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Chemosensory Behavior and Development of African Male Elephants (Loxodonta Africana)

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CHEMOSENSORY BEHAVIOR AND DEVELOPMENT OF AFRICAN MALE

ELEPHANTS (*LOXODONTA AFRICANA*)

by

Kathryn R. Bagley

(Under the Direction of Bruce Schulte)

ABSTRACT

African elephants are a polygynous species that raise offspring in a matriarchal society. Males disperse, spend time in male groups, and search for mates when mature. Urinary chemical signals play an important role in detecting reproductively active females. African male elephants develop movement, social and chemosensory behaviors over four major life changes. The first goal of this study was to compare behavior among four age classes of wild African male elephants in Addo Elephant National Park, South Africa. The second goal was to determine if adult captive African male elephants distinguish between urine from conspecific females in receptive and non-receptive estrous stages. Behaviorally, younger male elephants were more investigative, while older males exhibited more physical social interactions. The development of chemosensory behavior appeared to parallel general behavioral patterns in this polygynous species. Captive male elephants discerned between the two urine types, bolstering the pursuit to identify the estrous pheromone in African elephants.

INDEX WORDS: Chemical signals, Chemosensory, Development, Flehmen, *Loxodonta Africana*, Polygyny

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DEDICATION

This thesis is dedicated to the person who has always believed in me.

Thanks, Mom

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

GENERAL INTRODUCTION

Competition between males for reproductive females is common in polygynous

mating systems, such as resource and female defense polygyny (Emlen & Oring 1977,

Krebs & Davies 1993, Alcock 2001, Shuster & Wade 2003). Whether they are defending

groups of females or defending a territory containing females, males develop behaviors

that allow them to compete for female access (Krebs & Davies 1993, Alcock 2001,

Shuster & Wade 2003). In polygynous species in which females form matriarchal

groups, it has been suggested that males disperse in order to learn competitive and

reproductive behavior (Dobson 1982, Main & Colbentz 1990). With a variety of

different life changes and social environments experienced by these males, it is unclear

the age at which such behaviors needed for competition and reproduction are developed.

African elephants are a polygynous species that raise offspring in a matriarchal

society. Males disperse, spend time in male groups, and search for mates when mature.

Therefore, elephant male reproductive behavior involves locating estrous females.

Urinary chemical signals play an important role in detecting reproductively active

females. Asian male elephants are able to assess the estrous state of conspecific females

using a chemical signal, specifically a pheromone, found in female urine (Rasmussen et

al 1996, a, b, 1997). Developing investigatory behaviors that allow for reproductive

chemical signal assessment by elephant males is important in searching for estrous

females (Rasmussen & Hultgren, Rasmussen & Munger 1996, Rasmussen et al. 1996a).

This study compared movement behaviors, social interactions, and investigatory

trunk behaviors among four different age classes of African male elephants at Addo

Elephant National Park, South Africa. Understanding differences in behavior performed by males at AENP at these life stages may provide a clearer understanding of how competitive and reproductive male behavior develops in a variety of social environments. Furthermore, this study examined the importance of chemical signals in male discernment of female estrous state in African elephants. A specific reproductive chemical signal has not been identified in African elephants (Rasmussen 1998, Rasmussen & Schulte 1998). Therefore, male discernment between urine from receptive and non-receptive conspecific females would provide a probable location of a female-to-male signal and warrant further efforts to identify the properties of that signal (Goodwin et al. 2004, Schulte et al. 2004).

CHAPTER II

BEHAVIORAL MALE DEVELOPMENT IN A POLYGYNOUS SPECIES: THE
AFRICAN ELEPHANT (*LOXODONTA AFRICANA*)

INTRODUCTION

There are many forms of polygyny, such as female defense and resource defense,
in which males develop behaviors used in male-male-competition for access to females in
order to mate (Emlen & Oring 1977, Krebs & Davies 1993, Alcock 2001, Shuster &
Wade 2003). For example, adult male elephant seals physically compete for access to
reproductive females in a form of female defense polygyny (McCann 1981). On the
other hand, male Grevy's zebra defend small territories and attempt to mate with females
as they pass through male territories in a form of resource defense polygyny (Ginsberg
1988, 1989). Yet polygynous mating systems are not always easy to place categorically.
An unclear form of polygyny is demonstrated in elephants, in which there is no
pronounced breeding season and males are not territorial. Adult male elephants actively
search for ovulating females and compete with other males for mating rights (Buss 1961,
Laws 1969, Eisenberg et al. 1971, Douglas-Hamilton 1972). There are a variety of
behaviors that may be necessary for polygynous males to acquire in order to mate
successfully. For instance, Asian male elephants use investigative trunk behaviors to
assess female reproductive state and locate ovulating females (Rasmussen & Hultgren
1990, Rasmussen et al. 1996a, 1997, Rasmussen 1998). However, it is unclear how and
when such behaviors develop as males mature.

Differential social environments experienced by polygynous males as they mature
also may affect their behavioral development. Changes in male social environment are

demonstrated by polygynous species in which both males and females are born into matriarchal groups. Matriarchal groups occur when a group of females and subadult young of both sexes are led by a particular female (Krebs & Davies 1993, Alcock 2001, Shuster & Wade 2003). Males born into female societies commonly disperse upon reaching sexual maturity (Dobson 1982, Holekamp 1984, Johnson 1986, Koopman et al. 2000, Ji et al. 2001). Since females remain part of a matriarchal society throughout their lives (Krebs & Davies 1993, Alcock 2001, Shuster & Wade 2003), young females have continuous opportunities to learn behaviors from older members of the same sex. Males that are born into matriarchal societies lack as much opportunity to learn behaviors, especially those related to reproduction, from sexually mature males. Therefore, it is possible that males disperse from matriarchal societies in part to develop behaviors needed for adulthood. Main & Colbentz (1990) suggested this hypothesis among several others regarding reasons for mammalian sexual segregation.

Dispersal marks one of the major transitional stages in social environment and life stage experienced by sexually segregated species. Prior to dispersal, polygynous male mammals are dependent on their mothers. Furthermore, these males are becoming accustomed to their new environment while developing movement coordination (Moore 1985). After weaning males become less dependent on their mother and increase their level of interaction, such as play, with same age conspecifics (Alberts 1981). Similar events have been reported in other mammals. For example, Meaney and Stewart (1981) noted that once weaned, juvenile rats began initiating play such as jumping and chasing each other. This social interaction prepares polygynous males for dispersal from a female-dominated society to a male-male competitive environment. Male dispersal is

common among many sexually segregated species, as demonstrated by red deer (Darling 1937), white-tailed deer (Kammermeyer & Marchinton 1976), mountain sheep (Geist 1971), and Asian and African elephants (Buss 1961, Laws 1969, Eisenberg et al. 1971). With maturity, rats and many other mammals show a decrease in play behavior and an increase in sexual behavior (Meaney and Stewart 1981).

Sexual dimorphism in polygynous species becomes most apparent well after dispersal. Specifically, males tend to be much larger in size than females (Emlen & Oring 1977, Krebs & Davies 1993, Alcock 2001, Shuster & Wade 2003). Furthermore, males often possess particular physical accessories used for male-male competition, such as horns, antlers, and tusk (Krebs & Davies 1993, Alcock 2001). Elephant males demonstrate physical and social sexual dimorphism characteristic of polygynous species (Buss 1961, Eisenberg et al. 1971, Douglas-Hamilton 1972). For example, by the time African male elephants reach 45 years of age, they are twice the height and weight of adult females (Poole 1994). Male tusks, which are used to compete with other males for female access, are seven times longer and thicker than female tusks in adulthood (Eisenberg et al. 1971, Poole 1994).

African elephant calves of both sexes are born into complex, matriarchal family groups that consist of related females and other subadult offspring (Buss 1961, 1966, Eisenberg et al. 1971, Douglas-Hamilton 1972). Male and female calves receive protection and care from many females in the group and interact with the same individuals (Dublin 1983). However, suckling and milk intake rates are higher for male calves than for female calves (Lee 1986). Greater investment in male calves by mothers may result in sexual dimorphism as adults (Lee and Moss 1986).

As calves are weaned and begin to forage for themselves, males begin to spend less time with their mothers in comparison to females of similar age (Lee 1986). Juvenile males begin to prepare themselves for independence from their mothers and natal herds, although they still return to their mothers occasionally for nursing or contact. Lee (1986) found that juvenile males interacted with peers outside their natal herd more often than juvenile females, leaving the natal herd for long periods to play with other juvenile males.

The social dynamics of males and females continue to diverge as they reach puberty. Both males and females become sexually mature between 9 and 15 years of age (Eisenberg et al. 1971, Laws 1969). Females remain with their natal herd and typically produce offspring two years after reaching sexual maturity (Laws 1969, Dublin 1983). Males disperse from the natal herd to join loosely organized bachelor herds or roam alone. Although pubescent males are capable of producing offspring, typically they are not able to successfully compete with older, larger males for access to females until their mid-twenties (Eisenberg 1971, Poole 1989a, b, 1994).

As elephant males enter adulthood, they experience a phenomenon defined as musth (Poole 1989a, b). Musth is an annually occurring period of heightened plasma testosterone levels, typically lasting 2-3 months resulting in greater aggressiveness towards other males. Urine dribbling, temporal gland secretions, and concomitant changes in the chemistry of these exudates, along with weight loss and increased association with females are all common during this period (Jainudeen et al. 1972, Poole & Moss 1981, Poole 1987, Rasmussen et al. 1996b, Schulte & Rasmussen 1999a, Rasmussen & Wittemyer 2002). Elephants do not experience a true musth until their mid to late twenties (Jainudeen et al. 1972, Poole 1987, 1994). Musth acts as an honest signal

of male condition and fitness (Maynard-Smith & Price 1973 Poole 1989 a, b). As a result, musth males are dominant over non-musth males regardless of differences in body size and possibly are more appealing to adult females (Sukumar & Gadgil 1988, Poole 1989a, b, Schulte & Rasmussen 1999).

In order to find females and mate, a male elephant must be able to locate and interpret chemical signals of females. The highly sensitive olfactory and vomeronasal (VNO) systems of African elephants provide evidence that they regularly use chemical signals to communicate (Wysocki & Meredith 1987, Rasmussen & Hultgren 1990, Rasmussen 1998). Detection of chemical signals is accomplished with the aid of discrete behaviors of the trunk. These behaviors transport chemicals to the olfactory or vomeronasal receptors. The most significant and prominent chemosensory behaviors in elephants have been defined as sniff, check, place and flehmen response (Schulte & Rasmussen 1999b; see Table 2.4 for definitions of behavior; see appendix for illustration). Sniff occurs when the bottom of the trunk tip hovers over a substance but does not make contact. Check is defined as an elephant placing the "finger" of the trunk tip into a substance. Elephants perform place when they put the entire circumference of the trunk tip on the substance. Flehmen occurs when the trunk tip makes contact with a substance, which is directly followed by the trunk tip being placed near the VNO ducts located in the roof of the mouth.

Flehmen response is thought to provide males with means of evaluating estrous status and is seen in most ungulates (Hart 1983). Furthermore, flehmen appears to act as a tool in allowing Asian male elephants to process the female conspecific sex pheromone, *Z*-7-docecen-1-yl acetate (Rasmussen et al. 1996a, 1997). Chemosensory behaviors,

including flehmen, are generally performed by all ages of African elephants. However, it is unclear whether the development of these behaviors parallels changes in activity patterns and social interactions as African male elephants mature.

Elephant males share with other polygynous males the need to learn behaviors related to reproduction (Sukumar 2003). In addition, African male elephants exhibit varying social environments throughout their life, which is also common in other polygynous species. Therefore, the goals of my study were to compare behavior performed by African male elephants among 4 distinct life stages and determine if behaviors potentially relating to mate searching change with age. Based on the long-term study by Moss (1996), I used four major life stages (age classes) to examine behavioral changes in male African elephants, namely: calves (<1-4 years old), juveniles (5-9 years old), pubescents (10-19) years old), and adults (>19 years old). Behaviors compared among the age classes included movement, social interactions, and chemosensory behaviors.

METHODS

Study Site and Study Population

This study was conducted on a closed population of African elephants at Addo Elephant National Park (AENP) during August-November, 2003. AENP is located 72 km northeast of Port Elizabeth in the Eastern Cape Province of South Africa. This national park was founded in 1931 to protect the remaining 11 elephants left in the area (Whitehouse & Hall-Martin 2000). An electric fence was constructed around the park in 1954 to keep the elephants inside and reduce the risk of poaching (Whitehouse 2001, 2002). The total size of AENP is 700 sq km, but the fenced area holding the elephant

population is 103 sq. km (Whitehouse & Hall-Martin 2000, Whitehouse et al. 2001) (Fig

2.1). As of November 2003, there were approximately 340 elephants in 6 elephant

family groups in this population at AENP, which represented the study population.

Males ranging from newborn calves to adult bulls made up about half of the park's

population. The two oldest bulls in the park were 50 years old (Whitehouse 2001).

Another 60 elephants were located in a separate fenced area within the greater AENP and

not studied for this project.

Identification of Elephants

An identification file of the elephant population at AENP as well as family trees

of the elephants' lineage was established by Whitehouse (2001) and updated by Loizi

(2004). Individual African male elephants were identified by ear tears, ear wrinkles, eye

wrinkles, and other miscellaneous physical features such as presence or absence of tusks,

body marks, and tail length (Whitehouse & Hall-Martin 2000; Whitehouse et al. 2001).

In August 2003, H. Loizi provided training on individual male identification.

Most calves and many young juveniles were identified by first identifying the

mother and then confirming male age with the family tree. Many of the older juveniles

did not regularly associate with a particular female group, had no catalog picture in the

identification files, and few distinguishing features. These males were photographed

using a Minolta 35mm SLR camera or an Olympus 4040Z digital camera and then the

male in question was given an identification number. This allowed for a greater

probability of recognizing those males in the future. Most pubescents and adults could be

identified with picture identification (if they could not be identified, the same procedure

for unknown male identification was used).

Identified elephants were placed in age classes using their known age or morphological descriptions developed by Moss (1996, 2001; Table 2.1). In the nearly 30 year study of African male elephants at Amboseli National Park, Kenya, Moss (1996) has used age classes of five year intervals to delineate life stages. The first two five-year spans include pre-reproductive individuals. The next two five-year spans incorporate early reproductive females and, seemingly, socially non-reproductive males. In the current study, I used the first two five-year spans designated as calves (<1-4) and juveniles (5-9). I combined the next two age groups as pubescents (10-19) and considered all males 20 years of age and older as adults. The combined age spans provided a larger sample size while still capturing the important life stages in elephant behavior. The male age classes used in this study were comparable to those used by Loizi (2004) on the same population of elephants. Loizi provided assistance at AENP (2003) regarding placement of elephants in defined age classes.

Behavioral Observations

Behavioral observations of elephants were conducted at approximately 6-8 waterholes in the fenced park area (Fig 2.1). Waterholes were selected for observations because the lack of brush allowed for unobstructed viewing. Furthermore, water holes are high traffic elephant areas highly suitable for viewing of social interactions between elephants. African male elephants that were observed were chosen haphazardly based on elephant visibility. Attempts were made to observe a new focal male for each observation. However, if only previously observed males were present, behaviors performed by these males were recorded again and added to their prior observation. In short, males observed more than once still served as one focal male. Males that were

most visible to the observer were chosen to observe. Once identified, behaviors of African male elephants were recorded using a continuous focal sampling method (Altmann 1974, Marten and Bateson 1993). This form of sampling requires the observer to continually record behaviors performed by a single individual, known as the focal animal (focal male for this study). I attempted to observe each focal male for 20 minutes.

