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Sexual Dimorphic Social Development and Female Intrasexual Chemical Signaling of African Elephants (Loxodonta africana)

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SEXUALLY DIMORPHIC SOCIAL DEVELOPMENT AND FEMALE INTRASEXUAL CHEMICAL SIGNALING OF AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*)

by

JORDANA MEYER

(Under the Direction of Bruce A. Schulte)

ABSTRACT

African elephants are a polygynous species in which males and females carry out dimorphic lifestyles. Males search and compete for reproductively active females, while females care for offspring and facilitate group cohesion. The objectives of this study was a) to compare the development of sexually dimorphic behaviors and developmental trends between young male and female wild African elephants and b) to determine the ability of captive female African elephants to discern between the follicular and luteal phase of conspecifics through trunk-tip contacts and the investigation of urine, and whether the reproductive phase of the receiver affected the response to urine. For the first objective, focal animal observations were made on 83 female and 81 male elephants less than 11 years of age from June – October 2005 in Addo Elephant National Park, South Africa. For the second objective, 11 estrous and 10 non-estrous females were observed at nine zoos throughout North America from March – August, 2006. Sexually dimorphic behaviors and developmental patterns conducive to future adult lifestyles became apparent during social play, social interactions, and exploratory chemosensory behaviors of young male and female elephants. Captive elephants were able to discern estrous condition through direct contact to the urogenital region, increasing in rate with approaching ovulation, however, they did not distinguish between luteal and follicular urine from unfamiliar females.

INDEX WORDS: Captive, Chemosensory, Development, Female Intrasexual Communication, *Loxodonta africana*, Play, Sexual Dimorphism, South Africa

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CHAPTER I

SEXUAL DIMORPHISM IN THE DEVELOPMENT OF YOUNG AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*)

Abstract

African elephants are a sexually dimorphic species in which males and females carry out divergent lifestyles. Females reside in intricate matriarchal groups and males roam alone or form small bachelor herds with relatively loose social bonds compared to female groups. Females mature to reproductive status in their early teenage years while males are usually not viable mates until at least their mid-twenties. The difference in adult lifestyles and age of maturation should affect the developmental pattern for the two sexes. I hypothesized that behavioral patterns would show divergence in male and female elephants younger than 11 years old in preparation for the changes in social environment and reproductive opportunities experienced by pubescent elephants. Dimorphic adult behaviors were expected to develop through play, social interactions and exploratory behaviors. A cross-sectional study was conducted on male (N = 83) and female (N = 81) African elephants between 1 and 127 months of age at Addo Elephant National Park, South Africa from June to October of 2005 using continuous focal behavior observations. The hypothesis was supported by all three behavioral measures. Young males engaged in more agonistic play primarily with other males of equivalent age and at a higher rate and for a longer duration than did females. Males showed greater interest in indirect signal sources such as urine and feces, whereas juvenile females were more interested in direct contact to the genitalia of female conspecifics. Females increased nurturing behaviors across age and mainly interacted with calves. These sexually dimorphic developmental processes culminate in the emergence of the adult behavioral repertoire that may ultimately influences long-term reproductive success.

INDEX WORDS: Chemosensory, Development, *Loxodonta africana*, Play, Sexual Dimorphism, South Africa

Introduction

Sex differences in behavior and morphology can be explained through sexual selection (Darwin 1871; Trivers & Willard 1973; Lande 1980; Kelly 1988), and the degree of dimorphism is a direct result of the intensity of intrasexual competition (Alexander et al. 1979). Sexual dimorphism is defined as differences in traits, typically size, color, or behavior between male and female adults, and is most prevalent among polygynous species (Kelly 1988). In a polygynous system, males and females have distinct reproductive strategies that reflect fundamental evolutionary differences between the sexes (Lande 1980). The development of large body size, fighting abilities, and high social status is important in male mating success (Clutton-Brock et al. 1982). In contrast, adult females invest in parental care of their offspring. These sex-specific traits are likely to result in behavioral differences between the sexes that emerge during development.

Although behavioral development occurs throughout an individual's lifetime, the most sensitive time of development is during the pre-pubertal period, typically considered the infant or juvenile phase of life (Immelmann & Suomi 1981). It is hypothesized the sensitive phase is a specific age when an organism is most vulnerable to environmental stimulation (see review by MacDonald 1985). During this time, individuals practice and refine behaviors that facilitate survival and reproduction in adulthood (Mayr 1974; Geary 1999). Alterations in the social environment during the sensitive phase of development reveal the importance of this period in relation to later adult behaviors. For example, isolated juvenile rats (*Rattus norvegicus)* had a lower propensity for exploration than did non-isolated juveniles as adults (Arakawa 2005). However, isolation during the post-pubertal stage did not affect exploration behaviors among adults. This study and several

others (Gerall et al. 1967; Riopelle 1972; Van den Berg et al. 1999) suggest a critical age range in the development of behaviors that will be beneficial during adulthood. Because adult-like behaviors typically emerge as juveniles play, interact socially, and explore the environment (Geary 1999), sexually dimorphic behaviors should become pronounced during this sensitive phase in polygynous species.

Sex differences in early social interactions, such as play, are evident and often resemble action patterns used by adults of species with a polygynous reproductive strategy (Fagen 1981; Clutton-Brock et al. 1982; Bekoff 2001). Play is arguably the most prominent example of how immature individuals develop social and physiological skills (Bekoff 1974; Taylor 1980), however, play has been difficult to define. Bekoff & Byers (1981, p. 300) define play as "motor patterns from other contexts that may often be used in modified forms and altered temporal sequencing" and if directed towards an individual, it is termed social play. Play allows young individuals to practice adult behavioral patterns without their immediate functional consequences (Fagen 1981; Walters 1987; Bekoff 2001). Interactions through play equip young individuals with experience required for adult roles, such as intrasexual competition, parental care, group cooperation, reproductive proficiency, and communicative skills (Dublin 1983; Rothstein & Griswold 1991; Biben 1998; Pellegrini & Smith 1998). Species exhibiting dimorphism in play behavior include squirrel monkeys, *Sainiri sciureus,* (Baldwin 1969), cattle, *Bos taurus,* (Reinhardt 1983), horses, *Equus caballus,* (Waring 1983), reindeer, *Rangifer tarandus,* (Mathisen *et al.* 2002), and rats, *Rattus norvegicus,* (Pellis 2002). Males from these species have greater variance in reproductive success and do not typically invest in parenting (Clutton-Brock et al. 1983; Pellegrini 2004). Young males

acquire fighting tactics and assess ability through play fighting with individuals of the same sex and age (Thompson 1995; Pellis et al. 1997). Females of these species tend to avoid aggressive play behaviors, but form intricate social groups and invest in the care and security of their offspring (Clutton-Brock et al. 1982). Young females engage in more maternal interactions with all ages and sexes (Geary 1999), usually forming or strengthening bonds within the group (bonobos, *Pan paniscus,* Palagi 2006). Immature animals not only develop through social interactions, but also learn to function in highly dynamic social systems through sensory exploration of their environment (Palagi et al. 2002; Myers & Sclafani 2006).

In many mammals, the two primary senses responsible for maintaining social relationships and mate choice are smell (Gosling & Roberts 2001; Wyatt 2003) and touch (Dewsbury 1988). Differences in olfactory and tactile exploratory behaviors of immature animals may exist in association with dimorphic mating and social systems. In sexually dimorphic species, the male typically searches for sexually receptive females while the female chooses among available males (Clutton-Brock et al. 1982; Wyatt 2003). Olfactory stimuli, such as sexual signals, are carried as chemicals in individual excretions and secretions (Wyatt 2003). Interest in specific chemical cues and their sources changes as an individual develops (Alberts 1981). Juvenile males are interested in the genitalia of estrous females, yet have not learned to perform multipart sexual interactions such as pursuing, mounting, or mating that will develop through play and social interactions (Squirrel monkeys, *Saimiri sciureus*, Baldwin & Baldwin 1974; Bison, *Bison bison*, Rothstein & Griswold 1991; Mandrills, *Mandrillus sphinx*, Setchell 2003).

The meaning of a chemical signal is contingent upon the identities of the sender and the receiver. Males and females may exhibit different response behaviors to chemical signals; for instance, female lemurs, *Lemur catta* (Kappeler 2004) and Syrian hamsters, *Mesocricetus auratus* (Fischer & McQuiston 1991; Swann & Fiber 1997) counter-mark estrous urine, whereas males respond with copulatory behaviors to the sender. In addition, females tend to develop adult olfactory behaviors at a younger age than males. For example, female ring-tailed lemurs perform more olfactory investigative behaviors during infancy, while males perform more during the weaning and juvenile phase (Palagi et al. 2002). This may reflect the earlier age at which females reproduce, reducing the developmental window during which females learn to function in cooperative social relationships through olfactory and tactile investigatory behaviors (reviewed by Fagen 1981). The different selection pressures experienced by the sexes (males: mate searching, females: parental care and group cooperation) affect their chemical communication; therefore, olfactory behaviors are expected to show sexually dimorphic characteristics during the infant and juvenile phases of life.

Play, social interactions, and exploratory olfactory behaviors affect the acquisition of adult behaviors. For a socially complex species such as the African elephant (*Loxodonta africana*) that relies on chemosensory signals for sex-specific communication, these three modes of development may be instrumental in preparation for future adult lifestyles. African elephants are a long-lived, polygynous species in which adult roles are distinct (Douglas-Hamilton & Douglas-Hamilton 1975; Poole 1994). Adult females form tightly knit matriarchal groups that are composed of related females and their offspring and function in a fission-fusion society among multiple tiers

(Douglas-Hamilton 1972; Dublin 1983; Moss & Poole 1983; Wittemyer et al. 2005, Archie et al. 2006). Females nurture and care for the young (Lee & Moss 1986), create bonds within the group (Lee 1987; Lee & Moss 1999), signal estrus (Poole & Moss 1989), and choose mates (Moss 1983; Poole & Moss 1989). The male members of the family unit are offspring that disperse between the ages of 10-15 years to live in a highly dynamic world (Poole 1994). Males typically roam alone or form small bachelor herds with relatively loose social bonds compared to female groups (Poole & Moss 1989; Poole 1994). Throughout their adult lifetime, males search and compete for access to reproductively active females, but take no part in the rearing of offspring.

African elephant mating tactics consist of using long-distance communication via chemical signals and vocalizations (Poole & Moss 1989; Langbauer 2000). Males and females are spatially separated much of the time and require means to locate reproductive partners over large distances. African elephants announce sexual status through chemical signals emitted in urine, feces, and glandular secretions that are detected via chemosensory behaviors such as sniffing and flehmen (Poole & Moss 1989; Bagley et al. 2006). Flehmen in elephants occurs when the trunk tip presents possible chemical signals to the ducts of the vomeronasal organ in the roof of the mouth (Rasmussen et al. 1982). In the follicular urine of female Asian elephants (*Elephas maximus*), a pheromone identified as (*Z*)-7-dodecen-1-yl acetate signals approaching ovulation to conspecific males (Rasmussen et al. 1997). Although an estrous pheromone has yet to be isolated in the urine of African elephants, captive males can discern between follicular and luteal urine (Bagley et al. 2006), supporting the hypothesis that an estrous pheromone is released by female African elephants (Goodwin et al. 2006). Social context and

experience affect responses and decisions in intersexual mate choice (Lee & Moss 1999; Schulte et al. 2005), yet developmental patterns have not been described in detail.

Despite the obvious similarities in sexually dimorphic adult roles of other polygynous species, elephants differ in many ways. The combination of longevity, indefinite growth, delayed maturation, and the function of musth may factor into how elephants develop as they age (Sukumar 2003). Males and females continuously grow throughout their lifetime; however, males grow at a faster rate to become twice the size of adult females (Poole 1989). As subadults, males disperse from the natal herd (10-15 years old) and females begin to breed or have already had their fist calf by this time (Poole 1989; Sukumar 2003). Although both sexes are capable of producing gametes, males will not reach sexual maturity until several years later than females. Males typically will not father their first offspring until they are between 30 and 35 years of age when they are large enough to compete and sustain musth (Poole 1994; Vidya & Sukumar 2005; Archie et al. 2006).

Musth is an annual occurrence for adult males that has been associated to rutting behavior in ungulates (Poole & Moss 1981). At this time, males have a heightened interest in females and increased aggressiveness (Poole and Moss 1981; Hall-Martin 1987). Teenage male Asian elephants experience moda musth, during which they release a honey-smelling odor to avoid conflict with older males (Rasmussen et al. 2002). These younger, lower ranking adult males will drop out of musth if in the presence of an older, higher ranking musth bull (Vidya and Sukumar 2005). Because of this relationship between body size and rank in reproductive success, delayed maturation occurs within males (Poole 1994). Delayed maturation is thought to generate a difference in the rate of

development between male and female elephants before the subadult stage, which is the time when their social environment diverges.

Little is known about African elephants in their pre-pubertal period. There has only been one other study comparing play between male and female elephants. Lee (1986) reported a sex difference in the frequency of play bouts and play partner choice of calves ranging from 0-5 years of age. Lee's observations were conducted as scan data only and did not distinguish between types of play. The current study expanded the scope of ages by observing animals up to ten years of age and behaviors performed by African elephants, including type of play, social interactions and chemosensory exploratory behaviors. The juvenile phase, 5-10 year old, is an important time for understanding the development of sexually dimorphic adult behaviors because it precedes sub adulthood.

Recent studies at the Addo Elephant National Park, South Africa, have examined African elephant chemosensory and social behaviors. One study provided an overview comparison of the behavior of males and females (Loizi 2004), while two ensuing studies examined the behavior of males (Bagley 2004) and females (Merte 2006), respectively. This current study explored sexual differences of development during the sensitive period of infancy through juvenility.

Many behaviors of male and female elephants differ as adults; hence, these behaviors must diverge at some point of development. In this study, the developmental patterns for the two sexes were hypothesized to be dimorphic and to diverge before the social environment and reproductive opportunities of males and females began to differ. Because maturation occurs at different times for males and females, I predicted this to

affect the rate of development. I examined these hypotheses through the play, social and exploratory behaviors of male and female calves and juveniles. Sexually dimorphic developmental patterns were expected to be present in these three modes of development. Geary (1999) stated that in polygynous species the juvenile period will be longer for males than for females because male-male competition is more intense than female-female competition. Female elephant maturation is both physically and socially advanced over that of the males (Lee & Moss 1999); thus, I hypothesized that females would display adult skills at a younger age than males. In addition, I hypothesized that sex-specific chemosensory behaviors also would show dimorphic patterns. Given the gregarious, social nature of females compared to the often solitary, searching nature of adult males, I predict that females would direct a greater proportion of contact and chemosensory behaviors towards conspecifics, while males would direct a larger proportion of responses towards the excretions of conspecifics, namely their urine and feces. This study investigated the occurrence of sexually dimorphic behaviors and developmental patterns during the play, social interactions and exploration of young African elephants in relation to time of maturation and the dimorphic social setting and reproductive strategies of adult male and female elephants.

Methods

Study Site

The study site was located in the Eastern Cape of South Africa at the Addo Elephant National Park (AENP), 72 km northeast of Port Elizabeth. The AENP was originally founded in 1931 to protect the remaining 11 elephants left in the area

(Whitehouse & Hall-Martin 2000). Elephants in the Eastern Cape during the early 1900's were killed for ivory and because they were a nuisance to farmers, thus leaving a diminutive population to protect (Hoffman 1993). By 1954, a fence was constructed around the park to impede crop raiding and reduce the risk of poaching (Whitehouse 2001).

The study area now consists of 14,000 ha of habitat that ranges from sub-tropical succulent thicket to open, grassy plains (Low & Rebelo 1996 as cited in Whitehouse et al. 2001; Whitehouse & Hall-Martin 2000) (See Appendix A for map). Water within the park is pumped from a reservoir, ensuring a continuous water supply to five main waterholes. There are also natural water pans throughout the park that are filled by rainfall.

Study Animal

The elephant population used for this study consisted of approximately 360 elephants. Based on the terminology of Wittemyer et. al. (2006), the Addo population segregates into five distinct tier 3 or kinship (multi-family) groups (delineated by the letters A, B, P, R, and L) and one single family unit (H family) (naming system created by Whitehouse & Hall-Martin 2000). The H family only operates at the tier two (family) level because this group is not composed of multiple family units nor do these elephants regularly join up with other family units to comprise a kinship group). Only 2% of the females now have tusks because of a genetic bottleneck created from the original population size and conditions of the founding elephants. However, previous studies have shown that the general social structure of elephants at AENP is comparable to elephants from other regions of Africa (Whitehouse & Hall-Martin 2000; Loizi 2004). A

family tree of the elephant population was constructed from intense studies dating back to 1976-79 and ongoing since 1996 (Whitehouse et al. 2001). Elephants were identified through files consisting of photographs and descriptions of the individuals by ear tears, vein patterns in the ears, eye wrinkles, and other physical features (Whitehouse & Hall-Martin 2000; Whitehouse 2001). If an animal was not yet entered in the file, age was determined by estimations based on size and developmental stage (Moss 1996). Young individuals were identified by first determining the identity of their mother. For most individuals under age 12, the date of birth could be estimated to the month and in many cases, the week or day based on observations (Whitehouse 2001; Loizi 2004; Bagley 2004; Merte 2006).

Behavioral Observations

The project was conducted from June until early October 2006. A total of 85 days (500 hours) was spent in the field with 52.6 hours of focal data collected (including multiple focal observations on the same individuals). Elephants were easiest to locate and observe at the five waterholes throughout the park. Waterholes serve as areas of congregation, socializing, and information exchange (Merte 2006). In addition, I was most interested in social interactions and chemical signaling, which were easier to observe when elephants were not in the vegetation feeding; therefore, all observations occurred at these waterholes. Hapoor was the largest waterhole and used for 70% of the observations. Observations were conducted from a vehicle for safety. The elephants were habituated to vehicles in close proximity.

Before conducting observations, I generated a randomized list of the age and sex combinations for focal observations that day. Once elephants were found at a specific

waterhole, they were counted and identified before initiating a focal observation bout. I only performed observations with five or more individuals present because my interest was in social play and social interactions, which requires more than one individual. By using five, typically I would have more than one age class and sex present. Once five or more individuals were present at a waterhole, I haphazardly selected an individual from the first age/sex category to be observed that day. A haphazard sample is "one where the sample is chosen according to an arbitrary criterion such as availability of visibility" (Martin & Bateson 2001, p. 132). If no individuals matched this category, then I selected an individual from the next category, and so on. For subsequent observations, I started from the top of the list until all age/sex combinations were observed or it was time to leave the park.

Once an animal was identified, a 20-minute focal with continuous recording was conducted on the animal's activities and interactions (Altmann 1974). A full 20-minute focal observation was not always completed because an individual was lost from view among the group and or in the thick bush, or departed from the waterhole. Focals of five minutes or less were not used.

During the focal observation, I recorded all event behaviors during each state in order to examine the context in which social behaviors were performed (e.g. aggressive behaviors outside of play). State behaviors were measured by duration in seconds, whereas events were measured as a frequency, subsequently converted to rate per hour. State behaviors were long-lasting body movements with measurable duration such as walk, stand, drink, and play (Table 1.1) (Martin & Bateson 1993). Event behaviors were chemosensory behaviors to substrate and conspecifics, and social behaviors such as trunk

touches to and from elephants or body contacts to and from elephants (Table 1.2). The number of animals present, time of day, temperature, and location also were noted.

Social play bouts were defined as starting when two individuals were involved in reciprocal contact (Sharpe & Cherry 2003), such as trunk wrestling, sparing, rubbing, rolling, or mounting, and ending when animals separated for more then five seconds. Identities of initiator and recipient of social activity, such as play or physical interaction, were documented. Data were categorized as trunk tip touches to/from conspecifics or self, chemosensory behaviors to the environment, to urine/feces, and to conspecifics, and social contact to and from individuals. Specific event behaviors were grouped together to form general types of behavior, including nurturing, aggression, and chemosensory (Lee 1986) (Table 1.3). For the trunk over-back-behavior, late in the study I noticed differences in the direction of the trunk depending on the sender-receiver pairing. Typically, females would place the trunk over the side of younger individuals (transverse plane), while males would place the trunk over the back from behind (sagittal plane). Because these differences were not recorded for most of the study, trunk-over-back was considered a single behavior (see also Appendix B).

Chemosensory behaviors were recorded and then computed as the rate of responses to urine and feces, the environment, and conspecifics. Four main responses to urine, feces, or conspecifics were measured: sniff, check, place, and flehmen (Schulte & Rasmussen 1999). A sniff is when the trunk hovers over a substance without contact. Checks occur when the trunk-tip finger touches a substance such as feces or urine. When the end of the trunk flattens onto the substance to create a seal, this behavior was

recorded as a place. Flehmen is the characteristic placement of the trunk tip in the mouth onto the orifices of the vomeronasal organ ducts after contact with urine (see Table 1.2).

Sample Size

Focal animals were represented evenly across the five kinship groups and one family unit throughout the study (Table 1.4). I observed 81 females and 83 males for a total of 164 individual focal observations on young elephants. Eighty-two percent of the population that was ten years of age and younger was sampled (Fig. 1.1). The age range includes calves (≤4 years, 37 males and 40 females) who are still nursing to some extent and juveniles (5-10 years, 46 males and 41 females), who have been weaned but remain associated with the natal herd. The sample size is small for particular ages because of the number of available elephants in the population. As a result, each individual was aged to the nearest month at time of observation. This allowed the analysis of each focal by age in month using a regression approach, rather than grouping by year. Months were used for every analysis unless stated otherwise.

Data Analysis

Males and females were compared across the age of one to 127 months in order to determine if there were developmental trends and sex differences in the behaviors recorded. Behavior was measured by the rate and proportion of event behaviors and by the rate, duration and proportion of state behaviors. Rates for individual behaviors were calculated by dividing the frequency of event behaviors or the frequency of state bouts per individual by the total duration of focal length minus not visible time in minutes and then converted to an hourly rate. The true duration of a particular state behavior could not be determined for the first and last behaviors of focal observations as well as before

and after recordings of not visible because start and stop times were not obtained. Therefore, I removed these values for the analysis of state duration, defined as the duration of each occurrence of a complete state behavior. After this adjustment, two focal observations (one from each sex category) had no other state behaviors and were dropped from the analyses. Therefore, the sample size was 80 individuals for females and 82 individuals for males when calculating the mean bout duration of state behaviors. I calculated the mean bout duration of each state per individual and used this mean value for analyses so that each individual contributed only one value for a particular behavior. Mean bout duration was calculated by the total duration of a complete state behavior divided by the frequency of complete bouts. The proportion of time an animal spent in different states was calculated by the total duration of each state behavior divided by the total duration of focal length minus not visible time per individual. The proportion of event behaviors was based on number of times the behavior occurred per individual divided by the total number of event behaviors performed by that individual.

I sampled the ages and sexes haphazardly without replacement such that variations in the number of elephants present, air temperature and different water holes should have had no directional impact on the data obtained. I examined if relevant response variables were correlated with the number of elephants present or air temperature by using a Spearman's Rho Correlation. I also compared responses between the one water hole (Hapoor) that comprised 70% of the observations and all other waterholes to see if there was a waterhole effect using a Spearman's Rho Correlation.

The data for each behavioral category were examined in two ways using the JMP 4.0 statistical program for a PC. To investigate developmental trends, I used linear

regressions to determine if behavioral responses were affected by age of the individual (the null hypothesis was the slope would not differ from zero). To investigate differences in response by sex, I used analysis of covariance (ANCOVA) tests with age as the covariate. Parallelism of the slopes was tested before performing an analysis. If the parallelism test failed, this suggested that the rate of development by males and females differed across age for that behavior; therefore, a regression was used to examine the direction of the slope for each sex. An experiment-wise error rate of 0.05 was used for all analyses. Means (± SE) values are presented for each behavior. Each figure included in this study shows a trend line for male and female elephants only if it was significant across age or between the sexes.

Results

The results are presented in four sections based on the four main response types, namely state behaviors and three categories of developmental behaviors, to examine sexual dimorphism (sex) and developmental trends (ages 1 – 127 months) in the behavior of young African elephants. The average length of a focal bout was 15.3 min (+/-SE 1.20) (Fig. 1.2) with no significant difference between sex across ages 1-127 months (with age as the covariate) (ANCOVA: $F_{1,161}$=1.99, P=0.16). The mean age of the sampled population was 60.0 ± 4.3 months for females and 65.9 ± 4.2 months for males (ANOVA: $F_{1,162}$=1.0, P=0.31). Waterhole location, number of elephants present during an observation and air temperature on the measured responses by age and sex did not have an effect on the behavior of the focal animal (Table 1.5).

