Scent-Mark Identification and Scent-Marking Behaviour in African Wild Dogs (Lycaon pictus)

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Abstract

Scent-marking is common in mammals, but where signals are carried by urine and faeces, distinguishing between scent-marking and mere elimination is problematic. To do so, we documented behaviours and context variables associated with urination and defecation in free-ranging endangered African wild dogs (Lycaon pictus) and tested whether these were related to the responses of other dogs to deposits. We found that distinct postures were almost uniquely associated with deposits by dominant wild dogs, were more common during urination than defecation, and increased the likelihood that these deposits would be investigated by other wild dogs. The likelihood of investigation depended on the sex and dominance status of the depositor, the type of deposit and the substrate. Urine from dominant females was more likely to be investigated by other wild dogs than any other deposits, and deposits placed on vegetation were more likely to be investigated than those on bare ground. The likelihood that a deposit would be overmarked was affected by the deposit type and the sex and dominance status of the last depositor. Collectively, these results suggest that dominant wild dog urine is of greatest interest to other dogs. Our results show that some deposits by African wild dogs are not scent-marks and that detailed observations of behaviours and context variables during elimination events can be used to distinguish deposits that are likely to be of communication value.

Introduction

Scent-marking is almost ubiquitous among mammals (Macdonald 1980). While it may be relatively easy to recognise scent-marking when it involves elaborate behaviours while depositing secretions from specialist glands (e.g. aardwolf, Proteles cristatus, Sliwa & Richardson 1998), the use of urine and faeces as carriers of scent signals, which is particularly common in canids (see Kleiman 1966; Macdonald 1980), presents problems because all animals urinate and defecate, but not all such excretions are necessarily scent-marks. However, distinguishing between scent-marks and mere eliminations is critical in many contexts.

Functional interpretations of scent-marking behaviour and strategies require that scent-marks be distinguished from simple excretions that might have no communication function. Kleiman (1966) attempted to make this distinction on the basis of substrate characteristics by proposing that urine or faeces deposited in response to familiar landmarks or novel odours and objects, orientated towards a conspicuous object, or repeated frequently on the same object, were active scent-marks. In many species, scent-marks are placed in areas that are interpreted as likely to promote signal discovery and longevity (Alberts 1992; Bradbury & Vehrenkamp 1998). Deposits are commonly found on or near conspicuous landmarks such as rocks, trees or crossroads (for reviews see Eisenberg & Kleiman 1972; Macdonald 1985). For example, Iberian wolves, Canis lupus signatus, preferentially deposit faeces at trail junctions (Barja et al. 2004) and on specific vegetation (Barja 2009). Variation in the conspicuous placement of
deposits may suggest that some are active scent-marks, but are all deposits left in such locations scent-marks? Conversely, are all less conspicuous deposits simply inactive excretory eliminations? Specific postures adopted during deposition, and the token volumes deposited, may provide additional insights to a deposit’s communication potential. For example, scent-marking with urine in canids is often associated with a raised leg posture and high social status (e.g. grey wolf, Asa et al. 1990; Peters & Mech 1975).

For communication to occur, there must be a recipient, and subsequent responses such as investigation and overmarking may provide confirmation that a deposit constitutes a signal. Overmarking and counter-marking, where scent-marks are deposited on top of, or alongside, an existing mark (Johnston et al. 1994; Rich & Hurst 1999), are nearly ubiquitous among mammals and, regardless of their ultimate function (Ferkin & Pierce 2007), such secondary marking establishes that the original deposit was a signal and may help to distinguish active scent-marks from relatively inactive eliminations. Detailed observational data on both the circumstances of deposition and the responses of other individuals are therefore essential for functional interpretations of scent-marking, particularly in species where overt use of specialist glands does not occur.

