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# FEMALE REACTION TO MALE URINE SCENTS AS POTENTIAL INDICATOR OF MATE CHOICE IN CAPTIVE CHEETAHS (ACINONYX JUBATUS)

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by

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Submitted in Partial Fulfillment of the Requirements for the
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Genetic variation in the cheetah (*Acinonyx jubatus*) has become dangerously low because of dramatic population decline and fragmentation. Zoos throughout the world manage captive cheetahs with breeding programs to maximize genetic heterozygosity. Unfortunately, the zoo community has not accomplished consistent breeding success with cheetahs, possibly because of a general lack of information on sociosexual behavior. Currently, individual cheetahs are assigned mates based primarily on genetic relatedness; however, evidence from many species suggests that allowing animals to choose mates increases breeding success. When animals, primarily females, are allowed to choose mates they will often pick the best genetic match. I tested whether female cheetahs can determine their genetic relatedness to males by investigating their urine scents. Voided male urine was collected following scent marking. The female was offered scents from three different males: one from an unrelated male, a "good" mate choice (A), another from a male that was equivalent to a second cousin, an "average" mate choice (B), and one from a male that was closely related, equivalent to a brother/father, a "poor" mate choice (C). Every female was also offered "blank" gauze as a control (D). The scents were offered in a pair-wise forced choice paradigm for a total of six possible combinations. All behaviors displayed toward each scent (and their duration) were recorded. The estrous cycling of each female was monitored through fecal hormone

evaluation for approximately six weeks, including the weeks during scent trials. In every pairing except C vs. D, the females spent more time overall with the better mate choice in the pair; with three of the comparisons being significantly different (A>C, t=2.38, df=11, P=0.039; A>D, t=1.88, df=11, P= 0.087 and B>D, t=2.62, df=8, P=0.045). Proximity was the most frequently observed behavior and females spent more time in proximity to the most distantly related male scent in all pairings. They spent significantly more time in proximity with A in AC pairing (t=2.25, df=10, P=0.049) and with B in the BC and BD pairings (t=6.37, df=8, P=0.0002; t=2.46, df=6, P=0.049; respectively). Sniffing was the next most frequently observed behavior, and in all pairings (except CD) females spent more time sniffing the most distantly related male's scent; but was only significantly different for A in the AD pairing (t=2.31, df=7, P=0.055). Lag time of estradiol in fecal samples varies between individuals; therefore, the affect of daily estradiol concentrations on scent choices could not be determined. This is the first mate choice study using urine with a mammalian carnivore.

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# INTRODUCTION

## NATURAL HISTORY

Cheetahs (*Acinonyx jubatus*) are unique and highly endangered felids from Africa and parts of the Middle East. They have experienced a dramatic population decline, which has reduced the wild population from hundreds of thousands, that thrived as recently as a few hundred years ago, to between 7,000-12,000 individuals estimated today (Marker 1998, O'Brien 2003, IUCN 2009). All populations are listed as either critically endangered or vulnerable by the World Conservation Union (IUCN) Red List of Threatened Species (2009). Cheetahs are regulated by the Convention for International Trade in Endangered Species of Wild Fauna and Flora (CITES) as Appendix I, which means that they are at high risk of extinction in the wild (CITES 1992). The continued population decline has led to the disappearance of the cheetah from over three-fourths of its historic range, leaving the population sparse and fragmented (IUCN 2009).

The cheetah's current population decline can be linked to human land use practices, including the spread of agriculture and the construction of roads and buildings. Human development has also led to habitat loss, fragmentation, and a decline in the densities of the cheetah's prey populations (Marker-Kraus and Kraus 1990, Caro 1994). However, the main factor leading to the decline in the cheetah population is human exploitation (Caro 1994). Cheetahs are hunted for trophies and killed by ranchers and farmers as nuisance animals (Marker-Kraus and Grisham 1993).

## CAPTIVE HISTORY

There have been captive cheetahs in the United States since 1871 (Marker-Kraus 1997). The captive population in the United States is currently 251 cheetahs housed in 54 facilities (Long and Grisham 2008). The captive population has decreased several times during the last 137 years primarily because of declines in birth rates. To keep the population viable and healthy during the declines, several cheetahs were imported from zoos in other countries and caught from the wild. Capturing wild cheetahs to supplement the captive population is an unfortunate consequence for such an endangered species; nonetheless, it is a necessity until the captive population becomes viable and self-sustaining. The importation of these individuals contributed to several spikes in the captive population size and was followed by increased birth rates. However, since the 1870s the overall reproductive success in captivity has generally been poor (O'Brien et al. 1987, Long and Grisham 2008, Ziegler-Meeks 2009).

## POSSIBLE CAUSES OF POOR CAPTIVE REPRODUCTION

Zoos and related facilities, which house the captive population of cheetahs, have not accomplished consistent breeding success (Marker-Kraus 1997, Wielebnowski et al. 2002), a vital component of a self-sustaining population (Lees and Wilcken 2008). Many scientists (Newman et al. 1985, O'Brien et al. 1987, O'Brien 1994, 2003) believe that the cheetah's poor reproductive history in captivity is because of low genetic heterozygosity caused by two population bottlenecks: the first bottleneck occurred approximately 10,000 years ago near the end of the Pleistocene and the second has been occurring since the early 1900s (O'Brien et al. 1987). Bottleneck events cause a population to go from a

relatively large number of individuals to a much smaller number of individuals. Several studies have attempted to ascertain the effects of these bottlenecks. O'Brien (2003) and colleagues sutured skin grafts from one cheetah onto an unrelated individual's skin graft bed and then repeated this experiment with several cheetahs. Relation was determined by the cheetah's pedigree. The skin grafts were not rejected showing that cheetahs have severe genetic homozygosity, at least at the loci involved in reacting to the skin grafts. However, Wielebnowski (1996:353) "cast doubt on the overall significance of homozygosity" and its effects on the fecundity and breeding success of cheetahs by examining the difference in mortality rates of juveniles from related parents versus unrelated parents. She determined relatedness based on pedigree history using the cheetah studbook. It was hypothesized that the mortality rates would be similar because genetic variation is so low. However, she found that unrelated pairs had approximately 36% higher juvenile survival rate than inbred pairs. This suggests that there is enough genetic variation, specifically in loci that affect offspring survival, to cause a variation in juvenile survival rates (Wielebnowski 1996, Caro 2000). Another factor that may be caused by the cheetah's low heterozygosity, which has also been suspected of contributing to their lack of reproductive success, is high levels of abnormal sperm. Cheetah's percentage of morphologically abnormal sperm is 64.6% (Wildt et al. 1988). However, managers and researchers have witnessed female cheetahs in captivity becoming pregnant after a single mating and females in the wild becoming pregnant only three weeks after coming back into estrus, which indicates that sperm abnormalities have little effect on their reproduction (Laurenson et al. 1992, Caro 2000).

If genetic diversity was the only factor affecting reproduction then theoretically all facilities should have the same reproductive success. However, the same study that evaluated juvenile mortality rates found that they differed significantly across facilities (Wielebnowski 1996). Also, of the 35 institutions that attempted to breed cheetahs from 1970 to 1996, only 5 were highly successful (Wielebnowski 1996). Thus, poor reproductive success in captivity may not be related entirely to low genetic diversity, but could be attributed to certain management practices (Eaton 1974, Wielebnowski 1996, Caro 2000). Not all facilities that house cheetahs follow the same husbandry practices, and some of those practices have proven more successful than others. The unsuccessful husbandry practices primarily stem from the failure to use information on the natural behavior of cheetahs in the wild, including sociosexual behavior (Laurenson 1993, Caro 2000). One example is housing females in the same enclosure. In the wild, females would be solitary; housed together; most females go into anestrus (Wielebnowski et al. 2002). Another example is not providing adequate dens in off-exhibit areas to reduce stress and to supply adequate shelter (Laurenson 1993, Frank and Saffoe 2005).

## MATE CHOICE

During the last several decades, conservation of endangered species has become the cornerstone of numerous zoo programs (Olney et al. 1994) with captive breeding at the focal point. Zoos serve as a genetic reservoir for wild populations by maintaining genetic diversity in the captive population (Magin et al. 1994, Wielebnowski 1996). However, allowing individual animals to choose their mates is an important behavioral aspect of successful breeding (Moller and Legendre 2001, Roberts and Gosling 2004,

Gowaty et al. 2007, Clutton-Brock and McAuliffe 2009). Mate choice is defined as the behavior pattern of one sex that leads to their being more likely to breed with a certain member(s) of the opposite sex than others (Halliday 1983). Currently, individual cheetahs are assigned mates to preserve the genetic variability in the captive population (Long and Grisham 2008).

Association of Zoos and Aquariums (AZA) Species Survival Plans (SSP®) were established in 1984 for many endangered species, including the cheetah (Marker 1998). One of the primary functions of a SSP is to keep the genetic diversity above 90% for a given population to "insure the genetic and demographic health" of a captive population (Long and Grisham 2008:3). If genetic variation in a population decreases below the recommended 90%, several deleterious consequences can occur, including: an increase in cub mortality, a decrease in spermatozoa viability, a decrease in fecundity, and congenital and reproductive abnormalities (O'Brien et al. 1987, O'Brien 2003, Long and Grisham 2008). SSPs sustain genetic diversity and prevent inbreeding by arranging mate pairs with the lowest mean kinships. Mean kinship is how related individuals are to each other based on their pedigree history. The cheetah population is currently at about 98% genetic diversity.

Pairings based on genetic relatedness have the unfavorable consequence of preventing either sex from choosing their mate and managers' choices may not reflect the choices of the individual animals (Fisher et al. 2003). Unfortunately, for many species, that lack of choice has often led to unsuccessful breeding attempts and even aggression (Moller and Legendre 2001, Roberts and Gosling 2004). An individual should make the best genetic choice with the options they are given, because "mate choice amplifies the

chief advantage of sexuality, namely, genetic diversification" (Brown 1997:60). For example, when monogamous male oldfield mice (*Peromyscus polionotus*) were given a choice between two females, which differed only slightly from each other in their kinship to the male, the male consistently chose the more genetically distant female (Ryan and Lacy 2003).

Numerous studies have shown that sexual selection is determined by female choice (O' Donald 1983, Clutton-Brock and McAuliffe 2009). When females from various taxa were allowed unconstrained mate choice, not only did it result in successful reproduction, but several reproductive benefits also occurred, such as an increase in offspring survival (Drickamer et al. 2000, Moller and Legendre 2001, Gowaty et al. 2007, Clutton-Brock and McAuliffe 2009). The taxa in these studies included: mallards (*Anas platyrhynchos* [Bluhm and Gowaty 2004]), house mice (*Mus musculus* [Drickamer et al. 2000]), agile antechinus (*Antechinus agilis* [Parrott et al. 2007]), Japanese macaques (*Macaca fuscata fuscata* [Soltis et al. 1997]) and even humans (*Homo sapiens* [Wedekind and Furi 1997]). In the case of the agile antechinus, a small carnivorous marsupial, Parrot et al. (2007) offered females urine and body scents from two novel males, one being genetically similar and one dissimilar. Females consistently spent more time, showed more sexual and non-exploratory behaviors near the scents of males that were genetically dissimilar.

In another study, female house mice were allowed to discriminate between two males. The male that the female spent the most time with was considered her preferred mate, while the other male was considered the non-preferred mate. Approximately half of the females were placed with their preferred mate choice and half were placed with

their non-preferred mate choice. Females with the preferred mates were 22.2% more successful at producing litters. Furthermore, the resulting litters were more viable and exhibited a better quality of dominance behaviors, predator avoidance and nest building abilities (Drickamer et al. 2000). Even women, when given six T-shirts worn by different males, consistently picked the odor of a male who had significantly less MHC-alleles in common with themselves as the one they thought had the most pleasing smell (Wedekind et al. 1995). This study supports the theory that MHC genes are not just under natural selection but also sexual selection. MHC genes control self/non-self recognition, which is vital for immune response in vertebrates (Penn 2002). Having more heterozygosity in MHC-allele combinations improves immunological defenses against pathogens, and therefore gives offspring a better chance for survival (Wedekind et al. 1995, Wedekind and Furi 1997, Penn 2002). Mating preference based on MHC, which is picked up in olfactory cues, may play a key role in inbreeding avoidance (Penn 2002). But for most taxa, it remains unknown what specific factors or cues guide female mate choice decisions; their decision may be based on MHC genes, gene compatibility, on overall genetic relatedness or on seemingly non-genetically related qualities, such as territory size (Tregenza and Wedell 2000, Mays and Hill 2004, Parrot et al. 2007).

## EVIDENCE FOR MATE CHOICE IN CHEETAHS

The reason for the cheetah's poor reproductive history in captivity is unknown, but "has been attributed to disinterest on the part of females in numerous cases" (Caro 1994:77). There is evidence that mate choice is an important factor needed for a successful copulation. Several of the more successful breeding facilities offer a type of

mate choice by allowing the female to select a mate through what managers' call the "lover's lane" arrangement (Caro 1994). The lover's lane consists of a corridor (lane) that is bordered by several male enclosures (Figure 1). A female is allowed access to the lane and is able to choose from the surrounding males or visa versa (Karen Ziegler-Meeks, White Oak Conservation Center, Pers. Comm. 2008). Many facilities forego such methods of mate selection because they are expensive and time consuming. Current methods involve shipping males and females across the state, country or even from around the world in hopes of a successful reproductive pairing. Managers determine the pairs based almost solely on genetic relationship. Unfortunately, this has had several negative consequences, including: unnecessary stress for the animals being shipped (especially if the pairing proves unsuccessful), aggression within an incompatible pair, and a generally low reproductive success rate.

## URINE AS AN IDENTIFIER

Solitary animals, like the female cheetah, especially depend on olfactory signals to interpret information about other individuals (Brennan and Kendrick 2006, Clutton-Brock and McAuliffe 2009). It is primarily the male cheetah that scent marks in the wild and keeps a small territory (30-40 $km^2$) that is next to larger female territories (Eaton 1974, Caro 1994, Gottelli et al. 2007). In the wild, the average territory for a solitary female is approximately 830 $km^2$ (Eaton 1974, Caro 1994, Gottelli et al. 2007). Andersson (1994) suggests that scent marking not only attracts mates but stimulates their reproductive cycles as well. Cheetahs are polyandrous and induced ovulators that breed throughout the year (Laurenson et al. 1992, Wildt et al. 1993, Caro 1994, Brown et al.

1996B, Nowell and Jackson 1996, Gottelli et al. 2007) and go through estrus approximately every 12 days if not mated (Asa et al. 1992).

Urine is highly volatile and mammals, including cheetahs, use their vomeronasal system and the flehmen response to discern information from a particular scent (Eaton 1974, Caro 1994, Senger 1997, Brennan and Kendrick 2006). The vomeronasal organ is a sensory organ that consists of a pair of organs located in the roof of the mouth or at the base of the nasal septum (Meredith 2001). Because many factors about a male's condition can be identified via urine, including: sex, age, parasite load, nutrition, hormone levels, territory size, kinship, MHC genes and overall health and viability (Eaton 1974, O'Donald 1983, Caro 1994, Kavaliers et al. 2003, Bateson and Healy 2005), it may serve to relay information to the female. Information that relays genetic relatedness and influences pheromone levels, such as MHC, is consistently excreted in the greatest concentrations in urine (Singh et al. 1987, Novotny et al. 2007). Because scent marking with urine is one of the primary means of communication for the cheetah, it may be a factor in determining which individual would be the best mate choice.

## OBJECTIVES

The objective of this study was to determine if female cheetahs can make a mate choice decision based on urine scents from males of varying genetic relatedness. The specific objectives were:

1. To determine if female cheetahs can differentiate between closely related and distantly related males based on urine scent. A female's "preference" was based on the amount of time spent with each scent.

2. To compare the types of behaviors shown toward each scent to determine if any one behavior (or group of behaviors) was directed toward a particular scent.
3. To correlate stage of female estrous cycles with the investigation time and behaviors displayed toward the scents.

## HYPOTHESES

Because urine is an important means of communication between individuals in a mainly solitary species, and based on previous urine scent trials with several small mammal species, I hypothesized that females would spend more time with the most distantly related scent in a pairing. I also hypothesized that different types of behaviors, possibly indicating a choice (e.g., she spends more time in proximity to one scent) would be displayed more often toward urine scents from the most distantly related male in a pairing.

