

# Behavioral Correlates of Physiological Estrus in Cheetahs

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Historically, the cheetah (*Acinonyx jubatus*) has been known for its poor reproductive performance in captivity. Although breeding success has improved over the past decade, the percentage of breeders in the captive population is still low and successful propagation unpredictable. Estrus in this species has been reported as “silent” by some, therefore contributing to breeding problems by making appropriate timing of pair introductions difficult. To investigate whether any observable behavioral changes may be associated with estrus, we carried out quantitative behavioral observations and concomitant noninvasive monitoring of estradiol metabolites excreted in feces of 14 captive female cheetahs for periods of 5–22 consecutive weeks. We found that changes in fecal estradiol concentrations correlated significantly with variation in the occurrence of several types of behaviors, including rolling, rubbing, sniffing, vocalizing, and urine spraying. However, the number and types of correlated behaviors varied across females, revealing no single behavior indicative of estrus, but rather a constellation of behaviors that increased in frequency when estradiol concentrations were elevated. There was no significant difference in the overall average estradiol concentrations or peak values of the females that had previously mated and conceived compared to those of the females that had failed to breed. Successful breeders appeared to show significantly higher rates of rubbing and rolling than nonbreeders. However, rates of rubbing, rolling, and urine spraying also were found to increase with age, and older individuals were more likely to have bred. The results of this study indicate that estrus in the cheetah cannot be regarded as “silent” since the frequencies of some behaviors appear to covary with fluctuating estradiol levels. However, behavioral monitoring of estrus may nevertheless be difficult and time-consuming due to individual variation and subtle changes in behavioral frequencies rather than changes in the types of behaviors displayed. Zoo Biol 17:193–209, 1998. © 1998 Wiley-Liss, Inc.

**Key words:** *Acinonyx jubatus*; estradiol; estrous behavior; fecal steroids

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**INTRODUCTION**

Cheetahs have always had a reputation of being difficult to breed. Although cheetahs have been held in captivity for >1,000 years, successful captive propagation is only a recent phenomenon [Divyabhanusinh, 1995]. Aside from one birth in 1613 at the court of the Mughal Emperor Jahangir [Divyabhanusinh, 1995], no further captive births were reported until 1956 at the Philadelphia Zoo [van de Werken, 1968; Marker and O'Brien, 1989; Sunquist, 1992]. It was not until the early 1970s that births began to occur regularly, albeit only at a few facilities [Marker and O'Brien, 1989; Wielebnowski, 1996].

Today, because of the cheetah's endangered status in the wild and an increasing emphasis on the role of captive propagation as a conservation tool [e.g., Gibbons et al., 1994; Olney et al., 1994], successful breeding has become obligatory. However, breeding records for the North American cheetah population show that, although rates of reproduction have increased in recent years [Grisham and Marker-Kraus, 1993], only a relatively small fraction of the adult captive population reproduces (e.g., only 14% in 1986 and 18% in 1991) [Marker and O'Brien, 1989; Marker-Kraus and Grisham, 1993]. Reproductive performance is still unreliable and the circumstances surrounding successes and failures in captive breeding are poorly understood. The captive cheetah population, therefore, cannot be regarded as self-sustaining. An improved understanding of the cheetah's reproductive behavior and physiology is needed to achieve more consistent reproductive performance in captivity and to increase the number of breeding individuals in the population. A recent physiological survey of the North American captive cheetah population found no differences in reproductive tract anatomy, ovarian activity, semen quality, or pituitary function between breeders and nonbreeders [Wildt et al., 1993]. Although the cheetah is known to exhibit an unusually high number of abnormally shaped sperm [Wildt et al., 1983, 1987], the relationship of this trait to overall fertility remains unknown. Despite high abnormal sperm counts, it has been documented that copulation of adult cheetah males with an estrous female will usually result in fertilization [Lindburg et al., 1993]. Behavioral and management factors are now believed to be the main contributors to successful reproduction of this species in captivity [Caro, 1993; Laurenson, 1993; Lindburg et al., 1993; Wielebnowski, 1996].

One major impediment to captive propagation of the cheetah has been the inability to reliably identify when females are in estrus for appropriate timing of breeding introductions [e.g., Foster, 1977; Sarri, 1992]. Since cheetahs are largely solitary, males and females are usually housed in separate enclosures and are introduced to each other only for mating. However, moving large carnivores between enclosures is time-intensive and often logistically difficult. Moreover, animals may suffer serious injuries during first-time breeding introductions [K. Hughes and S. Wells, pers. comm.]. Although anecdotal information is available on the reproductive behavior of zoo-maintained cheetah, few studies have tried to examine behavioral estrus in a quantitative and systematic manner for this species [Foster, 1977; Sarri, 1992; Graham et al., 1995; Sorenson, 1995; S. Wells, unpubl. data]. A key problem in the study of behavioral estrus has been the concurrent identification of physiological events associated with the observed behaviors.