State Behaviors

The proportion of time, duration (Fig. 1.3), and rate of nursing drastically decreased in both males and females as they aged (Table 1.6, A-C:I, 4). Males and females showed no significant difference in the duration of nursing (Males: 27.2 ± 2.9 s, Females: 37.3 ± 4.2 s; ANCOVA: $F_{1,33}$=3.21, *P*=0.08). Interestingly, females seemed to wean earlier in life than males. Of the 36 elephants observed nursing (21 females, 15 males), the mean age for females (21.3 ± 2.7 months) was significantly less than for males (31.5 ± 3.6 months) (ANOVA: $F_{1,34}$=5.32, *P*=0.03). The oldest male observed nursing was 50 months of age and the oldest female was 40 months of age. Thus, on average, females were weaned ten months earlier than males. There were 97 elephants observed not nursing after these months (48 females, 49 males). The mean age for these females (85.7 ± 3.4 months) not significantly different from the age of the males (93.9 ± 3.1 months) observed in this study (ANOVA: $F_{1,95}$=3.21, *P*=0.08).

Developmental patterns were prevalent across age in both sexes. The mean bout length of drinking and standing lengthened as females matured (Table 1.6, B:I, 1-2) (Fig. 1.4). Younger males walked more frequently per hour than did older males, while the duration for each bout did not change (Table 1.6, C:I, 3). Males and females did not differ in the frequency, mean bout duration, or proportion of time spent drinking, standing, or walking (Table 1.6, A-C:II, 1-3).

Play Behavior

Males were predicted to take part in play (aggressive and non-aggressive) more often and play for a longer period of time (bout duration and later in life) than females. Of the 82 males observed, 40 (49%) took part in social play, while 23 of the 80 (29 %)

females were involved in social play (X^2=6.94, *df*=1, *P* =0.008). Ninety-two percent of total social play bouts involved males, while only 44% involved females (56% male-male, 36% male-female and 8% female-female). Males spent a greater proportion of time observed partaking in social play than females (Table 1.7, A:II, 3). When focal animals did participate in social play, males played (0.16 ± 0.02 /hour) twice as often (frequency of bouts/hour) as females (0.08 ± 0.01 /hour). The 40 focal males engaged in 109 play bouts as opposed to only 33 play bouts by the 23 focal females (Table 1.7, C:II, 3) (Fig. 1.5). Male elephants were observed playing more with intrasexual play partners (79 male-male and 30 male-female play bouts), whereas females played more with intersexual play partners (12 female-female and 21 female-male play bouts) (X^2=14.35, *df*=1, *P* =0.002). Across age categories (>1-10 year olds), males typically played with same age elephants throughout development, while females played most with calves up to eight years of age (Fig. 1.6). These patterns were evident by the pairs of elephants playing, regardless of which animal was under focal observation (Table 1.8a).

Social play was analyzed as aggressive play (trunk wrestling and sparring) and non-aggressive play. Thirty-four of the 82 male elephant focals participated in aggressive play compared to nine of the 80 female focals involved in aggressive play (X^2=19.70, *df*=1, *P* <0.0001). Eighty percent of male play bouts were aggressive (87/109, 71 M-M, 16 M-F), while females were aggressive in only 39.4% (13/33, 2 F-F, 11 F-M) of the play bouts (X^2=18.56, *df*=1, *P* <0.0001). Young males engaged in forceful agonistic play for longer bout durations and more frequently per hour than females when all focals were analyzed (Table 1.7, B-C:II, 1) (Fig. 1.7). However, when only those animals that were involved in aggressive play were considered, there was no sex

difference in the duration of bouts (Males: 28.0 ± 3.0s; Females: 21.9 ± 5.8s) (ANOVA: $F_{1,41}$=0.85, *P*=36). Females were not observed participating in aggressive play until after five years of age; whereas, males partook in aggressive play within the first year of life. From the start of first participation of each sex, the rate of aggressive play increased (Table 1.7, C:I, 1) (Fig. 1.8). For females, the proportion of observed time and the bout duration of aggressive play also increased from first play bout at five to ten years of age (Table 1.7, A-B:I, 1). Of the thirteen bouts in which females (*N*=9) were involved, ten were with male partners (Table 1.8b). Although not quite significant because of the small sample size, older males showed shorter bouts of aggressive play with male calves and longer bouts with juvenile males compared to young males (Linear Regression: R^2=0.10, *t*=1.96, *P*=0.057). By ages nine and ten, focal males were typically playing with pubescent males (≥10 years of age).

Males and female focal elephants engaged in similar duration and frequency of bouts per hour of non-aggressive play (Table 1.7, B-C:II, 2). The 23 females observed participating in social play spent 60.6 % (20/33, 10 F-F, 10 F-M) of the social play bouts involved in non-aggressive play (by 16 different females), but the 40 males only participated in non-aggressive play during 20% (22/109, 8 M-M, 14 M-F) of their social play bouts (by 12 different males). The frequency of non-aggressive play significantly decreased across age in females (Table 1.7, C:I, 2). Males exhibited a significant decrease in the bout duration and proportion of time involved in non-aggressive play from one to 127 months of age, while they showed a concomitant increase in the rate of play fighting (Table 1.7, A-B:I, 2).

Social Interactions

Nurturing contacts showed different rates of development between males and females and across age. Four contact behaviors were grouped together and classified as nurturing (Table 1.3). These behaviors were received at a higher rate by young male and female calves, compared to nine to ten year old animals (Table 1.9, A:I, 1) (Fig. 1.9). Females received nurturing behaviors at a higher rate than did males (Table 1.9, A:II, 1). When the focal animal was providing nurture behaviors, the developmental trend was inverted for females. Older females performed these behaviors at a significantly higher rate than female calves, while males maintained these behaviors at a very low rate over all ages from 1-127 months (Table 1.9, B:I, 1) (Fig. 1.10). This differential pattern of development in males and females resulted in a significant interaction across age (Table 1.9, B:II, 1).

Sending and receiving aggressive acts outside of play showed different rates between males and females and across age. Males were sending and receiving two times as much aggressive behaviors outside of play episodes (tusk, push, head butt, trunk over head) than females (Table 1.9, A-B:II, 2) (Fig. 1.11a). Only 30% (24/81) of the females acted aggressively compared to the 42% (35/83) of male elephants. Older juvenile females were more aggressive than young female calves. There was no developmental trend seen in males, however, this was attributed to one individual (Fig. 1.11b). A 21-month-old male exhibited an 11-fold higher rate (freq/hour) of aggression (30.22/h) than the average rate for males (2.71 ± 0.45/h). With the removal of the data from this individual, a significant difference still existed between the sexes (ANOVA: $F_{1,161}$=5.26,

P=0.02), and in addition, a developmental trend was present for male elephants (R^2=0.09, t=2.77, P=0.007) (Fig. 1.11b).

Exploratory Chemosensory and Tactile Behaviors

Skills for maintaining social equilibrium and investigating chemical signals were expected to develop through trunk tip touching and chemosensory behaviors during early development. Of the sampled population of elephants from one to 127 months of age, 100% (83/83) of males responded to the environment, 81.9% (68/83) to conspecifics, and 37.3% (31/83) to urine and feces. Ninety-eight percent (80/81) of females responded to the environment, 75.3% (61/81) to conspecifics, and 24.7% (20/81) to urine and feces. Males and females did not differ in the rate of chemosensory behaviors to the environment, conspecifics, or to urine and feces, nor show a developmental trend as they aged (Table 1.10, A:I-II, 1-3). Males, however, allocated a significantly greater proportion of their total chemosensory behaviors towards the excretions of conspecifics compared to females (Table 1.10, B:II, 3). Males directed 3.0 ± 0.6% of their chemosensory behaviors towards urine and feces as opposed to females who only directed 1.3 ± 0.3% of chemosensory behaviors to excretions (Fig. 1.12).

A sexually dimorphic pattern was not seen between the sexes for total rate of chemosensory behaviors (not divided into three categories as mentioned before), nor was there a developmental trend (Table 1.11, A:I-II, 6). The four main chemosensory behaviors, sniff, check, place, and flehmen were combined as a mean rate for each individual and no significance was found between the sexes or across age (Table 1.11, A:I-II, 7). Analyzing the behaviors separately as proportions revealed that males showed a decrease in the proportion of sniffs and an increase in checks and flehmens with age

(Table 1.11, B:I, 1-4) (Fig. 1.13). Females only increased in the proportion of checks across age. Although place did not change with age or show a sex difference proportionally, the rate of places performed was significantly higher for males (10.1 ± 1.6/h) than females (4.8 ± 1.6/h) (Table 1.11, A:II, 3) (Fig. 1.14). The rate of raised sniffs (horizontal and periscope sniff) significantly increased with age for females, but not for males (Table 1.11, A:I, 5) (Fig. 1.15).

Elephants explored conspecifics through trunk-tip touches, in which the rate significantly decreased across age from one to 127 months for both males (R^2=0.21, t=-4.59, P=<.0001) and females (R^2=0.15, t=-3.71, P=0.0004) (Fig. 1.16). Males decreased trunk touches at a faster rate than females (the slopes of the male and female linear lines across age were not parallel; $F_{1,160}$=4.14, P=0.04). For females less than 11 years of age, older females showed higher rates of contacting or sniffing the genitals of conspecifics (R^2=0.12, t=3.2, P=0.002) (Fig. 1.17). There was no relationship between the rate of genital contact and age of males. This difference in the developmental trend resulted in a statistical interaction by covariates (sex X age) ($F_{1,160}$=5.15, P=0.03). Females directed 66.7 % (44/66) of genital contacts intrasexually and males 62.9 % (56/89) intersexually (intra- versus inter-sexual contacts, (X^2=13.27, df=1, P = 0.0003). Therefore, both sexes were more likely to contact the genital area of females compared to males.

The combination of chemosensory and tactile behaviors directed to conspecifics is a means by which elephants investigate and communicate with one another. Very young males and females directed the greatest proportion of their chemosensory and trunk-tip behaviors to adult and pubescent females, but this interest waned with age (Table 1.12, A:I, 1,3). In contrast, the older the females, the greater proportion of their chemosensory

and trunk-tip to behaviors was directed towards calves, as opposed to males who showed no such change with age (Table 1.12, A:I, 7) (Fig. 1.18). The female proportion of chemosensory and tactile behaviors directed to calves was positively associated with the rate of nurture behaviors from females (Spearman's Rho Correlation: r_s=0.33, N=81, P=0.003). Conversely, males increasingly interacted with juvenile (Fig. 1.19) and pubescent (Fig. 1.20) males. Older males contacted and directed more chemosensory behaviors towards other pubescent males than did females (Table 1.12, A:I-II, 4).

Discussion

Social interactions and exploratory behaviors of immature African elephants functioned in the development of behaviors common to adult elephants. This supports the prediction that behavioral sexual dimorphism occurs early in the development of elephants, well before reproductive activity. Males nursed ten months longer than females, providing further evidence for the delayed maturation of male elephants as previously reported (Poole 1994). In females, nurturing behaviors (see Table 1.3) that may improve maternal skills for early reproduction showed an increase with age even though the female elephants studied were all pre-reproductive. The pattern of chemosensory behaviors was reflective of sexes that use different means to locate mates. Young male elephants showed greater interest to urine and feces than females, similar to the pattern shown by adult elephants (Loizi 2004; Vyas 2006). Behaviors that aid in detecting chemical signals, such as checks and flehmens, were developed across age in males from the current study. Females performed behaviors that are likely to facilitate social bonding within the kinship group, such as greater interest in direct contact to the

female genitalia of conspecifics. Females also directed investigatory trunk tip contacts and social interactions towards younger calves. Young males engaged in elevated agonistic social interactions with same age and sex individuals, reflective of adult intrasexual competition. The delayed maturation of males, early reproduction in females, and the dimorphic social setting and reproductive strategy of African elephants may shape the sexually dimorphic behaviors and developmental patterns evident in this study.

For animals that reside in sexually segregated groups, like elephants, sufficient social functioning requires an extensive and intricate repertoire of social behaviors. Adult social behavior can be characterized as the ability to respond with appropriate behaviors within the correct context at the right moment (Meaney and Stewart 1979). As elephants develop, adult behaviors were found to be practiced through social interactions and the investigation of the environment and conspecifics. Experience through these modes of development can be affected by many variables, including rank, family bonds, maternal experience, age and sex (Choleris & Kavaliers 1999). The current study focused on the latter two variables, age and sex. Age did have a significant affect on the pattern of specific behaviors; however, age only explained 10 - 30% of the variance in rates and durations. Difference between kinship groups may be attributing to the variability in different behaviors, which is under investigation at AENP (R. Esposito, pers. comm.).

Male and female elephants under the age of ten typically develop within the same natal social environment; however, they mature at different rates (Lee & Moss 1999). Between the ages 10-15, the social environment, reproductive abilities, and body size begin to change considerably. The finding of extended nursing emphasizes the delayed

maturation of male elephants. Males of many sexually dimorphic species, including elephants, may require a longer developmental period because of the requirements of large body size, fighting ability, and state of musth to mate successfully (Poole & Moss 1981; Clutton-Brock et al. 1982; Poole 1989). The delayed maturation also allows for refining physical, cognitive and behavioral skills that are related to reproductive demands of males in species with intense male-male competition (Geary 1999). These male skills related to dominance were practiced regularly in the frequency of aggressive play across age for the young elephants observed in the current study. Aggressive play was often initiated through several distinct behaviors among calf and juvenile elephants, similar to play bows in canids (Beckoff 1995). These included an initial aggressive act (e.g. tusk, push, trunk over-head, etc) to entice the other participant, followed (or also invited) by a raised trunk facing head to head. Sparring or trunk wrestling would then follow the invitation. In many polygamous species, play shows a bell shaped developmental pattern that peaks in the juvenile stage (Fagen 1981; Palagi et al. 2002). However, with male elephants there was a steady increase in this behavior, which suggests that the developmental period extends past the juvenile phase. Thus, aggressive play may reach a plateau in the early subadult phase and decline before reproduction.

Females have a shorter period of maturation because of early conception, sometime as young as seven years of age (Sukumar 2003), with the average age of conception at AENP being 11.2 years of age (Woodd 1999). During this study at AENP, female elephants weaned ten months earlier than did males. Females decreased the frequency of involvement of gentle play by age five, while older females (5-10 years of age) were more involved in providing nurture to calves. Providing nurture to young may

be more beneficial for maternal skill development than involvement in conspecific play; this may explain the observed inverse trend in the frequency of nurturing and gentle play behavior for females. Similar maternal skill development occurs in young female squirrel monkeys, in which females carry small infants for prolonged periods of time (review in Baldwin & Baldwin 1974). Young female elephants may be profiting through caring for the young of another if the experience increases the likelihood of successfully rearing their own offspring (Dublin 1983). A similar behavioral pattern was reported in a study on allomothering by elephants in Amboseli National Park, Kenya (Lee 1987). The caretaking relationships within families may act to enhance the stability of the group through time (Lee 1987). Thus, young females are potentially increasing their success in raising future offspring and strengthening bonds within the family or kinship group.

The highly dynamic and multi-tiered society of females, compared to the largely solitary and loosely associated lifestyle of male African elephants affects the type of social interactions during development (Moss & Poole 1983). Social play serves as a means for gaining experience to develop adult-like behaviors and skills in elephants, typical of other species with equivalent social structure (Walters 1987; Fagen 1981). The type of play or social interaction in which an animal partakes should be representative of its adult lifestyle. This was evident from the sexually dimorphic involvement of play fighting and aggressive acts observed in male elephants and by the degree of gentle play displayed by females. The age and sex combination of play partners also was representative of future adult lifestyles with males playing with same age partners while females played with calves. Studies on bighorn sheep, Siberian ibex, East African elephants and sable antelope have shown the same trends for males and females (Berger

1980; Byers 1980; Lee 1986; Thompson 1996). In such species, males appear to interact with the same age and same sex partners for the purpose of self-assessment (Thompson 1998). Males might play together to practice motor skills more effectively, physically train muscles, or enhance competency in social dealings with other males (Caro 1988). Play also has been hypothesized to prepare an individual for the unexpected, in unpredictable environments and situations (Spinka et al. 2001). Thus, the sex that experiences more unexpected occurrences in its environment, as do solitary adult male elephants, should play more frequently. This hypothesis was supported by the increased involvement of play for males, compared to females.

In the current study, the observed trend of increasing aggressive behaviors with age for females may be related to the adult hierarchy as the rank of female elephants rises with size and age (Dublin 1983; Archie et. al. 2006). Older females also partook in play fighting bouts more frequently than younger females, yet play fighting by females primarily occurred with male partners and based upon my notations, it was usually initiated by the male. Furthermore, both aggressive play and aggressive interactions were observed at comparatively lower rates than among males. It is interesting to note that both male and female focal animals played aggressively with males more than females. This trend of male-initiated play with females occurs in primates and mouflon sheep (*Ovis gmelini*); it has been attributed to males approaching others more often and initiating interactions (Lee 1986; Guilhem et al. 2006). In some cases, females may be more available as play partners as well. Merte (2006) reported aggressive acts to be greater in the female calf and juvenile phase compared to the subadult and adult phase of the AENP elephant population. Elephants may be developing a sense of place in the

social order before reaching the age of reproduction. Lee (1986) also found young females (from newborns to five year olds) to increase rates of aggression across time and attributed it to competition within the family group.

Male elephants navigate through their environment in a semi-solitary manner, while females reside in highly social groups. In preparation for this lifestyle, males and females should differ in develop chemosensory behaviors required for these dimorphic lifestyles. Proficiency in detecting chemical signals deposited by conspecifics should be favored in males to aid in navigation and eventually search for estrous females as adults (Schulte 2006). As predicted, males decreased the proportion of a more general chemosensory behavior, the sniff, while increasing the proportion of checks and flehmens across age and displaying places at a higher rate than females. This developmental trend was not evident in females. Flehmen is considered a more multifaceted behavior compared to a sniff, because the trunk must first be developed to bring a non-volatile chemical, possibly an estrous pheromone, to the vomeronasal organ for detection (Schulte et al. 2005). In many mammalian species, the flehmen is linked to male assessment of conspecific estrous state (Estes 1972; Hart 1983). The function of a chemosensory behavior may be affecting the dimorphic development between male and female elephants.

Females attract mates and interact directly with herd members (Vidya & Sukumar 2005), suggesting that direct contact with conspecifics would be more emphasized during female development then during male development. Both males and females decreased the rate of conspecific exploration through general trunk-tip touches with age; however, females increased their rate of touches to the genitalia area with age. For males and

females, the majority of genital contacts by the trunk tip were to females. Females may be assessing different chemical signals than males and using the trunk-tip genital contact for more than chemical signal reception. For example, the touching may strengthen social bonds by sending a signal such as appeasement or comfort as observed in bonobos, *Pan paniscus* (Palagi 2006). Chemical signal reception may permit the assessment of reproductive condition of females, which almost certainly occurs by male African elephants (Poole 1989; Poole & Moss 1989; Bagley et al. 2006). However, it is unknown whether female African elephants distinguish between different states of the estrous cycle through chemosensory input (see chapter two).

The reproductive strategies of African elephants are dependent on critically timed signaling and access to mates (Poole & Moss 1989; Poole 1999). Females are typically in estrus for only 2-10 days, once every 4-5 years (Moss 1996). Therefore, females invest in attracting mates, through auditory, visual and chemical signals, while males search for estrous females (Vidya & Sukumar 2005). The development of chemosensory behavior observed in this study could aid in detecting chemical signals over great distances. Despite the relatively small proportion of behaviors directed toward excretions, males directed more of their total behaviors towards urine and feces than did females. Because access to receptive females usually requires intense male-male competition (Poole 1989), the type and duration of play seen during the developmental phase in males was relevant to their future adult role. Young male elephants not only displayed behaviors that may assist them in finding reproductive females as adults, but they also showed play behaviors that may facilitate successful competition that is necessary within a polygamous mating system.

A polygamous mating system enhances the degree of sexual selection (Lande 1980), thus leading to increased dimorphism affecting the intensity of intrasexual competition (Alexander et al. 1979). If the mating system of a species were the cause of early developmental differences, then contradicting predictions would be made for the development of adult behaviors for monogamous versus polygamous species. Monogamous, monomorphic species with little dimorphism in adult roles and few sexual differences in dispersal should, and often do, develop behaviorally alike as young males and females (Bekoff 1974). Polygamous species would be expected to exhibit sexually dimorphic development patterns, as supported by the current study on young African elephants in Addo Elephant National Park, South Africa. Sex differences and developmental trends in social behavior, relationships and exploratory chemosensory and tactile behaviors are evident because of, and emphasize the differences in, the duration of the maturation period, social organization, and reproductive strategies of adult male and female elephants. This developmental process culminates in the emergence of adult behavioral repertoire that may ultimately influence long-term reproductive success.

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CHAPTER II

INTRASEXUAL CHEMICAL COMMUNICATION AND SOCIAL RESPONSES OF CAPTIVE FEMALE AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*)

Abstract

Female African elephants form tightly knit matriarchal groups composed of related females and their offspring. Intrasexual communication skills are important for social interactions that facilitate group cohesion. Contacts from conspecifics may serve to assess reproductive condition and reduce aggression within the group. Such a function would suppose that female African elephants can distinguish between the estrous (follicular) and non-estrous (luteal) phase, and possibly even between the anovulatory Lutenizing Hormone surge (LH1) and the second ovulatory LH surge (LH2). This study focused on the ability of captive female African elephants to discern between the follicular and luteal phase of known conspecifics through trunk-tip contacts and among urine from the LH1, LH2, and luteal phases of unknown conspecifics. Furthermore, I examined whether the estrous phase of the receiver affected responses to the urine samples. Behavioral observations and urine bioassays were conducted on 21 reproductively viable females at nine zoological facilities in North America. Estrous females received contacts to orifices that may be sources of chemical signals (genitals, anus, temporal glands) at a higher rate than non-estrous females and contacts increased with approaching ovulation. All elephants responded to the urine more than to the control, however, the rate of chemosensory responses to each sample did not differ, and the rates were not affected by the estrous phase of the receiver. Genital contacts among African elephants may be used as a multifunctional chemosensory and tactile behavior to assess reproductive condition and to facilitate group cooperation.

INDEX WORDS: Captive, Chemosensory, Genital Contact, Intrasexual Female Communication, *Loxodonta africana*

Introduction

Chemical signals are emitted through secretions and excretions of mammals (Brown 1979) that can be placed strategically on boundary lines of existing territories, deposited as trails, or positioned as announcements of status (Gosling & Roberts 2001). The body parts that emit chemical signals also are investigated directly by conspecifics. Direct contacts (e.g. touch) and indirect investigations (e.g. sniff) of the markings or source are performed to receive the chemical signal (Dethier 1987). Chemical signals elicit different response behaviors depending on characteristics of the sender and the receiver (Bigiani et al. 2005). When a chemical signal educes a specific behavioral or developmental response in a conspecific, it is termed a pheromone (Karlson & Lüscher 1959; Hildebrand 1995; Bigiani et al. 2005). Pheromones of mammals are directly related to hormone levels (Hasson 1994), and therefore provide an honest signal transmitting reliable information such as body condition or reproductive status of an individual (Zahavi 1975; Guilford 1995). Pheromones can be grouped as sex pheromones or social pheromones. Sex pheromones often are intersexual signals to assist in finding mates (common in many polygynous species) and to facilitate mate choice (bank mole, *Clethrionomys glareolus,* Kruczek 1997), while social pheromones are both inter- and intrasexual signals that function in group aggregations, social organization (marmoset, *Callithrix jacchus,* Barrett et al. 1990) and establishing territories (oribi antelope, *Ourebia ourebi*, Brashares & Arcese 1999). In effect, many social behaviors and reproductive strategies are mediated through chemical signals (Bigiani et al. 2005).

Species with a polygynous reproductive strategy often are sexually segregated (Greenwood 1980; Ruckstuhl & Clutton-Brock 2005), and may use chemical signals to

find or choose mates and maintain social groups (Kelly 1981). Typically, polygynous males invest little in offspring and experience natal dispersal, while females invest extensively in offspring and remain within the natal group (Packer 1975; Greenwood 1980; Silk 2002). Males search for estrous females by inspecting chemical signals, while females choose a mate according to mate condition or status also revealed through chemical signals. For example, mohor gazelle (*Gazella dama mhor)* and macaque (*Macaque macaque*) males mate with the most fertile females, detected through olfactory investigation of urine (Pickard et al. 2003; Engelhardt et al. 2004), while female elephants appear to prefer males that are in the high testosterone state of musth as revealed through chemical signals in the urine, breath and temporal gland (Poole 1989; Schulte & Rasmussen 1999; Rasmussen & Krishnamurthy 2000, 2001). The social organization of female groups is often more dynamic and complex than that of the searching or territorial males (wild house mice, *Mus domesticus,* Hurst 1990b; African elephant, *Loxodonta africana*, Poole 1994; wild goat, *Capra aegagrus*, Cote et al. 2001). This could be attributed to the more gregarious nature of females as is known of most diurnal primates (see review Sterck et al. 1997); hence, females depend more on intragroup communication than males.