# MATERIALS AND METHODS

## STUDY SITES AND STUDY ANIMALS

Twelve female cheetahs located at five different North American facilities were studied: Fossil Rim Wildlife Center (FR) in Glen Rose, Texas, females were observed 18-26 July 2008 (n=2); White Oak Conservation Center (WO) in Yulee, Florida, females were observed 4-16 December 2008 (n=4); Smithsonian National Zoological Park (NZP) in Washington, D.C., females were observed 5-16 May 2009 (n=2); NZP-Conservation and Research Center (CRC) in Front Royal, Virginia, females were observed 17-28 May 2009 (n=2); and the Saint Louis Zoo (STL) in Saint Louis, Missouri, females were observed 29 May through 11 June 2009 (n=2). The females ranged between 2 and 9 years of age (mean=5.2 ± 2.7) and were not pregnant or housed with offspring during the scent trials (Appendix I). Female cheetahs reach sexual maturity by about 2 years of age and have successfully reproduced up to 10 years of age (Eaton 1974, Nowell and Jackson 1996).

Females were fed either their entire diet or morning portion of diet prior to the start of the scent trial. Females were fed between 1.3-2.7 kg (± 0.8) daily. Water was provided *ad libitum*. Enclosure size ranged between 149 $m^2$-1,742 $m^2$ (1,026 $m^2$ ± 607 $m^2$). Scents were placed between 4.6 and 20.1 m apart (an average of 9.1 m [± 4.7 m]) depending on the size of the enclosure and availability of objects on which to secure them. The enclosure sizes, diet, distance of scent dispenser placement are located in Appendix II. Females at FR were supplemented with deer meat and bones a few times during the month and had “fast days” on Sundays. Females at NZP were supplemented

with bones a few times a month. One day a week, CRC females were fed a whole rabbit, and another day were fed only a femur bone.

All females were housed alone with the exception of the four females at WO. Females at WO were split into two groups of paired females, but both pairs were separated for the hour during the trials and did not have access to each other's male scents. Although some of the females in the study were housed next to males, none were males used for their scent trials. The one exception was Zuri at St. Louis Zoo. Zuri shared a fence line with her father, uncle, and brother (the only males that fit into the closely related male category), and therefore there was no alternative.

## URINE COLLECTION

Personnel at several different facilities collected urine from their male cheetahs. Two types of urine collectors were created for male cheetahs to account for the unique way that male cheetahs spray or mark objects. The first type of urine collector (Figure 2) was constructed from galvanized steel ductwork (15.24 cm diameter) placed into a galvanized steel reducer (7.62 cm circumference). The ductwork was secured to the reducer using two stainless steel nuts and bolts. At the bottom, narrow end of the reducer, the cap of a plastic container was glued to the reducer using Craftsman Industrial Grade Hot Glue®, so that a plastic container could be screwed securely into the reducer. The middle of the cap was cut out which allowed urine to flow freely down into the jar. Rounded hooks (3.8 cm x 5 cm) were inserted into the top two corners and in the middle sides of the collector and secured with stainless steel nuts and bolts. Bungee cords were

then hooked to the collector and used to secure it to a tree or fence approximately 13 cm off the ground.

A second collector was designed that could be placed on den boxes. It was constructed from a flat 60 cm x 60 cm, 18-gauge aluminum panel (Figure 3). The bottom of the panel was curled up 5 cm on one side and only 0.3 cm on the opposite side, creating a slope with a $30^{o}$ angle. Two stainless steel nuts and bolts held a 12-gauge copper wire covered in a black plastic sheath. The wire held a 3 cc syringe casing, which caught the urine as it flowed down the "trough." The collectors were screwed to the den boxes in an area where males were observed to mark frequently. All collectors were removed from enclosures after the collection period each day and washed.

Male cheetahs that were housed in coalitions were observed for the entire collection period to ensure that another male did not contaminate the urine sample. At the end of the collection/observation period the collectors were removed, and the urine was transferred to 3.6 mL cryovials, marked with the male's studbook numbers and the date of collection, and then immediately placed in an ultralow freezer. The veterinary staff at the Saint Louis Zoo determined the urine was safe to be presented to the females because they were never allowed to come into direct contact with the urine.

## MALE CHEETAH URINE

Urine scents from two males were presented during each trial in a pair-wise design in randomized combinations. The males used for this study were selected based on their relatedness to the females using the PM2000 Kinship Matrix, a program developed to analyze genetic relationship using pedigree history in captive wildlife

populations. Three categories of males were selected based on their relatedness to a female and the given ranges dictated by the PM2000 Kinship Matrix (Table 1) (Robert Lacy, Chicago Zoological Society, Pers. Comm. 2008). Nine females were presented urine scents from 3 different genetic categories of males: the first category was a "good mate choice," which consisted of a male that was not related to the female (A); the second category was an "average mate choice," which ranged between a second and first cousin (B); the third category was a "poor mate choice," a male that was closely related (e.g., a half-brother, brother, father, etc.) (C); and, a "blank" scent dispenser (to act as a control) was also presented to the females with only a clean piece of gauze inside (D). There were 6 possible scent combinations. Each female received each combination twice for a total of 12 days of scent trials in pair-wise, randomized combinations. Three additional females were only offered the good and the poor choice because either: no male in the current captive population fit the medium category, or urine was not collected from a male that fit in that category. For these females there were 3 possible scent combinations and each female received each combination twice for a total of 9 observation days (3 days of novelty control and 6 days of scent trials). All males used for the study ranged from 1 year and 10 months to 9 years in age (5.6 ± 2.2) (Appendix III); their relationship to each female is listed in Table 2. Male cheetahs reach sexual maturity between 1 and 2 years of age and have successfully reproduced up to 14 years of age (Nowell and Jackson 1996).

## URINE PREPARATION

Once the total amount of urine needed from a male was collected, separate vials of an individual's frozen samples were thawed to room temperature and pooled. The pooled urine was then divided into 1-ml aliquots, placed into labeled 1.5 mL cryovials stored at -70$^{o}$C, and they were kept frozen until the day of the trial. Pooling the urine eliminated any daily fluctuations in urine composition of an individual. A minimum of 30 minutes before the start of a trial, urine samples from two males were removed from the freezer and placed into individual zip lock bags allowing them to thaw. Immediately before the start of observations the 1-ml aliquot of urine was poured onto a 7.62 cm x 7.62 cm sterile gauze pad and placed inside the scent dispenser; then the process was repeated for the second scent dispenser (Figure 5). Disposable latex gloves were worn to reduce the transfer of human scent to the gauze or to the scent dispensers, and the gloves were changed after preparing the first scent dispenser to eliminate the transfer of the first male's scent to the second scent dispenser. After each trial the scent dispensers were soaked in a 10% non-scented Clorox® bleach solution for ten minutes and then rinsed with water. They were then scrubbed with Seventh Generation Free and Clear Natural Dish Liquid®, rinsed with water and allowed to dry overnight.

## SCENT DISPENSERS

Using Leonard's (2008) design, the scent dispensers were assembled from standard PVC pipe, which was 3.8 cm in diameter and 22.8 cm long (Figure 4). Several holes (1.7 cm diameter) were drilled around the circumference of each dispenser to allow the urine scent on the gauze inside the pipe to disperse without the cheetahs being able to

come into direct contact with the gauze. One end of the dispenser was closed with a PVC cap, and the other end was closed with a screw cap. On one side of the pipe, at either end, a 3.8 cm x 5 cm rounded hook was inserted into one of the holes and was secured with a nut at the base inside the pipe. Approximately 1.5 meters of steel chain was looped through the rounded hooks and the chain was linked with a double snap-end hook. The chain was used to secure the dispensers around a tree, log or fence inside the female's enclosure. Scent dispensers were placed were they could be observed at all times during the trial.

## BEHAVIORAL OBSERVATIONS

I conducted all behavioral observations. Because all females were housed individually, focal animal sampling was used to observe all of their interactions with the urine scents (Altmann 1974). The scent dispensers themselves were placed in the same two locations for each trial to control for positional bias. Because the females were offered each scent combination twice, the scents were switched from the first trial to the second to further control for positional bias. Prior to urine presentation, females were offered empty scent dispensers for three days to eliminate the novelty effect of the dispenser. The time of day each female was observed remained approximately the same throughout the trial period (all trials were conducted in the morning before 1200 hr). Females were given access to the scent dispensers and observed for approximately 1-hour. All behaviors directed toward the scent dispensers were recorded continuously, including the type of behavior exhibited (Table 3; e.g., Figure 6) and duration of each

behavior. The scent the female spent the greatest time with in a pair was considered the "preferred" male for that pairing.

Wind speed and direction were monitored and recorded using a Kestrel® 3000 Pocket Weather Station at the beginning, middle and end of each trial. If the wind speed was >1.6 kph, a wind corridor was established. If the cheetah passed through the corridor (walked downwind of the scent dispensers), then it was assumed that she was able to detect the scents. If she did not pass through the wind corridor and did not approach one or both of the scent dispensers, then it was assumed that she did not have a chance to detect the scents. She was offered that same combination again at the end of the trial period. Trials were cancelled if a heavy, steady rain was falling at the start of or during a trial. If at least 30 minutes of the trial was completed at the start of the rain and a female interacted with or had the opportunity to detect both of the scents (i.e., traveled downwind of both scents) then the trial was considered completed.

## HORMONE ANALYSES

### Female Fecal Sample Collection

Fecal samples were collected from females 4 to 7 times per week for approximately 1½ months including the 2 weeks during the scent trials. The fecal samples were placed in zip-lock bags, labeled with the female's studbook number and date collected and immediately frozen. The fecal samples were shipped to the Saint Louis Zoo on dry ice where they were stored at -70$^{o}$C until analyzed. If two females were housed together, food dye or another identifiable additive was placed in the individual female's daily diet. This method helped reliably distinguish to which female

the feces belonged. The fecal samples were collected between 0700 and 1700 hr and were known to be from the same day because enclosures were cleaned of all fecal matter on a daily basis.

This noninvasive method of hormone monitoring was used to prevent a change in hormone secretion and/or behaviors due to the stress of being handled (Wielebnowski and Brown 1998, Dehnhard et al. 2008). The trials were performed in the spring, summer and winter because captive and wild cheetahs go into estrus and successfully breed at all times of the year with no appearance of seasonality (Laurenson et al. 1992, Brown et al. 1996B, Wielebnowski and Brown 1998).

Female Fecal Estradiol Concentration

Felids excrete steroids almost exclusively in feces, which allows for the monitoring of estradiol levels through daily fecal collection (Brown 2006). I extracted steroids from feces according to the Saint Louis Zoo's standard operating procedure (Bauman 2007). Fecal samples were stored at -70$^{o}$C until the day prior to hormone extraction. At this time they were placed in a refrigerator at approximately 3$^{o}$C to allow the samples to thaw. Once thawed, approximately 0.5 g of wet feces was placed into plastic 20 mL scintillation vials, which had been previously weighed and labeled. Samples were taken from inside the center of the feces. I added 5.0 mL of Fecal Extraction Buffer II to the vial, which was then capped and shaken until the sample was well dispersed. The Extraction Buffer Solution II consisted of 50% methanol and 50% phosphate solution (700 mL water, 8.75 g NaCL, 5.55 g $NaH_2PO_4$ $H_20$, sodium phosphate monobasic, monohydrate), 8.87 g $Na_2HPO_4$ sodium phosphate dibasic

anhydrous, 1.00 g sodium azide, 0.5 mL Tween 20, 1.0 g BSA Bovine Serum Albumin RIA Grade (Bauman and Hardin 1998). The samples were then shaken overnight at 200 RPM. They were removed from the shaker in the morning and allowed to settle for a minimum of one hour. Then the liquid was decanted from the vial into a 12 x 75 polypropylene tube that was spun in a centrifuge for one hour at 4,000 RPM. The supernatant was decanted into clean cryovials and frozen at -70$^{o}$C until the day of assay. The scintillation vials were dried overnight at 100$^{o}$C. Once removed and cooled to room temperature the weight of the vial was recorded and used in calculations of the hormone levels.

Hormone extracts were assayed to determine the fecal estradiol levels for each sample. The endocrinologist at the Saint Louis Zoo, Joan Bauman, performed the radioimmunoassays of the fecal extracts. Quantification was performed according to previously set protocol (Valdespino et al. 2002, Asa et al. 2007) with the exception that reagents were obtained from Siemens Diagnostics, Los Angeles, CA. Serial dilutions of observed/expected = 101.6% ± 11.3. Recovery of added estradiol from fecal extracts was 107.3 ± 3.8%. Inter-assay variation of QC pools was 16.0 ± 3.1%; intra-assay variation of replicated was 6.6%. Fecal data are stated as ng per g of fecal dry weight.

Male Urinary Testosterone Concentration

Male urinary testosterone levels were compared as a factor that could potentially affect their attractiveness to females. A 1-ml sample of urine from each male was analyzed for testosterone levels according to the Saint Louis Zoo Endocrinology Laboratory protocol (Bauman 2007). Joan Bauman, endocrinologist at the Saint Louis

Zoo, performed the hormone analysis of the male urine samples. Urine was pre-treated with beta-glucuronidase-aryl sulfatase (Roche Molecular Biochemicals) to cleave conjugates, and then assayed for testosterone by RIA, using reagents from Diagnostics, Los Angeles, CA. All samples were analyzed in a single assay run.

## STATISTICAL ANALYSES

### Time Spent, Latency to Approach, Behavior Types and Age

For all statistical tests, the significance considered to be $\alpha < 0.1$ because of the small sample size. The magnitude of difference of the time spent, the behavioral durations, behavioral latencies and difference in age class data were analyzed using the non-parametric Wilcoxon matched-pairs signed ranks test (Siegel 1956) and Student's *t*-test using SAS statistical software version 9.1 for Windows. Time spent was defined as the duration of all observed behaviors combined for a specific scent in a pairing for each female cheetah. Latency was defined as the time delay between the start of the trial and when the first occurrence of a behavior started (Leonard 2008). Two age classes were defined as females over 4 years of age and under 4 years of age. Age was measured in years and months at the time when scent trials started for each individual female. The females used for this study were not skewed toward any specific age. Because the sample size was small and data were not normally distributed, data were log-transformed. Each female was presented a specific combination twice, for a total of 126 individual scent trials; however, for the purpose of data analysis, the two trials of each specific combination were combined for a total of 63 trials.

Estradiol Correlation with Time Investigating

Correlation between daily fecal estradiol levels and the total time spent investigating (regardless of which scent was investigated more) were analyzed using the Spearman rank correlation coefficient ($r_s$) (Siegel 1956). Felids have a lag time of approximately 24-48 hours from when steroids enter the bloodstream to the time they can be excreted in feces (Brown et al. 1994, Brown 2006). Several factors may cause the lag time to vary from individual to individual, including: variation in diet, metabolic rate and/or some health conditions (Brown et al. 1994, Wielebnowski and Brown 1998). Therefore, to determine if there was a correlation between a female's time spent investigating and her fecal estradiol levels, three different lag times were examined (no lag, 1 day of lag and 2 days of lag).

# RESULTS

I presented scents to 12 adult female cheetahs and collected 126 hours of quantitative behavioral data (combined to 63 trials). Nine of the 12 females were offered AB, AC, AD, BC, BD and CD combinations twice, and 3 of the 12 females were offered AC, AD and CD combinations twice.

According to her keepers, Zinga, a female located at WO, was hand-raised in a small litter (only her and her two brothers) and formed an unusually strong bond with her brothers (Karen Ziegler-Meeks, White Oak Conservation Center, Pers. Comm. 2009). Zinga's reaction to the scents supported these observations, because she spent more time with her brother's scent than with any other scents, and therefore was excluded from analyses, which reduced the sample size to 12 females.

## SCENT PREFERENCE

There was no difference in time spent with the scent pairs within individual females (female=110.81 and residual=2053.09) so the difference in time spent was pooled among females. The time spent was significantly different in the combination with the most genetic distance between the two scents (A>C, t=2.38, df= 11, P=0.039) and scents that were paired with the control (A>D, t=1.88, df=11, P= 0.087 and B>D, t=2.62, df=8, P=0.045 [Figure 7]). However, although the other pairings were not significantly different, the trend showed that in every pairing (except C vs. D), the total percentage of time spent overall by all females was greater with the more distantly related

male scent or "better" mate choice in the pair (A>B, 71%; A>C, 69%; A>D, 74%; B>C, 60%; B>D, 65% [Figure 8]).

