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Abstract: Cheetahs (Acinonyx jubatus) held ex situ can provide an important resource for obtaining new biological information that usually cannot be gleaned from free-living individuals. However, consistent captive propagation of the cheetah, a prerequisite for establishing a self-sustaining population, has not been accomplished so far. This study examined the effect of a husbandry regimen commonly used in ex situ facilities on female cheetahs. Although generally solitary in the wild, zoos frequently house cheetahs in pairs or groups. Using non-invasive hormone monitoring and quantitative behavioural observations, we studied the impact of such enforced social conditions on behaviour and ovarian/adrenal activity. Eight female cheetahs were evaluated for two consecutive 6-month periods, first while maintained in pairs and then as individuals. Subsequently four females were regrouped into two new pairs and monitored for another 6 months. Females in five of six pairings demonstrated prolonged anoestrus and displayed agonistic behaviours. After pair separation all females rapidly resumed oestrous cyclicity. Females in the sixth pair continued cycling throughout the year while consistently displaying affiliative grooming and no agonistic behaviours. Faecal corticoid patterns varied significantly among individuals, but appeared unrelated to behavioural or ovarian hormone patterns. Thus, data appear to indicate that same-sex pair-maintenance of behaviourally incompatible female cheetahs may lead to suppressed ovarian cyclicity. This suppression appears linked to agonistic behaviours but not to any particular adrenal hormone excretion pattern. Results clearly demonstrate the value of applying knowledge about in situ social behaviour to ex situ management practices. Conversely, however, non-invasive hormone monitoring conducted ex situ may help us to identify physiological phenomena of potential relevance for future in situ studies.

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Impact of social management on reproductive, adrenal and behavioural activity in the cheetah (*Acinonyx jubatus*)

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Abstract

Cheetahs (*Acinonyx jubatus*) held *ex situ* can provide an important resource for obtaining new biological information that usually cannot be gleaned from free-living individuals. However, consistent captive propagation of the cheetah, a prerequisite for establishing a self-sustaining population, has not been accomplished so far. This study examined the effect of a husbandry regimen commonly used in *ex situ* facilities on female cheetahs. Although generally solitary in the wild, zoos frequently house cheetahs in pairs or groups. Using non-invasive hormone monitoring and quantitative behavioural observations, we studied the impact of such enforced social conditions on behaviour and ovarian/adrenal activity. Eight female cheetahs were evaluated for two consecutive 6-month periods, first while maintained in pairs and then as individuals. Subsequently four females were regrouped into two new pairs and monitored for another 6 months. Females in five of six pairings demonstrated prolonged anoestrus and displayed agonistic behaviours. After pair separation all females rapidly resumed oestrous cyclicity. Females in the sixth pair continued cycling throughout the year while consistently displaying affiliative grooming and no agonistic behaviours. Faecal corticoid patterns varied significantly among individuals, but appeared unrelated to behavioural or ovarian hormone patterns. Thus, data appear to indicate that same-sex pair-maintenance of behaviourally incompatible female cheetahs may lead to suppressed ovarian cyclicity. This suppression appears linked to agonistic behaviours but not to any particular adrenal hormone excretion pattern. Results clearly demonstrate the value of applying knowledge about *in situ* social behaviour to *ex situ* management practices. Conversely, however, non-invasive hormone monitoring conducted *ex situ* may help us to identify physiological phenomena of potential relevance for future *in situ* studies.

INTRODUCTION

Wild cheetah (*Acinonyx jubatus*) populations are in decline throughout their native range, with probably fewer than 15,000 individuals remaining in Africa (Kraus & Marker-Kraus, 1992; Caro, 1994; Nowell & Jackson, 1996). The cheetah is well known for several of its fascinating biological features, including low amounts of genetic variation (O'Brien *et al.*, 1983) and consistently high proportions of pleiomorphic spermatozoa (Wildt *et al.*, 1983, 1987). Although these and other biological phenomena have been studied in free-living cheetahs, many explicit characterizations simply cannot be undertaken *in situ* for logistical reasons. Therefore, *ex situ* populations can serve as an important resource for controlled studies into mechanisms that are likely to be relevant to both *in situ* and *ex situ* conditions.

Interestingly, cheetahs held *ex situ* are well known to reproduce poorly (Lindburg *et al.*, 1993; Marker-Kraus & Grisham, 1993), unlike their counterparts in nature (Laurenson, Caro & Borner, 1992; Caro, 1994). In the case of the cheetah, we are interested in linking *in situ* scholarly knowledge to help produce a self-sustaining *ex situ* population. Such a strategy supports free-living cheetahs by (1) developing viable hedge populations as 'ambassadors' for conservation, (2) educating the public about the plight of wild counterparts and (3) establishing a 'research resource' for investigating phenomena that cannot be examined in individuals ranging in nature. The cause of poor reproduction in captivity has been studied extensively, and it is now apparent that there is no health, genetic, nutritional or other physiological difference between breeding and non-breeding

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cheetahs (*Zoo Biology* **12**, Special Issue on Cheetahs, 1993; Wildt *et al.*, 1993; Caro, 1994). Instead, inappropriate husbandry and management techniques are regarded as prime factors contributing to low breeding success (Lindburg *et al.*, 1993; Caro, 1994; Brown *et al.*, 1996).

In the wild, female cheetahs are usually solitary except when accompanied by dependent young (Caro, 1994). In contrast, most *ex situ* facilities keep females in pairs or groups since overt aggression is rarely observed. This husbandry strategy maximizes space utilization while providing perhaps a more interesting exhibit for the public. However, inappropriate social conditions are known to lead to social stress and adrenal activation in other species, which, in turn, may compromise the immune system and/or reproduction (Moberg, 1985; Von Holst, 1998; Mendoza, Capitanio & Mason, 2000; Jasnow *et al.*, 2001). Also, crowding and encounters with even moderately aggressive conspecifics can lead to chronic stress as evidenced by elevated blood cortisol levels (Fraser & Broom, 1990; Mendoza, 1991; Von Holst, 1998; Jasnow *et al.*, 2001). Jurke *et al.* (1997) found higher faecal corticoid concentrations in female cheetahs that were reproductively inactive compared to females that cycled regularly. Terio & Munson (2000) recently found that gastritis and other non-infectious diseases in zoo-held cheetahs may be correlated with elevated glucocorticoid excretion in faeces.

