Social and Reproductive Behaviours in the Cheetah (*Acinonyx jubatus*) in A Captive Population

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Chapter IV

Behavioural Cues for Oestrus in Captive Female Cheetah

Introduction

Throughout recorded history, the cheetah (*Acinonyx jubatus*) has been one of the few exotic animals that has had a long standing association with people (Hunter & Hamman 2003). Records have placed them as companion animals as far back as ancient Sumeria and Egypt, approximately 3000 BC (Marker 2000 and Saleh *et al.* 2001). Furthermore, documentation exists of cheetahs being kept in 'stables' by Maharajahs for hunting purposes (Divyabhanusinh 2002). In recent times, the cheetah has been officially kept in zoological parks and captive facilities since 1829 (Rawlins 1972). However, in spite of this long captive history the cheetah has had a remarkably poor breeding success rate (Marker & Grisham 1993 and Bircher & Noble 1997). The first successful breeding in modern times was reported in 1956 (Rawlins 1972), but it was not until 1970 that a litter of cubs was reared successfully. Since that time breeding has been sporadic, with few facilities reporting repeated success (Marker-Kraus & Grisham 1993 and McKay 2003).

Ex situ populations of cheetahs have come under a considerable amount of study from the 1960s to the early 1990s (Eaton 1974, Wildt *et al.* 1983, Caro & Collins 1986, Caro 1989, Caro *et al*. 1989, Laurenson *et al* 1992 and Caro 1994). Extreme differences in the breeding success of wild and captive cheetahs have been noted, with Caro (1994) and Laurenson *et al.* (1992) reporting a breeding success rate of 95% for wild females and Marker and colleagues (Marker-Kraus 1997, Marker 2000, 2002 and Marker & Linn 2009) reporting a breeding success rate of approximately 4% for females in captivity during the 1990s and 2000s.

Intensive physiological and genetic studies have been performed on wild cheetahs throughout the 1980s and 1990s (O'Brien *et al.* 1983, 1986 and Wildt *et al*. 1993). In 1988 a study of the cheetah by the Species Survival Plan (SSP) was developed in order to perform an extensive examination on the North American population of captive cheetah (Wildt & Grisham 1993). The work of the SSP was performed between January 1990 and June 1991 (Grisham 1997) and covered many areas of inquiry. Studies indicated that while there were a number of anomalies within the cheetah's reproductive system, these physiological problems were not preventing breeding (Wildt e*t al.* 1993). For female cheetahs, no obvious evidence of pituitary dysfunction was found and the reproductive tracts were anatomically sound (Howard *et al.* 1993). While approximately 50% of females had parovarian cysts, this did not appear to have an impact on the cheetah's ability to breed. In fact, of the 14 proven breeders examined in the study, 13 had parovarian cysts. Hence, the researchers concluded that the cysts were not likely to be playing a key role in the cheetah's inability to reproduce. Furthermore, Donoghue and colleagues (1992) found that there appeared to be little to no structural evidence of oocyte dysfunction, unlike the structural and functional problems observed with cheetah sperm (Wildt *et al.* 1993). The studies from the SSP illustrated the similarities of the captive cheetah population to the data recorded for the wild cheetah population (Laurenson 1993 and Caro 1993). These similarities provided evidence which supports the argument that behaviour, reproductive physiology and management are important to improving breeding in captive cheetahs.

In the last two decades the focus on cheetah breeding has diverged into two major streams of inquiry: assisted reproduction techniques (Wildt *et al.* 1993, Howard *et al.* 1992, 1997 and Wildt & Roth 1997) and behaviour, examining captive management techniques, environmental factors and methods to improve breeding success (Caro 1993, Wildt *et al.* 1993, Brown *et al.* 1996, Brown & Wielebnowski 1998, Wielebnowski 1998, Wielebnowski & Brown 1998, McKay 2003 and Terio *et al.* 2003 and 2004).

Assisted reproduction techniques for the cheetah have examined the use of artificial insemination and hormone therapy for stimulating ovulation and female receptivity (Wildt *et al.* 1981, 1993, Wildt & Roth 1997, Howard *et al.* 1992, 1997 and Brown *et al.* 1996). Wildt *et al.* (1993) found in their extensive study of 68 females that more than 50% of the surveyed females were reproductively inactive at the time of examination. Their study (based on laproscopic examinations and single assay of ovarian steroids) tentatively concluded that the female cheetah was primarily a stimulated ovulator. Wildt and his colleagues did report that some females would have occasional non-stimulated ovulation periods. The ability of the cheetah to spontaneously ovulate was also found by Asa *et al.* (1992). Wildt and colleagues (1993) also reported that the female cheetah displayed periods of anoestrous. This finding is different from most other species of large cats, with lions *Panthera leo* (Schmidt *et al.* 1979), tigers *Panthera tigris* (Seal *et al.* 1985) and leopards *Panthera pardus* (Schmidt *et al.* 1988) all having a regular defined oestrous cycle, ranging from 20-30 days.

Due to these findings Wildt and his colleagues (1993) found that the cheetah was an ideal candidate for assisted reproduction techniques and that these procedures were valuable in order to utilise animals that would not otherwise have the opportunity to breed. One of their main concerns with cheetah breeding, was that while there had been an increase in breeding success in recent times (Marker-Kraus & Grisham 1993), the actual number of breeding individuals was reducing. In particular, facilities were only holding one breeding male with the other males remaining idle and not passing on their genetic material (Wildt *et al.*1993). Assisted reproduction would allow for collection of non-utilised male and female gametes to enable an increased genetic bank of information that could be used in the future. This would provide more options to conservation managers.

Management techniques have also been explored in an attempt to solve reproductive problems with the cheetah, as well as many other species (e.g. Allendorf 1993, McCormick 2003 and Mitchell & Chasmer 2003). Studies on management and behaviour in captivity are important, not only to determine breeding techniques, but also to create optimal housing conditions, feeding schedules and to provide activities to stave off boredom (Brand 1980, McKeown 1991 and Bircher & Noble 1997). By creating natural environments, researchers are able to liken their captive research populations to wild populations, which could be very useful in order to learn more about a species. Wildlife managers and researchers attempt to produce a natural repertoire of behaviour within zoos by providing behavioural enrichment activities for animals to break up their daily routine, such as natural feeding regimes using carcass feeds and novel ways of delivering the daily meal (Augustus *et al.* 2006). By examining individual cheetahs in captivity, vital information is obtained for management practices. Wielebnowski (1999) has described differences in temperament/personality in individual cheetah in captivity. This idea of individual variation among animals could be of great importance to areas such as captive management (McDougall *et al.* 2006), as differing techniques may need to be implemented when handling specific individuals in a stressful environment. McKay (2003) also reported the value in examining individual ‘personalities’ among cheetahs and the benefits that were achieved when keepers had close relationships with the animals, which included increased breeding success. Understanding key behaviours and providing for specific animals needs is now seen as a part of good captive management.

As a result, zoos traditionally prefer non-invasive techniques for managing husbandry problems. Unfortunately, this conservativeness can result in zoos using a ‘wait and see’ approach to breeding. There is a general reluctance in zoo communities to undertake any practice or procedure that may place a perceived undue risk on an endangered species (Wildt *et al.* 1993). With this in mind, the ability to create efficient non-invasive methods of oestrus detection is crucial for captive breeding and management (Asa *et al.* 1992, Brown *et al.* 1996, 1997, Wielebnowski *et al.* 2002 and

Terio *et al.* 2003). Hence, understanding the mechanisms for reproductive cycles, the factors that influence those cycles and the behavioural and physical manifestations that they take are integral to sustained captive breeding. Many environmental factors, such as seasonality, photoperiods and lunar cycles can affect the expression of oestrus (Roy *et al.*1980, Hansen *et al.* 1983, Hansen & Hauser 1984, Orihuela 2000 and Dixon *et al.* 2006) with external time cues, or zeitgebers, playing a vital role for all organisms and environments (Sharma & Chandrashekaren 2005). Sociality and housing of species can also affect biological rhythms, causing oestrus suppression (Wielebnowski *et al.*2002) and oestrus synchronisation (Orihuela 2000 and Say *et al.*2001). Captive managers need to be aware of many factors that can influence the reproductive cycles of their animals and in recent times there has been a trend towards biological research in conjunction with behaviour to provide further information.

Reproductive cycles have been investigated using vaginal cytology of hand reared cheetah (Asa *et al.* 1992). While this may be a successful form of monitoring, it may not be a valuable technique for facilities that maintain a ‘hands-off’ approach to cheetah management. Faecal steroid monitoring is becoming more widely used, as it is a completely non-invasive way to follow the hormonal fluctuations of animals. This technique has been developed for cheetah and used with high levels of success (Brown & Wildt 1997, Jurke *et al.* 1997, Brown *et al.* 1996, Terio *et al.* 2002 and Wielebnowski & Brown 1998). The study by Brown *et al.* (1996) indicated that faecal steroid analysis is a very useful technique. Unlike the invasive laparoscopic examination of the female ovaries and single sample blood assays that Wildt *et al.* (1993) used, faecal steroids can be collected and compared over long periods of time. The faecal steroid analysis found cyclicity in hormones and reported a period of 12-14 days between cycles, with additional periods of anoestrus where hormone levels failed to fluctuate and remained low. Asa *et al.* (1992) also reported a 12-14 day cycle and supported the idea of regular oestrus cycles in the cheetah.

Wielebnowski and Brown (1998) examined changes in faecal steroids that mark oestrus in female cheetahs while observing changes in behaviour that could be associated. Females were observed for 5 to 22 weeks. This study found that there was not one single behaviour linked to oestrus, instead a number of behaviours showed subtle changes within multiple individuals. Until Wielebnowski and Brown's work, detailed research on captive cheetah's behaviour was uncommon. In the past, only anecdotal reports were generally published on the breeding behaviour of cheetahs (e.g. Florio & Spinelli, 1967, Manton 1970, Vallat 1971, Rawlins 1972 and Benzon & Smith

1975). It is only recently that research has been undertaken on captive cheetah behaviour (Brown & Wielebnowski 1998, Ruiz-Miranda *et al.* 1998, Wielebnowski 1999, McKay 2003, Augustus *et al.* 2006 and McDougall *et al.* 2006)

Further work by Wielebnowski *et al.* (2002 and Brown & Wielebnowski 1998) suggests that social management of the cheetah may have an impact on their behaviour and physiology, with evidence for oestrus suppression observed when female cheetahs are housed together in un-natural situations. Hence, Wielebnowski and Brown (1998 and Howard *et al.* 1992) report that by using faecal analysis to track female reproductive cycles, an accurate measure of female oestrus can be achieved. While the use of faecal steroids to determine oestrus cycles is certainly accurate (Brown *et al.*1996), it is not always a practical or cost effective way to determine the receptivity of cheetahs in small zoos and breeding centres, particularly over extended periods of time (Wildt *et al.* 1993). The time taken to collect samples and analyse results is valuable time lost for breeding introductions. Therefore, the need for a behavioural cue is important in order for management personnel to make instantaneous decisions on breeding introductions (Wielebnowski & Brown 1998).

Aims

The current study examines whether there is a reliable behavioural cue to oestrus in the cheetah. Such a cue could be used to aid in the appropriate timing of introductions between male and female cheetahs, hence removing the guesswork from pairings in a captive facility.

The primary focus in this study is to perform a comprehensive analysis of female cheetah behaviour over an extended period of time. Previous studies that have examined the possibility of behavioural markers to oestrus have typically covered only a subset of behaviour. Asa *et al.* (1992) performed a short behavioural study in conjunction with vaginal cytology. They examined behaviours such as calling, licking and spraying, yet did not observe tail movements. Wielebnowski and Brown (1998) performed a study of female behaviour in conjunction with their faecal steroid analysis work. This study covered a wider variety of behaviours than Asa and her colleagues, but only tail flicks (Tail Twitches in this study, see Chapter 3) were analysed out of all the tail movements displayed by the female cheetah. These studies were also performed over a relatively short period of time, with cheetahs examined between 1 and 5 months. Here, I explore whether there may be behavioural markers that coincide with the oestral cycle by investigating a wider range of female cheetahs' behaviour and how these behaviours change over extended periods of time.

As a further goal, I also aim to explore any negative consequences for cheetah housing. As the female cheetah is solitary in the wild, I examine if there is an impact of housing female cheetah in seemingly unnatural social situations, such as in pairs, trios or with males. It is known that different animal species vary in how well suited they are to captivity and breeding programs, due to their general temperament and behaviour (Novak & Suomi 1988, Sabalones 1995, McKay 2003 and McDougall *et al.* 2006). So therefore the current study also investigates not only differences in the behavioural repertoire of female cheetahs, but the differences between individual animals when determining cues to oestrus.

Methods

Animals and facility

The primary study animals were four female captive-born, adult cheetahs (ages ranging between 3 years 5 months and 5 years 5 months at age of first recording). Three of these animals (Pinda, Lula and Bopha) were parent-reared at Hoedspruit Endangered Species Centre (South Africa) and maintained at Monarto Zoological Park (MZP), South Australia for the majority of the study. The fourth female (Zilkaat) was hand raised at the De Wildt Breeding Centre (South Africa) and maintained at Perth Zoo, Western Australia. The Perth-based female was held on breeding loan at MZP for two short periods during 2001 and 2002. Bopha was transferred out of MZP in April 2002 on breeding loan.

The majority of observations, manipulations and data collection were performed between January 2001 and February 2003, with occasional short breaks taken during this time. Four female cheetahs were observed for a total of 1032 hours. These observations were primarily on Pinda and Lula, as they were housed at MZP for the entire study period. Each of these animals was observed for over 370 one-hour study periods. Bopha was also housed at MZP for a considerable period of time, and observed during 235 one-hour study periods. Zilkaat, the female cheetah from Perth Zoo was studied for 48 study periods within her two short stays. As Bopha and Zilkaat’s data sets are not as comprehensive as those of Pinda and Lula, they were not included in all analyses.

Four social conditions were used during the study. These were social with males and females (where the sexes were housed together permanently both on and off exhibit), social with females (where all females were housed together both on and off exhibit), solitary with minimal male contact (where females were kept off exhibit in individual lock-away and night-yards and were only able to view males as they moved on or off exhibit) and solitary, with a 30 minute observation period of males (where males were introduced to the females' lock-away yards to explore and examine scents left by the females). Females were further isolated from each other at the end of 2001 with the construction of visual barriers between their night-yards. However, the Perth female Zilkaat was held in a lock-away and night-yard by herself. She was only allowed contact with other cheetah through chain link fences (see Chapter 2 for a description of the enclosure).

Monarto Zoological Park held five male cheetahs during the study period. For the majority of the study, the sexes only had limited visual contact with each other, usually restricted to the time when males were moving to and from their exhibit (less than 2 mins at 10:30 h and 16:30 h each day). During the study, major male to female contact occurred within the first 6 months, when males and females were housed together in various combinations. After this time, male and female contact consisted of a parading period during parts of the study where the males had visual access to the females for approximately 30 minutes each morning (occurring between 7:30 h and 10:00 h). This contact occurred primarily through chain-link fences, allowing full visual access between the sexes. On these days the males also had their usual short period in the afternoon for visual contact (less than 2 mins at 16:30 h) when they were brought off of exhibit. At this time they were not paraded passed the females, but there momentary visual contact (less than 2 minutes) could occur as at other times. At all times males were housed within auditory range of the females. Occasionally, during the later periods of the study, males were introduced to females for short times (1-3 days). A full description is given in Chapter 6.

Parallel data were collected for the males at the beginning of the study and then at various times during the remainder of the study (see Chapter 5), to provide a comparison between the sexes. As one of the primary areas of interest was to determine if there were any obvious behavioural changes over time that could be attributed to oestrus, it was also important to track male behaviour to identify any behavioural changes that could not be attributed to sex.

Apparatus/Materials

The husbandry practices and enclosures operating during this study are described in detail in Chapter 2-General Methods. Equipment used for the observation of the cheetahs was a *Realistic®* Minisette – 20 tape recorder and an *Olympus®* C-740 digital camera. Charts of lunar cycles and the moon's luminescence for the MZP area during the study period were obtained from the Bureau of Meteorology (http://www.bom.gov.au).

Procedure

Collection of Behavioural Data

Initially I used focal sampling, with each animal being observed for seven hours (not included in the current statistical analysis) to determine a general list of behaviours. Observation times were sampled over the day, with the earliest starting at 06:30 h and the latest finishing at 19:00 h. The method of recording was continuous (Martin and Bateson 2000 further details are given in Chapter 2) and the results of this study are described in Chapter 3.

