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Spontaneous discrimination of urine odours in wild African lions (*Panthera leo*)

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Olfactory communication is the primary mode of communication for many mammals, yet research on this form of signalling is still largely descriptive in most species. Thus, despite the apparent importance of scent-marking in the social lives of wild felids, experimental studies directly investigating the function of olfactory communication are lacking. We conducted scent presentation experiments to investigate whether wild African lions can discriminate another lion's social group and sex from a sample of its urine. Our results indicated that lion urine has the potential to signal depositor sex and social group, and that lions can use urine to discriminate males from females and residents from non-residents. The response of lions to urine was also dependent on both the sex and age of the subject receiving the presentation. Female lions responded less frequently to urine from resident females than to either non-resident females or resident males. Males responded more strongly to urine from resident males than resident females, but did not appear to differentiate urine from non-resident and resident females. Observations of flehmen and further scent-marking responses from lions provide additional evidence that lion urine functions in scent-marking. These results establish that urine scent-marks contain sufficient information for receivers to discriminate the sex and social affiliation of the signaller, and are the first to experimentally demonstrate the functional relevance of scent-marking in African lions.

Keywords: Felids; Group discrimination; Mammals; Olfactory communication; Scent-marking; Scent presentation; Sex discrimination.
Olfactory communication is the primary means of communication among many mammals (Brown and MacDonald, 1985). Semiochemicals in urine, faeces and glandular secretions play a pivotal role in mediating social and sexual interactions (Bradbury and Vehrencamp, 1998; Wyatt, 2003; Brennan and Kendrick, 2006; Apps, 2013). The ability to assess the sex and residence status of conspecifics through scent-marks may be particularly adaptive for wide-ranging territorial species that are under intense selection pressure to deter territorial intruders and locate mating partners (Bradbury and Vehrencamp, 1998). These selection pressures should apply in many felid species (Kleiman and Eisenberg, 1973), but little is known about the function of felid scent-marking in the wild (Soso, Koziel, Johnson, Lee and Fairbanks, 2014; Vogt, Zimmermann, Kölliker and Breitenmoser, 2014; Allen, Wallace and Wilmers, 2015; Vogt, Boos, Breitenmoser and Kölliker, 2016).

It is thought that felids make extensive use of olfactory communication (Kleiman and Eisenberg, 1973). Cats have numerous specialised scent glands and also use urine for scent-marking (Brown and Macdonald, 1985; Asa, 1993; Mellen, 1993; Pageat and Gaultier, 2003; Vogt et al., 2016). Both male and female cats either spray urine backwards onto vegetation, or rake their feet through urine on soil (Eaton, 1970; Kleiman and Eisenberg 1973; Verberne and De Boer, 1976). Although olfactory communication is apparently important in felid social systems, research results are scarce because cats are elusive and nocturnal (Vogt et al., 2014). Whilst detailed data on marking behaviour is accumulating for wild felids (e.g. Vogt et al., 2014; Allen, Whittmer and Wilmers, 2014; Allen et al., 2015; Allen, Yovovich and Wilmers, 2016), experimental investigations of the response of felids to potential scent-marks have been limited to the puma (Puma concolor: Allen et al., 2014). To understand the functions of scent-marking, we need to consider the information content of olfactory signals and investigate the responses of individuals to scent-marks. Previous behavioural (Schaller, 1972; Asa, 1993; Brahmachary and Singh, 2000; Lehmann, Funston, Owen and Slotow,
chemical (Andersen and Vulpius, 1999; McLean, Hurst, Gaskell, Lewis and Beynon, 2007; 
Umapathy et al., 2007; Poddar-Sarkar, Chakroborty, Bhar and Brahmachary, 2008; 
Umapathy, Kumar, Kabra and Shivaji, 2013) work has suggested that scent-marking plays an 
important role in the social and sexual behaviour of lions (*Panthera leo*). For example, 
chemical analysis indicates the potential for lion urine to signal individual identity and sex 
(Andersen and Vulpius, 1999). We therefore conducted scent presentation experiments to 
investigate whether wild lions can discriminate another lion’s sex and social group from a 
sample of its urine.