Female survivorship is reliant on group cooperation in resource acquisition, defense, care of offspring and the mediation of aggression (Silk et al. 2003). Efficient communication through chemical signaling is evident amongst females and complements social behaviors supportive of group organization (McClintock 1981). Defense of resources and border maintenance can be upheld through scent marking that provides intragroup information as seen in a female-philopatric species such as the ring-tailed

lemur (*Lemur catta*) (Mertl-Millhollen 2006). Social factors and olfactory cues are important in the control of several aspects of female reproductive physiology. One such aspect is the regulation of the timing of reproduction so that mortality of the offspring is minimized, while maintaining maximal reproductive output (McClintock 1984; Post et al. 2001). Estrous regulation is present in plains bison (*Bison bison*), where reproductive synchrony is achieved through the use of olfactory cues by unmated females before estrus (Berger 1992). The converse of estrous regulation also occurs, in which reproductive abilities are suppressed within a social group by breeding or dominant cohorts (Epple & Katz 1984). Asynchronous estrus within a social group also arises and functions to maximize the efficiency of mate choice in female ring-tailed lemurs (*Lemur catta*) in order to avoid competition, as suggested by Pereira & Weiss (1991).

Female competition does occur within groups for access to resources (Silk 1993). Mates are often a resource, causing the greatest competition between females in strict matrilineal hierarchies (Kevles 1986; Dublin 1983). To avoid conflict between females, counter-marking scents of lower ranking females is common, such as in mice (Hurst 1990a) or ring-tailed lemurs (Kappeler 1998; Palagi et al. 2004). Females may monitor hormonal changes of conspecifics that could predict targeted aggression or for the purpose of synchronizing estrous cycles (McClintock 1978; Kappeler 1998). These signals are received by sniffing the chemical deposit or direct contact to the source. For instance, ring-tailed lemurs (*Lemur catta*) sniff scent markings while bonobos (*Pan paniscus*) directly contact the genitalia to investigate both condition of the female and to sustain social relationships (Kappeler 1998; Hohmann & Fruth 2000). How the reproductive state of the sender and receiver affects the response to the chemical signal(s)

or to an individual, may help to understand better how group dynamics are mediated by chemical communication (McClintock 1981).

Highly dynamic social groups, such as elephants, provide a useful model for examining the interaction of the effects of hormones on female intrasexual chemical communication and associated behaviors. Female African elephants form tightly knit matriarchal groups that are composed of related females and their offspring (Douglas-Hamilton 1972; Dublin 1983; Moss & Poole 1983; Archie et al. 2006b). There is a linear dominance rank relationship within the families, such that older, larger females consistently dominate smaller females (Archie et al. 2006a). Family groups are led by a matriarch, typically the largest and oldest female, who directs the family to vital resources and coordinates group defense (Dublin 1983; McComb et al. 2001). Although a linear rank is present within groups, competition between female elephants in the wild and in captivity still occurs when resources or breeding opportunities are limited (Schulte et al. 2000; Slade et al. 2003; Archie et al. 2006a). During this time of limited resources, dominant female elephants may use aggression (Dublin 1983; Slade 1999) or chemical signals (suggested for African elephants, Sikes 1971) to suppress reproduction in subordinates. Under favorable conditions, females within a related group would benefit from a group member successfully reproducing (Archie et al. 2006b), suggesting that an estrous female may have preferential access to resources. However, the ability or function of discerning estrous state is unknown amongst female African elephants.

Female intrasexual communication, through auditory, tactile or chemical, is important for maintaining social cohesion within this complex and fluid society (Langbauer 2000). Captive female African elephants were found to vocalize more often

during their anovulatory follicular phase (Leong et al 2003), suggesting possible relevance to other females rather than to males (Leong et al 2005). Frequent tactile behaviors to the urogenitals among elephants suggest this area to be an important signal source for communication between females (Rasmussen and Schulte 1998; pers. obs.). In a study on four captive Asian elephants (*Elephas maximus*), such contacts may have served to assess reproductive condition and prevent or diffuse aggression within the group (Slade 1999). Also in this study, the degree of aggressive or submissive interactions depended on the reproductive condition of the sender or receiver (Slade 1999). Furthermore, Slade et al. (2003) found that the ability to distinguish between follicular and luteal phase urine samples was greater when females were in their estrous phase. Thus, in Asian elephants, the reproductive state of a female appears to affect social dynamics. In female African elephants, dominance rank changes with reproductive state (Dublin 1983); however, the interaction of estrous state and associated behaviors in chemical communication has yet to be examined.

The estrous cycle of both Asian and African elephants is 14-16 weeks in duration, with a 4-6 week follicular phase and an 8-12 week luteal phase (Poltka et al. 1988; Brown 2000). During the follicular phase, two lutenizing hormone (LH) peaks occur, a phenomenon that appears unique among mammals (Hodges et al. 1997; Brown 2000). The first peak is anovulatory (LH1), and the second ovulatory peak (LH2) occurs 21 days later. The exact function of the first LH peak is not known, however, it has been speculated that nonovulatory follicles form accessory corpora luteal that produce progestins needed for ovulation (Brown 2006). In the follicular urine of female Asian elephants, a pheromone identified as (*Z*)-7-dodecen-1-yl acetate signals approaching

ovulation to conspecific males (Rasmussen et al. 1997), but females do not show interest in the pheromone (Rasmussen 1999). Although a chemical has yet to be isolated in the urine of African elephants, bioassay results from Bagley et al. (2006) support the hypothesis that an estrous pheromone is released by female African elephants (Goodwin et al. 2006).

The current study examined the ability of captive female African elephants to discern between the follicular and luteal phase of conspecifics through trunk tip contacts to familiar group members and to the urine from unfamiliar females. The influence of the reproductive phase of the receiver on the investigative behaviors to the urine samples was examined. Estrous females were predicted to receive more trunk tip touches to signal-emitting urogenital regions and temporal glands than non-estrous females. If supported, this would suggest that female African elephants have the ability to distinguish between estrous phases. Urine from both the LH1 peak and LH2 peak were used for the follicular phase, along with luteal urine. Although an estrous pheromone is likely to be present, the chemical compound has not been identified (Bagley et al. 2006; Goodwin et al. 2006). Thus, the exact timing of the release during the follicular phase is not known. In mice, the odor of urine from two different phases during the estrous phase (follicular and ovulatory odor) had opposing effects on the receiver, either shortening or lengthening the cycle of the female receiver (McClintock 1984). Previous studies had missed the effects of two confounding estrous chemosignals because the two urine types were combined for bioassays (Champlin 1971 as cited in McClintock 1984). This supports the testing of urine from the two LH points during the follicular phase. The reproductive state of females also was expected to affect interest in the urine. Estrous females were predicted

to be most interested in the follicular urine, specifically LH1, because the next three weeks before ovulation may be critical for resource acquisition in preparation for mating.

Methods

Study Sites and Population

Observational data were collected from 25 captive African female elephants at nine zoological facilities in North America from March – July 2006 (Table 2.1). Twenty-one of the females were showing regular estrous cycles. At the time I visited the particular facilities, 10 females were in estrus, 11 females were in their non-estrous phase, and three were considered flatliners (non-cyclers). One female, at the Montgomery Zoo, was impregnated prior to my arrival. This was not known until several months after the visit. The female was categorized as non-estrus because at the time of the observations she was in the early stages of the luteal phase when progestins are similarly elevated. The ages of the elephants ranged from 21 to 37 years (mean = 26 ± 4.7 years). All zoological facilities had an outdoor enclosure where elephants were exhibited for 6 – 9 hours throughout the day. During the study, the elephants were never moved off exhibit for weather purposes. Four facilities were holding a bull elephant; however, only two facilities (North Carolina and Montgomery Zoo) allowed the adult male to have free access to the females while on exhibit. The bull did not interfere with the bioassays and contacts from the bull to a female were not included in data analysis.

Reproductive Status

There are 230 female African elephants in the United States; however, only 38 of these females are known via hormonal monitoring to be cycling and paired with one or

more other cycling female (pers comm. with Dr. Janine Brown, Smithsonian Institute's Conservation Research Center). I chose my study sites for these two main criteria. The visits were timed such that at least one female elephant was in the follicular phase based on hormonal data over the preceding year. Serum progesterone (pg/ml) levels from weekly blood samples obtained from each individual were analyzed by Dr. Brown or by Dr. Schmitt at Missouri State University. Projected estrous cycles were calculated from the hormone profiles, and zoo visits arranged accordingly. During periods of data collection, the exact state of estrus of the female elephants was unknown, whenever possible, to avoid observer bias (Martin & Bateson 2001).

After the study was complete, updated hormone profiles were used to determine the exact state of estrus during the time I conducted the observations at each zoological facility. There were three facilities (North Carolina, Knoxville, and Lee Richardson Zoo) that did not have the blood samples analyzed for reproductive status. At two of these locations, the behavior of the bull elephant revealed which female was in estrus. For the third facility (Lee Richardson Zoo), three to four complete hormone cycles of the females in the previous year were very consistent, providing strong support for the categorization of one female in the estrous phase and the other female in the non-estrous phase during the bioassays.

Urine Collection and Storage

Urine was collected from eight captive African female elephants that were demonstrating normal estrous cycles (Table 2.2). The cycles of each female was predetermined by hormone analysis of weekly serum progesterone levels that were taken by staff and analyzed by the Smithsonian Institute Conservation Research Center. It was

determined from the progesterone and lutenizing hormone (LH) levels when each female was in the luteal or follicular phase (specifically the nonovulatory LH 1 peak and ovulatory LH 2 peak). The elephant staff collected urine at peak progesterone levels (mid-luteal phase) and during the follicular phase (specifically around the first and second LH peaks). Urine was collected directly into stainless steel containers and then stored in 250 ml jars and placed in a freezer at –80 C within 30 minutes of collection (Bagley 2004).

Samples

Four samples were used for each bioassay: follicular urine (LH 1, LH 2), luteal urine, and a vanilla extract/water control. Minuscule amounts of vanillin can be found in Asian elephant urine, and both species perform low but regular rates of chemosensory behaviors to this control (Schulte & Rasmussen 1999; Bagley et al. 2006). To reduce potential confusion for the elephants, each urine sample was from a different female, given that two states of estrus from a single female would not occur simultaneously in a natural setting. Therefore, a combination of the eight females at different stages of their reproductive cycle was formulated for each of the nine facilities. The combination of urine samples was unique to each facility. To the best of our knowledge, females observed had no previous contact with the females of the urine samples. Thus, urine presented to the females was from three novel females. This would insure females not to be responding to the individual but actually the chemical cues within the urine. The nine urine samples (three for each bioassay) were shipped to appropriate bioassay sites on dry ice as needed. Once received by the facility or myself, the samples were placed directly in an –80 C freezer or kept on dry ice.

Behavior Observations

Behavior observations took place when the elephants were first released on exhibit in the morning for two hours and in the afternoon for two hours before the elephants were brought back into the barn. If possible, elephants were observed at the same time for five consecutive days. Routine training in the morning sometimes delayed the release of the elephants on exhibit. Behavior data were recorded from a public viewing area or an area where elephants were habituated to the presence of humans.

Two types of data were collected to aid in understanding the social dynamics and daily time budget of the female elephants at each facility. An all occurrence of selected social behaviors (Table 2.3) and one-minute scans of selected state behaviors (Table 2.4) were recorded for each observation session (Altmann 1974). During the morning sessions of bioassays, social interactions were recorded only for the second hour of observation. I could not reliably record social interactions in the first hour because I was filming the high level of responses to the samples only, not the entire yard. The identification of the sender and receiver was recorded with every social interaction. For state behaviors, a stopwatch was set to go off every minute and on that minute, the state was recorded. A total of 120 scans were taken for each observation session (one for every minute of a two-hour session) unless an elephants was brought off exhibit for a blood draw or routine training. Time not visible was subtracted from total focal length for each session. The final behavior observation session was missed at the Lee Richardson Zoo because of a scheduling mishap. For the 25 females observed, the average focal length was 118 ± 0.29 min for a total of 490.5 hours of focal data (*ca.* 10 two-hour sessions for each individual).

Bioassays

Morning bioassay sessions were performed across three days at each facility. The three days were preceded by a day of baseline behavior observation and followed by the same to test for an effect of samples on behaviors. The three urine samples were thawed overnight and the control sample was mixed on the morning of a bioassay. To prevent observer bias, the samples were numbered and placed in the enclosure by an assistant from the facility. Urine samples and the control were numbered 1 – 4 and recorded in a notebook by the assistant. Before the elephants went into the exhibit, I marked where the samples were to be placed (3 meters apart) and the assistant poured each sample into the appropriate location. The sample area was chosen before the bioassays. I selected an area with sufficient substrate (not mud or rock) and where the females would pass but not regularly congregate. A video camera was positioned to see all of the samples at once. Time was started when all the elephants were released into the exhibit and visible. I verbally stated every behavior directed to the sample (Table 2.5), identifying elephant and minute of focal to the video camera. I also noted when an animal was in proximity (one body length) or near (one trunk length) to the sample. All samples were in the same designated sample area of the yard for each day of the study, however, samples did not overlap samples from previous days. Samples were washed away with water if available or dug up and removed from the yard each night, filling in the holes with nearby substrate from the exhibit. The tapes were reviewed and the data transcribed onto paper and entered into a computer. Because elephants could be at different samples simultaneously, video was the most accurate means of recording data and was used for the entire two hours.

Analysis

The measures of behavior were compared between estrous and non-estrous females to test each hypothesis. Social interactions were separated into sender and receiver categories and the frequency of each behavior was divided by the total duration of focal, excluding the time not visible, to obtain the rate of behavior per minute. Rates were then converted into frequency of behavior per hour. State behaviors were calculated as a proportion, dividing the number of scans in a state by the total number of scans for which an individual was visible. The rates of social interactions and proportion state behaviors were averaged across the five days and between morning and afternoon sessions for each female. Each female then had one value for rate of social behaviors and proportion of scans spent in a particular state. The proportion of the estrous cycle a female was in at the time of observation was calculated from the 13 hormone profiles received post observations. For each individual, length in weeks of the reproductive cycle was determined. The week of behavior observations was pinpointed to the exact week of the estrous cycle. The week of observation was divided by the total duration (in weeks) of the females' complete cycle. This gave a proportion of where each female was in her reproductive cycle during behavior observations (0.0 being the beginning of the non-estrous phase and 1.0 representing ovulation (LH2)) (see Schulte & Rasmussen 1999 for comparable methods). Social behaviors were compared across the estrous cycle. The social interactions and proportion state behaviors also were compared across the morning sessions of the five-day study. This comparison was done to determine whether the samples had an effect on the social and activity level (see Appendix C for further detail).

Chemosensory behaviors were measured as frequency to each of the four samples divided by the total duration of focal length. Thus, there was a rate of each behavior to each sample for each bioassay (three per female). Chemosensory behaviors were analyzed as main chemosensory behaviors (sniff, check, place, flehmen) grouped together, just check and place together (comparable to Slade et al. 2003), and accessory trunk behaviors (see Table 2.5).

Data for social interactions and state behaviors were examined using the JMP 4.0 statistical program for a PC and the chemosensory response to the urine samples were examined using Statistica software (StatSoft 1999). All behaviors were tested for normality using the Goodness-of-Fit test and for equal variance using Levene's test. If assumptions of parametric analysis were not met, comparable non-parametric techniques were used. All tests were two-tailed with $\alpha = 0.05$.

The interaction between estrous phase and sent and received contacts was analyzed using a two-way ANOVA, but a Tukey HSD post-hoc test failed because of lack of power as a result of the experimental design. The unbalance in the design precluded determining the interaction between sender and receive estrous state for the trunk tip contacts. There were four females in their estrous phase exhibited with a second female in her estrous phase, while nine females in estrus had a luteal female in her group. All ten females in their non-estrous phase were observed with a female in estrus, but three of these females were at the same facility so they had a non-estrous female with them as well. Thus, most of the females who could contact an estrous female were in their non-estrous phase, while most of the females who could contact a non-estrous

female were in their estrous phase. For analyses, I ignored estrous state of the female that was performing the contact by using a one-way ANOVA.

A linear regression analysis was used to determine the relationship between approaching ovulation and social interactions. Chemosensory response behaviors to the four samples (control, LH1, LH2 and Luteal) were compared between estrous and non-estrous females across the three-day trials by a Repeated Measures Analysis of Variance and Tukey HSD.

Results

Behavior Observations

The reproductive state of a female affected the receiving of trunk-tip contact behaviors. Females in their estrous phase received approximately twice as many direct contacts to the genital area (1.3 ± 0.2/h) compared to non-estrous females (genital: 0.7 ± 0.2/h; ANOVA: $F_{1,19}$=6.66, P=0.02) (Fig. 2.1). Genital contacts accounted for the most frequent social interaction (34%, 366/1084), followed by trunk to mouth (30%, 326/1084). There was no difference in the rate of receiving trunk-tip to mouth behaviors (Table 2.6). Combining behaviors to chemical emitting orifices (anus, genitals, mouth, temporal glands) showed estrous female to receive more contacts per hour (2.7 ± 0.4/h) than non-estrous females (1.6 ± 0.3/h; ANOVA: $F_{1,19}$=4.68, P=0.04). When trunk to mouth was removed (because this behavior could be used for inspecting food not signals related to estrous phase), there was a greater difference in trunk tip contacts to estrous (1.6 ± 0.2/h) and non-estrous (0.9 ± 0.2/h) females (ANOVA: $F_{1,19}$=6.89, P=0.02) (Fig. 2.2).

The rate of received genital contacts increased with approaching ovulation (Regression: R^2=0.40, t=2.68, P=0.02) (Fig. 2.3). The trend was similar for trunk-tip to orifices with contacts to the mouth omitted (Regression: R^2=0.39, t=2.66, P=0.02) and for overall social interactions that a female received related to her approaching ovulation (Regression: R^2=0.32, t=2.26, P=0.045) (Fig.2.4). Comparing the reproductive state of individuals, estrous females notably received more total trunk tip contacts (see Table 2.3 for descriptions) than non-estrous females, but the difference was not significant (P = 0.06, Estrous: 3.1 ± 0.4/h; Non-estrous: 1.2 ± 0.4/h). Levels of aggression were low for both estrous (sent: 0.7 ± 0.3/h; received: 0.4 ± 0.1/h) and non-estrous (sent: 0.5 ± 0.2/h; received: 0.5 ± 0.1/h) females, showing no difference in sending (Mann-Whitney: U=-0.49, P=0.62) or receiving (Mann-Whitney: U= 0.56, P=0.57). The activity level of estrous and non-estrous females was measured, however because it was not a specific hypothesis, it was included in Appendix C.

Bioassays

All elephants responded to each of the three urine samples at a higher rate than to the control (Sample effect: P=<0.001; Tukey-HSD, P<0.05) (Fig. 2.5) (See Table 2.7 for all repeated measure ANOVA results). The response to samples significantly decreased across the three days of bioassays (Sample*Day effect: P<0.001, Tukey-HSD, P<0.05) (Fig. 2.6: all females; Fig. 2.7: estrous and non-estrous females separate). Reproductive state of female elephants did not influence the response to urine samples across the three bioassays in any chemosensory categories (Fig. 2.7)). Compared to estrous females, non-estrous females tended to respond more to LH2 urine, but the difference was not statistically significant (Estrous: 0.3 ± 0.1/min; Non-estrous: 0.5 ± 0.1/min; ANOVA:

$F_{1,19} = 3.82$, $P=0.07$). Overall, flehmen behavior accounted for only 0.24% (15/6223) of the chemosensory responses. Females in their estrous phase displayed four times as many flehmen responses (13 versus 3); six different estrous females and two different non-estrous females performed flehmens. There was no pattern to what sample elephants performed a flehmen.

Discussion

In the present study on captive female African elephants, the effect of the reproductive state on social contacts and responses to unfamiliar follicular and luteal urine was investigated. The ability to discern between the follicular and luteal phase of conspecifics was evident through contacts to the genital, anal and temporal gland orifices of familiar females. However, these females did not distinguish via chemosensory behaviors between Luteal, LH1 and LH2 urine from unfamiliar females. Estrous females received the most investigatory contacts to the genital region when compared to non-estrous females, and the contacts increased with approaching ovulation. Both estrous and non-estrous females investigated the genitalia of others; however, the experimental design did not permit an equal balance of females in the two estrous phases to be together. That is, females in the estrous phase were primarily observed with females in the luteal phase and vice versa, although same phase groupings did occur in four cases for estrous-estrous and three cases for luteal-luteal. If only cross-phase pairings were observed, then greater rates of contact to estrous females also could be viewed as greater rates of touching by luteal females. However, because contact rates by estrous and luteal females did not differ (Figs. 2.1 and 2.2), the greater rate of contact to estrous females

supports the prediction that estrous females will be investigated through trunk tip touching more than luteal females. This pattern was not evident when considering aggressive interactions. Levels of aggression were very low compared to all other social behaviors and the reproductive phase of females did not affect aggressive interactions. If evaluation of estrous state is related to competition, then such competition is not immediately detectable through aggressive interactions. This is not unusual, for even in the wild rates of aggression between female African elephants are low (0.14 ± 0.02/h – 0.05 ± 0.01/h depending on relationship within a family unit: Archie et al. 2006a).

Female African elephants were hypothesized to respond more frequently to urine from the first lutenizing hormone peak (LH1), however, this study did not support that prediction. Instead, the general trend of response across the three days of bioassays implies non-estrous females to be more interested in the LH2 phase of estrous urine compared to estrous females. However, there was a lack of significance ($P = 0.07$) in the response to LH2 urine. These results contradict what was found in Asian elephants, where estrous females responded more frequently to follicular urine than did non-estrous females (Slade et al. 2003). In the Asian study, the variability of responses was much higher to unfamiliar than familiar urine (Fig. 1, Slade et al. 2003), so perhaps a larger sample size or the use of familiar urine would lead to significant results. In addition, African and Asian elephants have multiple differences, ranging from morphology (Sukumar 2003) to physiology (Meyer et al. 2004), and the evolution of intrasexual communication also may be species specific. In the study by Slade et al. (2003), estrous female Asian elephants showed the greatest rates of response to LH2 urine; whereas, in the current study, non-estrous female African elephants showed a trend to respond the

most, but also to LH2 urine. Thus, pre-ovulatory urine appears to be of greatest interest to elephants, but further work is needed to clarify the importance of receiver estrous status on the biological significance of this interest.

In the wild, female African elephants reside in groups comprising mainly of related individuals and encounter unknown or unrelated (foreign) elephants that may elicit defensive response in order to protect resources. Although this study focused on captive females, they too reside as a group with limited resources (allotted food, not *ad libitum*); thus, urine from an unknown female may be considered a threat. Because females responded to the control significantly less than to the samples, it can be concluded that females are not responding at increased rates solely because of sample novelty. When females did discover the foreign urine in their exhibit during the first trial, they were observed trumpeting to group mates and at times bunching together in a defensive mode, or displaying aggression. This is emphasized by the greatest response to the urine on the first day of bioassays in which investigation decreased thereafter. By the third trial, the response to urine decreased almost to control level. This decrease in response could be akin to what has been shown for some territorial animals that may mark territories following the predictions of the scent match hypothesis. This hypothesis predicts that the intensity of the response to the scent mark will decrease if the sender of the scent mark is not present (Sun & Muller-Schwarze 1998). This idea was substantiated in reports of a decreased response of beavers to odors of unknown beavers across multiple days when scents were not reinforced by the presents of the signaler (Sun & Muller-Schwarze 1998). Female elephants also respond similarly to unknown urine, with the decrease in response across trial, possibly due to unenforced signals. Therefore,

females may be investigating the urine of a foreign female initially for competitive purposes instead of seeking information on estrous state. However, the heightened interest in the urine samples relative to the control and the increase in the rate of contact to the vaginal area of estrous females suggest a mechanism for the detection of a reproductive chemical signal in these elephants.