Throughout the experiment, behaviours were recorded using a small tape recorder. Behaviours were spoken into the recorder and then transcribed as soon as possible after the session. All recordings were taken from the enclosure fence line (see Chapter 2), moving to different positions to maintain visual access to the study animal. My movement along the fence-line was a normal occurrence for cheetahs, to the point where I was almost always ignored. Before data collection for this current study, I performed an additional four hours of observations on both Bopha and Lula from a distance of 100 metres using binoculars. Non-statistical comparisons of these data with the initial ethogram data suggested no obvious differences in activity type or frequency. The examination of behaviours also included noting their activity levels for each study period. General activity levels were assessed based on the entire hour of recording. Each hour was given a score out of 3 based on low, medium and high levels of movement and behaviour (Martin & Bateson 2000 and Wielebnowski 1999).

I observed each animal four times per week as a minimum, for one hour per observation period. The females Pinda, Lula and Bopha were observed between January 2001 and April 2002. Pinda and Lula were observed between April 2002 and February 2003. Zilkaat was observed during October and December 2001 as well as November and December 2002.

Hormonal Component

Artificial Insemination

In 2001 MZP and Perth Zoo decided to collaborate on an artificial insemination project using the Perth female (Zilkaat), one MZP female (Lula) and males from MZP. Between the 29th of November and the 2nd of December 2001, Lula and Zilkaat were given injections of pregnant mare's serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) as a part of this project. The procedures used here were the same as described in Howard *et al*. (1992). These injections provided an opportunity to compare any behavioural effects of the treatment since two females were given the treatment and two were used as a control. Observations were performed on all females during these days.

A small number of faecal samples were collected during the artificial insemination project so that steroidal assays could be used to assess cheetah's reproductive status. However, these samples were accidentally destroyed while in the care of MZP veterinary staff.

Statistical Analysis

Behavioural data were analysed using SPSS (Statistical Package for the Social Sciences) Version 16. If assumptions for parametric tests (such as normality or homogeneity of variances) were not met then non-parametric analyses were used.

A problem arises in behavioural data analysis as a large number of different behaviours were exhibited and it is likely that different behaviours are not expressed independently of each other. As a consequence, separate analyses of all the different types of observed behaviours could entail substantial Type I error problems. To help overcome this problem, I used Principal Components Analyses (PCA) to determine if any underlying factors influenced the expression of multiple behaviours that might result in correlations of behaviours. When carrying out PCA, principal component scores for each observation for each individual were saved as regression scores and these were used to explore any temporal patterns in correlated sets of behaviours. Where appropriate, subsequent and more-detailed analyses then focussed on a small set of behaviours that were loading most strongly onto the principal components of interest. In particular, the Shannon-Weaver Index was used to examine the diversity in behaviours. While initially developed to measure diversity in populations (Krebs 1989), it is also useful when measuring diversity in behaviour (McCormick 2003) as it examines evenness in the distribution compared to richness of the sample.

Results

Primary Analysis

Multivariate behavioural analyses

Pinda

I began my analyses of Pinda's behaviour using Principal Components Analysis (PCA). I used the entire data set (from the 3rd of February 2001 to the 6th of February 2003), comprising 375 separate observational periods. The PCA was used to extract factors with eigenvalues >1 and the initial solution was subjected to Varimax rotation. The resulting four principal components are listed in Table 1 along with their eigenvalues, proportion of variation and cumulative variation explained.

Table 1. Principal *components extracted with eigenvalues and per cent variation and cumulative variation explained for Pinda.*

Principal Component	Eigenvalue	Variation Explained	Cumulative Variation Explained
1	2.405	19.780	19.780
2	1.358	11.202	30.982
3	1.111	9.566	40.548
4	1.091	9.167	49.715

Behavioural variable loadings for the rotated PCA solution are given in Table 2, where positive and negative loadings are given for each variable with the four principal components for Pinda's behaviour.

Table 2. *Rotated Component Matrix for Pinda. The key variables loading onto each component in bold type.*

Behaviours	Components			
	1	2	3	4
Tail Swishes	**0.932**	-0.030	0.046	-0.138
Tail Rolling	**0.929**	-0.011	0.065	-0.130
Rolling	0.472	0.202	0.168	0.160
Activity Level	0.426	-0.180	0.003	0.096
Rubbing Body	0.330	0.148	-0.112	0.096
Calling	0.052	**0.705**	-0.180	-0.002
Lip Licking	0.003	**-0.695**	-0.262	-0.042
Grooming	0.087	0.113	**0.616**	-0.279
Rubbing Face	0.184	0.091	**0.578**	0.188
Spraying	0.252	0.235	**-0.532**	-0.063
Scratching	-0.036	0.218	0.034	**0.748**
Tail Twitches	-0.142	0.379	0.022	**-0.584**

Inspection of the first principal component (PC1) in Table 2 suggests that for most behaviours with absolute loadings greater than 0.1, loadings were positive. This indicates that these variables were positively correlated. This may reflect variability in overall activity level. This component had very high loadings from Tail Rolling and Tail Swishes, with lower loadings from Rolling and Activity Level. There was an even lower loading from Rubbing Body and weak loadings from the other behaviours.

The two behaviours that loaded most heavily onto the second principal component (PC2) were Calling and Lip Licking (Table 2). These two variables have opposite loadings, such that as Calling increases, Lip Licking decreases. Grooming, Face Rubbing and Spraying behaviours loaded most heavily onto the third principal component (PC3), but whilst Grooming and Face Rubbing had positive loadings, Spraying had a negative loading. Finally, the fourth component (PC4) received its highest loadings from Scratching and Tail Twitching. These loadings were in opposite directions which indicates that these behaviours tend to be negatively correlated.

Behaviours Over Time

Due to the large number of variables and the very large number of observation periods, I began this analysis by exploring how key behaviours associated with the principal components discussed above varied over time. I started by looking at the variables most strongly associated with PC1. This component showed extremely heavy loading for Tail Rolling and Tail Swishing.

These two behaviours are highly correlated (Pearson's Correlation, $r = 0.972$, $P < 0.01$, 2-tailed) (Figure 1). In fact, Tail Swishing was never observed without Tail Rolling co-occurring, but there were many times when a Tail Swishing event was associated with multiple Tail Rolling events. Due to this close relationship, I used Tail Rolling in the following analysis.

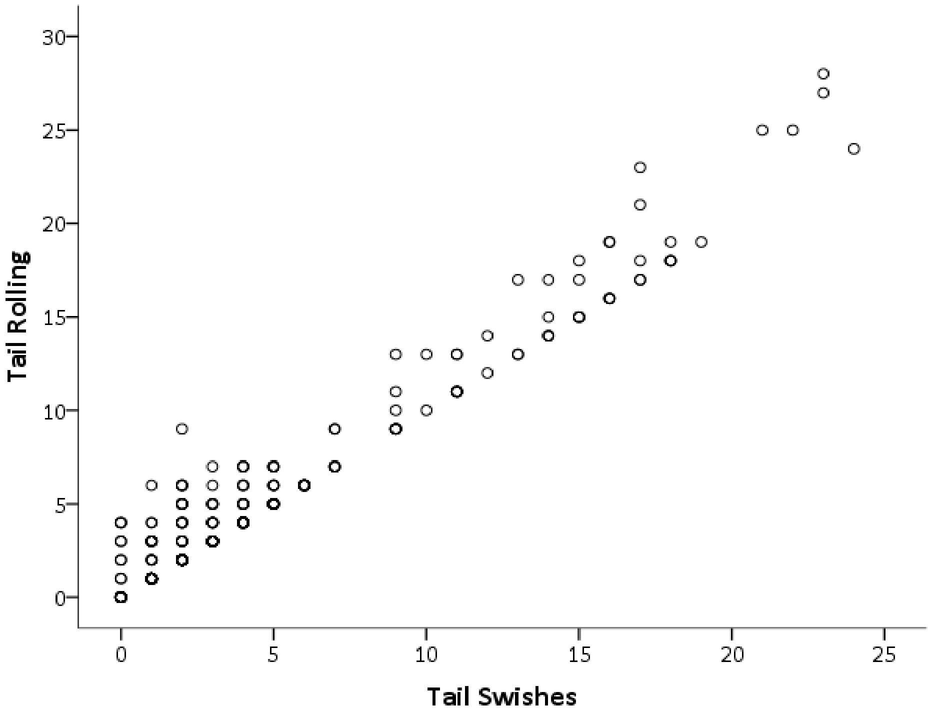

Figure 1. Scattergram of absolute counts for Tail Rolling with Tail Swishes by Pinda. Note that many circles represent more than one observation and the total number of data points plotted is 750.

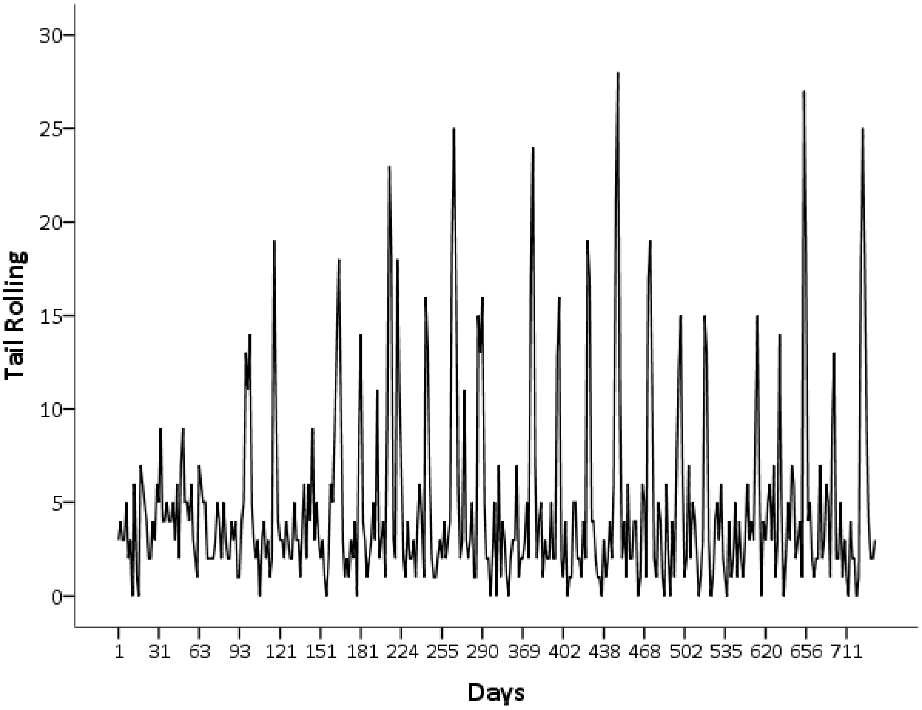

Figure 2. *Absolute counts for Tail Rolling by Pinda, plotted against days since recording began (recording started on the 3rd of February 2001 and finished on the 6th of February 2003).*

Figure 2 shows Tail Rolling plotted over time, where each case represents a day's observation. Tail Rolling was observed throughout the entire study for Pinda. However, spikes in behaviour were observed to occur periodically, and cycled repeatedly.

To examine how consistent the cyclical nature of Tail Rolling was over time, I performed an autocorrelation analysis for Tail Rolling by Pinda, using lag periods of 1-60 days. The resulting autocorrelation coefficients for each lag period indicate that Tail Rolling is indeed cyclical, with peaks and troughs showing a periodicity of about 12-14 days (Figure 3). The duration, in terms of lag times, is much shorter for positive correlations than for negative correlations (Figure 3). This change in time periods corresponds to the duration of increased Tail Rolling and suggests that increased tail movement occurs over shorter periods than low tail movement. Secondly, there appears to be some bimodality in the periods of negative correlation, indicating that further temporal patterns occur during periods of low Tail Rolling (Figure 3).

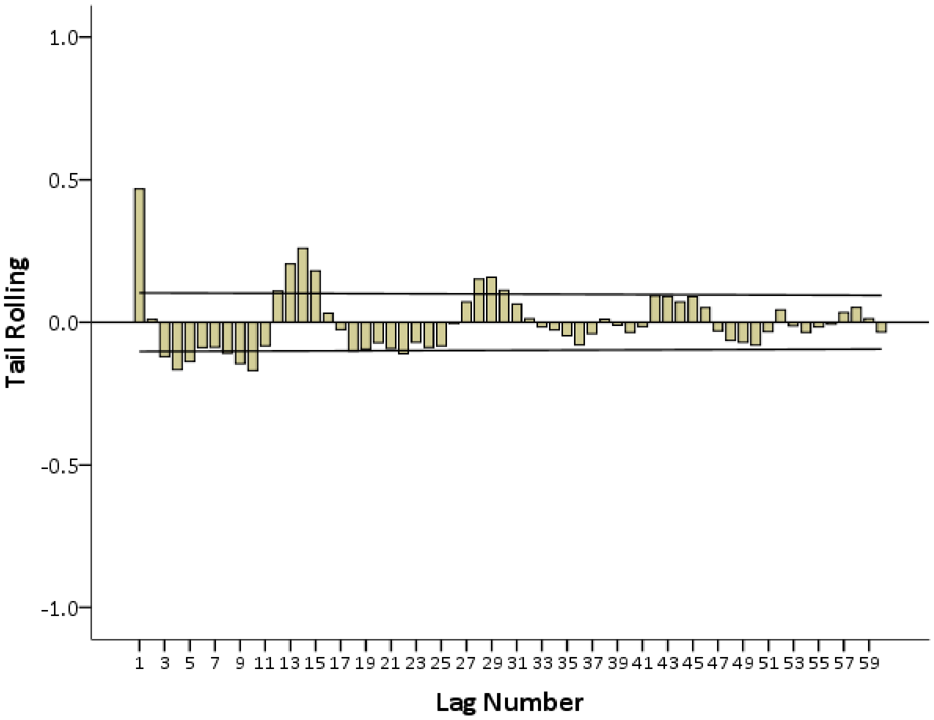

Figure 3. *Autocorrelation of Tail Rolling by Pinda over lag time. The y-axis is in units of the correlation coefficient and the x-axis is in units of lag days. The two horizontal lines indicate the upper and lower 95% confidence limits for statistically significant correlation coefficients, so that lag times with bars extending beyond these lines can be regarded as exhibiting significant positive or negative correlations.*

The autocorrelation analysis of Tail Rolling was then further broken down into the two separate years (2001 and 2002) of the study. During 2001 a large number of husbandry changes occurred for Pinda. During the first half of 2001 Pinda was moved around the facility, being housed in various social conditions with males and females (see Methods above). For the second half of 2001, Pinda was housed in semi-solitary conditions in an individual enclosure with only visual access to all other females. Throughout 2002, Pinda continued to be held in solitary conditions, but visual access to other females was limited. The autocorrelation analysis for 2001 (Figure 4) and 2002 (Figure 5) indicates significant cyclicity in Tail Rolling. However, the longer-term stability in periodicity is greater in 2002, as shown by the more even spacing of peak correlation values as a function of lag time. This suggests a much higher level of cyclicity in 2002 than 2001. This may be the result of housing changes as Pinda experienced much less change to her environment in 2002 and was visually removed from male and female cheetahs within the facility.

