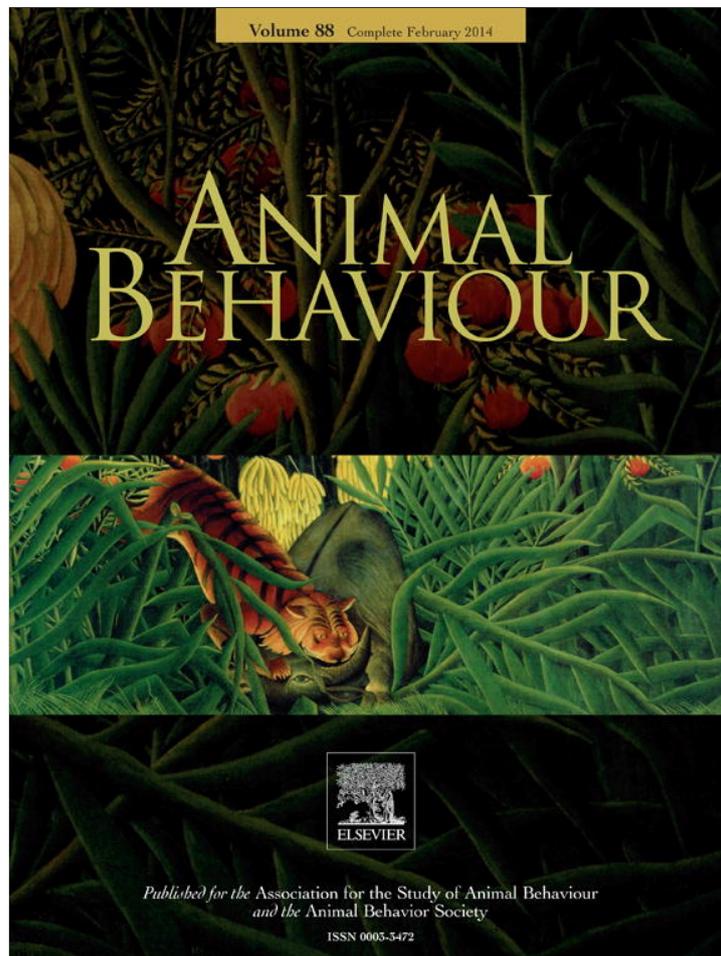


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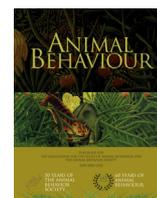
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## Top marks from top dogs: tandem marking and pair bond advertisement in African wild dogs

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Tandem marking occurs when both members of a pair scent-mark the same location in quick succession. This widespread behaviour, common in canids and monogamous antelopes, is generally believed to be involved in pair bond formation and advertisement. Despite their potential utility in determining tandem mark function, observations of individual contributions to tandem marking within pairs are rare. To this end, we made detailed observations of free-ranging dominant African wild dogs, *Lycaon pictus*, uncovering differences in the tandem-marking behaviour of pairs depending on their relatedness. In all packs, dominants were more likely to overmark their partner's scent mark than their own, but dominant-initiated scent marks were more likely to become tandem marks in related (full-sibling) pairs than in unrelated pairs. Despite this, females were more frequently on top at the end of marking bouts in related pairs than in unrelated pairs, because females in related pairs were more likely to overmark initial scent marks left by males, and less likely to have their scent marks subsequently overmarked by males. Scent-marking bout length was also significantly longer in related pairs. These differences suggest that advertising the presence of a mated dominant pair may be the main function of tandem marking in African wild dogs, but when mating opportunities are absent within the pack, contributions to tandem marking are altered, with individual advertisement perhaps becoming more important.

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Scent marking is often associated with dominance (Ralls, 1971); being performed almost exclusively (e.g. grey wolf, *Canis lupus*, Asa, Mech, Seal, & Plotka, 1990) or at higher rates (e.g. meerkat, *Suricata suricatta*, Jordan, 2007) by dominants than by subdominants. Scent marking commonly peaks at or immediately preceding the breeding period (e.g. leopard, *Panthera pardus*, Bothma & Coertze, 2004), suggesting a potential role in mate acquisition and defence, and generally males scent-mark at higher rates than females (Ralls, 1971) often with sex-specific scents (e.g. banded mongoose, *Mungos mungo*, Jordan, Manser, et al., 2011). In many pair-bonded canids however, males and females contribute similarly to scent marking (e.g. coyote, *Canis latrans*, Gese & Ruff, 1997), and although comparisons have been made between individuals of different dominance status (e.g. Peters & Mech, 1975), detailed observations of the marking behaviour within a dominant pair are less frequently described, and may provide important information on the function(s) of scent marking generally.