Vaginal secretions may emit a stronger signal of reproductive status to females than the urine of unknown females used for this study. Bagley et al. (2006) suggest the chemical signal revealing reproductive status in female African elephants showed greater interest in the genitalia of other females from age 1 to 127 months (~1-10 y), possibly developing the behavior of genital inspection for use as adults. Across the same ages, females showed lower likelihood of investigating urine and feces than males. These findings are similar to social interactions between captive female Asian elephants, in which genitals were contacted at the highest frequency to anywhere else on the body (Slade 1999). Such interest in genital secretions has been observed in other species. The vaginal discharge of female hamsters transmits a pheromone signal coinciding with estrus and elicits investigatory and copulatory behaviors in males (Macrides et al. 1984). In addition, sexually experienced male mice responded more to vaginal smears as opposed to urine from estrous females (Hayashi & Kimura 1974). In elephants, urine and feces may be a more conducive signal for searching males to find mates over great distances than for females to monitor the status of group members. Conversely, a chemical signal in vaginal secretions (also suggested by Slade 1999) may be more pertinent to stimulate a possible multifunctional tactile behavior for female intrasexual communication, relevant to social structure.

Female social structure varies systematically across species and taxa. Social organization is driven by resource competition in the sense that the social structure is dependent on the strength or weakness of within-group contest competition and between group contest competition for a resource (Sterck et al. 1997). Depending on the level of resource competition, either strong female dominance matrilineal hierarchy (high levels of competition) versus egalitarian dominance (low levels of competition) relationships will emerge (Silk 2002). Elephants reside in a matrilineal society in which resources can be scarce and intragroup competition consequently occurs (Dublin 1983; Archie et al. 2006a). Younger, subordinate females are less likely to compete successfully with the dominant females when resources, such as food, water, and mates are limited (Dublin 1983). The decreased access to resources and possible increased aggression may in turn lower reproductive success of subordinate females (Sikes 1971). Heightened levels of aggression in relation to the state of estrus have been observed in wild African elephants (Dublin 1983) and in captive Asian elephants (Slade 1999). Therefore, female elephants might benefit from the detection of an estrous pheromone to avoid intragroup conflict. Although this study found no difference in levels of aggression between estrous and non-estrous females, there was interest in the signal source of a possible estrous pheromone.

Competition may be avoided within the group through genital contacts for appeasement purposes as seen in bonobos, *Pan paniscus* (Palagi 2006) or as a mechanism to detect estrus for prevention of aggression. Savannah baboons, *Papio cynocephalus*, have a similar social structure to African elephants in that females reside within the natal group for life, while males disperse (cited within Silk et al. 2003). Female sociality within these natal groups, through contacts and group integration, were found to enhance

fitness of female baboons (Silk et al. 2003). The genital contacts among female elephants also may serve to maintain social bonds within the group. Again, a multifunctional tactile behavior may exist to not only detect estrus through vaginal secretions, but to ultimately increase group cooperation.

Recently, Archie et al. (2006b) found kinship groups of African elephants in Amboseli National Park in Kenya to be genetically related, thus, females within the group would benefit from mutualistic relationships that promote cooperative behaviors in acquiring resources. Therefore, if a female within the group comes into estrus, the group may facilitate the female in acquiring access to resources (i.e. food and mates) in order to indirectly increase individual fitness. The estrous female would be changing the group dynamics by disrupting the hierarchy. This occurs in adult male elephants, in which the largest male typically is dominant, however, smaller males can jump the hierarchy when in musth (Hall-Martin 1987; Poole 1989; Poole & Moss 1989). Perhaps both sexes possess the means of avoiding harmful aggressive interactions by assessing the reproductive status of same-sex conspecifics.

Females within this study were found to increase contacts to the genital area of the female or females in their social group as ovulation approached. This same relationship, between ovulation and genital contacts, was found occurring among *Lemur catta* females (Palagi et al. 2004). Palagi et al. (2003) also found that dominant females sniffed the genitals of others more frequently and received less genital sniffs than subordinate females. The authors suggest that this increase in genital sniffs facilitates the detection of an estrous pheromone, for asynchrony purposes, in order to be the first to ovulate within the group (Palagi et al. 2004). In the current study on female African

elephants, dominance was not measured or considered because of the relatively small sample size. However, I would predict dominant females to be the most interested in the reproductive condition of subordinates, because the dominant female rank would be susceptible to change. Hence, both rank and reproductive condition, including pregnancy, should be considered in further studies on an elephant group dynamics.

The reproductive state of female African elephants does not appear to have an effect on the response to LH1, LH2 and Luteal urine from unfamiliar females. The reproductive state of known females may be of greater interest and importance within a social group. In this study, the increased genital contacts to estrous females were the most supportive evidence for intrasexual communication through chemical signals. These findings were similar to the developmental trends for females from the study on the development of wild African elephants (Chapter 1). Young females showed little interest in urine and feces compared to males and increased genital contacts as they aged. Thus, assessing the reproductive phase between a sender and a receiver through direct contact or possibly urine may be of importance in the communication among elephants. However, to better understand the mechanism of female assessment of reproductive condition through genital contacts and the function of the signal within a related group of elephants, research on a wild population for which reproductive status was known or could be measured would be beneficial.

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Table 1.1. Ethogram used to record state behaviors performed by 1-127 month old male and female African elephants during continuous focal observations at AENP from June to October, 2005.

State Behavior Categories	Definition
Chasing	One elephant is pursuing another
Defecate	Release feces
Drink	Taking water into the trunk and immediately placing water into the mouth
Dust	Using the foot or trunk to place dirt particles on the body.
Eat	Taking nutrients into the mouth via the trunk
Gentle Play	Individuals contacting in an equivalent manner, pushing, rubbing, rolling (see Table 1.2)
Mud	Using the trunk to throw mud particles on the body or moving body rapidly in a mud hole
Object Play	Using the trunk to manipulate an inanimate object or splashing the tip of the trunk into water
Sparring	Trunks down, heads together and if animal has tusks they will be interlocked and pushing against another (trunk are not intertwined)
Stand	Remains in the same location for at least two seconds
Suckle[1]	Nipple contacts separated by less than 30s of time off nipple
Trunk Wrestling	Pushing against another individual while trunks are intertwined
Urinate	Release urine
Walk	Leaves location while all four legs are moving in a steady pace
Other	Behavior not defined in ethogram
Not Visible	Elephant has moved out of sight

[1]Suckling behavior derived from Lee 1986.

Table 1.2. Ethogram used to record event behaviors performed by 1-127 month old male and female African elephants during continual focal observations at AENP from June to October, 2005.

Event behavior categories and defined event behaviors	**Definition**
Trunk to Ground Substrate or Conspecific[1]	Behaviors presented in order representing increasing interest by elephants in substrate or conspecific
Sniff	Nasal openings hover over ground or conspecific without contact
Check	Touch ground with either finger of the trunk tip
Place	Entire nasal opening is placed on ground or conspecific and held momentarily
Flehmen	Tip of trunk touches substrate or conspecific then placed on the VNO ducts in the roof of the mouth
Trunk Tip to/from other Elephant	
Anus	Anal region
Body	Torso or areas not listed
Feet	Area below ankle
Genital	Penis or vulva
Head	Forehead and superior most point of head
Mouth	Trunk tip inserted into mouth
Tail	Touches or grabs tail
Temporal gland	Contact with temporal gland or secretion
Trunk	Portion of trunk starting from mouth area, down to tip

Body Contact to/from other Elephants	
Back into	Intentionally walks backward into the body of another individual
Body rub[3,4]	Using the torso to brush against another individual's torso
Head butt[2]	Quickly using the head to make contact with the body of another individual
Head into[3,4]	Lean on other animal with head, not a push
Kick[2]	Using legs to strike at another
Lean[3,4]	Placing body weight on the body of another individual.
Mount	Standing on hind legs, forelegs resting on body of a standing elephant
Present	Turn backside toward another
Push[2]	Using the body to displace another elephant from their location
Rolling	One elephant is on the ground, while other is on top
Trunk over Back[3,4]	Placing the entire length of the trunk across back and holding position for at least two seconds
Trunk on Head[2]	Placing the entire length of the trunk over head and holding position for at least two seconds
Tusk[2]	Using the end of the tusks to push another animal

[1]Chemosensory definitions derived from Schulte & Rasmussen (1999)
[2]Categorized together as Aggressive behaviors
[3]Categorized together as Provide Comfort if done as sender (trunk-over-back) or accepted as receiver (head into, lean, rub)
[4]Categorized together as Seek Comfort if done as sender (head into, lean, rub) or accepted as receiver (trunk-over-back)

Table 1.3. Subcategories of event behaviors incorporating multiple individual behaviors of African elephants ages 1-127 months at AENP South Africa from June to October, 2005.

Behavior Subcategories	Event Behaviors
Aggressive	Tusk, kick, push, head butt, trunk-over-head
Provide Nurture	Trunk-over-back, From others: head into, rub, lean
Receive Nurture	Head into, rub, lean, trunk-over-back (from other)

Table 1.4. The number of available and the number of observed male and female African elephants that were ten years of age and younger within each kinship group and one family unit at AENP from June to October, 2005.

		Available Population							**Observed Population**						
Sex	**Age**	**A**	**B**	**H**	**L**	**P/M**	**R**	**Total**	**A**	**B**	**H**	**L**	**P/M**	**R**	**Total**
Female	**<1**	4	3	0	1	1	0	**9**	4	3	0	1	1	0	**9**
	1	1	2	1	3	1	0	**8**	1	2	1	2	1	0	**7**
	2	2	1	0	2	4	1	**10**	1	1	0	2	3	1	**8**
	3	3	1	1	2	2	5	**14**	3	0	0	0	3	4	**10**
	4	0	2	0	0	1	0	**3**	0	2	0	0	1	0	**3**
	5	2	1	0	0	3	0	**6**	2	1	0	0	3	0	**6**
	6	3	3	1	1	0	0	**8**	2	2	1	0	0	0	**5**
	7	4	2	0	0	2	4	**12**	4	3	0	0	2	3	**12**
	8	1	1	0	2	3	0	**7**	1	1	0	2	3	0	**7**
	9	2	2	2	0	5	1	**12**	2	2	1	0	5	1	**11**
	10	3	0	0	0	0	0	**3**	3	0	0	0	0	0	**3**
Total Females		**25**	**18**	**5**	**11**	**22**	**11**	**92**	**23**	**17**	**3**	**7**	**22**	**9**	**81**
Male	**<1**	0	1	0	1	2	0	**4**	0	1	0	1	2	0	**4**
	1	0	3	1	0	3	1	**8**	0	3	1	0	3	1	**8**
	2	1	1	1	0	1	2	**6**	1	1	1	0	1	2	**6**
	3	4	3	1	3	4	3	**18**	4	2	2	0	0	1	**9**
	4	4	3	1	2	3	0	**13**	4	2	1	0	3	0	**10**
	5	1	0	0	0	2	2	**5**	1	0	0	0	2	1	**4**
	6	3	4	2	4	4	1	**18**	2	2	1	1	3	1	**10**
	7	1	0	2	2	2	1	**8**	0	0	2	2	2	1	**7**
	8	0	1	1	1	3	1	**7**	0	2	0	1	2	1	**6**
	9	2	1	0	0	0	3	**6**	2	1	0	0	0	3	**6**
	10	1	0	0	2	4	2	**9**	1	0	1	1	4	1	**8**
Total Males		**17**	**17**	**9**	**15**	**28**	**16**	**102**	**15**	**14**	**9**	**6**	**22**	**12**	**78**[1]
Percent of Groups Observed									**90**	**89**	**86**	**50**	**88**	**78**	**82**
Percent of Female Focals from Each Group									**28**	**21**	**4**	**9**	**27**	**11**	
Percent of Male Focals from Each Group									**19**	**18**	**12**	**8**	**28**	**15**	

[1]The kinship group of five males were unknown.

Table 1.5. The impact of waterhole location, number of elephants present during an observation, and air temperature on the measured responses of African elephants ages 1-127 months at AENP South Africa from June to October, 2005. Spearman's Rho correlation was used to test for the effect the independent variables.

		Temperature		**Number of Elephants**		**Location**	
	N	**r_s**	**P-value**	**r_s**	**P-value**	**r_s**	**P-value**
State Behavior (duration)							
Aggressive Play	162	0.02	0.84	0.08	0.29	0.03	0.69
Gentle Play	162	0.04	0.60	0.06	0.42	-0.02	0.81
Total Play	162	0.12	0.16	0.002	0.98	0.05	0.52
Drink	162	-0.06	0.43	-0.15	0.06	0.05	0.49
Stand	162	-0.06	0.48	-0.12	0.14	-0.09	0.28
Walk	162	-0.03	0.74	-0.04	0.64	-0.09	0.28
Event Behaviors (rate)							
Aggressive	164	-0.10	0.19	0.08	0.29	0.09	0.24
Nurturing	164	0.02	0.76	0.02	0.76	0.09	0.24
Total Chemo	164	-0.13	0.10	-0.013	0.87	-0.02	0.82
Total Trunk to	164	0.03	0.68	-0.06	0.42	-0.07	0.36

Table 1.6. Results of linear regressions for developmental trends and ANCOVA to determine sex differences in state behaviors of 82 male and 80 female African elephants ages 1-127 months at AENP South Africa from June to October, 2005.

State Behavior			**I-Developmental Trend**			**II-Sexual Dimorphism**		
	Sex	**Mean ± SE**	**R^2**	**t-Ratio**	**P**	**df**	**F**	***P***
A. Proportion (Total Focal-Not Visible Time)								
1. Drink	Male	0.11±0.02	0.008	0.81	0.42	1,161	1.03	0.31
	Female	0.13±0.02	0.04	1.7	0.09			
2. Stand	Male	0.44±0.2	0.007	0.23	0.82	1,161	2.19	0.14
	Female	0.39±0.02	0.05	2.14	0.04*			
3. Walk	Male	0.19±0.01	0.03	-1.64	0.11	1,161	0.02	0.88
	Female	0.19±0.02	0.01	-1.06	0.29			
4. Nurse	Male	0.02±0.007	0.11	-3.15	0.002*	1,161	0.07	0.79
	Female	0.03±0.006	0.22	-4.67	<.001*			
B. Mean Duration of Complete Bouts (seconds)								
1. Drink	Male	37.66±6.41	0.002	0.42	0.67	1,158	0.83	0.36
	Female	44.13±6.46	0.07	2.42	0.02*			
2. Stand	Male	79.79±7.00	0.014	1.08	0.28	1,158	1.95	0.16
	Female	63.96±6.36	0.14	3.61	<.001*			
3. Walk	Male	43.49±4.04	<.001	0.01	0.99	1,158	3.24	0.07
	Female	34.91±2.54	0.004	-0.55	0.59			
4. Nurse	Male	4.97±1.28	0.16	-3.87	<.001*	1,158	4.24	0.04**
	Female	11.35±2.33	0.20	-4.44	<.001*			
C. Frequency of bouts/hour								
1. Drink	Male	0.077±0.01	0.003	0.51	0.61	1,161	2.42	0.12
	Female	0.09±0.01	0.03	1.64	0.11			
2. Stand	Male	0.37±0.03	0.08	0.008	0.19	1,161	0.05	0.83
	Female	0.37±0.03	0.01	0.33	0.27			
3. Walk	Male	0.25±0.02	0.08	-2.64	0.01*	1,161	0.69	0.41
	Female	0.27±0.02	0.005	-0.66	0.51			
4. Nurse	Male	0.05±0.01	0.13	-3.43	0.001*	1,161	0.03	0.87
	Female	0.05±0.01	0.21	-4.62	<.001*			

*P-value < 0.05 indicating slope of best-fit linear regression was significantly different from zero.
** P-value < 0.05 indicating a significant sex difference across age.

Table 1.7. Results of linear regressions for developmental trends and ANCOVA to determine sex differences in play state behaviors of 82 male and 80 female African elephants ages 1-127 months at AENP South Africa from June to October, 2005.

State Behavior			**I-Developmental Trend**			**II-Sexual Dimorphism**		
	Sex	**Mean ± SE**	**R^2**	**t-Ratio**	**P**	**df**	**F**	***P***
A. Proportion (Total Focal- Not Visible time)								
1. Aggressive	Male	0.03±0.006	0.04	1.8	0.07	1,161	17.69	<.001**
	Female	0.003±0.002	0.09	2.76	0.007*			
2. Non-Aggressive	Male	0.01±0.004	0.07	-2.38	0.02*	1,161	0.29	0.59
	Female	0.006±0.002	0.01	-1.04	0.30			
3. Social Play	Male	0.04±0.007	0.006	0.69	0.49	1,161	15.83	<.001**
	Female	0.009±0.002	0.003	0.51	0.61			
B. Mean Duration of Complete Bouts (seconds)								
1.Aggressive	Male	11.41±1.97	0.04	1.82	0.07	1,159	15.13	<.001**
	Female	2.33±0.99	0.06	2.13	0.04*			
2. Non-Aggressive	Male	4.72±1.57	0.06	-2.26	0.03*	1,159	0.042	0.83
	Female	5.61±1.67	<.001	-0.09	0.93			
3. Social Play	Male	9.72±1.67	0.002	-0.36	0.72	1,159	1.11	0.29
	Female	7.09±1.81	0.002	0.41	0.68			
C. Frequency/hour								
1.Aggressive	Male	0.07±0.01	0.05	2.1	0.04*	1,161	17.45	<.001**
	Female	0.01±0.04	0.08	2.64	0.01*			
2. Non-Aggressive	Male	0.02±0.005	0.02	-1.44	0.15	1,161	0.83	0.36
	Female	0.01±0.004	0.16	-3.89	<.001*			
3. Social Play	Male	0.08±0.01	0.026	1.46	0.15	1,161	18.49	<.001**
	Female	0.02±0.005	0.003	-0.49	0.63			

*P-value < 0.05 indicating slope of best-fit linear regression was significantly different from zero.
** P-value < 0.05 indicating a significant sex difference across age.

Table 1.8. a) Count and percent of total sex and age combination of social play partners and b) count and proportion of bouts of aggressive and non-aggressive play with different age and sex of play partners of 82 male and 80 female African elephants ages 1-127 months at AENP South Africa from June to October, 2005.

a)

	Focal Animals							
[1]**Non-Focals**	**CF**	**CM**	**JF**	**JM**	**PF**	**PM**	***Total***	***Percent***
AF	1	0	1	0	0	0	**2**	**1.4**
CF	6	8	1	3	1	0	**19**	**13.4**
CM	3	14	8	7	0	0	**32**	**22.5**
JF	0	2	1	11	0	1	**15**	**10.6**
JM	0	4	7	29	2	8	**50**	**35.2**
PF	0	0	1	3	0	2	**6**	**4.2**
PM	0	0	1	7	0	10	**18**	**12.7**
Total	**10**	**28**	**20**	**60**	**3**	**21**	**142**	
Percent	**7.0**	**19.7**	**14.1**	**42.3**	**2.1**	**14.8**		

[1]Focal animal play partner: female (F) and male (M): Adults 20+ years (AF, AM), Pubescents 11-19 years (PF, PM), Juveniles 5-10 years, (JF, JM) and Calves 0-4 years (CF, CM).

b)

Social Play	**Male Aggressive**		**Female Aggressive**		**Male Non-Aggressive**		**Female Non-Aggressive**	
	Bouts	**Proportion**	**Bouts**	**Proportion**	**Bouts**	**Proportion**	**Bouts**	**Proportion**
[1]***Play Partner***								
CF	2	0.02	0	0.00	9	0.41	8	0.40
CM	17	0.20	5	0.38	4	0.18	6	0.30
JF	10	0.11	1	0.08	4	0.18	0	0.00
JM	38	0.44	5	0.38	3	0.14	4	0.20
PF	4	0.05	0	0.00	1	0.05	1	0.05
PM	16	0.18	1	0.08	1	0.05	0	0.00
AF	0	0.00	1	0.08	0	0.00	1	0.05
Total	**87**		**13**		**22**		**20**	

[1]Focal animal play partner: female (F) and male (M): Adults 20+ years (AF, AM), Pubescents 11-19 years (PF, PM), Juveniles 5-10 years, (JF, JM) and Calves 0-4 years (CF, CM).

Table 1.9. Results of linear regressions for developmental trends and ANCOVA to determine sex differences in social contact behaviors of 83 male and 81 female African elephants ages 1-127 months at AENP South Africa from June to October, 2005.

Contact Behavior			**I-Developmental Trend**			**II-Sexual Dimorphism**		
	Sex	**Mean ± SE**	**R^2**	**t-Ratio**	**P**	**df**	**F**	***P***
A. Receive/hour								
1. Nurture	Male	6.36±0.95	0.23	-5.1	<.001*	1,161	14.15	<.001**
	Female	11.80±1.03	0.15	-3.79	<.001*			
2. Aggressive[1]	Male	5.21±0.81	0.00	-0.08	0.93	1,161	4.816	0.05**
	Female	3.24±0.53	0.05	1.88	0.06			
3. Trunk over back	Male	1.29±0.33	0.09	-2.65	0.01*	1,161	2.71	0.10
	Female	2.42±0.48	0.20	-4.07	0.0001*			
4. Trunk over head	Male	1.56±0.34	0.00	0.04	0.97	1,161	0.42	0.52
	Female	1.09±0.33	0.01	0.89	0.38			
B. Send /hour								
1. Nurture	Male	3.05±0.49	0.0003	-0.15	0.88	1,160	7.98	0.005***
	Female	4.18±0.53	0.16	3.9	<.001*			
2. Aggressive[1,2]	Male	3.03±0.56	0.02	1.43	0.16	1,161	6.36	0.01**
	Female	1.40±0.28	0.05	2.0	0.048*			
3. Trunk over back	Male	1.27±0.33	0.004	0.57	0.57	1,161	3.59	0.06
	Female	2.10±0.37	0.10	2.88	0.005*			
4. Trunk over head	Male	1.07±0.29	0.005	0.66	0.51	1,161	4.18	0.04**
	Female	0.37±0.13	0.12	3.27	0.002*			

*P-value < 0.05 indicating slope of best-fit linear regression was significantly different from zero.
** P-value < 0.05 indicating a significant sex difference across age.
*** P-value < 0.05 indicating interaction by covariates (gender x age); failing parallelism test.
[1]Aggressive behaviors observed outside of play.
[2] The removal of one male that had a 11-fold greater rate of aggression compared to the average resulted in a significant increase in the behavior across time (Regresion:R^2=0.09, t=2.77, P=0.007).

Table 1.10. Results of linear regressions for developmental trends and ANCOVA to determine sex differences in three categories of chemosensory behaviors of 83 male and 81 female African elephants ages 1-127 months at AENP South Africa from June to October, 2005. No zeros were included for the rate.

Chemosensory			**I-Developmental Trend**			**II-Sexual Dimorphism**		
	Sex	**Mean ± SE**	**R^2**	**t-Ratio**	**P**	**df**	**F**	***P***
A. Frequency/hour								
1. To Environment	Male	141.36±9.38	<.001	0.25	0.80	1,160	1.24	0.28
	Female	125.99±8.34	0.03	1.57	0.12			
2. To Conspecifics	Male	11.95±1.41	0.03	-1.44	0.15	1,159	0.01	0.91
	Female	11.73±1.55	0.001	1.06	0.29			
3. To Urine/Feces	Male	20.41±5.57	<.001	0.14	0.89	1,48	2.84	0.09
	Female	8.62±1.50	<.001	-0.09	0.92			
B. Proportion (among 3 categories)								
1. To Environment	Male	0.88±0.01	0.01	0.013	0.01*	1,160	0.84	0.36
	Female	0.88±0.02	0.003	-0.5	0.62			
2. To Conspecifics	Male	0.09±0.01	0.03	-1.45	0.15	1,160	0.02	0.88
	Female	0.09±0.01	0.001	0.31	0.76			
3. To Urine/Feces	Male	0.03±0.007	0.004	0.54	0.59	1,160	5.11	0.03**
	Female	0.01±0.003	0.008	0.81	0.42			

*P-value < 0.05 indicating slope of best-fit linear regression was significantly different from zero.
** P-value < 0.05 indicating a significant sex difference across age.

Table 1.11. Results of linear regressions for developmental trends and ANCOVA to determine sex differences in chemosensory behaviors of 83 male and 81 female African elephants ages 1-127 months at AENP South Africa from June to October, 2005.