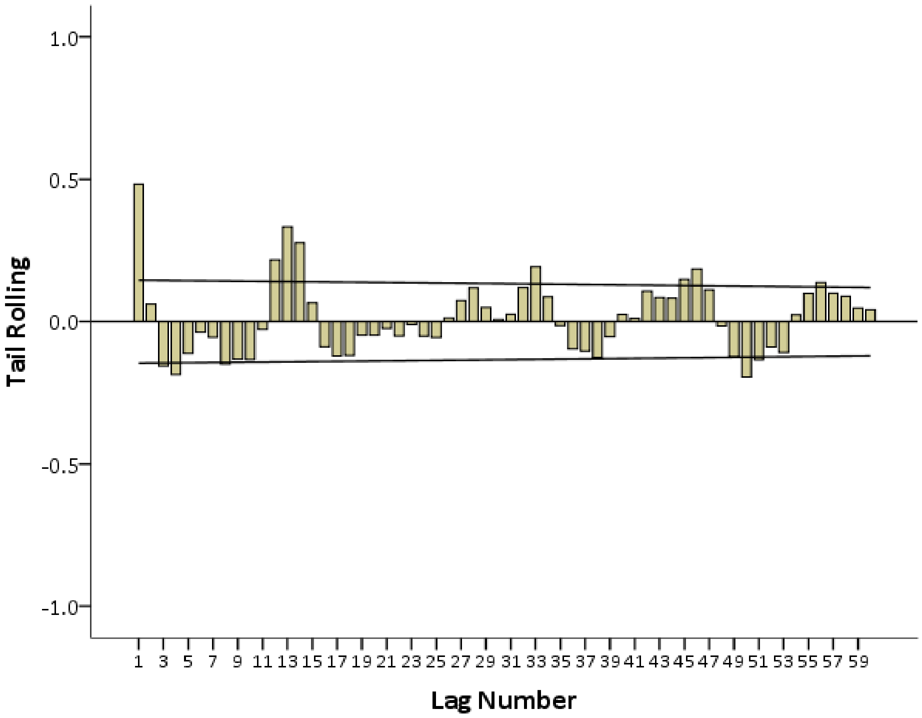

Figure 4. *Autocorrelation of Tail Rolling by Pinda in 2001 over lag time. The y-axis is in units of the correlation coefficient and the x-axis is in units of lag days. The two horizontal lines indicate the upper and lower 95% confidence limits for statistically significant correlation coefficients, so that lag times with bars extending beyond these lines can be regarded as exhibiting significant positive or negative correlations.*

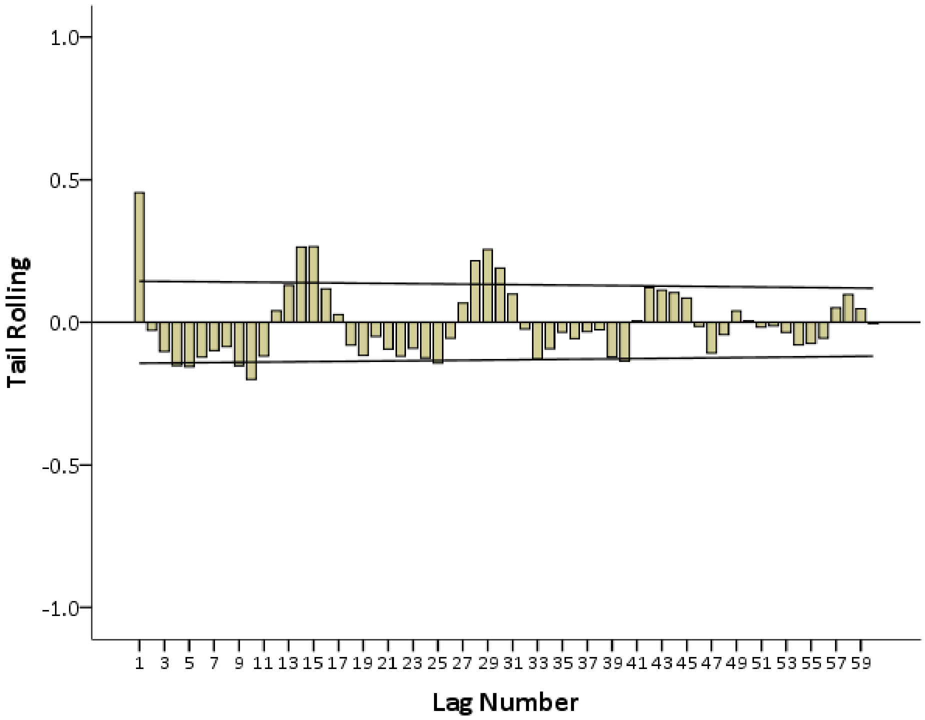

Figure 5. *Autocorrelation of Tail Rolling by Pinda in 2002 over lag time. The y-axis is in units of the correlation coefficient and the x-axis is in units of lag days. The two horizontal lines indicate the upper and lower 95% confidence limits for statistically significant correlation coefficients, so that lag times with bars extending beyond these lines can be regarded as exhibiting significant positive or negative correlations.*

Calling was the behaviour most heavily loaded onto PC2, over time for Pinda (Figure 6), but it does not show the marked cyclical pattern that was seen for Tail Rolling. However, there are some spikes in this behaviour that do not appear to be random events. In particular, on day 654 (13th of November 2002) Pinda is seen to call five times. On that particular day she was introduced to a male – Ndonda. This introduction was performed based on his behaviour, not on hers, and resulted in him frequently chasing her around the night-yards. While Calling did not appear to show any patterns, evidence for a true cyclical pattern is further explored below using an autocorrelation.

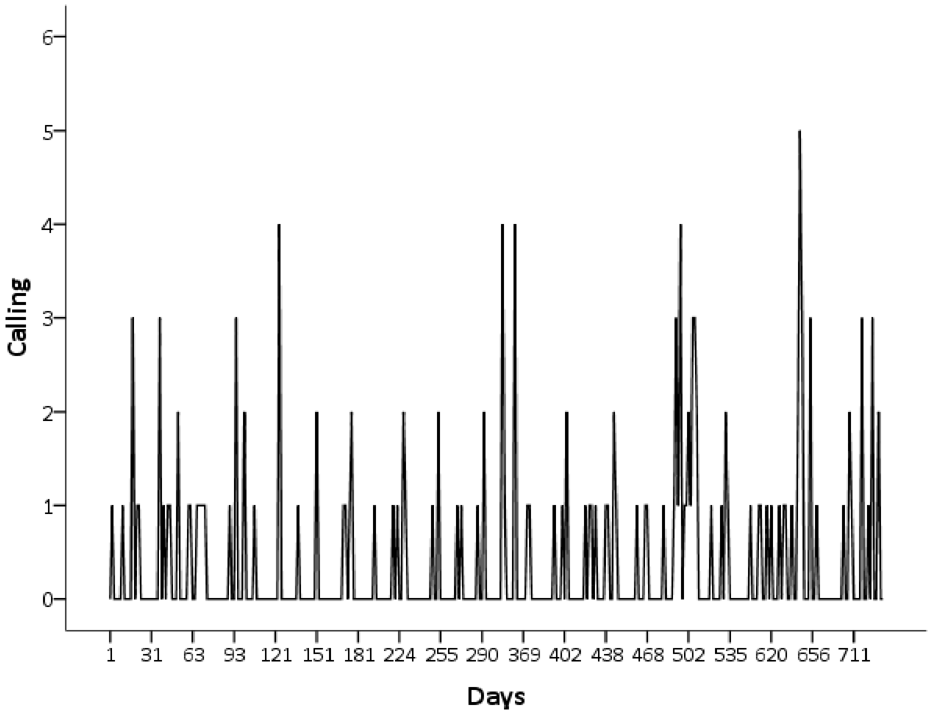

Figure 6. *Absolute counts for Calling by Pinda, plotted against days since recording began (recording started on 3rd of February 2001 and finished on 6th of February 2003).*

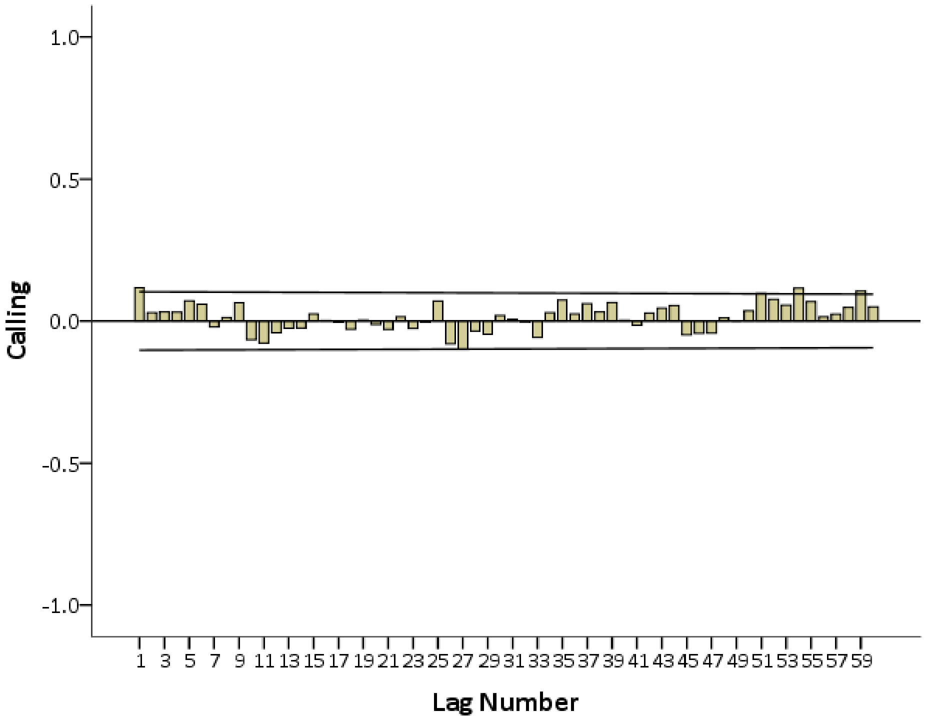

Figure 7. *Autocorrelation for Calling (with 95% Confidence Limits) by Pinda over lag time. The y-axis is in units of the correlation coefficient and the x-axis is in units of lag days. The two horizontal lines indicate the upper and lower 95% confidence limits for statistically significant correlation coefficients, so that lag times with bars extending beyond these lines can be regarded as exhibiting significant positive or negative correlations.*

The autocorrelation analysis did not indicate any clear patterns as correlations only marginally exceed the confidence limits in a few lag cases, and there was no evidence of clear periodicity in correlation values (Figure 7).

As was seen for Calling, there was no apparent cyclicity observed within Grooming (Figure 8), though the incidences of grooming vary enormously throughout the study. Grooming was the behaviour most heavily loaded on principal component 3. There is one period (approximately days 360-460) where grooming is elevated. These days represent March through to May 2002. An autocorrelation analysis performed for Grooming found no indication of any periodicity or any significant correlations.

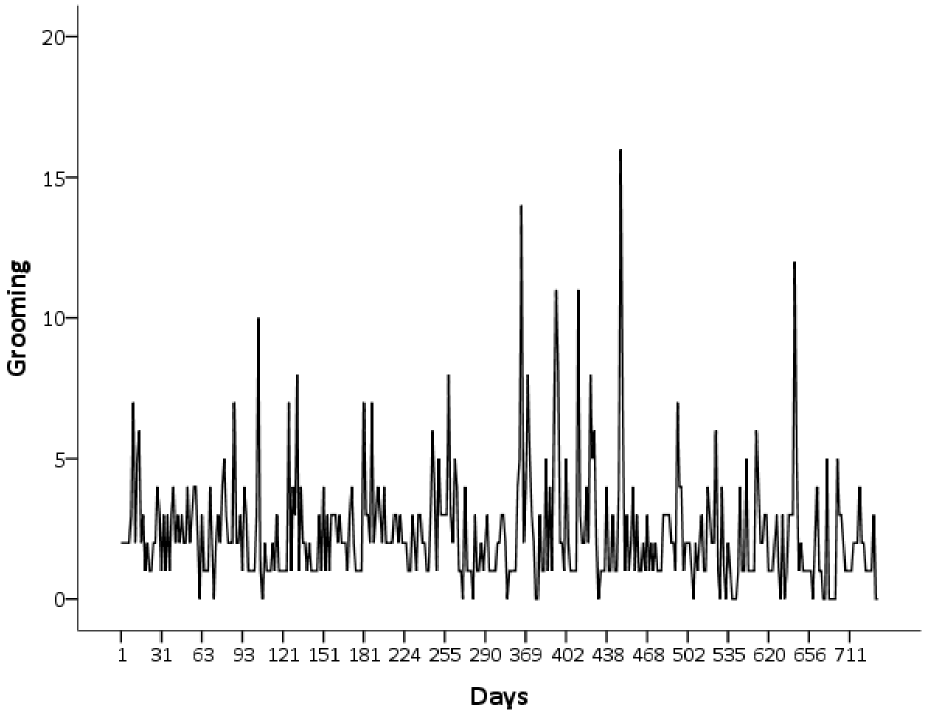

Figure 8. *Absolute counts for Grooming by Pinda, plotted against days since recording began (recording started on 3rd of February 2001 and finished on 6th of February 2003).*

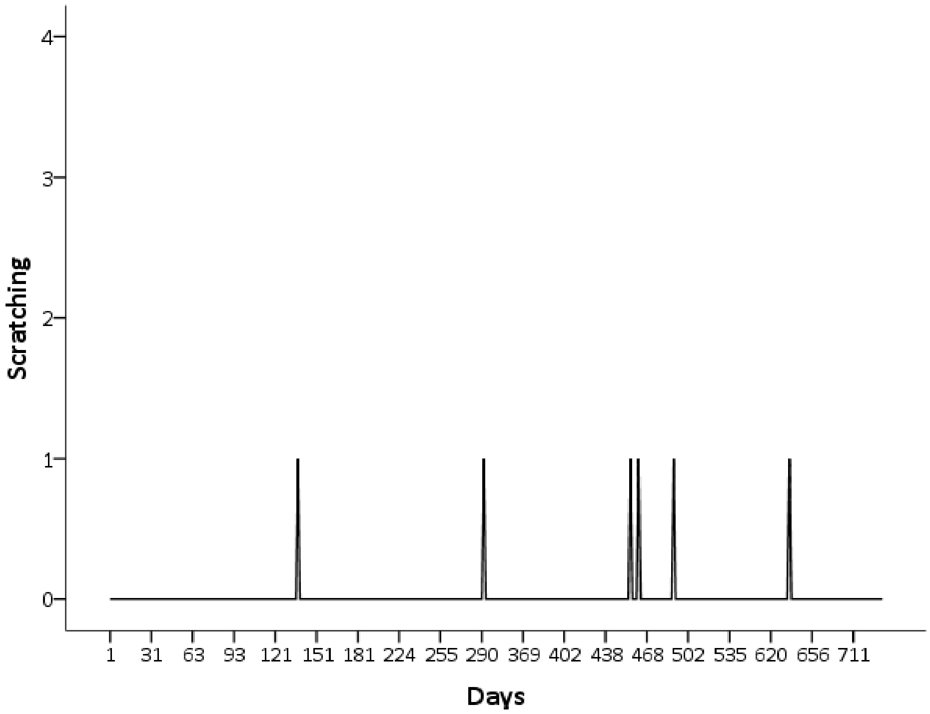

Figure 9. *Absolute counts for Scratching by Pinda, plotted against days since recording began (recording started on 3rd of February 2001 and finished on 6th of February 2003).*

Scratching was the behaviour most strongly loading onto principal component 4 (Figure 9). This behaviour was only seen very occasionally, with each example occurring in isolation. There is no obvious temporal pattern within this behaviour and due to the limited occurrences, no autocorrelation was performed.

Lula

I began my analyses of Lula's behaviour using Principal Component Analysis (PCA). The entire data set (from 3rd of February 2001 to 7th of February 2003), comprising 374 separate observational periods, was included in this analysis. As with the data on Pinda, PCA was used to extract factors with eigenvalues >1 and the initial solution was subjected to Varimax rotation. The four principal components for Lula are listed in Table 3 along with their eigenvalues and proportion of variation and cumulative variation explained.

Table 3. *Principal components extracted with eigenvalues and per cent variation and cumulative variation explained for Lula.*

Principal Component	Eigenvalue	Variation Explained	Cumulative Variation Explained
1	2.725	22.705	22.705
2	1.255	10.455	33.161
3	1.180	9.830	42.990
4	1.113	9.273	52.264

Behavioural variable loadings for the rotated solution (Table 4) indicate the primary positive and negative loadings for each of the four components for Lula's behaviour.

Table 4. *Rotated Component Matrix for Lula. The key variables loading onto each component are in bold type.*

Behaviours	Components			
	1	2	3	4
Tail Swishes	**0.974**	0.069	-0.007	-0.023
Tail Rolling	**0.969**	0.064	-0.005	-0.013
Rolling	0.297	0.080	0.013	-0.018
Activity Bevel	0.182	0.154	0.087	-0.091
Rubbing Body	0.005	0.025	0.019	-0.021
Calling	-0.041	-0.014	0.009	**0.993**
Lip Licking	0.128	-0.006	-0.045	0.018
Grooming	0.017	0.047	0.007	0.035
Rubbing Face	0.106	**0.980**	-0.003	-0.015
Spraying	-0.016	0.002	-0.006	-0.027
Scratching	-0.010	-0.002	**0.995**	0.009
Tail Twitches	0.003	0.038	-0.040	-0.048

The first component had very high loadings from Tail Rolling and Tail Swishes (Table 4), which was very similar to analysis of Pinda’s behaviour. Lower loadings were obtained for Rolling, Activity Level and Lip Licking. All other behaviours had even lower loadings towards this component. The behaviour that loaded most heavily on the second component was Rubbing Face (Table 4). No other variables loaded strongly onto this component. The third component had significant loading of Scratching. Finally, the fourth component shows a high loading for Calling. As was also seen from PC 2 and 3 for Lula, there was only one key variable within the component, with all other behaviours showing low correlations to the key component behaviour. While PC1 showed similarities to what was found for Pinda, the remaining three components are quite dissimilar to Pinda’s analysis.

Behaviours Over Time

Again, due to the large number of variables and the very large number of observation periods I explored how the principal components extracted for Lula’s behaviours varied over time. I started by looking at the two variables strongly associated with PC1, Tail Rolling and Tail Swishing. I performed a correlation between Tail Rolling and Tail Swishing by Lula.