Lions live in a fission-fusion social system in which group members collectively defend 
territories or access to mates from other same-sex groups (Schaller, 1972; Packer, Scheel and 
Pusey, 1990; Grinnell, Packer and Pusey, 1995). Maintaining territories is essential for 
breeding, with fights between territory rivals often leading to injury and death (Schaller, 
1972; Packer et al., 1990; Heinsohn and Packer, 1995). Lions can detect potential mates and 
territorial threats from the long-distance calls of conspecifics based on caller sex, familiarity 
and group membership (McComb, Pusey, Packer and Grinnell, 1993; McComb, Packer and 
Pusey, 1994; Grinnell et al., 1995). We hypothesised that olfactory communication in lions 
will also extend to sex and social discrimination. We predicted that lions presented with urine 
from resident adult males and females, will be more likely to respond (e.g. sniff, lick or 
overmark the urine), and will spend longer responding to urine from opposite sex 
conspecifics. In this way, lions could assess the reproductive condition of mates (Charlton, 
2014; Tinnesand et al., 2015). We expected that olfactory sex discrimination will be most 
pronounced in adult lions than in sexually immature subadults and cubs. With the ability to 
discriminate between the scents of resident and non-resident individuals, females could detect 
the presence of potential rivals for their territory. We predicted that female lions will be more
likely to respond, and will spend longer responding to urine from non-resident females than from resident females. In contrast, male lions would not show a strong difference in response to resident and non-resident female urine as both signal the presence of a potential mate. Since lions of all ages are at risk during territory take-overs, we hypothesised that there should be no clear age differences in the ability to discriminate the urine of resident from non-resident females.

METHODS:

Study Population and Site:

The study area (ca. 1500 km² 19°31’S, 23°37’E; elevation ca. 950 m) was bordered by the Okavango Delta and included the Moremi Game Reserve and its surrounding Wildlife Management Areas (for further details see: McNutt, 1996). Life histories and demographic data were available on the study population since 2007, and all individuals were habituated to close approaches by vehicles. Lions were located using spoor- or radio-tracking, with up to three individuals in each social group fitted with VHF-GPS radio collars (Royal Veterinary College, U.K; < 1080 g) or VHF radio collars (African Wildlife Tracking, Pretoria, South Africa; < 780 g). Individual lions were identified from their unique whisker-spot patterns, and were divided into demographic categories based on their sex and age, with age categories defined as follows: cubs (below 18 months), subadults (18-48 months), adults (four years and above) (Packer et al., 1988). Where the birth date was not known, the individual was aged using body size, teeth wear, male mane development, and the observation of sexual activity (Whitman and Packer, 2007).

Sixty-eight scent presentation experiments were conducted between May 2014 and December 2015 on 52 free-ranging lions from four female prides and three male coalitions. A pride was defined as a minimum of two sexually mature females that defend a shared territory, and
includes all offspring of pre-dispersal age. A coalition was defined as a group of post-dispersal males who attempt to associate with female prides (Schaller, 1972).

Scent Sample Collection:

Urine-soaked soil deposited with the following five body postures was collected for presentation: 1) Spray: backwards spray onto vegetation and surrounding soil whilst standing (N = 5 samples), 2) Scrape: downwards spray onto soil whilst scraping the urine soaked soil with hind paws (N = 28), 3) Squat: urination onto soil with genitals lowered to soil and no paw scraping (N = 22), 4) Stand: urination onto soil whilst standing with no paw scraping (N = 5), and 5) Lie: urination onto soil whilst lying down (N = 1). In the statistical analyses (see below), we further grouped the body postures of the urinating lions into ‘urine-only’ postures (spray, squat, stand and lie postures) and scrape postures. In contrast to urine-only postures, scrape postures could involve deposits of both urine and secretions from pedal (feet) glands.

Pedal glands are known to deposit important scent-marks for some mammals (white tailed deer (Odocoileus virginianus): Gassett et al., 1996; polar bear (Ursus maritimus): Owen et al., 2015; brown bear (Ursus arctos): Clapham, Nevin, Ramsey and Rosell, 2014), and felids also have inter-digital glands and sweat glands on their feet (Pageat and Gaultier, 2003). It is not known whether lions deposit semiochemicals from pedal glands.

Urine was collected from lions in different social (alone or in a group), sexual (oestrous or non-oestrous), and activity states (resting or mobile). Urine-moistened soil was collected using a clean metal spoon and placed into sterilised glass jars with aluminium foil-lined lids. Following collection, all samples were immediately stored in a 12 V cooler box (to keep contents 15 °C below the ambient temperature), and were later (within a few hours) frozen at -20 °C until thawed for presentation (average number of days frozen before presentation ± SD = 129 ± 112 days; see discussion for the effect of freezing). Sixty-one urine samples from 20 adult lions were used. Non-resident urine (see below) was collected from lions in social
groups with territory directly adjacent to the subjects receiving the presentation (determined through GPS radio-telemetry collars: Gilfillan, n.d).