In an extensive survey of the North American *ex situ* cheetah population, it was determined that most adult females had normal reproductive tract anatomy and pituitary function (Wildt *et al.*, 1993). Yet, more than 50% of females appeared acyclic on the basis of observing inactive ovaries (via laparoscopy) and parallel, one-time measurements of low circulating estradiol. A follow-up study longitudinally assessed follicular (oestrogen) and luteal (progestogen) activity by means of faecal steroid analysis in 26 females for up to 24 months (Brown *et al.*, 1996). Twenty-five per cent of female cheetahs failed to exhibit any ovarian activity, and, of the 75% that did, none cycled continuously. Rather, cyclicity was interrupted by prolonged periods of anoestrus, often months in duration. No obvious environmental or seasonal factor was associated with this sporadic reproductive activity.

However, most of the cheetahs evaluated in the latter two studies were housed in pairs or groups. We therefore speculated that cohabitation might have a suppressive effect on reproductive function. In the current study we test the hypothesis that housing female cheetahs together suppresses ovarian activity and that this effect is reflected by parallel increases in agonistic behaviours and glucocorticoid excretion (as an index of adrenal activation, or 'stress'). A secondary priority was to test the utility of faecal corticoid excretion as an index of stress in an intensively managed, controlled environment. Faecal corticoid monitoring has been touted as useful for assessing adrenal activity of animals in captivity (e.g. Palme & Möstl, 1997; Schatz & Palme, 2001; Wielebnowski *et al.*, 2002) and in nature (e.g. Wasser *et al.*, 1997; Wasser *et al.*, 2000; Foley, Papageorge & Wasser, 2001; Harper & Austad, 2001). Similarly, non-invasive monitoring of reproductive hormones has been identified as a valuable conservation tool for investigating ecological parameters in free-ranging populations (Berger *et al.*, 1999). Thus, we speculated that this study may provide new information on the potential of non-invasive hormone monitoring for future studies of wild cheetahs, including assessing the impact of ecological variables and human and environmental disturbance on health and reproduction.

METHODS

Animals and facilities

Study animals were eight captive-born, adult female cheetahs (mean ± SEM, 7.5 ± 2.6 years of age; range, 3.5–11.9 years) maintained at the White Oak Conservation Center (Yulee, Florida, USA), a private facility closed to the public. At study onset, the Center maintained a total of eight male and ten female cheetahs in large outdoor chainlink enclosures. For this investigation, single and paired females were maintained in enclosures of about 2000 m^2 that were immediately adjacent or in visual proximity to female conspecifics. All enclosures had a grass substrate and mature trees, were similar in design, and offered equal numbers and quality of hiding opportunities. The diet was a commercial, ground-horsemeat-based product (Nebraska Brand Canine Diet; Central Nebraska Packing Co., North Platte, Nebraska, USA) supplemented occasionally with venison and ox tails. Animals were fed once daily, and water was provided *ad libitum*.

Experimental design

Each female was housed separately for at least 2 weeks before study onset and then randomly assigned to a pair. Once established, pairs were maintained for 6 months, then the females were separated and housed alone for 6 months. At the end of the second 6-month period, four of the females were regrouped to form two new pairs for another 6 months of monitoring. Thus, the entire study comprised six different pairs (Table 1). Two were mother–daughter pairs (pairs 2 and 3), and all others were unrelated. Enclosures housing study females were dispersed randomly throughout the cheetah facility. None of the females (pairs or singletons) was completely isolated from other male and female conspecifics, and

Table 1. List of study females and pairings

Pairs and identification numbers	Studbook numbers	Months spent as pair
Pair 1: CH-1 & CH-2	2124 & 508	July–January
Pair 2: CH-3 & CH-4*	405 & 2983	July–January
Pair 3: CH-5 & CH-6*	1902 & 2920	July–January
Pair 4: CH-7 & CH-8	1907 & 1834	July–January
Pair 5: CH-5 & CH-7	1902 & 1907	June–December
Pair 6: CH-6 & CH-8	2920 & 1834	June–December

* Mother–daughter pairs

all females had visual, auditory and occasional olfactory contact with at least one adult male. However, no direct physical interaction occurred between males and females during the study.

Behavioural data collection

Quantitative behavioural data were collected twice weekly on each female in 30-minute observation sessions between 0700–1100 hours. During intervals of paired housing, data were collected on both females simultaneously. Types and definitions of recorded behaviours were consistent with previously published ethograms (Sorensen, 1995; Wielebnowski & Brown, 1998). Continuous time sampling was used for measuring behavioural event frequencies, and instantaneous or interval sampling (1-minute time intervals) was used to estimate time spent in any behavioural state. A check sheet, timer and stopwatch were used, and most observations were carried out by two of the authors (NCW and KZ) with occasional assistance by two other trained observers. Inter-observer reliability was assessed during six observation sessions in which the four observers recorded behaviours simultaneously and independently on the same female. Observer agreement, as measured by the Kendall Coefficient of Concordance (W) (Martin & Bateson, 1993), was 96% ($W = 85$ to $W = 100$, $P < 0.05$).

Faecal sample collection and steroid analysis

Daily faecal samples were collected from all females from July to July over 12 consecutive months and then from June through November in the following year for the two sets of re-paired females (Table 1). Green food colouring (~1–2 ml) or uncooked corn (2 tablespoons) and rice (1 tablespoon) were added to the diet of one of the paired females to identify individual faecal samples. Faecal samples were collected in 50-ml polypropylene conical vials that were labelled with animal number and date and stored frozen at –20°C. Samples were shipped on dry ice to the Conservation and Research Center for analysis at the end of each month.