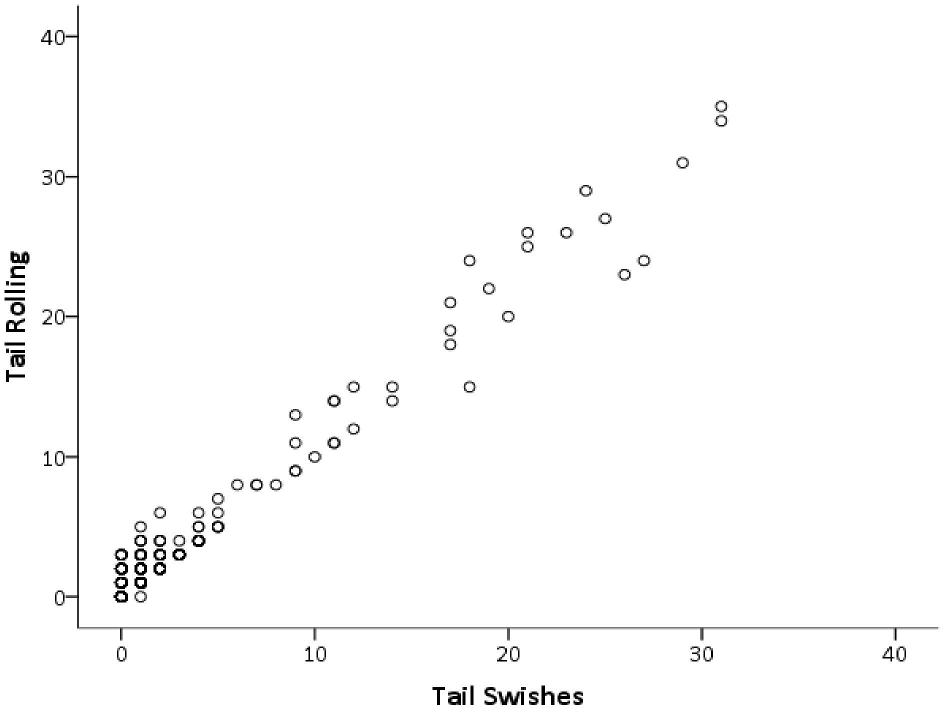

Figure 10. *Scattergram of absolute counts for Tail Rolling with Tail Swishes by Lula. Note that many circles represent more than one observation and the total number of data points in this graph is 746.*

Tail Rolling and Tail Swishing are highly correlated (Pearson's Correlation r = 0.980, P < 0.01, 2-tailed) (Figure 10). Due to the very close relationship between these two variables, I used Tail Rolling in the following analysis.

Spikes of Tail Rolling by Lula occur at different times within the study (Figure 11). Periods of cyclic behaviour were apparent (particularly between days 420-580) and some peak events appeared to be associated with husbandry practices for the cheetah at MZP (see also Figure 25). These events appear to relate an attempt at an artificial insemination (2nd of December 2001 seen as approximately day 300 in Figure 11), where Lula began the hormone therapy consistent with this process (see results further below). Peaks of Tail Rolling also coincided with animals being removed from the park (Bopha was removed on approximately day 420 and Zilkaat was removed on approximately day 700). A reduction in Tail Rolling behaviour coincided with the arrival of animals into the park (with Zilkaat coming back into the facility at approximately day 640). The final peak of Tail Rolling on day 720 corresponded with the time of courtship and mating on the 7th of February 2003 and the end of the current data collection.

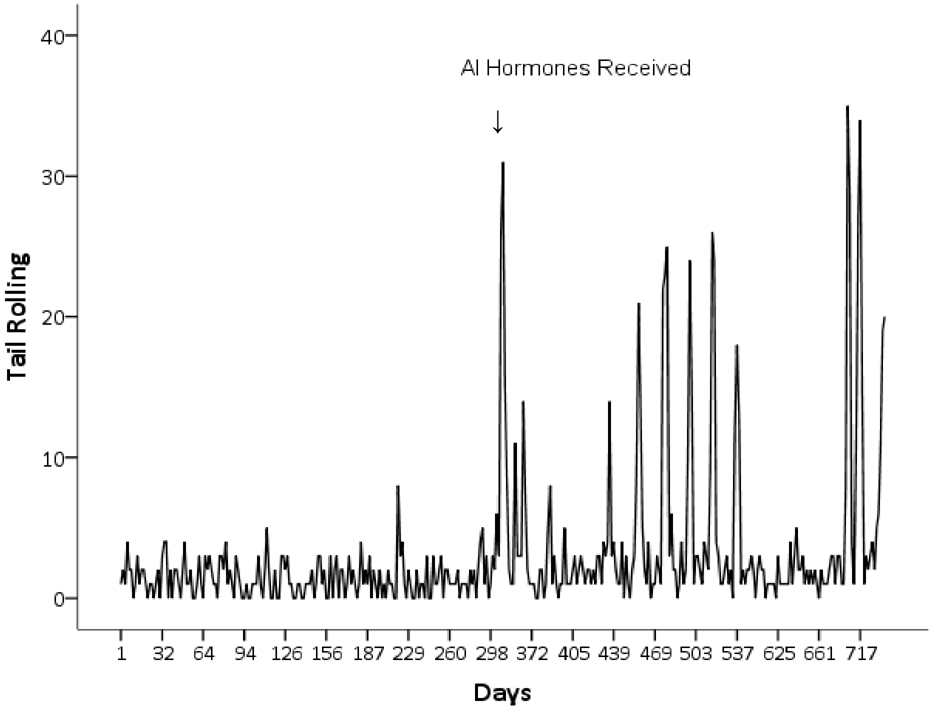

Figure 11. *Absolute counts for Tail Rolling by Lula, plotted against days since recording began (recording started on 3rd of February 2001 and finished on 7th of February 2003 with the artificial insemination (AI) procedure occurring on 2nd of December 2001).*

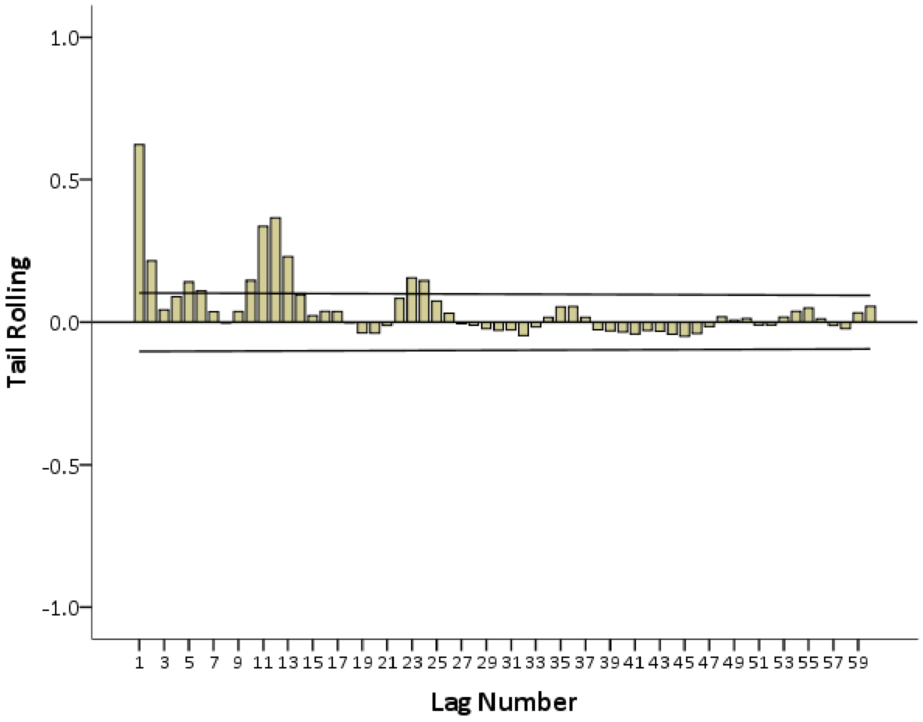

Figure 12. *Autocorrelation of Tail Rolling by Lula over lag time. The y-axis is in units of the correlation coefficient and the x-axis is in units of lag days. The two horizontal lines indicate the upper and lower 95% confidence limits for statistically significant*

correlation coefficients, so that lag times with bars extending beyond these lines can be regarded as exhibiting significant positive or negative correlations.

As for Pinda's Tail Rolling analysis, I analysed autocorrelations on Tail Rolling by Lula (Figure 12). This analysis strongly indicates Tail Rolling cyclicity. As was seen with Pinda (Figure 3), Tail Rolling cycles, with peaks occurring at intervals of about 12 days. Conversely, there is much less structuring than seen with Pinda, with longer periods of positive correlation and no periods of statistically significant negative correlation.

Due to the vast differences in Lula's Tail Rolling behaviour between 2001 and 2002, I performed the autocorrelation analysis for each year separately. The autocorrelation analysis indicates a lack of patterns in Tail Rolling by Lula during 2001, with only two instances where correlation coefficients exceeded the values required for statistical significance, and with no evidence of any obvious behavioural cycles (Figure 13).

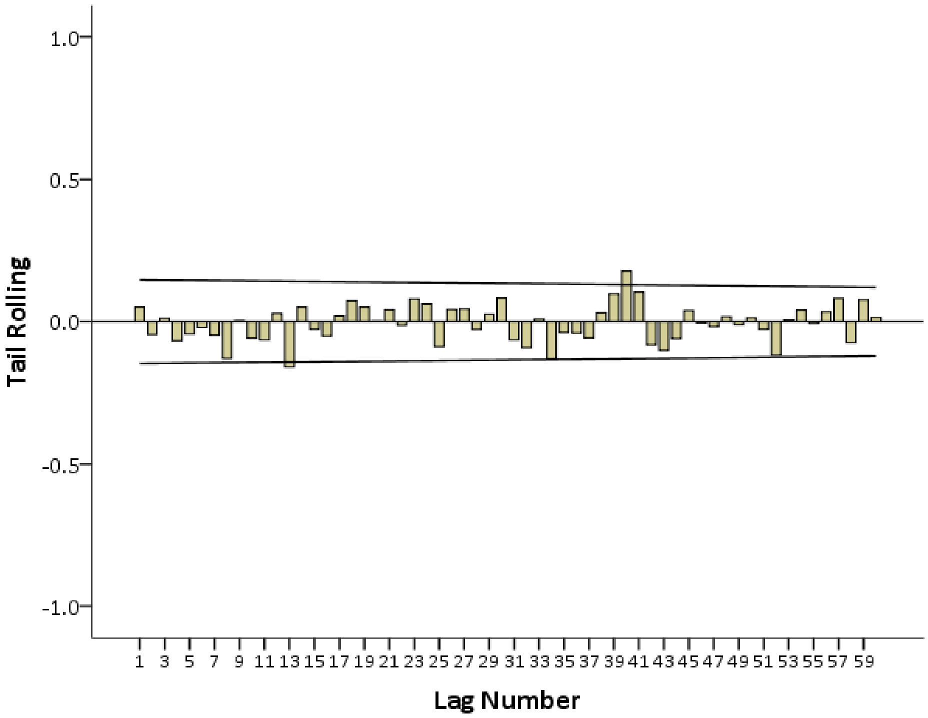

Figure 13. *Autocorrelation of Tail Rolling (with 95% Confidence Limits) by Lula in 2001 over lag time. The y-axis is in units of the correlation coefficient and the x-axis is in units of lag days. The two horizontal lines indicate the upper and lower 95% confidence limits for statistically significant correlation coefficients, so that lag times*

with bars extending beyond these lines can be regarded as exhibiting significant positive or negative correlations.

In contrast to the analysis for 2001 behaviour, the autocorrelation analysis of behaviour in 2002 suggests a much higher level of structuring and evidence of cycling in Tail Rolling (Figure 14). Here we see a strong cycling between positive and negative correlations up until a lag time of about 23 days. Interestingly, longer lag times mostly involved negative correlations, and while not statistically significant, this is likely due to the appearance and then disappearance of obvious cycling in 2002 (Figure 11).

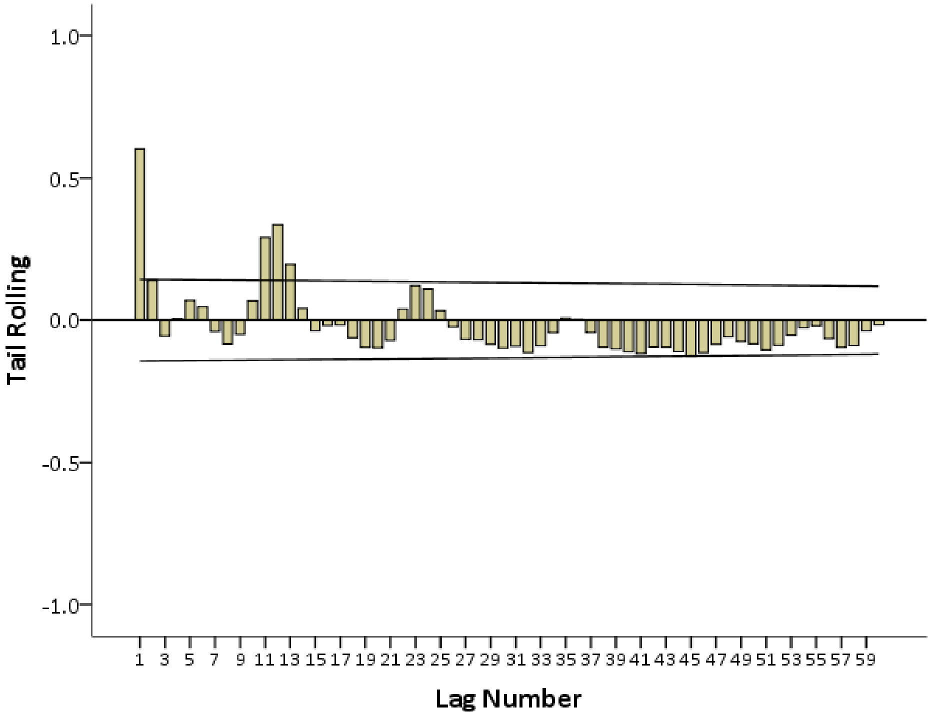

Figure 14. *Autocorrelation of Tail Rolling (with 95% Confidence Limits) by Lula in 2002 over lag time. The y-axis is in units of the correlation coefficient and the x-axis is in units of lag days. The two horizontal lines indicate the upper and lower 95% confidence limits for statistically significant correlation coefficients, so that lag times with bars extending beyond these lines can be regarded as exhibiting significant positive or negative correlations.*

The most heavily loading behaviour onto PC2 was Rubbing Face (Figure 15). It is apparent that while Rubbing Face is quite frequent and also variable in expression, there is no apparent cyclicity as was found for PC1. This behaviour increases in frequency in the second half of the study (from January 2002), corresponding to the time when Lula was involved in the artificial insemination trial.