The recent development of fecal steroid assays for assessing ovarian function in cheetahs [Gross, 1992; Möstl et al., 1993; Czekala et al., 1994; Brown et al.,

1994; Graham et al., 1995] makes it now possible to systematically study endocrine parameters in combination with behavior. Noninvasive fecal hormone monitoring avoids possible perturbations in hormone secretion and behaviors due to handling or restraint procedures. A recent study has revealed that although cheetahs are poly-estrous and exhibit estradiol excretion peaks at  $13.6 \pm 1.2$  (range 5–30 days) day intervals, reproductive cyclicity is punctuated by prolonged periods of ovarian inactivity that are unrelated to season [Brown et al., 1996]. Cheetahs, like most felids, appear to be primarily induced ovulators with ovulation occurring only after a copulatory stimulus [Bertschinger et al., 1984; Wildt et al., 1993; Graham et al., 1995; Brown et al., 1996]; spontaneous ovulation appears to be a rare event [Asa et al., 1992; Wildt et al., 1993; Czekala et al., 1994; Brown et al., 1996]. Estradiol levels fluctuate in nonmated females reflecting waves of follicular growth and regression, whereas progesterone concentrations remain at baseline until mating and subsequent ovulation occurs [Czekala et al., 1994; Graham et al., 1995; Brown et al., 1996]. Estrous, courtship, and mating behaviors are reported to be rather uniform across felid species and similar to that of the domestic cat [Ewer, 1973]. Common behaviors associated with estrus include rolling, object rubbing, vocalizing, pacing, locomotor activity, grooming, urination, urine spraying, investigative activity (e.g., sniff), and lordosis [Michael, 1961]. Descriptions of cheetah estrous behavior [Eaton and Craig, 1973; Eaton, 1974; Wrogemann, 1975] as well as anecdotal and qualitative reports from zoos [e.g., Florio and Spinelli, 1967; Grisham, 1988] confirm that typical signs of feline estrus also may occur in cheetahs. However, there appears to be considerable variability in the expression and occurrence of estrous behavior among individuals and it has been hypothesized that estrus may be “silent” in some females [Wildt et al., 1981; Sarri, 1992; Sorenson, 1995].

This study is unique in that we were able to monitor a comparatively large number of females for signs of behavioral estrus at several breeding facilities while simultaneously collecting near daily fecal samples for the analyses of ovarian activity reflected in estradiol excretion profiles. The project was designed to answer the following two questions: (1) do fluctuations in estradiol excretion patterns correlate with changes in the rates of occurrence of measured behavioral events for individual females? and (2) are there specific behaviors that consistently reflect increased estradiol concentrations in female cheetahs and may therefore serve as indicators of estrus?

## METHODS

### Animals

Fourteen captive-born, adult female cheetahs ranging from 2–12 years of age ( $5.2 \pm 0.7$  years) were studied (sexual maturity of female cheetahs is reached by ~2 yr of age [e.g., Eaton, 1974]). Animals were housed at three North American facilities: Sacramento Zoo, CA ( $n = 2$ ), White Oak Conservation Center, FL ( $n = 6$ ), and Wildlife Safari, OR ( $n = 6$ ). Females were kept in outdoor enclosures ranging from 450 m<sup>2</sup>–24,400 m<sup>2</sup> in size. Males and females were housed separately. However, females were frequently kept in enclosures adjacent to males. Two of the females in this study were housed together constantly (Studbook # 2440 and 2437), whereas several others (SB# 483, 2241, 270, 556, 2424, 2421, 508, 2439) were occasionally housed with other females during the study period. The remaining females (SB# 405, 2143, 1886, 586) were housed alone (Table 1). All animals were fed once a day

**TABLE 1. Studbook numbers, breeding status, age, facility, number of samples, and observations collected, and overall mean ( $\pm$  SEM) and range of fecal estradiol concentrations for female cheetahs**

Studbook #	Breeding status	Age (in years) at start of data collection	Facility	Study duration (wk)	Fecal samples collected (n)	Behavioral observations (hr)	Overall fecal estradiol (ng/g)	Fecal estradiol range (ng/g)
405	B	8	WO	5	31	20	82.1 $\pm$ 7.3	28.5–185.0
1886	B	4	WS	6	30	32	135.3 $\pm$ 17.3	47.3–408.1
483	B	8	WS	10	47	37	81.7 $\pm$ 5.4	35.9–211.5
586	B	6	WS	10	51	35	72.6 $\pm$ 5.6	26.0–231.3
2241	N	4	WS	10	54	35	66.3 $\pm$ 3.2	27.0–137.7
2439	ND	2	WO	10	59	30	55.5 $\pm$ 3.9	25.6–165.0
270	B	12	WO	10	62	30	80.1 $\pm$ 4.4	19.6–205.4
2440	B	3	WS	10	59	37	56.3 $\pm$ 2.6	17.9–114.4
2437	N	3	WS	10	59	37	79.5 $\pm$ 4.5	35.5–182.8
556	ND	5	WO	15	94	56	96.4 $\pm$ 5.7	31.5–323.1
2143	ND	5	WO	19	123	58	74.7 $\pm$ 3.4	20.5–200.9
508	B	7	WO	20	129	64	102.8 $\pm$ 4.9	29.8–336.7
2424	N	3	SZ	22	101	68	93.0 $\pm$ 5.2	27.6–390.3
2421	N	3	SZ	22	107	68	80.8 $\pm$ 3.8	28.8–229.1
Total	n.a.	5.2 $\pm$ 0.7	n.a.	179	1007	607	n.a.	n.a.

B, breeder; N, nonbreeder; ND, not determined; n.a., not applicable; SZ, Sacramento Zoo; WO, White Oak Conservation Center; WS, Wildlife Safari.

with one fast day each week. The diet consisted of Nebraska Canine (North Platte, NE) supplemented weekly with bones and horse ribs at the Sacramento Zoo and at the White Oak Conservation Center. Carcass meat (horse, cow, deer, chicken, turkey) supplemented with calcium and vitamins was fed at Wildlife Safari. Water was provided ad libitum.