Oestradiol, progestogen and corticoid metabolites were extracted from samples as previously described (Brown *et al.*, 1994, 1996; Graham & Brown, 1996). Briefly, samples were lyophilized and pulverized, and about 0.2 g of well-mixed powder was boiled in 5 ml aqueous ethanol (90% v/v) for 20 minutes. After centrifuging at 500 *g* for 10 minutes, the supernatant was recovered and the pellet resuspended in 5 ml 90% ethanol, vortexed for 1 minute and recentrifuged. Both supernatants were combined, taken to dryness and redissolved in 1 ml methanol. Extracts were vortexed (1 minute), placed into an ultrasonic cleaner for 30 seconds to free particles adhering to the vessel wall and revortexed briefly (15 seconds). Extract dilutions were made in assay buffer (0.01 mol PO_4 l^{-1}, 0.14 mol NaCl l^{-1}, 0.01% NaN_3, pH 7.4) before analysis by radioimmunoassays (RIA) (1:40 for oestradiol, 1:800–1:8000 for progestogens, 1:20–1:100 for corticoid metabolites). Extraction recovery of [^{3}H] oestradiol, [^{14}C] progesterone, or [^{3}H] cortisol (New England Nuclear, Boston, MA) added to faecal samples prior to extraction exceeded 85%. Faecal oestradiol, progestogen and corticoid metabolites were quantified using RIAs validated for the cheetah. The oestradiol RIA uses an antibody raised in rabbits against oestradiol-17β 6-*o*-carboxymethyloxime: bovine serum albumin (provided by Dr Sam Wasser, University of Washington, Seattle, Washington, USA) (Risler, Wasser & Sackett, 1987), a ^{3}H-labelled oestradiol tracer (New England Nuclear, USA) and oestradiol standards (Brown *et al.*, 1994, 1996). The antibody cross-reacts 100% with oestradiol-17β, 77% with 6-keto-oestradiol, 2% with 17α-hydroxyprogesterone and oestrone and < 1% with oestriol, 5-androstene-3β-17β-diol, testosterone, cholesterol and cortisol. Assay sensitivity, based on 90% of maximum binding, was 5 pg per tube.

The progesterone RIA relies on a monoclonal antibody produced against 4-pregnen-11-ol-3, 20-dione hemisuccinate: BSA (331; provided by Dr Jan Roser, University of California, Davis, California, USA), an ^{125}I-labelled progesterone tracer (ICN Biomedical Inc., Costa Mesa, California, USA) and progesterone standards (Brown *et al.*, 1994, 1996). The antibody cross-reacts 100% with progesterone, 96% with 5α-pregnane-3β-ol-20-one, 36% with 5α-pregnane-3α-ol-20-one, 15% with 5β-pregnane-3β-ol-one, 15% with 17β-hydroxyprogesterone, 13% with pregnenolone, 7% with 5β-pregnane-3α-ol-20-on, 5% with 5β-pregnane-3α,17α-diol, 20α-one and < 1% with pregnanediol-3-glucuronide, androstenedione, testosterone, oestrone, oestradiol, oestriol, 21-hydroxyprogesterone, 20α-hydroxyprogesterone and cortisol. Assay sensitivity was 7.5 pg per tube.

Concentrations of corticoid metabolites were quantified using a double antibody ^{125}I-corticosterone RIA (ICN Biomedicals Inc., Costa Mesa, California, USA) (Graham & Brown, 1996; Terio, Citino & Brown, 1999). The antiserum had the following crossreactivities: 100% corticosterone; and < 1% with desoxycorticosterone, testosterone, cortisol, aldosterone, progesterone, androstenedione, dihydrotestosterone, cholesterol, dehydroepiandrosterone sulfate, 11-desoxycortisol, dihydroprogesterone, oestrone, oestradiol, oestriol, pregnenolone, hydroxypregnenolone, and hydroxyprogesterone. Fifty µl of faecal extract (diluted 1:10 in assay buffer) were added to 50 µl of steroid diluent (provided in the assay kit) for analysis. Assay sensitivity was 12.5 ng/ml.

Intra- and interassay coefficients of variation were 9.3% and 13.6% for oestradiol, 7.6% and 10.7% respectively for progestogen, and 7.8% and 12.1% for corticosterone RIAs, respectively. All faecal data are expressed as per gram dry weight.

Statistical analysis

StatViewSE & Graphics (version 551+), EXCEL (version 5.0) and JMP (version 3.1) for Macintosh were used to analyze data.

Recorded behaviours were converted into rates. First, the observed behavioural frequencies were adjusted for the time the focal animal was out of sight by subtracting the number of observation intervals during which the animal could not be seen from the total number of observation intervals (a total of 30 1-minute intervals per observation session). Then the behavioural frequencies were divided by the remaining number of observation intervals. Based on these calculations, the number of resulting behavioural rates for each 6-month period was added and divided by the number of observation sessions during each housing treatment (n = 52 sessions/6 months).

