Responses to Femoral Gland Secretions by Visually Adept Male and Female Collared Lizards

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Abstract

Although visually transmitted social signals are well documented in many diurnal iguanians, including collared lizards, secretory femoral glands also suggest a role of chemical signals in intraspecific communication. We conducted laboratory trials to test the extent to which male and female collared lizards responded by tongue-flicking femoral gland secretions, neutral (water), and odoriferous (cologne) control substances, males distinguished self-secretions from those produced by unfamiliar rival males, and females distinguish secretions from unfamiliar vs. familiar males. Both males and females spent similar amounts of time in four arena quadrants each with a Petri dish treated with one of the four test compounds. Males gave more tongue flicks/trial to secretions produced by unfamiliar rivals and cologne than they tongue-flicked their own secretions and water. By contrast, the number of tongue flicks by females on control substances and familiar and unfamiliar males was similar. Results support the hypothesis that femoral gland secretions function in intrasexual signaling among male collared lizards, perhaps allowing them to distinguish unfamiliar rivals. Females tongue-flicked secretions from familiar and unfamiliar males with similar frequency that was high relative to that of males, suggesting a possible role of secretions in assessment of males. Ours is the first evidence of a signaling role of femoral gland secretions in collared lizards and adds to a growing body of evidence that chemical signaling has evolved in diurnal lizards that also have highly developed visual-based signaling.

Introduction

Intraspecific communication is essential in vertebrate social systems structured by sexual selection (Maynard-Smith & Harper 2003). The signaling modalities that vertebrates use may depend on several factors, including limitations on transmission efficacy, detection sensitivity, and signal content (Alberts et al. 1992). It is common for terrestrial tetrapods to signal involving more than one sensory modality (Searcy & Nowicki 2000; Hews & Martins 2013). For example, although acoustic communication is highly developed in many birds and anurans, signals that transmit visually are also sometimes important in both taxa (Narins et al. 2003; Arkay et al. 2011). The relative importance of signal modality in fishes often depends upon water clarity, with selection for visually transmitted coloration and/or stereotypical postures in clear water species, whereas non-visual signals sometimes evolve in species occupying turbid waters (Endler 1980; Bass & McKibben 2003). Although a few nocturnal lizards vocalize (Tang et al. 2001), most diurnal lizards signal visually, chemically, or using both modalities (López & Martín 2004; Baird 2013a).

The relative importance of visual vs. chemically based social signaling in lizards is often hypothesized to depend upon the extent to which species use each of these sensory modalities to their detect prey (Wigers & Horne 2009). Olfaction is the detection of airborne chemical cues that strike the nasal epithelia...
When air is inspired, whereas vomeronalction involves detection of chemicals transferred by the tongue to the vomeronasal organ (Burghardt 1970; Evans 2003). Lizards having highly developed vomeronasal organs (e.g. Lacertidae, Scincidae, Teiidae, Tropiduridae) often locate their prey by following concentration gradients of chemicals cues from prey (Cooper 2004). Perhaps as a consequence of their well-developed vomeronasal abilities, selection has also promoted highly specialized social signaling based upon chemical secretions in these lizards (Labra et al. 2001; López & Martín 2004). For example, experimental studies have shown that chemical secretions function in species recognition, advertisement of resource holding power to same-sex rivals, courtship signals to potential female mates, recognition of familiar vs. unfamiliar rivals, and even recognition of individuals (Martín & López 2007; Carazo et al. 2008; Font et al. 2012; López & Martín 2012). Chemoreception may be less well developed in iguanian lizards that employ their highly developed vision to detect approaching prey while scanning from elevated perches that are then captured using short chases (Evans 2003; Wilgers & Horne 2009). In many iguanians, selection has favored social signals involving conspicuous coloration and stereotypical displays that are transmitted visually (Shine 1990; Baird et al. 2012), perhaps as a consequence of selection for the acute vision required for sit-and-wait foraging tactics.