Calves and juveniles were difficult to observe for the full time because of their relatively small size and large number of family members surrounding them. Pubescents and adults were easier to observe for the full time because they were larger than most females and had more distinguishing physical marks, making it easier to keep track of them in a group of elephants. The mean observation duration (min) was significantly different among focal male age classes because of these factors (Kruskal-Wallis: df=3, H=34.26, P<0.05) (Table 2.2). Calves and juveniles were observed for a mean duration of approximately 12 minutes (calves: 11.77 ± 1.27, juveniles: 12.11 ± 1.35) while pubescents and adults were observed for a mean duration of approximately 20 minutes (pubescents: 18.36 ± 1.41, adults: 21.84 ± 1.20).

Independent variables such as approximate age of the focal male, date, location, and air temperature were recorded at the start of each observation. In addition, the number of elephants seen in the area and neighbors within ten body lengths were noted. If known, the family of the focal male was noted. Once the observation started, each occurrence and/or length of a behavior was recorded. All behaviors performed by focal males during observations were placed in the following categories: 1) state behaviors and 2) event behaviors. State behaviors are long-lasting body movements and trunk behaviors with measurable duration (Marten & Bateson 1993). Examples of state

behaviors are walking, standing, and drinking. Event behaviors are quick behaviors in which duration cannot be measured, such as trunk touches and body contacts to other elephants (Marten & Bateson 1993). The age and sex of elephants that focal males touched with their trunk or contacted with their body were recorded. The sex and age of elephants that touched focal males were also recorded, but were not analyzed due to small sample size. Chemosensory behaviors were recorded as event behaviors, but were analyzed separately (Tables 2.3 and 2.4).

Data Analysis

The frequency of event behavior and the duration of state behavior performed by focal males were recorded during observations. The first and last state behaviors of a focal session were not used in analysis involving duration because their full duration was not recorded. Since observation time differed among focal individuals, frequency and duration of behaviors were standardized as follows: the frequencies and durations of state behaviors were calculated as mean proportions, mean rates (frequency/min), and mean duration per bout. A bout was defined in this study as an occurrence of behavior. Event and chemosensory behaviors performed by focal males were calculated as a mean rate. Not all focal males performed all event behaviors during observations. Therefore, I additionally analyzed specific event behaviors using only those males that exhibited said behaviors. Because of small sample sizes for each age and sex class, recipients of trunk touches and body contacts performed by focal males were classified as male and female and analyzed. Two of the 16 focal pubescents and five of the 24 focal adults were observed in male only groups; these males were not included in analysis regarding female interaction.

JMP 4.0 for Macintosh systems was used for all statistical analyses. Analysis of Variance (ANOVA) within a 95% confidence level was performed to determine if any significant effect of focal male age class was exhibited on state behaviors, event behaviors, and defined chemosensory behaviors to ground substrate. For data that did not meet normal distribution and equal variance assumptions for ANOVA, non-parametric Kruskal-Wallis tests were performed to determine focal male age class effect on behaviors (Sokal & Rohlf 1995). Tukey-Kramer *a posteriori* tests that include an adjustment for multiple comparisons were used to compare all pairs when age class had a significant effect on behavior. The Tukey-Kramer test is a conservative test that protects against Type I error. Hence, the pair-wise comparisons could be all non-significant despite a significant overall difference among age classes in the general ANOVA. Results were significant when P was less than or equal to 0.05. Unless otherwise noted, all descriptive statistics are presented as means ± standard error.

RESULTS

Calves varied from pubescents and adults in their movement and trunk behavior. African male elephant calves observed in this study stood at a higher mean rate (F/min ± SE) than pubescent and adult males (calves: 0.52 ± 0.08, pubescents: 0.33 ± 0.04, adults: 0.27 ± 0.03) (Kruskal-Wallis: N=78, df=3, H=13.36, P<0.05; Tukey-Kramer *a posteriori*, P<0.05) (Fig 2.2). Play activity performed by male elephants corresponded with age class. Calves played at a significantly higher mean rate than pubescents (calves: 0.15 ± 0.04, pubescents: 0.02 ± 0.01) (ANOVA: $F_{3,74}=4.13$, P=0.01; Tukey-Kramer *a posteriori*, P<0.05; Fig 2.3). They also touched female elephants proportionally more than pubescents and adults (mean proportion of trunk-to-female behavior for calves: 0.30

± 0.11, pubescents: 0.04 ± 0.02, adults: 0.08 ± 0.05), (ANOVA: $F_{3,66}$=1.35, P=0.04; Tukey-Kramer *a posteriori*, P<0.05; Fig 2.4). Furthermore, of the males that performed chemosensory behavior to ground substrate, calves performed the highest mean rate of flehmen (calves: 0.36 ± 0.06, juveniles: 0.14 ± 0.02, pubescents: 0.10 ± 0.01, adults: 0.09 ± 0.01) (ANOVA: $F_{3,49}$=5.67, P<0.05; Tukey-Kramer *a posteriori*, P<0.05). The rate of sniff, check, and place did not vary among the age classes (Fig 2.5 a, b.).

Juvenile behavior rarely varied from the other male age classes, except that they stood at a higher mean rate than adults (juveniles: 0.45 ± 0.04, adults: 0.27 ± 0.03; (Tukey-Kramer *a posteriori*, P<0.05; Fig 2.2). Movement behavior, social interactions, and chemosensory behaviors performed by juveniles resembled the activity of both calves and the older male age classes, although typically intermediate between them.

While pubescent and adult male elephants varied behaviorally from calves, and occasionally juveniles, there was also some differential behavior between the two older age classes. Pubescents performed trunk behaviors longer per bout (min/bout) than adults (pubescents: 1.60 ± 0.46, adults: 0.60 ± 0.17) (ANOVA: $F_{3,74}$=3.22, P=0.03; Tukey-Kramer *a posteriori*, P<0.05; Fig 2.6). Furthermore, pubescents contacted other males with their body proportionally more than adults (mean proportion: pubescents: 0.18 ± 0.05, adults: 0.05 ± 0.02) (ANOVA: $F_{3,74}$=2.89, P=0.04; Tukey-Kramer *a posteriori*, P<0.05; Fig 2.4).

The mean rate of walking among the age classes also varied with age class (ANOVA: $F_{3,74}$=3.14, P=0.03). However, a pairwise comparison of the age classes demonstrated no significance (Tukey-Kramer a posteriori, P>0.05; protection of Type I error; Fig 2.2). While the rate and duration per activity of state behaviors varied, the

mean proportion of observation time dedicated to the various behavioral categories did not vary among the age classes (Fig 2.7). The age classes also did not differ in the rate of total trunk-to-ground behaviors (Fig 2.8). Chemosensory trunk behaviors were the vast majority of all trunk-to-ground behaviors, ranging from 0.79 ± 0.21 to 0.91 ± 0.06 (Fig 2.9). Behaviors making up the remainder of trunk-to-ground behavior were post-chemosensory behaviors such as trunk and head shakes.

DISCUSSION

Elephant calves experience a highly interactive environment in comparison with that of reproductive males. This is demonstrated in this study by the high rate at which calves touched females with their trunks. Male elephants that live separately from females typically contact inter- and intrasexual individuals only in regards to reproductive or competitive interactions (Buss 1961, Eisenberg et al. 1971, Douglas-Hamilton 1972, Laws 1969). However, like many young animals, elephant calves are constantly interacting with family members and becoming familiar with their surroundings (Alberts 1981).

This period of constant interaction and investigation has been described in altricial young as the "socialization period" (Williams & Scott 1953). Terranova and Laviola (1995) approximated this period in rodents from the opening of the eyes until weaning. This period is characterized by environmental exploration, individual feeding, and more sophisticated interaction with peers. Laviola and Terranova (1998) suggested that early patterns of social stimulation in altricial young prepare them for adult interactions. The high rate of standing and walking demonstrated by elephant calves suggests frequent change in movement in order to interact with peers and explore the

environment. While investigatory chemosensory behavior was common among all male age classes in this study, calves performed the highest rate of flehmen. This behavior directly involves the vomeronasal organ (Estes 1972, Wysocki & Meredith 1987). Large use of flehmen by male calves suggests the need to become more familiar with chemical signals in their environment.

As juveniles, African male elephants interact frequently with peers outside their family groups (Lee 1986). Juvenile African male elephants spend less time with their mothers, but these males are not completely segregated and are still often in contact with females. The current study indicates a lack in behavioral difference between juvenile males and other age groups. The similarity of juvenile behavior among younger and older age classes suggests a transitional phase. However, once males are totally independent of the natal herd, they need to establish dominance in a male society in order to have access to reproductively active females (Poole 1989a, b).

An ideal way to gain reproductive and combative skills in a new social dynamic is to wrestle with peers (similar to juvenile behavior). Since this behavior is typically non-aggressive, it allows male elephants with a common interest to gain competitive skills at low risk costs. The greater body contact rate between males by pubescents compared to adults in this study further supports this idea. In addition, pubescents performed trunk states longer per bout than adults. One of the behaviors included in this category was wrestling (see Table 2.3). Although the sample size of those that wrestled was not large enough to analyze separately, this behavior may have contributed to a developmental difference on overall trunk states between pubescents and adults.

Male elephant calves in this study conducted higher rates of movements, touched females more, and performed flehmen more when compared to older conspecific males. Juvenile African male elephants were transitional in their behavior when compared to calves and older age classes. Concurrently, when comparing the two oldest age classes, pubescents performed more physical behavior towards other males. Adult males demonstrated a trend of longer movement and less physical contact. The results of this study suggest that African male elephants are developing investigative behavior as calves. The juvenile life stage of African male elephants appears to serve as an intermediate stage of behavioral development. Furthermore, the results of this study imply that African male elephants are developing physical behaviors as pubescents, which likely aid in establishing dominance as adults. The behaviors demonstrated by the age classes of African male elephants at AENP correspond with their social environments.

Table 2.1. Age classes of African male elephants at AENP (Aug-Nov 2003) based on known ages and on descriptions of height, tusk size, body and social descriptions published by Moss (1996, 2001).

Age class and specific age (years old)	Height	Tusks	Body and social description
Calves			
<1	shoulder reaches elbow of adult female	none	body usually visibly hairy
1	should slightly taller than breast level of adult female	none	head and ears in proportion with body
2	reaches armpit of adult female	may begin to show (2 cm)	
3	reaches lower ear of adult female	6 cm	decreased suckling
4	reaches anal flap of adult female	16 cm	suckling drastically reduced
Juveniles			
5	1/4 size of adult	22 cm	begin to spar, time spent with mother reduced
6	shoulder taller than middle ear of adult female	22 cm	tusks begin to turn outward
7	shoulder at level of eye of adult female	22 cm	looking more like a small adult
8	overall size 1/2 of adult female	27 cm	
9	overall size 3/4 of adult female	27 cm	larger than females of same age; spending less time with family
Pubescents			
10-16	overall size 3/4 of adult female	27 cm	larger than females of same age; spending less time with family
16-19	taller than adult females but small compared to older males		
Adults			
>19	shoulder height still increasing, taller than females by 40 years old	tusks thick at lip	head broadens, body heavy set

Table 2.2. Mean (±SE) duration and sample size of focal continuous observations conducted with wild African male elephants at AENP (Aug-Nov 2003). The four age classes were calves (<1-4 years), juveniles (5-9), pubescents (10-19), adults (>19).

Age Class	Sample size (N)	Mean observation duration (min)	±SE (min)
Calves	15	11.77	1.27
Juveniles	23	12.11	1.35
Pubescents	16*	18.36	1.41
Adults	24**	21.84	1.20

* 2 of these males were observed in male only groups. These males were not included in data analysis of behavior directed towards females.
** 5 of these males were observed in male only groups. These males were not included in data analysis of behavior directed towards females.

Table 2.3. Ethogram used to record state behaviors performed by age classes of wild African male elephants during focal continuous observations at AENP (Aug-Nov 2003).

State Behavior categories and defined state behaviors	Definition
Stand	Remains in the same location for at least two seconds with none of the following trunk behaviors observed.
Walk	Leaves location while all four legs are moving in a steady pace with none of the following trunk behaviors observed.
Trunk Behavior	
Drink	Taking water into the trunk and immediately placing water into the mouth.
Eat	Taking nutrients into the mouth via the trunk.
Playing	Using the trunk to manipulate an inanimate object or splashing the tip of the trunk into water.
Rest trunk	Placing approximately ¼ of the lower trunk on the ground and allowing it to remain there for at least two seconds.
Wrestle	Pushing against another individual while trunks are intertwined.
Care	
Dust	Using the foot or trunk to place dirt particles on the body.
Lay	One side of the torso in contact with the ground.
Mud	Using the trunk to throw mud particles on the body or moving body rapidly in a mud hole.
Other	Behavior not defined in ethogram.

Table 2.4. Ethogram used to record event behaviors performed by age classes of wild African male elephants during focal continuous observations at AENP (Aug-Nov 2003).

Event behavior categories and defined event behaviors	Definition
Trunk to Ground Substrate	
Sniff*	Nasal openings hover over ground without contact.
Check*	Touch ground with tip of either finger.
Place*	Entire nasal opening is placed on ground and held momentarily.
Flehmen*	Tip of trunk touches ground then placed in the VNO ducts in the roof of the mouth.
Trunk to Females/Males	Places trunk on any area of a male or female's body.
Body Contact to Females/Males	
Body rub	Using the torso to brush against another individual's torso.
Head butt	Quickly and aggressively using the head to make contact with the body of another individual.
Push	Using the body to displace another elephant from their location.
Lean	Focal male placing his body weight on the body of another individual.
Trunk on Head/Back	Placing the entire length of the trunk on the head/back and allowing to hold position for at least two seconds.