### Behavioral Observations

All observations were conducted prior to feeding between 0700 and 1100 hr. Data collection at the Sacramento Zoo took place during the months of January, February, and March in 2 consecutive years for a total of 22 weeks per female (Table 1). At the White Oak Conservation Center, data collection occurred during the months September through November (SB# 405, for 5 weeks, SB# 508, for 10 weeks) and April through July (SB# 508, 2143, 2439, 270, 556, 2143, for 10 weeks each). Data at Wildlife Safari were collected between April and July for 10 weeks for each female (Table 1). Based on previous studies and captive breeding records, there appears to be no seasonality in reproduction of captive and free-ranging cheetahs, with successful breedings and physiological estrus occurring at all times of the year (Laurenson et al., 1992; Brown et al., 1996). Overall, each female was observed for 5–22 weeks, with observations carried out 3–5 times per week. Measured behaviors were chosen on the basis of previous descriptions of estrous behavior for the domestic cat [Michael, 1961] and for the cheetah [Florio and Spinelli, 1967; Eaton, 1970; Eaton and Craig, 1973; Eaton, 1974; Foster, 1977; Sarri, 1992; Sorenson, 1995]. Definitions of behaviors (Table 2) were formed during preliminary observations. Lordosis was deleted from the analysis because none of the observed females exhibited this behavior. Observations outside this study on breeding introductions revealed that lordosis only occurred when a soliciting male was present in the enclosure.

The senior author conducted behavioral observations at the White Oak Conservation Center and Wildlife Safari, whereas most observations at the Sacramento Zoo were carried out by two other trained observers. The interobserver reliability between the senior author and each of the observers was measured during five separate observation sessions using Spearman rank correlations. Correlation coefficients ranged from  $r_s = 0.88$  to  $r_s = 1.0$ ,  $n = 5$  (all  $P \leq 0.05$ ) for each of the 12 behaviors. A total of 607 hr of quantitative behavioral data were collected in combination with fecal data for all 14 females.

Observation periods lasted 1 hr and behavioral events (i.e., behaviors that usually are very brief in their duration; see Table 2) were recorded continuously, whereas behavioral states (i.e., behaviors that can continue for relatively long time periods; see Table 2) were recorded every 2 min using instantaneous time sampling (i.e., an observation period, in this case 1 hr, is divided into regular time intervals, in this case 2-min intervals. The behavioral state of the observed animal is recorded at the moment when the 2-min interval ends). This type of recording technique allows the observer to estimate the proportion of time spent in a particular state [Martin and Bateson, 1993]. The proportion of time spent in any one of the measured behavioral states during an observation period was then calculated by dividing the number of occurrences of the behaviors during the observation period by the number of instantaneous samples ( $n = 30$ ) taken.

**TABLE 2. Behavioral definitions**

States	
Pace	Repetitive walking or trotting (most often along fence line).
Active	Locomotor activity consisting of running, pacing, and walking.
Events	
Object rub	Rubs face, head, neck, or flanks on object (e.g., on fence, tree).
Roll	Rolls on back, rubbing the back on the ground while all paws are in the air, or rolls from one side to the other (each roll is then recorded as one occurrence).
Object sniff	Olfactory examination of ground (e.g., urine or feces) or structures.
Flehmen	Grimace with open mouth, wrinkled nose, retracted lips, and tongue may or may not protrude over lips. Head is raised. This behavior is commonly seen after olfactory investigation of urine or other scent marks.
Urine spray	Urinating in standing position with tail raised against a vertical structure (frequently trees or huts). Visually the same as male urine spraying; however, females are not able to spray directionally like males.
Meow-chirp	Meow: a soft call, low-pitched, similar to domestic cat. Occasionally chirps would be emitted together with meows. Chirps were more high-pitched than meows and very short. However these chirps were not as high-pitched, short, and loud as the chirps emitted when threatened.
Stutter	Soft, repetitive call usually emitted by an excited individual. Most commonly emitted by males during courtship.
Growl	Low-pitched, drawn-out "snarling" sound.
Groom	Self-grooming by licking or nibbling fur.
Tailflick	Moving tail vigorously from side to side while lying, sitting, standing, or walking.
Lordosis	Female copulatory posture. Female elevates hindquarters while resting most of the weight on her forepaws and chest. Tail is usually averted to one side.

### Fecal Sample Collection and Steroid Analysis

Fecal samples were collected 4–7 times a week for periods of 5–22 weeks (Table 1). When two females were housed together, 2 ml of green food color (Schilling, McCormick & Co.) were added to the daily diet of one of the females. Dyed feces could be reliably distinguished after 12–18 hr.