In this study, we investigate in African wild dogs whether context variables associated with excretion events and subsequent investigation by other individuals can identify which deposits are active scent-marks as confirmed by overmarking. African wild dogs are cooperative breeders, and packs typically consist of a dominant breeding pair and their offspring (Malcolm & Marten 1982). Dispersal is delayed, and mature offspring assist their parents to rear subsequent litters (Malcolm 1979). Although wide ranging, wild dog packs are territorial (Mills & Gorman 1997; Parker 2009; Woodroffe 2011). They deposit urine and faeces throughout their home ranges (Parker 2009), but which of these deposits are scent-marks is uncertain. To determine which African wild dog deposits are scent-marks, we first explore the frequencies of urination and defecation, and the sex and dominance status of the depositor. We then explore the factors affecting the likelihood that a deposit will be: (1) encountered and investigated and (2) overmarked by a conspecific, which are both indicators that deposits are active scent-marks. Our findings are discussed in the context of scent-mark identification and the study of scent-mark function in general.

Methods

Study Population and Site

Data were collected between May 2011 and Apr. 2012 from 9 packs of free-ranging African wild dogs in Botswana. The study area (approx. 2600 km²; 19°31’S, 23°37’E; elevation approx. 950 m) is bordered by the Okavango Delta and includes the Moremi Game Reserve and Wildlife Management Areas. Further details can be found in McNutt (1996). This subpopulation of African wild dogs has been studied since 1989. Immigrant dogs first identified as adults (n = 2) were aged on the basis of tooth wear and pelage. Each individual was identified by its unique tricolour pelage pattern, and distinctive ear notches and tail stripes, all of which were drawn and photographed on first encounter and updated as appropriate. Packs were radiotracked from the air and from a vehicle, with one to four individuals in each pack fitted with GPS radio-collars with a VHF tracking pinger (Vectronic Aerospace GmbH, Berlin, Germany; <280 g) or VHF radiocollars (Sirtrack, Havelock West, New Zealand; <180 g) following procedures described elsewhere (Osofsky et al. 1996).

In this study, a pack was defined as a group containing at least one adult male and female forming a potential reproductive unit (cf. Malcolm 1979; McNutt 1996). Independent of the mutual overmarking behaviour of dominant wild dogs (see Estes 1997), the dominant pair was easily identified within established packs. Only the dominant female bred in each pack and was closely guarded by the dominant male, who licked her vulva and lifted her with his shoulders during her brief receptive period in Mar./Apr. This male also repelled all other individuals from her side during this period. Outside the breeding period, the dominant pair also typically lay together during resting periods, and they were the focus of social activity prior to pack movement. Excluding the individual(s) that made the kill, the dominant pair also fed first at kills and repelled others from the carcass when pups were present. No other individuals in the pack displayed this combination of behaviours. During the study, only the dominant female became visibly pregnant (heavily swollen abdomen and distended teats). The mother of each litter was therefore easily identified, and she was the only female to nurse the pups (4/4 packs followed through breeding season). Using these criteria, mothers were known for all pups born during the current study and, as this study is part of a project that has run continuously since 1989, the mothers of 96.8% (60/62) of individuals were known.
overall. Paternity was less certain, but likely relatedness was determined by assuming that the dominant male in a pack during the dominant female’s oestrous fathered the entire litter that year, although some extra-pair paternity is known in this species (Spiering et al. 2010). Importantly, immigration events leading to the formation of the packs followed in this study involved either unknown (and therefore not closely related) individuals from outside the study population (1 pack) or individuals of known origin that were not closely related to the opposite-sex residents of the pack into which they immigrated (8 packs). On the basis of these criteria, and known relatedness within the pack, individuals were divided into three dominance status categories: DB- dominants with intra-pack breeding opportunities (unrelated to dominant partner); SB- subdominants with intra-pack breeding opportunities (unrelated to opposite-sex dominant); SN- subdominants with no intra-pack breeding opportunities (offspring, full sibling or parent of opposite-sex dominant).