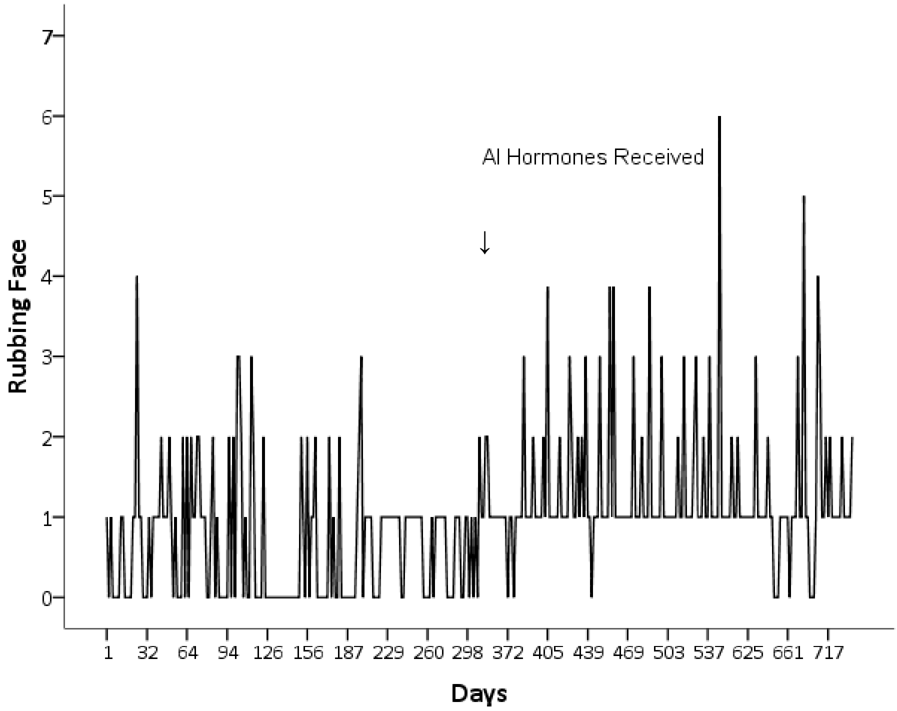

Figure 15. *Absolute counts for Rubbing Face for Lula, plotted against days since recording began (recording started on 3^{rd} of February 2001 and finished on 7^{th} of February 2003 with the artificial insemination (AI) procedure occurring on 2^{nd} of December 2001).*

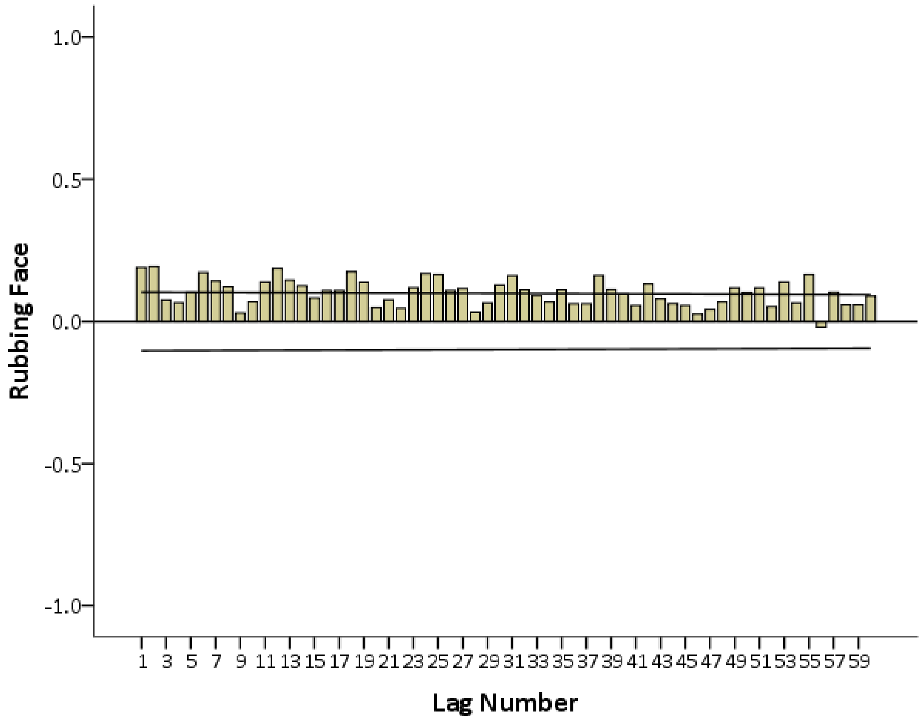

Figure 16. *Autocorrelation of Rubbing Face (with 95% confidence limits) by Lula over lag time. The y-axis is in units of the correlation coefficient and the x-axis is in units of*

lag days. The two horizontal lines indicate the upper and lower 95% confidence limits for statistically significant correlation coefficients, so that lag times with bars extending beyond these lines can be regarded as exhibiting significant positive or negative correlations.

The autocorrelation indicates some mild cycling, but this relationship is not as strong as for Tail Rolling (Figure 16). Rubbing Face only shows significant positive correlation across the lag times but frequently meets the 95% confidence limits for the analysis.

The most heavily loading behaviour for principal component 3 was Scratching. This behaviour was seen more frequently for Lula (Figure 17) than for Pinda (Figure 9), and there was no discernible cyclic pattern observed within this component. The behaviour does increase in frequency from approximately day 305, as seen with Tail Rolling and Rubbing Face. Due to the small number of observations, an autocorrelation was not performed for this component.

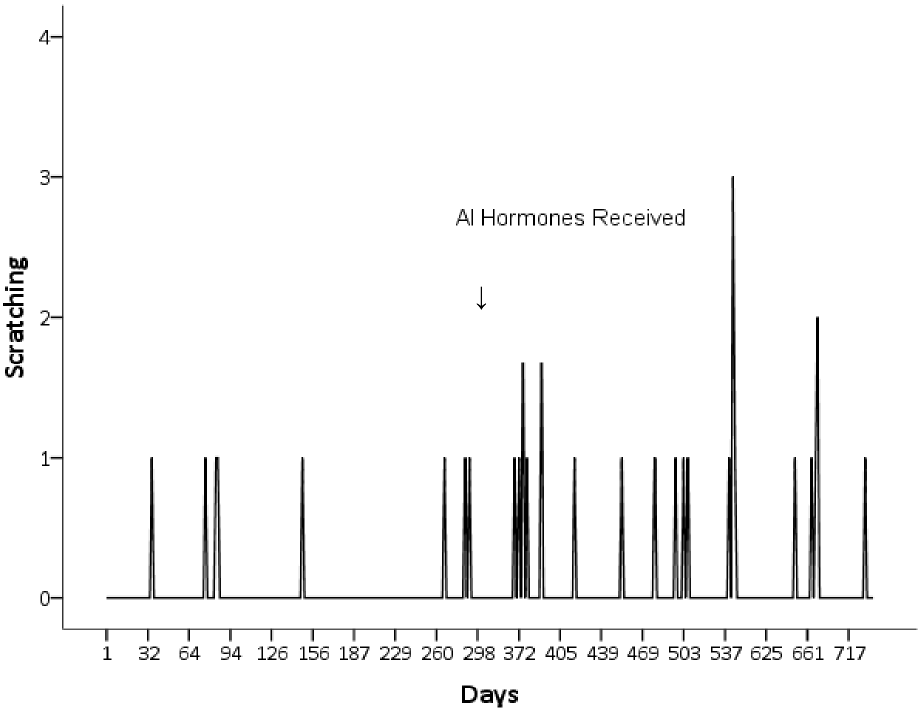

Figure 17. *Absolute counts for Scratching by Lula, plotted against days since recording began (recording started on 3rd of February 2001 and finished on 7th of February 2003 with the artificial insemination (AI) procedure occurring on 2nd of December 2001).*

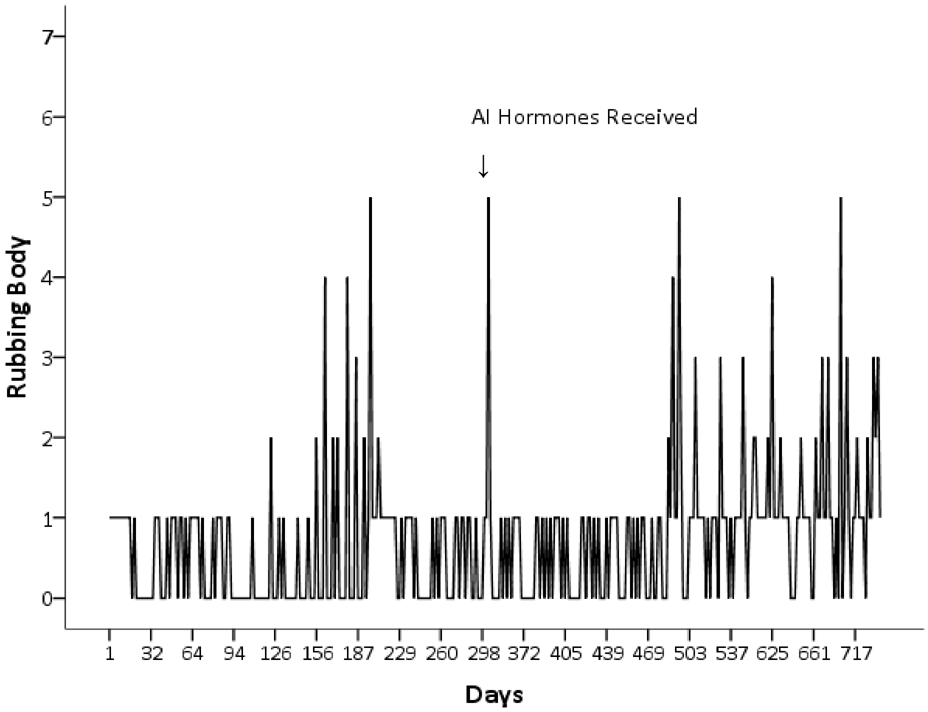

Figure 18. *Absolute counts for Rubbing Body by Lula, plotted against days since recording began (recording started on 3rd of February 2001 and finished on 7th of February 2003 with the artificial insemination (AI) procedure occurring on 2nd of December 2001).*

Finally, the most heavily loaded behaviour for principal component 4 was Rubbing Body. This behaviour was observed frequently, but there was no apparent cyclicity observed (Figure 18). Peaks of behaviour occurred during July-August 2001 and June 2002 to February 2003 (the end of recording). At the end of June 2001, female cheetahs were isolated from both male and female cheetahs on a permanent basis, with each female being housed in their own enclosure. As seen with other behaviours in the previous components, the frequency of Rubbing Body increases in the later part of the study, from approximately day 300, which is considerably different between 2001 and 2002.

An autocorrelation was performed for Rubbing Body in order to examine any possible cyclical expression of this behaviour. The autocorrelation did not indicate any clear evidence of cycling, and although there are some lag periods where correlation coefficients exceed critical values, these do not seem to have any clear periodicity to them (Figure 19).

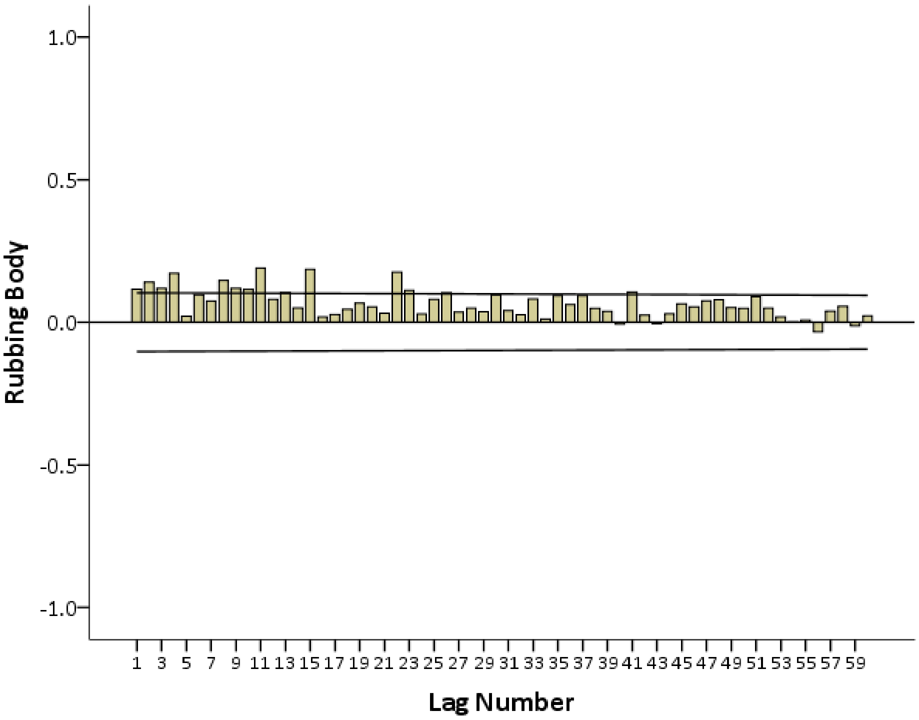

Figure 19. *Autocorrelation of Rubbing Body (with 95% confidence limits) by Lula over lag time. The y-axis is in units of the correlation coefficient and the x-axis is in units of lag days. The two horizontal lines indicate the upper and lower 95% confidence limits for statistically significant correlation coefficients, so that lag times with bars extending beyond these lines can be regarded as exhibiting significant positive or negative correlations.*

Changes in Behaviour Associated with Artificial insemination

The above analyses suggest that for all four representative behaviours (i.e. associated respectively with each of the four PCs) by Lula increased considerably in frequency in the second half of the study. This increase in behaviours seemed to correspond to Lula receiving the hormone treatment for the artificial insemination procedure. As these changes appear to involve a wide spectrum of behaviours, involving all four principal components, I explored the behavioural variation further by taking a behavioural diversity-over-time approach. I used the Shannon-Weaver Index (Krebs, 1989) for this exploration.

As I noted in Methods, the Shannon-Weaver Index was developed to measure diversity in populations by looking at species numbers and their relative abundance (Krebs 1989), but it can also be used to measure diversity in behaviours (McCormick 2003). It not only looks at the number of behaviours, but the evenness in how they are distributed and it is useful in comparing the richness/evenness in sample composition.

In order to calculate the Shannon-Weaver Index, observation days were grouped into 50-day periods, starting from day 1 of observation. Therefore all observations from observation day 1 to 50 formed period 1, and observations occurring between days 51 and 100 formed period 2. Observations were grouped in this way through to observations past 700 days from the beginning of the study, period 15.

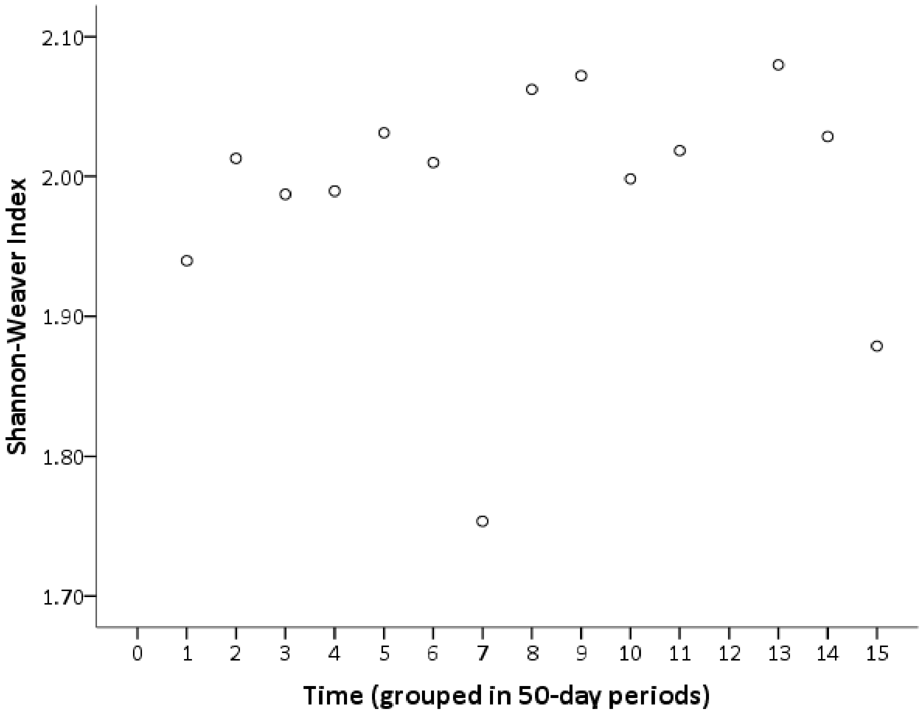

Figure 20. *Shannon-Weaver Index for Lula plotted against days grouped into 50-day time periods.*

The Shannon-Weaver Index over the 50 day periods for Lula suggests that almost all scores are grouped between 1.93 and 2.10, showing a relatively high richness and evenness of behaviour (Figure 20). The exception to this is for observation period 7 and, to a lesser extent, period 15, where the richness of behaviour is considerably reduced. These periods correspond to artificial insemination (period 7) and mating (period 15).

As Tail Rolling and Tail swishing were previously found to be associated with AI treatment and mating, I decided to explore which changes in behaviour corresponded to these decreased Shannon-Weaver values. Lula's Tail Rolling and Swishing combined, is contrasted with all other behaviours combined for each of the 50-day time periods in Figure 21. Again, a marked change in behaviour can be seen during period 7. Here we see that while there is an increase in Tail Rolling and Swishing there is a substantial decrease in all other behaviours The other unusually low value for the Shannon-Weaver Index is period 15 and this also corresponds to an increase in Tail movements and a decrease in all other behaviours combined (Figure 20). However, the increase in tail movements is much higher than for period 7. An increase in Tail Rolling and Swishing was also seen during period 10. However, unlike periods 7 and 15, equal increases in all other behaviours were seen at the time. This period corresponds to the time Bopha was removed from the facility.