**Experiment Procedure:**

Before an experiment, a clean metal spoon was used to place approximately 80 g of the frozen sample into a sterilised glass jar (with aluminium foil-lined lid). The sample was then allowed to thaw. A resting group of lions was approached with a vehicle. From the vehicle the urine sample was tipped out of the jar in a single pile on top of short grass or bare soil 7-25 m from the lions, and the vehicle was reversed away. Lions that moved to within 2 m of the sample were videoed with a Bell + Howell DNV16HDZ (North Carolina, U.S.A) digital camcorder. Observations stopped when the lions moved away from the area (Figure 1). Scent presentation experiments were conducted within two hours of sunset, when lions begin to become active. The sample was not re-collected. We put the samples upwind of the lion group as a whole, but for an observer sitting in an open-top vehicle judging whether any individual lion was downwind at any particular time was impossible.

To test whether lions can discriminate another lion's social group and sex from a sample of its urine, we presented three urine treatments from the following donors: 1) Absent adult female residents ("Female Resident"), 2) Absent adult male residents ("Male Resident"), and 3) Adult female non-residents ("Female Non-Resident"). Since the non-resident individual in treatment 3 was never present, urine samples from absent group members were used in Treatments 1 and 2 so that donors were absent in all three treatments. To further standardise the presentations, urine samples were presented to unisex (i.e. all adults were of the same sex) lion groups (two or more adult lions) that were not feeding. Two presentations (out of 68; 2.9%) were made to a nomadic coalition of two males who were attempting to take over a
pride. Only one sample was presented during each trial, but 98.1% of subjects were presented with at least two treatment categories spread over the study period, and 55.8% were presented with all three. Whilst no control treatment was used, the experimental design controlled for the handling, storing and presentation methods by comparing the response of lions to different treatments, whilst also largely controlling for individual differences between lions by using a repeated measures design.

**Ethical Note:**

The collaring of lions and presentation experiments were performed under permits granted by the Botswana Ministry of Environment Wildlife and Tourism (8/36/4 XXV (8)), and the University of Sussex (Non-ASPA 4 – November 2013).

**Behavioural Analysis of Response:**

Video recordings of lion responses (Table 1) were analysed frame-by-frame (frame = 0.033/0.034 s) on a Fujitsu Siemens Amilo Pi2515 (Munich, Germany) laptop using Avidemux 2.6.9 (Mean Development Team, 2015) video analysis software. Distances between each lion and the urine were estimated by sight during the experiment.

**Statistical Analyses:**

All statistical analyses were conducted using the R statistical software (v. 3.3.1; R Core Development Team, 2016). Fisher’s exact tests were used to compare the likelihood that lions of different age or sex would countermark or perform flehmen when responding to the urine. Where multiple comparisons were performed, the Bonferroni correction was applied. To assess what factors determine whether lions responded to urine samples, we ran a series of generalised linear mixed models (GLMMs) with a binomial error distribution (0 = sample
ignored, 1 = sample investigated/countermarked/overmarked) and a logit link function. The
identity of the test subject (N = 52) was included as a random term to account for multiple
data from the same lions. Regarding the fixed effects, the global model was coded as follows:
Respond or not ~ Treatment + Recipient Age + Recipient Sex + Number of Days Frozen +
Time Between Deposition and Collection (mean ± SD = 18.8 ± 18.1 minutes, range = 5-72
minutes) + Donor Posture (urine-only posture = 33 samples; scrape posture = 28 samples) +
Closest Distance to Sample (m, see Table 1) + Treatment * Recipient Sex + Treatment *
Recipient Age. To assist model convergence, the number of days each sample was frozen
before presentation, and the time between deposition and collection were scaled and centered
(using the ‘scale’ function in R) prior to running the models. The fixed effects were not
strongly correlated (measured using the variance inflation factor and the Condition Number
test). The GLMMs were run using the ‘lme4’ package (Bates, Maechler, Bolker and Walker,
2015), and the dredge function in the ‘MuMIn’ package (Barton, 2016) was used to create a
list of candidate models from the global model. We used Akaike’s information criterion with
a correction for small sample sizes (AICc) for model selection, where lower AICc values
corresponded with better support for a given model. Akaike weights were calculated to show
the relative importance of each candidate model (Akaike, 1974). Conditional model
averaging (MuMIn package) was performed on the models within 2 AICc of the optimal
model (lowest AICc) to extract averaged parameter estimates and their 95% confidence
intervals (Symonds and Moussalli, 2011). Tukey’s HSD post-hoc tests were run on the
optimal model using the ‘multcomp’ package (Hothorn, Bretz and Westfall, 2008).
For those individuals that responded to the urine, we ran a series of generalised linear mixed
models (GLMMs) with a Gaussian error distribution to assess what factors determine the
duration of response (seconds). The cube-root transformation was applied to the response
duration to correct for non-normality in the response variable. Regarding the fixed effects, the
global model was coded as follows: Treatment + Recipient Age + Recipient Sex + Number of Days Frozen + Time Between Deposition and Collection + Donor Posture + Treatment * Recipient Age + Treatment * Recipient Sex. The fixed effects were not strongly correlated. We incorporated the identity of the test subject ($N = 36$), the sample number ($N = 31$), and the recipient’s social group ($N = 7$) as random terms to account for multiple data from the same lions, when presented with the same sample of urine. As above, model averaging was performed on candidate models using AICc. Tukey’s HSD post-hoc tests were run using the ‘multcomp’ package on the optimal model.