**Behavioural Observations**

Packs were located by radiotracking and observed from a vehicle while resting (at distances of 3–40 m) and travelling (at 20–200 m, depending on terrain, vegetation and visibility). All deposits, investigations and overmarking were recorded on video or directly on datasheets by critical incident sampling (Altmann 1974), with overmarking defined as the placement of a deposit on an existing deposit so that the two were at least partially overlapping (sensu Johnston et al. 1994). When a dog deposited at a location, its posture, deposit type (urine or faeces), the substrate (vegetation or bare ground) and the identity of the individual that deposited were recorded. Each location was given a unique identifying number (the ‘Unique site ID’), and all behaviour by subsequent visitors to each site was recorded, until the pack moved away from the site. Individuals ‘investigated’ a deposit when they sniffed (muzzle directed at, and lingering within 30 cm of, deposit) or licked it (made direct contact using the tongue). Leg postures included cocked leg (single hind leg raised/cocked once); raised leg (both hind legs raised independently at least once); and handstand (both hind legs raised simultaneously in a hop). Squats (i.e. no leg posture) involved lowering the hindquarters with all legs on the ground. The first deposit at a site was termed the ‘original deposit’, and the deposit most recently left on a site was termed the ‘top deposit’. To avoid including sites at which the first deposit had not been observed, sites were excluded when the first behaviour observed was investigation.

To investigate relationships between context variables, we used binomial tests of proportions with continuity correction and chi-squared tests to compare the frequencies that different deposit types (urine and faeces) were deposited by different classes of individuals (sex, dominance status) in conspicuous locations (or not) and with postures (or not).

To investigate the factors affecting whether or not deposits were investigated by other individuals, we ran a series of generalised linear mixed models (GLMMs) with a binomial error distribution (0 = deposit not investigated by another dog following deposition, 1 = deposit subsequently investigated) and a logit link function. We included the identity of the individual that deposited (‘depositor identity’) and ‘pack’ identity as random terms to account for multiple data from the same individuals and packs. We used Akaike’s information criterion (AIC) to select the most plausible models from a set of credible options including all combinations of the likely terms (depositor type, depositor status, depositor sex) and their two-way interactions. Lower AIC values correspond with better relative support for each model (Akaike 1974), and models where delta AIC differed by less than two with fewer parameters were rated equally (Burnham & Anderson 2004). Akaike weights were then calculated to show relative importance (Akaike 1974) between these models.

For all investigated deposits, we evaluated the factors affecting whether or not they were overmarked by the investigator. We ran another series of GLMMs with a binomial error distribution (0 = not overmarked, 1 = overmarked) and a logit link function. We included ‘pack’, ‘unique site identity’, ‘recipient identity’, ‘top depositor identity’ and ‘original depositor identity’ as random terms. We used Akaike’s information criterion (AIC) as described previously to select the most plausible model from a set of credible options.

**Results**

**General Scent-Marking Behaviour**

A total of 1179 deposits were observed at 715 unique sites by 62 wild dogs from 9 packs. Each of 15 dominants was observed to defecate 4.60 ± 5.87 (x ± SD) times and urinate 45.93 ± 61.49 times. Defecation by 51 subdominants was observed 4.27 ± 4.43 times per individual, and urination was observed 3.98 ± 5.10 times per individual. At 66.4% of all sites, the first
deposit was urine, while 75.7% of all (1179) deposits were urine. 29.9% of all sites were overmarked at least once; these 215 sites received an average of \(3.17 / \sigma_{1.52} \) deposits.

Of 676 initial sites where the substrate could be clearly identified, a single defecation and 12 urinations were deposited directly into standing water by 13 different subdominants. A significantly greater proportion of urinations (79.5% of 449) than defecations (58.1% of 227) was placed on vegetation as opposed to on bare ground (binomial test of proportions with continuity correction: \( \chi^2_{(1)} = 33.319, p < 0.0001 \)). A significantly greater proportion of deposits from dominants (87.3% of 324) than subdominants (58.5% of 352) was placed on vegetation (binomial test with continuity correction: \( \chi^2_{(1)} = 255.613, p < 0.0001 \); Fig. 1). In dominants, leg postures were significantly more likely during urination than defecation (binomial test of proportions with continuity correction: \( \chi^2_{(1)} = 424.968, p < 0.0001 \)). No leg posturing occurred during defecation by subdominants (n = 218), and postures were adopted during only 13.1% of 61 dominant defecation events. Leg posturing during urination by subdominants was also rare (15.3% of n = 203), but postures were common during dominant urinations (89.4% of 689). Of all (892) urinations, 56.4% occurred with raised legs, 14.7% with a cocked leg and 1.5% with handstands.

