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Effects of sex, social status and gonadectomy on countermarking by domestic dogs, *Canis familiaris*

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To investigate the social roles of countermarking in dogs, we measured tail base position (TBP, a measure of social status), adjacent-marking and overmarking responses of male, nonoestrous female, gonadectomized male and gonadectomized female dogs to controlled presentations of urine from unfamiliar dogs and social groupmates. We also recorded dog sex, TBP, countermarking and urine investigation at a park. In urine presentations, only males overmarked, intact males (but not gonadectomized males) preferentially overmarked intact female urine, overmarking males had higher TBP than males that did not countermark and urine familiarity did not affect overmarking. In contrast, dogs adjacent-marked only unfamiliar samples, and neither sex nor TBP significantly affected adjacent marking. Gonadectomy did not significantly change the likelihood of countermarking. In dog park observations, males and females marked at and investigated 'scent posts' comprised of serial countermarks, often associated with visual landmarks. Males and females were equally likely to countermark and investigate urine and countermarks made up a similarly large portion of countermarking for males and females. Males and females with higher TBP urinated, investigated urine, and countermarked more than same-sex dogs with lower TBP. These studies suggest that although intact males may overmark female urine in part to guard mates as previously hypothesized, both sexes, intact and gonadectomized, likely countermark competitively. Social and sexual patterns also suggest that overmarks and adjacent marks may be distinct signals.

Countermarking, scent marking on (overmarking) or near (adjacent marking) previous scent marks, occurs in social and asocial species and serves multiple functions. Habituation tests suggest that some asocial rodents preferentially recall overmarks over bottom marks (*Microtus pennsylvanicus*, *Microtus ochrogaster*, *Mesocricetus auratus*, *Mus musculus*: Ferkin 1999; Johnston 1999; Cohen et al. 2001). Although intersexual overmarks may function in mate solicitation (Johnston 1999), these rodents also exhibit intrasexual competitive countermarking. Competitive countermarks, used to indicate competitive success or capability, follow predictable patterns of use: they are performed more frequently by higher-status individuals and directed at potential rivals (particularly intrasexual overmarks of unfamiliar nonrelatives). Competitive countermarks also may be used to aid in resource defence, territory establishment or maintenance, and as a general signal of competitive capability (Ferkin 1999; Hurst & Rich 1999; Johnston 1999), aiding assessments of prospective mates and competitors (Hurst & Rich 1999; Johnston 1999; Cheetham et al. 2007). The functions of countermarking in strongly social mammals, however, have not been adequately tested.

Canids with varied social systems countermark using urine and faeces within and between sexes, social groups, and in some cases even between species (*Canis aureus*, *Canis familiaris*, *Canis latrans*, *Canis lupus*, *Cerdocyon thous*, *Lycaon pictus*, *Otocyon megalotis*, *Speothos venaticus*, *Vulpes velox*: Rothman & Mech 1979; Bowen & Cowan 1980; Macdonald 1985; Ryon & Brown 1990; Darden et al. 2008). Despite extensive descriptions of countermarking, the social functions of canid countermarks are poorly understood. The best-established contexts for canid countermarking are intersexual countermarking during courtship and between members of an established pair (Rothman & Mech 1979; Porton 1983; Macdonald 1985), hypothesized to aid in courtship, the formation and maintenance of pair bonds, mate guarding and territory demarcation (Rothman & Mech 1979; Dunbar & Buehler 1980; Macdonald 1985; Asa et al. 1986; Mertl-Millhollen et al. 1986; Asa & Valdespino 1998), although these putative functions remain untested.

Canids may also countermark, particularly intrasexually, to indicate status or competitive capability. Competitive countermarking has

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not been demonstrated empirically in canids; however, anecdotal evidence suggests that it may occur widely in the family. In many canid species, including dogs, intra- and intersexual countermarking occurs outside of female oestrus, and outside of courting or bonded pairs and social packs (Fuller & Dubois 1962; Anisko 1976; Dunbar 1978; Dunbar & Carmichael 1981; Creel & Creel 2002). For some canids, social status affects urination rates, postures and investigation patterns (Barrette & Messier 1980; Biben 1982; Asa et al. 1985; Mertl-Millhollen et al. 1986; Asa et al. 1990; Ryon & Brown 1990; Peterson et al. 2002; Lisberg & Snowdon 2009). In established grey wolf packs, dominant males and females countermark more than lower-status wolves (Mertl-Millhollen et al. 1986; Ryon & Brown 1990), and testosterone may interact with social status in both males and females to increase urine marking during the breeding season (Asa et al. 1990).

As in asocial rodents, competitive countermarking in canids may allow individuals to assess competitors and to evaluate potential mates. Social canids might also use competitive countermarks to help establish and maintain social relationships, including social hierarchies. If countermarking is indicative of social status or competitive capability, canids could assess countermarks to determine whether and how to approach unfamiliar individuals that are potential mates, competitors and/or social group members.