Chemosensory			**I-Developmental Trend**			**II-Sexual Dimorphism**		
	Sex	**Mean ± SE**	**R^2**	**t-Ratio**	**P**	**df**	**F**	***P***
A. Frequency/hour								
1. Sniff	Male	45.97±3.97	0.05	-2.0	0.049*	1,161	0.85	0.36
	Female	41.04±4.35	0.001	0.4	0.69			
2. Check	Male	29.13±4.12	0.02	1.21	0.23	1,161	0.29	0.59
	Female	31.35±4.04	0.03	1.41	0.16			
3. Place	Male	10.08±1.89	<.001	-0.01	0.99	1,161	6.01	0.02**
	Female	4.76±1.10	0.017	-1.16	0.25			
4. Flehmen	Male	1.36±0.58	0.03	1.62	0.10	1,161	2.97	0.08
	Female	0.46±0.21	0.005	0.64	0.52			
5. Raised Sniff	Male	65.53 ±4.11	0.003	0.53	0.60	1,161	2.38	0.12
	Female	57.82±4.78	0.07	2.39	0.02*			
6. Total Chemo	Male	155.85±9.90	0.003	-0.5	0.62	1,161	1.96	0.16
	Female	136.13±9.10	0.03	1.58	0.12			
7. Total SCPF	Male	86.52±6.79	<.001	-0.28	0.78	1,161	0.89	0.35
	Female	77.61±5.88	0.014	1.06	0.29			
B. Proportion (among four behaviors)[1]								
1. Sniff	Male	0.61±0.034	0.14	-3.63	<.001*	1,158	0.80	0.37
	Female	0.57±0.04	0.04	-1.83	0.07			
2. Check	Male	0.30±0.03	0.12	3.29	0.002*	1,158	2.93	0.09
	Female	0.37±0.03	0.07	2.42	0.02*			
3. Place	Male	0.08±0.01	0.002	-0.15	0.88	1,158	1.49	0.22
	Female	0.06±0.01	0.02	-1.38	0.17			
4. Flehmen	Male	0.014±0.005	0.12	3.25	0.002*	1,156	8.49	0.004***
	Female	0.003±0.001	0.02	1.22	0.23			

*P-value < 0.05 indicating slope of best-fit linear regression was significantly different from zero.
** P-value < 0.05 indicating a significant sex difference across age.
*** P-value < 0.05 indicating interaction by covariates (gender x age); failing parallelism test.
[1]Animals that did not perform any of these behaviors were excluded, males (N=79) and females (N=79).

Table 1.12. Results of linear regressions for developmental trends and ANCOVA to determine sex differences in proportion of chemosensory plus trunk-tip behaviors that were directed from the focal animal to different age and sex categories at AENP South Africa from June to October, 2005.

Chemosensory + Trunk to			**I-Developmental Trend**			**II-Sexual Dimorphism**		
	Sex	**Mean ± SE**	**R^2**	**t-Ratio**	**P**	**df**	**F**	***P***
A. Receiver								
1. Adult Female	Male	0.29±0.04	0.07	-2.02	0.049*	1,107	2.37	0.13
	Female	0.22±0.03	0.07	-2.04	0.048*			
2. Adult Male	Male	0.05±0.02	0.01	-0.83	0.41	1,107	0.087	0.77
	Female	0.05±0.01	0.04	1.52	0.13			
3. Pubescent Female	Male	0.12±0.02	0.09	-2.31	0.02*	1,107	1.086	0.30
	Female	0.17±0.03	0.26	-4.39	<.001*			
4. Pubescent Male	Male	0.16±0.03	0.15	3.04	0.004*	1,107	3.96	0.049**
	Female	0.07±0.02	0.02	1.06	0.29			
5. Juvenile Female	Male	0.04±0.01	<.001	0.00	0.99	1,107	1.00	0.32
	Female	0.05±0.01	0.003	0.38	0.70			
6. Juvenile Male	Male	0.09±0.02	0.07	2.00	0.05*	1,107	0.82	0.37
	Female	0.06±0.01	0.10	2.42	0.02*			
7. CM + CF[1]	Male	0.17±0.03	0.003	-0.4	0.68	1,106	6.06	0.02***
	Female	0.29±0.03	0.14	3.02	0.004*			
8.Kinship Group	Male	0.09±0.02	0.02	1.14	0.26	1,107	1.67	0.09
	Female	0.09±0.01	0.03	1.27	0.21			

*P-value < 0.05 indicating slope of best-fit linear regression was significantly different from zero.
** P-value < 0.05 indicating a significant sex difference across age.
*** P-value < 0.05 indicating interaction by covariates (gender x age); failing parallelism test.
[1]Calves were combined because they had the same result for each sex.

Table 2.1. Dates of study, housing facility, age and reproductive phase of captive female African elephants that participated in bioassays conducted with captive female African elephant urine.

Date	Institution	Cow Name	State of Estrus	Age as of 2006
3/13-3/17/06	Riverbanks Zoological Garden	Tumpe	Non-estrous	23
		Penny	Estrous	26
		Bell	Flatliner	28
3/20-3/25/06	North Carolina Zoological Park	Rafiki	Estrous	25
		Little Diamond	Estrous	28
3/27-3/31/06	Knoxville Zoo	Ellie	Estrous	23
		Jana	Non-estrous	25
6/6-6/10/06	Pittsburg Zoo	Moja	Non-estrous	24
		Savannah	Estrous	23
		Tosh	Flatliner	28
6/12-6/17/06	Toronto	Thika	Estrous	26
		Tara	Non-estrous	37
		Tessa	Non-estrous	37
		Tequila	Non-estrous	36
6/19-6/23/06	Roger Williams Park Zoo	Alice	Non-estrous	21
		Ginny	Estrous	21
		O.S. Kate	Estrous	21
6/26-6/30/06	Lee Richardson Zoo	Msichana	Non-estrous	24
		Mokala	Estrous	24
7/9-7/13/06	Zoo Atlanta	Tara	Estrous	24
		Kelly	Flatliner	23
		Dottie	Non-estrous	24
7/15-7/19/06	Montgomery Zoo	Star	Flatliner	28
		Tina	Estrous	21
		Mary	Non-estrous	21
	Zoos = 9	**Cycling cows = 21**		**Avg. Age = 26**

Table 2.2. Captive African female elephants that supplied urine for bioassays conducted with captive female African elephants. Housing facility, birth year, and type of urine supplied by each female are included.

Female	Housing facility	Female birth year	Type of urine
Asali	Memphis	1975	LH2
Dolly	Baltimore	1971	LH2, Luteal
Kiba	Nashville Zoo	1982	LH1, LH2, Luteal
Kubwa	Indianapolis Zoo	1976	LH1
Mikki	Louisville	1979	LH2
Renee	Toledo	1977	Luteal
Tava	Six Flags Marine World	1978	LH1
Tombi	Indianapolis	1982	Luteal

Table 2.3. Ethogram of record all occurrence social behaviors performed by captive female African elephants during behavior observations. The trunk tip to other and body contact to other were used to record social interactions between captive female elephants.

Event behavior categories	**Definition**
Approach	One elephant walks towards another
Displace	One elephant approaches another elephant within 1 body length, approached elephant moves away within 1 minute of approach
Trunk Tip to/from other Elephant (body part contacted)	
Anus	Anal region
Body	Torso or areas not listed
Feet	Area below ankle
Genital	Penis or vulva
Head	Forehead and superior most point of head
Mouth	Trunk tip inserted into mouth
Tail	Tail
Temporal gland	Temporal region or secretion
Trunk	Portion of trunk starting from mouth area, down to tip

Body Contact to/from other Elephants	
Back into	Intentionally walks backward into the body of another individual.
Body rub	Using the torso to brush against another individual's torso.
Head butt	Quickly using the head to make contact with the body of another individual.
Head into	Lean on other animal with head, not a push
Kick	Using legs to strike at another
Lean	Placing body weight on the body of another individual.
Present	Turn backside toward another
Push	Using the body to displace another elephant from their location.
Trunk over Back	Placing the entire length of the trunk across back and holding position for at least two seconds
Trunk on Head	Placing the entire length of the trunk over head and holding position for at least two seconds
Tusk	Using the end of the tusks to push another animal

Table 2.4. Ethogram of state behaviors performed by captive female African elephants during one-minute scan data collection.

State Behavior Categories	Definition
Contact	Physically touching other animal with trunk-tip
Defecate	Release feces
Drink	Taking water into the trunk and immediately placing water into the mouth
Dust	Using the foot or trunk to place dirt particles on the body
Eat	Taking nutrients into the mouth via the trunk.
Mud	Using the trunk to throw mud particles on the body or moving body rapidly in a mud hole
Stand	Remains in the same location for at least two seconds
Sway	Stereotypic behavior of standing in one place and continually rocking back and forth
Urinate	Release urine
Walk	Leaves location while all four legs are moving in a steady pace
Other	Behavior not defined in ethogram.
Not Visible	Elephant has moved out of sight

Table 2.5. Ethogram of event behaviors performed by captive female African elephants to bioassay samples. Specific behaviors were categorized into Approach, Chemosensory, Accessory Trunk, and Other (Bagley 2004).

Behavior categories and defined behaviors	**Definition**
Approach	
Proximity	Female within one body length of sample
Near	Female within one trunk length of sample
Chemosensory	
Sniff[1]	Nasal openings hover over sample without contact
Check[1]	Touch sample with tip of either finger
Place[1]	Entire nasal opening is placed on a sample and held momentarily
Flehmen[1]	Tip of trunk touches sample then placed in the VNO ducts in the roof of the mouth
Accessory	
Blow	Performed after inspecting a sample. Air is expelled quickly from nasal openings of trunk; usually audible and mucus expelled usually visible
Dig	Elephant used trunk tip or foot to displace ground at sample area
Pinch	The two fingers of trunk pick up dirt around the sample.
Suck	Same trunk position as Place accompanied with trunk contraction; usually audible
Trunk Flick	Performed after inspecting a sample. Bottom ¼ of trunk moves up and down rapidly
Wriggle	Performed after inspecting a sample. Trunk twists and then untwists once at a moderate pace (slower than trunk flick)
Other	
Vocalize	Elephant vocalizes after investigating sample
Defecate/Urinate	Elephant releases expressions in sample area or on sample after contacting the sample
Other	Behaviors exhibited that are not defined in ethogram

[1]Chemosensory definitions derived from Schulte & Rasmussen (1999)

Table 2.6. Results of one-way ANOVA comparing between the rate (Freq/hour) of social behaviors that estrous (N=11) and non-estrous (N=10) female African elephants sent and received at nine zoological facilities from March – July, 2006.

1-Way ANOVA btwn Estrous and Non-estrous Females Mean ± SE (Freq/hour)					
Behavior	**DF**	**F Ratio**	**Prob > F**	**Estrous**	**Non-estrous**
Trunk-tip to Genitals					
Sent	1, 19	1.13	0.30	1.12 ± 0.18	0.83 ± 0.20
Received	1, 19	6.66	0.02*	1.34 ± 0.18	0.73 ± 0.15
Trunk-tip to Mouth					
Sent	1, 19	0.42	0.52	0.89 ± 0.15	0.89 ± 0.22
Received	1, 19	1.34	0.26	1.04 ± 0.20	0.69 ± 0.21
Trunk-tip to Anus, Genitals, Temporal Glands					
Sent	1, 19	0.64	0.43	1.27 ± 0.18	1.03 ± 0.25
Received	1, 19	6.89	0.02*	1.64 ± 0.23	0.87 ± 0.17
Trunk-tip to Anus, Genitals, Mouth, Temporal Glands					
Sent	1, 19	0.74	0.40	2.17 ± 0.25	1.75 ± 0.42
Received	1, 19	4.68	0.04*	2.68 ± 0.39	1.57 ± 0.32
Total Trunk-tip Touches					
Sent	1, 19	0.50	0.49	1.38 ± 0.25	2.16 ± 0.49
Received	1, 19	3.86	0.06	3.08 ± 0.42	1.95 ± 0.36
Total Rate of Social Behaviors					
Sent	1, 19	0.50	0.49	2.97 ± 0.28	2.56 ± 0.53
Received	1, 19	3.86	0.06	3.40 ± 0.44	2.28 ± 0.34

Table 2.7. Results of repeated measures ANOVA comparing the rate (Freq/min) of chemosensory behaviors to the control and urine samples (LH1, LH2, and non-estrous) from estrous (N=11) and non-estrous (N=10) female African elephants across three bioassays at nine zoological facilities from March – July, 2006.

Rate of Chemosensory Behaviors (Freq/min)				
Behavior	**Effect-df**	**Error-df**	**F**	**p-level**
Sniff, Check, Place, Flehmen				
Phase	1	19	0.01	0.90
Sample	3	57	7.24	<0.001*
Day	2	38	51.35	<0.001*
*Phase*Sample*	3	57	0.65	0.58
*Phase*Day*	2	38	1.23	0.30
*Sample*Day*	6	114	3.94	<0.001*
*Phase*Sample*Day*	6	114	0.27	0.94
Check, Place				
Phase	1	19	0.11	0.74
Sample	3	57	6.90	<0.001*
Day	2	38	51.52	<0.001*
*Phase*Sample*	3	57	0.59	0.62
*Phase*Day*	2	38	1.20	0.31
*Sample*Day*	6	114	3.70	0.002*
*Phase*Sample*Day*	6	114	0.21	0.97
Accessory				
Phase	1	19	0.32	0.57
Sample	3	57	4.09	0.01*
Day	2	38	35.79	<0.001*
*Phase*Sample*	3	57	0.70	0.55
*Phase*Day*	2	38	1.04	0.36
*Sample*Day*	6	114	3.17	0.01*
*Phase*Sample*Day*	6	114	0.16	0.98
Total				
Phase	1	19	0.06	0.81
Sample	3	57	6.42	<0.001*
Day	2	38	51.13	<0.001*
*Phase*Sample*	3	57	0.65	0.58
*Phase*Day*	2	38	1.27	0.29
*Sample*Day*	6	114	3.79	<0.001*
*Phase*Sample*Day*	6	114	0.22	0.96

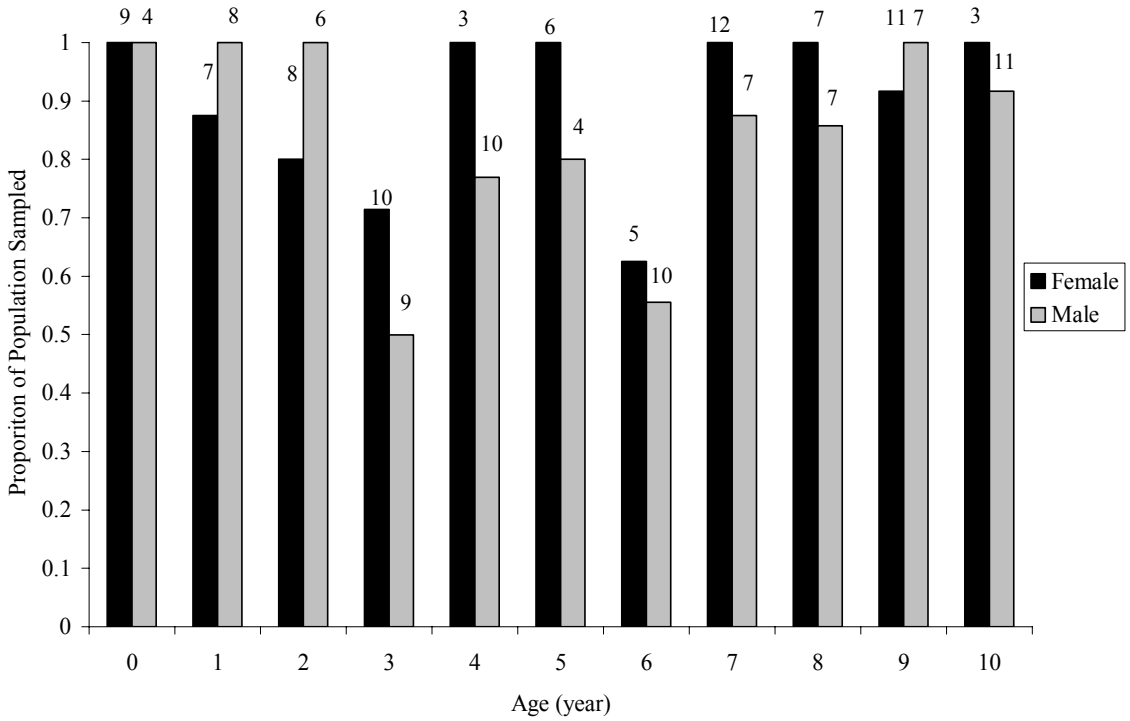

Figure 1.1. Proportion of the population that was sampled per age class for individual male ($N = 83$) and female ($N = 81$) elephants at AENP from June to October, 2005. Sample size is above each age category.

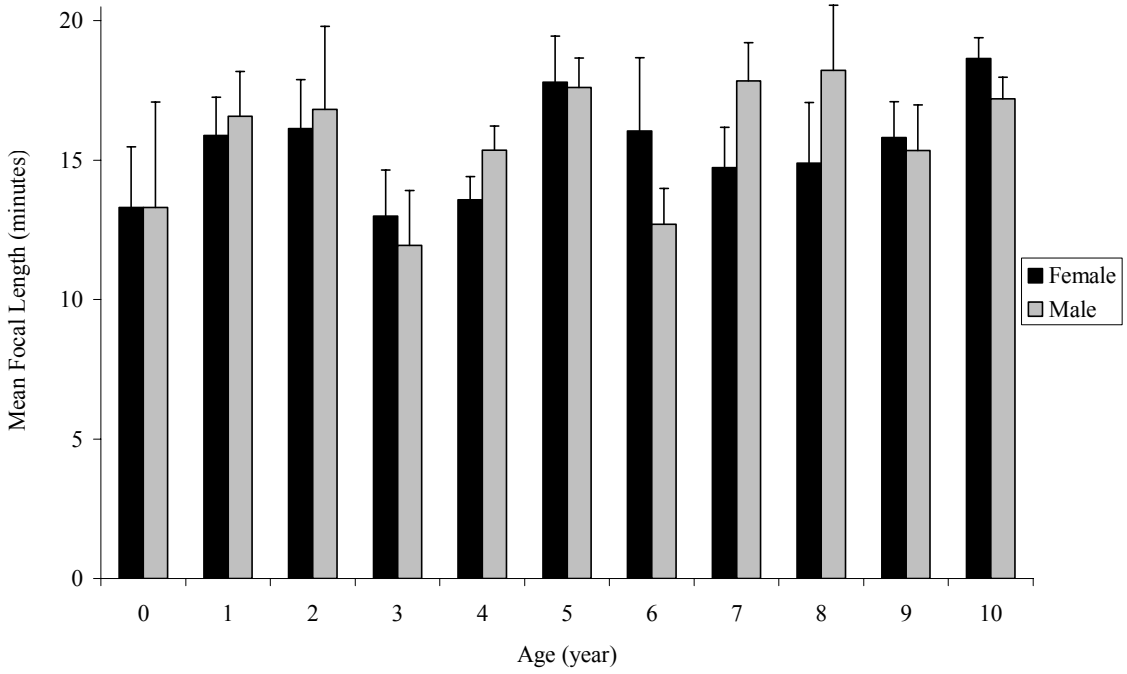

Figure 1.2. Mean (±SE) focal length (min) per age class for individual male (N = 83) and female (N = 81) elephants at AENP from June to October, 2005. Time not visible during focal was removed.

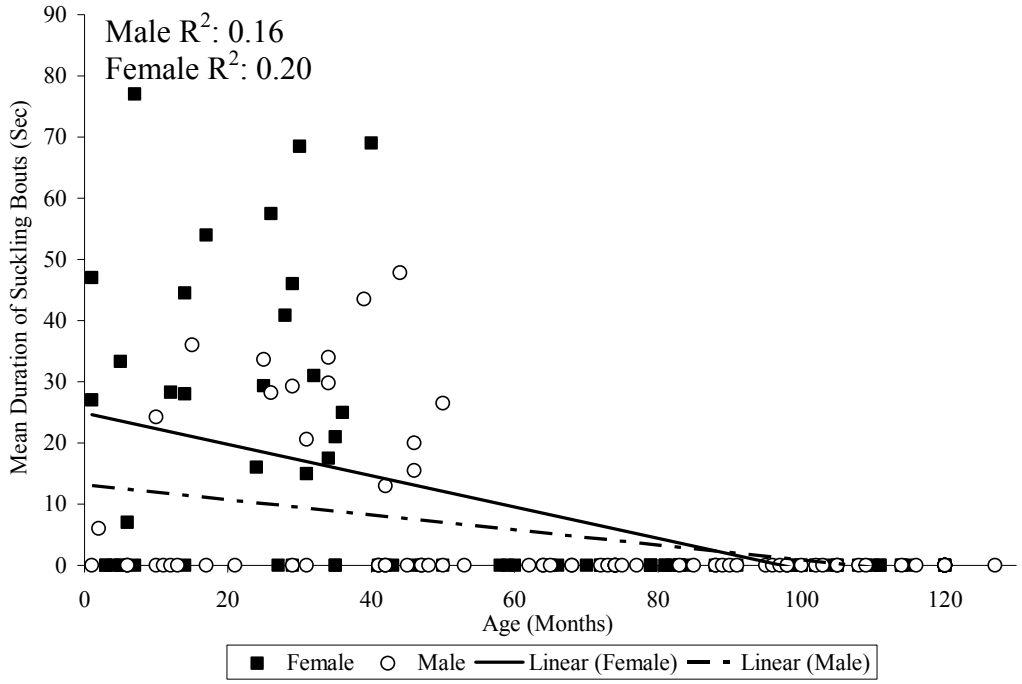

Figure1.3. Mean duration (sec) of suckling bouts for individual male (*N* = 82) and female (*N* = 80) elephants at AENP from June to October, 2005. Statistical analysis for this graph can be found in Table 1.6, B:I-II, 4.

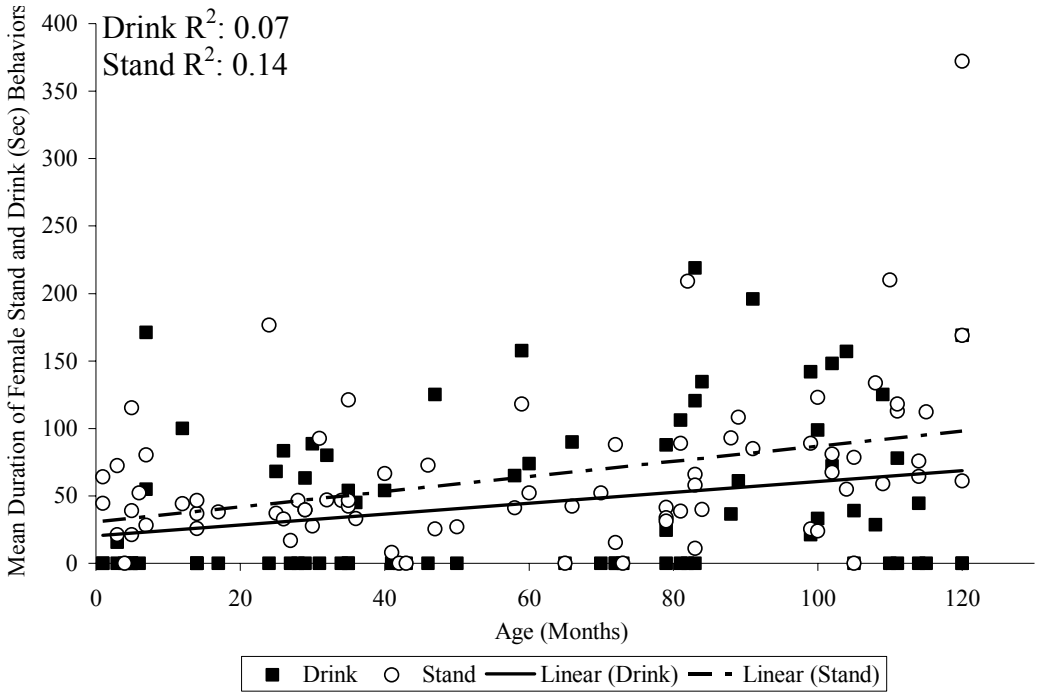

Figure 1.4. Mean duration (sec) of time spent standing and drinking per individual female (N = 80) elephant at AENP from June to October, 2005. Statistical analysis for this graph can be found in Table 1.6, B:I 1, 2.