Considering dominant deposits only, males urinated significantly more often than females (63.4% of 689 dominant urinations; chi-squared test: \( \chi^2_{(1)} = 49.674, p < 0.0001 \)). In contrast, faeces deposition did not differ significantly between dominant males and females (males produced 58% and females 42% of 69). In dominants, leg postures were significantly more likely during urination than defecation (binomial test of proportions with continuity correction: \( \chi^2_{(1)} = 45.786, p < 0.0001 \); Fig. 1), although both did so often (dominant males during 96.1% of 437 events; females during 77.8% of 252). In 9.4% of all 203 observed subdominant urinations, a dog urinated directly in its resting spot while lying on its side.

Factors Affecting Investigation

33.2% of 711 sites were investigated by at least one individual during observation sessions. Dominant scents deposited with a posture were more likely to be investigated by others (61.2% of 250) than
those deposited without a posture (43.2% of 74; chi-squared test: \( \chi^2_{(1)} = 6.801, p = 0.009 \)). Original deposit type had a significant effect on the likelihood that a deposit was subsequently investigated (Model 1, Table 1), with sites initiated with urine more often investigated (42.7% of 472) than sites initiated with faeces (14.2% of 239). In addition, both the dominance status and the sex of the original depositor affected the likelihood that a deposit would be investigated (Model 1, Table 1). The deposits of dominants were significantly more likely to be investigated than deposits of subdominants, and female deposits were more likely to be investigated than male deposits.

Factors Affecting Overmarking

At 259 unique sites, 60.3% of all 579 investigations resulted in overmarking. The likelihood of overmarking was affected by an interaction between the sex of the investigator and the sex of the last individual to deposit there (Model 1, Table 2; Fig. 1a). Investigators of both sexes were more likely to overmark scents where the top deposit was left by an opposite-sex individual (69.2% overmarked opposite-sex top deposits [\( n = 399 \]): 40.6% overmarked same-sex top deposits [\( n = 180 \)]). Although the model showed that males (45.2% of 104) and females (66.9% of 178) were similarly likely to overmark sites where the top deposit was from a male, male investigators (71.0% of 221) were more likely than female investigators (34.2% of 76) to overmark sites where the top deposit was from a female.

Overmarking likelihood was also affected by an interaction between the sex of the investigator (Model 1, Table 2; Fig. 1b). Dominants (79.9% of 398) were more likely than subdominants (17.1% of 181) to overmark a deposit. Within dominants, males (82.8% of 238) and females (75.6% of 160) were similarly likely to overmark, but female subdominants (25.5% of 94) were more likely to overmark than subdominant males (8.0% of 87). Subdominant females with breeding potential in the pack were also more likely to overmark deposits (38.1% of 21) than females with no intra-pack breeding opportunities (21.9% of 73).

Finally, overmarking likelihood was affected by the top deposit type (Model 1, Table 2; Fig. 1c). Sites where urine was last deposited were more often overmarked (65.0% of 506) than sites where faeces were last deposited (27.4% of 73).

Discussion

African wild dogs are one of many species for which scent signals are assumed to be incorporated with urine and faeces. Results presented here suggest that active scent-marks can be distinguished from mere waste elimination by characteristics of the deposit the depositor, the nature of the substrate and whether the deposit was investigated by a conspecific. Additionally, specific overmarking responses were used to confirm that certain deposits were scent-marks with communicatory significance.