Tandem marking (Rothman & Mech, 1979), where both members of a pair scent-mark the same spot (overmarking sensu Johnston, Chaing, & Tung, 1994), is common in pair-bonded canids (e.g. coyote, Gese & Ruff, 1997; domestic dog, *Canis familiaris*, Lisberg & Snowdon, 2011; grey wolf, Peters & Mech, 1975) and antelopes (e.g. Kirk's dikdik, *Madoqua kirkii*, Brotherton, Pemberton, Komers, & Malarkey, 1997; klipspringer, *Oreotragus oreotragus*, Roberts & Dunbar, 2000). The two most prominent, although not mutually exclusive, functions proposed to explain this widespread behaviour are (1) pair bond formation and (2) advertising the presence of a mated pair. Captive wolves provide some support for the pair bond formation hypothesis, because newly formed pairs scent-marked at the highest rates, and eventually decreased their rates to those of established packs (Rothman & Mech, 1979). Indeed, tandem marking forms part of the courtship behaviour of many canids (coyote, Bekoff & Diamond, 1976; domestic dog, Fox & Bekoff, 1975; red fox, *Vulpes vulpes*, Macdonald, 1979; bush dog, *Speothos venaticus*, Porton, 1983). In African wild dogs and golden jackals, *Canis aureus*, tandem marking occurs almost immediately after the pair first meet (Frame & Frame, 1976; Golani & Keller, 1975), while in newly formed wolf pairs it occurs within 5 days (Rothman & Mech, 1979). Together, these patterns suggest that tandem marking plays a role in pair formation.

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However, the persistence of tandem marking beyond initial pair bonding in some species (e.g. coyote, Gese & Ruff, 1997; grey wolf, Peters & Mech, 1975) suggests that tandem marking may also contribute to long-term pair bond maintenance and advertisement. In both of these hypotheses the mated pair are the focus, but it may be more informative to consider the individuals that make up these pairs, individuals that would be expected to behave selfishly to an extent that depends on social circumstances.

In evaluating the potential function(s) of tandem marking, the order in which scent marks are deposited is important. Scent marks placed on top of previous marks are most important in affecting subsequent responses at those sites (e.g. banded mongoose, Jordan, Manser, et al., 2011; African wild dog, Jordan, Golabek, Apps, Gilfillan, & McNutt, 2013), perhaps because recipients attach greater significance to the top marks at such sites than lower marks (e.g. Johnston, Sorokin, & Ferkin, 1997). It is important therefore to consider individual contributions to tandem marking, particularly scent-marking order and which individual/sex has its scent mark on top at the end of a scent-marking bout. Although some of the most detailed work on tandem marking in canids was conducted remotely by snow tracking wolves (Peters & Mech, 1975), circumstances that made it impossible to determine the order of deposition within the pair, some ungulate studies provide additional resolution. For example, plains zebra stallions, *Equus burchelli*, overmarked mares (Klingel, 1974) and female klipspringers initiated most tandem-marking bouts, but males marked more during these bouts and usually left the final mark at a site (Roberts & Dunbar, 2000). Such patterns of marking, with males generally emerging on top, suggest a role in mate guarding, but as only high-quality mates may be able to afford the costs of repeatedly overmarking their mate (Gosling & Roberts, 2001; Rich & Hurst, 1998) tandem marking may also be a means of testing the competitiveness or 'quality' of a mate.

To investigate the function of tandem marking, we investigated its occurrence within dominant pairs of free-ranging African wild dogs in northern Botswana. African wild dogs live in packs in which a single pair typically monopolizes breeding and parents are assisted in pup rearing by mature offspring that have delayed dispersal (Girman, Mills, Geffen, & Wayne, 1997). African wild dogs scent-mark using urine (Frame & Frame, 1976), with the majority of scent marks in a pack being deposited by the dominant pair (Jordan et al., 2013; Parker, 2009). We investigated factors that potentially affect overmarking within the dominant pair. Despite earlier reports to the contrary (Frame & Frame, 1976), both sexes of wild dog disperse (McNutt, 1996), and therefore both male and female breeders are vulnerable to being usurped by same-sex immigrants. As a result, if the pair bond maintenance hypothesis applies in this species, both sexes might be expected to contribute similarly to tandem marking and dominant males and females might be expected to initiate and finish a similar number of marking bouts. We also investigated whether the details of tandem marking depended on relatedness within the dominant pair. In some study packs, the social role of a dominant that died was taken over by an opposite-sex sibling of the surviving dominant. In such circumstances neither social dominant has (out)breeding opportunities within the pack; both of them would benefit from an opposite-sex immigrant as a mate, and therefore, as in unrelated pairs, both of them are vulnerable to displacement by a same-sex immigrant. If tandem marking is involved in maintaining the pair bond, we might expect that closely related socially dominant pairs would show lower rates of tandem marking or perhaps a cessation of tandem marking altogether as each sex shifts to self-advertisement to attract an immigrant of the opposite sex. Alternatively, within related pairs the need to avoid having a same-sex immigrant attracted by the opposite-sex

dominant's marks might lead to greater competition to leave the top mark, and thus an increase in tandem marking.