## LATENCY TO APPROACH AND AGE EFFECT

There was no difference in latency to approach within or among females. They did not approach one scent in a pair significantly faster than the other scent. However, there was a trend to approach the scents that were paired with the control, and in one case the scent from the most distantly related male in the pairing, faster in AD, BC, BD and CD pairings (Figure 9). When the females were separated into two age classes, females above 4 years and below 4 years of age, and tested for difference in time spent with the scents in a pair, there were no significant results.

## BEHAVIORS DISPLAYED

Proximity and sniffing behaviors were seen more frequently, more consistently, and in longer duration than any other behaviors. There was no difference in the time that "proximity" and "sniff" behaviors were displayed between individuals, so the difference in time spent was pooled and analyzed among all 12 females. Females spent more time in proximity to the most distantly related male scent in all pairings; however, they spent significantly more time in proximity to A in AC pairing ($t=2.25$, $df=1, 10$, $P=0.049$) and to B in the BC and BD pairings ($t=6.37$, $df=1, 8$, $P=0.0002$; $t=2.46$, $df=1, 6$, $P=0.049$; respectively) (Figure 10). Females only spent significantly more time sniffing A in the AD pairing ($t=2.31$, $df=1, 7$, $P=0.055$) (Figure 11). Pawing behaviors appeared to be shown more toward A and C when paired with D by most females, but were not

significantly different. The total duration, of all females combined, for proximity and sniff behaviors were greater for the more distantly related male in all pairings; with the exception of C vs D in sniff (Figure 12-17). If a female did not display proximity or sniffing behavior with both scents in a pairing, she was not included in the analysis. Other behaviors were displayed by four or less females, and because of the small sample size were not analyzed (Figures 12-17).

HORMONES

In males, androgen levels found in the male urine samples were extremely low in all samples, ranging between 0.06ng/g to 0.27ng/g. Fecal estradiol concentrations in some females, when compared to time spent investigating male scents, suggested a very slight lag time while others appeared to have a lag of 1-2 days (Figures 18-23). Because of possible variability in lag time, when all females were analyzed together for no lag effect, 1 day of lag time, and 2 days of lag time there were no correlations ($r_s$=0.22, $r_s$= 0.15 and $r_s$=0.23 respectively) between time spent in investigative behaviors versus daily estradiol concentrations. Lag time can vary between individuals and within an individual. However, the lack of correlation could be because there was no relationship between estradiol and investigation time, not because of individual variation in lag time.

# DISCUSSION

Female cheetahs in this study generally spent more overall time with the "better" mate choice or more distantly related male in each pairing, which indicates that they could discern the genetic relatedness of a male through olfactory cues in the volatile components of urine. However, only the pairs that had the most genetic distance between them, or scents that were paired with the control, were significantly different. It is not known if the females would mate with the "preferred" male. However, previous studies that defined "preference" of a scent or individual as the one that the choosing animal spent more time with (Drickamer et al. 2003, Gowaty et al. 2003, Kavaliers et al. 2003, Ryan and Lacy 2003, Bluhm and Gowaty 2004, Parrott et al. 2007) found that the individuals that bred with preferred mates bred successfully and produced more viable offspring (Drickamer et al. 2000, Drickamer et al. 2003, Gowaty et al. 2003, Bluhm and Gowaty 2004). With house mice, when two males were placed in front of a female house mouse the one that she spent the most time with was considered the preferred male. Some females were allowed to mate with their preferred choice and others with the non-preferred choice. The preferred pairings showed higher reproductive success in several areas, including: a greater number of pups weaned, quicker time to first litter, birth-to-weaning viability, increased pup body weight at birth and weaning, and the growth rate of pups was greater. Offspring of preferred pairings also showed a significantly better ability to build nest, sons were more dominant during aggression trials and they were better at avoiding predator (Drickamer et al. 2000).

There are several potential factors that could influence mate choice in cheetahs, including behavior, genetic relatedness, phenotype, health status, parasite load, physiology, hormone levels, MHC and/or others. This research was a preliminary study to determine if females could ascertain relatedness based on the volatile components of male urine. Because urine is an important means of communication between individuals in a largely solitary species, I hypothesized that females would spend more total time with urine of the male that was the least related to her, possibly indicating a preference. The results support my hypothesis.

There was an increase in types and durations of specific behaviors displayed by the female cheetahs toward the urine scents with relation to genetic distance. However, only sniffing and proximity behaviors were exhibited by all females in all scent pairings, and in every pairing more time was spent performing those behaviors with the most distantly related male urine scent. Proximity was the most frequently observed behavior, and, in a few pairings, significantly more time was spent in proximity with the more distantly related male (A>C, B>C and B>D). Sniffing was also seen with every pairing, but time spent sniffing was only significantly different in one pairing (A>D), presumably because there was a lack of any urine scent to investigate in the D dispenser, while the other dispenser contained the most distantly related ("best" mate choice) male's urine. For other pairings, females may not have needed to sniff one scent longer than another to get the information needed from the volatile components of the urine. Sniffing may represent a largely investigative behavior (with limited time needed to gain olfactory cues), whereas proximity is a behavior resulting from choice. Once females receive the

information from sniffing, they then can choose to stay in proximity to the preferred male's scents.

There were no significant differences in time spent when females were separated into the two age classes of greater or less than 4 years of age. This implies that there was no age effect because one class did not spend more time with the scents compared to the other class. Wielebnowski and Brown (1998) found behaviors displayed in comparison to daily estradiol levels increased significantly with age and were not affected by prior breeding experience. Data from my study does not support that there is difference in behaviors displayed between the two age classes. However, in my study the lack of significant differences between females separated into two age classes may be attributed to the small sample size.

Increases in estradiol concentration correlate with an increase in certain behaviors exhibited by females (Wielebnowski and Brown 1998), suggesting that there should be a correlation between the total time a female spent investigating male scents each day and her daily estradiol concentrations. However, each female may differ in the time between hormones entering the bloodstream and being excreted in feces. This "lag time" varies between individuals and even within an individual because many factors affect the rate at which hormones are excreted such as diet, health and metabolic rate (Brown et al. 1994, Wielebnowski and Brown 1998, Brown 2006). In some females, there seemed to be a possible correlation between graphic representation of total time spent investigating scents and the daily estradiol concentrations. However, because of the variation of lag time between and within females, determining if there was a correlation was not possible.

Testosterone levels were very low in all of the male urine samples, which is consistent with previous reports that, in felids, steroids are almost exclusively excreted in feces with very small amounts being released in urine (Brown et al. 1996A, Young et al. 2004, Brown 2006). Therefore, testosterone levels in male urine should not have affected the female's response to any urine scents.

## MATE CHOICE

Currently, the management strategy of the cheetah SSP is to assign mates based almost solely on genetic relatedness to preserve the genetic variability by maximizing heterozygosity in the captive population. However, there is a great deal of evidence that females in many taxa *do* make good genetic mate choice decisions—often better decisions regarding individual fitness than those a manager would assign. Allowing individual animals to choose their mates is an important behavioral aspect needed for successful breeding (Soltis et al. 1997, Moller and Legendre 2001, Gowaty et al. 2007, Parrott et. al. 2007).

Very little is known about cheetah mating behavior or how mates are chosen in the wild (Eaton 1974, Caro 1994, Gottelli et al. 2007). This is because cheetahs are elusive and travel constantly around their territory (Eaton 1974). Like most mammals, cheetahs are not monogamous but are polyandrous (Gottelli et al. 2007, Clutton-Brock and McAuliffe 2009). Polyandry may be one of several ways to avoid inbreeding (Gottelli et al. 2007). Females, although solitary, maintain large territories surrounded by several smaller male territories (Caro 1994, Gottelli et al. 2007). Visiting several small male territories and encountering roaming males may offer a female a wide selection of

mates from which to choose. Male cheetahs primarily use urine to mark their territories and mark more frequently than females, which may be the best means of communicating with the opposite sex in this particular type of physical and social environment (Eaton 1974). It is unknown, but seems probable, that female cheetah mate choice decisions are based on investigating these scent marks. By investigating a male's scent, a female may be able to discern if he is a good mate choice (including relatedness); if he is a poor choice, she may keep traveling until she finds a suitable mate or more likely his scent. Because of the size of a female's territory it seems unlikely that she would encounter an actual male when she comes across his scent. Therefore, the proximity behavior exhibited by the captive females in this study may be representative of a natural behavior—to stay in proximity to a preferred males scent in order to increase the chances of encountering and mating with him.

Inbreeding avoidance can help increase offspring survival (Ryan and Lacy 2003, Hoffman et al. 2007, Sherborne et al. 2007). Although cheetah sibling groups disperse before or at sexual maturity, sisters and brothers often remain in or near their natal range, and there is evidence they occasionally meet after dispersal (Caro 1994). The ability to discern unrelated vs. related males may be an important component of mate choice for cheetahs in this type of social situation, especially with regard to inbreeding avoidance (Clutton-Brock and McAuliffe 2009).

Most animals seem to choose mates based on genetic compatibility, but that may just be one factor used in their decision-making. Qualities that do not seem to be genetically related, such as territory size or behavior, could also play a role in mate choice decisions (Tregenza and Wedell 2000, Mays and Hill 2004, Parrot et al. 2007).

Constraining pairs in captivity to a mate, based on what managers decide is a good mate, could potentially affect the way captive animals evolve. What long-term affects could this have on a population? We could be selecting for animals that are less "choosy" in terms of mate choice. Choosiness, as Jennions and Petrie (1997: 286) define it is "the effort or energy an individual is prepared to invest in mate assessment." Keeping populations genetically healthy is the main priority, but natural behaviors have evolved over eons, enabling these animals to survive and thrive in the wild.

Although release programs have been established for several species, currently there is not a program to reintroduce cheetahs to the wild; but if one comes to fruition, it should be taken into consideration that human-controlled mate choice decisions for a species might not mimic nature. Animals in captivity may be facing different types of selection pressures because of the artificial environment compared to the natural selection that their wild counterparts are simultaneously undergoing (Kunzel et al. 2003). This has an overall effect on their behavior, morphology and physiology (Darwin 1859, Clutton-Brock 1989, Kunzel et al. 2003, O'Regan and Kitchener 2005). Allowing natural behaviors, such as mate-choice, or parents rearing offspring (instead of being human-reared), can benefit the species and should be included in management practices as often as possible.

## MANAGEMENT IMPLICATIONS

As stated by Lees and Wilcken (2008:2), "…animal collections must be demographically robust, genetically representative of wild counterparts and able to sustain these characteristics for the foreseeable future." Zoo personnel need to manage

captive animal populations in a way that maintains population viability (Lees and Wilcken 2008). This approach attempts to minimize inbreeding and maximize heterozygosity. Although a female should want to avoid inbreeding, she would be making her decisions based on her own heterozygosity, by choosing a male of optimal genetic distance and compatibility to her own genotype.

In the case of the cheetah, the current management approach is to place a male and a female together based primarily on genetic relationship, and if a pair fails to reproduce, then the recommendation is to change the pairing. However, this process may take several years, e.g., the recommendation is made to put a specific male and female together, then it takes several months of coordinating to ship the male to the facility where he undergoes quarantine; then he gets acclimated; then the male and female are placed together several times over the course of several months. When this proves unsuccessful then managers wait until the next SSP meeting to decide which male they will pair with the female next, and the process is repeated. For an animal that is only reproductively viable for about a decade, every year is important. SSPs for several different species are starting to monitor the pairings to determine possible cause(s) of unsuccessful reproduction; however, few examine the potential role that mate choice may have on a successful pairing.

Mate choice may be important for successful reproduction and should be incorporated into breeding recommendations of captive cheetahs. Because time spent with a scent or individual can indicate a preference, then a possible strategy could be to ship urine from several recommended or potential “good” mates to a facility and present them to a female, allowing her to make a choice. This will ensure sound genetic

management while simultaneously permitting the female to make the most genetically compatible choice (or what she chooses as the best choice).

Other benefits of this strategy include:

1) Reduced stress because of shipping one or several incompatible mates as only the preferred male would be shipped.

2) Reduced danger of pairing incompatible animals.

3) Reduced time and expense involved with shipping incompatible mates.

4) Increased efficiency and productivity of mated pairings could lead to more success in the captive breeding program.

5) Successful reproduction in captivity would reduce the number of individuals that would need to be captured from the wild or shipped from other countries to supplement captive populations.

6) Successful births could potentially increase public awareness of an endangered species while creating positive publicity for the zoos.

## FUTURE RESEARCH

One of the main challenges working with large mammals is having a large enough sample size. Perhaps having a larger sample size for this study would have shown more significant results. Also, some chemosignals are highly volatile, while others are non-volatile; cheetahs, like most mammals, have a vomeronasal accessory olfactory system and a main olfactory system that are used in combination to detect both volatile and non-volatile components (Brennen and Kendrick 2006, Keller et al. 2006, Martínez-Ricós et al. 2008). It is possible then, that females also needed access to the non-volatile

components of urine. In this experiment they did not have access to the urine itself or the non-volatile components. Licking the scent dispensers (suggesting that licking the actual urine was necessary to discern all of its olfactory cues) often preceded the flehmen response, which indicates that perhaps direct access to the urine may have yielded more significant results.

Possible research that could complement this study, in the continuing process of learning about cheetah mate choice, would be to give urine scents from several recommended potential mates to the females. Many factors may be taken into account and weighed by a female, and there may not be one "best" male for a female (Hoffman et al. 2007, Parrott et al. 2007, Clutton-Brock and McAuliffe 2009). However, females in this study were able to determine genetic distance of a male and indicated that preference in a quantifiable manner, and it has been shown in other species that time spent equates to a mate preference (Drickamer et al. 2003, Gowaty et al. 2003, Kavaliers et al. 2003, Ryan and Lacy 2003, Bluhm and Gowaty 2004, Parrott et al. 2007). It is possible, then, that a female could choose a mate from urine scents, and show that preference by spending more time with a "preferred" male. The next step would be to allow the female physical access to the same males, including the "preferred" male, to see if she make the same choice and successfully mate with him.

And lastly, another aspect that should be evaluated is if MHC plays a role in the mate choice decisions of cheetahs. This study used the pedigree history of the captive population to determine relatedness of individuals. However, mice show a preference for odor from individuals that are dissimilar at the MHC-loci (Penn 2002). MHC should be

determined for the different males offered to the female (as well as the female) to see if she chooses the male with the most MHC-dissimilarity.

Table 1. Genetic relationship values based on PM2000 Kinship Matrix Software.

| Range | Genetic Relationship to Individual |
|---|---|
| 0 | No relationship |
| 0.0313 | Second cousin (or equivalent) |
| 0.0625 | First cousin (or equivalent) |
| 0.125 | Half-sibling, aunt or uncle |
| 0.25 | First order kin such as full sibling, parent or offspring |
| 0.50 | One's relationship to oneself (which can be higher if an individual is inbred) |

Table 2. Studbook numbers of male cheetahs, whose urine was offered to each female. Genetic relationship values to the female according to the PM2000 Kinship Matrix Software are in parentheses.

| Female Name | Good Choice | Medium Choice | Poor Choice |
|---|---|---|---|
| Simika | 5457 (0) | None | 4257 (0.2539) |
| Sethunya | 6180 (0) | 5480 (0.0363) | 5496 (0.1624) |
| Zazi | 6359 (0) | 5478 (0.0285) | 4452 (0.2734) |
| Sunshine | 6359 (0) | 5990 (0.0469) | 6180 (0.1875) |
| Tumai | 5480 (0) | 5990 (0.0469) | 6180 (0.3125) |
| Azizi | 6359 (0) | 5478 (0.0229) | 4224 (0.1646) |
| Amani | 4224 (0) | 6503 (0.0507) | 6338 (0.25) |
| Scarlet | 6015 (0) | None | 6359 (0.1875) |
| Pia | 6015 (0) | None | 6359 (0.1875) |
| Zuri | 6359 (0) | 6338 (0.0507) | 5458 (0.2586) |
| Krapinka | 6359 (0) | 5458 (0.0352) | 5991 (0.1338) |
| Ally | 4257 (0) | 5504 (0.0313) | 6590 (0.25) |

Table 3. Ethogram of all behaviors exhibited by female cheetahs toward male urine scents that were observed and recorded at zoos and related facilities.

| Behavior | Description |
|---|---|
| Proximity | The female comes within one body length of the scent dispenser but does not touch it or perform any other behaviors toward it. The behavior ends when the animal moves at least one body length away from the scent dispenser. |
| Sniff | The female brings her nose to or within one head length of the scent dispenser. |
| Lick | The female makes contact with the scent dispenser with her tongue. |
| Bite | The female places her mouth around the scent dispenser. |
| Paw (i.e., Figure 6) | The female makes contact, using one or both front paws, with the scent dispenser. |
| Flehmen | Female raises her head to the horizontal plane, holds opens her mouth approximately 3-7 cm after smelling or licking the scent dispenser. |
| Scent Roll | The female simultaneously rolls and rubs with her entire body on the ground or on the scent dispenser or within one body length. |
| Urinate | The female releases urine within one body length of the scent dispenser. |
| Hiss | The female brings her head to or with in one head length of the scent dispenser, opens mouth and forcibly expels air towards the scent dispenser. |

Figure 1. A cheetah “lover’s lane” at Fossil Rim Wildlife Center located in Glen Rose, TX. A female cheetah is being guided down a corridor that is surrounded by several male enclosures which she is allowed to investigate for potential mates.

