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Development of Chemosensory Behaviors in African Elephants (*Loxodonta Africana*) and Male Responses to Female Urinary Compounds

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THE DEVELOPMENT OF CHEMOSENSORY BEHAVIORS IN AFRICAN
ELEPHANTS (*LOXODONTA AFRICANA*) AND MALE RESPONSES TO FEMALE
URINARY COMPOUNDS

by

Helen Loizi

(Under the Direction of Bruce Schulte)

ABSTRACT

Chemosignals play a vital role in the social and reproductive interactions of elephants. As with many social, sexually dimorphic mammals, elephants experience major developmental stages of nursing, weaning, pubescence and adulthood. Some stages occur at different ages for males and females. I observed the trunk tip, chemosensory behaviors of male and female African elephants in each of the four major developmental stages at Addo Elephant National Park, South Africa. Young female elephants showed the highest rates of chemosensory behavior among females; whereas, pubescent and adult males showed the highest rates among males. The sexes differed in the prevalence of chemosensory behaviors. I also performed bioassays of female urinary compounds with captive elephants at Riddle's Elephant Sanctuary and wild elephants to isolate the estrous pheromone released by female African elephants. The five compounds used did not elicit high responses from conspecific males, but responses were similar by wild and captive elephants.

INDEX WORDS: *Loxodonta africana*, Chemosensory Behaviors, Development,
Pheromones

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by

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B.S., Georgia College & State University, 1997

M.Ed., Georgia College & State University, 1999

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial
Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

STATESBORO, GEORGIA

2004

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ELEPHANTS (*LOXODONTA AFRICANA*) AND MALE RESPONSES TO FEMALE
URINARY COMPOUNDS

by

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Electronic Version Approved:
July 2004

DEDICATION

This thesis is dedicated to my grandfather and my mother:

Find Peace.

ACKNOWLEDGEMENTS

I wish to thank my major professor, Dr. Bruce Schulte for his continuous support, advice and encouragement throughout this project. Furthermore, I would like to thank my committee members, Dr. Alan Harvey and Dr. Steve Vives for their advice and support.

Thanks to Dr. Tom Goodwin and Dr. Bets Rasmussen for comments on my thesis, and Dr. Ray Chandler for help on statistics. Scott and Heidi Riddles and the staff at Riddle's Elephant Sanctuary, I thank you for helping me with bioassays and giving me my first contact with elephants. I extend my gratitude to Augustus Oil Ltd. for donating chemical compounds for the assays.

In South Africa, I am deeply grateful to John Adendorff and Lucius Moolman for the opportunities and experiences they gave me, and to AENP rangers and staff. I would like to thank my Addo friends, Anban, Ilze, Ivan and Michelle for their moral support, Ralf Edler for allowing me stay in his "wendy". To my assistants, Amy Gray and Maureen Correll, my gratitude for all their help. Special thanks to Dr. Anna Whitehouse for showing me the ropes in Addo and allowing me to use the ID files, and Dr. Graham Kerley (TERU) for all his help in Port Elizabeth.

For the funding of the project, I would like to thank the National Science Foundation (grant # 0217068) and Georgia Southern University.

Last but not least, I would like to thank Craig Weyer, my partner in life, who has stood by me through the good and the bad. Thank you for supporting me in living my dream and never holding me back. You have always believed in me even at my worst. I am forever grateful to you.

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Chapter 1

The Development of Chemosensory Behaviors in African elephants

(Loxodonta africana)

INTRODUCTION

In polygamous and polygynous societies, reproductive success in males is dependent on body size, fighting skills and developing a high social status (Clutton-Brock et al. 1982). Females, on the other hand, invest time and care in their offspring and maintain social cohesiveness. Such sexual dimorphism evinces behavioral and developmental differences between males and females (Mathiesen et al. 2002).

Males and females of many species of mammals live separate or isolated lives until it is time to reproduce. Attracting or sexually arousing a mate may be done by visual, vocal or chemical cues that may eventually lead into tactile stimuli as part of the arousal process (Dimond 1970). Chemical signals or semiochemicals (Law & Regnier 1971) are an integral part of mammalian communication. Pheromones are those semiochemicals secreted by an individual into the environment and eliciting a specific behavioral or developmental response in a conspecific (Karlson & Luscher 1959). Chemical signals are used for relaying information on reproductive state, providing species recognition, deterring rivals and marking of territories (Vandenbergh 1983, Sillero-Zubiri & Macdonald 1998). For example, scent secretions released by Eurasian deer contain information that is unique to individuals and from which identity, age, sex and population of origin can be determined by conspecifics (Lawson et al. 2000, 2001). A combination of cues also may be used. Rams use both visual and tactile cues to stimulate

ewes to ovulate (Pearce & Oldham 1988). Males of many species such as meadow voles (Ferkin & Johnson 1995), cattle (Reinhardt 1983), horses (Crowell-Davis & Houpt 1985), and elephants (Moss 1983, Poole 1989a, 1989b) use chemical and tactile communication when they approach a female and sniff, check (elephants) or lick (other ungulates) her anogenital area to determine reproductive state. A sniff behavior can be viewed as an olfactory function and is an initial behavior to the chemical sequence of behaviors in mammals. A lick may involve substrates or a conspecific to transfer chemical compounds to the mouth. A check, as seen in elephants, is the trunk touching a substrate or a conspecific.