**RESULTS:**

Thirty-six lions (out of 52 lions; 69.2%) responded to at least one urine sample. The average ($\pm$ SD) distance from which lions first appeared to detect the urine (as evidenced by changing the position of the head in the direction of the sample location) was $2 \text{ m} \pm 4 \text{ m}$ (range: 0-20 m, mode: 1 m), but since this can be difficult to measure, lions were scored as investigating the sample only when within 0.5 m of the sample (consistent with Jordan, Golabek, Apps, Gilfillan and McNutt 2013; Allen et al., 2014; Allen et al., 2015). A typical response (93.1% of responses) involved sniffing the sample, with flehmen following sniffing in 59.7% of cases. The average ($\pm$ SD) duration of response was $35.3 \pm 34.1$ seconds. Overmarking was never observed, whilst countermarking was observed nine (12.5%) times (resident female urine = 4, non-resident female urine = 4, and resident male urine = 1). Countermarking involved defecation twice (22.2% of countermarks) and urination seven times (77.8% of countermarks), all within 2 m of the urine (Table 2). The average ($\pm$ SD) time it took the urine to elicit a response from a lion was $27 \pm 20$ minutes. The average ($\pm$ SD) time that individual lions ignored the urine (irrespective of whether other group members responded) was $66 \pm 18$ minutes.
When responding to urine, males and females were as likely to flehmen (Two-tailed Fisher’s exact test: $P = 0.106$), and to countermark the sample (Two-tailed Fisher’s exact test: $P = 0.999$). Adults were significantly more likely to countermark the urine than either subadults (Two-tailed Fisher’s exact test with Bonferroni correction of alpha: $P < 0.001$), or cubs (Two-tailed Fisher’s exact test with Bonferroni correction of alpha: $P < 0.001$). However, there was no significant age-class difference in the likelihood of performing flehmen after investigating the urine (Two-tailed Fisher’s exact test: $P = 0.950$).

The optimal model of the likelihood of lions responding to the urine included the closest distance the lion moved to the sample, the donor posture, the age of the recipient, the sex of the recipient, the urine treatment, and the interaction between recipient sex and urine treatment. Model averaging of the best GLMMs revealed that the closest distance the lion moved to the sample, the donor posture, the age of the recipient, and the interaction between recipient sex and urine treatment are the best predictors of whether or not test subjects responded (Table 3). Post-hoc tests revealed that females were significantly less likely to respond to resident female urine than either resident male urine ($P = 0.008$; Figure 2; Table 4), or non-resident female urine ($P = 0.039$; Table A2 in Appendix). The likelihood of males responding was independent of both the donor’s sex ($P = 0.869$) and social group ($P = 0.409$). Adults were as likely to respond as subadults ($P = 0.150$) or cubs ($P = 0.729$), but subadults were significantly more likely to respond than cubs ($P = 0.027$; Table A3 in Appendix). Lions were more likely to respond to urine deposited with a scrape posture (45 out of 152 trials; 29.6%) compared to a urine-only posture (27 out of 167 trials; 16.2%).

Finally, the time between urine deposition and collection, and the number of days the urine was frozen before presentation were weak predictors of response likelihood (relative importance < 0.5; as in Dala-Corte, Becker and Melo, 2016): lions were more likely to respond to urine that had been collected more quickly after deposition, and were less likely to
respond to urine that had been frozen for longer.