*Chemosensory definitions derived from Schulte & Rasmussen (1999b).
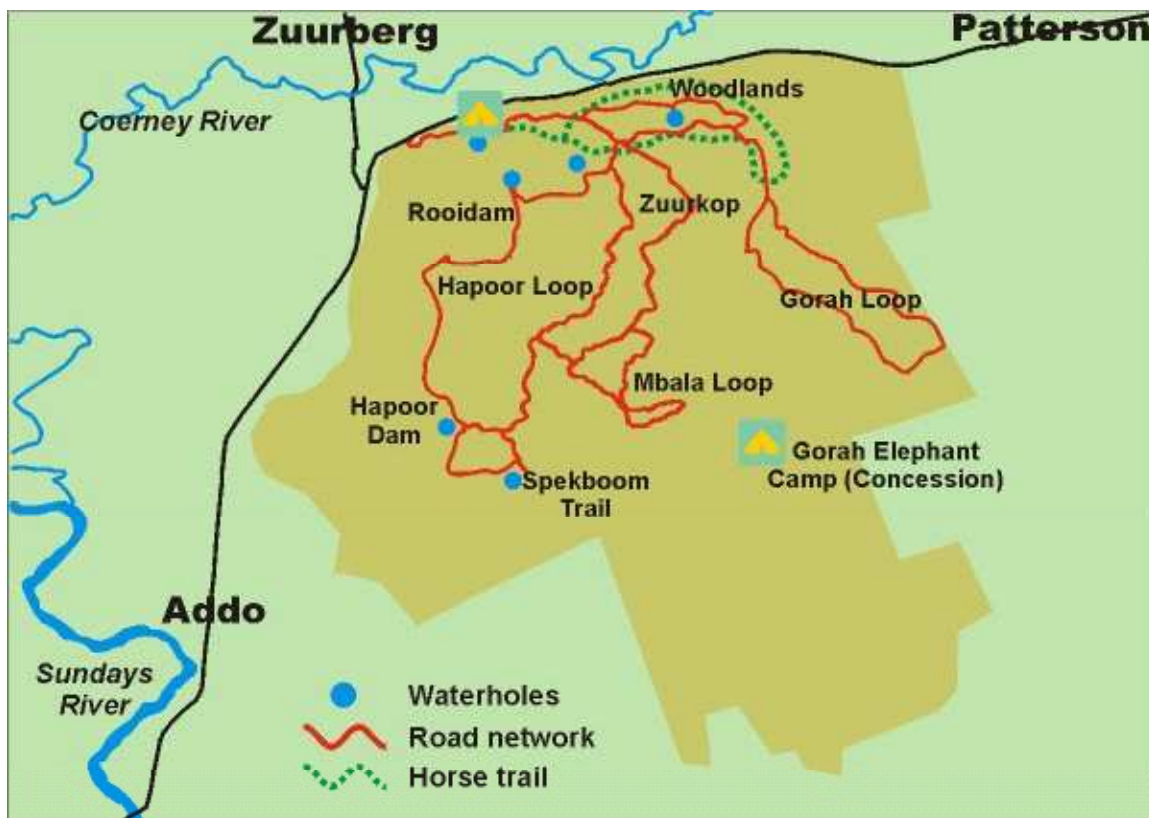
Figure 2.1. Map of the fenced area (103 sq km) at Addo Elephant National park containing the African elephant population observed in this study (Aug-Nov 2003; map taken from http://www.addoelephantpark.com/images/AENP_roads_maps.jpg
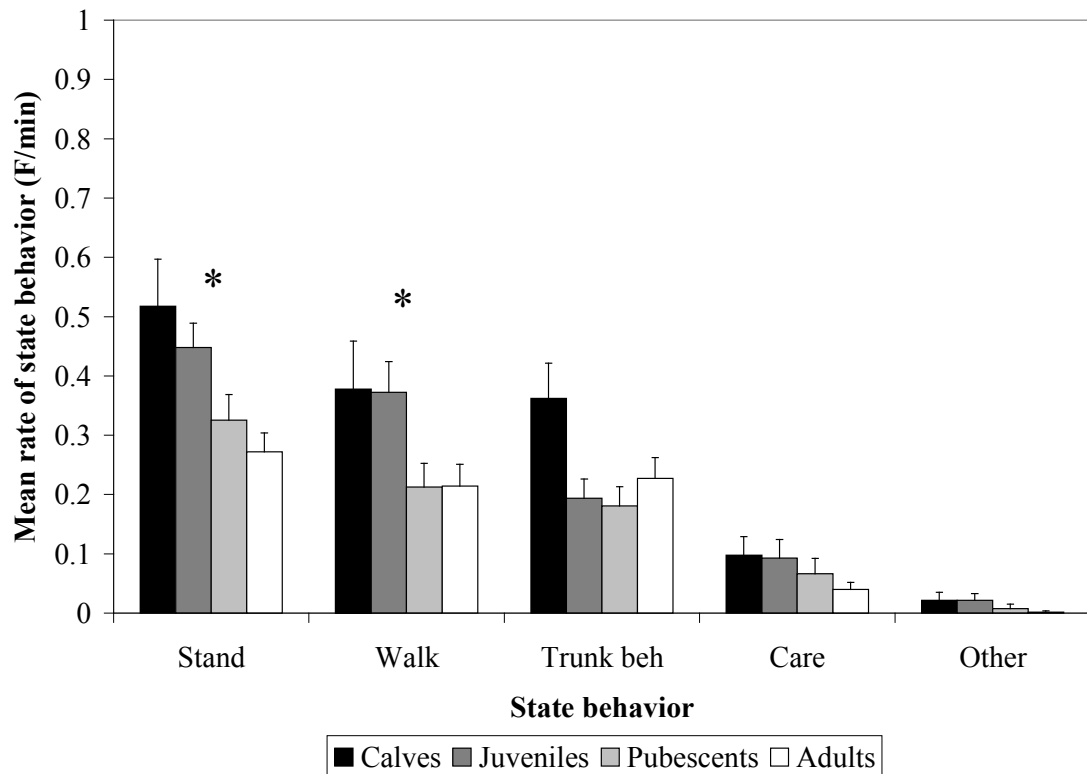
Figure 2.2. The mean (±SE) rate (bout/min) of state behaviors performed by focal males in each of the four defined age classes at AENP (Aug-Nov 2003). State behaviors were categorized as Stand, Walk, Trunk Behavior (Trunk beh), Care, and Other (see Table 2.3 for definitions of behavior; * significance $P<0.05$). (Tukey-Kramer *a posteriori* $P<0.05$: Stand: calves>pubescents & adults, juveniles> adults; Walk: no significance between age classes).
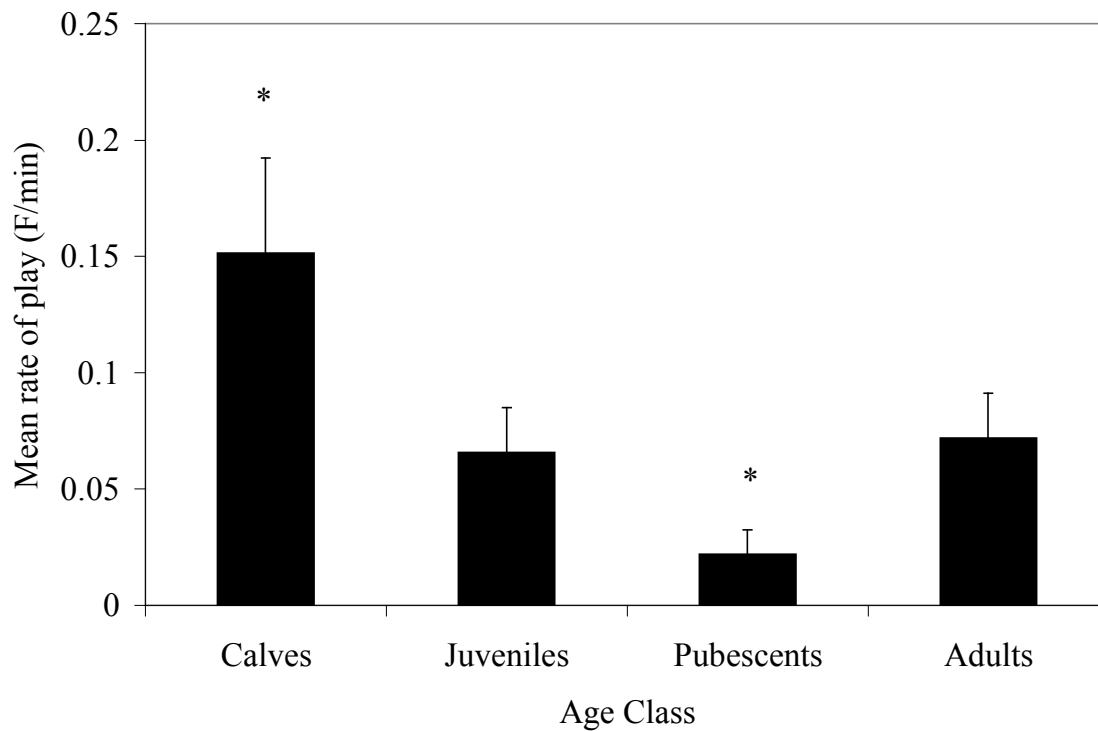
Figure 2.3. Mean rate (±SE) of play behavior performed by defined age classes of African male elephants at AENP (Aug-Nov 2003). *calves performed a higher mean rate of play compared to pubescents (Tukey Kramer *a posteriori,* $P<0.05$).
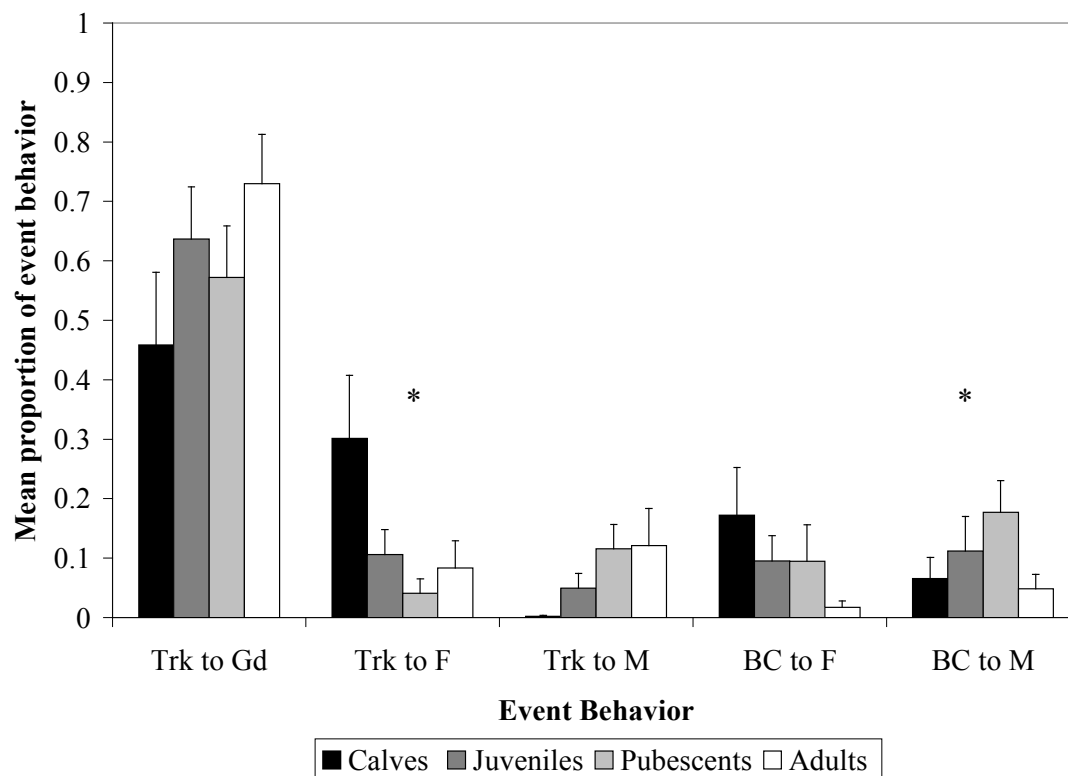
Figure 2.4. Mean (±SE) proportion of event behaviors performed by focal male age classes at AENP (Aug-Nov 2003). Event behavior was categorized as Trunk to Ground (Trk to Gd), Trunk to Female (Trk to F), Trunk to Male (Trk to M), Body Contact to Female (BC to F), Body Contact to Male (BC to M) (see Table 2.4 for definitions of behavior; *significance P<0.05). (Tukey-Kramer *a posteriori* P<0.05: Trk to F: calves>pubescents & adults; BC to M: pubescents>adults).
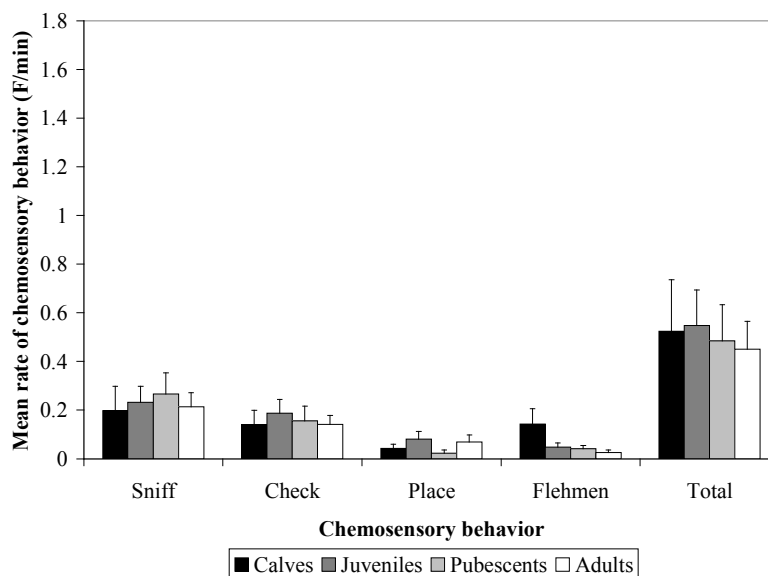
Figure 2.5 a. Mean (±SE) rate of defined chemosensory behaviors performed to ground substrate by all focal males at AENP (Aug-Nov 2003).
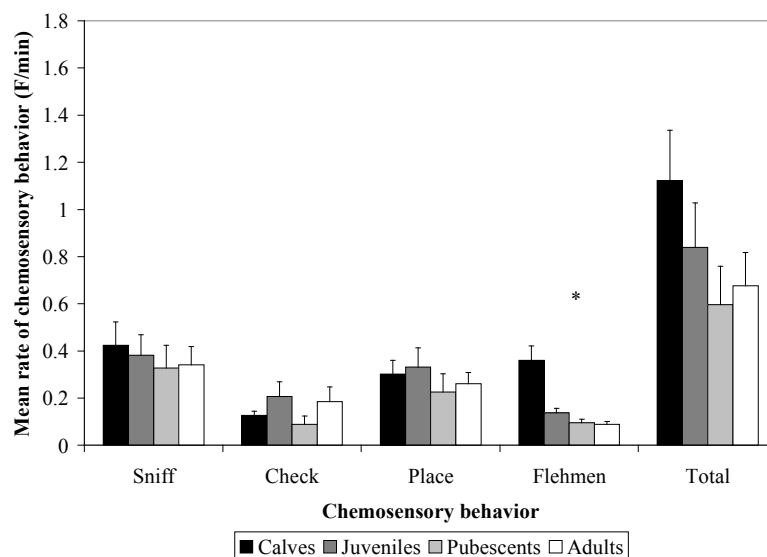
Figure 2.5 b. Mean (±SE) rate of defined chemosensory behaviors performed to ground substrate by all focal males that exhibited specified behaviors at AENP (Aug-Nov 2003) (see Table 2.4 for definitions of behavior; * significance $P<0.05$). (Tukey-Kramer *a posteriori* $P<0.05$: calves> juveniles, pubescents & adults).
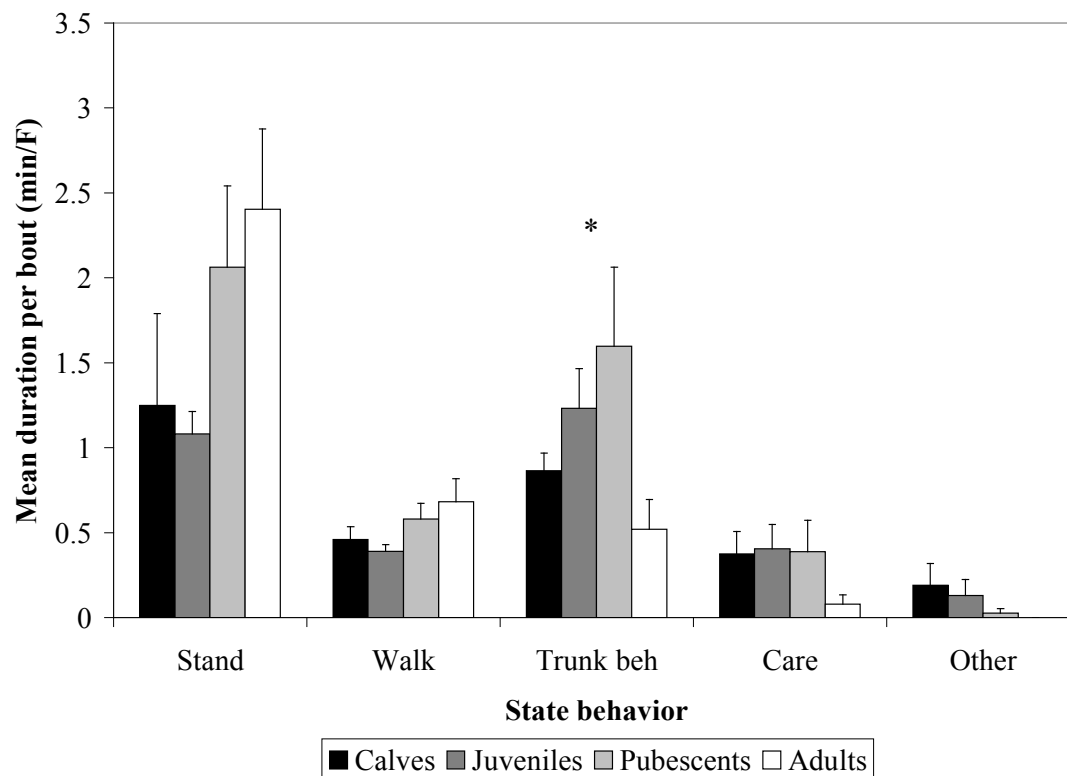
Figure 2.6. Mean (±SE) duration (min) per bout of state behaviors performed by focal male age classes at AENP (Aug-Nov 2003). State behaviors were categorized as Stand, Walk, Trunk Behavior (Trunk beh), Care, and Other (see Table 2.3 for definitions of behavior; * significance $P<0.05$). (Tukey-Kramer *a posteriori* $P<0.05$: pubescents>adults).
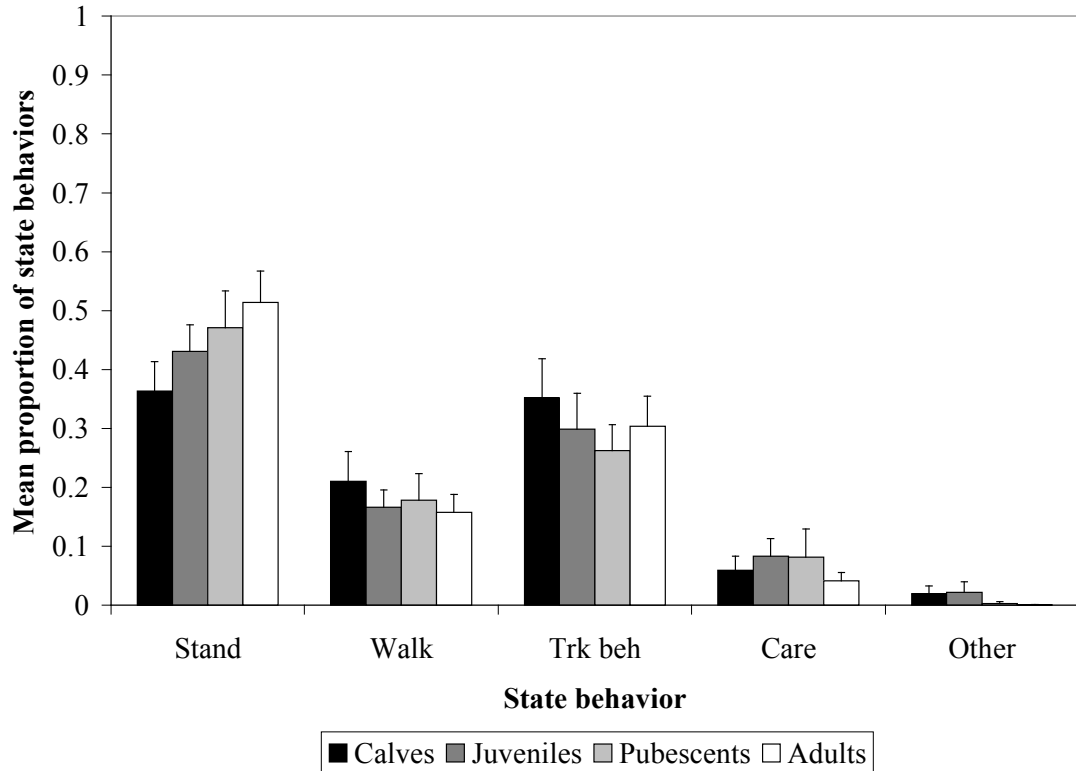
Figure 2.7. Mean (±SE) proportion of observation time spent conducting state behaviors for males in the four age classes at AENP (Aug-Nov 2003). State behaviors were categorized as Stand, Walk, Trunk Behavior (Trk beh), Care, and Other (see Table 2.3 for definitions of behavior).
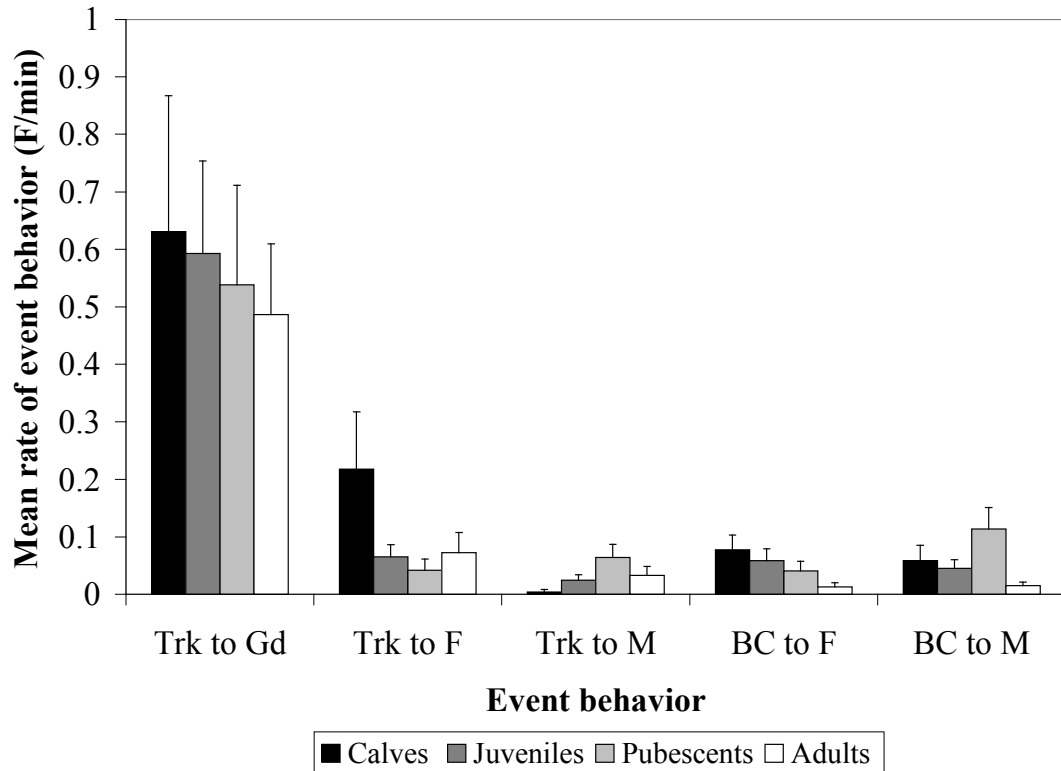
Figure 2.8. Mean (±SE) rate of event behaviors performed by focal male age classes at AENP (Aug-Nov 2003). Event behavior was categorized as Trunk to Ground (Trk to Gd), Trunk to Female (Trk to F), Trunk to Male (Trk to M), Body Contact to Female (BC to F), Body Contact to Male (BC to M) (see Table 2.4 for definitions of behavior).
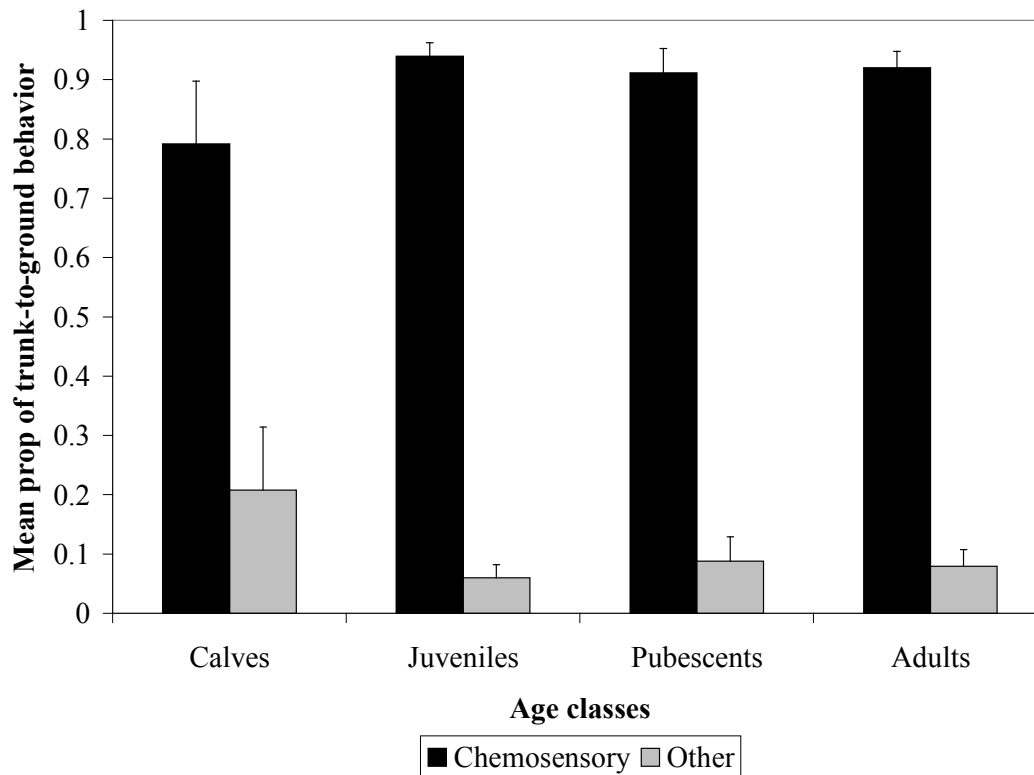
Figure 2.9. Comparison of mean (±SE) proportion of chemosensory and other behaviors (see Table 2.3) performed to ground substrate by focal males at AENP (Aug-Nov 2003) that exhibited Trunk to Ground (Trk to Gd) behavior.