Samples were collected between 0700 and 1700 hr and were known to be from the same day since enclosures were cleaned of all fecal matter on a daily basis. The samples were placed into 8 × 13-cm zip-lock bags and put into an ice cooler (~4°C). Within 2 hr of collection, the samples were transferred into a freezer (-20°C) and subsequently transported on dry ice to the University of California, Davis, where they were lyophilized and pulverized to eliminate varying sample consistency and moisture content. Dried feces then were placed into 12 × 75-mm conical polypropylene vials (Perfector Scientific: #2382) and transported on ice to the Conservation and Research Center, National Zoological Park, Smithsonian Institution (Front Royal, VA) for estradiol metabolite analysis according to a protocol previously detailed by Brown et al. [1994, 1996]. To summarize the procedure briefly, ~0.2 g of each well-mixed pulverized fecal sample were weighed out and boiled in 5 ml aqueous ethanol 90% for 20 min. After centrifuging at 500 g for 10 min, the supernatant was recovered and the pellet resuspended in 5 ml 90% ethanol, vortexed for 1 min and re-centrifuged. Both ethanol supernatants were then combined, dried completely, and redissolved in 1 ml methanol. The resulting extractants were vortexed for 1 min, placed into an ultrasonic cleaner for 30 sec, and revortexed (15 sec). Samples

were diluted (1:40) in phosphate-buffered saline prior to analysis. Estradiol metabolite concentrations were quantified using a radioimmunoassay validated for cheetahs as described by Brown et al. [1994]. The assay relied upon an antibody provided by S. Wasser (Center for Wildlife Conservation, Seattle, WA) (Risler et al., 1987), a  $^3\text{H}$ -labeled estradiol tracer (New England Nuclear, Boston, MA) and estradiol standards. The assay specifically quantified estradiol with minimal crossreactivity ( $\leq 2\%$ ) with other fecal estrogen metabolites (estradiol sulfate and estrone). Assay sensitivity based on 90% of maximum binding was 5 pg per tube. Intra- and interassay coefficients of variation were  $<10\%$ . All fecal data are expressed as ng per g dry fecal weight.

### Age and Breeding Status

Females were defined as having bred (breeder) if they had previously produced offspring, or failed to breed (nonbreeder) after having been introduced to a male repeatedly (on at least three different occasions) with introduction periods of at least 1 hr each. However, all females identified as nonbreeders in this study had been introduced to at least two different males on numerous occasions ( $>15$  introductions) and in some cases where fecal samples had been collected during such introductions, elevated estradiol concentrations indicated estrus could be detected when males were present. Three females had never been introduced to a male (SB# 2439, 2143, 556) and were therefore excluded from any subsequent analysis investigating the relationship of breeding status and behavioral events, and breeding status and estradiol levels. Seven females had produced offspring previously, whereas four had not mated or produced offspring (Table 1). Age was measured in years at the time when data collection started for each female. It should be noted that the chosen sample of females was not highly skewed toward any specific age (Table 1).

### Statistics

Little is known about assumed lag-times between fecal hormone concentrations and the occurrence of any associated behaviors. Due to variation in metabolic rate, diet composition and health status, lag-time might be expected to vary among and within individuals [e.g., Shille et al., 1990; Brown et al., 1994]. In this study, behavioral data were collected 3–5 times a week and fecal data 4–7 times a week, varying per female and week. Thus to allow for comparison of all females, weekly averages were calculated for estradiol values and number of behavioral occurrences. Data are presented as mean  $\pm$  SEM. Spearman rank-order correlation coefficients,  $r_s$ , were used to test for association between weekly estradiol values and rates of behavioral occurrences within females and to determine the association between estradiol concentrations, rates of behavioral occurrences, and age across females [Siegel and Castellan, 1988]. A Mann-Whitney U-test was used to compare breeders versus nonbreeders [Siegel and Castellan, 1988]. All analyses were carried out using the software program StatviewSE & Graphics [Abacus Concepts, 1988].

## RESULTS

### Estradiol Concentrations and Behavioral Measures

When individual females were examined, weekly estradiol fluctuations were found to correlate significantly with changes in weekly average rates of behavioral occurrences for 9 of the 12 behaviors measured (Table 3, Fig. 1). The behaviors that

**TABLE 3. Spearman rank-order correlations between weekly estradiol concentrations and weekly rates of behavioral occurrences for female cheetahs**

Stud book #	Behaviors												No. of significant correlations per female
	Object sniff	Roll	Meow- chirp	Rub	Urine spray	Stutter	Flehmen	Growl	Groom	Tailflick	Pace	Active	
405 N = 5	r=0.01 P=0.84	r=1.0 P=0.05*	r=0.18 P=0.73	r=0.90 P=0.07	r=0.95 P=0.05*	-	r=0.40 P=0.42	-	r=0.03 P=0.96	r=0.90 P=0.07	r=0.30 P=0.55	r=0.10 P=0.84	2
1886 N = 6	r=0.89 P=0.05*	r=0.03 P=0.95	r=0.89 P=0.05*	r=0.26 P=0.58	r=0.03 P=0.95	-	r=0.66 P=0.14	r=0.60 P=0.18	r=0.53 P=0.24	r=0.83 P=0.06	r=0.22 P=0.61	r=0.14 P=0.75	2
483 N = 10	r=0.21 P=0.53	r=0.09 P=0.80	r=0.15 P=0.66	r=0.07 P=0.84	r=0.77 P=0.02*	r=0.46 P=0.17	r=0.32 P=0.34	-	r=0.39 P=0.24	r=0.23 P=0.48	r=0.44 P=0.19	r=0.65 P=0.05*	2
586 N = 10	r=0.68 P=0.04*	r=0.71 P=0.03*	r=0.08 P=0.81	r=0.25 P=0.46	r=0.75 P=0.03*	-	r=0.20 P=0.55	r=0.42 P=0.20	r=0.48 P=0.15	r=0.32 P=0.34	r=0.07 P=0.83	r=0.17 P=0.62	3
2241 N = 10	r=0.12 P=0.73	r=0.72 P=0.03*	-	r=0.21 P=0.53	r=0.62 P=0.07	-	r=0.29 P=0.40	-	r=0.42 P=0.20	r=0.25 P=0.46	r=0.37 P=0.27	r=0.32 P=0.33	1
2439 N = 10	r=0.24 P=0.49	r=0.78 P=0.02*	r=0.13 P=0.70	r=0.83 P=0.01**	-	-	r=0.51 P=0.12	r=0.21 P=0.53	r=0.22 P=0.51	r=0.14 P=0.68	r=0.02 P=0.96	r=0.25 P=0.34	2
270 N = 10	r=0.85 P=0.01**	r=0.70 P=0.04*	r=0.16 P=0.64	r=0.54 P=0.11	r=0.65 P=0.05*	-	-	r=0.10 P=0.76	r=0.49 P=0.14	r=0.62 P=0.06	r=0.09 P=0.79	r=0.09 P=0.79	3



