

## The Influence of Social and Endocrine Factors on Urine-Marking by Captive Wolves (*Canis lupus*)

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Although serum hormones varied seasonally in all adult animals, only dominant male and female wolves urine-marked. Serum testosterone and urine-marking rates, which increased during the fall/winter breeding season, were positively correlated in both male and female dominant wolves. Estradiol, which increased in conjunction with proestrus and estrus, was not correlated with female urine-marking. These findings suggest that hormonal influence on urine-marking in the wolf is modulated by social factors and contrast with those for both domestic dogs and coyotes, two other members of the genus *Canis*. © 1990 Academic Press, Inc.

Of the numerous investigations of canid scent-marking, none have examined possible physiological influences (Kleiman, 1966; Peters and Mech, 1975; Golani and Keller, 1975; Henry, 1980; Rothman and Mech, 1979; Macdonald, 1979; Barrette and Messier, 1980; Bowen and Cowan, 1980; Harrington, 1981; Wells and Bekoff, 1981; Porton, 1983; Asa, Mech, and Seal, 1985). However, studies of domestic dogs have demonstrated an important role for gonadal hormones in the initiation of urine-marking (Sprague and Anisko, 1973; Beach, 1974; Hart, 1974; Anisko, 1976). We have found changes in volatile constituents of wolf urine related to gender and season (Raymer, Wiesler, Novotny, Asa, Seal, and Mech, 1984), but more importantly these constituents varied in response to exogenous steroid hormones (Raymer, Wiesler, Novotny, Asa, Seal,

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TABLE 1  
Sex and Age Composition of the Wolf Packs

Male number	Date of birth	Date of death	Female number	Date of birth	Date of death
North pack					
21	April 1969	June 1981	60	April 1973	
85	April 1978		89	April 1978	September 1982
86	"		90	"	May 1982
88	"		140	April 1981	
			147	"	
South pack					
82	April 1978		31	May 1977	October 1983
202	April 1982		204	April 1982	
204	"		207	"	

and Mech, 1986). These results suggest that urine-marking also may be influenced by maturational or seasonal changes in gonadal hormones.

The wolf is particularly appropriate for the study of endocrine influences on urine-marking. Distinctive raised- and flexed-leg postures are used for urine-marking by both males and females (Peters and Mech, 1975; Asa *et al.*, 1985), but perhaps more importantly, social rank influences urinary posture and behavior. Raised- or flexed-leg urine-marking is restricted to dominant individuals and appears to increase in frequency during the breeding season (Peters and Mech, 1975; Asa *et al.*, 1985). These observations suggest an interaction of social and endocrine influences on urinary behavior. The present study used two captive wolf packs to investigate this relationship.

## METHODS

### *Animals*

Two wolf packs (Table 1) each were maintained in 0.16 ha ( $57 \times 18.5$  m) enclosures containing natural vegetation (grass and trees) and surrounded by chain-link fencing. They were fed dog chow *ad libitum* as well as eviscerated deer (*Odocoileus virginianus*) carcasses, as available, supplemented with vitamins and minerals. "North Pack" wolves were studied from April, 1980 through April, 1983 (888 morning and 826 evening observation periods) and "South Pack" wolves from October, 1982 through June, 1984 (500 morning and 476 evening observation periods).

Packs were observed for social, sexual, and urinary behavior for 15 min at sunrise and at sunset ( $\pm 1$  hr) times determined in earlier studies to include their most active periods, 4 to 7 days per week (mode = 6 days). Urinary postures, defined in Asa *et al.* (1985), consisted of raised-leg (RLU) or standing (STU) urination by males, and flexed-leg (FLU)

or squatting (SQU) urination by females. We considered RLU and FLU as urine-marking and STU and SQU as merely urination.

Relative dominance was assessed by tail positions during interactions, i.e., a wolf with tail up was considered more dominant than one with tail down (Schenkel, 1947). Hierarchical positions were confirmed by results of agonistic encounters such as competition for food or displacement from a resting site. Pair-bond formation was evaluated by proximity-related behaviors, e.g., moving, feeding, or sleeping in proximity (Mech and Knick, 1978).