Overmarking was conducted predominantly with urine and occurred on only 27.4% of encountered sites where faeces were the top deposit compared with 65.0% of sites with urine on top. That around two-thirds of scats had no confirmed scent-marking
function was predictable from context variables when they were deposited; 97.1% were deposited with no leg posture; 41.9% were deposited on bare ground rather than vegetation; and faeces were investigated only one-third as often as urine and overmarked less than half as often. Only urine was deposited in token quantities and at elevated frequencies; while rates of defecation were similar for both sexes and for dominants and subordinates. Urination accounted for >90% of all deposits by dominants, and dominants urinated an order of magnitude more frequently than subdominants, probably because urination by dominants occurred in smaller volumes. Token urination by dominants is consistent with other canids (see Macdonald 1985) and has been associated with scent-marking generally. During boundary patrols, Ethiopian wolves, C. simensis, deposited token urinations but rarely defecated, and raised leg urinations were more likely than scats to be directed at other scent-marks (Siller-Zubiri & Macdonald 1998). In contrast to previous work (Parker 2009), these results suggest that the majority of African wild dog faeces may be of limited importance as scent-marks. Scent-marking among canids is typically carried out by dominant animals, including territory holders (coyote, C. latrans, Gese & Ruff 1997; wolves, Rothman & Mech 1979; Ryon & Brown 1990; domestic dogs Lisberg & Snowdon 2011; Cafazzo et al. 2012). Among mammals in general, responses to previous deposits are commonly related to the relative dominance status (meerkat, Suricatta suricata, Jordan 2007) or competitive ability (house mouse, Gosling et al. 1996) of depositors and investigators. We found the same for African wild dogs; a greater proportion of dominant than subdominant urines was confirmed as scent-marks by being overmarked, and dominants were significantly more likely than subordinates to overmark encountered deposits. As well as urinating more frequently than subdominants, dominants were also significantly more likely than subdominants to adopt postures while urinating, and these deposits were more likely to be investigated by other dogs than those deposited with no posture. Of all initial

### Table 2: GLMMs with binomial error distribution and logit link function investigating the factors that predict the likelihood of an investigated scent-mark being overmarked (N = 259)

<table>
<thead>
<tr>
<th>Model</th>
<th>Description</th>
<th>Estimate</th>
<th>AIC</th>
<th>k</th>
<th>Δi</th>
<th>wi</th>
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<tbody>
<tr>
<td>Basic</td>
<td>Investigator sex</td>
<td>Male</td>
<td>1.981</td>
<td>580.4</td>
<td>6</td>
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<tr>
<td>1</td>
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<td>544.6</td>
<td>14</td>
<td>0.0</td>
<td>0.591</td>
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<tr>
<td></td>
<td>Investigator dominance status</td>
<td>DB</td>
<td>0.000</td>
<td>539.6</td>
<td>16</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>SB</td>
<td>−1.786</td>
<td>539.6</td>
<td>16</td>
<td>0.0</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>SN</td>
<td>−2.238</td>
<td>539.6</td>
<td>16</td>
<td>0.0</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Top deposit type</td>
<td>Urine</td>
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<td>539.6</td>
<td>16</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Faeces</td>
<td>0.000</td>
<td>539.6</td>
<td>16</td>
<td>0.0</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Investigator sex:Top depositor sex</td>
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<td>−1.867</td>
<td>539.6</td>
<td>16</td>
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</tr>
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<td>539.6</td>
<td>16</td>
<td>0.0</td>
<td>0.000</td>
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<tr>
<td></td>
<td>Investigator sex:Investigator dominance status</td>
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<td>1.105</td>
<td>539.6</td>
<td>16</td>
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</tr>
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<td>Female</td>
<td>0.000</td>
<td>539.6</td>
<td>16</td>
<td>0.0</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Pack identity (n = 9), unique site (n = 579), investigator identity (n = 49), top depositor identity (n = 39) and original depositor identity (n = 36) were included as random terms. Model 1 best fits the data with the fewest explanatory parameters and lowest AIC. k = parameters, Δi = AICi − AICmin, wi = Akaike weights. Dominance status categories: DB- dominants with breeding intra-pack breeding opportunities; SB- subdominants with intra-pack breeding opportunities; SN- subdominants with no intra-pack breeding opportunities. In models 2–8, * refers to the minimal model with additional (+) or removed (−) terms.
scent samples, urine deposited by dominant females was the most likely to be investigated and overmarked. This may be because the dominant pair spends much of their time in a loose form of consort, lying together during resting periods and associating closely during pack movements and hunts (Creel & Creel 2002), allowing dominants to regularly check on and overmark their mate. However, work on spotted hyaenas (Hyaena hyaena) demonstrated that females were more likely to investigate and anoint themselves with scent from more dominant females (Burgener et al. 2009), suggesting that remote discrimination of dominance status from scents could also occur.