CHAPTER III

THE ROLE OF CHEMICAL SIGNALING IN THE REPRODUCTION OF A
POLYGYNOUS SPECIES, *LOXODONTA AFRICANA*

INTRODUCTION

Polygynous species are those in which males attempt to mate with as many

females as possible, while females are selective in choosing mates (Emlen & Oring

1977). Sexes of these species typically remain segregated except for times of mating

(Clutton-Brock 1989, Krebs & Davies 1993). There are several hypotheses suggested by

Main & Colbentz (1990) that attempt to explain reasons for sexual segregation. Two of

theses hypotheses are based on differential nutritional needs and resource location. For

example, male bighorn sheep feed in smaller groups and in areas with more vegetation

when compared to conspecific female groups (Mooring et al. 2003). Another hypothesis

suggests that sexes must segregate to develop sex-specific reproductive skills. Dobson

(1982) stated that males of polygynous species are more likely than females to disperse

from their mothers or natal groups. Male dispersal occurs in a number of polygynous

species, including white-tailed deer (Kammermeyer & Marchinton 1976) and red deer

(Darling 1937), and elephants (Buss 1961, Laws 1969, Douglas-Hamilton 1972).

Species in which males and females are in constant contact are able to use visual,

auditory, and chemical signals to communicate sexual status. Chemical signaling likely

provides a reliable, honest source of communication for sexually segregated species

(Maynard-Smith and Price 1973). Chemical signals are long lasting and remain after the

individual has departed. Therefore, they can be assessed by a receiver even though the

sender is not present (Alberts 1992, Krebs & Davies 1993). Conveying sexual receptivity

can be conveyed through chemical signals (Alcock 2001, Wyatt 2003). For instance, two

pheromones that mediate reproductive and social interactions have been discovered in Asian elephants (Rasmussen 1998, Rasmussen 2001, Rasmussen et al. 1996a, Rasmussen et al. 1997).

Evidence of chemical signal use by Asian elephants also is indicated by the highly sensitive and complex olfactory and vomeronasal organ (VNO) systems (Rasmussen & Hultgren 1990, Rasmussen et al 1996b, 1997, Rasmussen 1998, Rasmussen 2001). Two ducts leading to the VNO are located in the roof of the mouth. These ducts have mucus-filled tubes in the nasal septum, dorsal to the hard palate (Estes 1972; Hart et al. 1988). Previous research has linked the VNO to the assessment of reproductive chemical signals (Wysocki & Meredith 1987). The complexity of theses systems suggests that elephants readily use their olfactory network and VNO to process chemical signals (Rasmussen 1998).

Chemical signal investigation is performed by the dexterous trunk. Particular trunk movements allow for signal recognition by elephants (Rasussen & Munger 1996). These trunk behaviors are often performed by elephants to areas that may possess chemical signals, such as the mouth, temporal glands, the genital area, and excrement. Primary chemosensory behaviors include sniff, check, place, and flehmen (Schulte & Rasmussen 1999b; see Table 2.4 for behavior definitions; see appendix for illustration). A specific trunk behavior, identified as flehmen, is directly related to reproductive signal transmission from the signal matrix to the VNO (Rasmussen 1998). Flehmen is defined in elephants as the trunk tip making contact with a substance and then bringing the trunk tip to the ducts of the vomeronasal organ, which is located in the roof of the mouth (Rasmussen et al. 1982, 1993, Rasmussen & Munger 1996, Rasmussen 1998).

Asian male elephants perform high rates of flehmen and sexual behavior to a specific pheromone that has been isolated in conspecific female urine. This behavior provides evidence that this pheromone, (Z)-7-dodecen-1-yl acetate (Z7-12: Ac), acts as a female-to-male signal (Rasmussen et al. 1996a, 1997). Specifically, Z7-12: Ac serves as an indicator of impending ovulation to conspecific males (Rasmussen et al. 1996a, 1997, Rasmussen 1999). The pheromone reaches detectable levels in urine approximately at the midpoint of the follicular (ovulatory) phase of the estrous cycle. Concentrations of Z7-12: Ac increase in the follicular phase until females reach ovulation (Rasmussen et al. 1997, Rasmussen 2001).

The Asian elephant female-to-male sex pheromone has not been isolated in African female urine (Rasmussen 1998, Rasmussen & Schulte 1998). In addition, African male elephants do not demonstrate significantly increased rates of chemosensory behavior to Z7-12: Ac. However, African male elephants perform investigative responses similar to that of Asian male elephants to conspecific female urine (Rasmussen 1998). Adult males of this species will usually perform flehmen to females when they come into contact (Hall-Martin 1987). Poole & Moss (1989) observed that African male elephants continuously inspected the genital areas of females. These females would often back into the male after they being inspected and urinate. Males would also inspect female urine as the females departed (Poole & Moss 1989).