Both African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants live in matriarchal societies consisting of an older female, the matriarch, along with closely related females and their respective offspring. Male elephants stay with their natal group until the age of 12-15 years, when they leave to form loosely knit bachelor groups where they associate temporarily with female herds (Eisenberg et al. 1971, Estes 1991, Poole 1994). Males primarily associate with females to reproduce, where the older, larger males are the most likely mates.

Male and female African elephants continue to grow throughout their lives, but males grow at a faster rate and by the age of 20, there is a marked difference in weight and height (Lee & Moss 1986). At the age of 25 and older, males are much larger than females and begin to compete for access to estrous females. Such sexual dimorphism suggests that African elephant mothers invest more in male calves than female calves (Lee & Moss 1986). Male calves spend more time suckling than female calves; females are weaned sooner than males; and mothers with male calves have longer interbirth

intervals. As males and females grow, their display rates of some behaviors such as play and aggression differ (Lee 1986). A difference also is seen in the reproductive development of male and female elephants. Male and female elephants reach sexual maturity at similar ages (around 7 years old in captivity). In the wild, female elephants begin reproducing from the age of 10 –15 years old (Whitehouse and Hall-Martin 2000, Moss 2001), but males, although they produce sperm, are usually unsuccessful at reproducing until their mid-20's when the first full musth occurs (Eisenberg et al. 1971 & 1980, Poole 1989 & 1994).

Musth is described as a condition of heightened aggressiveness in male Asian elephants (Jainudeen et al. 1972a) and more recently in male African elephants (Poole & Moss 1981, Hall-Martin & van der Walt 1984). Musth has been likened to the rut behavior seen in other ungulate species (Poole 1987), but elephants do not all “rut” simultaneously. It is associated with high levels of testosterone, temporal gland secretions and dribbling urine from the penis (Jainudeen et al. 1972b, Poole et al. 1984, Hall-Martin 1987). Females prefer larger, older male elephants in musth as their mate of choice, and Asian females will reject younger ‘moda’ musth males (‘moda’ musth is a youthful musth experienced as young as 8 years old) (Eisenberg et al. 1971, Rasmussen et al. 2002). In elephant populations where older adult males do not exist, young African male elephants have been seen experiencing full musth as early as 18 years old for periods of 3 to 5 months (Slotow et al. 2000, Slotow & van Dyk 2001). In demographically balanced populations, male elephants will begin to experience more predictable, frequent musth events from age 25 and older (Poole 1982). Female Asian elephants have shown increased responses to temporal gland secretions and specifically to cyclohexanone which

may provide information to the female about the state of musth (Perrin & Rasmussen 1994). Males in musth temporarily gain dominance in rank and generate increased attention from females; they become the preferred mate if the female is reproductively ready regardless of the males' age and size (Poole 1989b).

Females of many mammal species use chemical signals in their urine to advertise their reproductive state. Although chemicals are released by mammals from several different sources such as preorbital, metatarsal and interdigital glands, the most common source of intraspecific chemical signals is urine (Lawson et al. 2000, 2001). Once an animal comes into contact with a chemical source, it is transferred to their nasal passage and the mouth where the chemical information is received through the olfactory epithelium and the vomeronasal organ (VNO), respectively. This enables both inter- and intrasexual communication in many species of mammals. Cannulation of the VNO in male goats showed that liquids only reach the VNO after a flehmen was performed and not by the fluid simply being passed through the oral cavity (Melese-D'Hospital & Hart 1985).

Male Asian elephants exhibit different responses to chemical stimuli depending on their developmental stage and social setting (Rasmussen et al. 1986). Asian elephant calves demonstrate flehmens as young as three months old and react more frequently to their mother's urine than to other related or non-related females (Rasmussen & Krishnamurthy 2000). In the presence of an older dominant male, younger males, described as sexually but not socially mature, back away from (Z)-7-dodecen-1-yl acetate (Z7-12:Ac), which has been identified as the sex pheromone in female Asian elephant follicular phase urine (Rasmussen et al. 1996). When tested without the presence of the

dominant male the young males respond with multiple flehmens (Rasmussen et al. 1997). Asian female elephants have shown to be more responsive to male musth urine, but their behavior also depends on their own reproductive status (Schulte & Rasmussen 1999).

Female intrasexual behaviors also depend on reproductive status of sender and receiver (Rasmussen & Schulte 1998; Slade et al. 2003). In the matriarchal society communication between female elephants is important in the survival of calves by means of allomothering and by nulliparous females gaining the experience of rearing calves (Lee 1986). Chemical communication plays a role in maintaining the hierarchical structure of the society, where the dominance of the female is dependent on age and reproductive status (Eisenberg et al. 1971). Female Asian elephants in the follicular phase of their estrous cycle respond more to follicular phase urine than do female elephants in the luteal phase of the estrous cycle (Slade et al. 2003).

Although flehmen and other chemosensory behaviors have been reported in elephants of most ages, documentation of the developmental stages for both sexes does not exist for elephants. The development of the flehmen behavior has been studied in several ungulates and has been seen in horses as early as a day old for fillies and 2 days old for colts (Crowell-Davis & Houpt 1985). In a population of semi-wild cattle, mounting and flehmen were occasionally seen in calves that were older than one week. By the age of 4 months, flehmen became more frequent and seen more often in male than female calves (Reinhardt 1983).