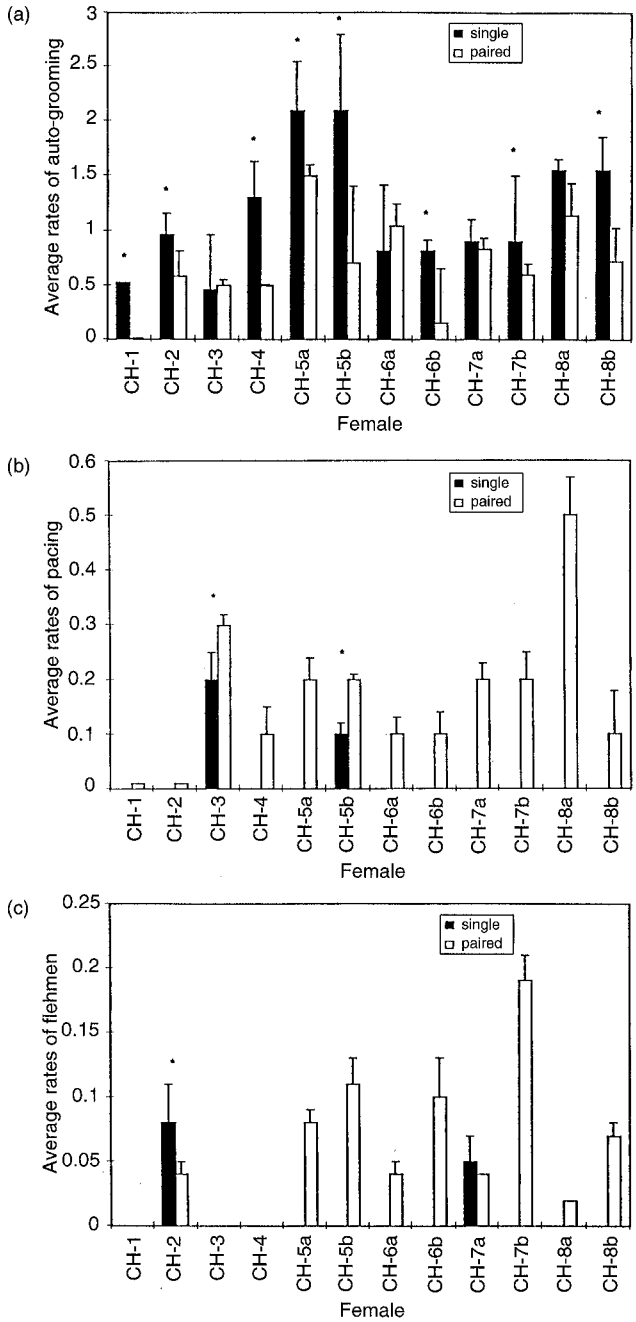

Fig. 1. Comparison of average rates (mean ± SEM) of auto-grooming (a), pacing (b) and flehmen (c) observed in females while housed as pairs versus singletons (n = 52 observation sessions for each housing condition). An asterisk denotes significant differences ($P < 0.05$) within a female. Females labelled 'a' or 'b' were monitored during two pairings: 'a' represents data collected during the first pairing and 'b' represents data collected during the second pairing.

To compare differences in behavioural rates for each female and for all females combined during the two housing treatments, a Wilcoxon matched-pairs rank test was used (Siegel & Castellan, 1988). Differences in behavioural rates among pairs were tested using a Kruskal–Wallis one-way nonparametric analysis of variance (Sokal & Rohlf, 1995).

Significant increases in faecal oestradiol and corticoid metabolite concentrations were determined by an iterative process in which high values exceeding the mean plus 1.5 standard deviations (SD) were excluded (Brown *et al.*, 1996). Baseline values were those remaining after excluding all high values. Individual average baseline concentrations were calculated from these remaining values. A peak was considered the highest point in a group of significantly (> 1.5 SD) elevated values. Overall average concentrations included all values for each female. Oestrous cycle length was calculated as the number of days between peaks in oestradiol concentrations (presumed to be associated with oestrus) for periods not exceeding 30 days (i.e., twice the estimated oestrus cycle duration based on other studies; Eaton & Craig, 1973; Asa *et al.*, 1992). Inter-oestradiol peak intervals of > 30 days were considered periods of anoestrus (Brown *et al.*, 1996). Because cheetahs are primarily induced ovulators (Wildt *et al.*, 1993; Brown *et al.*, 1996), progestogen increases were not expected. Thus, this analysis was done retrospectively to determine the incidence of any spontaneous (non-mating induced) ovulation (defined as an increase in progestogen metabolite concentrations that exceeded the mean plus 2 SD and remained elevated for at least 1 week). A Student's t-test was used to compare baseline, peak and overall averages of faecal oestradiol and corticoid concentrations for each female when housed in pairs versus alone; and also to compare hormone concentrations of dominant versus subordinate individuals. Prior to analysis, data were tested for normality using the Shapiro–Wilk Test and for homoscedasticity using the Levine Test to ensure that the application of parametric statistics was appropriate (Sokal & Rohlf, 1995). Average values are presented as mean ± standard error (SEM).

RESULTS

Behavioural observations

Comparing behavioural frequencies among all females before and after pair separation revealed significant differences in the incidence of grooming (P = 0.003), pacing (P = 0.04) and flehmen (P = 0.02) (Fig. 1(a–c)). Grooming activity decreased ($P < 0.05$, Fig. 1(a)), whereas pacing (Fig. 1(b)) and flehmen (Fig. 1(c)) increased ($P < 0.05$) when females were housed in pairs. In ten of 12 cases, cheetahs only paced when pair-housed