Although the relative importance of vomeronalction vs. vision in social signaling correlates with foraging mode in some lizards, this may be a misleading consequence of experimental approaches that have tended to focus on only one mode based upon traditional views of the importance of the two signaling modes. Other results suggest that this paradigm is too simple. Use of both visual and chemical cues has been demonstrated in several species (Alberts 1993; Hews & Martins 2013). Dual modality signaling seems likely in others where chemical signals are well established, but pronounced sexual dichromatism that is often associated with visual signaling is also well developed (Pérez I de Lanuza et al. 2013).

Collared lizards (Crotaphytidae) are a good example of the biased evaluation of social signaling modalities based on foraging mode and cues used to detect prey. Eastern collared lizards use their highly developed vision to detect arthropod prey which they ambush from elevated perches (Best & Pfaffenberger 1987). They also use visually transmitted displays to signal conspecifics (Baird 2013b). The possible role of chemical secretions in collared lizard social signaling has received little attention, even though males possess actively secretory femoral glands (Cole 1966), and males in our study population tongue-flick the substrate as frequently as 0.43 times/min (\( \bar{x} + SE = 0.06 + 0.03, N = 16 \)), which may enhance their ability to sample chemical cues that they or rival males have deposited. Males also sometimes drag their hind legs while crawling in a circular pattern (T.A. Baird unpubl. obs.), which may be involved in depositing secretions from the femoral glands. Use of chemical cues in crotaphydid lizards is suggested further because male and female C. collaris responded differently to chemical cues in fecal pellets relative to neutral and odoriferous controls (Wilgers & Horne 2009), and chemical analysis of femoral gland secretions produced by a congener (C. bicintores) revealed compounds that have been implicated in chemical communication in scleroglossan lizards (Martín et al. 2013).

As a first step toward investigating a possible social signaling role of femoral gland secretions in collared lizards, we conducted laboratory trials to investigate the hypotheses that (1) male and female C. collaris distinguish femoral gland secretions from neutral and odoriferous control substances, (2) males differentiate secretions produced by non-familiar rival males from their own secretions, and (3) females distinguish secretions produced by the male whose territory overlapped the home ranges of those females from secretions produced by unfamiliar males occupying distant territories.

**Methods**

**Study Population**

We obtained male and female C. collaris subjects for laboratory trials (described below) and male donors of femoral gland secretions from the population at the Arcadia Lake Dam Spillway (hereafter AL) located on State Hwy 66, 9.6 km east of Edmond, OK. Our laboratory has studied the behavior of the AL population since 1990 (Baird 2013b). At AL, collared lizards occupy four discrete patches of rock and broken concrete that have been used to construct flood-control spillways and drainage ditches. All individuals used in this study were captured as hatchlings, given a unique toe-clip for permanent identification, and uniquely colored paint dots (non-toxic acrylic) were applied to the dorsal surface for identification from a distance. From subsequent recapture of marked lizards, we know the ages, as well as the social/spatial histories of all individuals in this population (Baird 2013b).
Experimental Protocol

Laboratory trials involved adult lizards for which the social and spatial behavior had been determined for other on-going studies. Trials were conducted in 2010 and 2012–2013 during the reproductive season (May and June) when females produce up to three successive clutches of eggs, and males advertise and defend breeding territories (Baird 2013b). Experiments were conducted in an isolated room at the University of Central Oklahoma Recreation Facility at Arcadia Lake (State Hwy 66, 0.5 km west of the field site). We converted a portion of this facility into a temporary laboratory space by setting up a wooden experimental arena (117 × 78 × 40 cm, L × W × H) behind a visual blind of opaque plastic sheeting. The arena was painted (white non-toxic acrylic) to seal the wood making it easy to wash, equipped with a wire mesh lid, a substrate of indoor–outdoor carpeting, and two incandescent lamps (60 W) set using timers to mimic the natural photoperiod and to maintain ambient temperature in a range that promotes collared lizard activity (Baird et al. 2001). A window at the front of the arena allowed us to videotape lizard behavior from behind the blind. The arena and the carpet substrate were thoroughly cleaned between trials using water and 70% ETOH, and allowed to dry.