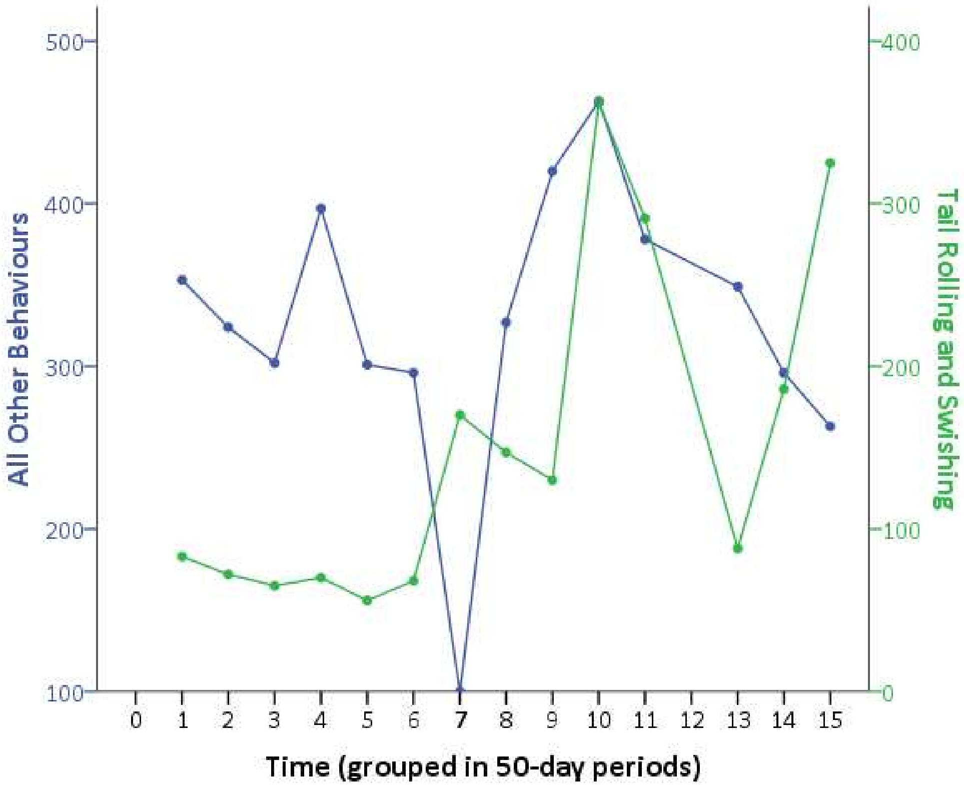

Figure 21. *Absolute counts for Tail Rolling and Swishing for Lula contrasted with All Other Behaviour plotted against days grouped in 50-day time periods.*

These results for Lula were then compared against measures of behavioural diversity using the Shannon-Weaver Index for Pinda to examine whether the changes observed in Lula, seemingly influenced by the hormone treatment, were absent in Pinda where no hormone treatment was administered.

The Shannon-Weaver Index for Pinda, also calculated from daily records pooled into 50-day periods is shown in Figure 22. There is a lower index observed for Pinda than Lula, and the 50-day scores show less variation for Pinda than Lula. The index over these periods also shows a higher degree of general variability than was seen for Lula, where scores were tightly grouped.

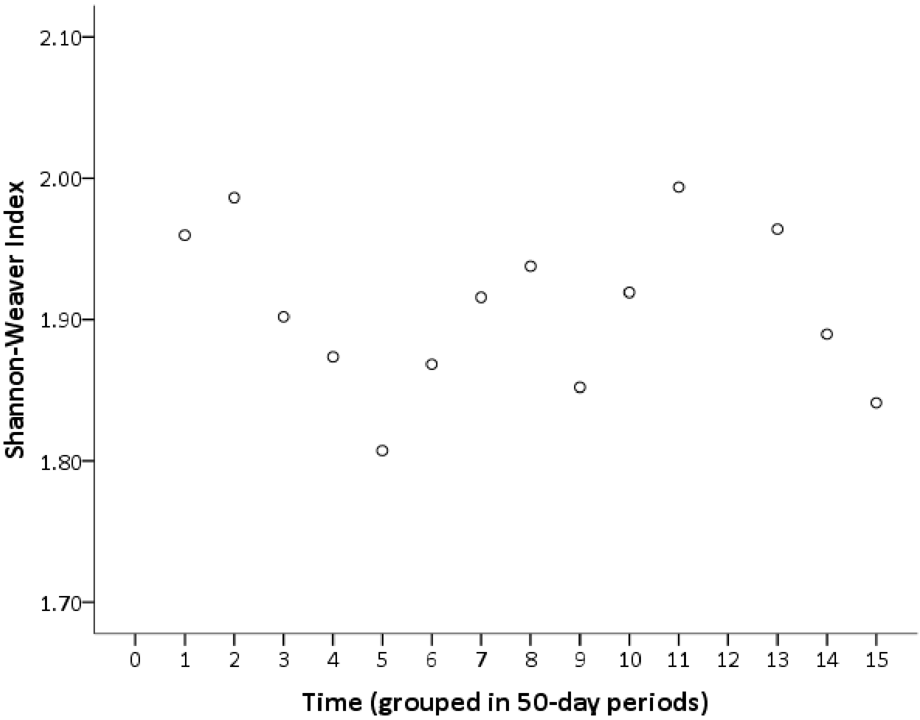

Figure 22. *Shannon-Weaver Index for Pinda plotted against days grouped into 50-day time periods.*

Pinda’s Tail Rolling and Swishing was contrasted with all other behaviours for each of the 50-day time periods and a different pattern was seen for Pinda (Figure 23) than was for Lula. Tail Rolling and Swishing as well as all other behaviours have almost ceased in period 7, with very little behaviour being displayed. A reduction in all behaviours in seen in period 15, although not to the same extent as in period 7. While Lula’s behaviours opposed each other at these periods, Pinda’s behaviours all trend in the same direction.

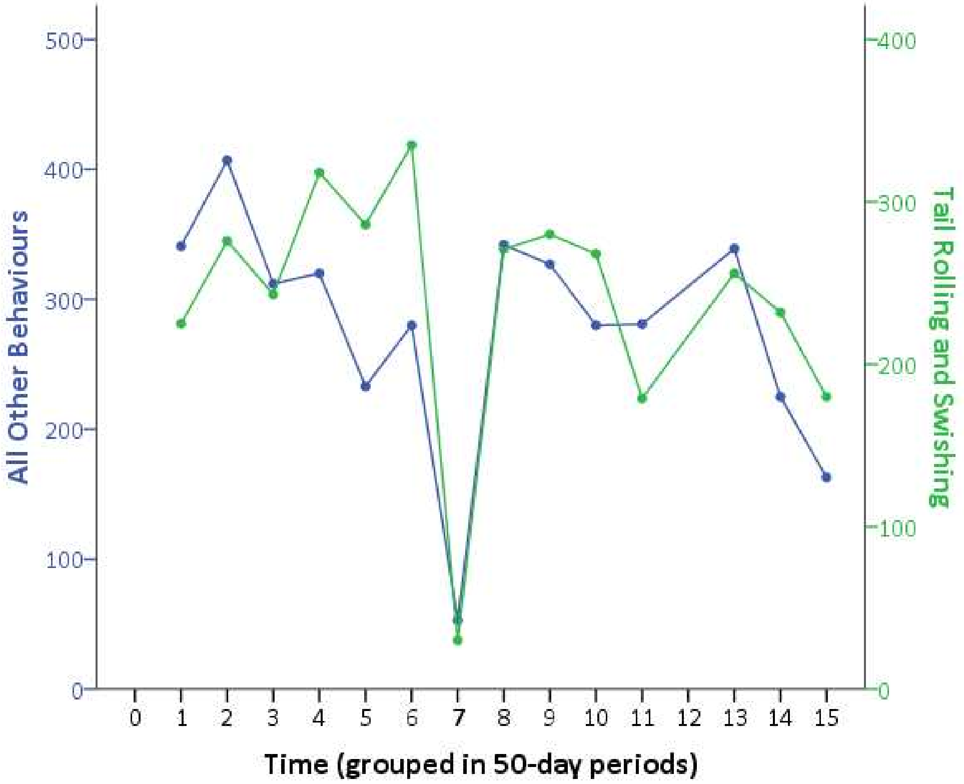

Figure 23. *Absolute counts for Tail Rolling and Swishing contrasted with All Other Behaviour by Pinda plotted against days grouped in 50-day time periods.*

Female Tail Movement Behaviours

Each female displayed a large variation in tail movement behaviours. These behaviours included twitching the entire tail, twitching the tail tip, rolling the tail from the base and Tail Swishing. Each female cheetah had a distinctive tail swish pattern. Each of these tail swish patterns was specific to the individual and was repeated at various times during the study. As a component to Tail Swishing, each female showed Tail Rolling, where the tail was rolled away from the anal-genital region. Tail Rolling was observed in isolation to other behaviours for three of the four female cheetah, but it was always seen as a component of each individual’s tail swish patterns. It is therefore important to look at Tail Swishing as well as Tail Rolling as it is an exaggerated movement and visible from the distance.

Components to the Tail Swish

I defined three body regions where the various elements of the tail swish could take place. These regions were referred to as the upper, mid and lower regions. The upper region was defined as anywhere above the rump/back of the cheetah, the mid region was the rump, including the area from the top of the rump to the area where the hind legs visibly separate, and the lower region was the area from the bottom of the mid-section to the ground/end of the tail. Observations were conducted from a direct rear view of cheetahs when the region was determined.

Tail Swishes all began from the relaxed position, with the tail hanging between the legs. Tail swishes consisted of large rhythmic motions, exaggerated motions or fast flicks to the side. Rolling the tail from the base (to the side) and exposing the anus was linked to swishes in all females. Individual Tail Swishing patterns were observed in all four females, with each female displaying a separate and distinct pattern. One swish was considered to be complete if the tail remained in the relaxed position for at least two seconds. Patterns of swishing were seen to vary between animals, with peak swishing lasting between 2 and 4 days. Further details on individual’s behaviour are described below.

Individual Tail Swish Descriptions

Pinda

Pinda’s tail swish changed by becoming more abbreviated once the males began to be paraded as a group past the female enclosures (early October 2001, see Chapter 6). Originally she moved her tail through all three regions, but this was abbreviated to include only the mid and lower regions. Each swish exposed her anus/genital region as the tail was lifted and rolled to the right, paused and then fell down moving to the left of her body and then back to the right before returning to the relaxed position. In the abbreviated form, the tail would roll from the base and then be swished around to the side of the body (often slapping the body) in the mid region. It would then fall to the opposite side, through the lower region and then back to the relaxed position. Pinda was seen to always roll her tail with every swish (See Figure 2), and as these behaviours have been shown to be strongly correlated, Tail Rolling has been used for the following analysis. Pinda’s Tail Rolling was plotted against days and key events, when she was isolated from other animals, are noted using arrows (Figure 24).

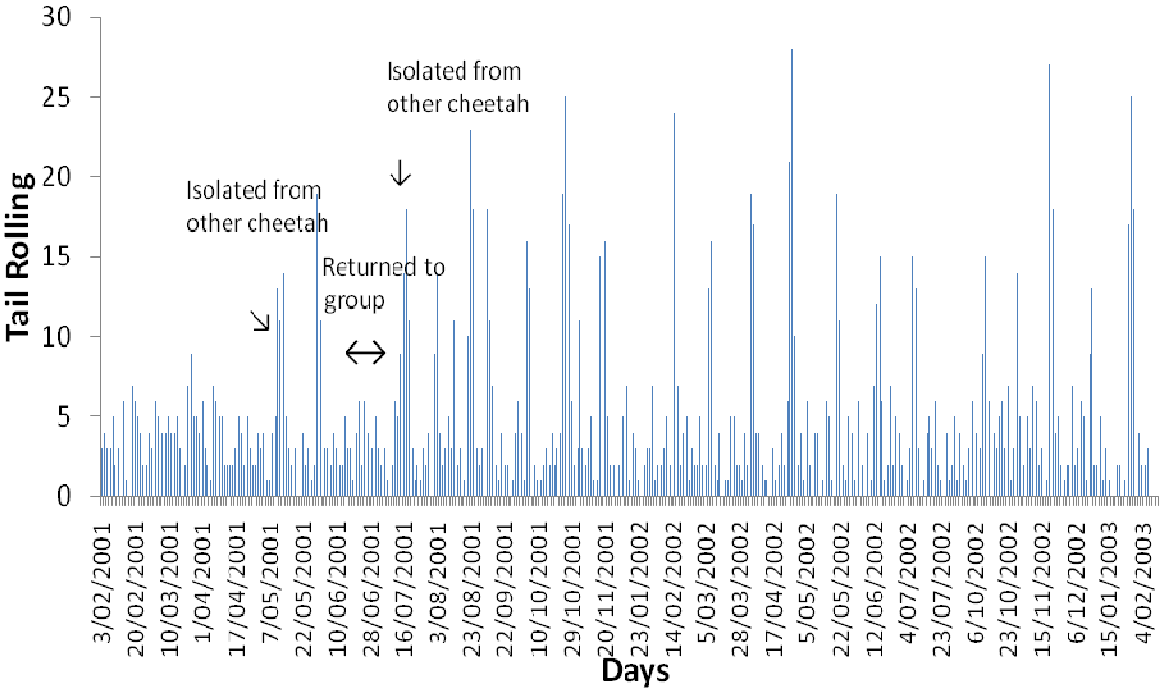

Figure 24. *Absolute counts for Tail Rolling by Pinda plotted against days. Initially, Pinda was housed with a group of both males and females. It was not until July 2001 that she was isolated.*

Lula

Lula's tail swish occurred within the mid and lower regions and consisted of rolling from the base and then holding the tail to the side while twitching and curling the lower quarter of the tail. The twitching was very rapid, almost as in a vibration. Lula also twitched her tail tip without the swish behaviour. However, this did not correspond with Tail Rolling or Tail Swishing. Lula's Tail Rolling was plotted against days as well as key husbandry events during data recording (Figure 25). Tail Rolling spiked dramatically when she was given the AI hormone therapy (PMSG and hCG) as well as when animals were moved out of the facility.

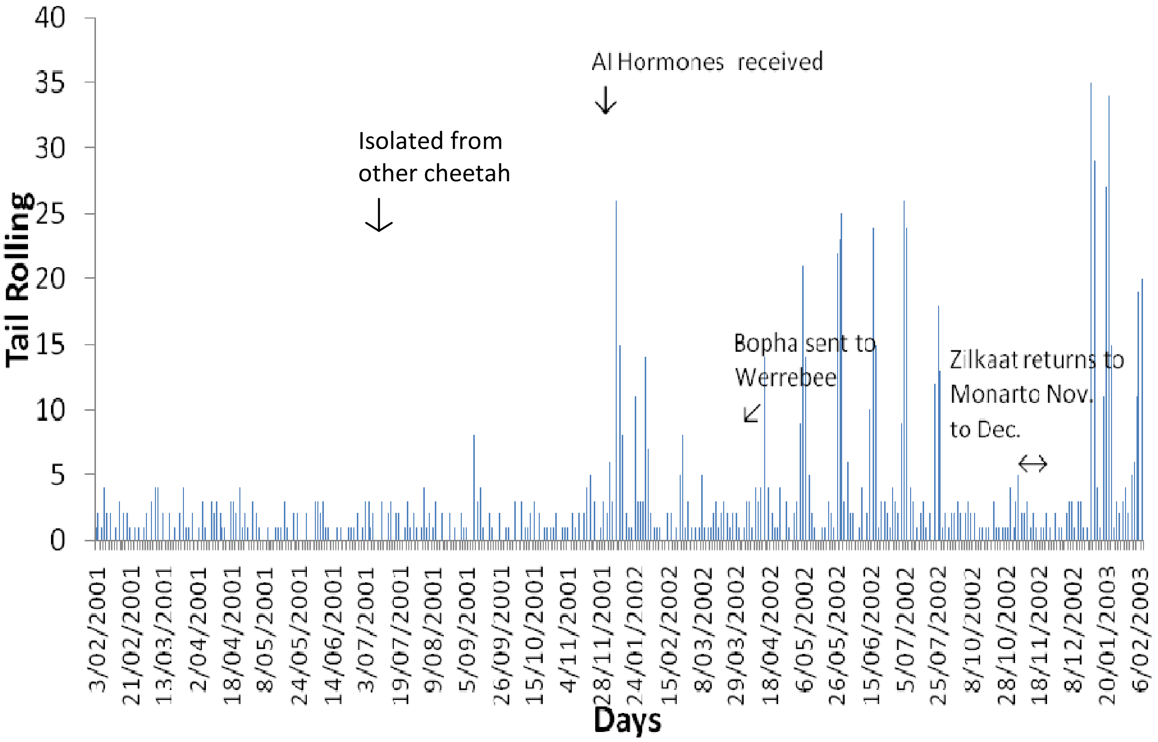

Figure 25. *Absolute counts for_Tail Rolling by Lula plotted against days. Initially Lula was housed with a group of both males and females. It was not until July 2001 that she was isolated.*

Bopha

Bopha’s tail swish moved through all three regions. From the relaxed position, her tail would roll from the base and typically swish to the right (occasionally seen to the left), usually up and partly over her back. The tail would then fall back through the mid-section to the lower region, twitching after it passed the relaxed position and on to the left side of her body. The tail would then return to the relaxed position. As was seen with Lula, Tail Twitching occurred at other times and was not correlated with Tail Swishing or Tail Rolling. Bopha’s Tail Rolling was plotted against days (Figure 26) and she was removed from the facility at the end of March 2002.

