicited per minute. The duration of time that males moved away from the swab was discounted in calculations of TF rates. Latency period and nondirected TFs were not normally distributed (Shapiro–Wilk test $W<0.84$, $P<0.01$ in all cases) and were analyzed using nonparametric statistics (Friedman ANOVA by ranks and nonparametric a posteriori multiple comparisons;

Siegel and Castellan 1988). Numbers of directed TFs and total TFs (square root transformed for the experiment on femoral secretions and log-transformed for the experiment on plugs) were normally distributed (Shapiro–Wilk test $W>0.98$, $P>0.20$ in all cases). Variances were not significantly heterogeneous between treatments (Hartley's test $F_{\max}>2.0$, $df=2$, $P>0.10$ in all cases). Directed TFs and total TFs were analyzed using parametric statistics (repeated measures ANOVA and Newman–Keuls a posteriori multiple comparison tests).

To examine whether males discriminated the dominance status of other males (or the morphological attributes associated with dominance status) based on chemical cues arising from the femoral secretions and plugs, we studied TF behavior toward swabs bearing femoral secretions and plug products of other males according to the dominance status relationship between test and donor males. We developed GLMs of total TFs (transformed as above) based on the difference in dominance scores and in the morphological attributes (all those measured) between test and donor males. We included a categorical variable with two states that described whether the test male had contact with (for up to 5 min) the donor male in previous staged encounters or with the donor male's femoral secretions before the experiment on discrimination of plugs. GLMs were developed using the same procedures as above.

Results

Male dominance status

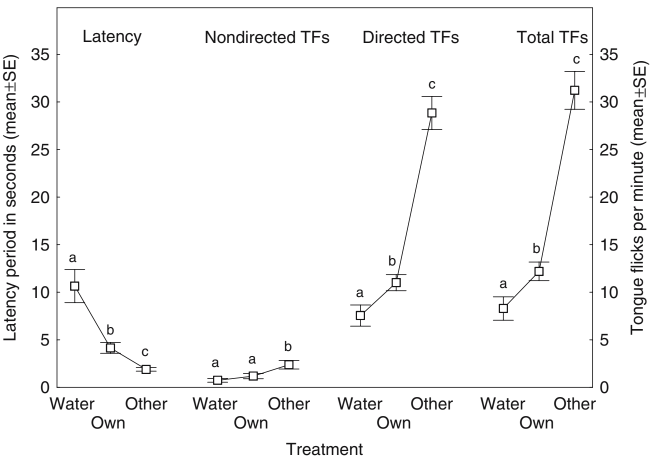
In 15 (27%) of the 56 staged encounters, chases or combats between male pairs did not occur. Attacks, followed by chases or combat, occurred in the remaining 41 (73%) encounters. In 27 encounters, the winning male attacked and chased its opponent, which immediately ran away. In 14 encounters, males engaged in combat. In ten combats, one male won and four combats resulted in a tie (both males retreated in two cases and combat lasted for more than 5 min in two cases). Attacks were frequently (78%, 32 of 41 cases) preceded by threat displays in which males approached their opponents while raised on four legs with the body compressed laterally and the throat inflated. In combat, males inflicted bites to the opponents' heads and attempted to turn them over.

Results from GLM models (final model with head width, head height, and body mass as independent continuous variables: adjusted $R^2=0.37$, $F_{3,24}=6.23$, $P=0.003$) show that male dominance score correlated positively with the average difference between a male's head width and that of its four opponents ($F_{1,24}=6.24$, $P=0.020$, Coeff.=0.95), and with the average difference between a male's body mass and that of its four opponents ($F_{1,24}=6.62$, $P=0.017$, Coeff.=0.55). Male dominance score did not correlate significantly with any of the other morphological variables (head height $F_{1,24}=3.46$, $P=0.075$, Coeff.=−1.15; $P>0.10$ for the remaining variables).