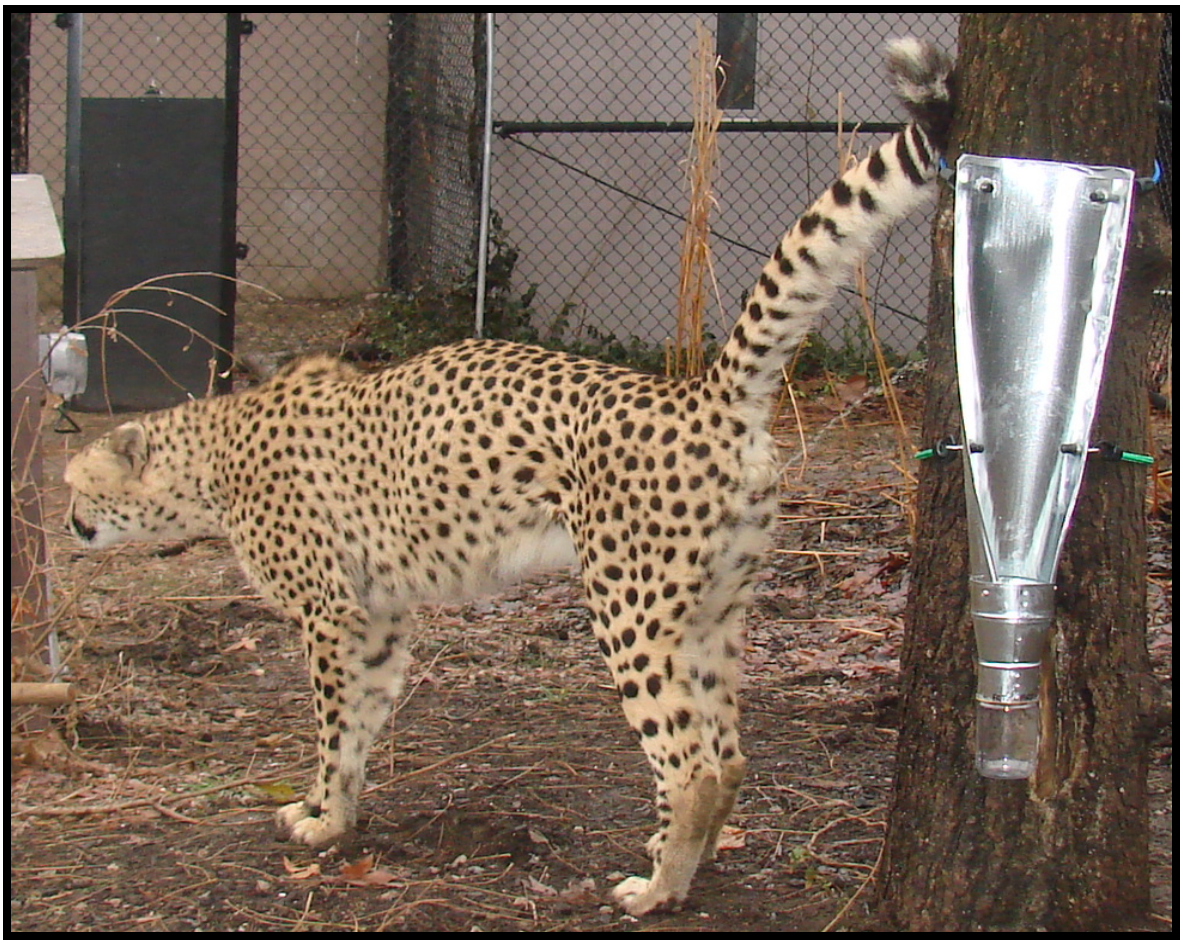


Figure 2. Sabi, a male cheetah, scent marking/urinating next to a tree collector at the Saint Louis Zoo.

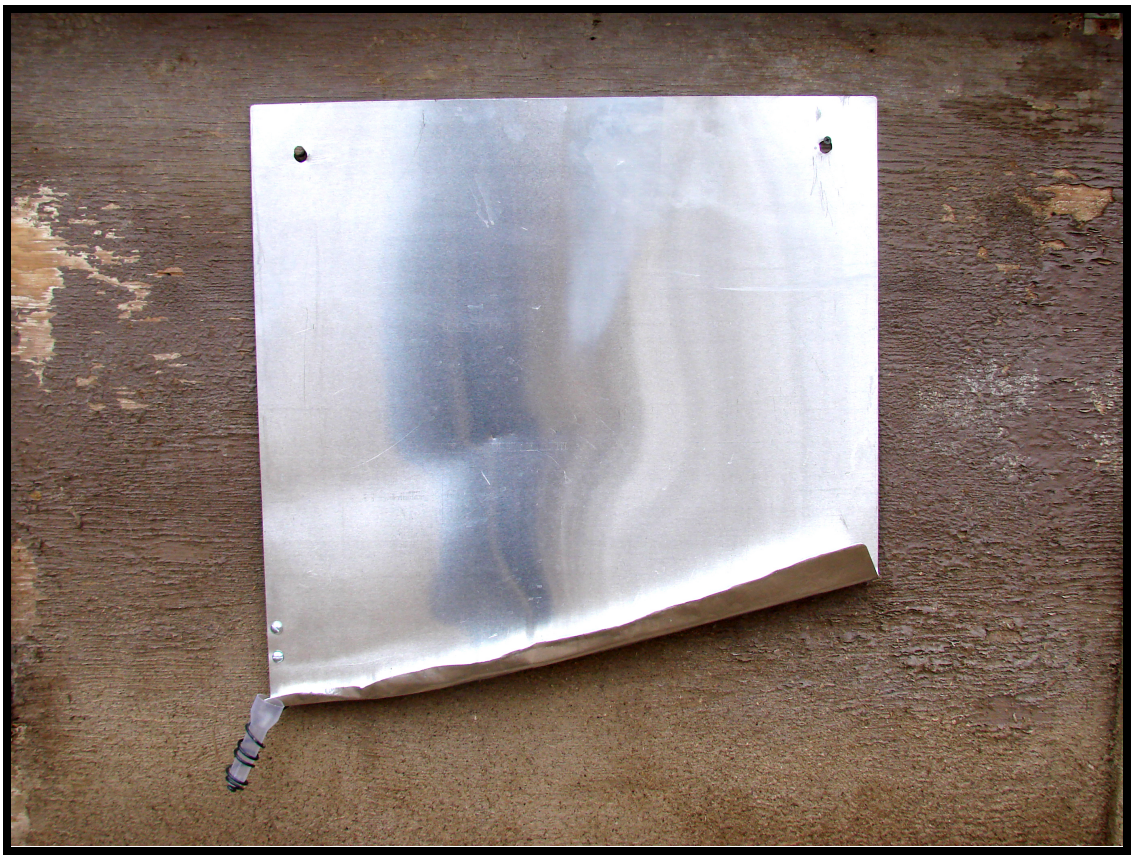


Figure 3. A flat urine collecting device on a cheetah den box with a collection cup in place.

Figure 4. Scent dispenser with gauze soaked with male cheetah urine inside.

Figure 5. 1mL of male cheetah urine being poured on gauze immediately before scent trial.

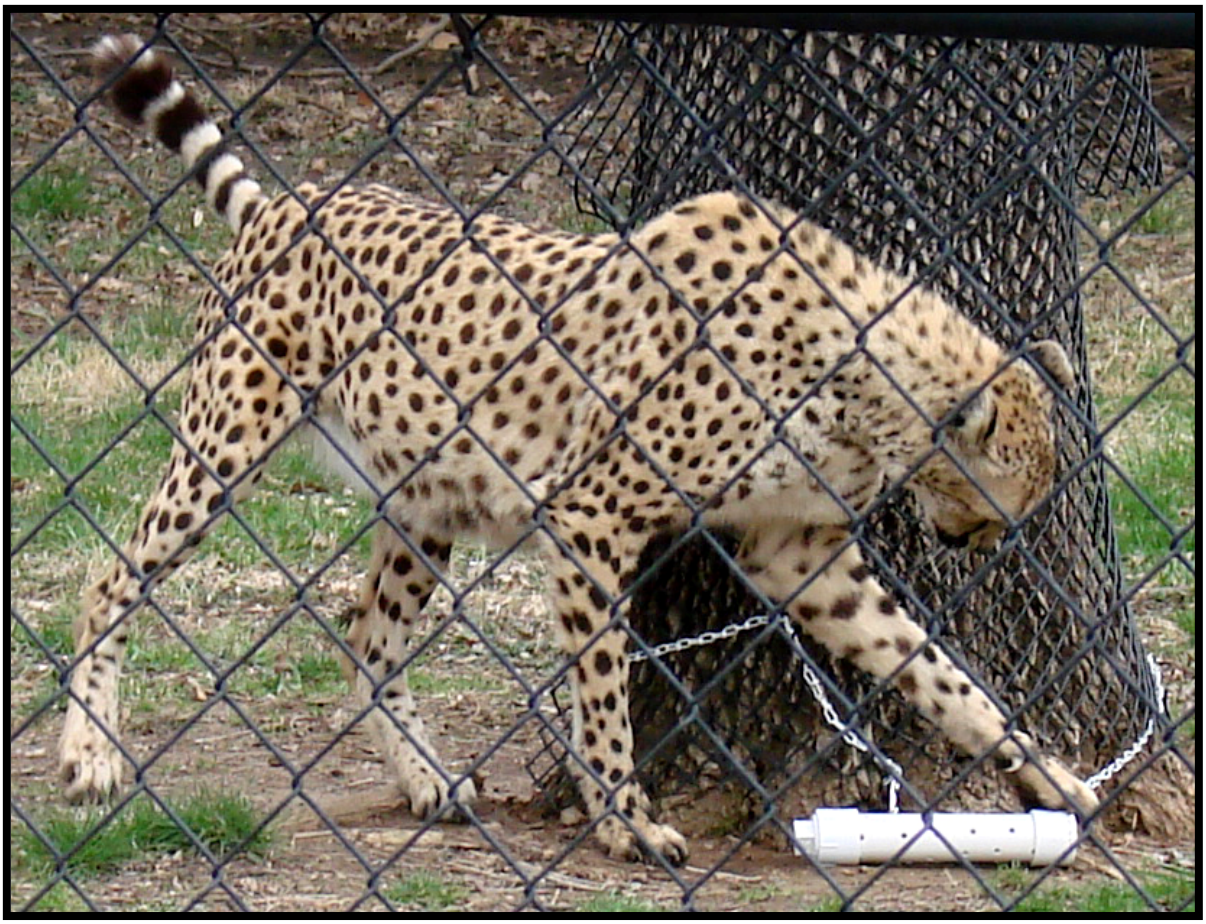


Figure 6. Sethunya, an adult female cheetah at the Saint Louis Zoo, exhibiting “paw” behavior toward scent dispenser containing male urine.

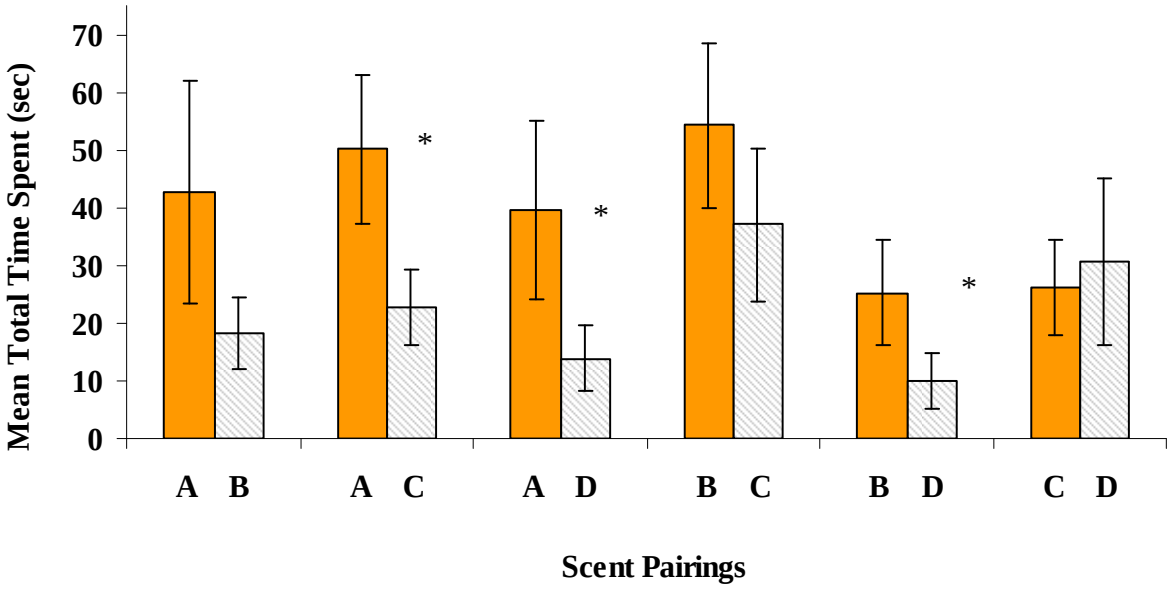



Figure 7. Mean time, spent with different male cheetah urine scents ("good" mate choice: genetically unrelated males=A, an "average" mate choice: equivalent to a second cousin=B, a "poor" mate choice: equivalent to a brother/father =C and a control: no scent=D). Mean total time was defined as the average time spent with a scent (regardless of behavior). For AC, AD and CD n=12 and AB, BC and BD n=9. Asterisk indicates a significant difference in time spent.

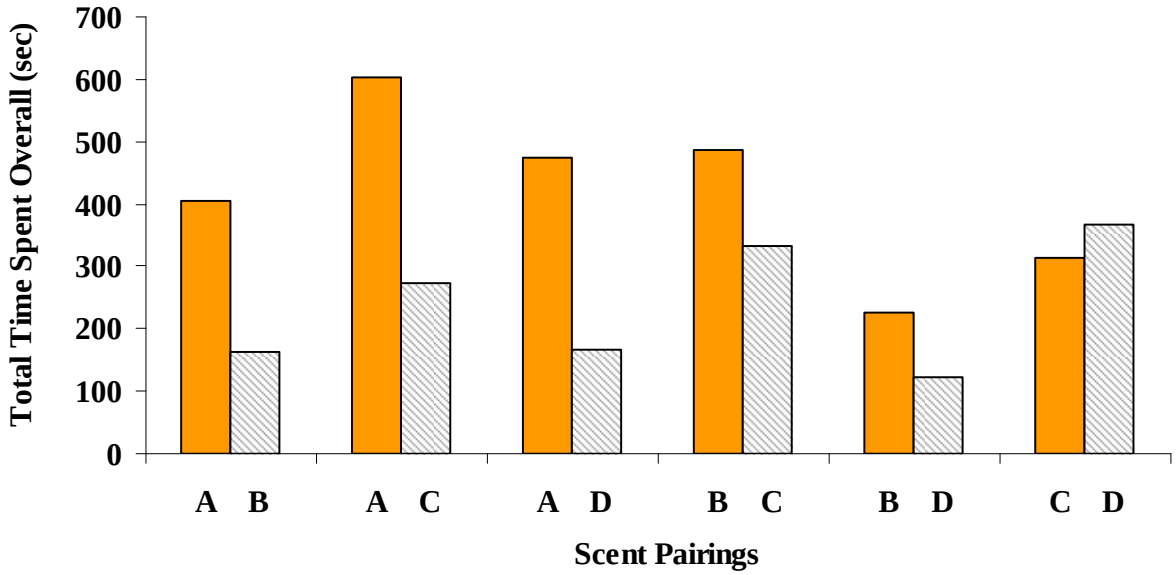



Figure 8. Total time (all behavior durations combined) for all 12 female cheetahs spent with different male cheetah urine scents. A, B, C, D as in Figure 7.