Subject lizards were captured by noose, transported to the laboratory, and allowed to acclimate to the arena for 24 h prior to trials. No more than 2 h before trial onset, lizards from which femoral gland secretions were collected in the field (hereafter donors) were captured by noose, and secretions were collected by applying gentle pressure to both sides of femoral pores (5–8) on one leg causing extrusion of one pellet/pore, which we transferred to a capped centrifuge tube using clean forceps. Secretions were held on ice until used in trials. The test arena was subdivided into four equally sized quadrants (58.5 × 39 cm, L × W). One of four treatment substances (described below) was used to coat a 1-cm² surface on the center of inverted Petri dishes (9 cm diameter, also cleaned between trials), placed in the center of each quadrant. We used 2–3 pellets for both of the femoral secretion treatments, and one drop for the cologne and water treatments. The positions of the four substances were randomized for all trials.

For trials using male subjects (n = 31), we tested femoral gland secretion collected from those same subject males, secretions collected from an unfamiliar rival territorial male, a novel odoriferous control (cologne; undiluted ‘Swiss Army for Men’), and a neutral control (distilled water). For trials using female subjects (n = 20), in addition to cologne and water controls, we tested secretions from the male that overlapped the female’s home range and that we had previously observed engaged in courtship behavior, and secretions from a territorial male located distant (defined below) from that female. Each male and female was used only once as test subjects. A few (15%) of the same non-familiar secretion donors were used in trials for two different subject males, but new secretions were collected from repeat donors from the opposite leg, and these males were not used as repeat donors for at least 7 d following their first donation. Most non-familiar male donors occupied territories or home ranges on a habitat patch that were separated from the patch of the test lizard by an expanse (≥70 m) of grass, plus additional separation across contiguous rock habitat. For the few lizards that lived on the same rock patch, their territories were ≥80 m away from the closest boundaries of the home ranges (females) or territories (males) of subject lizards.

One hour before trials, we placed subject lizards underneath an inverted opaque plastic pot located in the center of the arena, equidistant from the four Petri dishes. Immediately before trials, chemical substances were applied to the Petri dishes, and subject lizards were released by remotely lifting the pot using an attached line and pulley. Even though test subjects probably transferred their scent to the arena during the 24-h habituation period, our protocol controlled for this possibility because we only recorded tongue flicks directly on Petri dishes. Our protocol also prevented contamination of the dishes prior to trials because they were not placed in the arena until immediately prior to the onset of trials when subject lizards were restrained. We then videotaped the behavior of test lizards. We recorded the behavior of subject males for 2 h. Because male trials conducted mostly in 2010 and 2012 revealed far more activity during the first hour of trials, we shortened trial duration for female tests to 1 h. Behavior was recorded between 1300 and 1530 h when arena substrate temperature ranged from 32 to 38°C, which is optimal for collared lizard activity (Baird et al. 2001). From videotapes, we quantified the total time that subject lizards spent in each quadrant to ascertain that they had equal opportunity to react to all four chemical treatments, and the number of tongue flicks on petri dishes as our measurement of the extent to which lizards inspected the four chemicals.

All procedures were approved by the University of Central Oklahoma Institutional Animal Care and Use Committee (permit # 130008), and the Oklahoma...
Department of Wildlife (permit # 5553). Experiments required keeping lizards in captivity for 30 h maximum, after which they were returned to the precise capture location. Lizards were in good health when returned to their home ranges, and subsequent monitoring for the balance of each activity season confirmed no ill effects on test subjects or secretion donors.

Statistical Analyses

Data were analyzed using the Program R version 3.01 (R Development Core Team 2013). Separately for experiments with males and females, we used generalized linear mixed models (Bates et al. 2014) (GLMM using a repeated-measures design) using lizard identity as the random effect to compare the percentage of time that test subjects spent in each of the quadrants, and the number of tongue flicks on each petri dish treated with different chemicals. Because the time spent in quadrants is a continuous variable, we used Gaussian error structure and identity-link function, whereas we used Poisson error structure and log-link function for the discrete variable the number of tongue flicks on dishes per trial. We used the package ‘compute.es’ to calculate standardized (\( \bar{x} = 0 \), standard deviation = 1) effect sizes (Cohen’s d) and associated 95% confidence intervals (CI) for all models (Del Re 2010). Using these analyses, statistical significance is indicated when the 95% CI of independent variables did not include zero.