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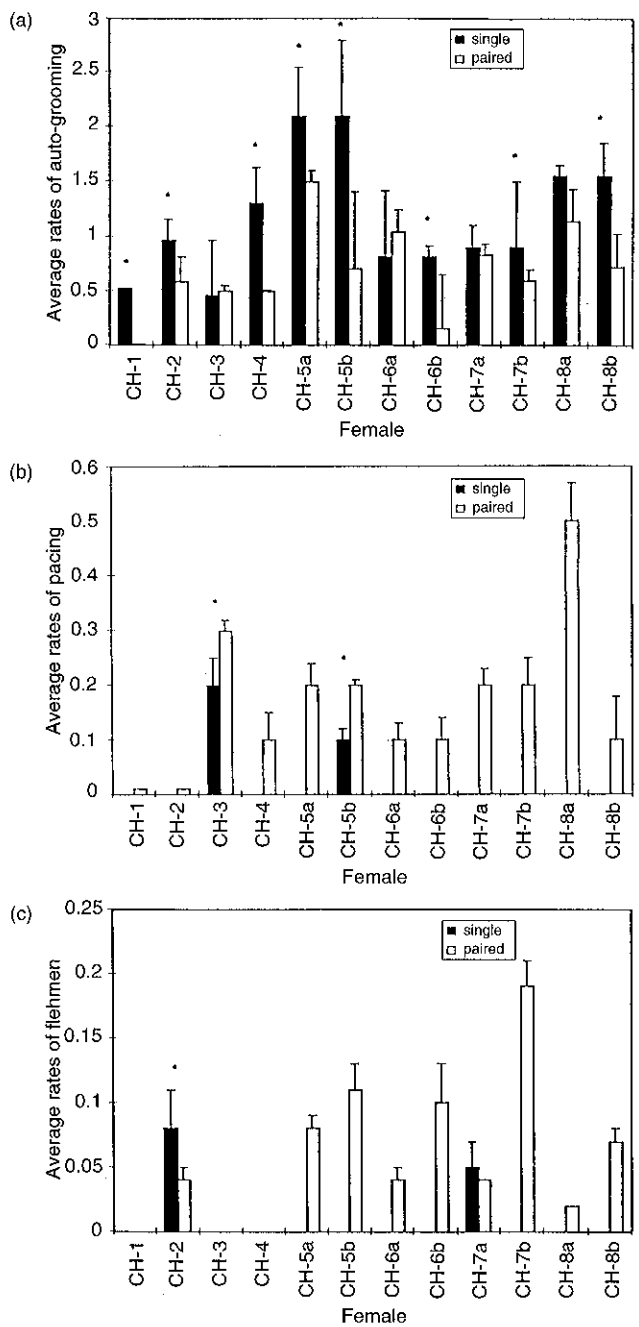

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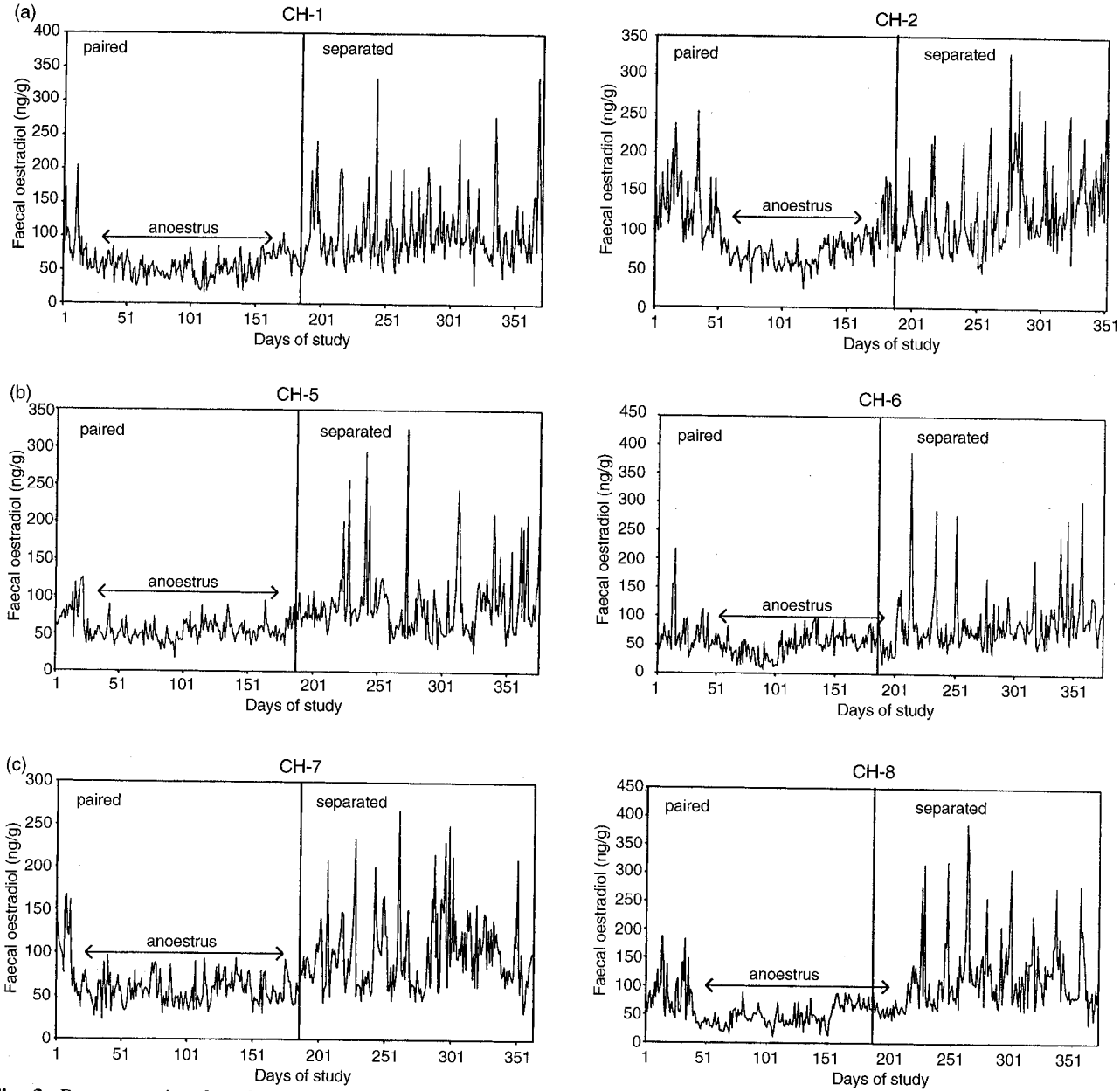


Fig. 3. Representative faecal oestradiol profiles of female cheetahs that showed prolonged anoestrus while housed as pairs. Pairs: (a) CH-1 and CH-2; (b) CH-5 and CH-6; (c) CH-7 and CH-8.

increased aggression). Although pairing cheetahs resulted in altered behaviours and reproductive steroid patterns in females, findings could not be correlated to faecal corticoid excretion, a potential index of physiological stress. Particularly interesting was that separating females into individual enclosures rapidly reversed ovarian suppression with re-cycling beginning almost immediately. These findings also emphasized the importance of the solitary nature of cheetahs in the wild. Taken one step further, these results indicate the need to maintain sufficient habitat size to avoid constrictions that might lead to excessive female-to-female encounters that, in turn, may lead to reduced reproductive fitness.