2440 N = 10	$r=0.76$ $P=0.03^*$	$r=0.29$ $P=0.39$	$r=0.27$ $P=0.42$	$r=0.36$ $P=0.28$	$r=0.24$ $P=0.48$	–	$r=0.33$ $P=0.32$	–	$r=0.23$ $P=0.49$	$r=0.23$ $P=0.48$	$r=0.48$ $P=0.15$	$r=0.25$ $P=0.46$	1
2437 N = 10	$r=0.77$ $P=0.02^*$	$r=0.52$ $P=0.12$	$r=0.03$ $P=0.93$	$r=0.44$ $P=0.18$	$r=0.83$ $P=0.01^{**}$	–	$r=0.04$ $P=0.90$		$r=0.20$ $P=0.56$	$r=0.19$ $P=0.57$	$r=0.29$ $P=0.39$	$r=0.40$ $P=0.23$	2
556 N = 15	$r=0.07$ $P=0.80$	$r=0.42$ $P=0.12$	$r=0.53$ $P=0.05^*$	$r=0.75$ $P=0.005^{**}$	$r=0.33$ $P=0.21$	–	$r=0.29$ $P=0.29$	$r=0.27$ $P=0.32$	$r=0.09$ $P=0.75$	$r=0.37$ $P=0.17$	$r=0.03$ $P=0.91$	$r=0.16$ $P=0.56$	2
2143 N = 19	$r=0.11$ $P=0.65$	$r=0.36$ $P=0.12$	$r=0.51$ $P=0.03^*$	$r=0.33$ $P=0.16$	$r=0.44$ $P=0.06$	$r=0.51$ $P=0.03^*$	$r=0.04$ $P=0.85$	$r=0.53$ $P=0.03^*$	$r=0.10$ $P=0.67$	$r=0.31$ $P=0.19$	$r=0.14$ $P=0.55$	$r=0.16$ $P=0.52$	3
508 N = 20	$r=0.59$ $P=0.01^{**}$	$r=0.68$ $P<0.01^{**}$	$r=0.54$ $P=0.02^*$	$r=0.85$ $P<0.001^{***}$	$r=0.31$ $P=0.17$	$r=0.53$ $P=0.02^*$	$r=0.23$ $P=0.32$	$r=0.56$ $P=0.01^{**}$	$r=0.21$ $P=0.36$	$r=0.15$ $P=0.52$	$r=0.15$ $P=0.51$	$r=0.34$ $P=0.14$	6
2424 N = 22	$r=0.51$ $P=0.02^*$	$r=0.69$ $P<0.01^{**}$	$r=0.46$ $P=0.04^*$	$r=0.73$ $P=0.001^{***}$	–	$r=0.54$ $P=0.02^*$	$r=0.61$ $P<0.01^{**}$	–	$r=0.07$ $P=0.77$	$r=0.22$ $P=0.32$	$r=0.21$ $P=0.23$	$r=0.28$ $P=0.35$	6
2421 N = 22	$r=0.56$ $P<0.01^{**}$	$r=0.40$ $P=0.06$	$r=0.77$ $P<0.001^{***}$	$r=0.74$ $P<0.001^{***}$	–	$r=0.27$ $P=0.20$	$r=0.52$ $P=0.02^*$	–	$r=0.18$ $P=0.40$	$r=0.01$ $P=0.98$	$r=0.25$ $P=0.22$	$r=0.28$ $P=0.21$	3
no. of significant corr. per behavior	8	7	6	5	5	3	2	2	0	0	0	0	

Shaded areas indicate significant  $P$  values: \* $P\leq 0.05$ , \*\* $P\leq 0.01$ , \*\*\* $P\leq 0.001$