Results

Males spent similar amounts of time in the four arena quadrants (GLMM: \( \chi^2_3 = 4.29 \), Cohen’s d = 0.44, 95% CI = -0.30 to 1.18; Table 1). Although the mean time that females spent in the water quadrant was small, it was not statistically lower (GLMM: \( \chi^2_3 = 4.31 \), Cohen’s d = 0.56, 95% CI = -0.40 to 1.51, in all cases CI’s included zero) than time spent in any of the other quadrants (Table 1). There was no significant effect of chemical treatment on the number of tongue flicks per trial by females (GLMM: \( \chi^2_3 = 6.53 \), Cohen’s d = 0.80, 95% CI = -0.18 to 1.78). By contrast, the number of tongue flicks by males was influenced by chemical treatment (GLMM: \( \chi^2_3 = 32.50 \), Cohen’s d = 2.33, 95% CI = 1.38–3.29; Table 1). Males gave fewer tongue flicks on water than on the other three treatments (self-secretions, \( p = 0.02 \), secretions from stranger males, \( p < 0.001 \), cologne, \( p < 0.001 \)). On average, males tongue-flicked cologne 1.6 times more frequently (\( p = 0.03 \)) than their own secretions, whereas they tongue-flicked secretions from unfamiliar rival males 1.4 times more frequently (\( p = 0.03 \)) than their own secretions (Table 1).

Discussion

Similar time spent in the four arena quadrants confirmed that both male and female subjects had opportunities to respond to all chemical treatments. Males performed more tongue flicks on the novel chemical cues (secretions from unfamiliar rivals and cologne) than on familiar (self-secretions) or neutral chemical cues. Moreover, males showed the greatest interest in secretions produced by unfamiliar same-sex rivals, but they also were attracted to the odoriferous, novel cologne control. The relative strong response to cologne is interesting in light of the strong male response to secretions produced by unfamiliar same-sex rivals, but they also were attracted to the odoriferous, novel cologne control. The relative strong response to cologne is interesting in light of the strong male response to secretions produced by novel, unfamiliar males. Use of cologne as an odoriferous control has

Table 1: (A) Responses of male collared lizards to their own femoral gland secretions, secretions from an unfamiliar male, an odoriferous control (cologne), and a neutral control (water). (B) Responses of female collared lizards to femoral gland secretions from a familiar male, a stranger male, an odoriferous control (cologne), and a neutral control (water). Data are means and 1.05E in parentheses.

<table>
<thead>
<tr>
<th>Chemical treatment</th>
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<tbody>
<tr>
<td>Variable</td>
<td>Self secretion</td>
<td>Unfamiliar male secretion</td>
<td>Cologne</td>
<td>Water</td>
</tr>
<tr>
<td>(A) Male trials</td>
<td></td>
<td></td>
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<tr>
<td>Time in quadrant (min)</td>
<td>31.7 (5.7)</td>
<td>25.8 (3.8)</td>
<td>23.9 (5.0)</td>
<td>38.0 (5.8)</td>
</tr>
<tr>
<td>Tongue flicks/trial</td>
<td>1.2 (0.3)</td>
<td>1.7 (0.4)</td>
<td>1.9 (0.6)</td>
<td>0.5 (0.2)</td>
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<td>Variable</td>
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<td>(B) Female trials</td>
<td></td>
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<tr>
<td>Time in quadrant (min)</td>
<td>12.3 (4.3)</td>
<td>19.8 (5.4)</td>
<td>23.4 (5.5)</td>
<td>5.9 (2.0)</td>
</tr>
<tr>
<td>Tongue flicks/trial</td>
<td>1.0 (0.4)</td>
<td>1.2 (0.6)</td>
<td>1.9 (0.5)</td>
<td>1.2 (0.6)</td>
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become standard protocol in studies of reptilian chemical communication. Femoral gland secretions produced by a congener, Crotaphytus bicinctores (Martín et al. 2013), contained several alcohol-based compounds, which may be similar to those in cologne. Although most reptilian studies report low responses to cologne (Font & Desfilis 2002), test subjects responded positively in some studies (López & Salvador 1992; López & Martín 1994). If alcohols found in cologne mimic femoral secretions in some ways, perfume may not be biologically irrelevant, even though it is novel.