An inappropriate social environment *ex situ* is known to inhibit reproduction in other species (Kleiman, 1980; Lindburg & Fitch-Snyder, 1994; Estep & Drewsbury, 1996); and more generally, overcrowding has been shown to reduce reproductive success in social as well as non-social species (Christian & LeMunyan, 1958; Boyd, 1986; Von Holst, 1998). For example, in an extensive study of small felids, Mellen (1991) found group size to be negatively correlated with reproductive success. For this reason, Mellen suggested that the preferred husbandry strategy for small-sized, wild felid species (genus *Felis*) is single housing with occasional introductions to potential mates.

Our findings on female cheetahs are important because zoos, in an effort to conserve space and create interesting exhibits, frequently house this species in same-sex pairs or small groups. Present data suggest that

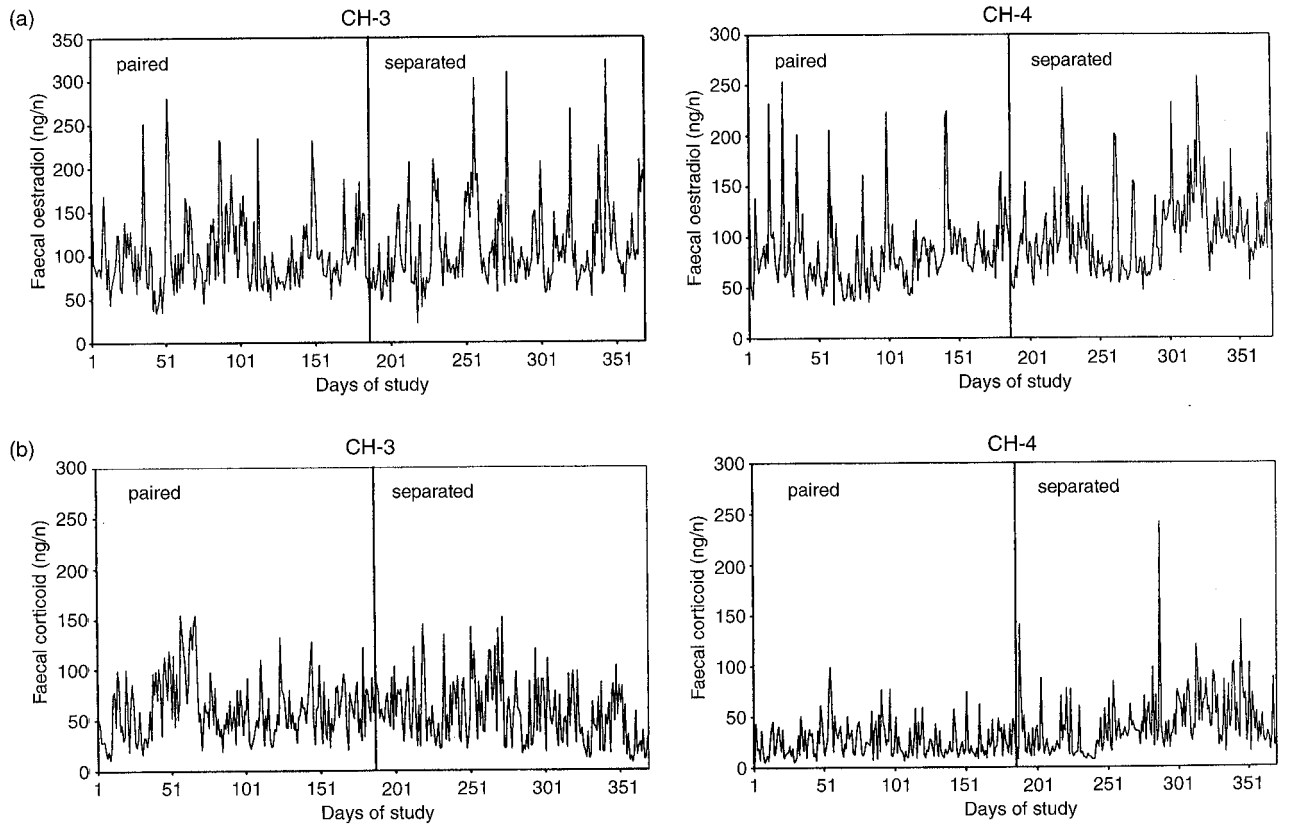
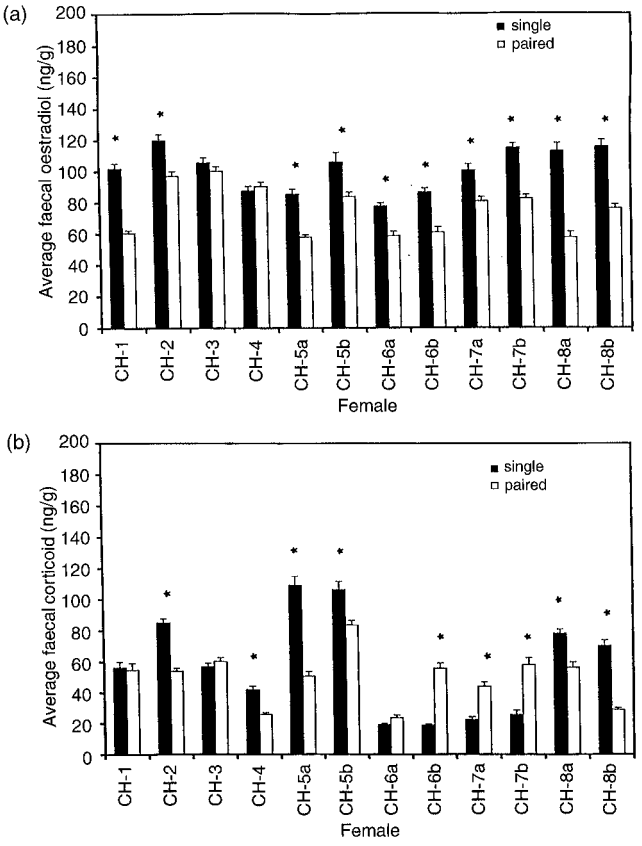