Such behavioral observations provide evidence that trunk investigation performed by African male elephants to females and female urine are important in assessing estrous status. The estrous cycle of female elephants lasts approximately 16 weeks (Hess et al. 1983, Plotka et al. 1988). This cycle is divided into two phases: luteal and follicular.

Females are non-receptive during the luteal phase, which lasts between 8 and 12 weeks.

High levels of progesterone, a female sex hormone, are present during this phase. The

follicular phase is a period of low progesterone levels and occurs the last 4 to 6 weeks of

the estrous cycle. Luteinizing hormone, which is a hormone emitted by the pituitary

gland, peaks 2 times during the follicular phase. The first peak (LH1) occurs three weeks

before ovulation, which corresponds closely with the second LH peak (LH2) (Kasputin et

al. 1996, Brown et al. 1999, Czekala 2003). The Asian reproductive pheromone, Z7-12:

Ac is highest in concentration at the periovulatory, LH2 peak (Rasmussen et al. 1993,

1996 a, b, 1997).

Similarities among the Asian and African elephants such as sociality, physiology

(including similar female estrous patterns), and trunk behavior provide support for the

use of reproductive chemical signaling in African elephants (Rasmussen & Hultgren

1990, Rasmussen & Munger 1996). Therefore, the goal of this study was to determine if

captive African male elephants can discern between conspecific follicular and luteal urine

using investigative trunk behaviors such as flehmen. If discernment occurs, it supports

the hypothesis that African female elephants release a pheromone in urine that advertises

reproductive status. I predicted that males would perform more investigative trunk

behavior to follicular urine, specifically urine collected during the LH2 peak, than to

luteal urine. Rasmussen et al. (1996) noted that responses to novel substances by male

Asian elephants subsided with repeated testing, but this habituation did not occur to

natural signals such as follicular urine. Therefore, I predicted that African male elephants

would discern between follicular and luteal urine at least by the end of testing when the

urine was more familiar.

METHODS

Study Sites and Study Subjects

Observational data were collected from captive African male elephants at 6 zoological facilities in the United States. The ages of the males ranged from 20 years to 30 years (Table 3.1). All of the males, except one (Bulwagi), were born into wild populations and brought to captivity at young ages. Bulwagi exhibited the lowest amount of behavior to the urine when compared to the other 8 males. Being born in captivity may have affected his responsiveness. Males were housed in the same facilities at night with conspecific females, but were not allowed free contact with them. About half of the males observed shared daily display areas with females. To prevent females from influencing male response to urine and control samples, resident females were either kept inside or moved to a separate holding area during the experimental trials. Bulwagi was the only male that was observed with females present, which also could have affected his responsiveness to urine.

Urine Collection

Urine was collected from 7 different captive African female elephants housed at facilities in the United States (Table 3.2). These females were demonstrating normal estrous cycles (Meyer et al. 2004). Estrous cycles were monitored by weekly serum progesterone levels taken from the females by the staff of the facility where they were housed. Drs. Goodwin, Rasmussen and Schulte used the serum progesterone levels to confirm female reproductive status at the time of the study. Knowing female progesterone levels allowed the elephant husbandry staff to collect urine during the two different phases of the estrous cycle: the luteal phase and the follicular (specifically

LH2). Luteal urine was collected around peak progesterone levels several weeks after ovulation. Follicular urine was collected at the second luteinzing hormone peak. Urine from each female was collected directly into stainless steel containers. The urine was then stored in 500 ml jars and placed in a freezer at -80ºC within 30 minutes of collection and shipped to bioassay sites as needed.

Bioassay Protocol

Three samples were used for each bioassay: follicular urine, luteal urine, and a vanilla extract/water control. Small amounts of vanillin can be found in Asian elephant urine, and both species perform low but regular rates of chemosensory behaviors to a vanilla/water mixture (Schulte & Rasmussen 1999a, Slade et al. 2003, Loizi 2004). For each male tested, the female origin of follicular urine was different than that of luteal urine. To the best of our knowledge, the males observed had no previous contact with these females. Therefore, urine presented to the males was from two novel females. This was done to ensure that males were exhibiting genuine responses to each sample and not to a particular female. Furthermore, presenting a male with urine from a single female in two states of estrus would not occur in natural settings. We attempted to give the same combination of urine to two males for repeatability, but this was not always possible because of difficulties in locating and collecting urine from cycling females (Table 3.3).

The combination of urine used for each male was determined before bioassays were conducted. Frozen urine samples were completely thawed before each bioassay. Blind testing protocol was used to place the samples (follicular urine, luteal urine, and vanilla extract/water control) in the yard to avoid observer bias (Marten & Bateson 1993). Samples were placed approximately three meters from one another and poured next to a

marker, such as stone or stick. This enabled observers to recognize sample location.

Each bioassay was recorded with a video camera, which prior to testing, was placed at a

location that would not disturb the male. Tapes were later watched by observers and data

collected by hand was inspected for confirmation.

Two observers, one scoring behavioral data and one recording video data, were

present for all bioassays. Each bioassay began once the male entered the holding area

and was free to examine the samples. A method of continuous focal observation was

used to record behaviors exhibited by males to samples (Altmann 1974, Marten &

Bateson 1993). During focal continuous observations, behavior that males performed to

the samples was continuously recorded throughout the duration of the bioassay. Both

observers recorded behavior for 1-2 hours depending on elephant and time constraints of

the facilities. Behaviors recorded were defined in an ethogram constructed before the

study and categorized accordingly after all bioassays were complete (Table 3.4). This

bioassay procedure was repeated for three trials over the course of three days (one trial

per day). Mean (±SE) duration (hours) of male observation time was similar for each

trial (Trial 1: 1.44±0.14, Trial 2: 1.45±0.14, Trial 3: 1.42±0.15). After each bioassay

trial, water was poured over the samples in order to prevent an interaction effect with

samples during the next trial.

Data Analysis

Nine African male elephants were observed in this study. Captive males

observed in this study were presented with urine from unfamiliar females. Since

unfamiliar urine may have presented a novelty effect, I looked at male discernment on

three separate trials. Because of unavailability, one male (Artie) could only be observed

for two trials. Therefore, he was not included in the analyses of trial 3. I calculated a proportion of behavior performed to follicular urine, luteal urine, and vanilla extract/water control from the total frequency in order to demonstrate the prevalence of chemosensory behavior in urine investigation. Other behaviors categories observed and recorded were accessory trunk, penis, and other (see Table 3.4). Accessory trunk behaviors were subdivided as trunk movement, accessory chemosensory, and other. "Other" accessory trunk behavior was not analyzed because of the small sample size.

The mean rate of approach (proximity and near; see Table 3.4 for definitions of approach) to bioassay samples on each trial was calculated to further analyze chemosensory and accessory trunk behavior per approach to bioassay samples. A mean rate per hour of total and individual chemosensory behavior to the samples on each trial was calculated. The mean rate per hour of accessory trunk behavior also was analyzed on each trial.

A one-tailed Page's order test (Sigel & Castellan 1988) was conducted with data on each trial to determine if the hypothesized relationship of the response variables occurred. Specifically, the Page order test determined if chemosensory behavior per approach and rate of behavior per hour were highest to follicular urine than to luteal urine and lowest to the control (F>L>C). For this test, failure to reject the null hypothesis only indicated that the order of responses was not greatest to follicular urine and least to the control. If the Page order test demonstrated significance on trial 3 for behaviors analyzed, a matched pairs t-test was conducted to determine if rate of behavior was higher to follicular urine than to luteal urine (Sokal &Rohlf 1995; response to control was not included in this analysis).

RESULTS

Chemosensory related behavior played a large role in African male elephant investigations of bioassay samples. Of the total behavior frequency performed by males to bioassay samples, the proportion of chemosensory behaviors ranged from 0.72 to 0.76. The mean rate of approach (proximity and near) per trial displayed by males was not significantly greater to follicular urine than to luteal urine, nor to the control (k=3, N=9,8, all L< 116 for N=9; L<104 for N=8; P>0.05; Fig 3.1 & Fig 3.2). However, chemosensory behavior performed by males per approach (proximity and near) was higher to follicular urine than to luteal urine than to the control on all three trials (k=3, N=9,8, P<0.01; Fig. 3.3 & 3.4). Furthermore, when comparing differences between chemosensory behavior per response to the urine types, this rate was significantly higher to follicular urine than to luteal urine on trial 3 (chemosensory per proximity: df=7, t=4.29, P<0.01; chemosensory per near: df=7, t=2.89, P=0.02).

When analyzing total chemosensory behavior (SPCF) to bioassay samples, males performed a higher mean rate per hour to follicular urine than to luteal urine than to the control on trials 2 and 3 (k=3, N=9,8, P<0.001; Fig 3.5). Comparison of chemosensory rate between the urine types on trial 3 demonstrated that this rate was significantly higher to follicular urine than to luteal urine (df=7, t=2.80, P=0.03). In fact, all males observed on trial 3 performed a higher rate (F/hour) of total chemosensory behavior to follicular urine than to luteal urine (Fig. 3.6).

Males performed sniff, check, and place at a higher mean rate per hour to follicular urine than to luteal urine than to control on trial 3 (k=3, N=9,8, P<0.01; Fig 3.7, 3.8, and 3.9). Furthermore, the mean rate per hour of flehmen was higher to follicular

urine than to luteal urine than to the control on trial 2 in addition to trial 3 ($k=3$, N=9,8, P<0.001; Fig 3.10). The rate of chemosensory responses per hour did not follow the predicted order (F>L>C) when urine samples were completely unfamiliar to the males observed (trial 1). Comparison of defined chemosensory behaviors between urine types demonstrated that flehmen was the only behavior performed more to follicular urine than to luteal urine on trial 3 (df=7, t=2.61, P=0.03).

The remaining 0.22 to 0.23 of behaviors performed by males was attributed to accessory trunk behaviors (Table 3.5). Trunk movement per proximity were higher to follicular urine than to luteal urine than to control on trial 2 ($k=3$, N=9, P<0.01; Fig.3.11). This pattern of behavior was seen for all 3 trials of trunk movement per near ($k=3$, N=9,8, P<0.001; Fig.3.12). Accessory chemosensory behavior per approach (proximity and near) did not follow the predicted pattern (F>L>C) on any trial ($k=3$, N=9, L<116; N=8, L<104). Accessory trunk behavior (trunk movement and accessory chemosensory) per hour was higher to follicular urine than to luteal urine than to control on trials 2 and 3 regardless of approach rate ($k=3$, N=9,8; Figs. 3.13 & 3.14). The rate of trunk movement performed was nearly significantly greater to follicular than luteal urine and was significantly greater for accessory chemosensory behaviors on trial 3 ($k=3$, N=9,8; trunk movement: df=7, t=2.37, P=0.06; accessory chemosensory: df=7, t=2.38, P=0.05).

DISCUSSION

While males did not approach follicular urine more than luteal urine, the chemosensory behavior performed per approach differed significantly. Males performed more total chemosensory behavior per approach to follicular urine than to luteal urine on every trial of testing. These findings suggest that males can discern between follicular

and luteal urine even when urine is unfamiliar. Even though a specific reproductive chemical signal has not been isolated in African female elephant urine (Rasmussen 1998), the high amount of chemosensory investigation to follicular urine per approach offers evidence that one exists. Asian male elephants perform similar behavior to conspecific female follicular urine, which contains the identified sex pheromone Z-7-dodecen-1-yl acetate (Rasmussen et al. 1996a, 1997, Rasmussen & Schulte 1998, Rasmussen 1999). This pheromone increases in concentration as females approach ovulation (Rasmussen et al. 1997, Rasmussen 2001). Since Asian and African female elephants share similar estrous patterns, it is likely that an African female elephant sex pheromone would be found in follicular urine. This idea is further supported by the differential chemosensory behavior performed per approach and per hour by African male elephants in this study.

Chemosensory behaviors performed by captive African male elephants played the most important role in sample investigation. Approximately 75% of all behavior displayed to the samples was chemosensory. This relatively large proportion of chemosensory behavior further suggests the importance of chemical signaling in African elephants. Chemosensory investigation by adult African male elephants is apparent when they come into contact with conspecific female groups. These males regularly inspect the genital regions of many females (Hall-Martin 1987). Furthermore, they perform chemosensory behavior to urine and feces as a female group leaves (Poole & Moss 1989). The investigation via chemosensory trunk behaviors of conspecific females and their excrement indicates the general interest by males in sources of potential chemical signals. In the current study, males did not approach follicular urine more than luteal, nor

did they approach the urine more than the control. Even as males became familiar with female urine, approach to ovulatory urine was not significantly higher. However, their rate of chemosensory responses per approach differed. This suggests that male discernment between reproductive and non-reproductive signals take place after males have approached and have conducted trunk investigation.

While rates per hour of total chemosensory behavior (SCPF) and the individual chemosensory behaviors were higher to follicular urine than to luteal urine, this typically did not happen until the second or third trial. This was when males were more familiar with the presented female urine. The only defined chemosensory behavior that was higher to ovulatory urine than to non-ovulatory urine on both trials 2 and 3 was flehmen. This particular behavior is linked to male assessment of conspecific female reproductive state in many species (Estes 1972, Hart 1983). In several species, flehmen is performed to follicular urine or directly to ovulating females, such as stallions (*Equus caballus*) and bovine bulls (Estes 1972, Hradecky et al. 1983, Anderson et al. 1996). The results of this study support previous studies, thus providing evidence that males are using chemosensory behaviors, particularly flehmen, to aid in the reproductive assessment of conspecific females (Wysocki & Meredith 1987, Rasmussen 1998).

The results of this study suggest that a reproductive chemical signal is emitted in African female elephant urine. Once captive African male elephants observed in this study approached the bioassay samples, they were able to discern between urine from females of different estrous stages using chemosensory behaviors. Of additional interest, males also exhibited higher rates per hour of trunk movements and accessory trunk behaviors associated with chemosensory behaviors to follicular urine compared to luteal

urine and the control (F>L>C). These behaviors included trunk flicks and wriggles, as well as loud exhalations (blows) and trunk tip pinches. Such behaviors may have no particular function, but they may be related to clearing the trunk or isolating headspace of potential chemosignals. Considering all behaviors, these males responded more to follicular urine than to luteal urine. In addition, there appeared to be a staggered effect of behavioral response to bioassay samples by males from start to end of testing. The chemosensory behaviors that are most related to the VNO and assessing reproductive state, which are place and flehmen, remained relatively constant to follicular urine throughout the trials. This pattern of behavior was not demonstrated to luteal urine. Hence, chemical investigation to identify the estrous pheromone in female African elephants clearly is warranted (Goodwin et al. 2004, Schulte et al. 2004).

Table 3.1. Housing facility and birth year of captive African male elephants that participated in bioassays conducted with captive female African elephant urine in 2003 and 2004.

Male	Housing facility	Male birth year
Artie	Riddle's Elephant Sanctuary	1983
Machito	Miami Metro Zoo	1979
Tuffy	Riddle's Elephant Sanctuary	1984
Ali	Jacksonville Zoo	1981
Bulwagi	Lion Country Safari	1981
Solomon	Riddle's Elephant Sanctuary	1983
C'sar	North Carolina Zoo	1974
Tonka	Knoxville Zoo	1979
Willie	Riddle's Elephant Sanctuary	1979

Table 3.2. Captive female elephants that supplied urine used in bioassays conducted with captive African male elephants in 2003 and 2004. Housing facility and birth year of each female are included.