Regarding the duration of response of lions to the urine, the optimal model included the sex of the recipient, the urine treatment, and the interaction between recipient sex and urine treatment. Model averaging on the best GLMMs revealed that the urine treatment, the sex of the recipient, and the interaction between recipient sex and urine treatment are the best predictors of response duration (Table 5). Post-hoc tests revealed that males responded for longer to urine from resident males than from resident females ($P = 0.017$; Figure 3; Table 4), but had similar response durations to urine from resident and non-resident females ($P = 0.995$; Table A5 in Appendix). The response duration of females was independent of both the donor’s sex ($P = 0.994$), and social group ($P = 0.844$). Time between urine deposition and collection, and the number of days the urine was frozen before presentation were weak predictors of response duration: lions responded for longer to urine that was collected more quickly after deposition, and to urine that had been frozen for longer.

**DISCUSSION:**

Our scent presentation experiment indicated that lion urine has the potential to signal depositor sex and social group, and that lions can use urine to discriminate males from females and residents from non-residents. The response of lions to the urine also depended on both the sex and age of the subject receiving the presentation. These results provide evidence that lions perceive certain urine deposits as scent-marks with a communicatory significance.

Female lions were more likely to respond to resident male urine than to resident female urine. Females cooperate to defend cubs and territory and often hunt together (Packer et al., 1990). In contrast, male lions typically take food from females (Schaller, 1972) and consequently a high level of aggression can be seen between females and resident males (pers. obs.). It may be adaptive for females to use olfactory cues such as urine to monitor the movement and...
presence of males, potentially allowing them to avoid moving through and hunting in areas
with signs of male presence. Infanticidal non-resident males also pose a significant threat to
females and their cubs (Pusey and Packer, 1994), and it may be adaptive for females to
respond to male urine in order to establish the residency status (and thus risk of infanticide)
of the urine donor. We were unable to test this hypothesis directly, but we predict that female
lions will show elevated responses towards non-resident male urine compared to resident
male urine.

We had hypothesised that male lions would show stronger responses towards resident female
urine, but males responded more strongly to resident male urine. These results could reflect
the fact that we had insufficient data to test for a three-way effect of recipient age, recipient
sex and urine treatment. It seems likely that adult and sexually immature (subadults and cubs)
males will respond differently to urine. Male lions disperse at sexual maturity (Schaller,
1972). Adult males are aggressive towards related juvenile males (pers. obs.), and unrelated
adult males pose a significant infanticidal threat to juveniles. Consequently, juvenile males
could be expected to show strong responses to male urine.

We would still expect adult males to show stronger responses to female urine than resident
male urine. Competition between adult males for reproductive opportunities is intense (West
and Packer, 2002). Males are attracted to the urine of oestrous females in some mammals
(e.g. Swaisgood, Lindburg, and Zhang, 2002; Charlton, 2014). We were unable to account for
the oestrous state of female urine donors in our experiment, and urine from anoestrous females
could be less interesting to males (Charlton, 2014). Keeping track of male companions
(potentially through scent-marking) is also likely to be a key aspect of coalition formation
and function, enabling males to cement and maintain social bonds with their coalition
partners. Coalition cohesiveness would likely influence male tenure in prides and thus
ultimately reproductive success.
As predicted, female lions, but not males, were more likely to respond to non-resident female urine than to resident female urine. For resident female lions, rival females (and their prides) pose a significant territorial threat (Schaller, 1972; Packer et al., 1990). Since mammalian scent-marking often functions for territory demarcation (Roberts and Gosling, 2001, Christensen, Kern, Bennitt and Radford, 2016), it would be adaptive for females to pay attention to the scent-marks of territory rivals (non-residents) to detect when their territory ownership is being challenged. While we provide evidence that female lions could use urine to determine the residency status of conspecifics, further research would be required to sufficiently test this in males. Non-resident adult male lions compete aggressively with resident males for access to reproductive females (Grinnell et al., 1995), and it may be adaptive for males to use scent-marking to distinguish coalition partners from non-resident males.