Domestic dogs provide an experimentally flexible model for testing the functions of countermarks and the relationships between gonadal hormones, sex, social status and countermarking outside of the intrapair countermarking that has been the focus of previous studies on canids. In controlled urine presentations with laboratory beagles housed together, males overmarked more than females (Dunbar 1978), predominantly overmarked oestrous female urine (Dunbar 1977, 1978) and were no more attracted to simulated male/female overmarks than to male urine, suggesting that the primary function of countermarks within established social groups of dogs may be to hide oestrous females from rival males (Dunbar & Buehler 1980; but see Discussion). Although these studies establish one important context of countermarking in dogs, they may not fully represent countermarking in dogs. No study has compared countermarking among gonadally intact and gonadectomized male and female dogs. No prior study has tested countermarking among unfamiliar dogs for which competitive countermarking may be more significant. Previous studies on canid countermarking have not considered social status as a factor, nor has adjacent marking been considered separately from overmarking. If competitive countermarking occurs in dogs, we expected that high-status dogs would countermark more than low-status dogs, and that gonadally intact and gonadectomized of both sexes would countermark same-sex and opposite-sex unfamiliar conspecifics.

To establish basic countermarking patterns and test the possible functions of countermarking, we used controlled experimental urine presentations to Labrador retrievers at their homes as well as direct observations of dogs of several breeds at an off-leash dog park to test the effects of sex, social status (approximated by tail base position: see Lisberg & Snowdon 2009), familiarity and gonadal hormones on adjacent marking and overmarking in dogs.

Ethical Note

Both studies were conducted under the approval of the University of Wisconsin-Madison College of Letters and Science Institutional Animal Care and Use Committee (protocol L00352).

COUNTERMARKING RESPONSES TO UNFAMILIAR URINE AT HOME

To test the effects of sex and gonadal status both of urine sources and of dogs investigating urine on overmarking, to determine whether dogs of higher social status countermark more or differently from lower-status dogs, and to establish basic sexual and social patterns of overmarking and adjacent marking within and outside of established social groups of dogs, we presented urine to Labrador retrievers outside their homes and measured the countermarking responses.

Methods

After first allowing each subject to urinate, we recorded the countermarking responses of 48 privately owned Labrador retrievers (13 intact males, 13 intact females, 10 gonadectomized males and 12 gonadectomized females) to water controls and to urine samples from unfamiliar 'donor' dogs of the same four sex and gonadal status categories. All subjects were at least 1 year of age at the time of the study. We excluded oestrous and pro-oestrous females based on owner identification (all gonadally intact dogs belonged to breeding stock) both as subjects and as urine donors to isolate the investigatory patterns of dogs outside of the breeding context. We did not differentiate between anoestrous and metoestrous females since females in both states are neither sexually receptive nor proceptive (Beach et al. 1982b) and are equally likely to investigate or countermark urine for mate attraction or identification. When adequate urine sources were available (30 subjects: 8 intact males, 8 intact females, 7 gonadectomized males, 7 gonadectomized females), we also recorded subjects' countermarking responses to urine from groupmates. To ensure a minimal level of previous social experience with conspecifics and their urine, all subjects lived with at least one other dog. Intact and gonadectomized dogs were housed similarly, 10 of 26 intact subjects were housed with 11 of 22 gonadectomized subjects, and all subjects were provided daily free social interactions with other dog(s) in their homes. Group sizes ranged from 2 to 14 (mean $\pm$ SD group size $= 5.25 \pm 3.00$). We collected unfamiliar urine samples from 51 dogs in total, including Labrador retrievers, greyhounds and border collies. Urine collection and storage methods are described in Lisberg & Snowdon (2009).

We presented urine and control (water) samples to subjects by leading them on-leash through a 'urine course', which included samples applied to upright individual wooden stakes 2.85 m apart, as described in Lisberg & Snowdon (2009). At the end of the course, we presented groupmate urine samples using the same methods. Responses were scored from videotapes.

We considered all urinations that occurred on the urine course following olfactory investigation of one stake but before the handler moved to the next stake as a countermark of the first stake. We further classified each countermark as an overmark (subject urinated within approximately 30 cm of the stake) or an adjacent mark (subject urinated farther than approximately 60 cm of the stake) with distance approximated from videotapes. No subjects urinated between 30 and 60 cm of the stake. We also recorded tail base position (TBP) as subjects approached the urine samples as low (1), medium (2), or high (3), and averaged the scores for each subject across the first six stakes of the urine course as an indicator of social status, with high TBP indicating high social status (Lisberg & Snowdon 2009).

To determine whether urinations observed on the urine course were responses to urine samples and not to the wooden stakes on which we presented the samples, we performed a binomial test on the frequency of unfamiliar urine stakes versus control stakes overmarked and, in a second binomial test, the frequency of urine stakes versus water control stakes adjacent-marked, taking the relative numbers of urine stakes and control stakes into account. We used two additional binomial tests (separate tests for overmarking and adjacent marking) to determine whether presentation