Fig. 4. Faecal hormone profiles of female cheetahs (CH-3 and CH-4) that showed no anoestrus periods while housed as pairs: (a) oestradiol concentrations, (b) corticoid concentrations.



this practice may be contributing to the overall poor reproductive success of cheetahs in captivity. Therefore, it is recommended that the Cheetah Species Survival Plan (Cheetah SSP) programme, which currently manages all cheetahs in accredited North American zoos, and other captive-propagation programmes incorporate this knowledge into its management protocols. According to our findings female cheetahs that are a high priority for mating should be maintained in solitary conditions to promote normal ovarian activity. It is interesting to note that a reversal in ovarian suppression occurred rapidly after pair separation, even when previously pair-housed females were only separated into adjacent enclosures. However, the long-term effect of such close proximity in single-housing situations on actual successful mating and conception remains to be tested.

Furthermore, it is noteworthy that only the use of intensive quantitative behavioural observations allowed us to detect the subtle antagonisms and the lack of affiliative interactions in pair-housed females. Frequently,

Fig. 5. (Left) Comparison of overall average faecal hormone concentrations in female cheetahs housed as pairs versus singletons: (a) oestradiol concentrations; (b) corticoid concentrations. Females labelled with 'a' or 'b' were monitored during two pairings: 'a' represents data collected during the first pairing and 'b' represents data collected during the second pairing.

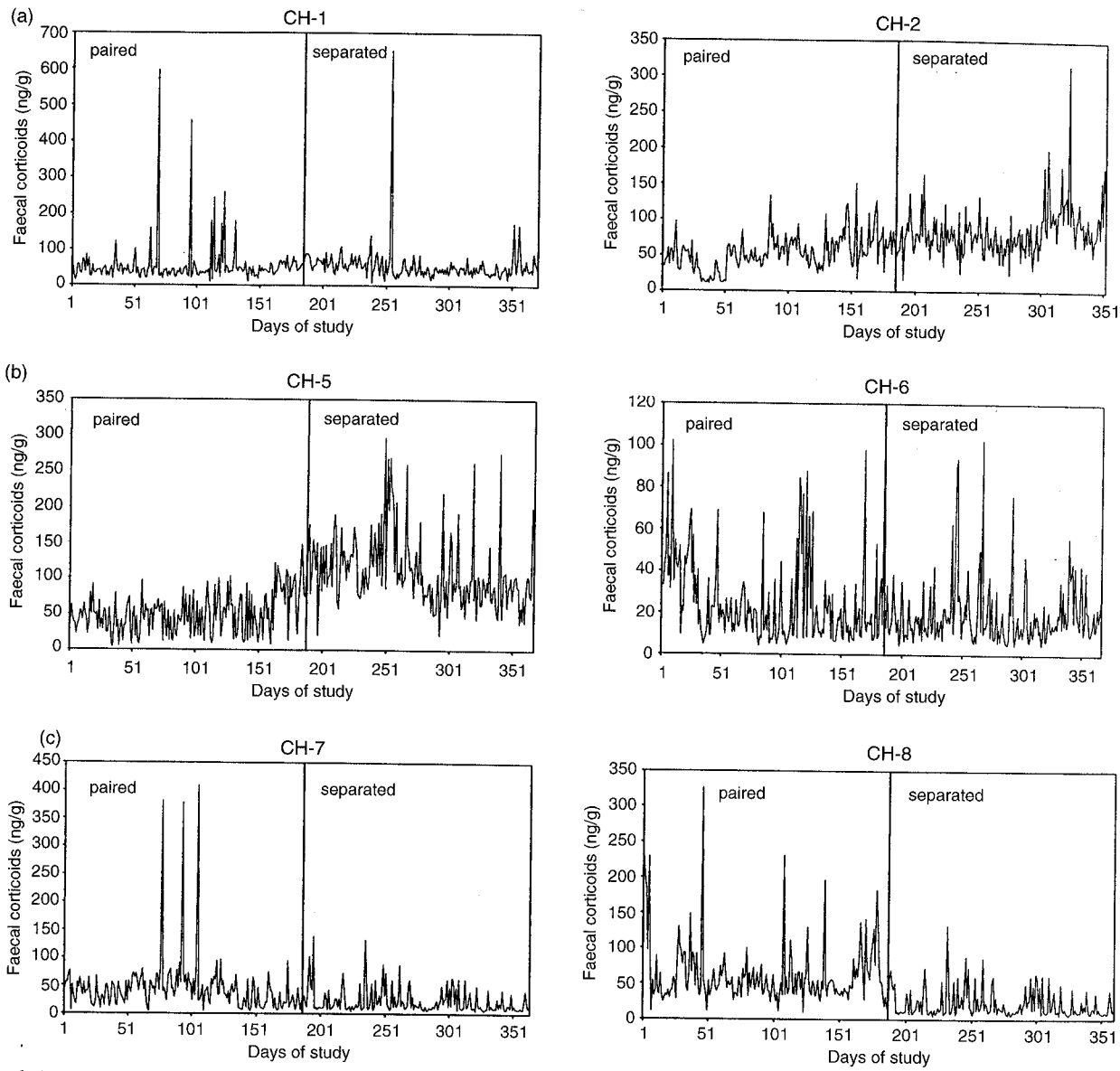


Fig. 6. Representative faecal corticoid profiles of female cheetahs that showed prolonged anoestrus and aggression while housed in pairs: (a) CH-1 and CH-2; (b) CH-5 and CH-6; (c) CH-7 and CH-8.

managers of captive cheetahs characterize minor agonistic behaviours as trivial or unimportant to reproductive health. However, our findings confirm that even subtle behavioural differences may be detrimental, translating into ovarian suppression. Interestingly, one pair showed neither agonistic interactions nor prolonged ovarian quiescence. Familiarity may diminish aggression between individuals (Randall, 1989; Mendoza, 1991) and thus the mother–daughter relationship here may have facilitated the continuous cyclicity and affiliative behaviours. None the less, because agonistic behaviours and ovarian suppression were observed in the other mother–daughter pair, such a relationship cannot always be anticipated between close relatives.