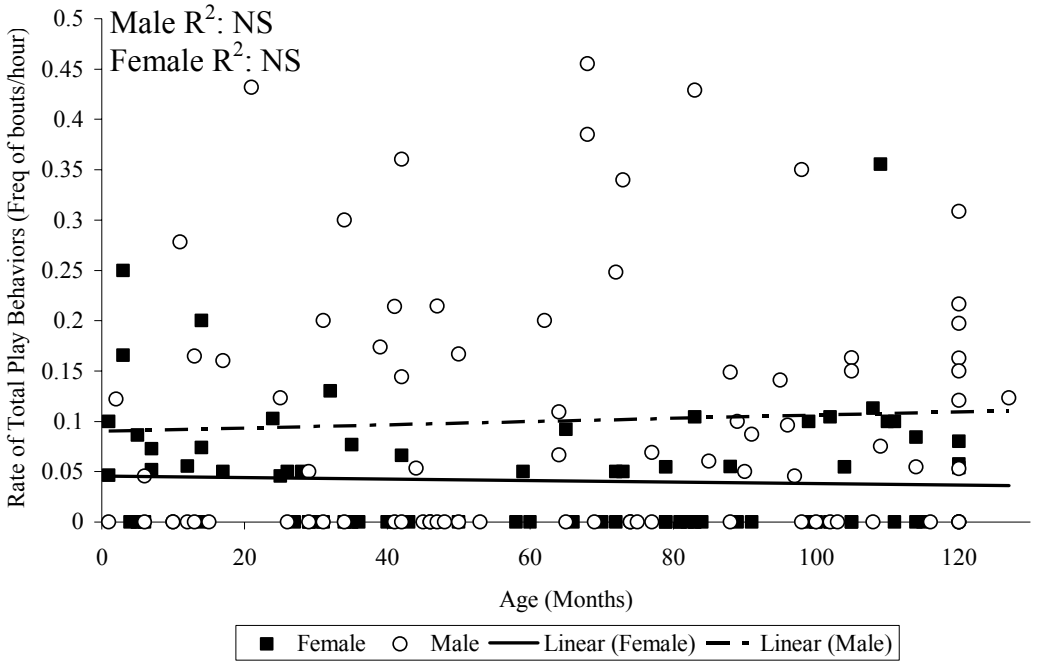

Figure 1.5. Rate (Freq of bouts/h) of total play for individual male (N = 83) and female (N = 81) elephants at AENP from June to October, 2005. Statistical analysis for this graph can be found in Table 1.7, C:II, 3 for the sex difference.

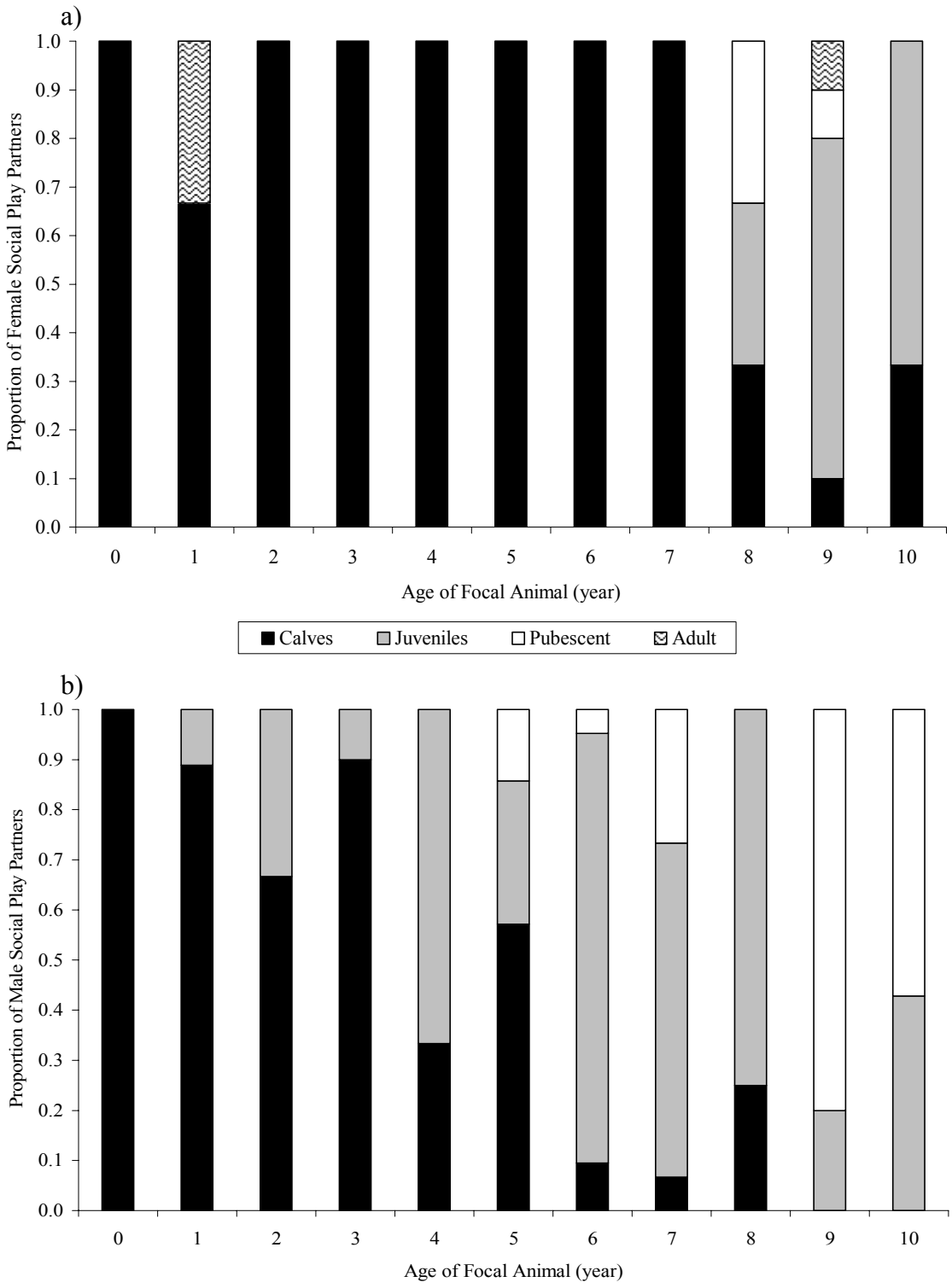

Figure 1.6. Proportion of the focal animals social play partner for each age (year) category. Females (a) were involved in 33 play bouts and males (b) in 109 play bouts at AENP from June to October, 2005. See Table 1.8 for proportions for each age category and sex of partner.

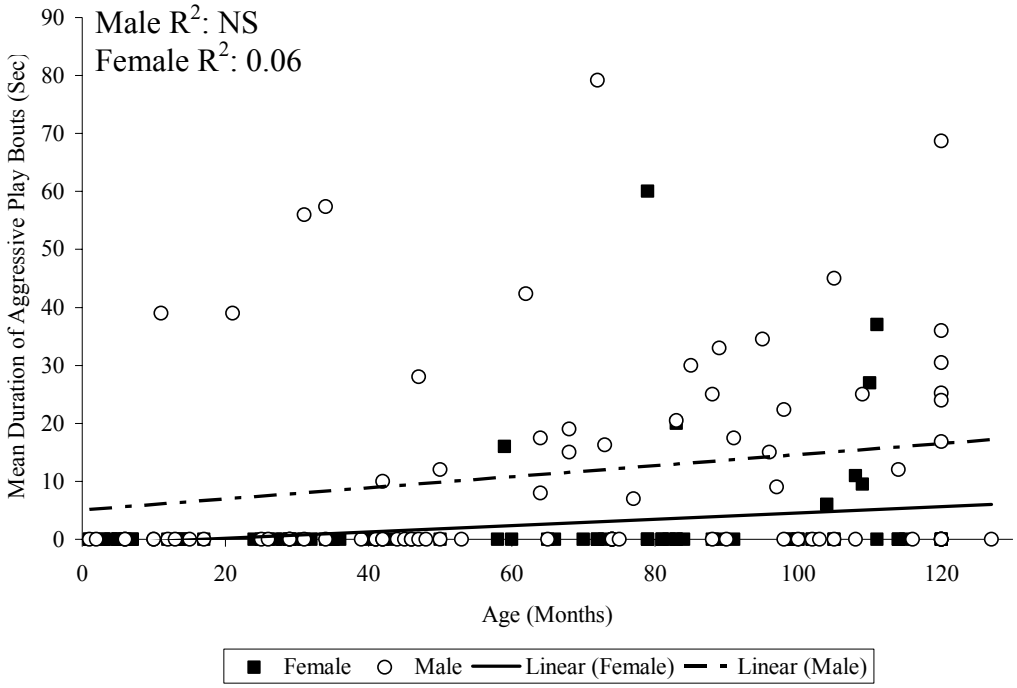

Figure 1.7. Mean duration (sec) of aggressive play bouts for individual male (N = 82) and female (N = 80) elephants at AENP from June to October, 2005. Statistical analysis for this graph can be found in Table 1.7, B:I-II, 1.

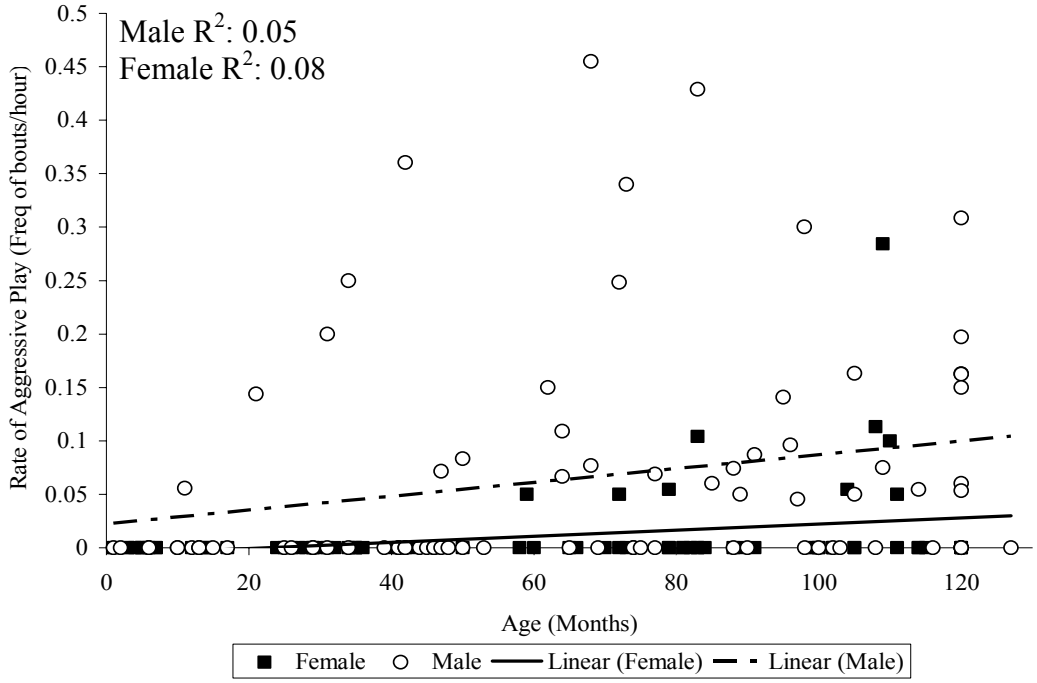

Figure 1.8. Rate (Freq of bouts/h) of aggressive play for individual male (*N* = 83) and female (*N* = 81) elephants at AENP from June to October, 2005. Statistical analysis for this graph can be found in Table 1.7, C:I-II, 1.

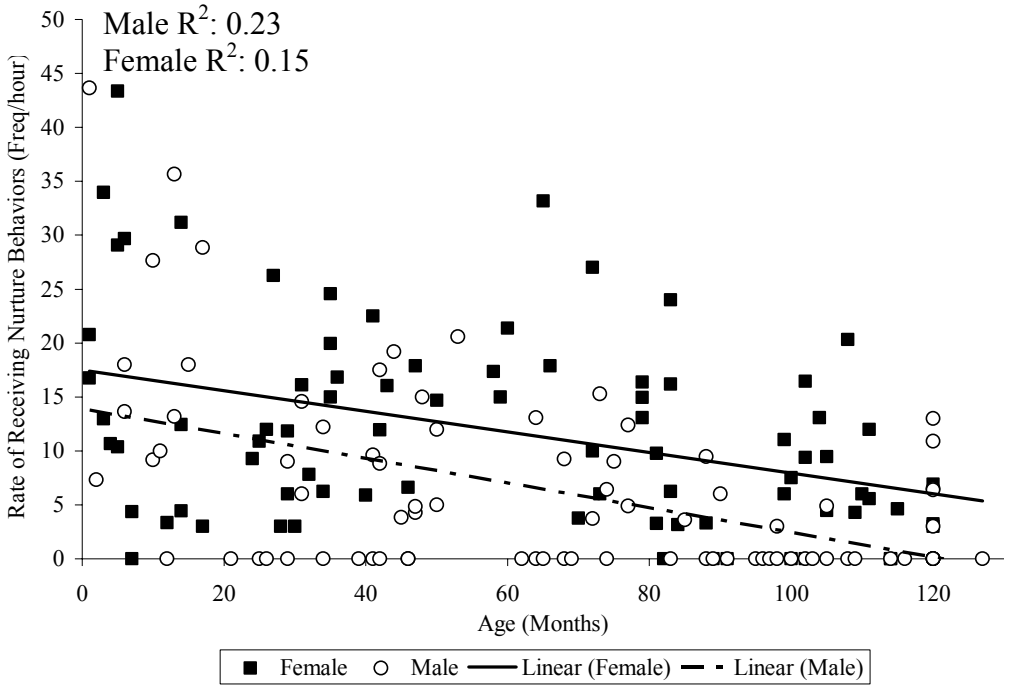

Figure 1.9. Rate (Freq/h) of receiving nurture behaviors per hour during focal observations for individual male (N = 83) and female (N = 81) elephants at AENP June to October, 2005. Statistical analysis for this graph can be found in Table 1.9, A:I-II, 1.

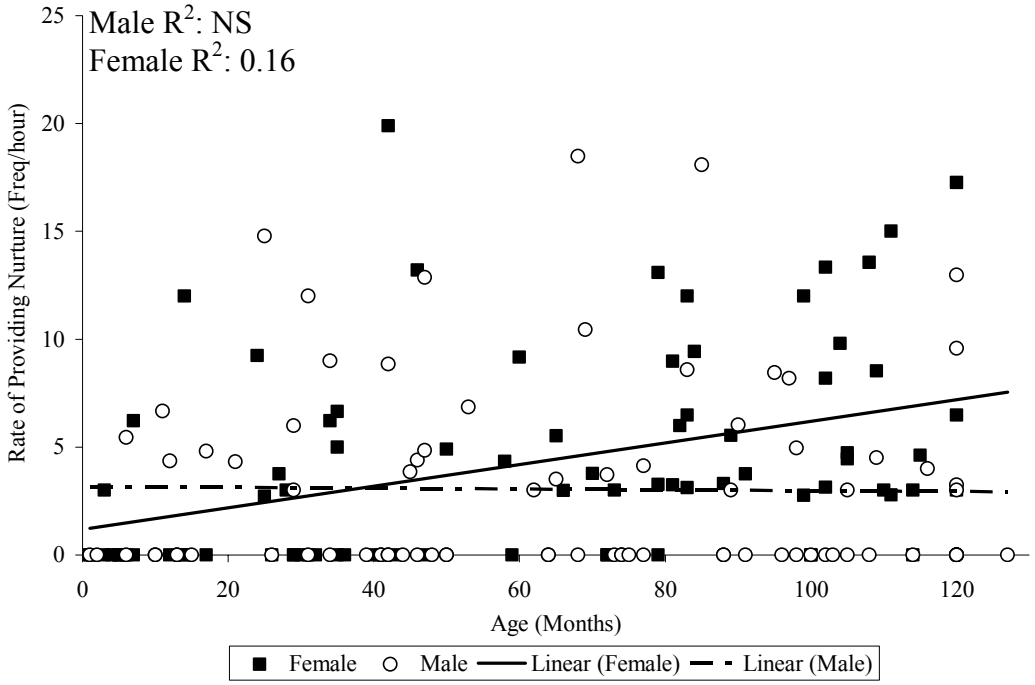

Figure 1.10. Rate (Freq/h) of providing nurture behaviors during focal observations for individual male (N = 83) and female (N = 81) elephants at AENP June to October, 2005. Statistical analysis for this graph can be found in Table 1.6, B:I-II, 1.

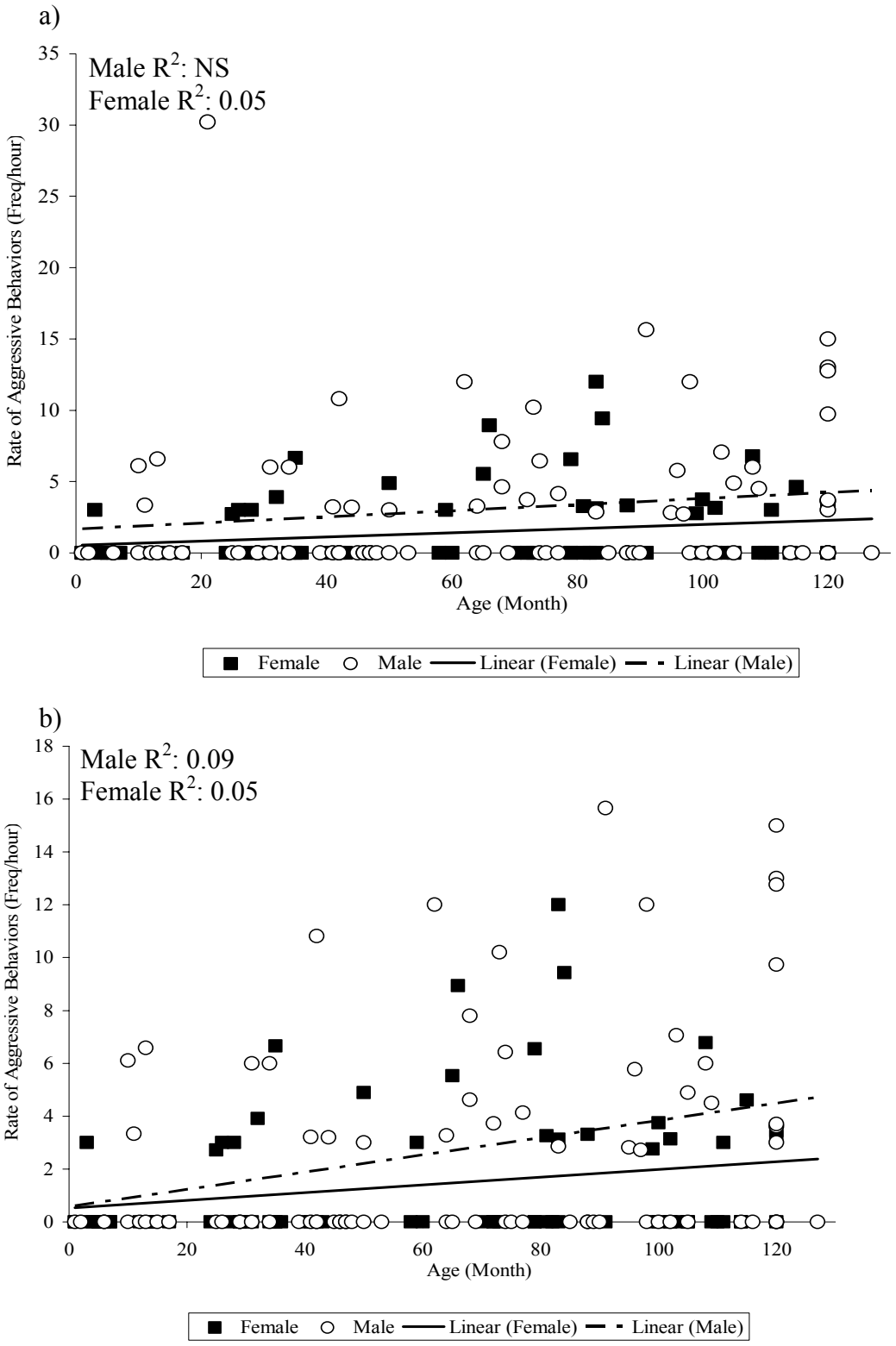

Figure 1.11. Rate (Freq/h) of sending aggressive behaviors per hour during focal observations. Figure (a) represents 83 male and 81 female elephants and Figure (b) represents the developmental trend with removal of one male outlier (10-fold increase from mean) at AENP June to October, 2005. Statistical analysis for this graph can be found in Table 1.9, B:I-II, 2. Note differences in y-axis scales for the two figures

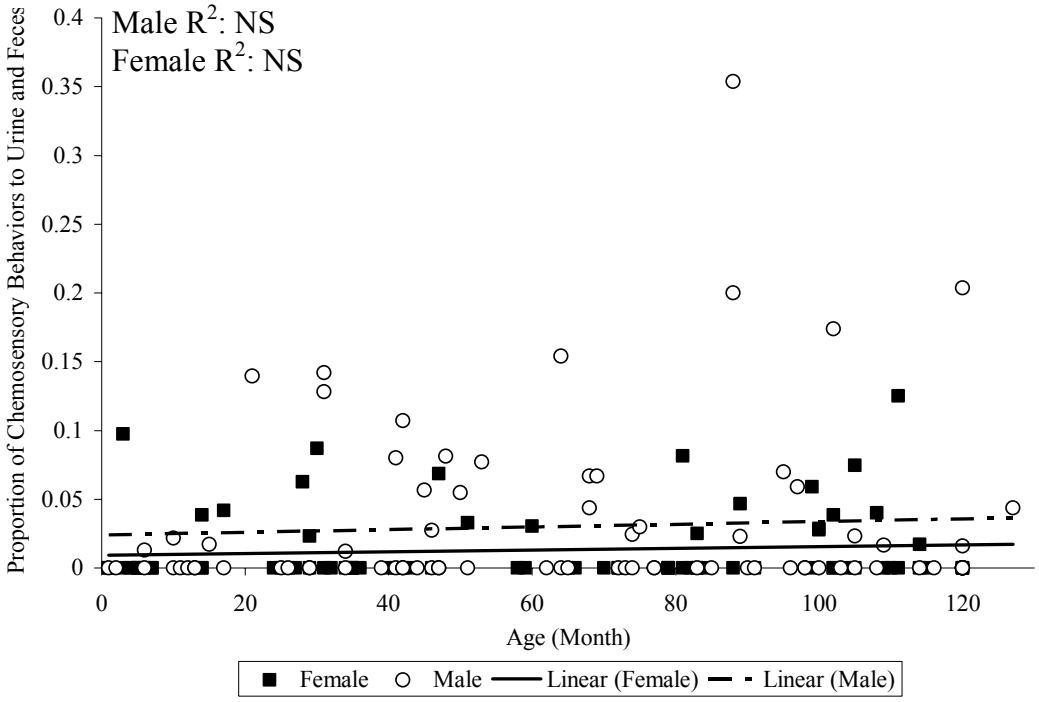

Figure 1.12. Proportion of chemosensory behaviors directed to urine and feces from individual male (N = 83) and female (N = 81) elephants at AENP June to October, 2005. Statistical analysis for this graph can be found in Table 1.10, B:II, 3.

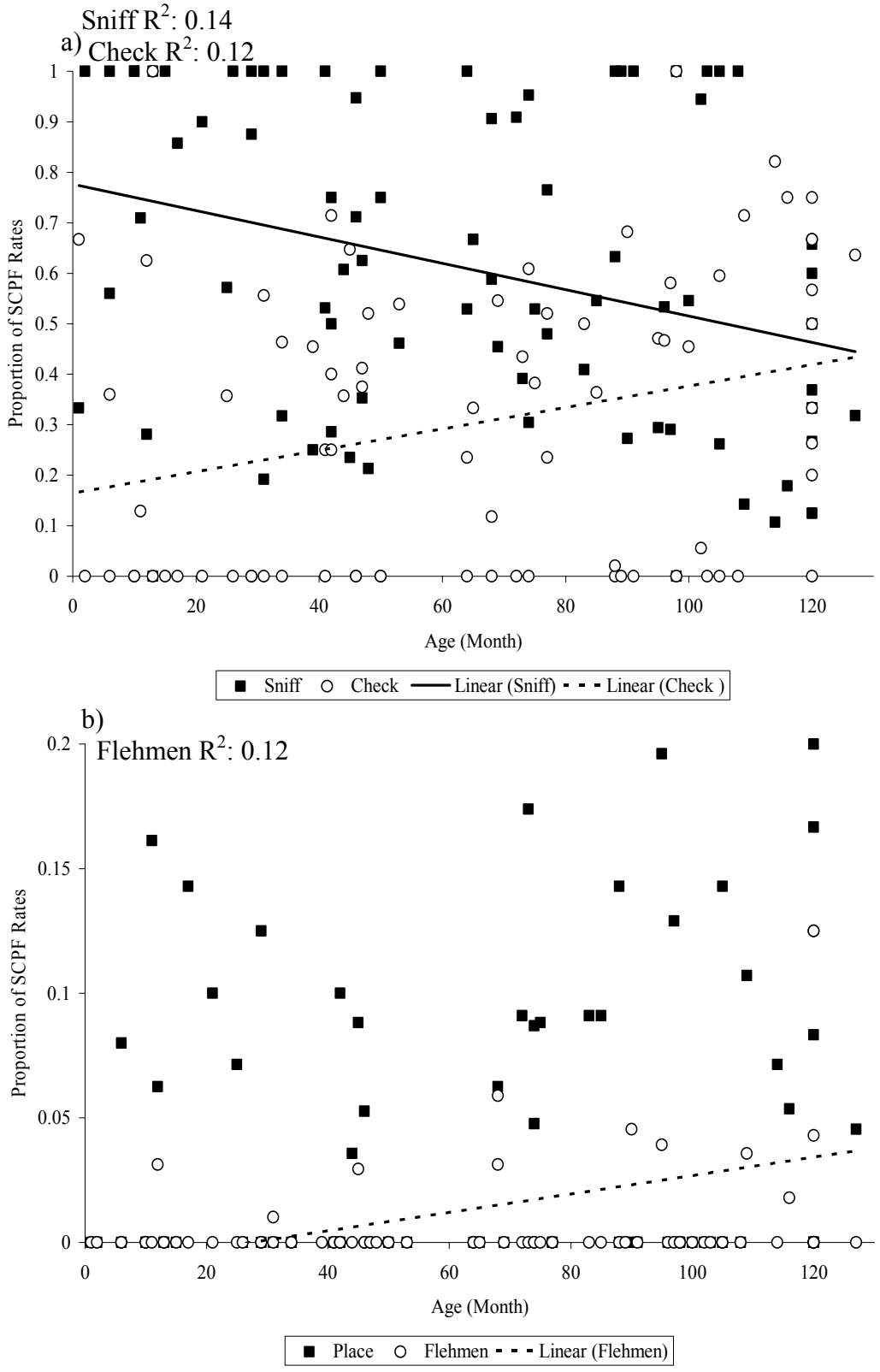

Figure 1.13. Proportion of (a) sniff, check and (b) place, flehmen behaviors (Place was developmentally not significant, thus trend line not shown) from individual male ($N = 81$) elephants at AENP June to October, 2005. Statistical analysis for this graph can be found in Table 1.11, B:I, 1-2,4.