### *Hormone Assays*

All wolves in each pack were anesthetized on an alternating 2-week schedule using ketamine hydrochloride (Ketaset, Bristol Labs, Syracuse, NY) and promazine hydrochloride (Sparine, Wyeth Labs, Philadelphia, PA). Blood samples were drawn from North Pack wolves from April, 1980 through April, 1983 and from South Pack wolves from October, 1982 through July 1984. Additional blood samples (June–September, 1982) from South Pack female 31 were assayed to delineate testosterone and estradiol patterns prior to the breeding season although behavioral data are not available for that time.

Because baseline serum testosterone concentrations in male wolves reflect pulsatile release (Seal, Plotka, Packard, and Mech, 1987), sequential luteinizing-hormone releasing hormone (LHRH) injections at 0 and 90 min were used to test the secretory capacity of the pituitary–gonadal axis. Blood samples drawn at 60 min after the second LHRH stimulation, determined in an earlier study to represent peak values (Seal *et al.*, 1987), were assayed for testosterone. Because female serum testosterone concentrations following LHRH stimulation were not different from baseline in the earlier study, only baseline testosterone was measured from single samples from females. All female samples were drawn between 8:30 and 9:30 AM; male samples were drawn between 11:00 AM and 12 PM.

Samples from female 60 for April, 1980 to October, 1981 and all male samples were extracted and measured by radioimmunoassay (RIA) for testosterone according to McMillin, Seal, Keenlyne, Erickson, and Jones (1974). The interassay coefficient of variation (CV) was 7.4% at the assay sensitivity of 0.1 ng/ml. Cross-reactivity with 5 $\alpha$ -dihydrotestosterone (DHT) was 28%; with 4-androstene-3 $\beta$ , 17 $\beta$ -diol 6%; 5 $\alpha$ -androstan-3 $\alpha$ , 17 $\beta$ -diol 6%; and with other steroids less than 1%. The remaining samples from female 60 and from all other females were assayed for testosterone using Coat-A-Count RIA kit (Diagnostic Products Corp., Los Angeles, CA). This direct assay has CV's between 9 and 13% at a sensitivity of 0.04 ng/ml. Cross-reactivity with DHT was 3% and with other naturally occurring steroids  $\leq$ 2%.