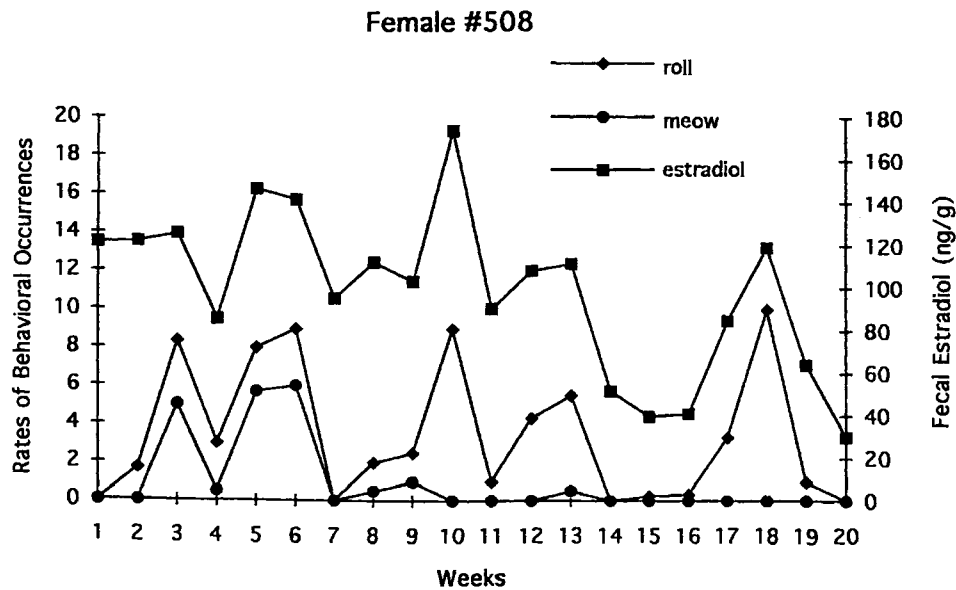
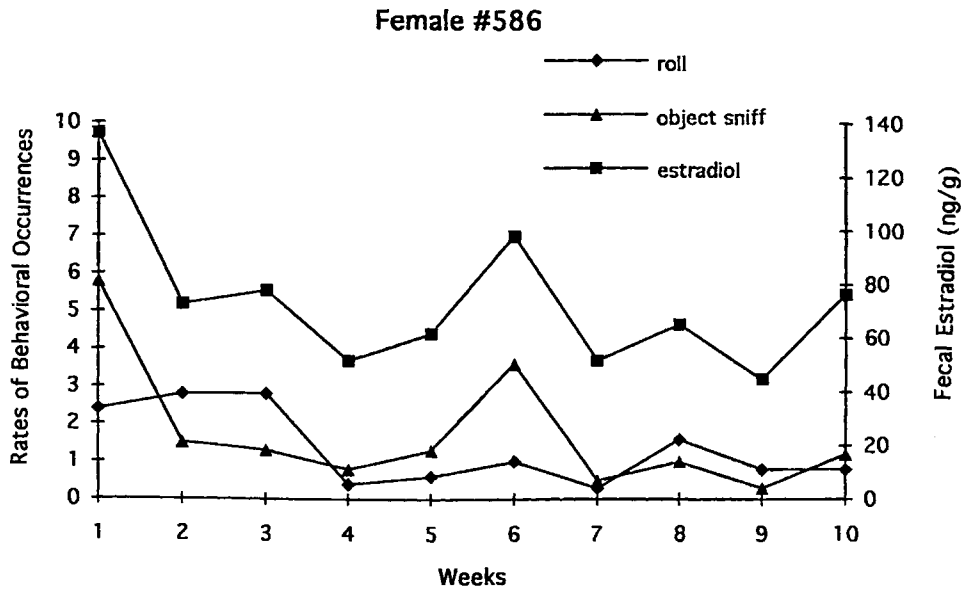


Fig. 1. Examples of behaviors, for which weekly average occurrence rates correlated positively and significantly ( $P \leq 0.05$ ) with fluctuations in weekly average estradiol concentrations, are shown for two females.

were most often significantly and positively associated ( $P < 0.05$ ) with increased estradiol concentrations were “object sniff” (8 of 14 females) and “roll” (7 of 14 females). An increase in the occurrence of the behavioral events “meow-chirp,” “rub,” and “urine spray” was also frequently (in at least 5 females; Table 3) significantly correlated with increased estradiol concentrations. The events “active,” “flehmen,” “growl,” and “stutter” were correlated with estradiol concentrations in only a few ( $\leq 3$ ) females (Table 3). Although significantly correlated behaviors varied for each female, some behaviors consistently showed no significant correlation with fluctuating estradiol: “groom,” “tailflick,” and “pace.” The trait “active” showed only one significant, but negative, correlation with estradiol concentration.

Several females never exhibited some of the measured behaviors: “flehmen,” “urine spray,” “meow-chirp,” “stutter,” and “growl” (Table 3). Only 5 of the 14 females stutter-called and only half of the females growled.

Overall, females displayed between one and six behaviors significantly correlated with estradiol. The same types of behaviors repeatedly were found to be significant, only in varying combinations for each female (e.g., female #405: “roll” and “urine spray”; #586 and #270: “object sniff,” “roll,” and “urine spray”; #2437: “object sniff” and “urine spray”).

### Comparisons Across Females: Average Estradiol Values, Average Rates of Behavioral Occurrences, Breeding Status, and Age

Across all females average estradiol concentrations did not differ with age (mean estradiol:  $r_s = 0.36$ ,  $P = 0.20$ ; estradiol peak:  $r_s = 0.12$ ,  $P = 0.68$ ) or breeding status (estradiol (ng/g): breeders  $87.3 \pm 9.6$ , nonbreeders  $799 \pm 5.5$ ,  $U = 11$ ,  $P = 0.57$ ; estradiol peak values (ng/g): breeders  $241.7 \pm 37.3$ , nonbreeders  $234.9 \pm 55.0$ ,  $U = 12$ ,  $P = 0.71$ ) (Table 1). However, the rates of several behavioral events were found to correlate significantly and positively with age and breeding status. The behaviors “rub,” “roll,” and “urine spray” showed a significant and positive correlation with age for all females combined (Fig. 2), and significant differences in the rates of “rub” and “roll” were found between breeders ( $n = 7$ ) and nonbreeders ( $n = 4$ ) (rub: average weekly rates of occurrences in breeders  $2.3 \pm 0.6$  and in nonbreeders  $0.6 \pm 0.2$ ,  $U = 2$ ,  $P < 0.05$ ; roll: breeders  $2.2 \pm 0.5$ , nonbreeders  $0.7 \pm 0.2$ ,  $U = 3$ ,  $P < 0.05$ ).