## METHODS

### *Study Population and Site*

The research was undertaken under permit from the Botswana Department of Wildlife and National Parks and adhered to the ASAB/ABS Guidelines for the Use of Animals in Research. Data were collected between May 2011 and September 2012 from 13 packs of free-ranging African wild dogs in northern Botswana. The study area (ca. 2600 km<sup>2</sup>; 19°31'S, 23°37'E; elevation ca. 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, and 95.5% of all ( $N = 112$ ) individuals observed in the current study were of known age and origin. Each individual was identified by its unique tricolour pelage pattern, distinctive ear notches and tail stripes, all of which were drawn and photographed (usually in the few weeks following emergence from the den or immigration). Immigrant dogs first photographed as adults were assumed to be unrelated to the residents. In this study, a pack is defined as a group containing at least one adult male and female. The dominant pair could be easily identified within established packs from behaviours in addition to their stereotyped tandem marking (Jordan et al., 2013). Only the dominant female bred in each of our study packs, and was closely guarded by the dominant male, who licked her vulva and lifted her with his shoulders during her brief receptive period in April/May. Outside the breeding period, the dominant pair also typically rested together, and were often the focus of social activity during rallies, when many individuals greeted and submitted to them prior to pack movement. Apart from young pups and the individual(s) that made the kill, the dominant pair also fed first at kill sites, and repelled others while doing so.

We determined relatedness by assuming that the male that was dominant during oestrus fathered the entire litter that year, although extrapair paternity is known in this species (Spiering, Somers, Maldonado, Wildt, & Gunther, 2010) and so mixed paternity is possible. Study packs were divided according to the relatedness of the dominant pair, which were either full siblings ('related',  $N = 2$  packs) or were less closely related ('unrelated',  $N = 11$ ). It was not possible to determine relatedness to any greater degree of accuracy, owing to both small sample sizes and the long-distance dispersal in this species meaning that relatedness between different coalitions of previously unknown immigrants was impossible to determine.

### *Behavioural Observations*

One to four individuals were radiocollared in each pack using Vectronic (Vectronic Aerospace GmbH, Berlin, Germany; <320 g) or Sirtrack (Sirtrack, Havelock West, New Zealand; <180 g) transmitters mounted on collars which allowed them to be located by radiotracking from the air and from a vehicle. To allow radiocollars to be fitted, wild dogs were darted from a vehicle from <15 m using Telinject (Telinject U.S.A., Inc., Agua Dulce, CA, U.S.A.) darting equipment with a mixture of ketamine HCl with xylazine and atropine (Osofsky, McNutt, & Hirsch, 1996), with an intramuscular reversal of anaesthesia with yohimbine. Drug dosages were based on weights given in Smithers (1983) and were adjusted based on visual estimates of relative size, as dogs were not weighed during immobilizations. Based on weights given in Smithers (1983), fitted collars represented 0.64–1.14% of body weight. Immobilized

animals were moved to the shade by hand and cooled using water if necessary. They were measured and radiocollared, and their temperature and heart rate were monitored at intervals throughout the 30–40 min procedure. A total of 18 ml of blood was drawn from the saphenous vein through a needle attached to two vacutainers. The interval to full recovery was approximately 1.5 h from darting. All dogs were seen to rejoin their pack within a few minutes after full recovery. Some radiocollars expired after about 12 months, and dogs were reimmobilized to replace them as needed. There were no observed negative effects on wild dogs from immobilization or wearing collars in this study, and this suggestion has been refuted elsewhere (Woodroffe, 2001).

All observations were conducted from a vehicle, at distances of 3–40 m while dogs were resting and at 20–200 m while dogs were travelling, depending on terrain, vegetation and visibility. All scent marks, investigations and overmarking were recorded on video or directly on datasheets by critical incident sampling (Altmann, 1974), until the pack moved away from the site. This study includes only urine scent marks deposited with specific leg postures by dominants (sensu Jordan et al., 2013). 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). Initial defecations and squat urinations (i.e. no leg posture) were excluded from analyses because previous work suggests that these are purely eliminatory (Jordan et al., 2013). Investigation of a scent mark was recorded when an individual sniffed (muzzle directed at, and lingering within 30 cm) or licked (made direct contact using the tongue) it. Overmarking occurs when an individual places a scent mark on an existing scent mark of another individual such that the two are at least partially overlapping (sensu Johnston et al., 1994). Tandem marking, as defined in this study, is a subset of overmarking in which one member of the dominant pair overmarks the other. Tandem marks contain at least one mark from each of the dominant pair, but may contain several. Each scent mark site was given an identifying number (the 'unique site ID'). The first scent mark at a site was termed the 'initial mark' and the scent mark most recently deposited on a site was termed the 'top mark'. All behaviour by subsequent visitors to each site was recorded until the pack moved away from the site. To avoid including sites at which the first deposit had not been observed, we excluded sites when the first behaviour observed there was investigation.