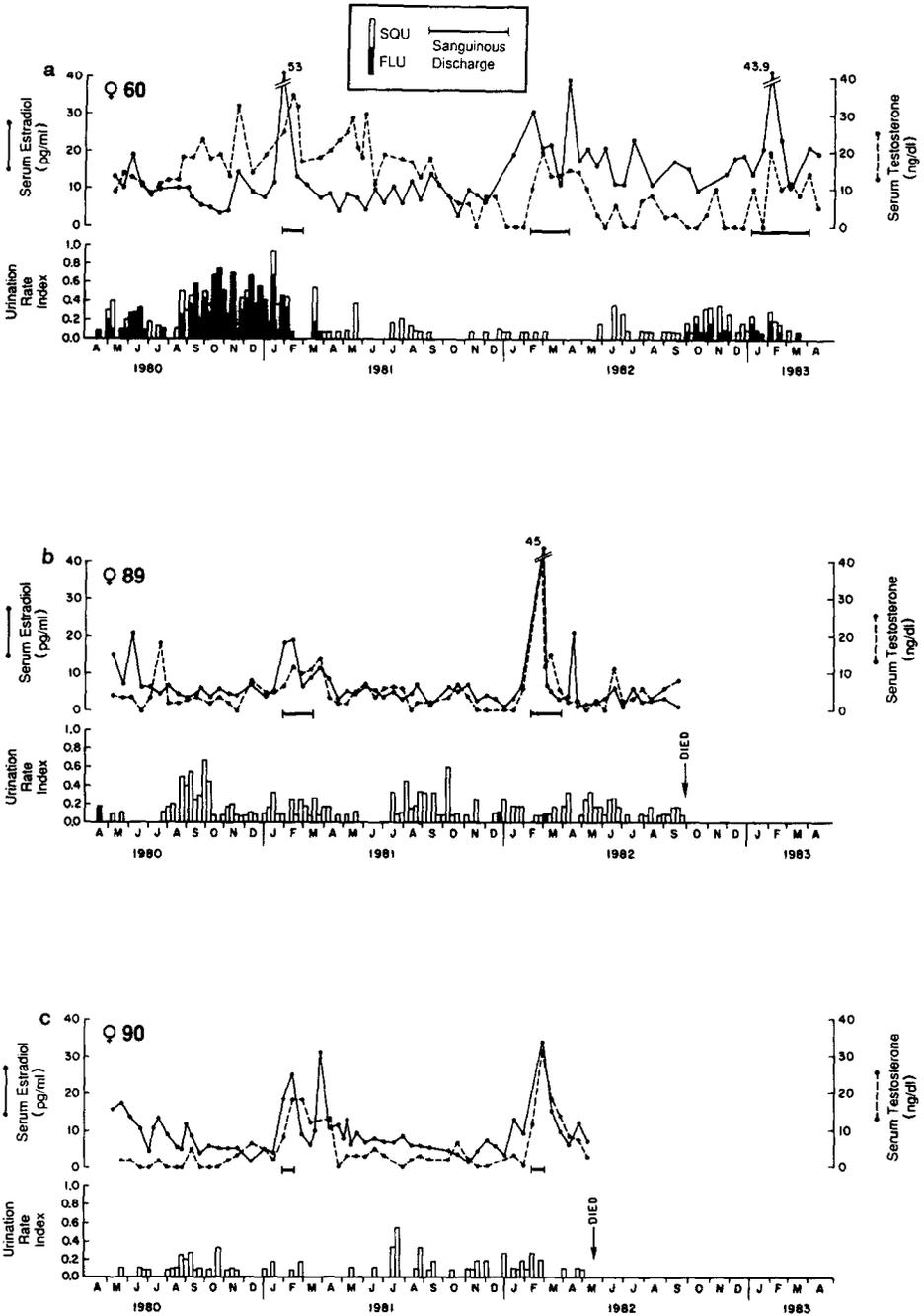


FIG. 1. Seasonal changes in serum estradiol and testosterone concentrations and in weekly index of urination rate (flexed-leg urination: FLU and squatting urination: SQU) in North Pack female wolves. (See Methods for definition of index of urination rate).