When the association of age and the above behavioral rates was examined in breeders and nonbreeders only (excluding the three females with undetermined breeding status), the strong and significant association between age and the rates of “rub,” “roll,” and “urine spray” remained (rub:  $r_s = 0.85$ ,  $n = 11$ ,  $P < 0.01$ ; roll:  $r_s = 0.88$ ,  $n = 11$ ,  $P < 0.01$ ; urine spray:  $r_s = 0.77$ ,  $n = 11$ ,  $P = 0.02$ ), suggesting a relatively strong effect of age rather than breeding status. Testing for age differences between breeders and nonbreeders did not show significance. However, most nonbreeders in this study were young animals (nonbreeders  $3.8 \pm 0.8$  yr, breeders  $6.6 \pm 1.2$  yr) and the results of the test bordered on significance ( $U = 4.5$ ,  $n = 11$ ,  $P = 0.07$ ).

## DISCUSSION

Estradiol is known to play an essential role in the control of estrous behavior in female mammals [Morali and Beyer, 1979; Bronson, 1989], and plasma concentrations of estradiol have been correlated with the display of sexual behaviors for many felid species (e.g., domestic cat, *Felis catus* [Shille et al., 1979; Wildt et al., 1981;

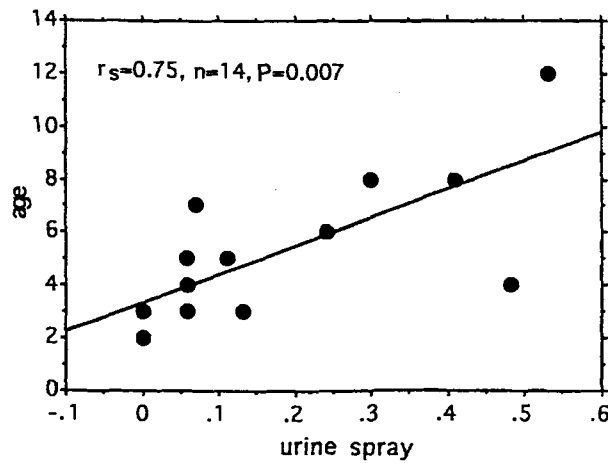
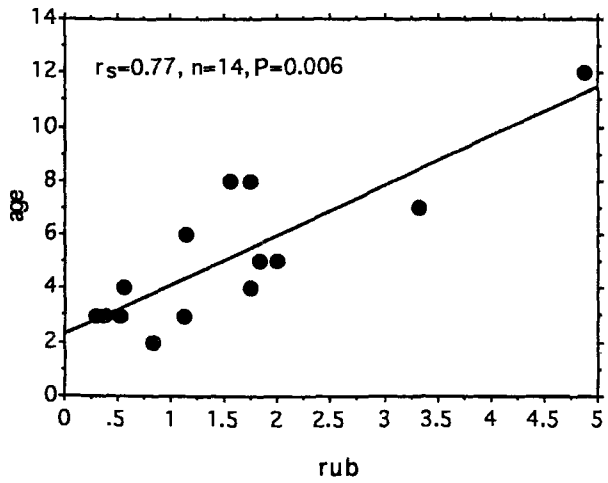
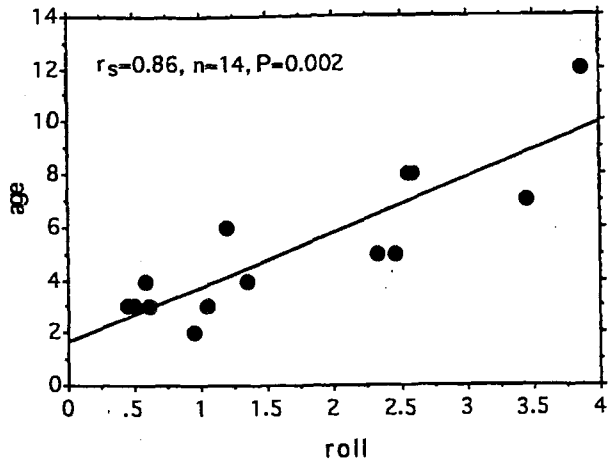


Fig. 2. The association between age (in years) and the occurrence of the behavioral events, "roll," "rub," and "urine spray" (measured as mean frequency of behavioral occurrences during the observation period for each animal) for all observed cheetah females ( $n = 14$ ) is depicted. Spearman rank-order correlation coefficients and P-values for each association are presented.

Goodrowe et al., 1989); jaguar, *Felis onca* [Wildt et al., 1979]; puma, *Felis concolor* [Bonney et al., 1981]; Siberian tiger, *Panthera tigris altaica* [Seal et al., 1987]; snow leopard, *Felis uncia* [Schmidt et al., 1993]).