In elephants, flehmen is identified as the trunk curling into the elephant's mouth and the trunk tip placed on the VNO openings known as the incisive ducts. The pair of incisive ducts is found in the anterior portion of the palate (Rasmussen et al. 1982).

When the trunk tip contacts urine or another substrate of interest, mucous from the trunk combines with the urine. Specific protein transporters transfer molecules to the VNO once a flehmen is performed, thereby informing the elephant of the conspecific's reproductive status (Rasmussen et al. 1998). The flehmen response of stallions to mare urine is influenced by the estrous cycle of the females that in turn result in a significant increase in flehmens on the day of copulation (Stahlbaum & Houpt 1989). Bland and Jubilan (1987) noted that ewes urinate more frequently in the presence of a ram, resulting in increased flehmen responses of the ram to the urine. Swaisgood et al. (1999) observed female pandas urinate more frequently while in estrus, presumably to advertise their reproductive status. This leads to increased urination by the male panda, after which both sexes exhibit close interest in the urine of the opposite sex. Female and male pandas also distinguish among individual male scents, suggesting that each individual scent may contain distinct chemical compounds. Elephants show similar behaviors. Upon approach of the male elephant, the female is stimulated to urinate while the male checks her genital area with his trunk. A flehmen results if she is approaching ovulation (Eisenberg et al. 1971, Rasmussen et al. 1993, Moss 1983, Poole 1989a, 1989b, Ortolani et al. in prep.).

Chemical communication plays an integral part in the reproductive success of elephants and is important in maintaining a cohesive social structure through the communication of all ages and sex. Suspected sources of chemical communication among elephants include urine, feces and secretions from the temporal gland, mouth, and ears with the latter three sources resulting in more tactile interactions between the elephants. Males and females respond to chemical cues with chemosensory behaviors such as sniffs, checks, places and flehmens (Fig.1). Other sexual arousal behaviors by

males include erections, mounting and tactile behaviors such as trunk to a female's mouth, back or head. Females also perform specific behaviors during the mating process, such as trunk investigations of the male and postural movements in preparation of mounting and intromission. This process is at least in part set in motion by complementary chemosensory behaviors and signals. As with most mammals, such behaviors are unlikely to be fully formed at birth, but probably arise during development (Moore 1985).

Like other mammals, elephants go through distinct developmental stages of nursing, weaned juveniles, pubescent or young adults, and mature animals. In elephants, calves (0-4yrs) are still suckling, while juveniles (5-9yrs) are being weaned and males are venturing further away from their mothers. At pubescent age (10-19yrs), females have begun to reproduce having their first calf and males have left their natal group to form bachelor herds of their own with males from other family groups. As adults (>19yrs), females have become successful breeders and males are much larger than the females and entering their first full musth. Because of the profound changes and resultant challenges at each of these stages, communicative and investigative behaviors, and for the current interest especially chemosensory ones, may show reflective alterations.

Because the study population of elephants at Addo Elephant National Park (AENP) stems from a founder population of 11 individuals, my results may not generalize to other elephant populations. Therefore, I first examined if two basic behaviors, walking and eating, occurred at comparable rates in young elephants as from elephants in a well-studied population in Kenya. The elephants at Amboseli National Park have been under observation for over 25 years (Moss 2003). Lee (1986) examined

the developmental patterns of 0-9 year old males and females, reporting on general activity levels. I examined some of the same behaviors in the young elephants from AENP for comparison.

The primary objective of this study was to examine the chemical and tactile behaviors of an African elephant population in order to determine the development of such behaviors across age and sex. My competing hypotheses for this project were that there will be differences in chemosensory behaviors of 1) calves (0-4yrs) to the rest of the population (>4yrs), 2) calves and juveniles (0-9yrs) to the rest of the population (>9yrs), and 3) calves, juveniles and pubescents (0-19yrs) to adults (>19yrs). These differences are predicted for both males and females, and that males and females would differ in the rate of chemosensory behaviors for each hypothesis. Because male and female elephants develop at different rates, I hypothesized that they would show different types and rates of chemosensory behaviors at the same stages, and differential changes in behavior over the four developmental stages. Specifically, I predicted that males would develop chemosensory behaviors at a younger age than females. Males would also show higher rates at older age groups compared to the females. Males also would exhibit trunk touches to other elephants at higher rates than would females. In addition, males also would be more likely to touch non-kin elephants as they venture further away from their mothers as juveniles and pubescent males form bachelor herds.