Female	Housing facility	Female birth year
Alice	Wildlife Safari Park	1970
Cinda	Sedgwick County Zoo	1971
Kiba	Nashville Zoo	1982
Kubwa	Indianapolis Zoo	1976
Tava	Six Flags Marine World	1978
Tembo	Cameron Park Zoo	1977
Timba	Seneca Park Zoo	1982

Table 3.3. Captive female African elephant origin of urine that was presented to captive African male elephants during bioassays in 2003 and 2004. The female's estrous stage was determined before bioassays took place.

Male	Follicular female origin	Luteal female origin
Artie	Kiba	Alice
Machito	Kiba	Alice
Tuffy	Cinda	Kiba
Ali	Cinda	Kiba
Bulwagi	Alice	Tembo
Solomon	Alice	Tembo
C'sar	Tava	Timba
Tonka	Timba	Kiba
Willie	Kubwa	Alice

Table 3.4. Ethogram used to record behaviors performed by captive African male
elephants to bioassay samples in 2003 and 2004. Specific behaviors were categorized
into Approach, Chemosensory, Accessory Trunk, Penis, and Other.

Behavior categories and defined behaviors	Definition
Approach	
Proximity	Male within one body length of sample.
Near	Male within one trunk length of sample.
Chemosensory	
Sniff	Nasal openings hover over sample without contact.
Check	Touch sample with tip of either finger.
Place	Entire nasal opening is placed on a sample and held momentarily.
Flehmen	Tip of trunk touches sample then placed in the VNO ducts in the roof of the mouth.
Accessory Trunk	
Trunk Flick (trunk movement)	Performed after inspecting a sample. Bottom ¼ of trunk moves up and down rapidly.
Wriggle (trunk movement)	Performed after inspecting a sample. Trunk twists and then untwists once at a moderate pace (slower than trunk flick)
Blow (accessory chemosensory)	Performed after inspecting a sample. Air is expelled quickly from nasal openings of trunk; usually audible and mucus expelled usually visible.
Suck (accessory chemosensory)	Same trunk position as Place accompanied with trunk contraction; usually audible.
Pinch (accessory chemosensory	The two fingers of trunk pick up dirt around the sample.
Periscope sniff (other accessory trunk)	Trunk is raised to air above head level and held for at least 2 seconds.
Dig (other accessory trunk)	Elephant used trunk tip or foot to displace ground at sample area.
Penis	
Penis drop	Penis is unsheathed after investigating sample. No urination directly follows unsheathing.
Penis pull	Male uses trunk in a swinging action to investigate their own genital region.
Belly hit	Unsheathed penis arches and hits underside of male's torso in a rapid motion.
Other	
Dust	Elephant throws dirt from sample area on body using trunk.
Vocalize	Elephant vocalizes after investigating sample.
Blow	Elephant expels air through nasal passages after investigating sample.
Ear Wave	Ears extend out and rapidly brought back to the body.
Motionless	Elephant exhibits no behavior for at least 5 seconds.
Other	Behaviors exhibited that are not defined in ethogram.

Table 3.5. The proportion of total behaviors observed in each behavior category performed by all captive African male elephants in bioassay trials in 2003 and 2004.

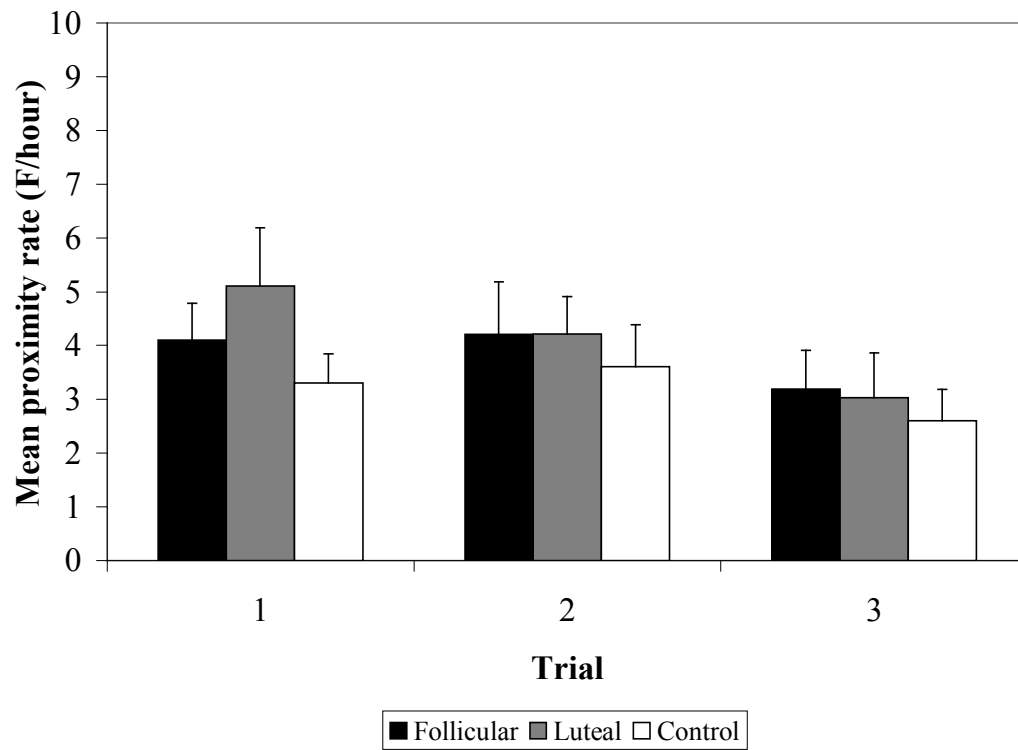| Sample	Total behaviors observed (N)	Chemosensory	Accessory Trunk Behavior	Penis Behavior	Other
Follicular	1399	0.73	0.22	0.01	0.04
Luteal	1030	0.76	0.22	0.00	0.01
Control	239	0.72	0.23	0.02	0.03

Figure 3.1. Mean rate (F/hour ± SE) of proximity (within one body length of sample) performed by captive African male elephants to bioassay samples on each trial of observation.
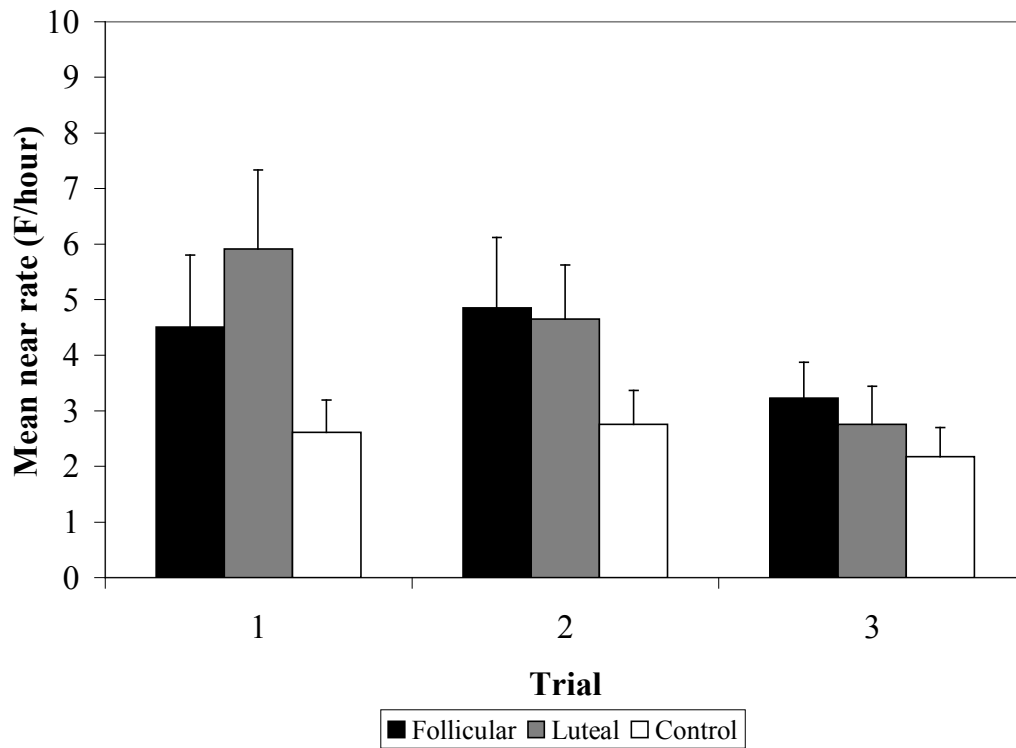
Figure 3.2. Mean rate (F/hour ± SE) of near (within one trunk length of sample) performed by captive African male elephants to bioassay samples on each trial of observation.
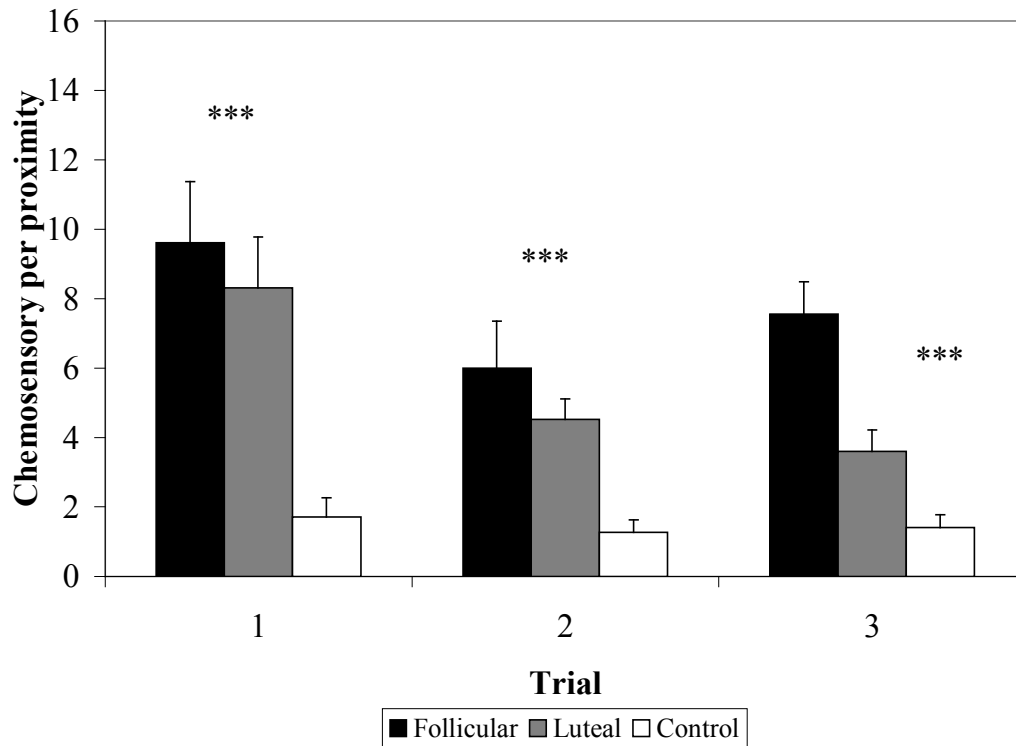
Figure 3.3. Total chemosensory behavior per proximity (±SE) performed by captive African male elephants to bioassay samples on each trial of observation in 2003 and 2004 (Page's order test: Trial 1: $k=3$, N=9, L=122>121; Trial 2: $k=3$, N=9, L=123.5>121; Trial 3:$k=3$, N=8, L=112>109) (H$_a$=F>L>C). ***significance (P<0.001).
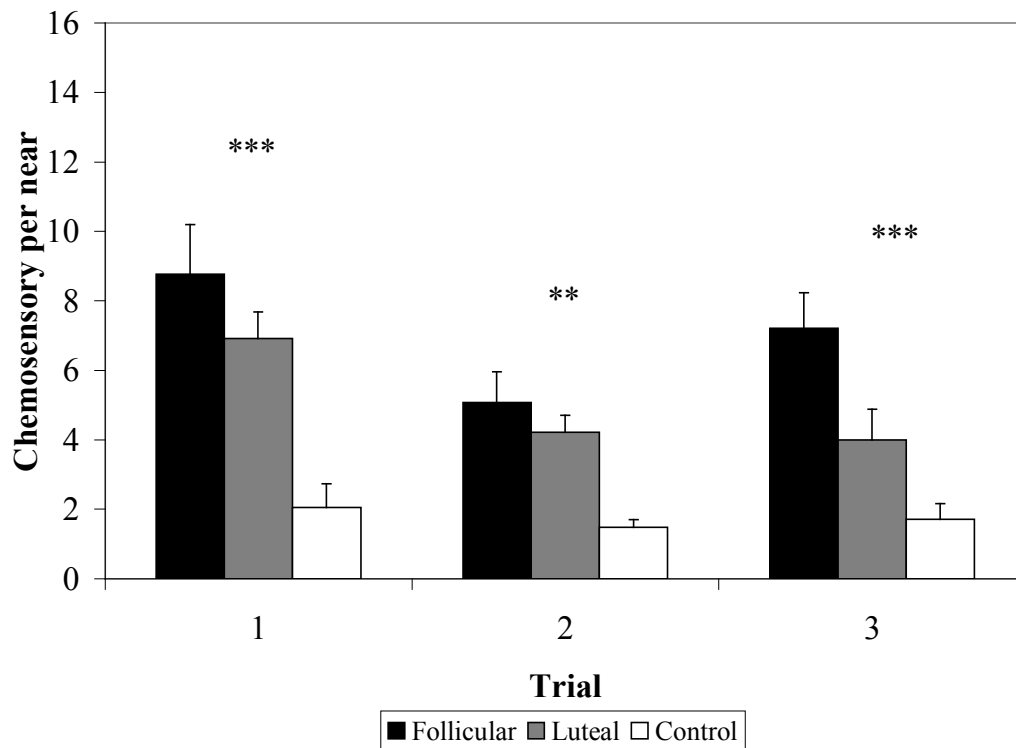
Figure 3.4. Total chemosensory behavior per near (±SE) performed by captive African male elephants to bioassay samples on each trial of observation in 2003 and 2004 (Page's order test: Trial 1: $k=3$, N=9, L=122>121; Trial 2: $k=3$, N=9, L=120>119; Trial 3: $k=3$, N=8, L=111>109) (H_a=F>L>C). ** significance (P<0.01); *** significance (P<0.001).

Figure 3.5. Mean rate (F/hour ± SE) of total chemosensory behavior performed by captive African male elephants to bioassay samples on each trial of observation in 2003 and 2004 (Page's order test: Trial 2: $k=3$, N=9, L=121=121; Trial 3: $k=3$, N=8, L=112>109) (H_a=F>L>C). *** significance (P<0.001).

Figure 3.6. Rate (F/hour) of total chemosensory behavior performed by individual captive African male elephants to bioassays samples on each trial of observation in 2003 and 2004.