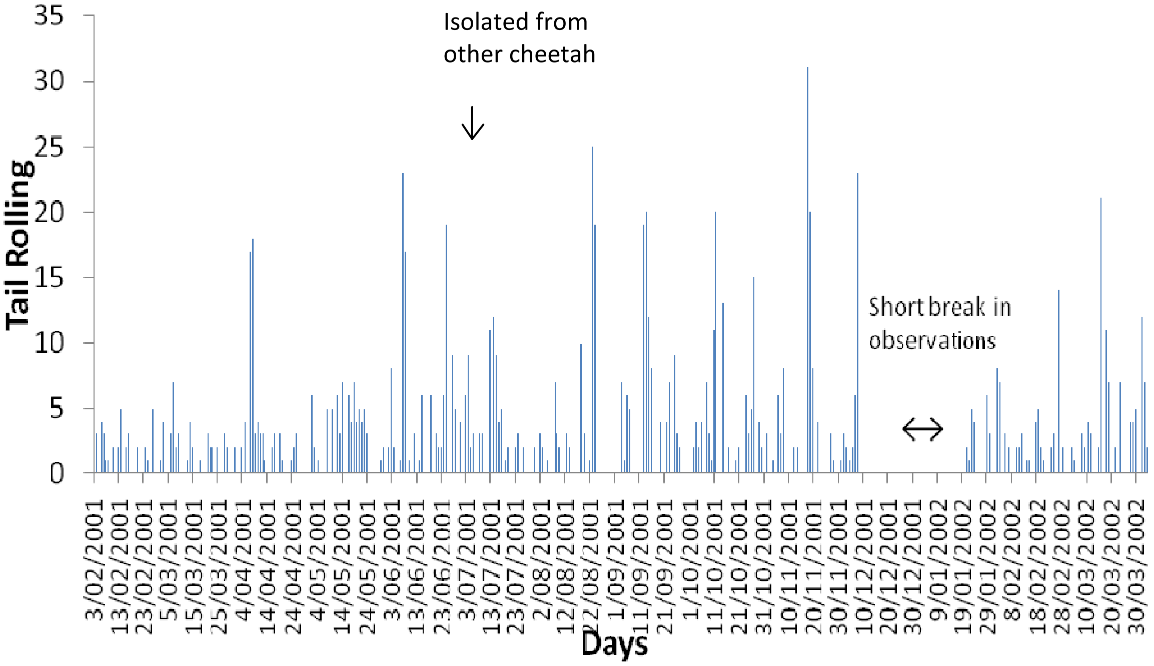

Figure 26. *Absolute counts for Tail Rolling by Bopha plotted against days. Initially Bopha was housed with a group of both males and females. It was not until July 2001 that she was isolated. (Observations for Bopha ended on 30th of March 2002 as she was sent to Werribee, Victoria on breeding loan).*

Zilkaat

Zilkaat's tail swish moved through the mid and lower regions. Beginning by rolling from the base, the tail would move to either side and be held slightly hooked on that side (usually with a twitch-like vibration) before moving back to the relaxed position. Zilkaat also twitched her tail at different times during the study, not related to Tail Swishing or rolling behaviour. The two short stays Zilkaat had at MZP as well as the AI procedure increased Tail Rolling dramatically (Figure 27).

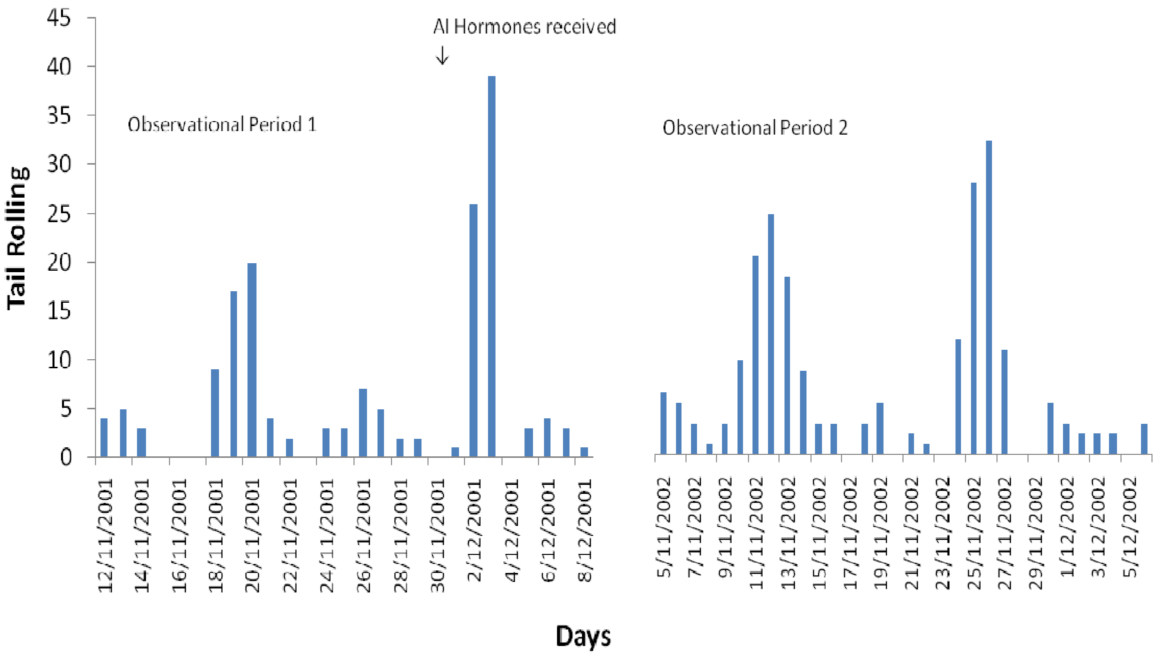

Figure 27. *Absolute counts for Tail Rolling by Zilkaat plotted against days. (Zilkaat was on breeding loan from Perth Zoo and had two short stays at Monarto Zoological Park where observations could be performed)*

Each female displayed varying levels of Tail Rolling behaviour. Pinda displayed regular cyclic behavioural events for the length of the study, while Lula displayed elevated periods of Tail Swishing related to events in captivity and changes in her social surroundings. Bopha displayed a lesser level of cyclicity in her Tail Rolling behaviour and Zilkaat showed cyclicity during her two short stays at MZP.

Lula's Tail Rolling behaviour differed strongly from the other females. Initially a spike in Tail Rolling was observed during the artificial insemination process, with a peak number of 31 tail rolls observed on 6th of December 2001. The second change occurred after Bopha was sent to Werribee Open Range Zoo in Victoria on breeding loan. Lula and Bopha had spent a considerable amount of their time housed together since their arrival at MZP, either in the same exhibit or in adjoining yards separated by a chain-link fence. While in these yards, the two females spent the majority of their days within five metres of each other, often lying against the fence next to each other. After the removal of Bopha, Lula appears to show cyclicity in her Tail Rolling behaviour. However, this cycling gradually declined over six months, ceasing altogether when Zilkaat was re-introduced into MZP. Finally after Zilkaat returned to Perth Zoo in December 2002, Lula was seen to show cyclicity in tail rolling up to 7th of February

2003. At this time she was mated. All Tail Rolling behaviour ceased soon after mating and shortly after it was ascertained that Lula was pregnant.

Luminescence

Periodicity in Tail Swishing and Tail Rolling was approximately 12-14 days (see above), which is close to half the duration of the lunar cycle. As there is evidence that lunar cycles might act as a zeitgeber in reproductive cycles of some mammals (e.g. Dixon et al. 2006 work on badgers), a possible coincidence between the lunar cycle and patterns of female cheetahs' tail movements was explored. I compared female cheetah tail movements with the levels of luminescence of the moon.

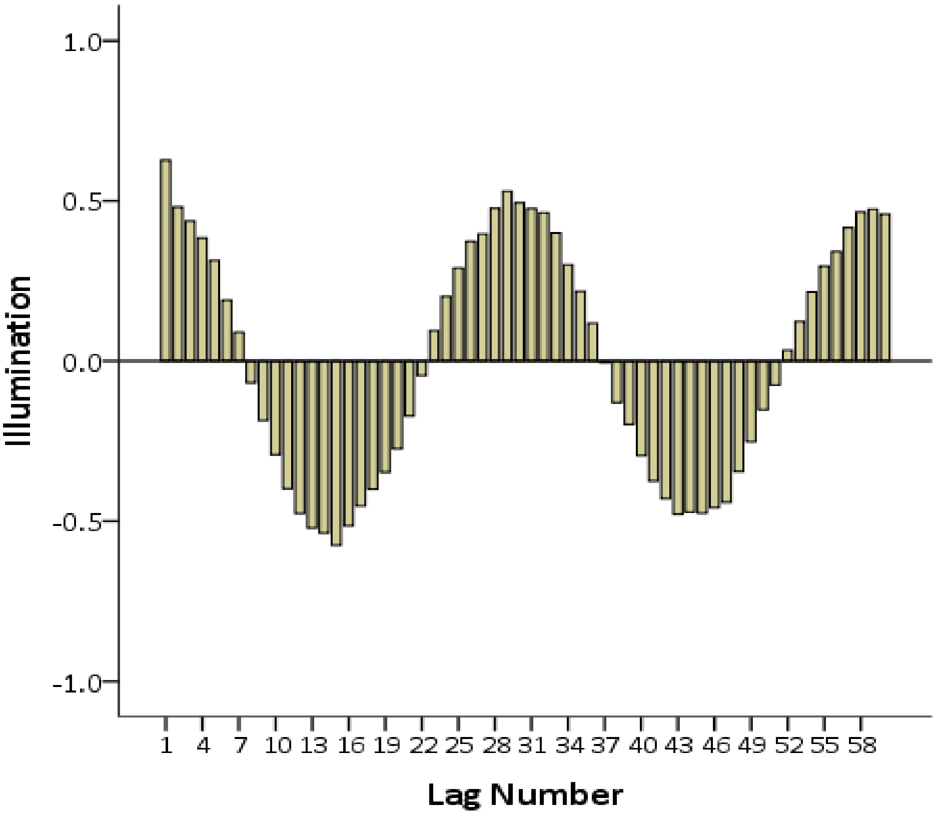

Figure 28. *Illumination of the moon plotted against lag time in days. (Data from the Bureau of Meteorology (http://www.bom.gov.au).*

The cyclic nature of the luminescence of the moon as measured by the Bureau of Meteorology for the MZP area was plotted against the lag number of up to 60 days (Figure 28). The periodicity of this cycle is strongly marked, as expected, with peak luminescence occurring every 29.5 days. This lag time is not seen with either Pinda or

Lula, with Pinda displaying a lag time of approxmately13.5 days (Figure 3) and Lula displaying a lag time of approximately12 days (Figure 12).

I then looked for a correlation between Tail Rolling and Illumination. Pinda had a Pearson's correlation coefficient of $r = 0.826$, $p < 0.01$, suggesting a correlation whereas Lula had a correlation coefficient of $r = 0.058$, $p = 0.316$, suggesting no evidence of a correlation. While the result for Pinda is suggestive of a correlation, it is possible that this represents non-causal coincidence resulting from an endogenous behavioural cycle that is approximately half that of the lunar cycle.

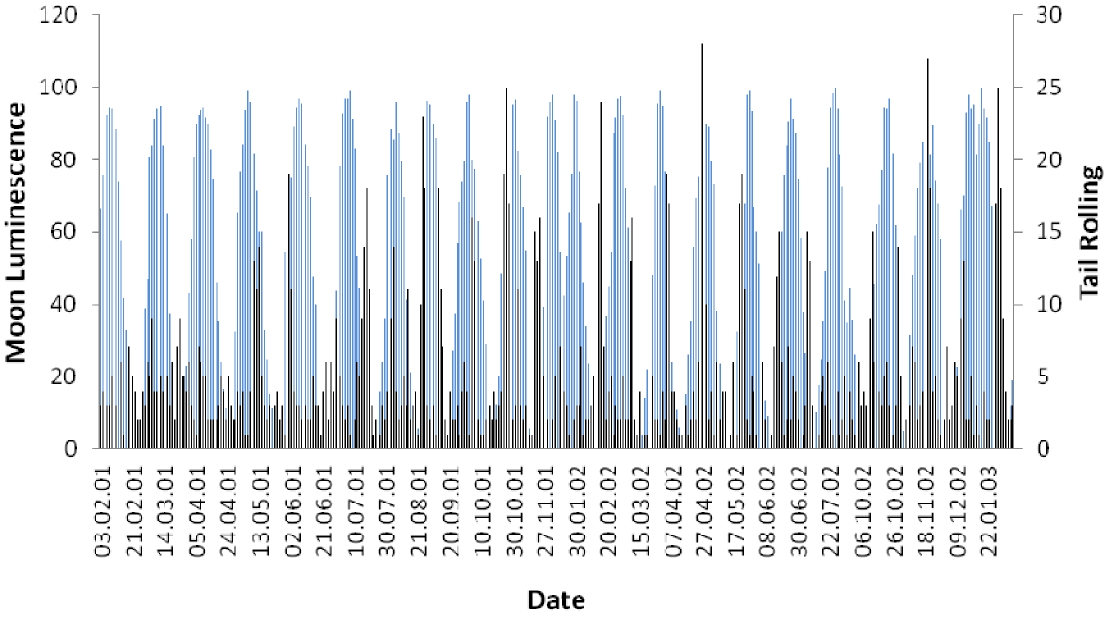

Figure 29. *Absolute counts for Pinda's Tail Rolling compared to luminescence for the study (where luminescence is represented by blue columns and Tail Rolling is represented by black bars).*

The lack of a causal relationship between the lunar cycle and the tail movement cycle is evident for Pinda (Figure 29) and for Lula (Figure 30). It can be seen that peaks in Tail Rolling occur at various times of the lunar cycle. Therefore lunar luminescence does not appear to impact on the increase or decrease of Tail Rolling behaviour exhibited by cheetahs. The significant correlation observed for Pinda is most likely due to a non-causal coincidence in the phase of both variables for part of the observational period.

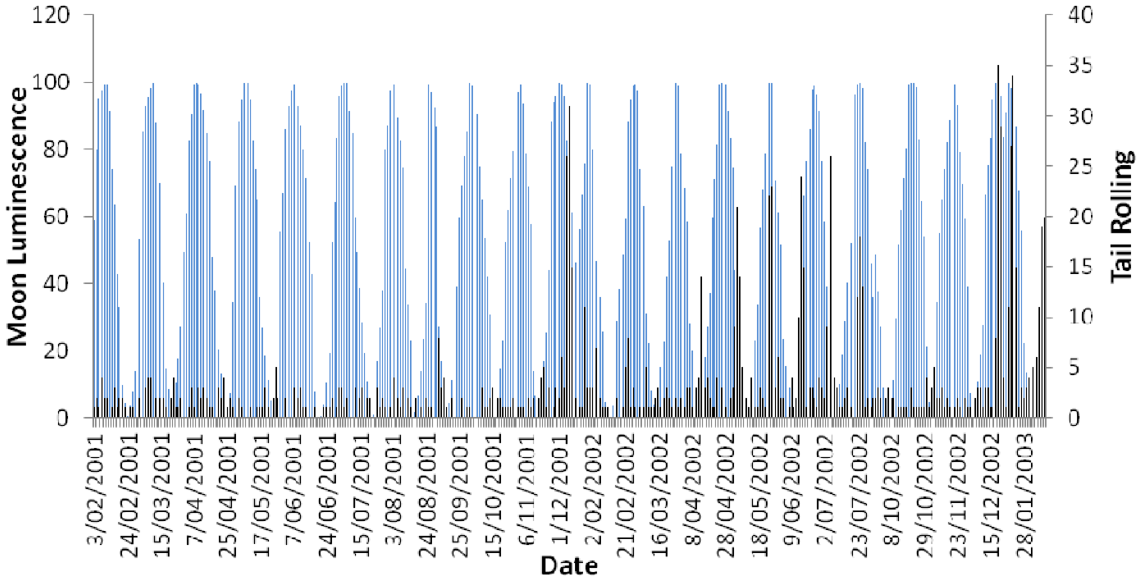

Figure 30. *Absolute counts for Lula's Tail Rolling compared to luminescence for the study (where luminescence is represented by blue columns and Tail Rolling is represented by black bars).*

Tail Movement Cycles Between Individuals

Finally, I examined the relationship of Tail Rolling and Tail Swishing between female cheetahs. I compared females as a final check against the possibility that the lunar cycle was driving their behavioural cycles. If this was the case, then all females should have oestrus cycles that are correlated over time, since they were all exposed to the same levels of luminescence stimuli. The relationships between animals (Pinda, Lula and Bopha) on both behaviours is outlined in Table 5. Zilkaat was excluded from analysis as data was only obtained for her over two short periods, as opposed to data collection for the other females, obtained over a considerably longer period of time.