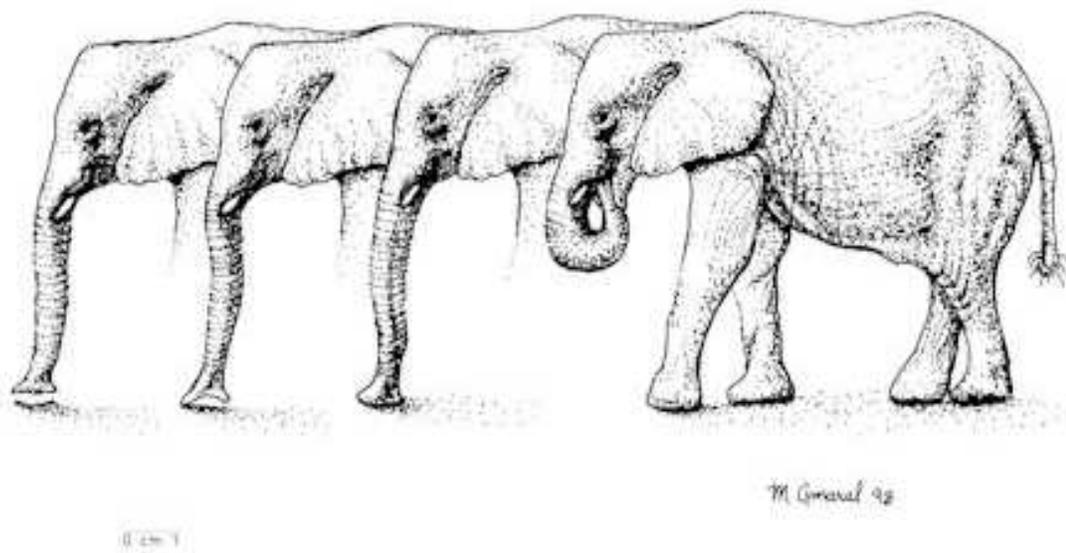


Figure 1. Indicates trunk tip chemical and tactile behaviors starting from left to right with **sniff** (nasal openings hover over substrate/substance without contact), **check** (touch substrate/substance with either finger of the trunk tip), **place** (end of trunk is placed flat on a substrate/substance), and **flehmen** (tip of trunk touches substrate then placed in the openings in the roof of the mouth).

METHODS

History of Study Site: Addo Elephant National Park

In the mid 1600's, the elephant population in South Africa was said to be *ca.* 100,000, but as settlers moved further inland, conflicts between elephant and humans increased (Hall-Martin 1992). By the early 1900's, the majority of the South African elephant population had been decimated for the ivory trade or for crop protection. In the Eastern Cape region there were approximately 130 elephants left, who were the cause of

major crop damage in the area. The authorities decided that the problem had to be taken care of and a hunter, Major P.J. Pretorius undertook this feat. The task began in July 1919, and by August 1920, Major Pretorius had killed close to 120 elephants. The very man that decimated this elephant population also was instrumental in saving the population from becoming extinct. With only 16 elephants remaining there was an outcry from the general public, press, farmers and Pretorius himself to spare the lives of these elephants (Hoffman 1993). In 1931 a small area in the Eastern Cape was proclaimed as the Addo Elephant National Park with only 11 elephants remaining. The founder population consisted of 8 females (six sexually mature) and three males, of which only two were mature (Trollope 1931). Within the next six years the two mature bulls had been shot. The growth of the population was slow and without secure fencing elephants continued to destroy crops. These elephants were killed and several others fatalities were caused by a train that runs along one of the boundaries of the Park (Whitehouse 2001). In 1954, an elephant-proof fence was constructed, which has proved to be very successful in securing the confinement of the elephants, the reduction of human-elephant conflict and the growth in the elephant population. Because of the selective hunting of tusked elephants and a founder population of 11 individuals, only 2% of the current female population has tusks (Whitehouse 2002).

Study Site

Addo Elephant National Park (AENP) is located in the Eastern Cape of South Africa *ca.* 70 km north of the coastal city of Port Elizabeth. Originally founded in 1931, the park consisted of 2237 hectare (ha) (Castley 2004). Currently the game area is 11708 ha (Hall-Martin 1992) and is expanding to reach the goal area of 492,000 ha., which is

known as the “Greater Addo” park. The elephant population consists of approximately 400 elephants. Most of these elephants roam in the original park area that was fenced in 1954 to prevent the elephants from damaging surrounding farms. In May 2003, nearly 60 elephants were moved into a new section of the park called Nyathi.

AENP has several waterholes dispersed throughout the area that are reliant on seasonal rains for replenishment. The average rainfall for the region is 445 mm annually (Paley & Kerley 1998). Park management regulates five additional water holes that are filled by a reservoir to ensure a continuous water supply. During the hot seasons, the elephants spend the warmer part of the day near waterholes. In wet seasons, the elephants are more difficult to find as more of the natural waterholes are filled and are located within the thick vegetation.

The Addo elephants have been extensively studied and all individuals have been identified and catalogued (Whitehouse & Hall-Martin 2000). These elephants are recognizable by individual ear notches and other physical characteristics. AENP’s elephant population consists of 6 family groups ranging from 19 to 106 individuals. Whitehouse (2001) determined the years of birth for all the elephants and each new birth is recorded accordingly.

Observations

The project was conducted from February to early September 2003. Fieldwork began every morning when the park opened and continued until late afternoon. Elephants were first located and a Global Positioning System (GPS) reading of their location was taken. After a count of the elephants was completed, the ID files were used to record the

family and the individual elephants within the group. The elephants were classified by age and sex and divided into four age classes as follows: calves (0-4 yrs), juveniles (5-9 yrs)(stop suckling), pubescents (10-19 yrs)(males have left their natal group and females have at least one calf by early teens) and adults (>19 yrs)(males are larger than the females and beginning to experience their first full musth). At the start of each day, focal animal observation with continuous recording was performed with the focal animal selected haphazardly according to age and sex (Altmann 1974, Martin & Bateson 1993). Therefore, when faced with a group of elephants, the first elephant of the predetermined observation bout to be spotted became the focal animal. Observations were carried out for 20 minutes or until the focal individual was no longer in sight, whichever came first. Figure 2 indicates the average length of a focal bout for each age class, with an overall average of 14.14 minutes (+/-SE 0.37). A total of 116 days was spent in the field with 65 hours of focal data collected. A significant portion of the day was spent locating elephants, then identifying and photographing the elephants for ID file update and determining the identity of the focal individual.