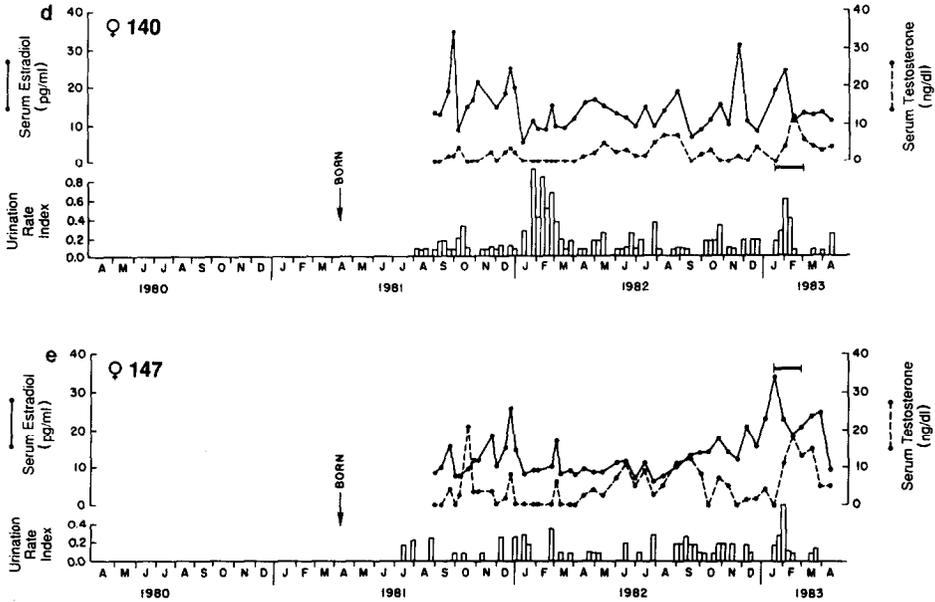


FIG. 1—Continued

Estradiol and progesterone were measured by RIA as described in Seal, Plotka, Packard, and Mech (1979). The progesterone assay was a modification of the method of Thorneycroft and Stone (1972) and had a CV of 17% at the sensitivity of 10 pg. The estradiol-17 $\beta$  assay was a modification of the method of Hotchkiss, Atkinson, and Knobil (1971) with a CV of 20% at 2 pg.

During immobilization, vaginal smears were taken from females to detect the sanguinous discharge associated with estrus and proestrus (Seal *et al.*, 1979).

### Data Analysis

The Wilcoxon sign test was used to evaluate AM versus PM urination rates. The weekly mean urination frequency per 15-min observation period (index of urination rate) was calculated by summing the number of urinations observed for each category that week (e.g., total SQU) and dividing by the total number of 15-min observation periods that week.

Patterns of urine-marking were compared with those of serum hormone concentrations by Pearson product-moment correlation (Number Cruncher Statistical System (NCSS), Kaysville, UT). Analysis of variance for repeated measures was used to compare breeding versus non-breeding season concentrations of testosterone and urine-marking rates (NCSS).

## RESULTS

Only wolves that were dominant (males 21, 82, 85; females 31, 60, 89, 204) or vying for dominance (male 88) urine-marked using the male raised-leg or female flexed-leg posture. When dominant, males urine-marked (RLU) during every month, but urine-marking rates were significantly higher during the fall and winter breeding season for both males and females ( $P < 0.01$ ). Alpha-male urine-marking rates were consistently higher than those of alpha females. Male STU's were equally distributed between AM and PM observation periods, whereas female SQU's were more frequent AM ( $P < 0.01$ ). Both male RLU's and female FLU's were more frequent PM ( $P < 0.05$ ).

Throughout the study all females past puberty, regardless of social rank, had yearly (1 to 3 years per female) increases in estradiol and sanguinous vaginal discharge during proestrus and estrus in January and February (Figs. 1 and 3). Females 140, 147, 204, and 207 reached puberty at approximately 22 months of age. (Puberty was defined as first ovulation, inferred from sustained serum progesterone concentrations of greater than 2 ng/ml through at least three samples following observed estrus, sanguinous discharge, or increased estradiol; progesterone concentrations are not shown.)

Urine-marking (FLU) rates by dominant females 60 and 204, but not 31, were correlated with serum testosterone concentrations (female 60:  $r = 0.245$ ,  $P < 0.05$ ,  $df = 80$ ; female 204:  $r = 0.481$ ,  $P < 0.01$ ,  $df = 25$ ; female 31:  $r = 0.277$ ,  $P = 0.17$ ,  $df = 24$ ). Serum estradiol also was correlated with serum testosterone ( $r = 0.784$ ,  $P < 0.001$ ,  $df = 25$ ) and with FLU ( $r = 0.650$ ,  $P < 0.001$ ,  $df = 29$ ) for female 204. Other females (all subordinates) did not urine-mark, nor were their serum hormone levels correlated with urination (SQU) rates.

For dominant male wolves 82 and 85, and beta male 88, seasonal patterns of urine-marking rate and of serum testosterone were correlated (male 82:  $r = 0.618$ ,  $P < .001$ ,  $df = 41$ ; male 85:  $r = 0.357$ ,  $P < 0.005$ ,  $df = 73$ ; male 88:  $r = 0.387$ ,  $P < 0.005$ ,  $df = 62$ ), and both were higher during the breeding season ( $P < 0.05$ ; Figs. 2 and 3) than the rest of the year. Subordinate males did not urine-mark despite seasonal patterns of testosterone comparable to those of dominant males.