Flehmen was consistently exhibited by all age/sex classes of lion in response to urine. It is notable that flehmen is typically performed by males in response to sexual olfactory stimuli (Rasmussen, Schmidt, Henneous, Groves and Daves, 1982; Hart and Leedy, 1987; Stahlbaum and Houpt, 1989), but we regularly observed flehmen by females and sexually immature lions in response to urine. Flehmen transports non-volatile chemical signals (especially proteins and steroid conjugates) from the oral cavity to the vomeronasal organ for chemosensory analysis (Hart and Leedy, 1987; Igbokwe, 2009), and its occurrence gives further evidence that lion urine functions in scent-marking.

Lions of all ages had a similar pattern of response to the urine, but subadults were more likely to respond than cubs. Whilst little is known about the development of scent-marking in carnivores, scent-mark investigation and overmarking often increase with age, and adults scent-mark the most (Ferkin, 2010; Clapham et al., 2014, Vogt et al., 2014; Allen et al.,
Subadult lions are approaching sexual maturity and male subadults disperse to locate mating partners (Schaller, 1972). Perhaps subadult lions pay particular attention to the olfactory signals of conspecifics to begin acquiring information regarding mating opportunities, and to assess the risk of intra-sexual competition (White, Swaisgood and Zhang, 2002; Clapham et al., 2014). However, only adult lions were observed to countermark the thawed urine ($N = 9$). Countermarking establishes that both the urine and the countermark are scent-marks (Jordan et al., 2013).

Lions were more likely to respond to urine deposited with a scrape body posture compared to a urine-only posture. Urine deposited in combination with scraping could elicit a greater likelihood of response because it contains semiochemicals from pedal gland secretions as well as urine (Gassett et al., 1996; Clapham et al., 2014; Owen et al., 2015). The mixing of chemical signals from multiple sources may increase the information content of deposits, and increase the response likelihood of conspecifics (Greene et al., 2016). Further research would be required to test this hypothesis, but lions still investigate and overmark pedal gland secretions in the absence of urine (Gilfillan, Golabek, Vitale, McNutt and McComb, n.d).

Finally, our results provide valuable methodological insights that might aid future scent presentation experiments. Lions were less likely to respond, and responded for shorter durations to urine that was exposed to the environment for longer before collection. While the time between urine deposition and collection was a weak predictor of how lions responded, our results suggest that some semiochemicals important to lions were degraded or diffused in the time between deposition and collection.

There is also conflicting evidence about the effects of freezing scent, with some evidence that freezing has a significant effect on the subsequent response of animals (Hoffmann, Musolf and Penn, 2009), and some evidence that freezing has little or no effect (Smadja, Catalan and
Ganem, 2004; Bagley, Goodwin, Rasmussen and Schulte, 2006; Kwak et al., 2009;
Lenochova, Roberts and Havlicek, 2009). In our experiments, the number of days that urine
was stored at -20 °C had a weak effect on the likelihood of response, and the duration of
response from lions. Lions were less likely to respond, but responded for longer to urine that
had been frozen for longer. These results suggest that some semiochemicals important to
lions were degraded over time in the frozen urine. Freezing may have had additional effects
on the urine that were not directly evident in our results – such as influencing the distance
over which lions were attracted to the scent, or leading to lower response rates than may have
been possible with fresh urine.

In conclusion, we provide the first direct experimental evidence that urine functions in social
and sexual communication in wild lions. Our results suggest that lions can use urine to
discriminate males from females and residents from non-residents. The response of lions to
urine was also dependent on the sex and age of the subject receiving the presentation. Further
research is required to reveal the specific semiochemicals in urine and their functions for
social and sexual scent communication in lions, as well as the longer-term behavioural
changes of lions following the detection of scent-marks.
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Peter Apps provided valuable comments on the manuscript, whilst Krystyna Golabek provided valuable statistical advice.
REFERENCES:


### Table 1- Behavioural measurements of lions after the presentation of urine.

<table>
<thead>
<tr>
<th>Behavioural Measurement</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respond to urine</td>
<td>A lion responded to the urine when investigating it, countermarking it, or overmarking it.</td>
</tr>
<tr>
<td>Investigate urine</td>
<td>Nose pointed towards the ground within 0.5 m of the urine. Investigatory behaviours included sniffing (with or without flehmen) and licking the sample. Flehmen is a key response of mammals to semiochemicals (Hart and Leedy, 1987) and involves a curled upper lip facial expression, exposing the front teeth.</td>
</tr>
<tr>
<td>Countermark urine</td>
<td>Pawing the ground, urinating, and/or defecating within 2 m of the urine, without the two deposits touching (Rich and Hurst, 1999).</td>
</tr>
<tr>
<td>Overmark urine</td>
<td>Pawing the ground, urinating, and/or defecating on top of the urine, so that the two deposits are at least partially touching (Johnston, Chiang and Tung, 1994).</td>
</tr>
<tr>
<td>Duration of response</td>
<td>The combined total time each lion was scored as investigating, countermarking and/or overmarking the urine.</td>
</tr>
<tr>
<td>Closest distance to the urine</td>
<td>The closest distance the test subject moved to the urine sample (in metres), irrespective of whether they responded to the urine. If the subject moved away from the sample from their resting place, the closest distance to the sample was equal to the distance that the sample was originally placed from the lion. The closest distance that the subject moved to the sample was chosen rather than the distance the sample was first placed from the subject, since in only 2 cases (out of 72) the subject showed signs of detecting the sample (e.g. sniffing the air) from their resting spot.</td>
</tr>
</tbody>
</table>