The behaviors recorded in each observation bout were divided into four main types as follows: 1) general state behaviors, 2) trunk tip contacts to non-elephant substrates such as urine, feces or their surroundings, 3) interactions between the elephants by trunk tip, 4) other body contacts not involving the trunk tip (Table 1). Most of the data of responses to urine and feces were collected at waterholes, where elephants gathered most frequently. The park contains ten waterholes with Hapoor being the largest. On several occasions, approximately 100-120 elephants were observed at this waterhole at the same time. This made it difficult to determine the ID of the individual that defecated

or urinated and there were also old feces that had accumulated around the waterhole. As such, the data presented represent responses to urine and feces in general, without knowing the identity of the donor elephant. The frequency and duration of all instances of general state behaviors such as walking, eating and drinking were recorded for all ages, but only eating and walking habits were analyzed for 0-9 year olds. These behaviors were recorded in order to compare rates of walking and eating by the young elephants in the Addo population with similarly aged elephants from the Amboseli study (Lee 1986). For the current study, the main focus was on all chemical and tactile trunk tip behaviors such as sniff, check, place, flehmen and others. The total number of focal observations collected ranged from 18 to 32 different individuals for different age classes of both sexes (Fig. 3). The proportion of the population for each age class that was sampled ranged from 36% to 72% (Fig. 4).

Table 1. Ethogram - list of behaviors and definitions of behaviors observed for each focal observation of elephants at AENP. The capital letter in bold indicates the code used during data collection.

| State Behaviors: | |
|-----------------------------------|--|
| DeF ecate | Release feces |
| U rinate | Release urine |
| DiG | Using trunk or foot to dig into ground |
| D rink | Drinking water |
| DuS T | Using trunk to throw dirt over body |
| E at | Consuming food, whether gathering with trunk, lifting to mouth or chewing |
| L ie | Body on side on ground |
| M ud | Either wallowing in mud/waterhole, or using trunk to spray water and mud over body |
| S tand | Stay in same spot |
| W alk | Moving forward |
| WR estle/ spaR ring | Face to face contact, trunks intertwined, pushing and shoving |
| O ther | Other behaviors observed, but not listed on ethogram |
| Not Visible | Elephant has moved out of sight |
| Trunk Tip To/From: | |

| | |
|-----------------------|---|
| Anal | Anal region of another elephant |
| Body | Torso or areas not listed |
| Ears | Hole |
| Feet | Area below ankle |
| Head | Forehead and superior most point of head |
| LeGs | Area between ankle and hip |
| Mouth | Trunk tip inserted into mouth of another |
| Pinnae | External portion of ear |
| TaiL | Trunk touches or grabs another's tail |
| Trunk | Portion of trunk starting from mouth area and down to the tip |
| TusK | Any visible part of the tusk |
| Temporal Gland | Point of TG secretion |
| Genital Check | Trunk touching genitals of another elephant |

Contact To/From:

| | |
|----------------------|---|
| Back Into | Moves backward into another elephant, intentionally |
| Body Rub | Walks past another elephant and rubs against them |
| Head Butt | Uses head to bump into another elephant |
| Incidental | Any contact that is not intentional |
| Kick | Lifts leg to touch/push another elephant |
| Trunk on Head | Place one's trunk on another's head |
| Trunk Wrap | Trunks are intertwined |
| Tail Touch | Tail outstretched to touch another elephant |

Trunk Chemosensory: (trunk tip behaviors)¹

| | |
|----------------|---|
| Sniff | Nasal openings hover over substrate/substance without contact. |
| Check | Touch substrate/substance with tip of trunk (either finger) |
| Place | End of trunk is placed flat on a substrate/substance |
| Rub | In place position, the trunk is moved in a circular motion |
| Flehmen | Tip of trunk touches substrate then placed in the openings in the roof of the mouth. |
| Other | Other behaviors associated with the trunk after coming into contact with a substrate. |

¹Based on Schulte & Rasmussen, 1999

Analysis

The data were first tested for normal distribution of the dependent variables using the JMP 4 statistical program. The dependant variables were separated into different categories. First I looked at differences in every instance of trunk chemosensory behaviors to substrates not including those behaviors to other elephants across sex and age classes. These behaviors were separated into trunk tip behaviors to general

surroundings and trunk tip behaviors to urine and feces. The calculation of the rate (number per minute) of chemosensory behaviors represented the trunk chemosensory behaviors listed in Table 1 including other behaviors such as aborted flehmen, trunk shake and flick, and periscope. The rate for all individual focal observations and the rate of individuals actually seen demonstrating trunk chemosensory behaviors were analyzed separately. Normality on some data was not indicated and therefore the data were transformed to achieve a normal distribution. Parametric analysis of variance (ANOVA) tests were used to determine behavioral differences between age, sex, and the interaction between the two. To compare the specific hypotheses, the data were grouped by age classes as follows: (1) calves (0-4 y) versus all elephants > 4 y; (2) calves and juveniles (0-9 y) versus pubescents and adults (> 9 y); and (3) pre-adults (0-19 y) versus adults (> 19 y). Separate ANOVA tests were performed for each comparison ($\alpha = 0.05$). If assumptions were not met after transformation, then nonparametric tests were used. Specifically, Kruskal Wallis was used to determine differences in age classes within a sex and Mann Whitney tests were used to determine differences between sexes. Tukey Kramer test was used for a posteriori analysis with alpha adjusted for multiple comparisons. In order to determine if there was a difference in the proportion of males and females exhibiting chemosensory behaviors, a Chi-Square test was performed.