Discrimination of femoral secretions

Males discriminated their own femoral secretions from those of other males (Fig. 1a). TF behavior was significantly different among chemical stimuli as shown by differences in latency period ($\chi^2_2 = 34.4$, $P<0.001$) and in numbers of nondirected TFs ($\chi^2_2 = 12.9$, $P<0.002$), directed TFs ($F_{2,54}=100.5$, $P<0.001$) and total TFs ($F_{2,54}=99.0$, $P<0.001$). Latency period, directed TFs, and total TFs differed significantly between the two stimuli consisting of femoral secretions and the water control (a posteriori tests $P<0.02$ in all cases), confirming that TF behavior toward swabs bearing femoral secretions differed from baseline TF rates. When males were presented with

a Femoral secretions



b Copulatory plug

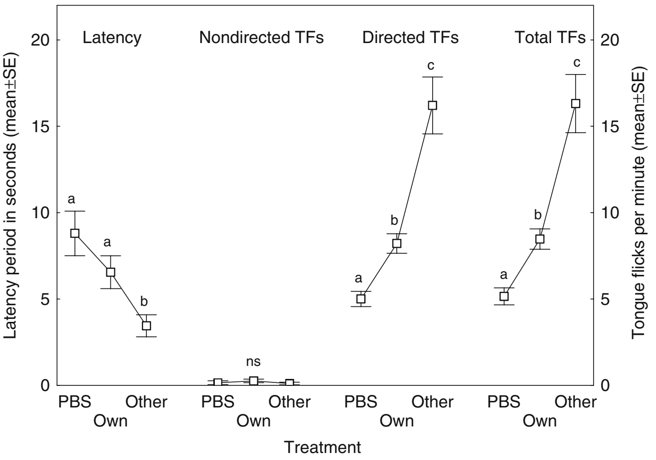


Fig. 1 Latency to first directed TF and numbers of nondirected, directed, and total TFs elicited per minute by male *Lacerta monticola* in response to chemical stimuli consisting of **a** cotton swabs bearing deionized water (*water*), the lizard's own femoral gland secretions (*own*), and the femoral secretions of another male (*other*) and **b** cotton swabs bearing PBS solution (*PBS*), the lizard's own copulatory plug products (*own*), and the plug products of another male (*other*). Different *letters* indicate statistically significant differences ($P<0.02$ in all cases) between treatments as determined by a posteriori multiple comparison tests. *ns* indicates statistically nonsignificant differences ($P>0.05$) among treatments

femoral secretions of other males instead of their own, latency period was significantly lower and numbers of nondirected, directed, and total TFs were significantly higher (a posteriori tests $P<0.02$ in all cases).

Males discriminated morphological attributes of other males that were associated with dominance status based on chemical cues arising from femoral secretions. Because males increased numbers of nondirected and directed TFs when presented with femoral secretions of other males, we used total TFs to address chemical discrimination of other males' dominance status. Results from GLM models (final model with male dominance score, head width and body mass as continuous independent variables and previous contact between test and donor males as a categorical variable: adjusted $R^2=0.27$, $F_{4,23}=3.43$, $P=0.02$) show that total TFs toward swabs bearing femoral secretions of other males increased, although results were not statistically significant, with the difference between the test male's dominance score and that of the secretions donor male ($F_{1,23}=3.51$, $P=0.074$, Coeff.=0.20). However, total TFs toward swabs bearing secretions of other males correlated with body mass ($F_{1,23}=5.85$, $P=0.024$, Coeff.=0.36) and head width ($F_{1,23}=9.64$, $P=0.005$, Coeff.=−1.11) differences between test and donor males. These two variables were themselves associated (see above) with male dominance score. Total TFs did not correlate significantly with any of the other morphological variables ($P>0.10$ in all cases) nor did it differ significantly according to whether the test male had contact with the donor male in previous staged encounters ($F_{1,23}=3.14$, $P=0.090$, $\text{Coeff.}_{\text{without previous contact}}=-0.29$, $\text{Coeff.}_{\text{with previous contact}}=0.29$).