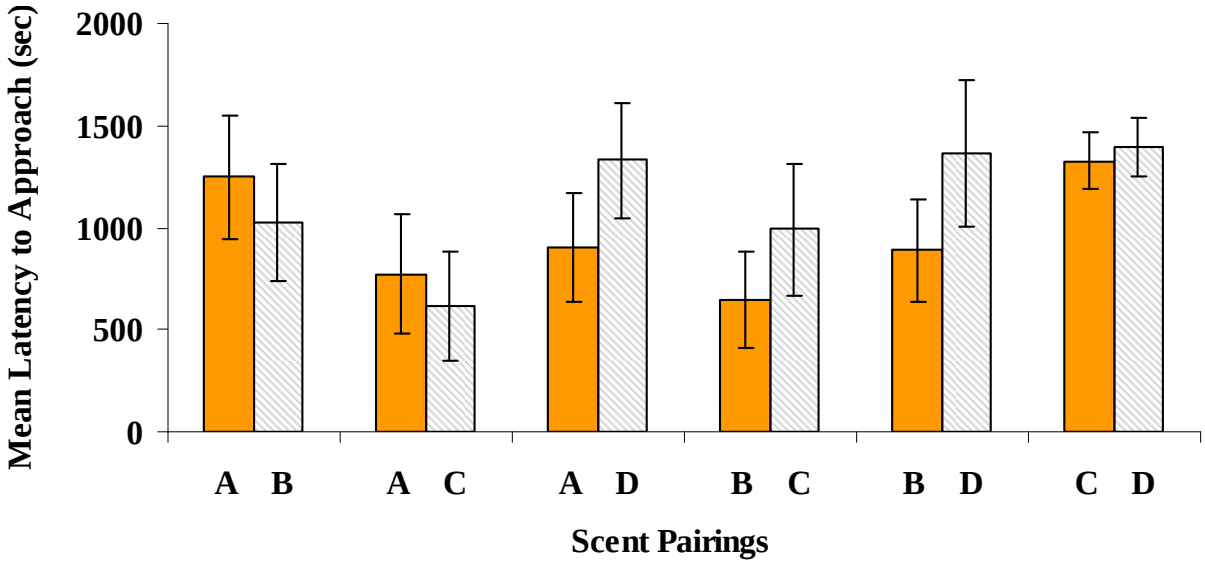



Figure 9. Female cheetah mean latency to approach to different male cheetah urine scents; there was no significant difference in which scent was approached first. A, B, C, D as in Figure 7.

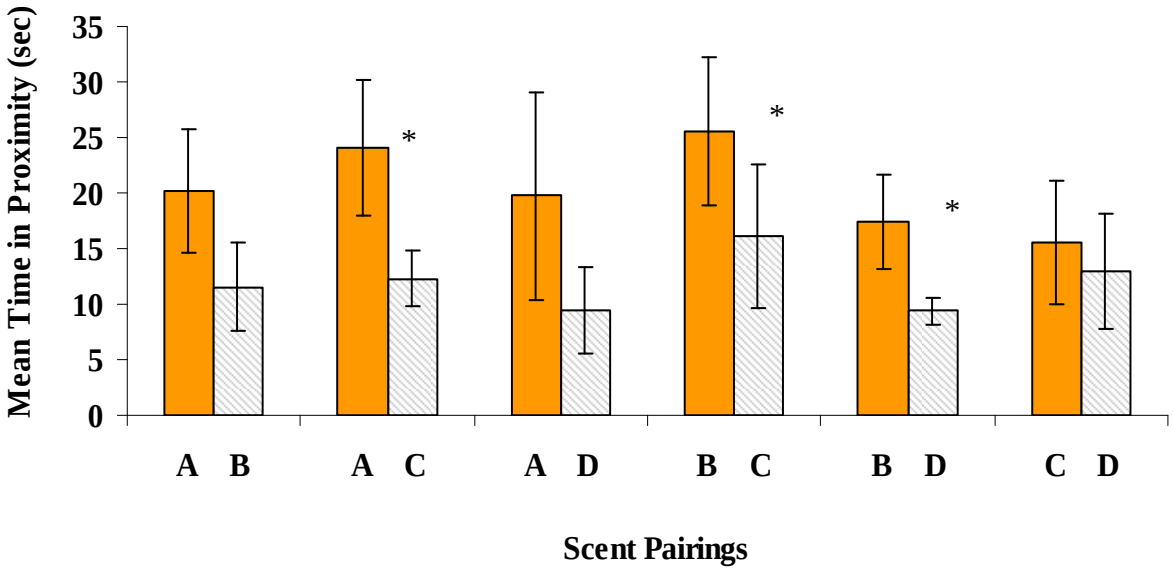



Figure 10. Mean time female cheetahs spent in proximity to different male cheetah urine scents. Asterisk indicates a significant difference in time spent in proximity. A, B, C, D as in Figure 7.

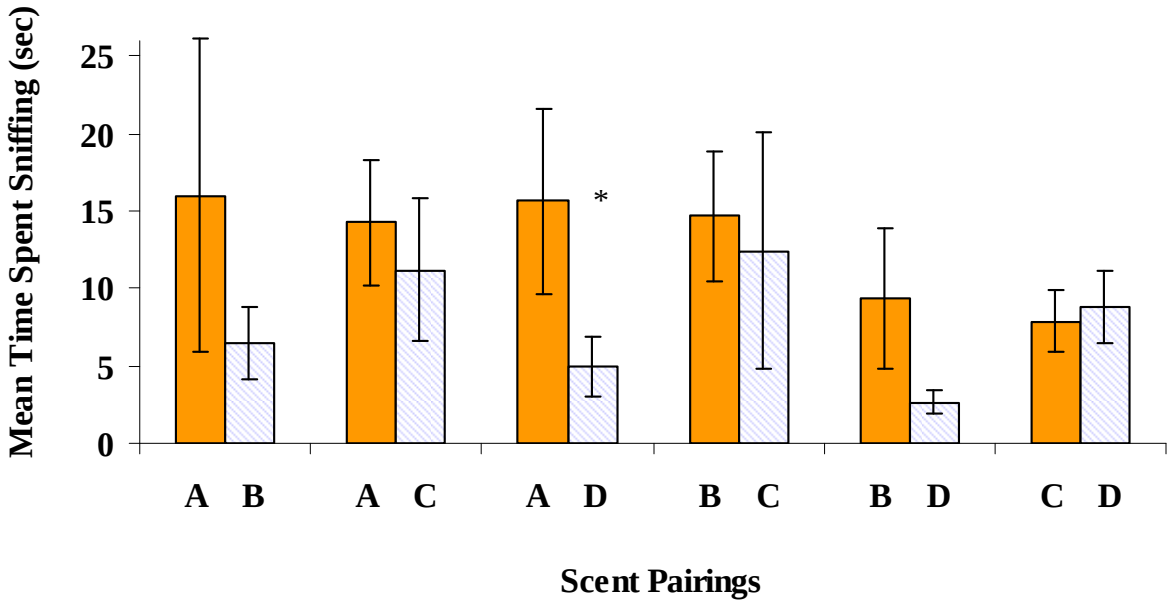



Figure 11. Mean time female cheetahs spent sniffing different male cheetah urine scents. A, B, C, D as in Figure 7. Asterisk indicates a significant difference in time spent sniffing.

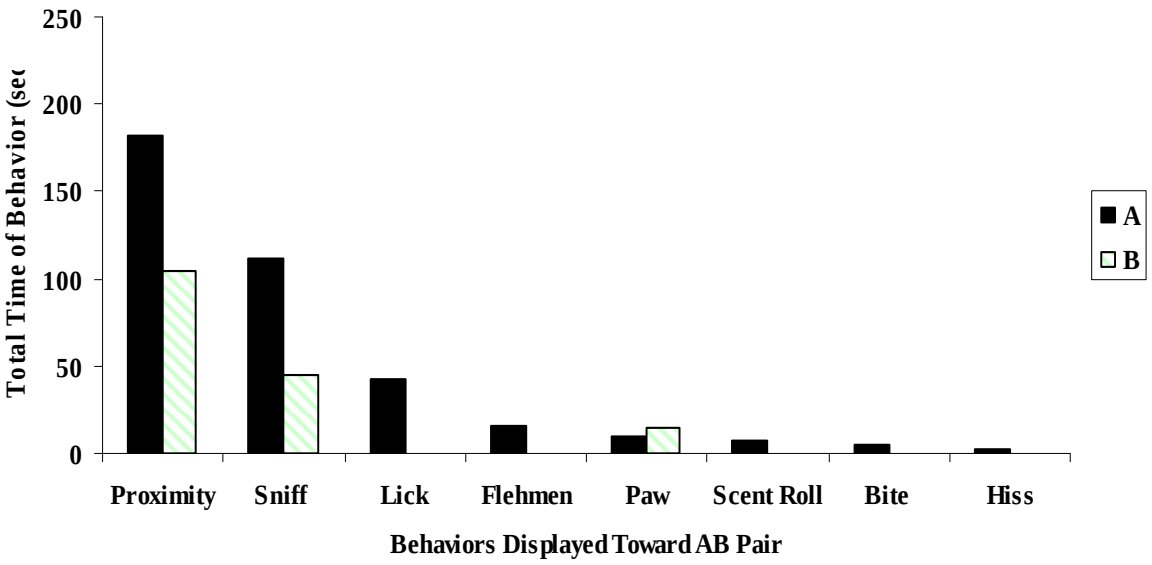



Figure 12. All behaviors, and the combined total time of those behaviors, female cheetahs (n=9) displayed toward the pairing of urine scents A and B as in Figure 7.

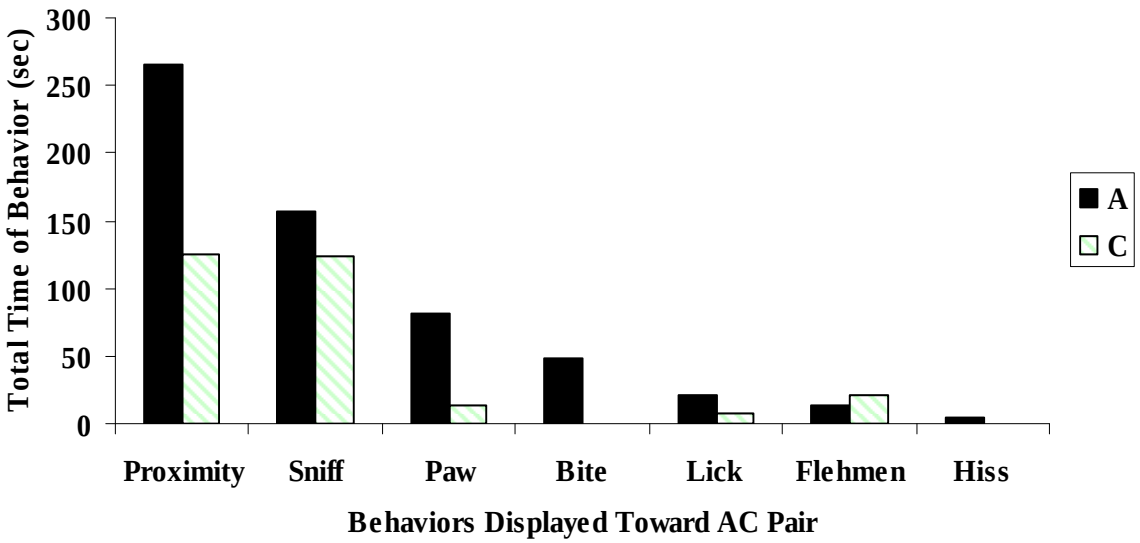



Figure 13. All behaviors, and the combined total time of those behaviors, female cheetahs (n=12) displayed toward the pairing of urine scents A and C as in Figure 7.

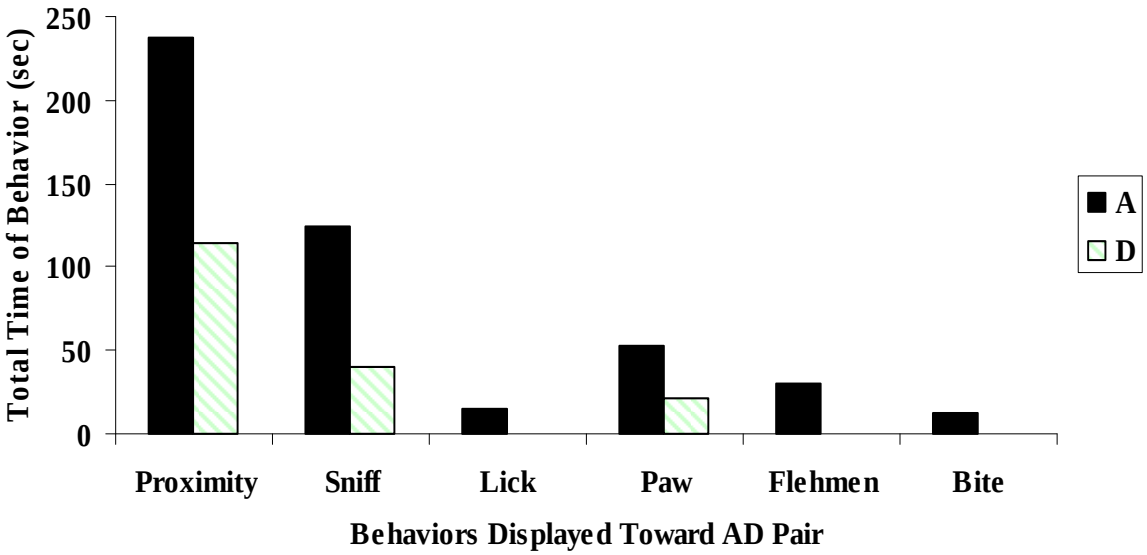



Figure 14. All behaviors, and the combined total time of those behaviors, female cheetahs (n=12) displayed toward the pairing of urine scents A and D as in Figure 7.

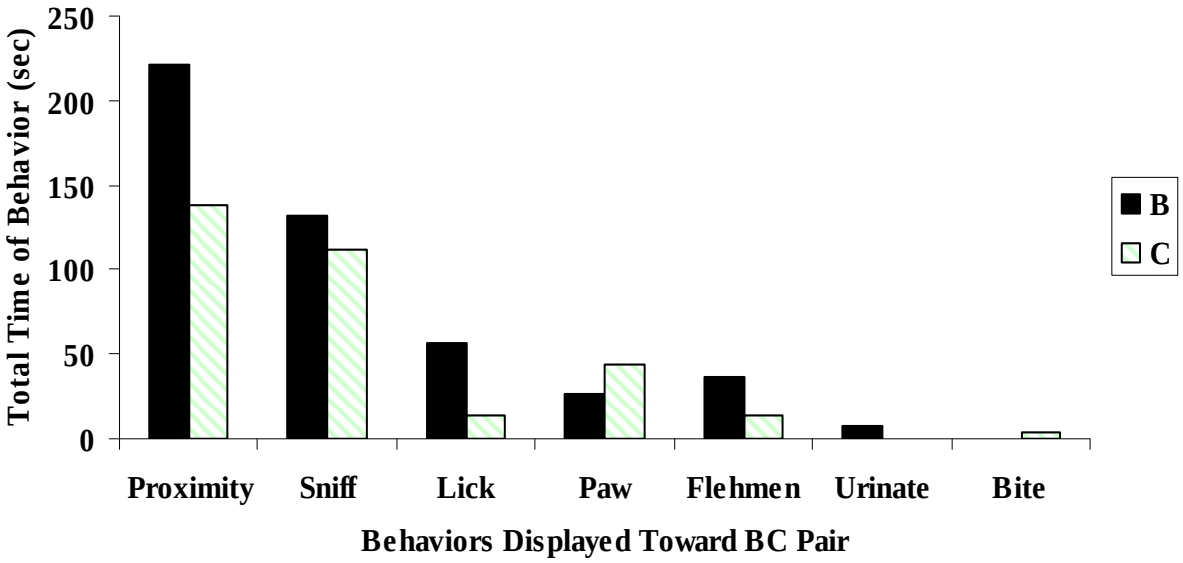



Figure 15. All behaviors, and the combined total time of those behaviors, female cheetahs (n=9) displayed toward the pairing of urine scents B and C as in Figure 7.