RESULTS

A. State Behaviors

State behaviors for young elephants were used to compare with studies conducted in Amboseli, Kenya. Young elephants (0-9 yrs) spent 49% of their daily activities eating and 24% of their time walking. There was no significant difference in the time spent walking by age class (2-way ANOVA: $F_{1,90} = 0.56$, $P = 0.46$), between sexes (2-way ANOVA: $F_{1,90} = 0.31$, $P = 0.58$) or the interaction between age and sex (2-way ANOVA: $F_{1,90} = 0.101$, $P = 0.32$) (Fig. 5). There was no significant difference in the time spent eating for age comparing 1-9 year olds (2-way ANOVA: $F_{1,55} = 0.14$, $P = 0.70$) or sex (2-way ANOVA: $F_{1,55} = 0.14$, $P = 0.71$), but there was a significant interaction between age and sex (2-way ANOVA: $F_{1,55} = 5.86$, $P = 0.02$) (Fig. 6). No data were available for female calves younger than one year old in this study for eating, so male calf data of this age were excluded for their comparison. For pre-pubescent males, time spent eating differed significantly by age (Kruskal Wallis: $H = 11.25$, $df = 5$, $P = 0.05$), but young female elephants up to the age of nine showed no such difference (Kruskal Wallis: $H = 4.16$, $df = 5$, $P = 0.53$). When a similar comparison by age within sex was performed for walking, males again differed by age (males: $H = 14.08$, $df = 6$, $P = 0.03$) but females did not ($H = 3.36$, $df = 6$, $P = 0.76$).

B. Chemosensory Behaviors

Three main hypotheses were examined by comparing the prevalence and the rate of chemosensory behaviors across all ages and sex. A total of 204 focal data were collected on different individuals, consisting of 112 focal observations on males and 92 focal observations on females. The overall average time males and females were

observed did not differ (mean=15.7 \pm 4.9; Mann Whitney: U= 5834, df = 1, P>0.05). The proportion of males and females demonstrating chemosensory behaviors did not differ between the sexes in the rate of chemosensory behaviors to general surroundings ($\chi^2 = 0.07$, df=1, p = 0.79, Fig. 7). There were, however, significantly more males than females responding to urine and feces ($\chi^2 = 5.89$, df = 1, P= 0.02, Fig. 8). Combining across age classes, 24.7% of males and only 10.5% of females responded to urine and feces.

Average chemosensory behaviors of all focal individuals

To examine the specific competing hypotheses, different age classes were compared in order to determine if there were developmental differences in chemosensory behaviors and if they also differed by sex. There were no significant differences between sexes and age classes for chemosensory responses to general surroundings (2-way ANOVA, sex: F = 0.12, df = 1,195, P = 0.73; age class: F = 0.64, df = 3,195, P = 0.59, Fig.9). For responses to urine and feces, there was a significant interaction between sex and age class (2-way ANOVA, F = 2.79, df = 3,161, P = 0.04, Fig.10 & Fig. 11). A *posteriori* tests showed significant differences between the female calves and adult females for responses to urine and feces, with calves (mean \pm SE = 0.38 \pm 0.26) responding more than the adult females (mean \pm SE = 0.02 \pm 0.01)(Fig. 10).

No differences were seen in responses to the general surroundings for the three main hypotheses (Fig. 12). Male elephants showed a significant increase in overall rate of chemosensory behaviors until pubescence, thereafter they showed a slight decline in these behaviors as adults (Kruskal Wallis: H = 10.88, df = 3, P= 0.01)(rates of behaviors per minute: calves = 0.16 \pm 0.06, juveniles = 0.44 \pm 0.24, pubescents = 0.61 \pm 0.14,

adults = 0.49 ± 0.13). Females did not show a difference in rate of chemosensory behaviors by age classes (Kruskal Wallis: $H = 3.56$, $df = 3$, $P = 0.31$)(females overall mean rate per minute = 0.37 ± 0.17).

Average chemosensory behaviors of focal individuals who showed a response

For elephants that exhibited chemosensory responses, the rate of responses by males to their general surroundings significantly differed by age class, but there was no difference in female responses by age class (1-way ANOVA: $F = 3.52$, $df = 3$, $P = 0.04$; $F = 1.24$, $df = 3$, $P = 0.31$, respectively). Pubescent males showed a consistent peak in the rate of responses (calves = 0.40 ± 0.14 , juveniles = 0.76 ± 0.34 , pubescents = 0.88 ± 0.15 , adults = 0.49 ± 0.12 per minute), whereas calf females exhibited higher rates than females from older age groups (calves = 1.47 ± 0.84 (N=19), juveniles = 0.82 ± 0.36 (N=24), pubescents = 0.72 ± 0.29 (N=17), adults = 0.49 ± 0.10 (N=32)). This trend was seen for responses to the general surroundings with a significant interaction between sex and age class (2-way ANOVA, $F = 3.06$, $df = 3, 94$, $P = 0.03$; Fig. 13). Although a similar trend was seen for responses to urine and feces, there were no significant differences for any effect (Fig. 14). *A posteriori* tests showed differences only between male calves and pubescent males for chemosensory responses to general surroundings (Fig. 13).