Discrimination of copulatory plugs

Males discriminated their own copulatory plug products from those of other males (Fig. 1b). There were significant differences among chemical stimuli in latency period ($\chi^2_2 = 15.1$, $P<0.001$) and in numbers of directed TFs ($F_{2,38}=82.0$, $P<0.001$) and total TFs ($F_{2,38}=74.8$, $P<0.001$), but not in nondirected TFs ($\chi^2_2 = 2.3$, $P=0.32$). Latency period did not differ significantly between males presented with their own plug products and the control (a posteriori tests $P>0.05$). Directed and total TFs differed significantly between the two stimuli consisting of plug products and the control (a posteriori tests $P<0.001$ in both cases). When males were presented with plug products of other males instead of their own, latency period was significantly lower and directed and total TFs were significantly higher (a posteriori tests $P<0.02$ in all cases).

Males discriminated the dominance status of other males based on chemical cues arising from plugs. Results from GLM models of total TFs (final model with male dominance score, number and FA of the femoral pores as continuous independent variables: adjusted $R^2=0.42$, $F_{3,16}=5.53$, $P<0.01$) show that total TFs toward swabs bearing plug products of other males correlated positively with the difference between the test male's dominance score and that of the plug donor male ($F_{1,16}=8.56$, $P=0.010$, Coeff.=0.06). Total TFs toward swabs bearing plug products of other males also correlated with the difference in FA of the femoral pores between test and donor males ($F_{1,16}=12.71$, $P=0.003$, Coeff.=−0.08). Total TFs did not correlate significantly with any of the other morphological variables nor did it differ significantly according to whether the test male had contact with the donor male in previous staged encounters or with the donor male's femoral secretions in the previous experiment on discrimination of femoral secretions (number of femoral pores $F_{1,16}=3.39$, $P=0.084$, Coeff.=−0.02; $P>0.10$ for the remaining variables).

Discussion

Results suggest that *L. monticola* femoral gland secretions and copulatory plugs bear chemicals that convey information about male identity and dominance status. Males discriminated their own femoral secretions from those of other males, as found in *L. cyreni* (Aragón et al. 2001b) and several other lizards (e.g., Alberts 1992b; Alberts and Werner 1993). TFs toward femoral secretions of other males increased, although results were not statistically significant, with the difference in dominance scores between test and donor males. However, TF behavior varied significantly with the difference between test and secretion donor males in head width and body mass, and these variables were themselves indicative of dominance status. These findings are consistent with the hypothesis that femoral secretions may signal chemically male dominance status in lizards (Alberts et al. 1992; López et al. 2002). Results also support the hypothesis that copulatory plugs play a role in intraspecific chemosensory communication (Volsøe 1944 cited in Fox 1977). Males discriminated their own plug products from those of other males and TF behavior toward plug products of other males differed according to the dominance status relationship between test and plug donor males. While lizard femoral secretions may allow scent-marking of territories (Alberts 1992a), we suggest that both copulatory plugs and femoral secretions may allow males to scent-mark the female body.

Wider heads and higher body masses were associated with dominance status as reported for other reptiles (see Olsson and Madsen 1998 for review). These traits may visually signal male fighting ability in *L. monticola* as agonistic interactions were frequently preceded by threat displays and may be implicated in resolution of combat when escalation occurs. Wider heads (and correlated bite strength; Verwaijen et al. 2002) and higher body masses likely confer an advantage during combat because males bite each other's heads and attempt to turn the opponent over when fighting. However, energetic and survival costs associated with high levels of aggressiveness (Marler and Moore 1988, 1989) often select for behavioral fighting rules and for establishment of social dominance hierarchies in reptiles (Olsson and Madsen 1998; López and Martín 2001). Selective pressures favoring such mechanisms seem to be particularly strong when species occur at high population densities and male encounters are frequent

(Zucker 1994), as is the case with *L. monticola* and *L. cyreni* (Aragón et al. 2001d, 2004; Moreira 2002). Marking of territories with chemical signals of a male's identity and dominance status may aid in the reduction of costs incurred through frequent agonistic encounters (Gosling 1990; Gosling and Roberts 2001).