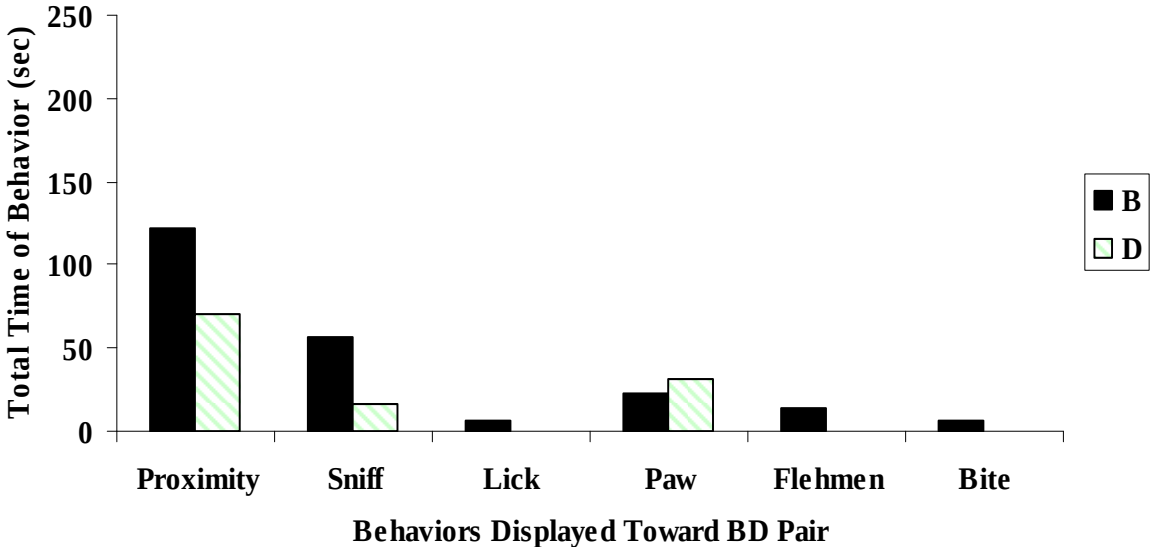



Figure 16. All behaviors, and the combined total time of those behaviors, female cheetahs (n=9) displayed toward the pairing of urine scents B and D as in Figure 7.

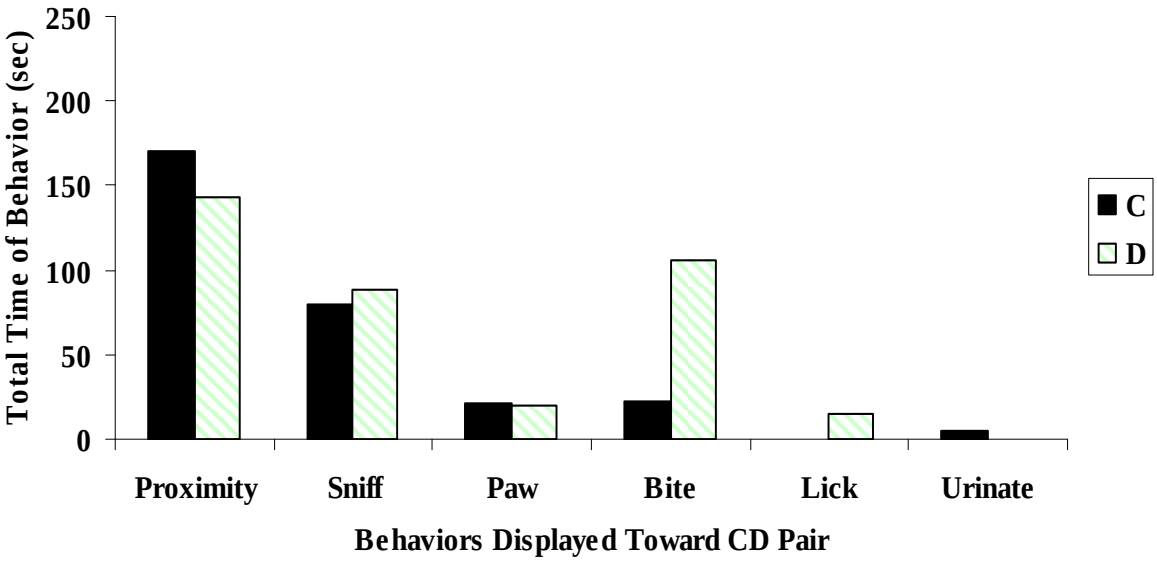
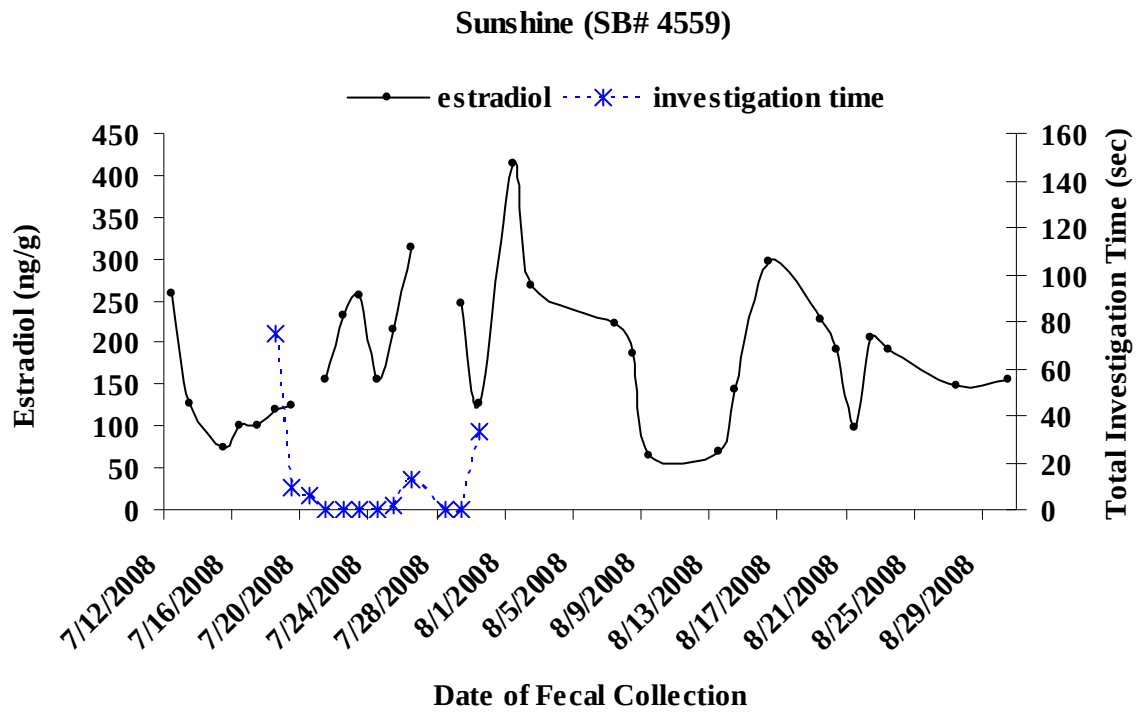



Figure 17. All behaviors, and the combined total time of those behaviors, female cheetahs (n=12) displayed toward the pairing of urine scents from males C and D as in Figure 7.

A.




B.

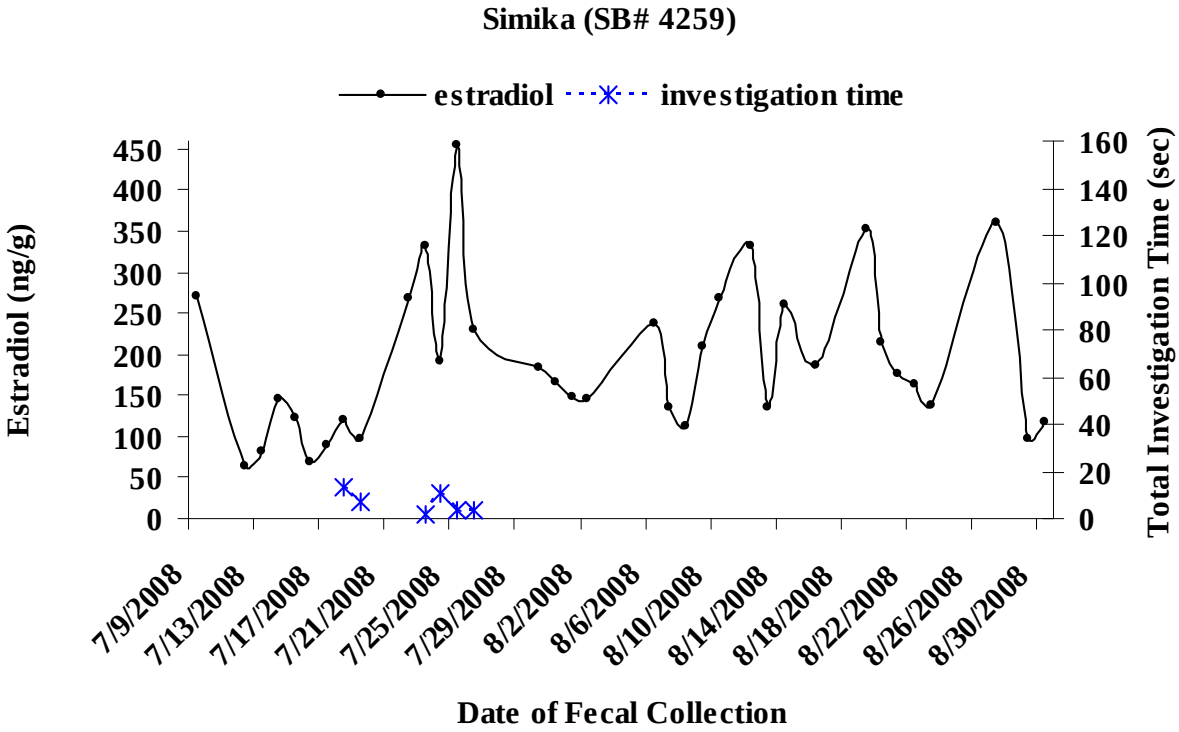
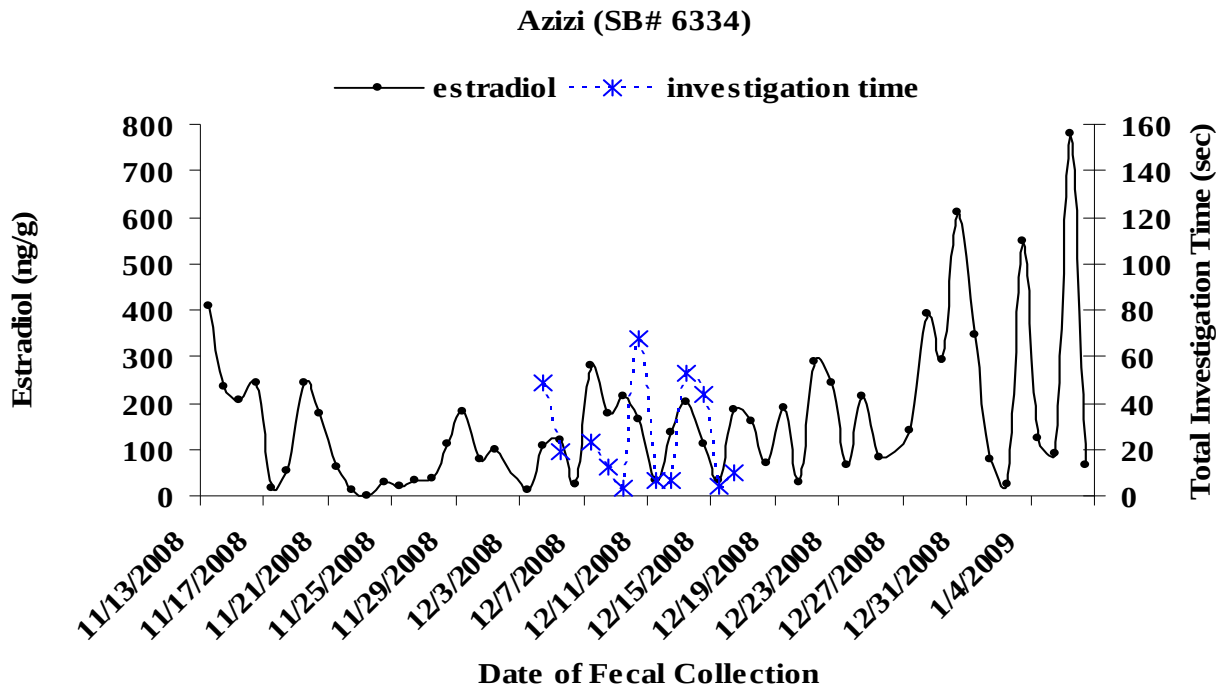



Figure 18. The daily fluctuations in fecal estradiol concentrations and total time spent daily investigating male urine scents of two female cheetahs that were located at Fossil Rim Wildlife Center, Glen Rose, TX. Average temperature and humidity during scent trials for A. Sunshine were 33°C and 57% and B. Simika were 37°C and 47%.

A.




B.

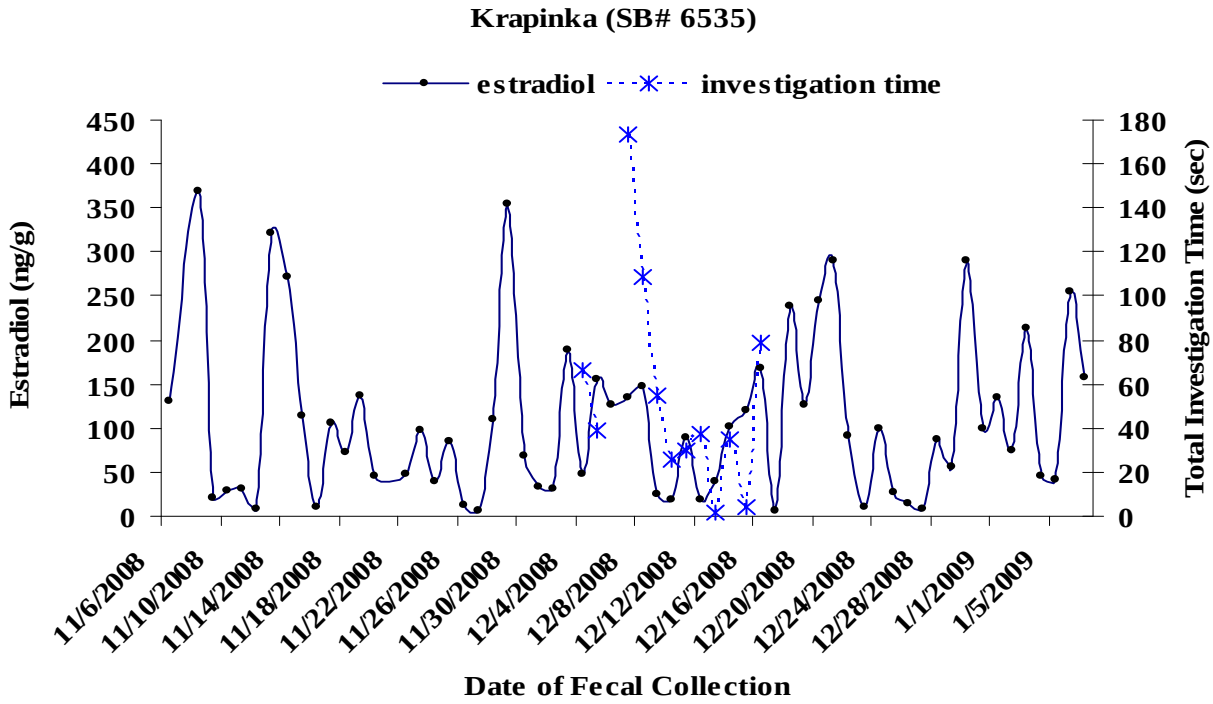
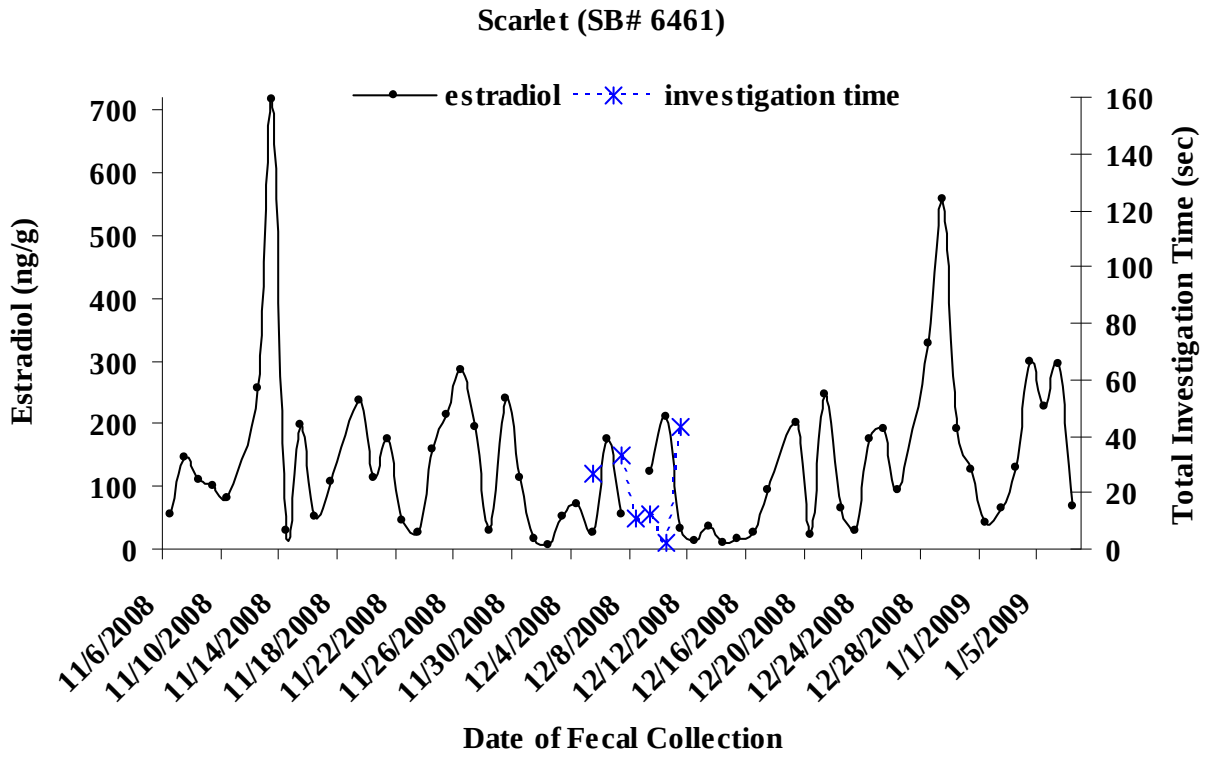