Glucocorticoid hormones have been implicated in mediating reproductive success in several social species. The resulting response mechanism has been termed 'social stress response', or in some, more extreme cases, 'psychological castration' (Brown, 1978; Creel *et al.*, 1997; Von Holst, 1998). Thus, social stress due to increased aggression has been hypothesized to constitute a mechanism for reproductive suppression (Packard *et al.*, 1985; Sapolsky, 1987; Fraser & Broom, 1990; Wingfield, Hegner & Lewis, 1991; Blanchard *et al.*, 1995). Many studies have demonstrated that acute or chronic emotional arousal or distress can lead to adrenocortical activation and increased glucocorticoid production (Mason & Brady, 1956; Mason, 1968; Ursin & Olff, 1993; Jasnow *et al.*, 2001). Jurke *et al.* (1997) have found that two out of seven zoo-held female cheetahs had high average faecal corticoid concentrations and also were acyclic. These authors speculated that social stress and variations in adrenal function might be contributing to variations in reproductive activity. However, we found no apparent relationship between ovarian and adrenal activity. Faecal corticoid concentrations were higher dur-

ing pair-housing in three cases and lower in six cases, and no difference was found in three cases. Interestingly, there was evidence that the same individual may respond similarly to the pairing manipulation. For example, when CH-5 and CH-8 were re-paired, corticoid concentrations again decreased, whereas levels for CH-7 increased, as during the first pairing. However, the fourth female, CH-6, experienced no difference in corticoid concentrations during the first pairing, but had higher corticoids during the second pairing. These results highlighted the individualistic nature of the stress response rather than any consistent trend among all females.

The disparate results from the faecal corticoid analysis also emphasized the challenges associated with defining and measuring stress. Others have pointed out that, because hypothalamo–pituitary–adrenal function is modulated by multiple behavioural and physiological variables, an inconsistent relationship between social stress and adrenal activity may not be surprising (Saltzman *et al.*, 1998; Wingfield & Ramenofsky, 1999). Individuals of the same species, sex and age may differ markedly in response to the same environmental stimulus, as their behaviour may be based on individual experiences with aversive stimuli (Miller, 1980). Therefore, measuring glucocorticoid concentrations alone may not always represent the best indicator of an individual's stress response, and examination of other indicators is advised.

Quantitative behavioural observations conducted in this study identified some behaviours potentially associated with stress. For example, stereotypic pacing, an indicator of suboptimal welfare for many captive species (Broom, 1983; Wiepkema, 1983; Broom & Johnson, 1993), was observed in all pair-housed females. Six of eight females only paced when in the same enclosure with another female. Furthermore, of the two females that paced while housed alone, both experienced increased pacing frequency when in a pair. The incidence of auto-grooming also declined for most individuals, possibly owing to the increased level of pacing and enhanced vigilance expended on monitoring activities of the other female in the same enclosure. The increased incidence of flehmen in seven of 12 cases may also have been related to enclosure mates needing to monitor each other's markings via urine sniffing (Ewer, 1973; Eaton, 1974). Animals housed alone rarely exhibit this behaviour. Pheromonal cues have been implicated in mediating reproductive suppression in some social species (Wasser & Barash, 1983; Barrett, Abbott & George, 1990). This effect seems unlikely in this study, because Pair 2 had equal olfactory exposure, yet experienced no ovarian suppression. However, it is interesting to note that Pair 2 did not exhibit flehmen behaviour.

In summary, ovarian suppression occurs in female cheetahs that are maintained together in the same enclosure. The severity of the effect, however, may depend on pair composition and behavioural compatibility. Although agonistic behaviour was observed in pairs that experienced ovarian suppression, increased glucocorticoid concentrations were not associated with observed aggression. This result may be due to (1) the idiosyncratic nature of the cheetah or the stress response itself, (2) marked individual variation with respect to behavioural and physiological characteristics and/or (3) different coping abilities and strategies of individuals. Furthermore, the observed increase in stereotypic pacing and concurrent decline of auto-grooming in pair-housed females indicated some level of social stress not adequately reflected by the measurement of faecal glucocorticoids. Therefore, future studies should focus on observed ovarian suppression in combination with other potential measures of captivity stress, including immune function and general clinical health status.

Lastly, this study reconfirmed the value of non-invasive faecal hormone monitoring as a powerful tool for examining the impact of environmental/social factors and potential environmental disturbances on reproductive function and adrenal activity in a wild species. However, to better understand overall stress sensitivity and the effects of various environmental parameters on reproductive biology and ecology of cheetahs, it is essential to carry out faecal steroid monitoring studies on wild populations. Recent studies on other species have already illustrated the feasibility of this method for monitoring the physiology/endocrinology of free-ranging populations (e.g. Creel *et al.*, 1997; Wasser *et al.*, 1997; Berger *et al.*, 1999; Cavigelli, 1999; Foley *et al.*, 2001; Harper & Austad, 2001). Measuring adrenal activity in wild cheetahs could be accomplished by closely monitoring radio-collared individuals to collect fresh scat or through obtaining faecal swabs or samples during capture for radio-collaring or relocation. One advantage is that the steroid hormone content of fresh faeces from captured or otherwise disturbed individuals represents the hormonal status of the individual 1–2 days before its capture, thus providing an accurate index of normative adrenal and reproductive hormone activity in truly wild individuals. Some of these studies are already in progress with colleagues working on wild cheetahs in Namibia. Overall, the availability of faecal hormone monitoring provides important research opportunities for conservation science, allowing us to obtain previously inaccessible and highly relevant biological information for both *in situ* and *ex situ* populations.

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