Table 5. *Correlations between individuals for Tail Swishing and Tail Rolling (values above the diagonal give correlations between individuals for Tail Swishing, and values below the diagonal give correlations between individuals for Tail Rolling) where the correlation coefficient is reported in plain font and probability is reported in brackets.*

	Lula	Pinda	Bopha
Lula	--	-0.066 (0.202)	0.087 (0.182)
Pinda	-0.083 (0.202)	--	-0.060 (0.363)
Bopha	0.070 (0.182)	-0.083 (0.363)	--

There are no significant relationships between the females for either Tail Rolling or Tail Swishing (Table 5). This suggests that each female's Tail Rolling and Swishing behaviours are independent of each other and that there was little or no synchronicity between the animals in their tail movement behaviours.

Discussion

While the breeding success of cheetahs in captivity has improved over the last few decades, this success has occurred mainly in larger facilities that hold many animals. Little is still known about behaviour linked to oestrus in female cheetahs, and even less is known about the ways to determine if a female may be receptive, other than by steroidal assays.

Previous studies have looked at the physiology and genetics of the cheetah to explain poor breeding success rates. Research by the Species Survival Plan (SSP) examined the North American cheetah population to determine the key problems for the cheetah (Wildt & Grisham 1993). It was discovered by Wildt *et al.* (1993) that while the cheetah had a number of reproductive abnormalities, this was not preventing them from breeding. In fact, there were no obvious differences observed between proven breeders and non-breeders. They concluded that the cheetah was an induced ovulator, typically needing stimulation or male interaction to come into oestrus. Further studies by Asa *et al.* (1992) and Brown *et al.* (1996), tracked hormone levels over time and found cyclicity in hormonal fluctuations that occurred every 12-14 days, with occasional periods of anoestrus where this cycling ceased and hormones remained low. These cycles have been shown to be stable for some females, while fractured cycles with periods of anoestrus have occurred in other females (Brown *et al.* 1996). Brown and Wielebnowski (1998) showed that housing females in unnatural social pairings led to the suppression of oestrus. Suppression of oestrus among individuals housed together appears to be a strong factor, hence potentially causing periods of anoestrus. Yet, while the knowledge of the female cheetah's oestrus cycles is increasing, a key behavioural marker of oestrus has remained elusive.

Principal Component Analysis and Correlated Behaviour

In an initial examination of female cheetahs' behaviour, I found high levels of variability in the frequency of behaviours. These fluctuations were seen over short periods of time, such as weeks, as well as over longer periods of time, allowing comparisons over several years. Different behaviours were observed to fluctuate differently between individuals and sometimes at different rates within individuals. My behavioural data was examined using Principal Component Analysis (PCA), which uncovered some interesting relationships and patterns that had not been otherwise apparent. In each female cheetah, the first four principal components combined explained about 50% of the observed behaviour. The first principal component for both Pinda and Lula's behavioural data was almost entirely associated with tail movements, particularly Tail Rolling and Tail Swishing. I will discuss this in more detail below, but firstly I will consider the three other components.

Principal Components Two to Four

There were some marked differences between Pinda and Lula in the behavioural composition of the 2nd to 4th principal components. There was a considerably higher level of structuring to Pinda's behaviours, with significant loadings from a number of variables within each principal component (PC). This result is very different to what is seen with Lula, with only one variable showing a significant loading in each of the principal components 2-4. The behaviours heavily loaded onto components 2, 3 and 4 do not correspond between individuals and components (Tables 2 and 4). This result is consistent with the findings of Wielebnowski and Brown (1998), who found a high level of variability in behaviour between individual animals. In their study, differences were found in behaviours correlated with faecal oestradiol concentrations but no single behaviour in common was observed to fluctuate among individuals.

Rubbing Face and Scratching were the only behaviours to have heavy loadings for each female in different components; components 3 and 4 for Pinda and components 2 and 3 for Lula. For Lula, these were the only behaviours loaded onto the components. However, Pinda showed a higher level of structuring in her behaviour, with component 3 indicating that Grooming increased with Rubbing Face while Spraying decreased. Component 4 for Pinda depicted a reduction in Tail Twitching with an increase in Scratching. Wielebnowski (1999) reported that there was no significant correlation between Tail Twitching and oestrus. The current results support this finding, with Tail Twitching not significantly loading onto any PC for Lula. The other behaviours loaded onto the components occurred at different frequencies and seem to correlate with each other in different ways, depending on the individual animal being assessed. Lula also showed a marked difference in the behaviours loaded onto components 2, 3 and 4 at different times of the study, with a general increase in all behaviours displayed from 2001 to 2002 (Figures 15, 17 and 18).

Principal Component One-Tail Rolling and Tail Swishing

Approximately 20% of the observed variation in behaviour for Lula and Pinda was explained by PC 1, and for both females this was very strongly linked to tail movements. Tail Rolling and Tail Swishing by females were observed to fluctuate over the study period and the strong correlations between Tail Rolling and Tail Swishing illustrated the importance of the relationship between these two behaviours. Tail Swishing rarely occurred without Tail Rolling, suggesting that they are functionally linked. The components extracted by PCA are statistically independent of each other, exhibiting no covariation (Sokal & Rohlf 1995). It is therefore interesting that both Tail Rolling and Tail Swishing showed very low loadings onto the 2nd to 4th principal components, and were also the only behaviours to load heavily onto PC 1. This indicates that that while these two behaviours strongly covary, they do not appear to be linked to other behaviours.

The autocorrelations for Pinda and Lula indicate large differences in Tail Rolling from the first to the second half of the study. During the first half of the study (2001), the animals were moved around considerably and housed in various combinations, housed socially in large groups and also with male cheetah. While Pinda's Tail Rolling appears to be cyclical throughout the study, the autocorrelation suggests that there is less structuring throughout 2001, with bimodality within the negative correlations. This bimodality indicated further structuring within Tail Rolling at this time. During 2002 however, this bimodality disappears and the evidence for cyclicity becomes stronger.

During 2001 there was almost no evidence of cyclicity in Lula's tail movement behaviour, in strong contrast to Pinda's results. Positive and negative autocorrelation values consistently failed to meet the 95% confidence limits, and values appeared to be sporadic. However, a cyclic pattern was forming in 2002. This change in Tail Rolling, from a seemingly haphazard pattern to a cyclic pattern occurred after artificial insemination, which suggests a possible link to the procedure and possibly oestrus.

Artificial Insemination

Further analysis was performed to determine if the cyclicity of Tail Rolling could be linked to oestrus. Patterns of behaviour were investigated in relation to an artificial insemination procedure. As there was no faecal steroid analysis performed for this study, the hormones given through the artificial insemination process provided a small gauge to compare changes in reproductive status with behaviour.

Two of the four female cheetahs were involved with the artificial insemination program. Lula and Zilkaat were given serums to stimulate ovulation, while Bopha and Pinda were not treated. Increased Tail Rolling and Tail Swishing behaviour was noted for the two females treated with the hormone injections. Examination of the animals determined the presence of fresh luteal scarring, which provided physical evidence that the hormones had taken effect (Pers. comm. C. Monaghan – Senior Veterinarian, Perth Zoo and S. Bigwood – Senior Veterinarian, MZP).

Lula showed a marked change in her behaviour from approximately day 300, which corresponds to the time that she was given hormones for artificial insemination (Figure 11). At this time, the frequency of behaviours increased dramatically and cyclic Tail Rolling and Tail Swishing began – behaviours that were rarely seen previously for Lula. This extreme change in behaviour is evidence to suggest that the hormones given to Lula had an impact on her behaviour. Similar results were observed for Zilkaat, with Tail Rolling behaviour increasing significantly after the hormone therapy. As she was hand-reared, it was possible to physically manipulate this animal throughout the process. After the hormone therapy, Zilkaat became very sensitive to any touching of the rump/tail area and repeatedly laid down in a ‘lordosis’ type position in the presence of her primary keeper.

Although hormonal treatment involved only a single instance for two females, it does allow a valuable comparison of Tail Rolling in these animals. The increases in Tail

Rolling and Swishing before and after, and the associated changes, support the possibility that tail movements are a cue to oestrus in cheetahs.

The artificial insemination also brought to light another interesting finding. After being given the hormone therapy, Lula's entire behaviour patterns changed. Lula had previously displayed lower overall levels of behaviour and less structuring than Pinda. Lula now began displaying behaviours that she had not previously exhibited, such as cyclic Tail Rolling (Figure 11), increased Face/Body Rubbing (Figures 15 and 18) and increased Scratching (Figure 17).

The sudden change in the behavioural repertoire suggests that Lula may have been in a long period of anoestrus. This may have been caused by suppression of her oestrus cycling behaviour as a result of being housed with males and other females for long periods of time. Similar findings have been reported by Brown and Wielebnowski (1998). The comparison of Lula to Pinda suggests that while Pinda's cycling behaviour was quite stable, only showing a possible short period of possible anoestrus when housed with the males, Lula appeared to be considerably more sensitive to changes in her social situation as well as her environment. Changes in her behaviour appear to coincide with a number of social changes throughout the study. When given hormone therapy, cyclicity of Tail Rolling occurred. Yet when Lula was housed next to Bopha, with which she was very social, there was no evidence of cyclicity. When removed from Bopha, cyclic Tail Rolling was again evident. Later in the study, cycling ceased when Lula was housed next to Zilkaat, but started again soon after Zilkaat was returned to Perth Zoo. The current results support the findings of Wielebnowski *et al.* (2002) who reported that social housing of female cheetah can lead to oestrus suppression. The current study provides evidence of long periods of suppression and anoestrus in Lula, while oestrus only appeared to be suppressed in Pinda when she was housed with males.

Wielebnowski and Brown (1998) studied the possibility of behavioural cues to indicate oestrus. They reported considerable variation of behaviour over their study, with some behaviours such as "rub, roll, object sniff, meow-chirp and urine-spray" correlating to increases in oestradiol concentrations. Yet they did not conclusively correlate any behaviour with key events such as oestrus. They found that there was considerable variation between individual animals as well as variation related to age. Their study did not explicitly examine tail movements such as Tail Rolling or Tail Swishing, but did focus on behaviours such as rolling, vocalisations and spraying. However, they did note that the "tail is usually averted to one side" (p. 198) in reference to lordosis and mating positions. This description is similar to the behaviour I call Tail Rolling.

I observed both tail movements, Tail Rolling and Tail Swishing, for each of the four female cheetah over the study. Each individual displayed the same rolling behaviour but a unique variation of the swish movement. These tail movements were observed frequently, but Tail Rolling was the only behaviour that could be consistently tracked over all four individual females. Tail Swishing was also observed without Tail Rolling in three of the four females, and therefore the behaviour of Tail Rolling was used predominantly in the analysis. However, due to the highly visible action of the Tail Swish it may act as a possible aid for captive managers to determine key events and to be used as a cue to oestrus.

Mating

Additional supporting evidence for Tail Rolling being a cue to oestrus came at the end of the study. On the 5th of February 2003, Tail Rolling began to increase considerably for Lula, and was elevated to 20 events per hour of observation from approximately 1-2 times per hour on previous days (Figure 25). Two days later she successfully mated.

Cyclicity Measured Against Luminescence

Lastly, the cyclic nature of the Tail Rolling was further analysed by comparing the cyclic patterns to the lunar cycle. Hansen and colleagues (1983), Sharma & Chandrashekaren (2005) and Dixon and colleagues (2006), have shown how external time cues, or zeitgebers, can influence the reproductive cycles of both nocturnal and diurnal species. As Tail Rolling was observed consistently at 12-14 days, I felt it was important to examine any possible external drivers to this behaviour. As Pinda was the only female that displayed regular cyclicity for the majority of the study, I could not eliminate the possibility of lunar cycles affecting behaviour. Tail Rolling analysis showed that while there was a consistent pattern similar to the lunar cycle, this similarity was due to the average periodicity for the female cycle being 12-14 days.

On closer examination of Tail Rolling for each female, it was seen that behaviours failed to match the lunar cycles over time. I also found differences between the individuals, with data points for Pinda, Lula and Bopha failing to show a correlation. The lack of correlation between females suggests that there was no common external cause that linked the females' Tail Rolling cyclicity. This further supports Tail Rolling as a possible marker for oestrus.

Tail Rolling as a Marker of Oestrus

Oestrus in most of the 'great' cats occurs cyclically (Schmidt *et al.* 1979, Bonney *et al.* 1981, Seal *et al.* 1985 and Schmidt *et al.* 1988) with cycles ranging from 20-30 days. The results observed from Pinda and Lula show strong cycling of Tail Rolling and Tail Swishing. This distinctive pattern is only seen for these behaviours. The cyclicity of tail rolling is consistent and observed over a 12-14 day period. This time period of behavioural fluctuation is consistent with the length of cycle reported over many studies on the cheetah, including those examining biological assays (Asa e*t al.* 1992 and Brown *et al.* 1996). Zilkaat also showed a cycle of 13 days for Tail Rolling over her 2 short stays at MZP. This cyclicity was also reported by her primary keeper in the months leading to her second stay at MZP (Pers. comm. Starr, K. 3rd of November, 2002). This result, teamed with the considerable rise in Tail Rolling behaviour seen during artificial insemination and when Lula was successfully mated, certainly suggests that Tail Rolling is a possible marker for oestrus in cheetah.

Finally, there were no correlations found between females for Tail Rolling. This indicates that there is no further influence of external practices or management within the facility on Tail Rolling. As all three females, Pinda, Lula and Bopha, were subjected to the same husbandry routine, if there were some underlying factor driving Tail Rolling, the behaviours would correlate. Instead, we see little to no synchronicity, with vast differences in Tail Rolling behaviour.

Cues to Oestrus for Captive Management

The cyclicity of Tail Rolling behaviour certainly suggests that this behaviour is a relevant marker for oestrus in the cheetah. Whilst Tail Rolling appears to be the most important factor in determining oestrus, it is usually not as visible as the highly demonstrative tail swish. Hence, as it was found that the behaviours are closely correlated, it is important to take into account the entire tail swish behaviour. For management of cheetah in captivity, the swish behaviour can help to provide the visual aid needed by staff to monitor their animals and perform introductions.

Conclusions

In the last 15 years, breeding successes have been more consistently noted in the larger South African facilities of Hoedspruit, Pretoria–de Wildt and Oudtshoorn (Marker *et al.* 2007 and Bertschinger *et al.* 2008). Yet these facilities not only have the benefits of large open spaces to house cheetah (closely representing their normal home ranges) but are typically not dependant on visits from the public (McKeown 1991). Hence, they can provide different conditions compared to the average suburban zoo where the majority of the captive cheetah's genetic diversity is held (Marker-Kraus & Grisham 1993).

While providing vital information on the cheetah, the research performed by the Species Survival Plan has still been unable to solve the mystery of captive cheetah breeding and achieve successful and frequent breeding throughout a range of facilities (Wildt *et al.* 1993, Terio *et al.* 2004 and Augustus *et al.* 2006).

The current study’s primary goal was to determine if a behaviour could be linked to oestrus in cheetahs. This would allow the development of a behavioural key to determine receptivity in the female cheetah. This key is important to assist management staff in performing introductions between the sexes with minimal risk to the animals. Reliable methods, such as behavioural indicators of oestrus are needed to improve the breeding chances of captive species. Wildt and his colleagues (1993) were concerned with the drop in the effective population size of the cheetah in captivity, with very few animals breeding and passing on genetic information. While some facilities are having breeding success, more and more of the studbooks depict the same individuals siring litters, rather than multiple individuals from within each facility (Marker *et al.* 2007).

The current study also aimed to investigate housing female cheetah in unnatural social situations. I found that housing females socially may cause oestrus suppression, varying from mild suppression when housed with males for some individuals (i.e. Pinda) to total suppression of behaviours in other females (i.e. Lula). Limiting visual access and providing females with separate enclosures could improve the levels of observed suppression, as was seen from manipulation of animals within MZP. The suppression of oestrus may be adding to the problems faced by captive cheetah and compound the problem of fewer individuals breeding successfully.

With the vast body of research on cheetahs over the last 20 years, we now know that the breeding problems faced by the cheetah have a significant social/behavioural component. Further research is needed to confirm a viable cue to oestrus and to determine at what point social housing becomes a problem for the female cheetah. This is crucial to the breeding success of cheetah in smaller facilities like suburban zoos.

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