Figure 19. The daily fluctuations in fecal estradiol concentrations and total time spent daily investigating male urine scents of two female cheetahs that were located at White Oak Conservation Center, Yulee, FL. Females were observed simultaneously. Average temperature and humidity during scent trials was 17$^{o}$C and 78%.

A.




B.

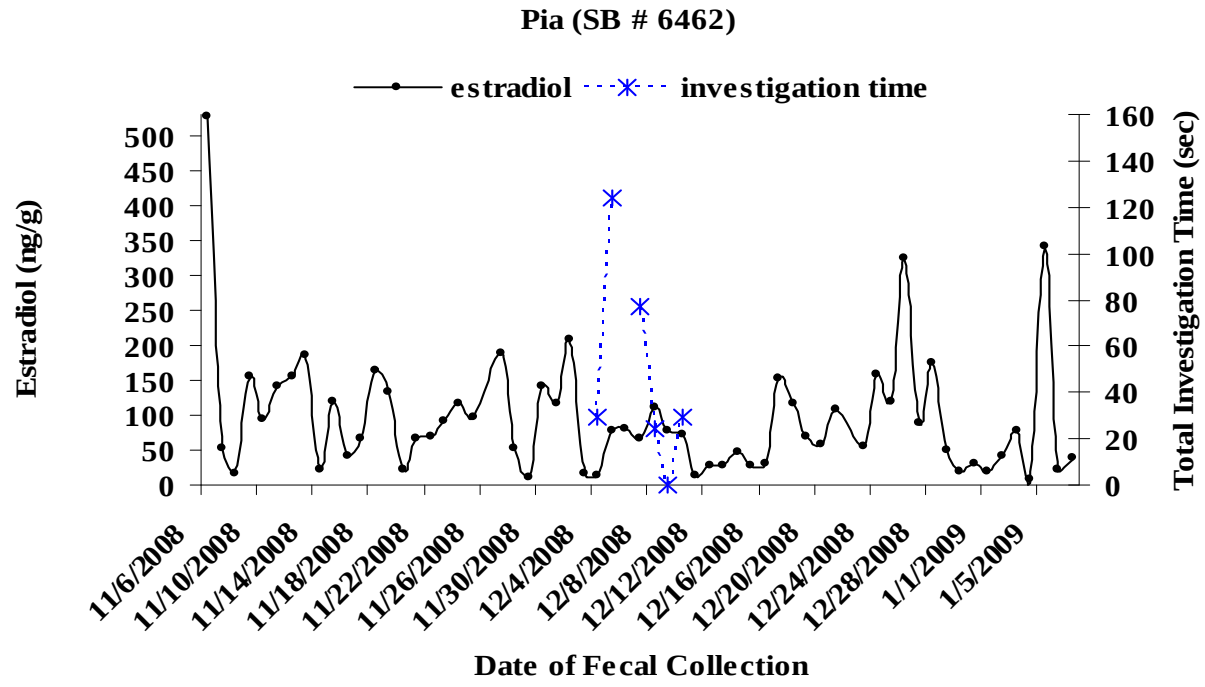
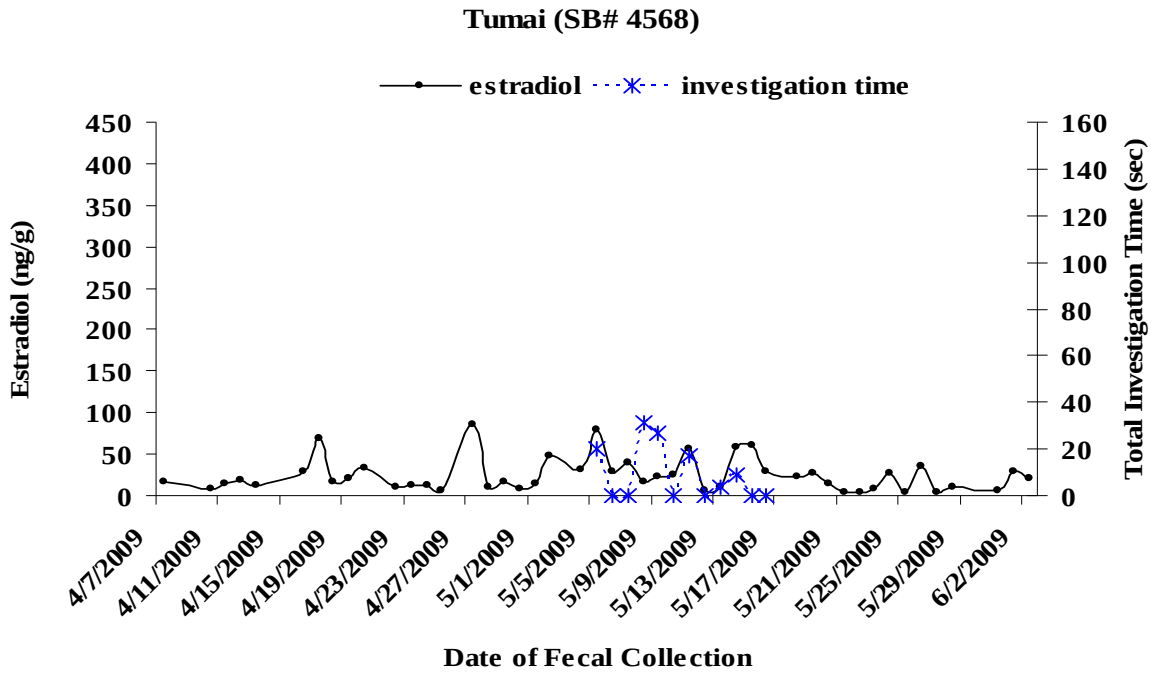



Figure 20. The daily fluctuations in fecal estradiol concentrations and total time spent daily investigating male urine scents of two female cheetahs that were located at White Oak Conservation Center, Yulee, FL. Females were observed simultaneously. Average temperature and humidity during scent trials was 17$^{o}$C and 67%.

A.




B.

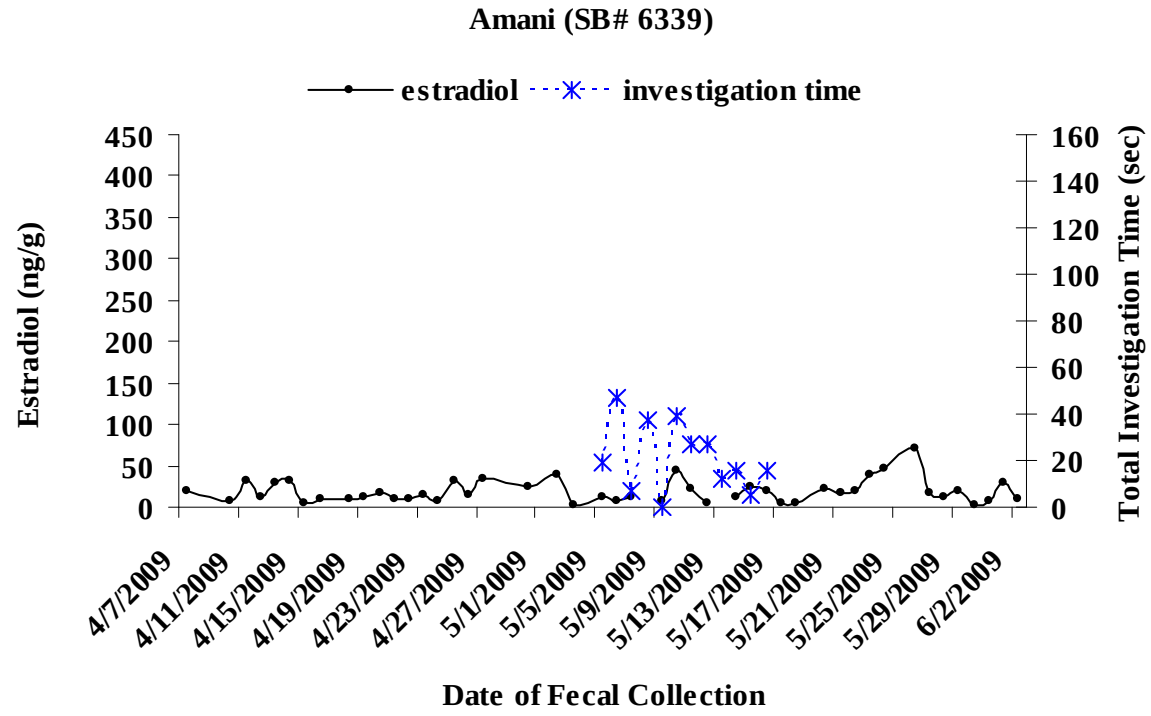
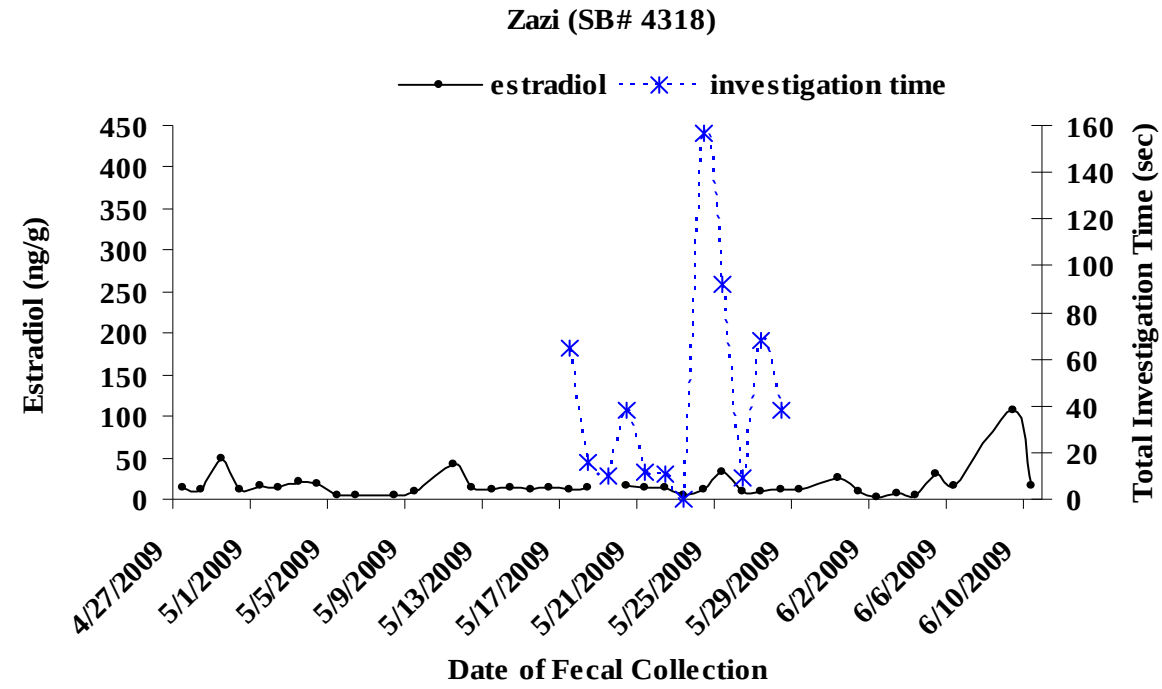



Figure 21. The daily fluctuations in fecal estradiol concentrations and total time spent daily investigating male urine scents of two female cheetahs that were located at the National Smithsonian Zoological Park, Washington, D. C. Average temperature and humidity during scent trials for A. Tumai were 21$^{o}$C and 66% and for B. Amani were 26$^{o}$C and 52%.

A.




B.

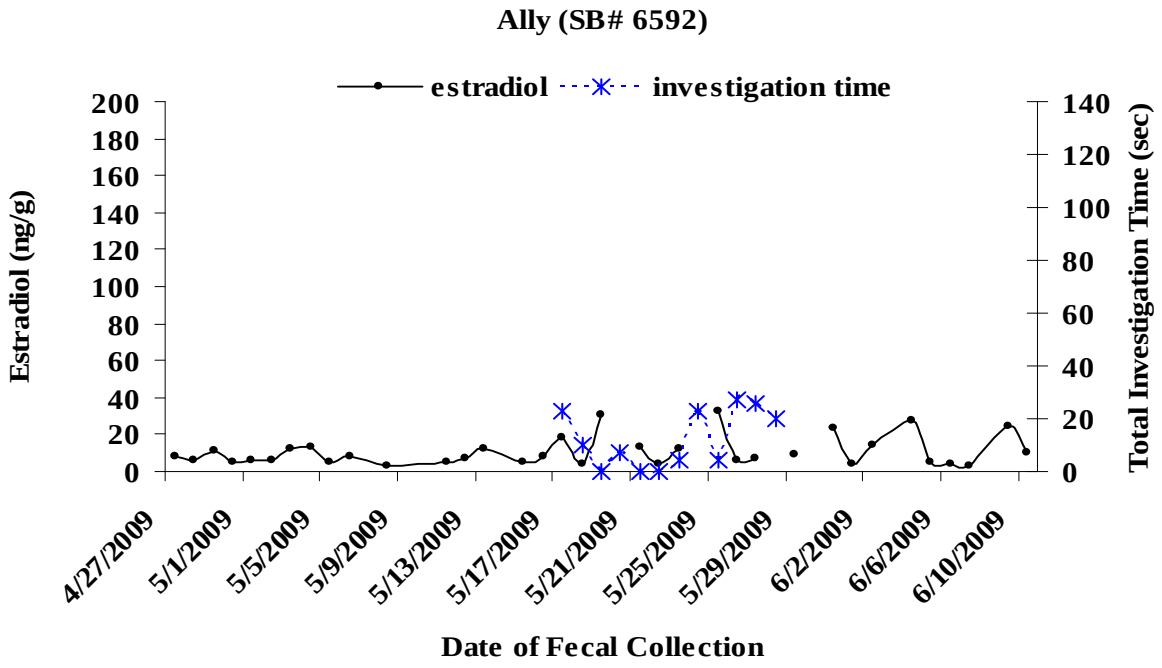
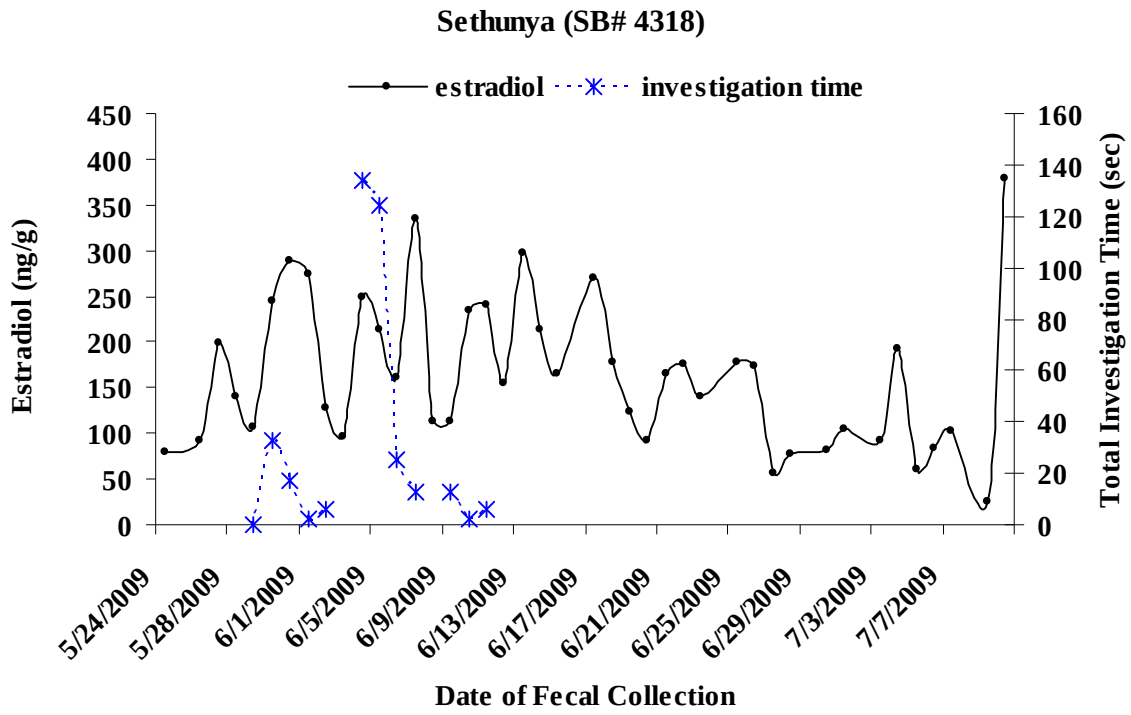