### Table 2- Summary of the raw data on the responses to urine for lions of different sex and age.

<table>
<thead>
<tr>
<th>Individual class</th>
<th>Number of trials</th>
<th>Number of responses</th>
<th>Responses involving sniffing</th>
<th>Responses involving flehmen</th>
<th>Responses involving a countermark</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recipient Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>99 (31.0% of total trials)</td>
<td>22 (22.2% of male trials)</td>
<td>21 (95.5% of male responses)</td>
<td>16 (76.2% of male sniffings)</td>
<td>3 (13.6% of male responses)</td>
</tr>
<tr>
<td>Female</td>
<td>220 (69.0%)</td>
<td>50 (22.7%)</td>
<td>46 (92.0%)</td>
<td>24 (52.2%)</td>
<td>6 (12.0%)</td>
</tr>
<tr>
<td><strong>Recipient Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>143 (44.8%)</td>
<td>23 (16.1%)</td>
<td>18 (78.3%)</td>
<td>10 (55.6%)</td>
<td>9 (39.1%)</td>
</tr>
<tr>
<td>Subadult</td>
<td>72 (22.6%)</td>
<td>25 (34.7%)</td>
<td>25 (100.0%)</td>
<td>15 (60.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Cub</td>
<td>104 (32.6%)</td>
<td>24 (23.1%)</td>
<td>24 (100.0%)</td>
<td>15 (62.5%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>319</td>
<td>72 (22.6% of total trials)</td>
<td>67 (93.1% of total responses)</td>
<td>40 (59.7% of total sniffings)</td>
<td>9 (12.5% of total responses)</td>
</tr>
</tbody>
</table>

### Table 3- Factors influencing whether lions responded to the urine ($N = 319$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>SE</th>
<th>CI</th>
<th>Relative importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.722</td>
<td>0.648</td>
<td>0.448-2.996*</td>
<td>-</td>
</tr>
<tr>
<td>Closest Distance to Sample (m)</td>
<td>-3.398</td>
<td>0.582</td>
<td>-4.544-2.253*</td>
<td>1.00</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female Non-Resident</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Female Resident</td>
<td>-1.761</td>
<td>0.694</td>
<td>-3.125-0.396*</td>
<td>1.00</td>
</tr>
<tr>
<td>Male Resident</td>
<td>0.669</td>
<td>0.727</td>
<td>-0.761-2.099</td>
<td>-</td>
</tr>
<tr>
<td>Recipient Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Male</td>
<td>-0.760</td>
<td>0.910</td>
<td>-2.551-1.030</td>
<td>1.00</td>
</tr>
<tr>
<td>Recipient Age</td>
<td>Adult</td>
<td>Subadult</td>
<td>Cub</td>
<td>Total</td>
</tr>
<tr>
<td>--------------</td>
<td>-------</td>
<td>----------</td>
<td>-----</td>
<td>--------</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
</tr>
<tr>
<td>Male * Female Resident</td>
<td>3.378</td>
<td>1.319</td>
<td>0.782:5.973*</td>
<td>-</td>
</tr>
<tr>
<td>Male * Male Resident</td>
<td>0.090</td>
<td>1.416</td>
<td>-2.695:2.875</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Recipient Sex * Treatment</th>
<th>Female * Female Non-Resident</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male * Female Resident</td>
<td>-1.028</td>
<td>0.550</td>
<td>-2.133:0.044</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Donor Posture</th>
<th>Scrape</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>0.80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine-only</td>
<td>-0.259</td>
<td>0.277</td>
<td>-0.804:0.286</td>
<td>0.21</td>
<td></td>
</tr>
</tbody>
</table>

| Time Between Deposition and Collection (scaled and centered) | -0.225 | 0.258 | -0.733:0.283 | 0.20 |

Model parameters were generated using model averaging on the best GLMMs (with binomial error distribution) selected using AICc (see Table A1 in Appendix). SE = Standard Error. CI = Confidence Interval. * indicates confidence intervals that do not cross zero.