Figure 3.7. Mean rate (F/hour ± SE) of sniff performed by captive African male elephants to bioassay samples on each trial of observation in 2003 and 2004 (Page's order test: Trial 3: $k=3$, N=8, L=106.5>106) ($H_a=F>L>C$). **significance ($P<0.01$)
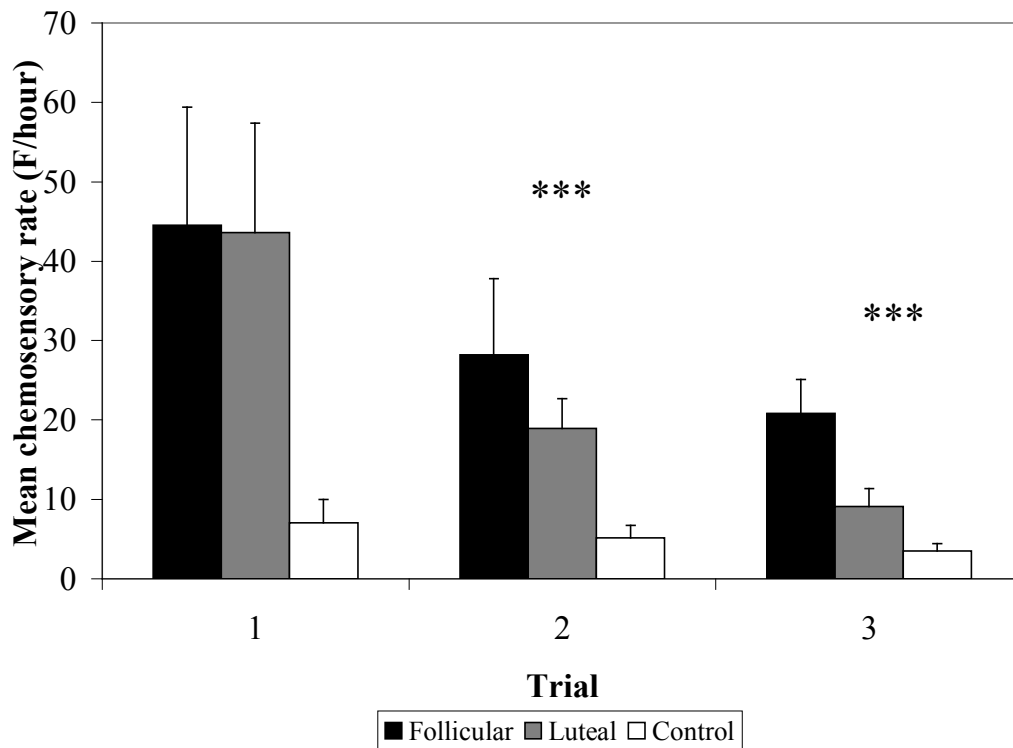
Figure 3.8. Mean rate (F/hour ± SE) of check performed by captive African male elephants to bioassay samples on each trial of observation in 2003 and 2004 (Page's order test: Trial 2: k=3, N=9, L=119=119; Trial 3: k=3, N=8, L=106.5>106) (H_a=F>L>C). **significance (P<0.01).

Figure 3.9. Mean rate (F/hour ± SE) of place performed by captive African male elephants to bioassay samples on each trial of observation in 2003 and 2004 (Trial 3: $k=3$, N=8, L=105.5>104) (H_a=F>L>C). *significance ($P<0.05$)

Figure 3.10. Mean rate (F/hour ± SE) of flehmen performed by captive African male elephants to bioassay samples on each trial of observation in 2003 and 2004 (Page's order test: Trial 2: k=3, N=9, L=122.5>121; Trial 3: k=3, N=8, L=109.5>109) (H_a=F>L>C). ***significance (P<0.001).
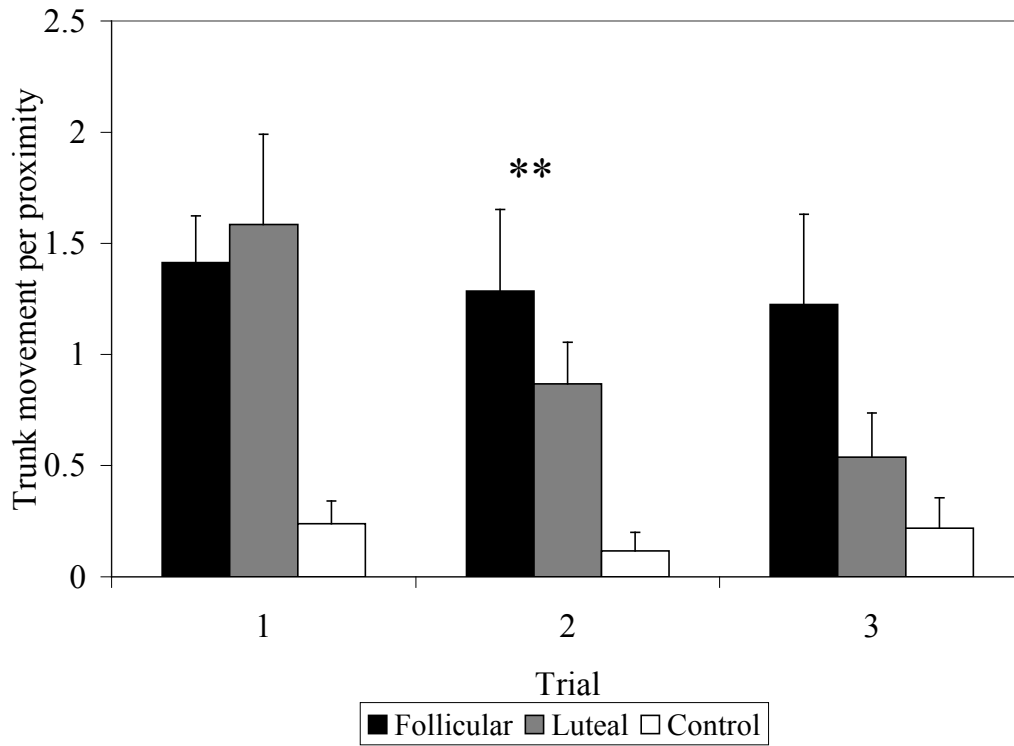
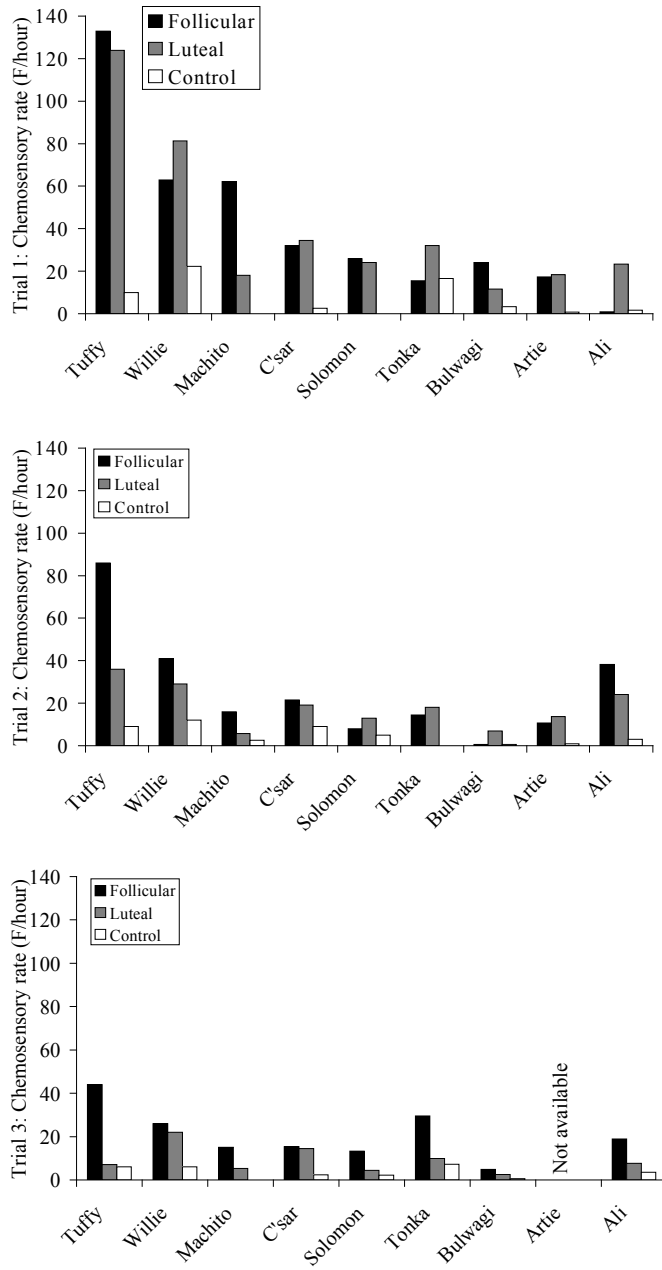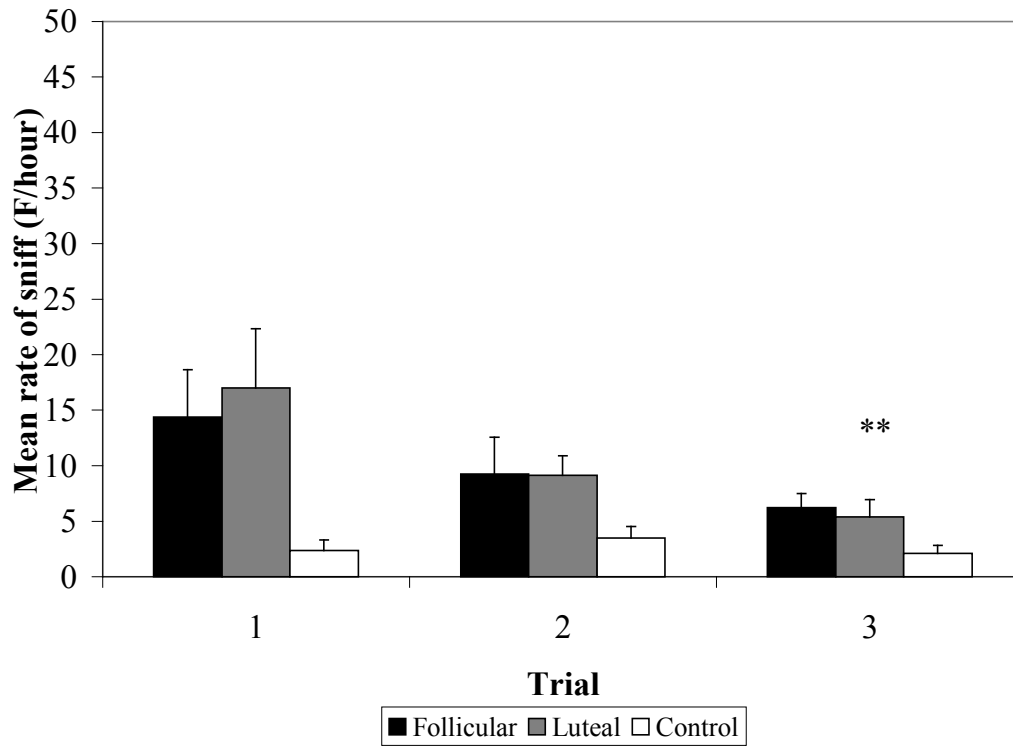Figure 3.11. Trunk movement per proximity (±SE) performed by captive African male elephants to bioassay samples on each trial of observation in 2003 and 2004 (Page's order test: Trial 2: k=3, N=9, L=119=119) (H$_a$=F>L>C). ** significance (P<0.01) See Table 3.4 for definition of trunk movements (flick and wriggle).
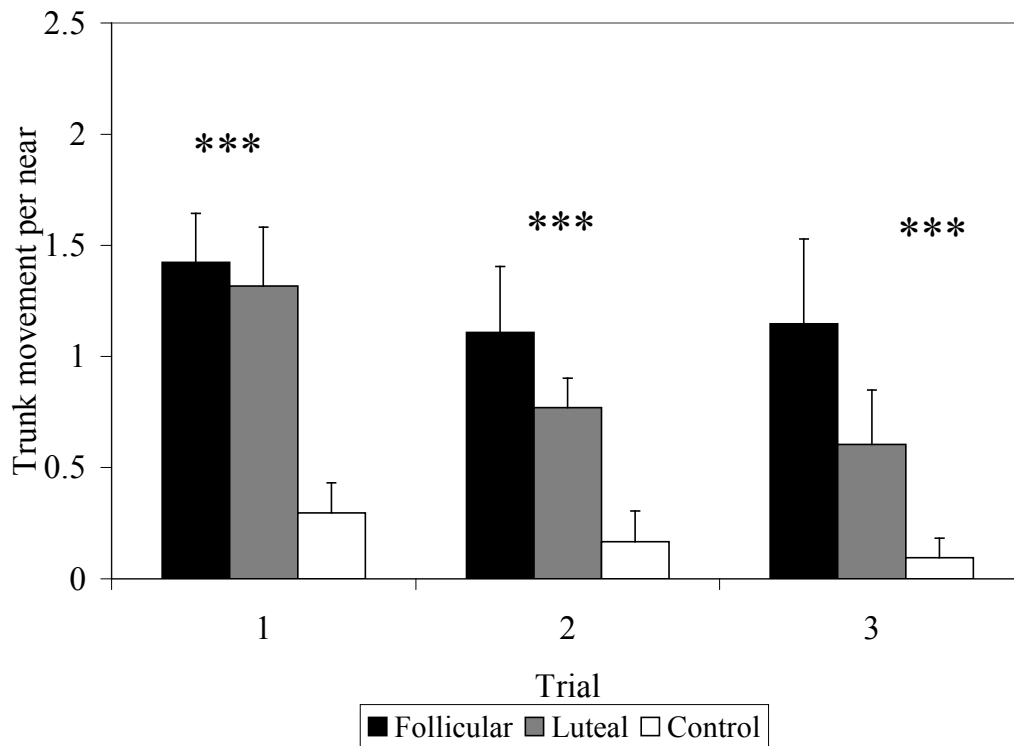
Figure 3.12. Trunk movement per near (±SE) performed by captive African male elephants to bioassay samples on each trial of observation in 2003 and 2004 (Page's order test: Trial 1: k=3, N=9, L=125>121; Trial 2: k=3, N=9, L=122>121; Trial 3: k=3, N=8, L=109=109) (H_a=F>L>C). *** significance (P<0.001). See Table 3.4 for definition of trunk movements (flick and wriggle).
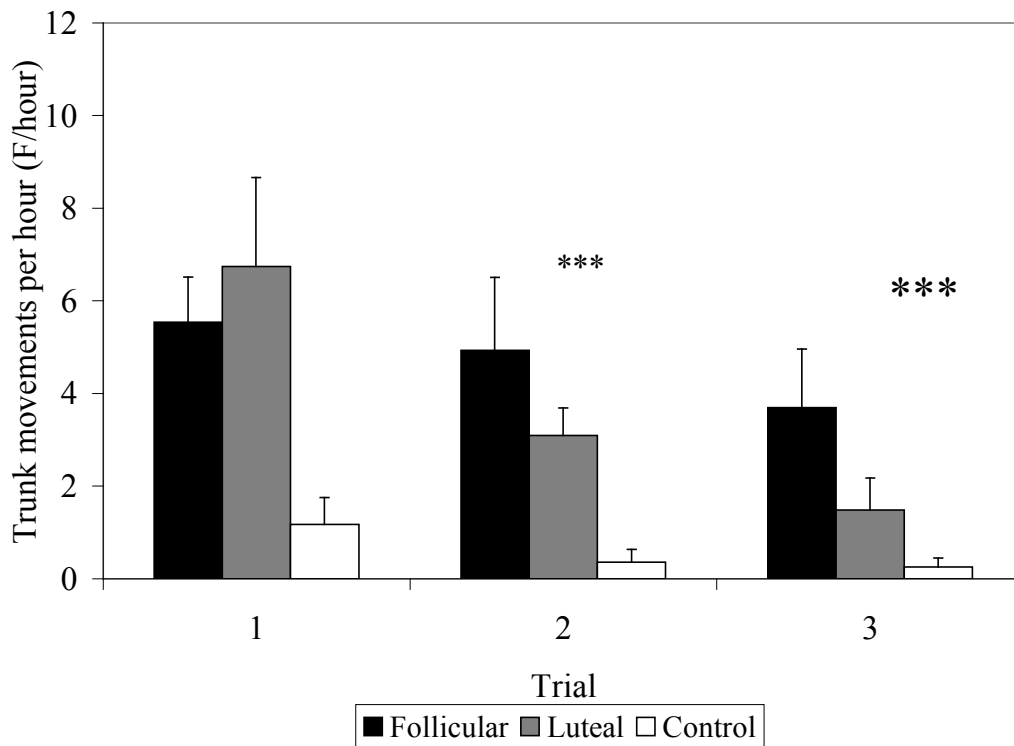
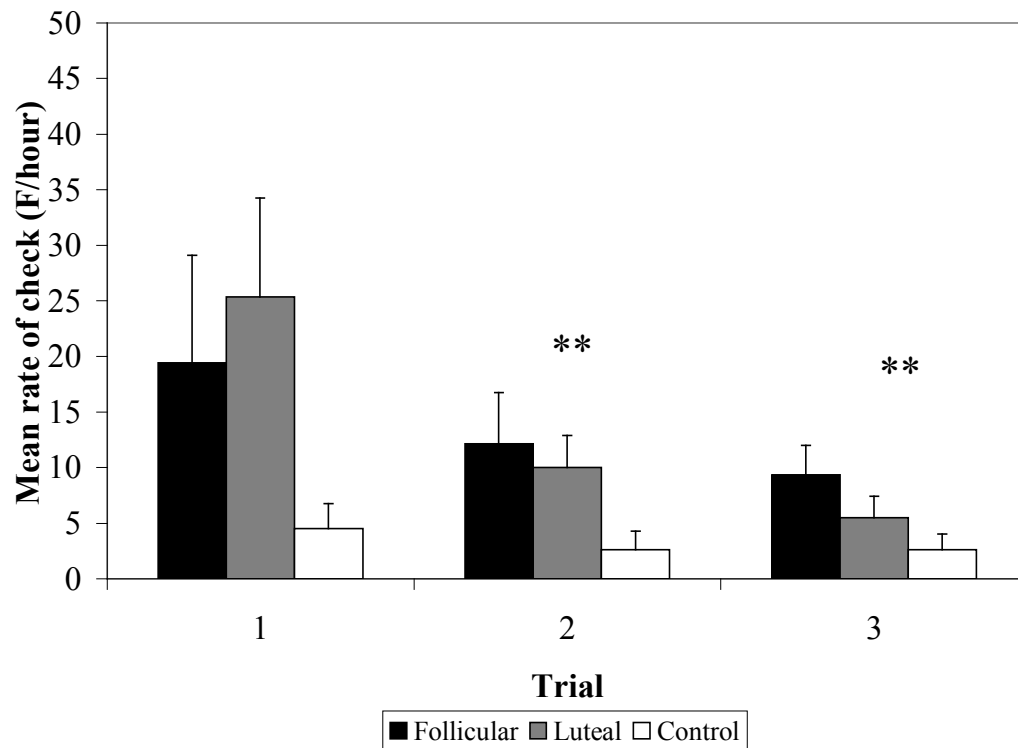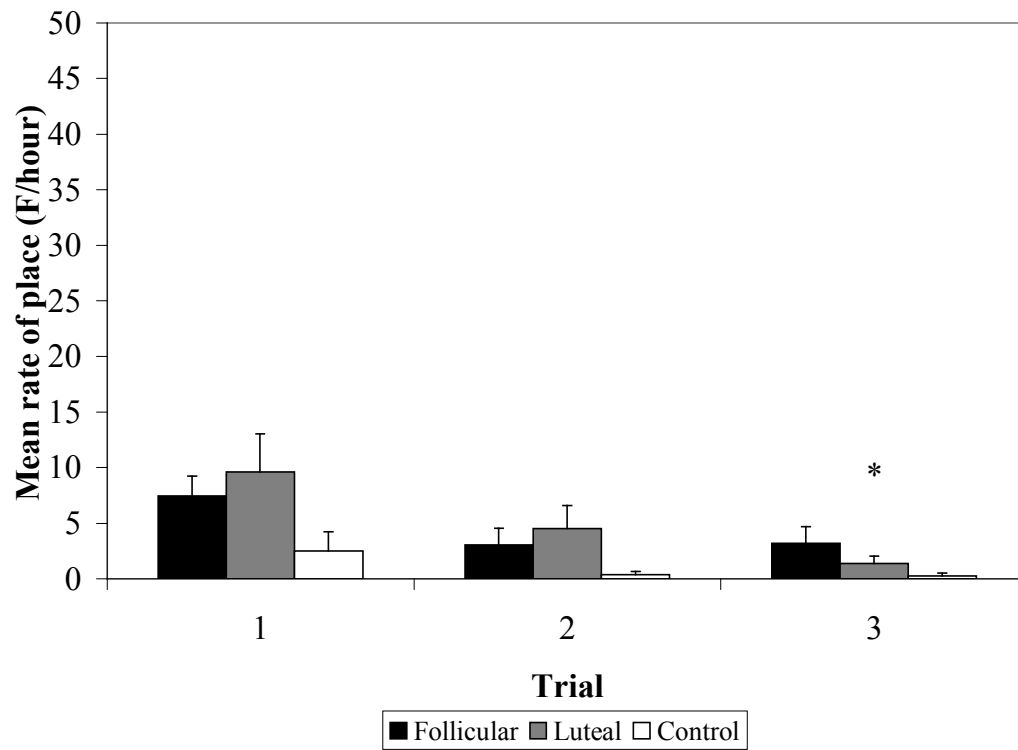Figure 3.13. Mean trunk movement rate (F/hour ± SE) performed by captive African male elephants to bioassay samples on each trial of observation in 2003 and 2004 (Page's order test: Trial 2: *k*=3, N=9, L=121=121; Trial 3: *k*=3, N=8, L=109=109) (H_a=F>L>C). ***significance (P<0.001). See Table 3.4 for definition of trunk movements (flick and wriggle).