#### Statistical Analyses

Statistical tests were carried out in 'R' (R Development Core Team, 2013). All parametric statistics were checked for homogeneity of variance, normality of error and linearity.

To investigate tandem-marking order, particularly which individual was on top at the end of a marking bout, we recorded the initial and subsequent markers at each dominant-initiated scent mark site. We looked at the likelihood that initial dominant scents would be tandem-marked at least once depending on relatedness (related or unrelated) within the pair, and we recorded tandem-marking bout length (total number of scent marks) at each site during the entire marking bout.

To investigate temporal patterns of tandem marking, we quantified by month the proportion of dominant-initiated scent marks that were tandem-marked by the dominant partner. Male-led and female-led bouts were investigated separately.

To investigate the factors affecting whether a dominant's scent marks would be tandem-marked, we ran a series of generalized linear mixed models (GLMMs) with a binomial error distribution (0 = scent mark not tandem-marked following investigation,

1 = scent mark tandem-marked following investigation) and a logit link function. We included the identity of the individual that deposited the initial scent mark ('initiator identity'), 'pack identity' and 'unique site ID' as random terms to account for repeated measures. We used Akaike's information criterion (AIC) to select the most plausible model from a set of credible options. All terms, including initiator sex, top mark sex, investigator sex and their two-way interactions, were removed from a saturated model. Terms were retained only if their removal inflated AIC by more than two (Burnham & Anderson, 2004), as lower AIC values correspond with better relative support for each model (Akaike, 1974). To validate that there was no improvement to the minimal model, all original terms were returned to the model one by one, creating our model set together with the basic model, containing only the intercept and the random term. Akaike weights were then calculated to show relative importance (Akaike, 1974) between these final models. As the Akaike weight of the best model was <0.9 (Grueber, Nakagawa, Laws, & Jamieson, 2011) and several models had deviance in the AIC lower than 7 units (Burnham, Anderson, & Huyvaert, 2011), we conducted model averaging using the MuMIn package in R (Bartoń, 2012). We selected the top models whose cumulative AIC weights were >0.95 to construct model-averaged estimates of the parameters (Grueber et al., 2011).

#### RESULTS

We recorded scent marking by dominant African wild dogs in 13 packs at 502 unique sites (mean  $\pm$  SD = 38.62  $\pm$  56.47 sites per pack). Scent marking at 69.32% of these sites was initiated by the dominant male, and dominant males left the top mark at 80.29% of sites.

#### Single Marks

Of 502 dominant-initiated marks, 47.21% were not tandem marked, and thus remained as single marks. Almost all (90.30%) of these 237 single marks were made by dominant males. Overall, dominant male-initiated scents were significantly less likely to become tandem marks (40.23% of 348 sites) than female-initiated scents (85.06% of 154 sites; binomial test of proportions with continuity correction:  $X^2_1 = 84.593$ ,  $P < 0.0001$ ). None of the dominant female single marks that were not tandem-marked had been investigated by the dominant male (0% of nine in related pairs; 0% of 23 in unrelated pairs); in other words, dominant males always tandem-marked female-initiated marks that they investigated. In contrast, dominant females appeared to 'choose' not to tandem-mark the initial scent of their dominant partner in some cases; of 214 dominant male single marks that did not become tandem marks, 9.81% had been investigated by dominant females, and this 'decision' not to tandem-mark occurred similarly frequently in related (13.79% of 29 sites) and unrelated pairs (9.19% of 185 sites;  $X^2_1 = 0.1929$ ,  $P = 0.661$ ).

Of all dominant scent marks that never became tandem marks, females deposited a significantly greater proportion in related dominant pairs (42.86% of 21 sites) than in unrelated dominant pairs (14.53% of 172 sites; chi-square test:  $X^2_1 = 11.3715$ ,  $P < 0.001$ ). There are two possible explanations for this that are not mutually exclusive: either females in related pairs were actively placing scents away from the male, or males were less motivated to investigate marks when they were related to the female. To tease these possibilities apart, we looked at the frequencies with which dominant males passed close to (<2 m) single scents of dominant females without investigating them. Males always investigated female marks that they passed close to, and conversely did not pass close to marks that they did not investigate, which suggests that

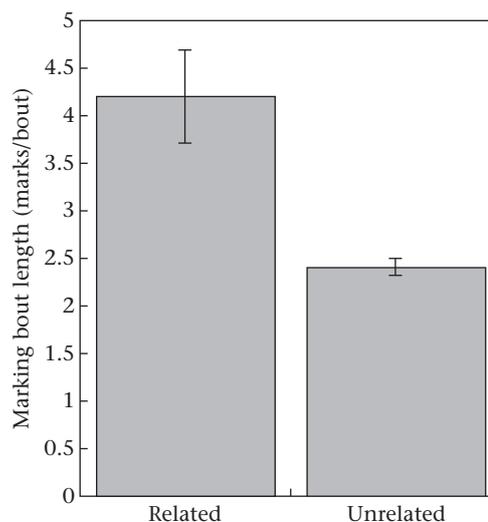
female marks that were not investigated had been placed where males would not pass close by. In contrast, dominant females passed within 2 m of 10.88% of the single scents of dominant males that they did not investigate ( $N = 193$ ), suggesting at least an occasional active decision by dominant females not to tandem-mark. Dominant females ignored dominant male scents in this way at similar rates regardless of relatedness (8.00% of 25 sites were ignored in related pairs versus 11.31% of 168 sites in unrelated pairs;  $X_1^2 = 0.023$ ,  $P = 0.880$ ).