*North Pack Wolves*

Female 60 urine-marked during the 1980–1981 and 1982–1983 breeding seasons when she held alpha status, but not during the 1981–1982 season

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FIG. 2. Seasonal changes in serum testosterone concentrations following LHRH stimulation and in weekly index of urination rate (raised-leg urination: RLU and standing urination: STU) in North Pack male wolves.



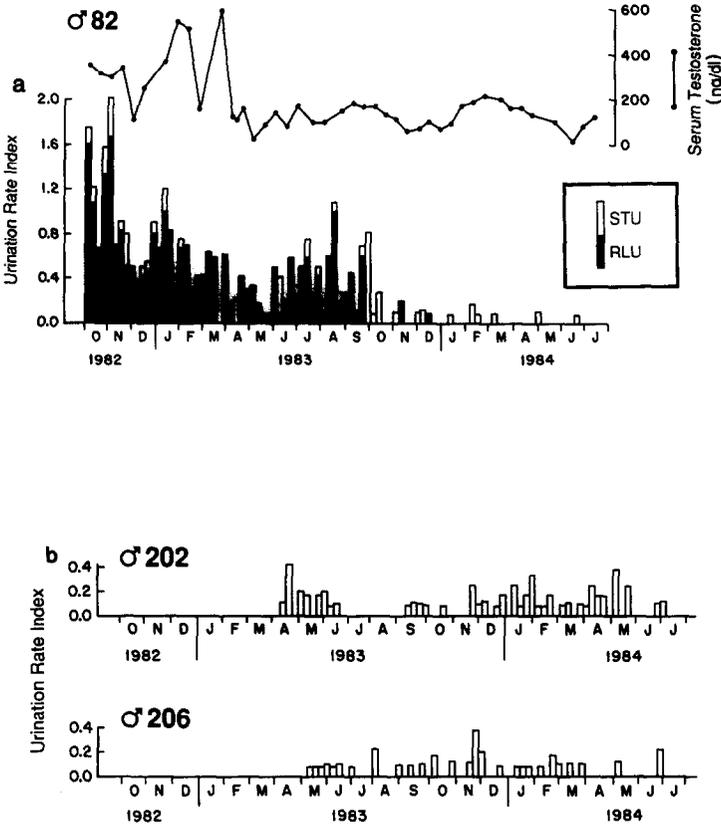


FIG. 3. Seasonal changes in serum hormone concentrations (males: LHRH-stimulated testosterone; females: estradiol and testosterone) and in weekly index of urination rate (males: RLU and STU; females: FLU and SQU) in South Pack wolves.

when she was beta (Fig. 1). Female 89, who clearly was dominant to 60 based on agonistic encounters (1981–1982), seldom urine-marked, possibly because she was anosmic (see Asa, Seal, Plotka, Letellier, and Mech, 1986). While she was dominant, female 60 urine-marked at a higher rate during the 1980–1981 season when the potential challenger, female 89, was present than during 1982–1983 following 89's death. Female 60 reassumed dominant status after 89's death without challenge.

In late 1980 and early 1981, 2-year-old males 85 and 88 began to urine-mark during their challenge to 11-year-old male 21 for dominance (Fig. 2). After the death of male 21, although 85 achieved alpha status, 88 continued for several months to contest 85's position.

#### *South Pack Wolves*

During the 1982–1983 breeding season, the urine-marking pattern of the dominant, adult pair (male 82, female 31) in the South Pack (Fig. 3)