For elephants' responses to the general surroundings, there was a significant interaction with sex and age class between both calves (0-4yrs), and calves and juveniles (0-9yrs) to the rest of the age classes (Fig.15). Female calves showed the highest rate of response to the general surrounds (mean \pm SE = 0.82 ± 0.19) with values decreasing at the older age groups (mean \pm SE = 0.58 ± 0.12). On the other hand, male calves had low

levels of chemosensory trunk behaviors to the general surrounds (mean \pm SE = 0.40 \pm 0.14) whereas older males, especially pubescent males (mean \pm SE = 0.88 \pm 0.15), exhibited higher rates. Similar trends were seen for responses to urine and feces and there was a significant interaction between age class and sex for rates of responses to urine and/or feces (2-way ANOVA, $F = 4.41$, $df = 1,26$, $P = 0.045$; Fig. 16).

C. Types of Chemosensory Behaviors

Overall, more males ($N = 75$ to general surroundings and $N = 27$ to urine and feces of 110 males observed) responded with individual types of chemosensory behaviors than females ($N = 53$ to general surroundings and $N = 8$ to urine and feces of 92 females observed). The proportion of males were not significantly more than females for responses to general surroundings ($\chi^2 = 2.40$, $df = 1$, $P = 0.12$), but there was a significantly greater proportion of males responding to urine and feces ($\chi^2 = 9.29$, $df = 1$, $P = 0.0023$).

Overall males exhibited 58.1% of the total types of chemosensory behaviors by males, whereas females only exhibited 41.9% of behaviors to general surroundings by females. Breaking it down into the individual types of behaviors, males performed 12.7% sniffs and 43.3% checks of total behaviors, but females exhibited 5.2% sniffs and 35.2% checks of the total behaviors. Place behaviors accounted for a small percentage of behaviors for males (1%) and females (1.4%). For flehmen behaviors, the percentages were also small with males exhibiting 0.9% and females only 0.1% (Table 2A). Similar trends were seen for responses to urine and feces. Males performed 8.1% sniffs and 44% check behaviors, whereas females only exhibited 4.8% sniffs and 20.7% checks of the

total behaviors. Males and females exhibited the same percentages of place (10% each) behaviors. Males demonstrated 1.5% flehmen behaviors, whereas females only exhibited 0.3% flehmens of the total behaviors (Table 2B).

D. Trunk Tip Behaviors to Other Elephants

Trunk tip behaviors to other elephants did not differ by age class, sex or their interaction. The sample sizes for each category were too small to detect any significant differences. Moms and calves did not touch each other more (mean rate per minute \pm SE = 0.09 ± 0.01) than close family members touched each other (mean rate per minute \pm SE = 0.1 ± 0.01) or non-family members (mean rate per minute \pm SE = 0.1 ± 0.02) (Fig. 17). Pubescent and adult males and females performed all trunk tip touches to non-kin (Fig. 17). In opposition to the stated prediction, males (mean rate per minute \pm SE = 0.12 ± 0.03) did not show significantly more trunk tip touches to non-kin than females (mean rate per minute \pm SE = 0.06 ± 0.01).

DISCUSSION

Eating and walking behaviors in African elephants at Addo Elephant National Park (AENP) indicated trends similar to those found in a previous study by Lee (1986) in Amboseli National Park, Kenya. Differences in the methods between the two studies, for example length of study, scan versus focal data collection and hence statistical analysis may explain the differences in levels of significance found in the current study compared to Lee's study. Male elephant calves spent more time eating at the older age groups than

females of the same age. The similarities in trends of state behaviors in these two studies leads me to believe that the results and findings of my study on the development of chemosensory behaviors can be generalized to other elephant populations.

Adult male and female elephants are sexually dimorphic in behavior and morphology. Thus, their developmental pathways are likely to differ in both rate and type of behavioral (and morphological) alterations. This study examined the developmental patterns of male and female African elephants for chemosensory and tactile behaviors, which are very important in the stability of their social structure and reproduction. I predicted that males and females would be sexually dimorphic in the rate of development and the specific types of chemosensory behaviors exhibited.

The rate of chemosensory behaviors across age classes and sex for this population of African elephants differed in prevalence and context. The behaviors were examined in two contexts: 1) elephant sources such as urine and feces, or other elephants; and 2) sources from the general surroundings such as the road, vegetation (with no indication of feeding) and other objects. The rationale at looking at individual chemosensory behaviors was to determine whether the expression of specific behaviors such as checks and flehmens differed by sex.

Males and females did indeed show different developmental pathways for chemosensory behaviors in rate and kind. Female calves had the highest rates among the female age classes and in fact, this rate was even greater than that observed by male calves, although the latter was not explicitly analyzed statistically. The rate of chemosensory behaviors for the male elephants increased from calves, peaked with pubescent males and then showed a slight decline for adult males. This trend was

significant for responses to general surroundings and was also seen in responses to urine and/or feces. A greater proportion of adult males and females compared to the younger age groups responded to sources from the general surroundings. For urine and feces, a greater proportion of males than females exhibited chemosensory behaviors.