### Tandem Marks

Of 502 dominant-initiated scents, 58.57% were encountered at least once by the opposite-sex dominant partner, which subsequently tandem-marked 88.10% of these encountered single scents. Scents were significantly more likely to be tandem-marked in packs with related (75.29% of 85 sites from two packs) than unrelated dominant pairs (60.05% of 418 sites from 11 packs; binomial test of proportions with continuity correction:  $X_1^2 = 6.379$ ,  $P = 0.012$ ). Opposite-sex dominants tandem marking their partner's initial scent marks resulted in 259 double marks. Of these double marks, 57.14% were encountered by the other dominant, which remarked 89.86% of these sites, producing a triple mark and re-establishing their own scent mark's position on top. Of the resulting 133 triple marks, 51.13% were encountered and 88.24% of these were marked again by the opposite-sex dominant to the one that left the previous top mark. Single, double and triple scent marks (of alternating sex) were encountered at similar rates by dominants of the opposite sex to the top mark depositor (chi-square test:  $X_2^2 = 1.020$ ,  $P = 0.600$ ), and were overmarked at similar rates ( $X_2^2 = 0.112$ ,  $P = 0.946$ ). In other words, investigation and overmarking were not significantly influenced by whether a scent was underlain by others. This confirms the importance of the top scent over previous scents in determining overmarking responses.

Overall, dominant male marks were on top at a significantly higher percentage of scent-marking sites when the dominant pair were unrelated (82.73% of 417 sites) than when they were related (68.24% of 85 sites; chi-square test:  $X_1^2 = 8.415$ ,  $P = 0.004$ ). This is because dominant female-initiated marks were more likely to be tandem-marked in unrelated pairs than in related pairs (see above); when scent marks that were not tandem-marked (single scents) were removed from analyses, similar percentages of dominant-initiated marks finished with males on top irrespective of whether packs had related (71.88% of 64 sites) or unrelated (79.20% of 250 sites) dominant pairs (chi-square test:  $X_1^2 = 1.1837$ ,  $P = 0.277$ ).

Mean scent-marking bout length (including single scents) was significantly longer in packs with a related dominant pair (mean  $\pm$  SE =  $4.20 \pm 0.486$  marks/bout from 85 bouts) than in packs with an unrelated dominant pair ( $2.41 \pm 0.086$  marks/bout from 427 bouts; Welch two-sample  $t$  test:  $t_{89.279} = 3.6259$ ,  $P < 0.001$ ; Fig. 1). Additionally, there was a strong tendency for dominant males to encounter a lower proportion of dominant female-initiated scents within related pairs (58.82% of 34) than in unrelated pairs (73.33% of 120 sites; binomial test of proportions with continuity correction:  $X_1^2 = 3.4792$ ,  $P = 0.062$ ). This contrasts with dominant male-initiated scents, where similar proportions were encountered by the dominant female regardless of relatedness (related: 49.02% of 51 sites; unrelated: 56.57% of 297 sites;  $X_1^2 = 0.3364$ ,  $P = 0.562$ ). Where a male's last behaviour at a scent site was to investigate it and walk away (rather than overmark), a similar proportion of these sites were sites with a female scent mark on top in packs in which the pair were related (25% of eight sites) or unrelated (28% of 25 sites;  $X_1^2 = 0.0$ ,  $P = 1$ ).



**Figure 1.** Mean scent-marking bout length (scent marks/bout), with SE bars, in related ( $N = 85$  sites from two packs) and unrelated ( $N = 427$  sites from 11 packs) dominant pairs.

Throughout the year, a higher proportion of dominant female-initiated scent marks than dominant male-initiated marks were tandem-marked each month (Fig. 2). During June/July, when the dominant female was confined to the vicinity of the den, almost all of the dominant female's scent marks were tandem-marked, while almost none of the dominant male's scent marks were tandem-marked.