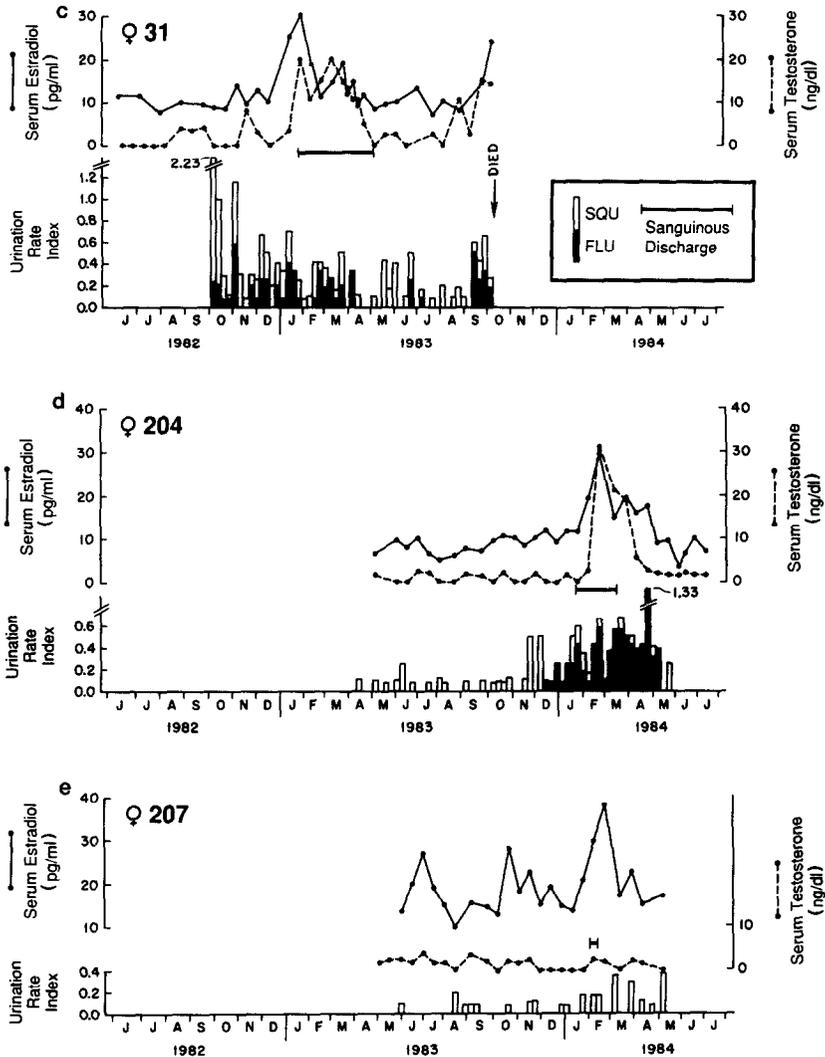


FIG. 3—Continued

was similar to that of the North Pack dominant pair, 85 and 60. However, following the death of female 31, adult male 82 ceased urine-marking or exhibiting other dominance-related behaviors such as a raised tail. Female 204 began urine-marking at age 20 months and assumed the dominant female position. Male 82's serum testosterone and urine-marking rate were elevated during the breeding season which encompassed late 1982 and early 1983 ( $P < 0.001$ ), but both remained depressed following his mate's death.

## DISCUSSION

Male wolves urine-marked (RLU) more frequently than did females (FLU), even during estrus. However, urination rates (male STU and female SQU) were not different for subordinate or prepubertal animals except at estrus when some females urinated more frequently. These results are similar to those for domestic dogs (Berg, 1944; Martins and Valle, 1948; Fox, Beck, and Blackman, 1975).

### *Role of Hormones*

Gonadal hormones appear to be necessary but not sufficient for stimulation of urine-marking in wolves. Evidence for a hormonal requirement is twofold: (1) urine-marking is not observed until after puberty, and (2) in dominant animals, seasonal changes in testosterone are accompanied by changes in urine-marking frequency.