Subdominant deposits were investigated less often than dominant deposits, suggesting that subdominants may sometimes deposit in locations unlikely to be investigated by pack-mates, or that subordinates’ deposits are less detectable, or perhaps less interesting than dominants’ deposits. Subdominants occasionally went to great lengths to avoid leaving detectable deposits, by depositing directly into water. Such apparently deliberate inconspicuous deposition is relatively rare but has been previously described in aardwolves (Polemonium aardwolf) and wild dogs (Kruuk & Sands 1972), which conceal their deposits by burying them. Additionally, subdominants occasionally urinated without moving from their resting spot, or even standing up, while dominants always roused themselves to distribute urine nearby. Urinating while lying down is rarely described in any species, but Smithers (1983) describes similar behaviour in a male lion, *Panthera leo*.

Urine rather than faeces and dominant rather than subordinate deposits were more often placed on vegetation. Placing deposits on vegetation or other prominent spots is common in the scent-marking behaviour of many species (e.g. Ethiopian wolf, Sillero-Zubiri & Macdonald 1998; Iberian wolf, Barja et al. 2005; *Canis familiaris*, Cafazzo et al. 2012) and is presumed to increase the likelihood of detection by conspecifics. Our data confirm that dominant urine deposited on vegetation is more detectable; a greater proportion of this urine was investigated and overmarked compared with that deposited on bare ground.

Following investigation of a scent, animals have the choice of adding to it with a scent of their own. Such overmarking (*sensu* Johnston et al. 1994) is common in many species (Ferkin & Pierce 2007), and it establishes unequivocally that both the initial deposit and the overmark are scent-marks. Most overmarking was undertaken by African wild dogs of the opposite sex to the previous individual to deposit there, as in other carnivores (e.g. banded mongoose, *Mungos mungo*, Jordan et al. 2011; meerkat, Jordan 2007), which may suggest a role in pair bond maintenance. The overmarking responses of African wild dogs being influenced by the attributes of the top deposit is consistent with data from golden hamsters (Johnston et al. 1994) and meadow voles, *Microtus pennsylvanicus* (Ferkin 1999), where top scent attributes appear to mask (or reduce the importance of) overmarked scents (e.g. Johnston et al. 1997). However, in wild dogs the most parsimonious explanation may be that each dominant preferentially over-marks its partner’s scent rather than its own.

Within dominant pairs of wild dogs, each sex was similarly likely to overmark encountered scents, while among subdominants, females were more likely to overmark than males. To our knowledge, such sex differences in the response of subdominants to scents have not been described previously. Interestingly, subdominant females with breeding potential in the pack were more likely to overmark than those without breeding opportunities, suggesting a link between reproductive access and scent-marking investment (Jordan 2007; Mares et al. 2011). These patterns might reflect competition for dominance status or the advertisement of availability or sexual status to potential mates both within and outside the pack.

Our results show that not all African wild dog deposits are scent-marks and those that are can be recognised by the adoption of specific leg postures and their being deposited on vegetation. The scent-marking role of dominant urine was confirmed by its being more likely to be investigated and then overmarked by other dogs. The data presented here establish that, even in a species where scent-marking exclusively involves urine and faeces, detailed observations of elimination can be used to determine potential scent-marks. Future work in scent-marking in other species must distinguish simple eliminations from scent-marks before assigning functional significance to deposits.

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