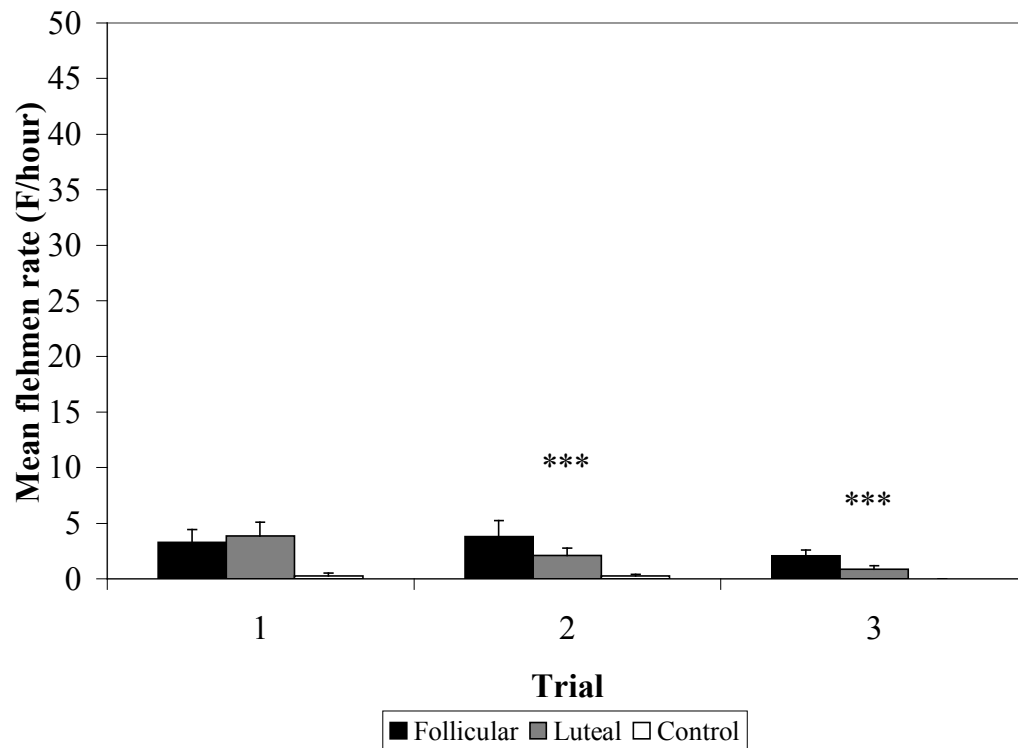Figure 3.14. Mean accessory chemosensory rate (F/hour ± SE) performed by captive African male elephants to bioassay samples on each trial of observation in 2003 and 2004 (Page's order test: Trial 2: k=3, N=9, L=125>121; Trial 3: k=3, N=8, L=107.5>106) (H_a=F>L>C). ***significance (P<0.001); ** significance P(<0.01). See Table 3.4 for definitions of accessory trunk behaviors (blow, suck and pinch).
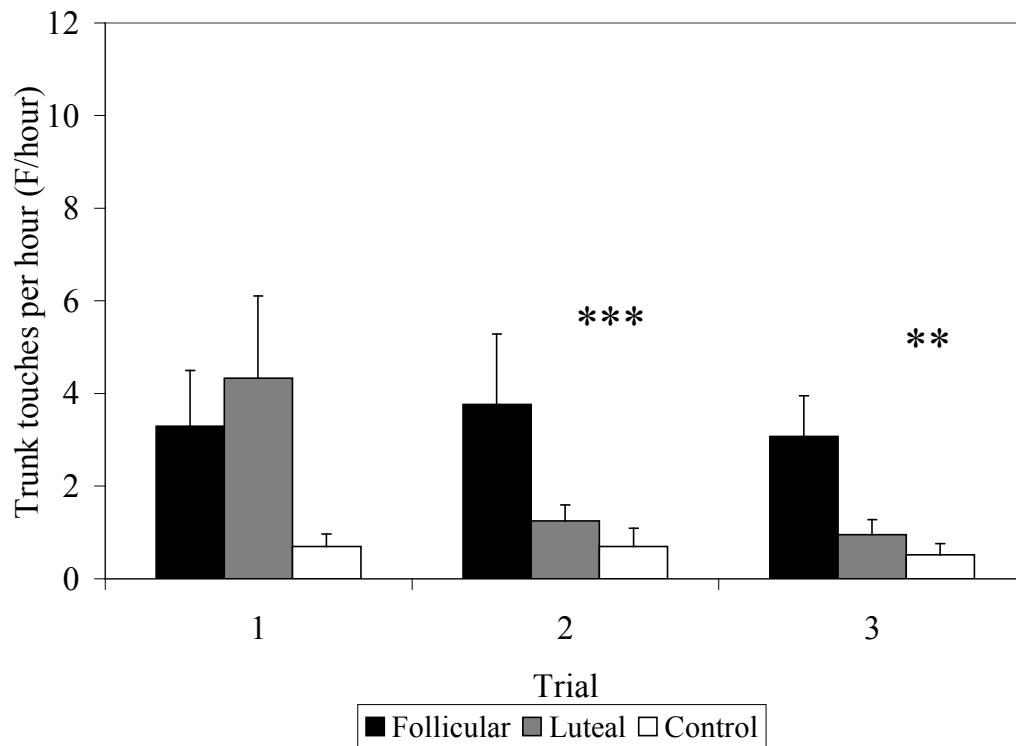
CHAPTER IV

CONCLUSIONS

This study demonstrated differences in how African male elephants perform behavior as they mature. As calves, African male elephants are more investigative in behavior when compared to older conspecific males. Flehmen, a behavior related to processing chemical signals, was performed the most by calves. These findings suggest that investigation and recognition of chemical signals is occurring during this life stage. Pubescent African male elephants in this study, which were new to a competitive male society, were relatively most physical in their behavior. As adults, African male elephants have established dominance and are likely to be reproducing. Reproductive males selectively approach and investigate conspecific females and female excrement (Hall-Martin1987, Poole & Moss 1989). This study demonstrated how adult African male elephants assess estrous state after they have approached conspecific female urine. Captive African male elephants behaviorally demonstrated recognition of a reproductive chemical signals released in female urine.

African male elephants vary in their behavior as they mature in a variety of social environments (Buss 1961, 1966, Laws 1969, Eisenberg et al. 1971, Douglas-Hamilton 1972, Poole 1994). Social environments are often variable for polygynous males, particularly those that are born into matriarchal societies. These males mature through adolescence in a female dominated society (Krebs & Davies 1993, Alcock 2001, Shuster & Wade 2003). Therefore, young African male elephants learn behaviors important to establishing dominance and reproducing largely without being able to model after older males. These two studies have demonstrated how African male elephants, a polygynous

species born into a matriarchal society, develop behaviors important to male dominance and reproduction. The development of the behaviors likely includes investigatory trunk behaviors, such as flehmen, that allow males to use chemical signals to assess estrous state and locate mates.

African elephants socially and physically represent the characteristics of sexually segregated species (Buss 1961, Eisenberg et al. 1971, Douglas-Hamilton 1972, Laws et al. 1975). Therefore, the results of this study also provide information to future studies regarding the use of reproductive chemical signaling in other polygynous species. Chemical communication is beneficial to the reproduction of sexually segregated species. Chemical signals are able to be received after the sender has released the message, which increases the chances of a receiver encountering the signal after the sender is gone (Alberts 1992, Krebs & Davies 1993). Furthermore, they provide honest signals of sender condition, such as female sexual readiness or male reproductive status (Maynard Smith & Price 1973). By quantifying chemical signal assessment by captive African male elephants, these benefits of chemical communication are further supported.

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Figure 2.1. Map of the fenced area at Addo Elephant National park containing the African elephant population observed in this study (Aug-Nov 2003): http://www.addoelephantpark.com/images/AENP_roads_maps.jpg)

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APPENDIX

ILLUSTRATION OF CHEMOSENSORY BEHAVIORS PERFORMED BY AFRICAN
MALE ELEPHANTS
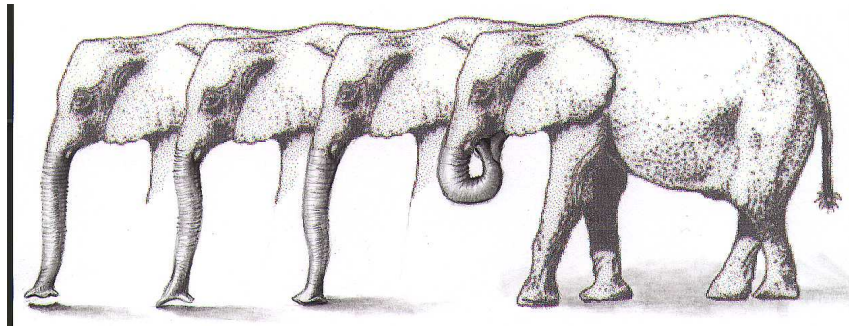
SNIFF   CHECK   PLACE   FLEHMEN

Illustration provided by Mary Amaral