The hypothesis for the endocrine requirement is supported by studies with domestic dogs. Prepubertal males stand and females squat to urinate (Berg, 1944; Martins and Valle, 1948; Beach, 1974) as do prepubertal and subordinate wolves (males: STU, females: SQU). Only at puberty does the RLU posture appear in male dogs (Martins and Valle, 1948), although prenatal, not pubertal, concentrations of testosterone may be sufficient (Ranson and Beach, 1985). Female dogs do not urine-mark before puberty, but because even postpubertal urine-marking is relatively uncommon in females (Martins and Valle, 1948; Sprague and Anisko, 1973), its onset has not been well-studied. However, in the female dog, urination frequency increases during estrus (Beach, 1974; Anisko, 1976) and following exogenous estrogen or testosterone (Beach, 1974).

The seasonal changes in urine-marking by both male and female dominant wolves were correlated with seasonal changes in testosterone. In contrast, no seasonal trend has been reported for urine-marking in the dog, perhaps because estrus can occur at any time of the year (Christie and Bell, 1971). Perhaps more importantly, concurrent testosterone is not necessary to support adult urine-marking in dogs: (1) neonatally castrated males adopt the RLU posture in adulthood (Ranson and Beach, 1985) and (2) adult castration is not followed by disappearance of the RLU posture or of sexual behavior, nor by decreased RLU rate (Beach, 1974; Hart, 1974). In the wolf, urine-marking may continue at a lower rate during summer. However, testosterone concentrations at that time failed to support a sexual response in males introduced to female wolves treated with exogenous hormones to induce estrus during the summer nonbreeding season. (Successful induction of estrus was indicated by the behavior of male dogs toward the treated female wolves: sniffing and licking of the vulva, mounting, and thrusting). These observations suggest that, in the male wolf, urine-marking might be less dependent on concurrent hormone support than is sexual response.

In two of three dominant, female wolves rates of urine-marking were correlated with testosterone. The lack of a significant correlation between these parameters for the third female may have been due to her limited data base. A transient increase in testosterone occurred in late summer/early fall in dominant females near the onset of seasonal increases in urine-marking. In all but female 204, who become dominant later in the year, the period of increased urine-marking commenced well before detectable increases appeared in estradiol associated with proestrus and estrus. These results suggest that, as with males, seasonal increases in urine-marking in dominant females are mediated by testosterone.

### *Role of Social Rank*

According to the domestic dog model, following puberty all male wolves should be physiologically stimulated to raised-leg urinate. In fact, only dominant males or those actively vying for dominance ever do. That virtually all adult male dogs RLU suggests that each considers itself alpha. Yet, even among adult male dogs, temporary reversions to the juvenile STU pattern may occur when the dog is frightened or maintained in confined quarters (Berg, 1944; Martins and Valle, 1948), suggesting some influence of social and situational factors.

Changes in social status in this study were accompanied by changes in urination posture for both males and females. Although hormone patterns within each sex were similar for dominant and subordinate animals, only those that were dominant urine-marked. The positive correlation between seasonal increases in testosterone and urine-marking rates in all three dominant males and two of three dominant females suggests hormonal influence on this behavior. The absence of urine-marking in subordinate wolves in spite of a comparable hormonal milieu indicates that subordinate status modulates endocrine stimulation. The absence of the seasonal testosterone rise in a dominant male following the death of his mate demonstrates that social factors also can influence gonadal hormones.

In contrast to wolves, all adult coyotes urine-mark, females as well as males (Barrette and Messier, 1980; Bowen and Cowan, 1980; Wells and Bekoff, 1981). Furthermore, although there are seasonal peaks, females as well as males urine-mark year-round (Wells and Bekoff, 1981). The differences in urine-marking patterns for dogs, coyotes, and wolves may well be related to their different social systems. The social organization of wolves is more complex, structured, and stable. This stability may be the result of a relatively rigid social hierarchy which is enforced in part by ritualized dominance displays such as the raised- and flexed-leg urine-marking postures. However, a proximate cause for restriction of urine-marking in dominant wolves is not apparent. Results of our

attempts to determine physiological correlates of dominance in wolves have been inconclusive.

Regardless of possible physiological influence, it appears that urine-marking in wolves is an important social symbol of self-assertiveness both toward other members of the pack and toward neighboring packs. Thus, it is not surprising that social factors tend to play such a pervasive role in mediating the behavior.

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