Femoral secretions that convey information about male identity may be hypothesized to aid in the establishment of *L. monticola* dominance hierarchies through scent-marking of territories as suggested for *L. cyreni* (Aragón et al. 2001a, 2001b, 2001c, 2003). Femoral glands are located on the underside of the hind legs and secretions are deposited on the substrate as lizards move through their home ranges (Alberts 1992a). Male *L. cyreni* discriminated the scent of familiar vs unfamiliar males (Aragón et al. 2001a, 2003) and settled more frequently in areas scent-marked by unfamiliar males (Aragón et al. 2003). Because costs incurred during agonistic encounters seem to be higher between unfamiliar males (Glinski and Krekorian 1985), those studies suggest that male *L. cyreni* recognize chemical signals of other males and assess the eventual costs and benefits expected in aggressive encounters using such signals (Aragón et al. 2001a). Our results suggest that in *L. monticola*, femoral secretions contain chemicals that convey information about male dominance status. Accordingly, it may be hypothesized that male *L. monticola* femoral secretions provide a means of signaling a male's competitive ability through scent-marking of the territory, thereby contributing to the establishment of dominance relationships while minimizing costs of agonistic encounters.

Scent-marking of territories with chemical signals of a male's dominance status is taxonomically widespread and occurs, for instance, in mice (*Mus musculus*; see Gosling and Roberts 2001 for review) and in red-backed salamanders (*Plethodon cinereus*; Mathis 1990; Wise et al. 2004). In mice, the urine's (used for scent-marking of territories) volatile compounds are androgen-dependent, differ qualitatively between dominant and subordinate males, and explain differential behavioral responses of dominant and subordinate males toward scent-marks of other males (Gosling and Roberts 2001). Studies in green iguanas suggest that androgen levels may provide a link between male dominance status and correlated chemical signals in lizards (Alberts et al. 1992). However, mechanisms allowing establishment of male dominance hierarchies in animals that scent-mark territories with signals of dominance status are diverse (Gosling and Roberts 2001) and remain to be tested in *L. monticola*.

L. monticola copulatory plugs may allow males to scent-mark females during copulation because plugs remain in the female cloaca for an average of 8 daytime hours (range 1–20 h; Moreira and Birkhead 2003). It is also plausible that males scent-mark females using femoral secretions. Males often climb on top of females during mate guarding events, and during copulation male femoral pores come in direct contact with the female body. Male scent-marking of the female body is documented in mammals (e.g., Taylor et al. 1984; Gosling 1990) and may explain higher male aggressiveness toward polyandrous than monogamous females in red-backed salamanders (polyandrous females "wear" the pheromone of their partner plus that of other males; Jaeger et al. 2004). However, to our knowledge, the adaptive function of male scent-marking of the female remains to be experimentally addressed.

Scent-marking of the female may be hypothesized to function as a cue to the signaling male himself, to other males or to females. Chemical cues from plugs may allow a male *L. monticola* to recognize and avoid displacing its own plugs and promote displacing plugs of other males (males displace a plug in the female cloaca, and possibly also sperm, during copulation; Moreira and Birkhead 2003, 2004). Scent-marking of the female could signal a male's reproductive association with a female and function as a mate-guarding mechanism (Gosling 1990), possibly deterring rival males from mating with a female (in the absence of the guarding male) due to costs incurred in eventual aggressive encounters with the guarding male. If females benefit genetically from mating with socially dominant males (Calsbeek and Sinervo 2002), signaling of male dominance status directly on the female body could favor female assessment of male quality and influence post-insemination female sperm choice (Olsson and Madsen 2001; Calsbeek and Sinervo 2002).

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