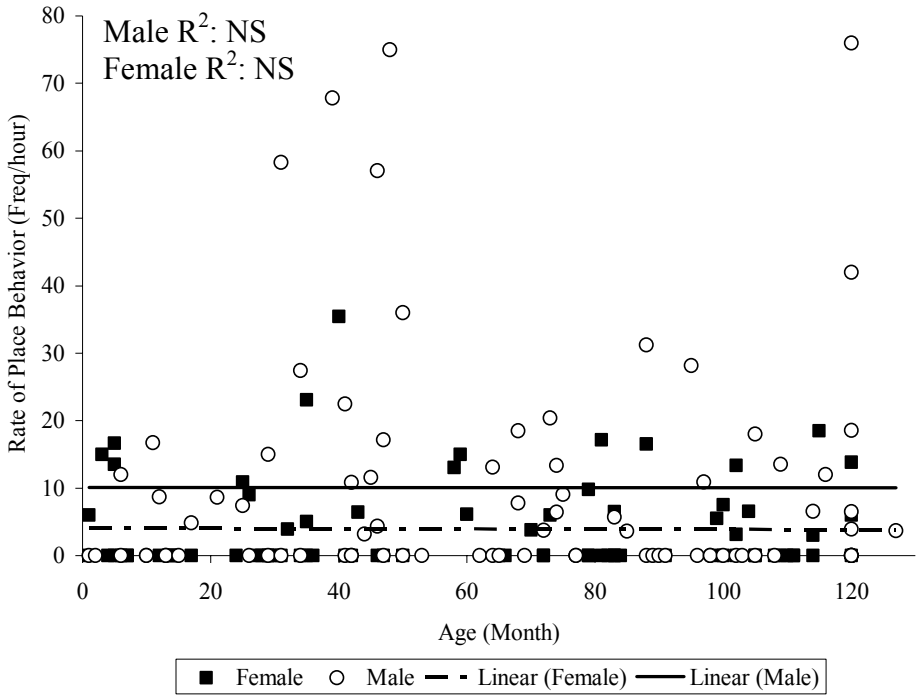

Figure 1.14. Total rate (Freq/h) of place behavior from individual male (N = 83) and female (N = 81) elephants at AENP June to October, 2005. Statistical analysis for this graph can be found in Table 1.11, A:II, 3.

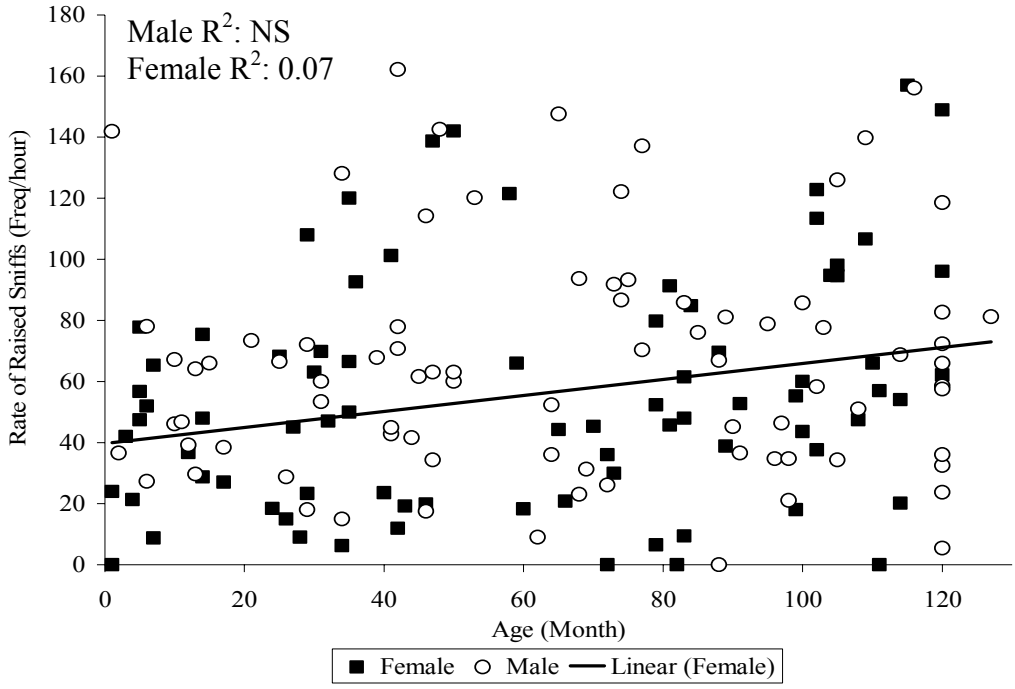

Figure 1.15. Total rate (Freq/h) of raised sniffs (horizontal + periscope) from individual male (N = 83) and female (N = 81) elephants at AENP June to October, 2005. Statistical analysis for this graph can be found in Table 1.11, A:I, 5.

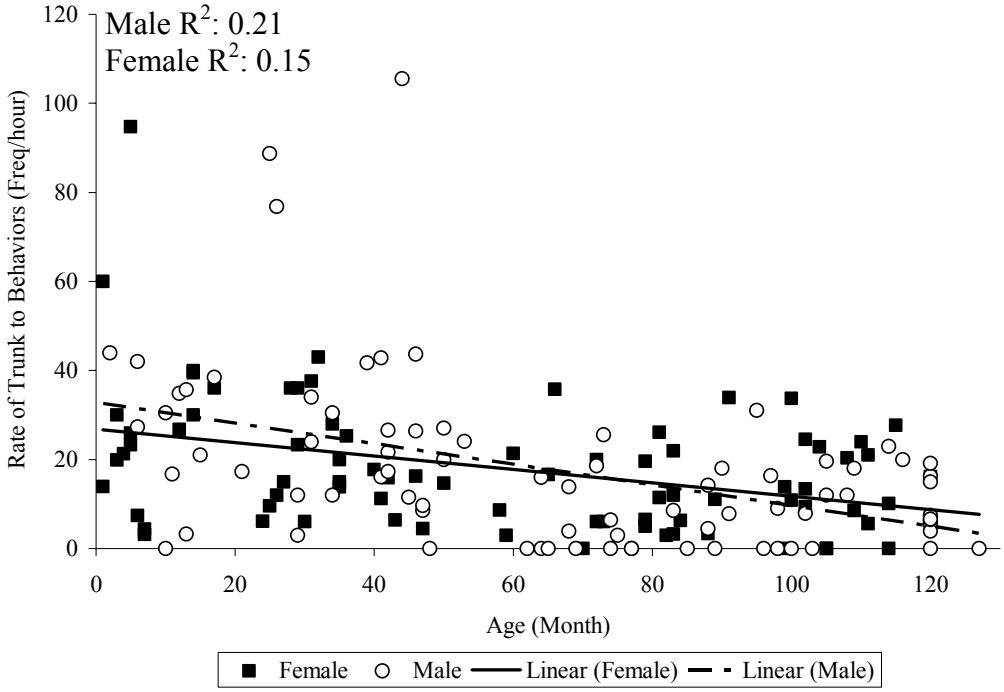

Figure 1.16. Total rate (Freq/h) of trunk-tip contacts to conspecifics from individual male (N = 83) and female (N = 81) elephants at AENP June to October, 2005. Both males (R^2=0.21, t=-4.59, P=<.0001) and females (R^2=0.15, t=-3.71, P=0.0004) decreased rate of trunk-tip contacts to conspecifics.

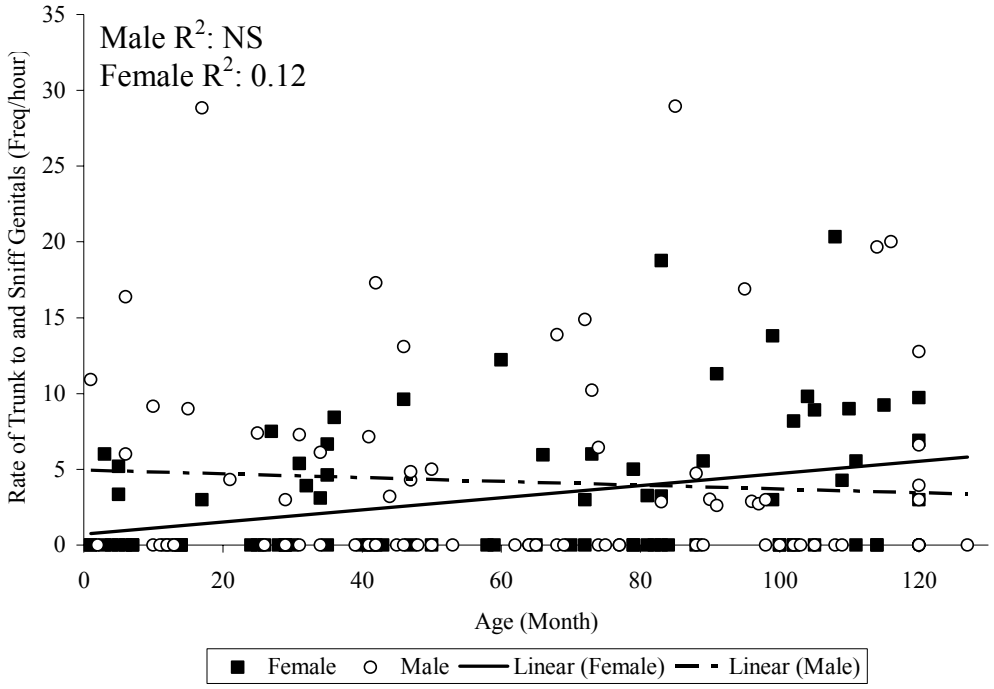

Figure 1.17. Total rate (Freq/h) of trunk-tip and sniff genitals of conspecifics from individual male (N = 83) and female (N = 81) elephants at AENP June to October, 2005. Females increase in rate of genital contacts across time (R^2=0.12, t=3.2, P=0.002). There was an interaction by covariates (sex x age) ($F_{1,160}$=5.15, P=0.025).

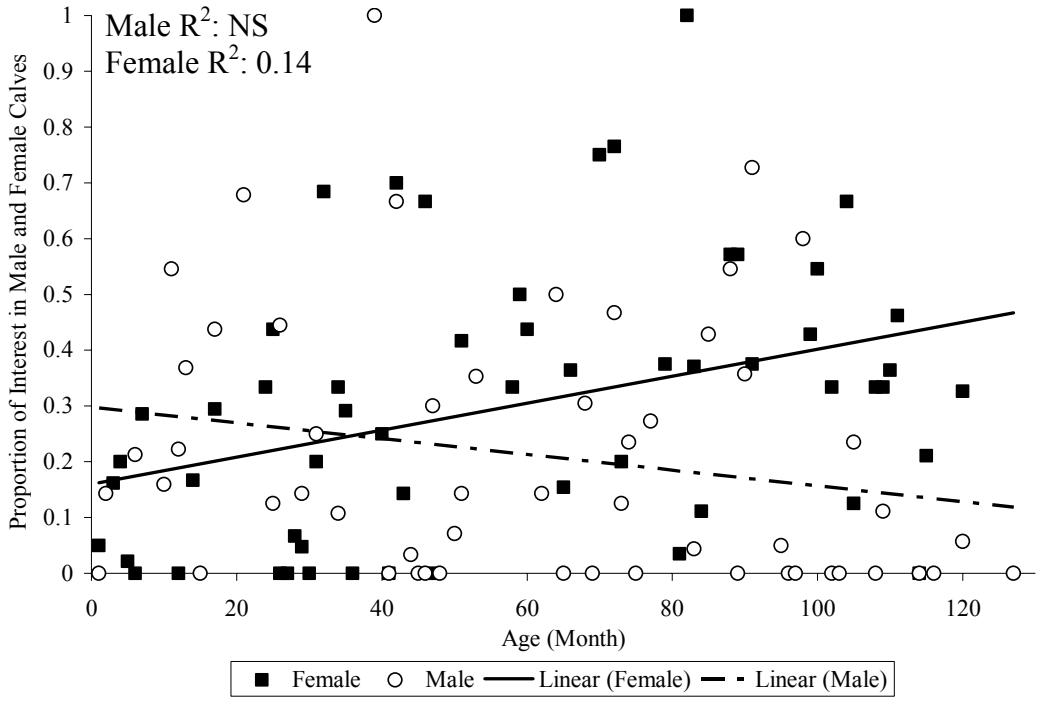

Figure 1.18. Proportion of chemosensory and trunk tip contact behaviors directed to male and female calves from individual male (N = 55) and female (N = 56) elephants at AENP from June to October, 2005. Statistical analysis for this graph can be found in Table 1.12, A:I-II, 7.

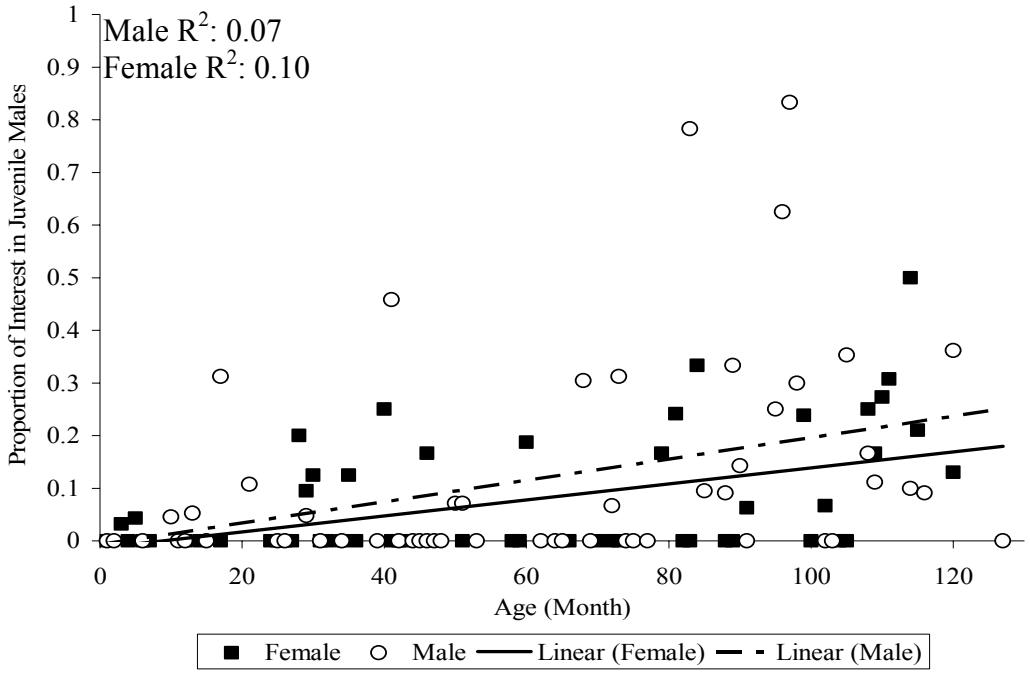

Figure 1.19. Proportion of chemosensory and trunk tip contact behaviors directed to juvenile males from male (N = 55) and female (N = 56) elephants at AENP from June to October, 2005. Statistical analysis for this graph can be found in Table 1.12, A:I, 6.

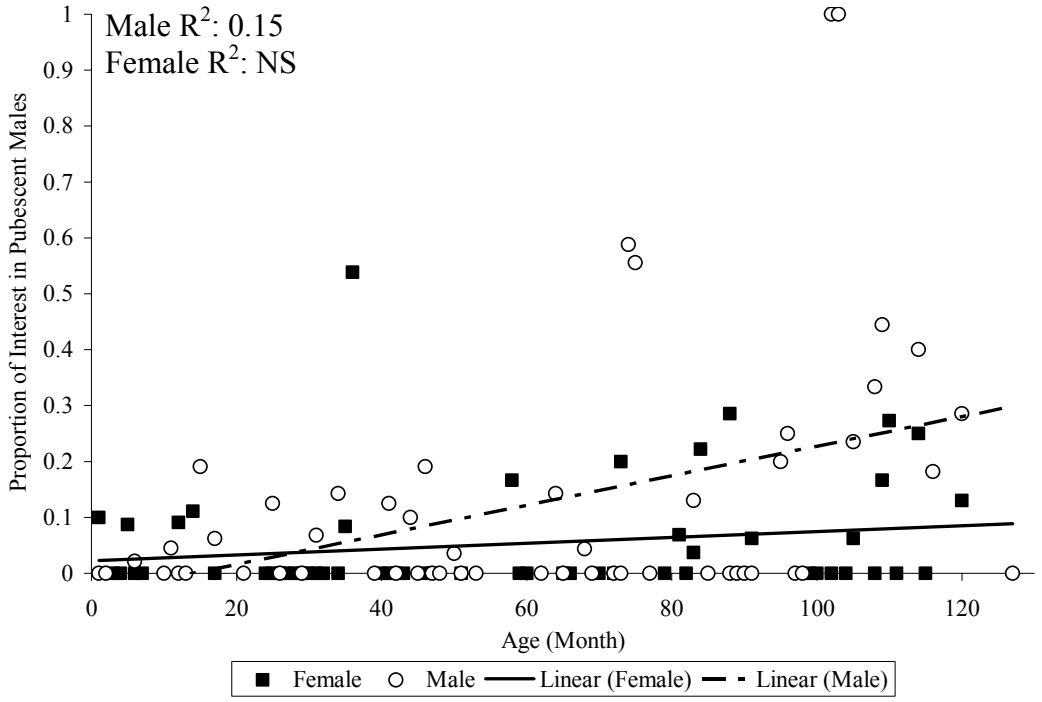

Figure 1.20. Proportion of chemosensory and trunk tip contact behaviors directed to pubescent males from male (N = 55) and female (N = 56) elephants at AENP from June to October 2005. Statistical analysis for this graph can be found in Table 1.12, A:I-II, 4.

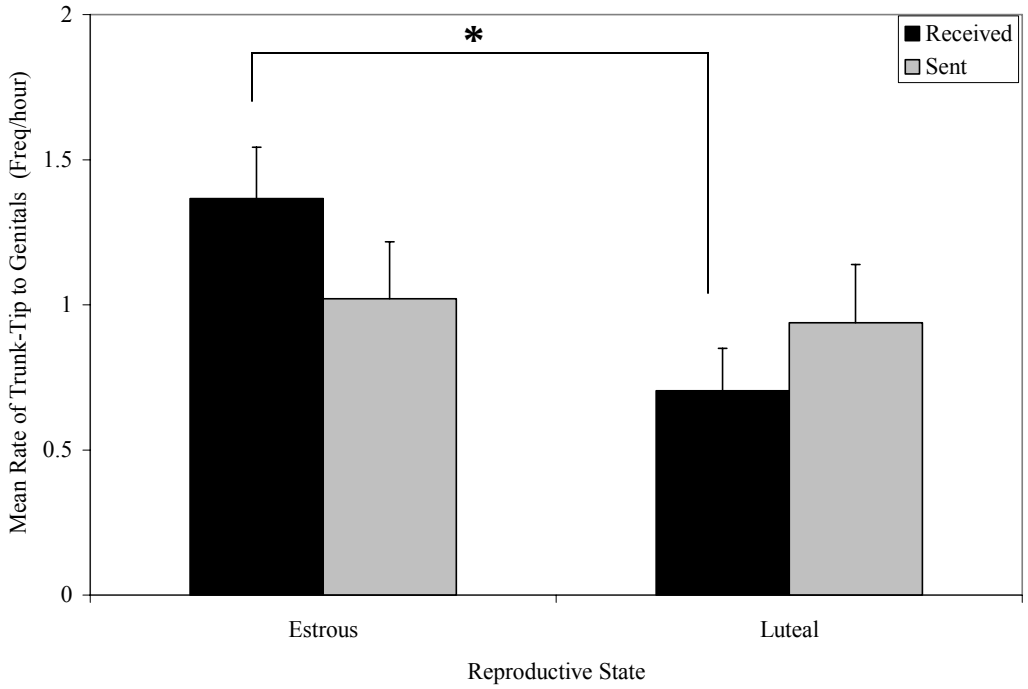

Figure 2.1. Mean (± SE) rate (Freq/h) of sent and received trunk-tip to the genitals between estrous (*N*=11) and non-estrous (*N*=10) females at nine zoological facilities from March – July, 2006. **P*<0.05 one-way ANOVA

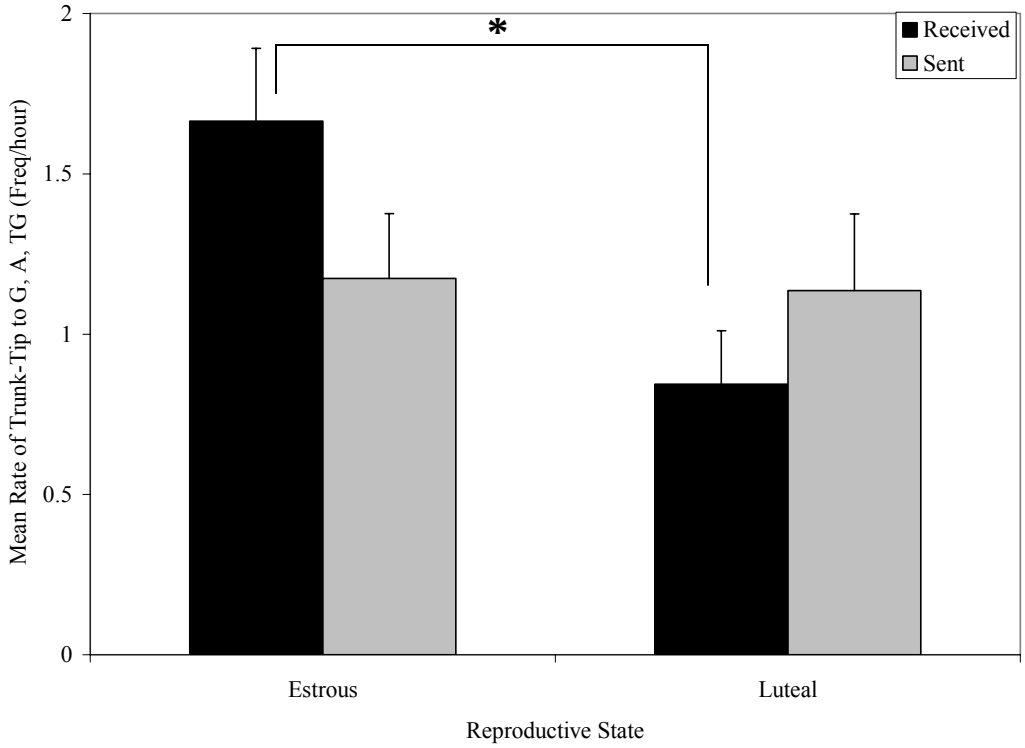

Figure 2.2. Mean (± SE) rate (Freq/h) of sent and received trunk-tip to the genitals, anus, and temporal glands between estrous (*N*=11) and non-estrous (*N*=10) females at nine zoological facilities from March – July, 2006. **P*<0.05 one-way ANOVA

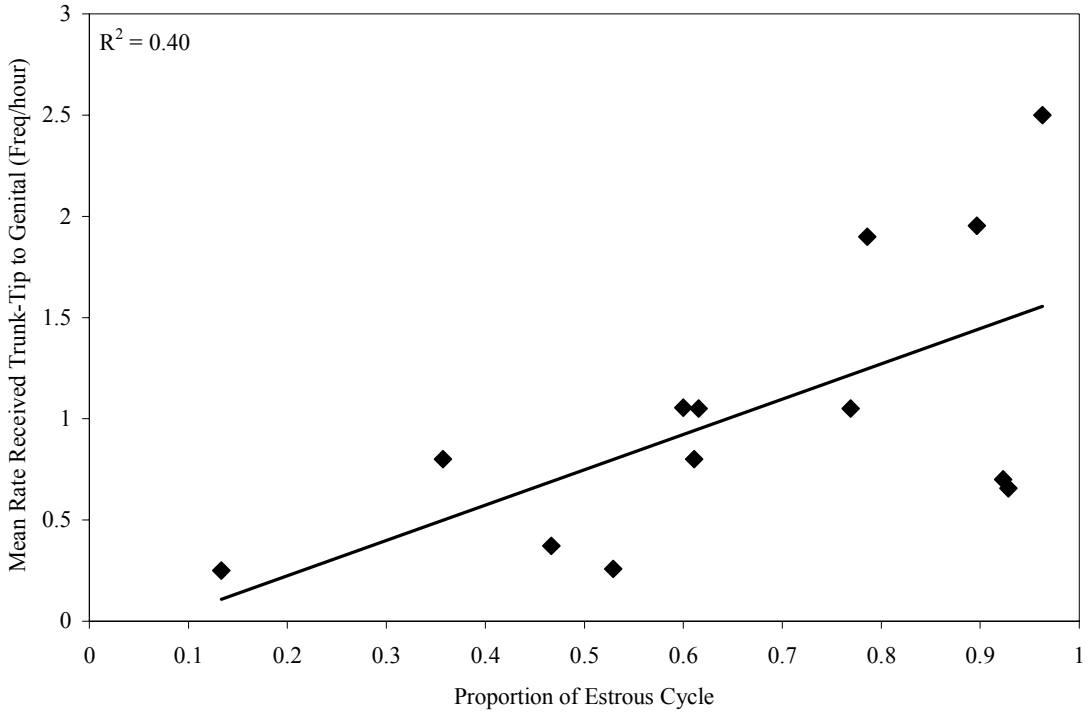

Figure 2.3. Mean rate (Freq/h) of trunk-tip to the genitals received by female African elephants (*N*=13) compared across proportion of each reproductive cycle (0 = beginning of the luteal phase to 1 = ovulatory LH2 peak).