Even though visual signaling is highly developed in collared lizards (Fox & Baird 1992; Baird 2013b), especially in males, chemically based communication among males may also be adaptive for advertisement of territory boundaries. Territory areas in the AL population are large (1000–1300 m², 60–90 m between borders). High rates of travel are required to patrol and advertise boundaries of such large territories to as many as 12 same-sex rivals that occupy neighboring territories and home ranges (Baird 2013b). Male rivals include not only neighboring territory owners (usually 2), but also several sexually mature males that use stealthy social tactics to avoid attacks by territory owners (York et al. 2014). Non-territorial males are under no pressure to defend particular areas, and the almost unlimited hiding places beneath the boulders at AL promote their ability to avoid attacks by territory owners without leaving areas near to females. As a consequence, non-territorial males sire similar numbers of offspring using stealthy tactics as males that defend territories that overlap females which they court frequently and repetitively (York et al. 2014; York and Baird 2015).

Dual modality signaling may be selected in males when habitat conditions, such as those at the AL site, make it costly for individual males to defend particular areas from large numbers of reproductive competitors. Because chemical secretions may continue to signal rivals even after patrolling males have moved, femoral gland secretions may function to advertise territory boundaries without diminishing the ability of males to advertise visually on different portions of their territories. The frequency of tongue flicking by males that were actively patrolling their large territories was much higher than rates observed in the laboratory. If secretions from several rival males have been deposited on surface rocks throughout the habitat, frequent tongue flicking of the substrate while patrolling is expected if territory owners use chemical cues to detect and evaluate intruders. Elevated rates of male tongue flicking on secretions from unfamiliar rivals may suggest that collared lizards use chemical cues, in addition to visual cues (Fox & Baird 1992; Husak & Fox 2003), to recognize classes of intruders as a mechanism that reduces the costs of defense against established neighbors. The dear-enemy phenomenon based on chemical cues has been demonstrated in several lizards, including some iguanians (Hanley et al. 1999; Bull et al. 2000; Aragón et al. 2001).

The total frequency of tongue flicks by females was similar to that of males even though trials were only one half as long. Nonetheless, tongue flick frequency by females did not differ based on chemical treatment, most especially the familiarity of male donors. One possible explanation of this result is that we did not fully detect female responses to male secretions because we only recorded tongue-flicking responses. Wilgers & Horne (2009) reported that direct presentation of fecal material on cotton swabs prompted gular pumping in female C. collaris. Because we did not observe gular pumping on the videotaped records of lizards that were allowed to voluntarily approach chemical sources nor have we observed this behavior in free-ranging lizards, this explanation of our results seems unlikely.

Courtship behavior in our population involves one male and one female engaging in prolonged physical contact (e.g. mounting, nudging, superimposition of the limbs and tail; described in Baird 2013b). Therefore, another possible explanation of similar female responses to secretions by familiar and unfamiliar males is that visual and tactile cues are more important than chemical cues in intersexual signaling. This explanation is not consistent with our observation that females tongue-flicked secretions from both familiar and unfamiliar males frequently. Instead, this response may suggest that female C. collaris use chemical cues to assess, and perhaps choose their mates. Several studies on lizards have suggested a role of male chemical cues in intersexual communication and female choice of mates (López & Martín 2004, 2012). This hypothesis is particularly intriguing for the collared lizards in our population. Even though males that control territories court females much more frequently than the stealthy non-territorial males on our site, females mated with multiple males employing both social tactics, such that territorial and non-territorial males sired similar numbers of offspring (York et al. 2014; York and Baird 2015). The polygynandrous social structure at AL may be a consequence of the interconnected labyrinth of crevices running beneath surface rock surface occupied by lizards in our population (York et al. 2014). These
crevices probably make it possible for females to secrete- 
vively interact with males independent of their social status. Because chemical cues can function at close range, and their detection is not limited by low light levels in crevices, it is entirely possible that femoral gland secretions of males play a role in establishing mating relationships between male and female collared lizards.

Acknowledgements

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