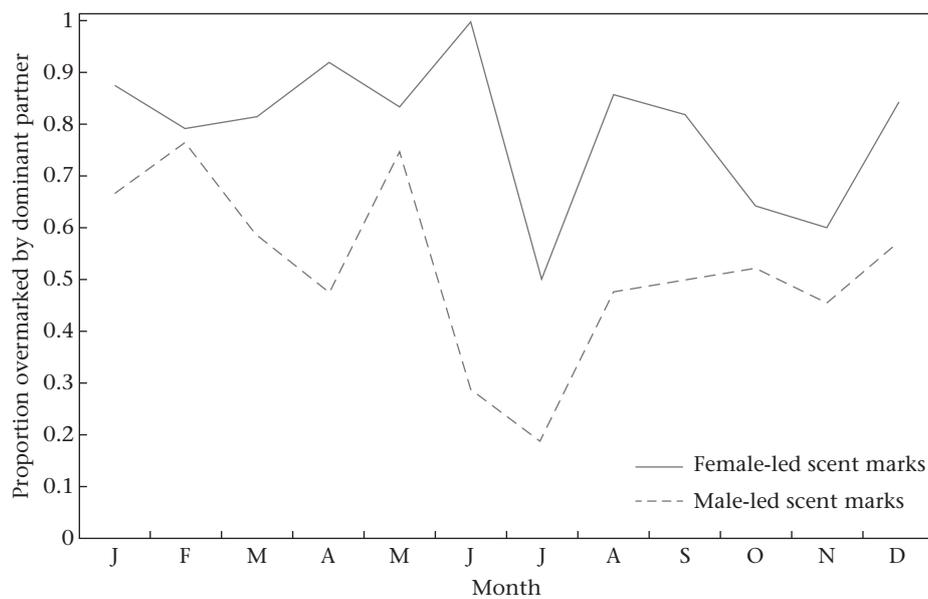
### Factors Affecting Tandem Marking by Dominants

In a GLMM analysis, the likelihood of an investigated dominant-initiated scent mark site being tandem-marked was affected by an interaction between the sex of the recipient and the sex of the last individual to mark at the site (Model 1, Table 1). Dominant recipients of both sexes were more likely to tandem-mark sites where the current top scent had been deposited by the opposite-sex dominant than sites where their own scent was already on top (Model 1, Table 1, Fig. 3).

## DISCUSSION

As in other mammals (e.g. meadow vole, *Microtus pennsylvanicus*, and prairie vole, *Microtus ochrogaster*, Ferkin, Mech, & Paz-y-Miño, 2001; Johnston et al., 1997; golden hamsters, *Mesocricetus auratus*, Johnston, Munver, & Tung, 1995) and African wild dogs generally (Jordan et al., 2013), a dominant wild dog's response to a scent mark depends upon properties of the last scent left at a site. Dominant wild dogs were more likely to overmark sites where this 'top scent' was left by their partner than sites with their own mark on top. Such top scent effects suggest that African wild dogs may be able to perceive which scent is on top, and are motivated to deposit their own scent mark in that 'primary' position. Overall, dominant males were more likely to tandem-mark dominant female top scents than dominant females were to tandem-mark dominant male top scents, perhaps indicating that advertisement of the pair bond is more important to males than to females.

In many pair-bonded canids (e.g. grey wolf, Rothman & Mech, 1979; Ethiopian wolf, *Canis simensis*, Sillero-Zubiri & Macdonald, 1998), some mongooses (e.g. meerkat, Jordan, 2007) and a range of ungulates (e.g. klipspringer, Roberts & Dunbar, 2000; oribi, *Ourebia ourebi*, Brashares & Arcese, 1999), males tend to cover the



**Figure 2.** The proportion of dominant-initiated marking bouts each month that were overmarked at least once by the dominant partner at 501 sites in  $N = 13$  packs. Dominant females initiated 153 bouts, dominant males 348.

scent marks of females with their own, a pattern usually attributed to males advertising the presence of a mated pair. This might be expected in many mammal species, because mates are generally a more limiting resource for males than for females (Clutton-Brock, 1988; Trivers, 1972). In wild dogs, and other pair-bonded species with paternal care, males are susceptible to being cuckolded by transient males gaining extrapair paternity through sneak copulations, whereas females must be usurped by female intruders to lose fitness. Indeed, we observed very few cases where dominant males passed up the opportunity to tandem-mark an encountered site where its partner's scent was currently on top (27.3% of 33 encounters were not tandem-marked). In situations where a male's last behaviour at a scent site was to sniff before walking away, most (ca. 75%) of these sites had male top marks, and these proportions were similar within related and unrelated pairs. In common with coyotes (Gese & Ruff, 1997), we also found a tendency for elevated levels of tandem marking by dominant males during the females' brief receptive period (April), further suggesting a mate-guarding function of tandem marking. In African wild dogs, however, the peak in the proportion of dominant female-initiated scent marks that were tandem-marked was during the denning season, when

the female was confined to the vicinity of the den and all female-initiated sites were tandem-marked. This may reflect the ease with which female scents are discovered and covered at this time.