**Table 4- Summary of the raw data on the responses to the urine of each treatment for lions of different sex and age.**

<table>
<thead>
<tr>
<th>Recipient Sex</th>
<th>Female Resident</th>
<th>Female Non-resident</th>
<th>Male Resident</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trials</td>
<td>Response(s)</td>
<td>Response duration (s ± SD)</td>
<td>Trials</td>
<td>Response(s)</td>
</tr>
<tr>
<td>Male</td>
<td>43</td>
<td>9 (20.9%)</td>
<td>48</td>
<td>11 (22.9%)</td>
</tr>
<tr>
<td>Female</td>
<td>79</td>
<td>9 (11.4%)</td>
<td>72</td>
<td>13 (24.4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Recipient Age</th>
<th>Adult</th>
<th>Subadult</th>
<th>Cub</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>58</td>
<td>32</td>
<td>32</td>
<td>122</td>
</tr>
<tr>
<td>Male * Female Resident</td>
<td>22</td>
<td>14</td>
<td>6</td>
<td>42</td>
</tr>
<tr>
<td>Male * Male Resident</td>
<td>22</td>
<td>12</td>
<td>7</td>
<td>41</td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>29</td>
<td>23</td>
<td>153</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Recipient Sex</th>
<th>Female</th>
<th>Male</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>3.198</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SE</td>
<td>0.375</td>
<td>0.422</td>
<td>-</td>
</tr>
<tr>
<td>CI (2.5:97.5%)</td>
<td>2.451:3.944*</td>
<td>-1.722:-0.033*</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>SE</th>
<th>CI (2.5:97.5%)</th>
<th>Relative importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>3.198</td>
<td>0.375</td>
<td>2.451:3.944*</td>
<td>-</td>
</tr>
<tr>
<td>Recipient Sex</td>
<td>Female</td>
<td>Male</td>
<td>-0.878</td>
<td>0.422</td>
</tr>
<tr>
<td>Treatment</td>
<td>Female Non-resident</td>
<td>-0.333</td>
<td>0.407</td>
<td>-1.147:0.481</td>
</tr>
<tr>
<td>Male Resident</td>
<td>-0.507</td>
<td>0.457</td>
<td>-1.420:0.406</td>
<td>-</td>
</tr>
<tr>
<td>Recipient Sex * Treatment</td>
<td>Female * Female Non-resident</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

± SD = standard deviation.

Table 5- Factors influencing the duration of response (s) of lions to urine (N = 72).
<table>
<thead>
<tr>
<th></th>
<th>Male * Female Resident</th>
<th>Male * Male Resident</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.472</td>
<td>2.409</td>
</tr>
<tr>
<td></td>
<td>0.553</td>
<td>0.560</td>
</tr>
<tr>
<td>Time Between Deposition and Collection</td>
<td>-0.017</td>
<td>0.001</td>
</tr>
<tr>
<td>Number of Days Frozen</td>
<td>-0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>0.011</td>
<td>-0.039:0.006</td>
</tr>
<tr>
<td></td>
<td>0.011</td>
<td>-0.001:0.004</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Model parameters were generated using model averaging on the best GLMMs (with Gaussian error distribution) selected using AICc (see Table A4 in Appendix). SE = Standard Error. CI = Confidence Interval. * indicates confidence intervals that do not cross zero.
FIGURE CAPTIONS:

Figure 1. Experimental procedure: approximately 80 g of thawed urine-soaked soil was deposited on the ground 7-25 m away from resting lions. Responses were observed from a vehicle.

Figure 2. (a) Predicted probabilities of responding to the treatments of presented urine for male and female lions. Estimates were derived from the averaged model presented in Table 3. (b) Mean probabilities of responding to the treatments of urine for male and female lions calculated from the raw data in Table 4. Bars are binomial standard errors.

Figure 3. (a) Predicted mean duration (\(\sqrt{s}\)) of response to the treatments of urine for male and female lions. Estimates were derived from the averaged model presented in Table 5. (b) Mean duration (\(\sqrt{s}\)) of response to the treatments of urine for male and female lions calculated from the raw data in Table 4. Bars are standard errors.