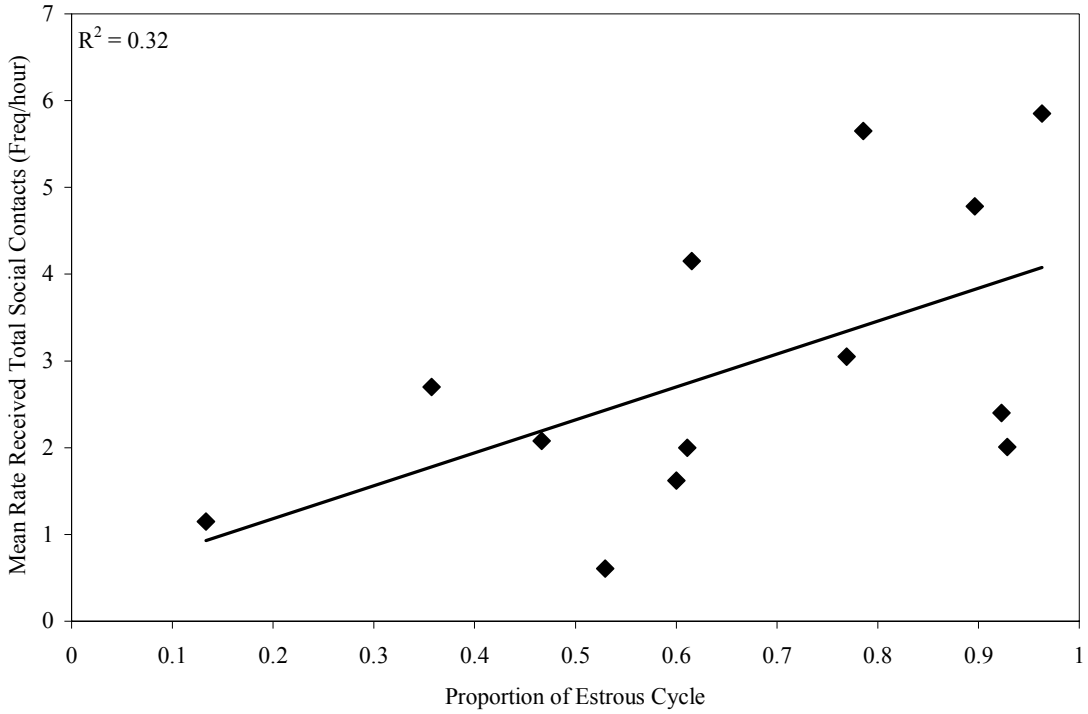

Figure 2.4. Mean rate (Freq/h) of total social interactions received by female African elephants (*N*=13) compared across proportion of each reproductive cycle (0 = beginning of the luteal phase to 1 = ovulatory LH2 peak).

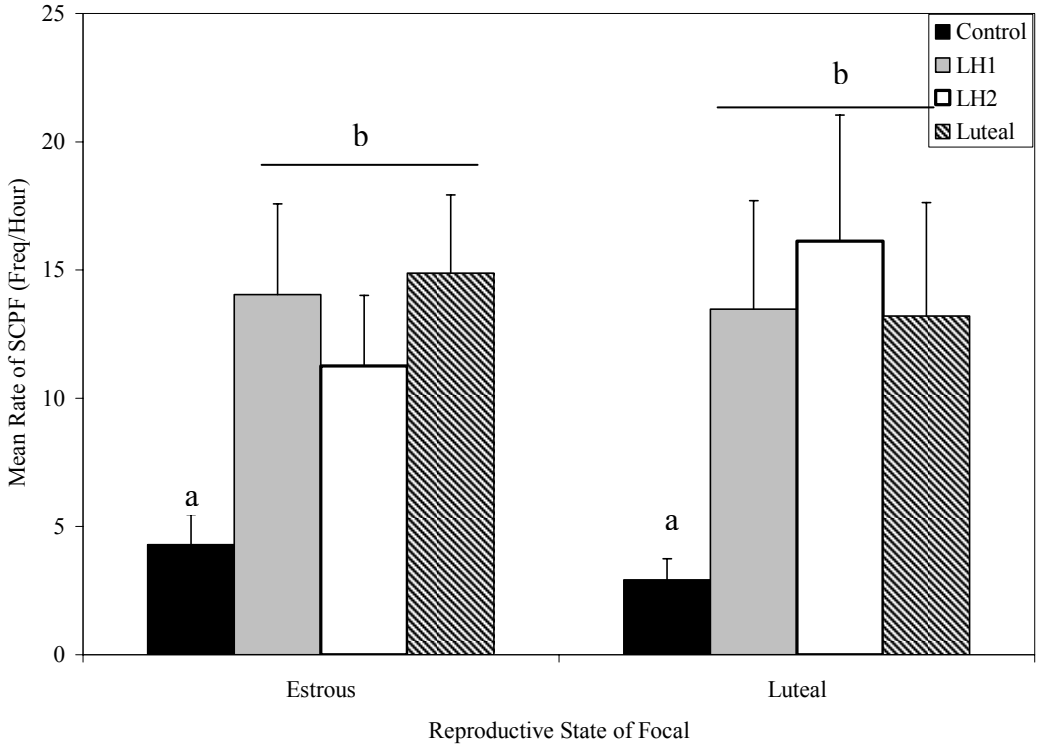

Figure 2.5. Mean (± SE) rate (Freq/h) of chemosensory behaviors (Sniff, Check, Place, Flehmen) to the control, LH1, LH2, and Luteal samples from females in their estrous (*N*=11) and non-estrous (*N*=10) phase of the reproductive cycle at nine zoological facilities from March – July, 2006. (Sample effect: *P*=<0.001; Tukey-HSD, *P*<0.0; see Table 2.7)

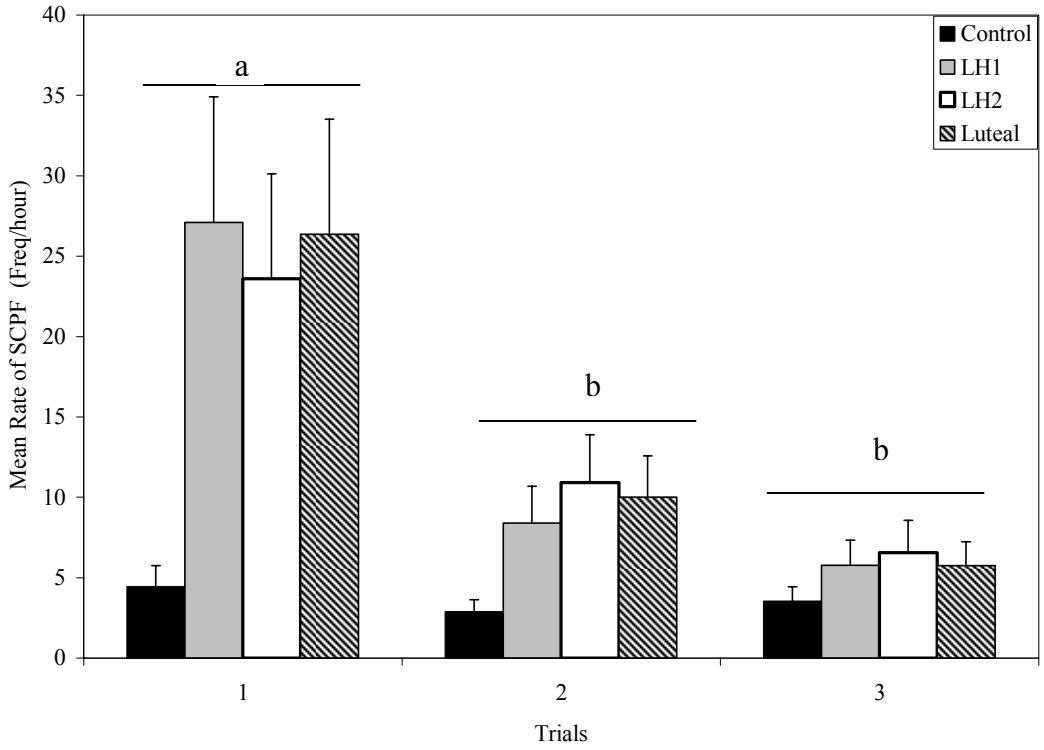

Figure 2.6. Mean (± SE) rate (Freq/h) of chemosensory behaviors (Sniff, Check, Place, Flehmen) to the control, LH1, LH2, and Luteal samples from reproductively active females (estrous + non-estrous phase) (*N*=21) across three trials at nine zoological facilities from March – July, 2006. (Sample*Day effect: $P<0.001$, Tukey-HSD, $P<0.05$; see Table 2.7)

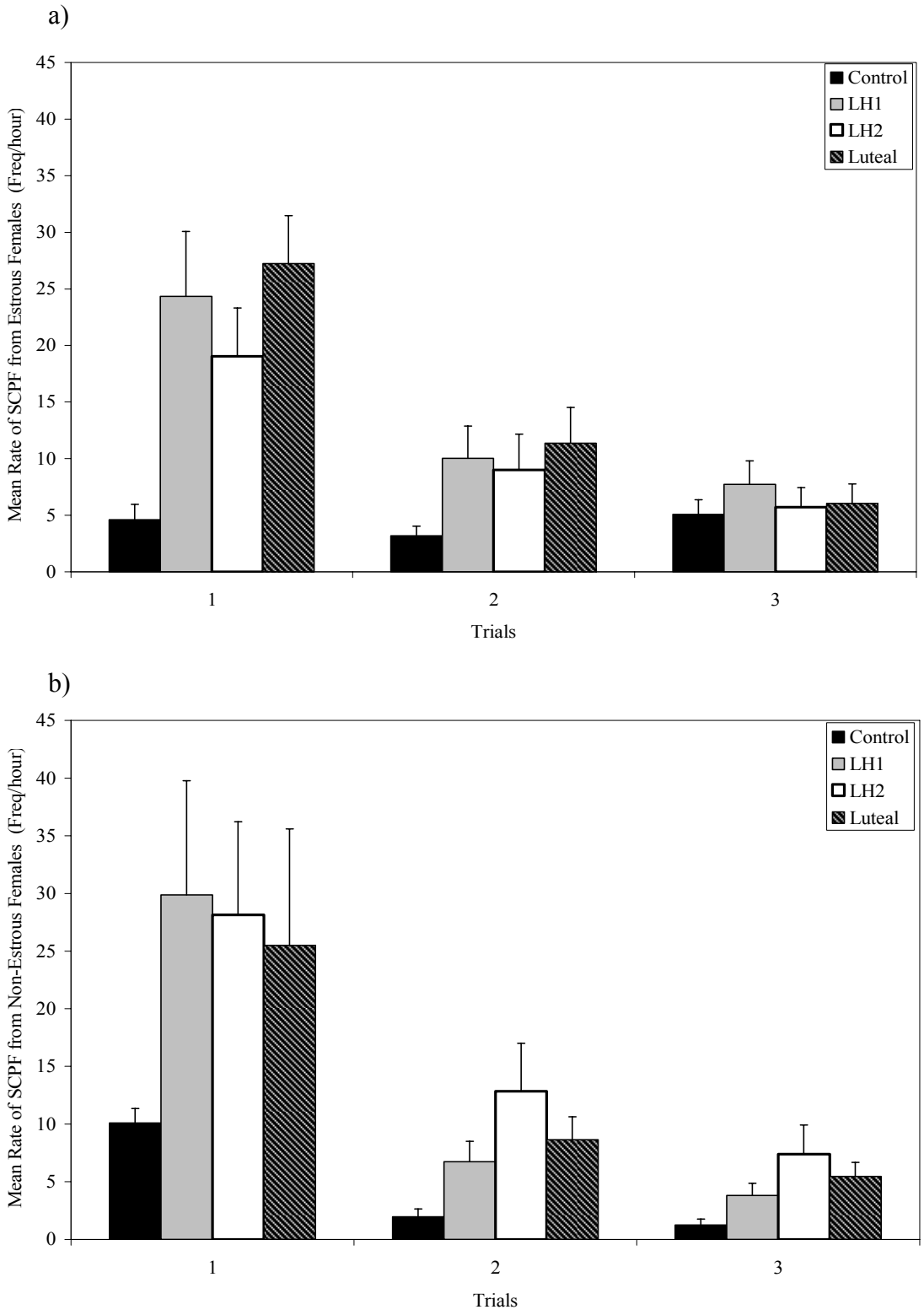

Figure 2.7. Mean (± SE) rate (Freq/h) of chemosensory behaviors (Sniff, Check, Place, Flehmen) to the control, LH1, LH2, and Luteal samples from (a) estrous (*N*=11) and (b) non-estrous (*N*=10) females across three trials at nine zoological facilities from March – July, 2006. (See Table 2.7 for statistical analysis)

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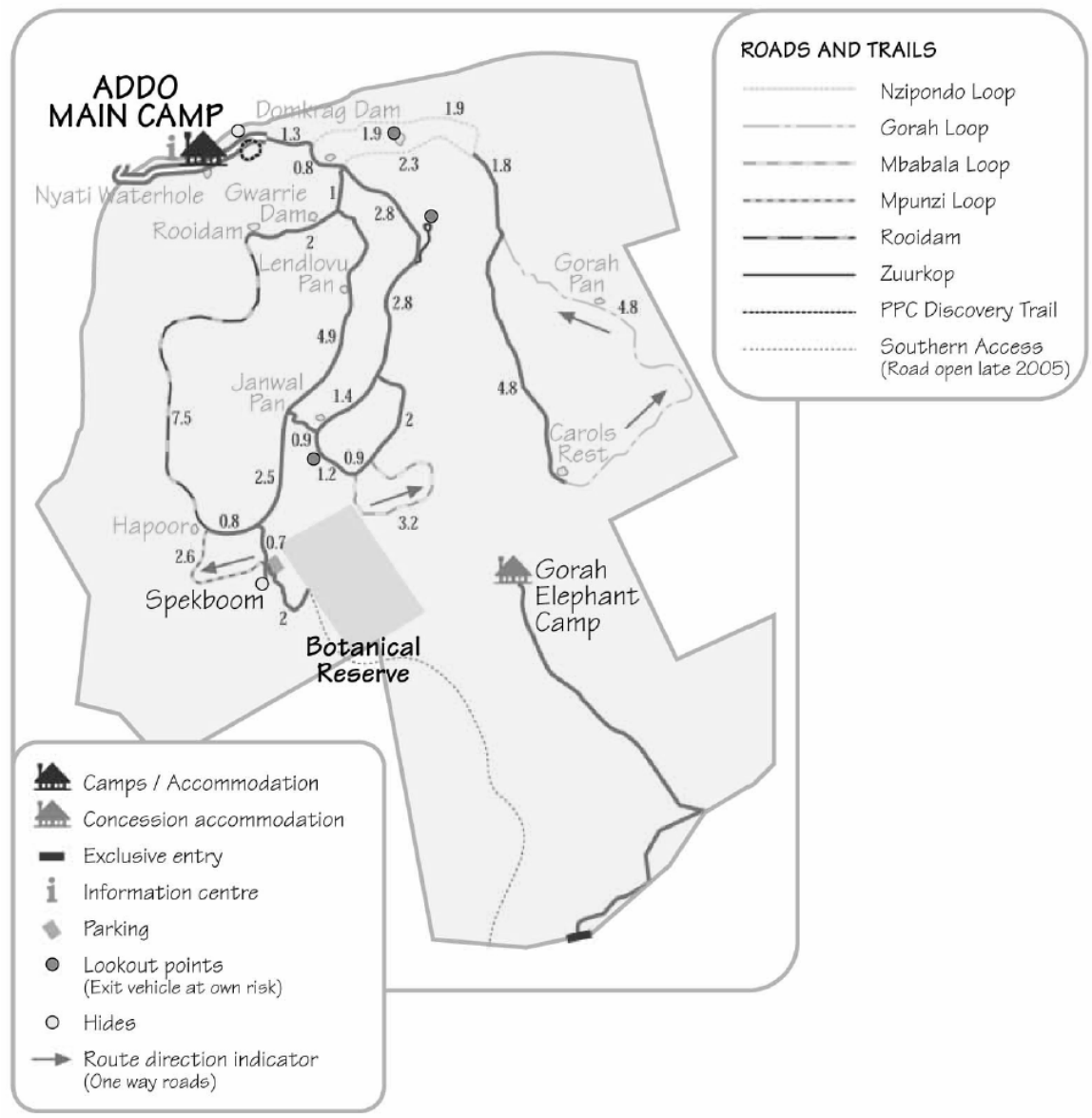
APPENDICES

Appendix A

MAP OF ADDO ELEPHANT NATIONAL PARK

Map shows main elephant camp, as reproduced from Addo Elephant National Park Official guide (2005). Map credits to Gillian Morgenrood, South African National Parks.



Appendix B

DIFFERENTIATION OF TRUNK OVER-BACK BEHAVIORS

Figure B. 1. Males often would place the trunk over another's back from behind (sagittal plane position), as shown above, while females would place or wrap the trunk over back from the side of an individual (transverse plane position).

Appendix C

ACTIVITY LEVEL OF ESTROUS AND NON-ESTROUS FEMALES

The activity level, measured through state behaviors (Table 2.4), was described in the methods of the second study; however, it was not relevant to the hypotheses. Female elephants spent the greatest proportion of the time observed eating (0.45 ± 0.03), walking (0.17 ± 0.01), standing (0.16 ± 0.02), and swaying (0.09 ± 0.04) compared to all other state behaviors. These four behaviors were analyzed with repeated measures ANOVA across the five days of the study while accounting for the effect estrous state. This determined if the level of activity varied across days with urine present in the yard (Days 2, 3, and 4) and days without urine in the yard (Days 1 and 5). Samples were only present during the morning sessions; therefore, only AM sessions were compared across the five days. The activity level during the morning observations significantly varied across day for eating and walking, however was similar between estrous and non-estrous females for all four behaviors (Table C.1). There was a significant difference in the activity level of walking and eating between Day 1 (baseline) and Day 2 (first day of bioassays) (Table C.1). Using a paired t-test to compare all 21 females (because there was no difference in behavior) between day one and day two also showed a significant difference between the proportion of time spent eating (decrease from Day 1 to 2: t-ratio= -3.22, df=20, P=0.004) and walking (increase from day 1 to 2: t-ratio=3.41, df=20, P=0.003) (Fig. C.1).

Table C.1. Results of a repeated measures ANOVA comparing the morning activity level (proportion of state behaviors during observation session) of estrous (*N*=11) and non-estrous (*N*=10) female African elephants across a five day study at nine zoological facilities from March – July, 2006.

Behavior		**DF**	**F Ratio**	**Prob > F**	**Tukey HSD P>0.05**
Eat	Day	4, 76	2.82	0.03*	Between Day 1 - 2
	Phase	1, 76	0.53	0.47	
Stand	Day	4, 76	1.95	0.11	
	Phase	1, 76	0.13	0.72	
Sway	Day	4, 76	0.78	0.54	
	Phase	1, 76	0.003	0.95	
Walk	Day	4, 76	3.55	0.01*	Between Day 1 - 2
	Phase	1, 76	1.62	0.22	

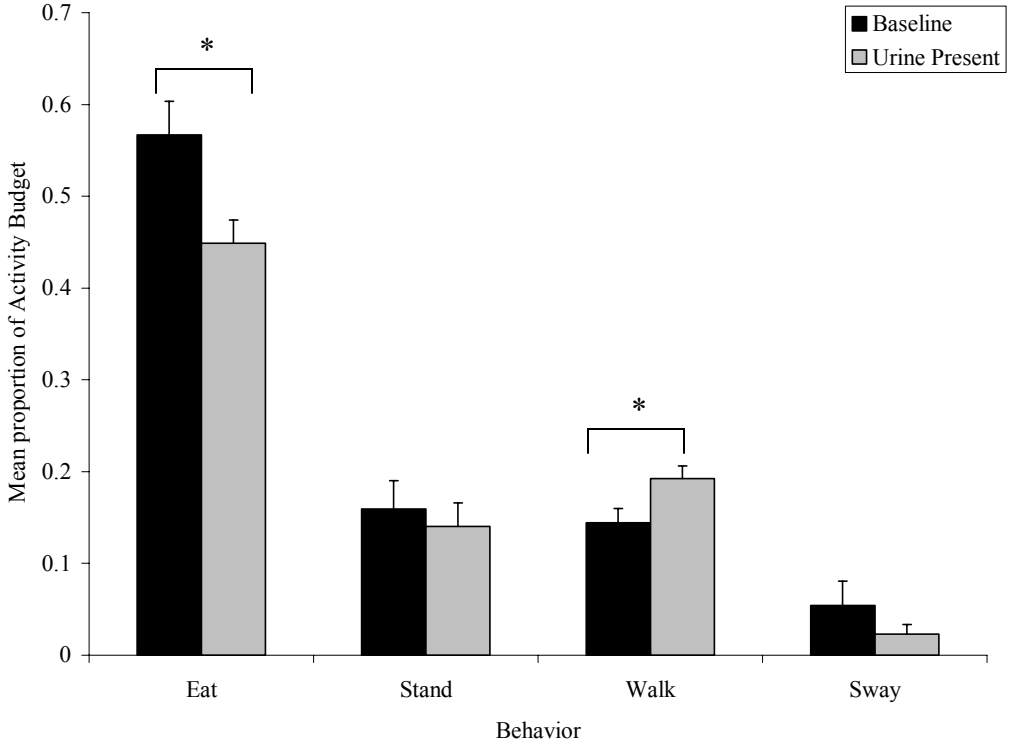


Figure C.1. Mean (± SE) proportion of focal scan spent eating, standing, walking and swaying. The activity budget of females (*N*=21) were compared between the morning baseline mean of the first day and the morning bioassay mean of the first trial. *P>0.01 using a Paired t-Test