This study showed that several of the behaviors associated with typical feline estrus covary with estradiol concentrations in female cheetahs and may therefore provide indicators of physiological estrus. For each of the females in this study, there was a significant correlation between elevated fecal estradiol concentrations and an increased occurrence of at least one of the following behaviors: "roll," "rub," "object sniff," "meow-chirp," and "urine spray." All of these behaviors have been noted in anecdotal accounts of cheetah estrus [Florio and Spinelli, 1967; Eaton and Craig, 1973; Eaton, 1974; Brand, 1980]. A few quantitative studies have also been able to detect significant correlations between the frequencies of some behaviors and either verified or suspected physiological estrus. For example, one study associating physiological and behavioral measures found a significant and positive correlation between nuclear epithelial cell count measured in vaginal swabs and "roll" and "back rub" in one female [Asa et al., 1992; Sarri, 1992], whereas another study found the rate of vocalization to be significantly and positively correlated with increased fecal estradiol concentrations for two females [Graham et al., 1995]. Two additional studies reported an increase in the behaviors rubbing, rolling, investigating (i.e., sniffing), vocalizing, and urine marking during suspected estrus [Foster, 1977; Sorenson, 1995]. In the wild, increased rates of urine-marking have also been observed in connection with cheetah estrus [Eaton, 1970; Frame and Frame, 1981]. Several other behaviors found to be positively associated with estrus by some observers were self-grooming [Foster, 1977; Sarri, 1992], tail-flicking [Herdman, 1973], and increased locomotor activity [Benzon and Smith, 1977; Sarri, 1992]. However, this study did not reveal any significant positive correlation with estradiol fluctuations for any of these three behaviors. Increase in locomotor activity has been associated with estrus in several mammalian species (e.g., the rat, *Rattus sp.* [Geral et al., 1973]; cow, *Bos taurus* [Kiddy, 1977]; pig, *Sus scrofa* [Signoret et al., 1975]; and domestic cat, *Felis catus* [Leyhausen, 1979]), and these behaviors have reportedly been linked with females seeking males [Morali and Beyer, 1979]. Contrary to such findings, activity level in this study was found to be significantly and negatively correlated with estradiol concentrations for one female and not significantly correlated for all other females.

All significantly correlated behaviors observed in this study also have been listed as proceptive behaviors for other female mammals, i.e., behaviors that initiate the establishment or maintenance of male sexual interaction [Beach, 1976]. Increased frequency of marking by rubbing skin areas containing odiferous glands, or increased rates of urination have been reported for rodents [Calhoun, 1962; Reynolds, 1971], ungulates [Fraser, 1968], canids [Kleiman, 1966], and primates [Epple, 1970], and increased frequency of vocalizations have been observed in felids [Michael, 1961], rodents [Floody et al., 1977], and ungulates [Fraser, 1968]. Since felids possess odiferous glands on the chin, lips, cheeks, and above the base of the tail among other areas [Kitchener, 1991], rubbing of any of these body parts on objects will lead to scent marking. Urine spraying in female felids, as opposed to the more usual squat urination, also may serve as an advertisement to conspecifics [Ewer, 1973]. Therefore, an increase in scent marking behaviors ("rub," "roll," "urine spray") and/or vocalizations ("meow-chirp") positively as-

sociated with estradiol concentrations, may represent an increase in female advertisement to attract males.

The significant positive correlation found between age and the behaviors "roll," "rub," and "urine spray" for captive female cheetahs appears to agree with previous studies in other species, since experience and age have been found to alter the display and intensity of estrous behavior in some mammals [Thompson and Edwards, 1971; Cooper, 1977]. All three behaviors, "rub," "roll," and "urine spray," may represent olfactory advertisements and may be particularly effective in eliciting male attention and arousal. The significant differences in the rates of "roll" and "rub" observed between breeders and nonbreeders may be largely related to age. Most nonbreeders in this study were relatively young animals and the association between age and these behaviors was strong and remained when breeders and nonbreeders alone were examined. However, further studies are needed to allow adequate testing of the possible origin of such differences in the rates of behavioral occurrences.

This study suggests that regular quantitative monitoring of a chosen set of behavioral events commonly associated with estrus in several other felid species may also serve as an indicator of estradiol fluctuations in cheetahs. Long-term quantitative recordings of the frequencies of "roll," "rub," "object sniff," "urine spray," and "meow-chirp," may allow us to identify increases in estradiol levels, and therefore provide a visual identification of the estrous state in female cheetahs. Continued studies over prolonged time-periods tracking both hormones and behaviors on a daily basis are required to further test the predictive ability of these behaviors for identifying cheetah estrus and for investigating potential causes of the behavioral variability found among individual females.

## **CONCLUSIONS**

1. Increases in estradiol concentrations were positively and significantly correlated with frequencies of several behavioral events ("rub," "roll," "object sniff," "meow-chirp," and "urine-spray") in captive female cheetahs.

2. There was considerable interindividual variation in the number and types of behaviors that correlated significantly with fluctuations in estradiol concentrations for each female, making it impossible to identify one or two behaviors that would provide sure signs of estrus for all females.

3. Increased age was found to be significantly and strongly associated with an increase in the rate of occurrence of three behaviors ("roll," "rub," and "urine spray"), and significantly higher rates of rubbing and rolling were measured for breeders versus nonbreeders. However, most breeders were older individuals and the behavioral differences found may be largely related to age rather than breeding status.

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