The potential effects of relatedness on marking behaviour within dominant pairs have not, to our knowledge, been assessed previously in any species. However, work on prairie voles shows that relatedness within sexes can affect overmarking behaviour; males deposited fewer over- and countermarks in arenas scent-marked by a male sibling than in arenas containing the scent marks of less related males (Kohli & Ferkin, 1999). Relatedness also seems to have an effect on the fine-scale scent-marking patterns within wild dog pairs, but in this case in an intersexual context. That dominant male scent marks were more likely to be on top at the end of a bout when the males were unrelated to their partner than when they were closely related might reflect a greater motivation of unrelated males to complement the scent of their female and advertise the presence of a mated pair in the territory. Alternatively, or perhaps additionally, females that are closely related to their dominant partner may have a greater motivation to elude being tandem-marked in order to advertise themselves to potential mates outside the pack. In other species, tandem marking is suggested to signal the presence of a mated pair (e.g. Brashares & Arcese, 1999). Indeed, a study of domestic dogs demonstrated that the addition of male urine decreased the 'attractiveness' of urine from oestrous females to other males (Dunbar & Buehler, 1980), a potential example of olfactory mate guarding by tandem marking despite the urine being mixed prior to application.

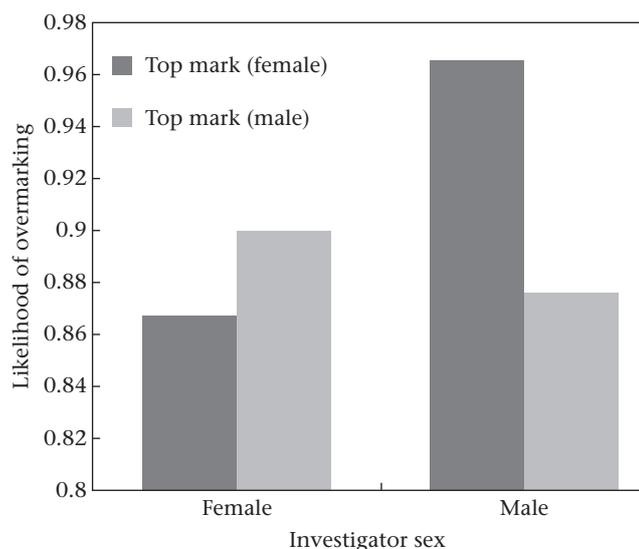
When single marks that remained as such (those never observed to be tandem-marked) were removed from the analysis in this study, males emerged on top of a similar proportion of sites regardless of relatedness. Ostensibly this is because a (three times) greater proportion of single marks are left by females in related pairs than in unrelated pairs, but the possibility that these results are a consequence of males being less motivated to tandem-mark females to which they are related is not supported by the data. Instead, our result suggests that females play a rather more active role in determining signalling outcome. Females were more likely to tandem-mark the initial scents of their partner when they were closely related to them, and they left a greater proportion of single

**Table 1**

GLMMs with binomial error distribution and logit link function investigating the factors that predict the likelihood of an investigated dominant scent mark being overmarked following investigation by a dominant individual ( $N = 725$ )

Model set	Description	$k$	AICc	$\Delta i$	$W_i$
Basic			607.73		
1	Investigator sex+Top mark sex+Investigator sex×Top mark sex	7	595.81	0	0.47
2	Initiator sex+Investigator sex+Top mark sex	8	597.36	1.55	0.21
3	Initiator sex+Investigator sex+Top mark sex	8	597.37	1.55	0.21
4	Top mark sex	5	599.56	3.75	0.07
5	Top mark sex+Investigator sex	6	601.04	5.23	0.03

Pack identity ( $N = 13$ ), investigator identity ( $N = 21$ ) and scent mark unique identity ( $N = 294$ ) were included as random terms. Model 1 best fits the data with the fewest explanatory parameters and lowest AICc.  $k$  = parameters,  $\Delta i = AIC_i - AIC_{min}$ ,  $W_i$  = Akaike weights.



**Figure 3.** Predicted effect of the interaction between the sex of the recipient and the sex of the last individual to mark the site (top mark sex) on the likelihood that an investigated dominant scent mark will be overmarked by a dominant individual, from Model 1, Table 1. Estimates for each term were derived by model averaging, presented in Table 2.

marks in related pairs. This suggests that females may have been more motivated to keep their scent signals separate, and to ‘cancel out’ male marks when they were related to the dominant partner. In African wild dogs, in which both sexes disperse and either sex can ‘inherit’ the natal territory by attracting an immigrant mate (McNutt, 1996), selection for strategies to attract opposite-sex dispersers may have evolved. The possibility that dominant females are more motivated to scent-mark in packs in which they are related to their dominant partner is additionally supported by the result that marking bouts are on average about two times longer (marks/bout) in related pairs than in unrelated pairs. This might be explained by competitive overmarking within the pair, with each sex vying to be on top in order to advertise their availability and/or to obliterate their partner’s advertisements. However, as dominant males were more likely to tandem-mark their partner’s scent than vice versa, dominant males can generally be considered responsible for creating the tandem mark which may signal the presence of a mated pair in the territory. Although both sexes are potentially vulnerable to being usurped by immigrants, the result that dominant males were more likely to tandem-mark their partner than were dominant females may be explained by asymmetries in the cost of intrusion depending on sex. While males may lose fitness by sneak copulations by intruding males, females must be fully usurped from the pack/territory to lose fitness, so that males are more