Figure 22. The daily fluctuations in fecal estradiol concentrations and total time spent daily investigating male urine scents of two female cheetahs that were located at the National Smithsonian Zoological Park Conservation and Research Center, Front Royal, VA. Average temperature and humidity during scent trials for A. Zazi were 18$^{o}$C and 66% and for B. Ally were 21$^{o}$C and 62%.

A.




B.

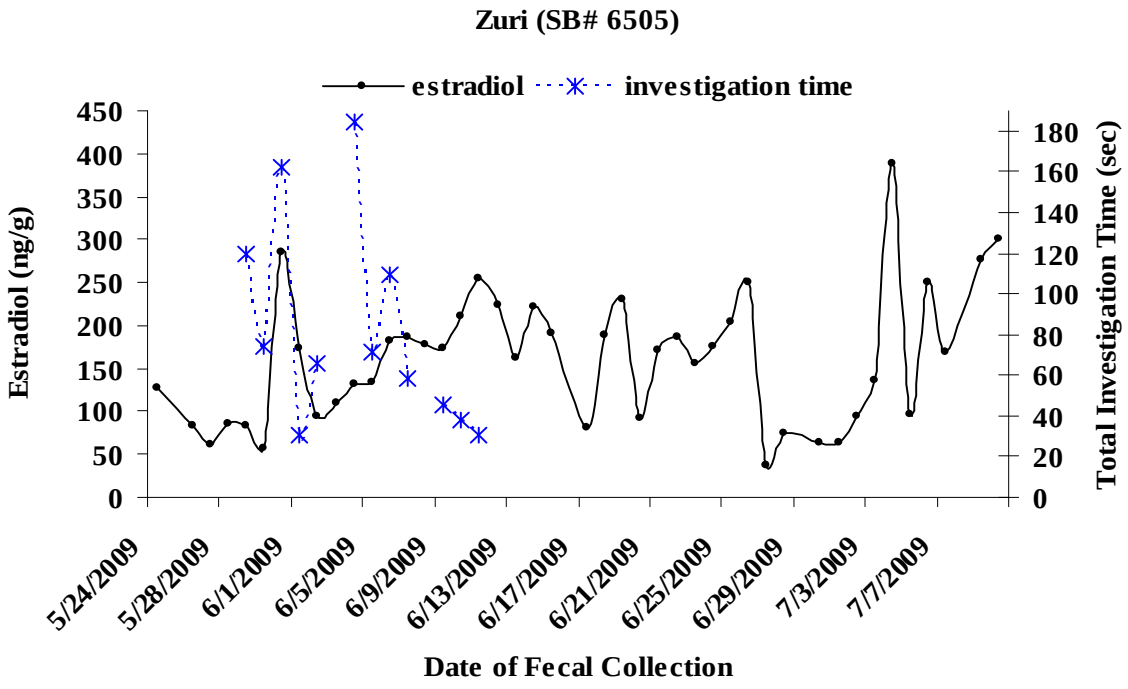



Figure 23. The daily fluctuations in fecal estradiol concentrations and total time spent daily investigating male urine scents of two female cheetahs that were located at the Saint Louis Zoo, St. Louis, MO. Average temperature and humidity during scent trials for A. Sethunya were 23°C and 65% and for B. Zuri were 24°C and 60%.

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# APPENDICES

APPENDIX I

Names, studbook numbers, location at time of scent trials and approximate ages during trials of female cheetahs.

| Female Name | Studbook Number | Birth Date | Approximate Age During Trials (Dates of Trials) | Location of Trials |
|---|---|---|---|---|
| Simika | 4259 | 02-Nov-00 | 7 years, 8 months (18-26 July 2008) | Fossil Rim Wildlife Center |
| Sethunya | 4318 | 27-Apr-01 | 8 years, 2 months (29 May-11 June 2009) | Saint Louis Zoo |
| Zazi | 4453 | 27-May-01 | 8 years (17-28 May 2009) | Smithsonian National Zoological Park's Conservation and Research Center |
| Sunshine | 4559 | 19-Apr-00 | 8 years, 3 months (18-30 July 2008) | Fossil Rim Wildlife Center |
| Tumai | 4568 | 19-Apr-00 | 9 years, 1 month (5-16 May 2009) | Smithsonian National Zoological Park |
| Azizi | 6334 | 23-Apr-05 | 3 years, 8 months (4-16 December 2008) | White Oak Conservation Center |
| Amani | 6339 | 07-Jun-05 | 4 years, 1 month (5-16 May 2009) | Smithsonian National Zoological Park |
| Scarlet | 6461 | 20-Jun-06 | 2 years, 6 months (4-11 December 2008) | White Oak Conservation Center |
| Pia | 6462 | 20-Jun-06 | 2 years, 6 months (4-11 December 2008) | White Oak Conservation Center |
| Zuri | 6505 | 10-Nov-06 | 2 years, 7 months (29 May-11 June 2009) | Saint Louis Zoo |
| Krapinka | 6535 | 12-Apr-05 | 3 years, 8 months (4-16 December 2008) | White Oak Conservation Center |

APPENDIX I (Continued)

Names, studbook numbers, location at time of scent trials and approximate ages during trials of female cheetahs.

| Female Name | Studbook Number | Birth Date | Approximate Age During Trials (Dates of Trials) | Location of Trials |
|---|---|---|---|---|
| Ally | 6592 | 01-May-07 | 2 years (17-28 May 2009) | Smithsonian National Zoological Park's Conservation and Research Center |

APPENDIX II

Location of female cheetahs within their respective facilities, enclosure size, amount of food fed daily and when it was fed in relation to the scent trials and the distance that scent dispensers were placed apart inside their enclosure.

| Female Name | Enclosure Number | Approx. Enclosure Size ($m^2$) | Feeding in Relation to Trials | Approx. Amount Fed | Distance Scents were Apart (m) |
|---|---|---|---|---|---|
| Simika | IMA Yard 2 | 1,129 | Full Prior | 1.4 kg[N] | 7.7 |
| Sethunya | Yard 5 | 604 | Full Prior | 1.4 kg[N] | 7.2 |
| Zazi | Yard 1 | 1,742 | Partial Prior | 2.7 kg [Na] | 14 |
| Sunshine | IMA Yard 1 | 1,417 | Full Prior | 1.4 kg[N] | 8.5 |
| Tumai | Yard 1 | 584 | Full Prior | 1.4 kg [Na] | 5.2 |
| Azizi | C21 | 1,980 | Full Prior | ~3 kg [Na T] | 5.5 |
| Amani | Yard 8 | 822 | Partial Prior | 1.3 kg [Na] | 20.1 |
| Scarlet | C17 | 1,137 | Full Prior | ~3 kg [Na T] | 6.4 |
| Pia | C16 | 828 | Full Prior | ~3 kg [Na T] | 9.5 |
| Zuri | Yard 3 | 149 | Full Prior | 1.8 kg[N] | 4.6 |
| Krapinka | C21 A/C | 176 | Full Prior | ~3 kg [Na T] | 6.4 |
| Ally | Yard 3 | 1,742 | Partial Prior | 2.7 kg [Na] | 14.2 |

[N] -Fed Nebraska Premium Feline Diet®
[Na]-Fed Natural Balance®
[T] - Fed Toronto Diet®

APPENDIX III

Male cheetah's birthdates, location and approximate ages at time of urine collection.

| Male Studbook Number | Birthdate | Age at Time of Urine Collection (Approximate Date Of collection) | Male Location at Time of Urine Collection |
|---|---|---|---|
| 4224 | 12-Aug-99 | 9 years, 3 months (November 2008) | Jacksonville Zoo and Gardens |
| 4257 | 02-Oct-00 | 7 years, 8 months (June 2008) | Dickerson Park Zoo |
| 4452 | 27-May-01 | 7 years, 9 months (February 2009) | Sunset Zoo |
| 5457 | 10-Aug-01 | 5 years, 8 months (April 2008) | Saint Louis Zoo |
| 5458 | 10-Aug-01 | 5 years, 8 months (April 2008) | Saint Louis Zoo |
| 5478 | 22-Mar-02 | 6 years, 4 months (July 2008) | Scovill Zoo |
| 5480 | 16-Apr-02 | 6 years, 2 months (June 2008) | White Oak Conservation Center |
| 5496 | 28-Apr-02 | 7 years (April 2009) | Safari West |
| 5504 | 30-May-02 | 7 years, 1 months (April 2009) | Binder Park Zoo |
| 5990 | 22-Jun-04 | 4 years (June 2008) | White Oak Conservation Center |
| 5991 | 22-Jun-04 | 4 years (June 2008) | White Oak Conservation Center |
| 6015 | 27-Jun-04 | 3 years, 10 months (April 2008) | Saint Louis Zoo |
| 6180 | 23-Nov-04 | 3 years, 7 months | Milwaukee County Zoo |

APPENDIX III (Continued)

Male cheetah's birthdates, location and approximate ages at time of urine collection.

| Male Studbook Number | Birthdate | Age at Time of Urine Collection (Approximate Date Of collection) | Male Location at Time of Urine Collection |
|---|---|---|---|
| | | (June 2008) | |
| 6338 | 07-Jun-05 | 3 years, 9 months (March 2009) | Wildlife Safari |
| 6359 | 15-Apr-05 | 3 years, 1 month (May 2008) | Smithsonian National Zoological Park |
| 6503 | 10-Nov-06 | 2 years, 5 months (April 2009) | Saint Louis Zoo |
| 6590 | 01-May-07 | 1 year, 10 months (March 2009) | Wildlife Safari |

APPENDIX IV

The combined total time each female cheetah spent with each male urine scent in all six pairings. Pairings that had a significant difference in time spent between the scents are highlighted in bold.

| Combination | Total Time Spent with First Scent (seconds) | Total Time Spent with Second Scent (seconds) | P Value |
|---|---|---|---|
| **Total time spent with A B** | | | |
| *All 12 females* | 406 | 164 | 0.254 |
| Sethunya | 0 | 13 | |
| Zazi | 183 | 12 | |
| Sunshine | 31 | 2 | |
| Tumai | 2 | 2 | |
| Azizi | 17 | 5 | |
| Amani | 20 | 44 | |
| Zuri | 80 | 39 | |
| Krapinka | 29 | 45 | |
| Ally | 44 | 2 | |
| **Total time spent with A C** | | | |
| *All 12 females* | 602 | 274 | **0.039** |
| Sethunya | 61 | 80 | |
| Zazi | 129 | 28 | |
| Sunshine | 0 | 0 | |
| Tumai | 17 | 10 | |
| Azizi | 66 | 6 | |

APPENDIX IV (Continued)

The combined total time each female cheetah spent with each male urine scent in all six pairings. Pairings that had a significant difference in time spent between the scents are highlighted in bold.

| Combination | Total Time Spent with First Scent (seconds) | Total Time Spent with Second Scent (seconds) | P Value |
|---|---|---|---|
| Amani | 55 | 8 | |
| Zuri | 109 | 31 | |
| Krapinka | 105 | 40 | |
| Ally | 13 | 14 | |
| Simika | 4 | 3 | |
| Pia | 18 | 35 | |
| Scarlet | 25 | 19 | |
| **Total time spent with A** | | **D** | |
| *All 12 females* | 473 | 167 | **0.087** |
| Sethunya | 10 | 2 | |
| Zazi | 66 | 13 | |
| Sunshine | 31 | 2 | |
| Tumai | 2 | 7 | |
| Azizi | 2 | 8 | |
| Amani | 22 | 12 | |
| Zuri | 144 | 71 | |
| Krapinka | 23 | 7 | |
| Ally | 2 | 12 | |

APPENDIX IV (Continued)

The combined total time each female cheetah spent with each male urine scent in all six pairings. Pairings that had a significant difference in time spent between the scents are highlighted in bold.

| Combination | Total Time Spent with First Scent (seconds) | Total Time Spent with Second Scent (seconds) | P Value |
|---|---|---|---|
| Simika | 6 | 0 | |
| Pia | 151 | 2 | |
| Scarlet | 14 | 31 | |
| **Total time spent with B** | | **C** | |
| *All 12 females* | 481 | 333 | 0.287 |
| Sethunya | 33 | 103 | |
| Zazi | 15 | 10 | |
| Sunshine | 63 | 12 | |
| Tumai | 26 | 5 | |
| Azizi | 37 | 28 | |
| Amani | 36 | 19 | |
| Zuri | 142 | 49 | |
| Krapinka | 106 | 104 | |
| Ally | 23 | 3 | |
| **Total time spent with B** | | **D** | |
| *All 12 females* | 227 | 121 | **0.040** |
| Sethunya | 17 | 10 | |
| Zazi | 40 | 10 | |

APPENDIX IV (Continued)

The combined total time each female cheetah spent with each male urine scent in all six pairings. Pairings that had a significant difference in time spent between the scents are highlighted in bold.

| Combination | Total Time Spent with First Scent (seconds) | Total Time Spent with Second Scent (seconds) | P Value |
|---|---|---|---|
| Sunshine | 0 | 0 | |
| Tumai | 0 | 0 | |
| Azizi | 4 | 7 | |
| Amani | 12 | 12 | |
| Zuri | 81 | 43 | |
| Krapinka | 53 | 32 | |
| Ally | 20 | 7 | |
| **Total time spent with C** | | **D** | |
| *All 12 females* | 315 | 367 | 0.797 |
| Sethunya | 14 | 33 | |
| Zazi | 8 | 2 | |
| Sunshine | 13 | 6 | |
| Tumai | 24 | 13 | |
| Azizi | 110 | 2 | |
| Amani | 9 | 3 | |
| Zuri | 32 | 168 | |
| Krapinka | 21 | 90 | |
| Ally | 2 | 2 | |

APPENDIX IV (Continued)

The combined total time each female cheetah spent with each male urine scent in all six pairings. Pairings that had a significant difference in time spent between the scents are highlighted in bold.

| Combination | Total Time Spent with First Scent (seconds) | Total Time Spent with Second Scent (seconds) |
|---|---|---|
| Simika | 21 | 3 |
| Pia | 45 | 32 |
| Scarlet | 16 | 13 |

# VITA

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