**Table 2**  
Effects of each parameter from Table 1 on overmarking responses within African wild dog dominant pairs

Parameter	Estimate	SE	CI (2.5–97.5%)	Relative importance
Intercept	1.644	0.598	0.475, 2.816	
Top mark sex (Male) <sup>1</sup>	0.3156	0.6142	–0.888, 1.519	1
Investigator sex (Male) <sup>1</sup>	1.4405	0.5733	0.309, 2.565	0.93
Investigator sex (Male) <sup>1</sup> × Top mark sex (Male) <sup>1</sup>	–1.6763	0.6067	–2.865, –0.487	0.89
Relatedness (Unrelated) <sup>2</sup>	0.2561	0.3623	–0.454, 0.966	0.21
Initiator sex (Male) <sup>1</sup>	0.2364	0.3429	–0.436, 0.909	0.21

<sup>1</sup>Female and <sup>2</sup>related were the reference categories.

susceptible to reduced fitness by ‘allowing’ competitors to intrude. Additionally, although both sexes do disperse, male wild dogs tend to disperse over greater distances (McNutt, 1996) while females, in common with other carnivores (e.g. banded mongoose, Nichols, Jordan, Jamie, Cant, & Hoffman, 2012), commonly set up home ranges adjacent to (McNutt, 1996) and often overlapping with (Jackson, Groom, Jordan, & McNutt, 2013) that of their natal pack. Therefore the likelihood of being usurped is greater for dominant males than for dominant females, because resident packs are expected to encounter dispersing males more often than dispersing females as a direct result of the greater distances that males travel during dispersal. Additionally, the level of threat associated with these encounters may be further sex biased, because the female-budding mode of dispersal may reduce the likelihood of unrelated females being encountered.

Although competitive scent marking between the sexes has been described in a number of species (e.g. females: house mouse, *Mus domesticus*, Hurst, 1990; banded mongoose, Jordan, Mwanguhya, et al., 2011; prairie vole, Wolff, Mech, & Thomas, 2002; males: house mouse, Rich & Hurst, 1999), intersexual competitive advertisement within pairs is relatively unknown. In African wild dog packs with an unrelated dominant pair, patterns of scent marking suggest that females might be employing a ‘test and assess’ strategy. If only high-quality males are able to cover the scents of their female effectively (see Gosling & Roberts, 2001; Hurst & Beynon, 2004; Rich & Hurst, 1998 for discussion of the costs of covering competitors’ scents), high levels of marking by females may help to ensure that they have access to the best possible mates. If tandem marking does signal the presence of a pair bond, it might be possible for intruding potential immigrants to assess the strength of this bond (and/or the quality of the male) by assessing the ratio of dominant single marks to dominant tandem marks (see Gosling & Roberts, 2001). Similar patterns of tandem marking are described in monogamous klipspringers, in which females initiated most tandem-marking bouts, but males usually left the final mark at a site (Roberts & Dunbar, 2000). However, when it pays to avoid advertising a functional pair bond, such as when the dominant pair is closely related, dominant female African wild dogs appear to scent-mark selfishly, both by attempting to keep their scent marks separate from those of their partner and by tandem marking more frequently than in unrelated pairs. Given that a similar proportion of encountered scents of their partner are tandem-marked by males in related and unrelated pairs, it appears that this shift according to relatedness is female driven. However, it is currently unclear why related males continue to scent-mark in a manner consistent with maintaining or advertising the pair bond. Perhaps potential immigrant females are attracted to males that have a proven ability to tandem-mark females consistently (cf. Johnston et al., 1997), or tandem-marked females are less attractive to males that might displace the resident male before it is optimal for them to disperse. Experimental presentations of single and tandem marks to potential immigrants are required to shed further light on these potential mechanisms.

**Conclusions**

Tandem marking in pair-bonded mammals is generally associated with pair bond formation, and/or maintenance and advertisement. The death of one member of a dominant pair followed by its replacement by a sibling of the surviving member of the pair creates a breeding vacuum in which pair bond maintenance is not expected, and provides a unique insight into the function of tandem marking generally. In African wild dogs, a small sample of related (full-sibling) dominant pairs exhibited tandem-marking behaviour different to that in unrelated pairs. These differences suggest that in

unrelated breeding pairs female wild dogs might employ a 'test and assess' strategy, advertising the presence of both a functional pair bond and the presence but unavailability of a high-quality male in the area, where the male is able to meet this challenge by consistently tandem marking. In related pairs our results suggest that females adopt a self-advertisement strategy, perhaps to encourage unrelated male immigration. However, these results are based on a small sample size of related pairs, and further work on this and other species will be necessary to test this hypothesis.

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