The increased rate of chemosensory behaviors for pubescent males to surroundings included dirt roads, which may include urine signals or other scents from elephants from days passed. These young males having left their natal group and out on their own without the guidance and security of their family may be more active to determine the possibility of a musth male in the vicinity. If so, these young males will be wary of a musth male, reducing the possibility of conflict. Scott (2002) suggests this concept as an explanation having found that subordinate and nonmusth Asian males were able to differentiate musth urine from nonmusth urine. Musth males are the dominant bulls regardless of age when confronted with other nonmusth males (Poole 1989b). Older males in Addo, when not in musth spent most of their time in the same area of the park, rarely associating with female family groups, unless at a waterhole together. This might explain the slight decline in the rate of chemosensory behaviors observed, as adults may have less new stimuli to investigate (Loizi pers. obs.).

Chemosensory behaviors may have to be mastered and refined quickly by females as they begin reproducing earlier than males. Some females in Addo have their first calf at 10-11 years of age, and the average age of conception in this population is 11.2 years old (Woodd 1999). Adult females showed a slightly higher rate of behaviors than pubescent females. This may serve to maintain the social structure and hierarchy within the family group. Older female matriarchs are repositories of knowledge, possibly

serving to guide and protect the family groups more efficiently than younger matriarchs (McComb et al. 2001). Because females live with closely related individuals throughout their lives (Buss 1961), the need to constantly explore and “check” each other using chemosensory behaviors may not exist. However, olfactory detection may be quite beneficial for intersexual communication. For instance, males release many odiferous signs of musth through urine dribbling, secretions from their temporal gland and a general body odor (Poole 1987). It may be possible that females make use of their olfactory system and therefore need less chemosensory verification of the signals than males through trunk tip contact behaviors. Females may have a more refined use of their accessory olfactory system, performing flehmen in only very particular situations.

Males and females differed in the types of behaviors they exhibited. Pubescent and adult male elephants demonstrated more flehmen behaviors than younger males and females of all ages. Similar patterns have been observed in other mammals. Ungulate pubescent and adult males, cattle (Reinhardt 1983), and horses (Crowell-Davis & Houpt 1985) exhibited more flehmen behaviors than younger males and females. In a semi-wild population of cattle, males exhibited more flehmens than females. Bull cattle (*Bos indicus*) demonstrated the flehmen behavior more frequently than younger males, suggesting the adult bull's sensitivity towards estrous female signals (Reinhardt 1983).

Flehmen is a behavior that is performed prior to mating and instrumental in the reproductive assessment of a conspecific (Rasmussen et al 1997). Adult males become experienced breeders; therefore, they become selective in the types of behaviors they exhibit (Rasmussen & Greenwood 2003). A greater proportion of males exhibited these chemosensory behaviors than females. Many pubescent and adult females in Addo were

lactating or may already be pregnant, and such females may not be interested in receiving chemical cues as they are not reproductively ready. As adults, males go through predictable periods of musth in which they are actively searching for females. Once located, the males will actively check females and their urine assessing their reproductive state (Poole 1989a, 1989b).

In this study, males and females did not discriminate in their relationship to other elephants they touched with the tip of the trunk. This may be because of the small sample size. One interesting trend that came from these observations was that pubescent and adult males and females initiated all trunk tip behaviors exhibited to non-kin. A compound found in temporal gland secretions (TGS) of Asian male elephants has recently been identified as the Asian male pheromone (Rasmussen & Greenwood 2003). The pheromone, known as frontalin, is only found in musth males and is present in their urine, breath and TGS (Rasmussen 1998). A further look into the data of this study indicated that all pubescent and adult female trunk tip touches were to pubescent and adult males, but the males' touches were geared toward pubescent and adult males, and adult females. As suggested by Rasmussen and Greenwood (2003), females can assess the musth male reproductive condition and maturity because the longer a male is in musth, the more frontalin is secreted. Younger and nonmusth males may use this information to avoid possible conflicts (Rasmussen et al. 2002).

This study has given us a better understanding of elephant development and chemical communication. In polygynous societies, selection favors female elephants to develop chemosensory behaviors at a faster rate than males in order to begin reproducing

at an earlier age. Male elephants are able to reproduce with several females during a musth period and continue to do so throughout their lives.

This study has given us a better understanding of elephant development and chemical communication. It is probable that in most polygynous societies, selection favors females to develop chemosensory and other adult behaviors at a faster rate than males in order to begin reproducing at an earlier age. For elephants and many males in polygynous societies, peak male reproductive success may occur later in life, permitting a longer period for the development of adult behaviors. From this study on African elephants, it appears that the development of chemosensory behaviors reflects the general development of reproductive morphology, physiology and behavior.

Chapter 2

Male elephant responses to female urinary compounds in a captive population and a wild population

INTRODUCTION

African and Asian elephants live in matriarchal societies that consist of very close knit related females and their offspring. Males leave their natal group between the ages of 12-15 years old to form bachelor herds and as they get older may lead solitary lives, infrequently associating with females primarily to reproduce (Eisenberg et al. 1971, Estes 1991, Poole 1994).

The gestation period of elephants is approximately 22 months and thereafter females will lactate for three to four years (Poole & Moss 1989, Moss 2001). The interbirth interval is longer after birthing male than female calves as greater energy and time is invested in male calves (Lee 1986). Therefore, females may only ovulate once every four to five years, making their timing of ovulation and successful mating very important.

Female elephants have an estrous cycle lasting approximately 16 weeks. The cycle consists of two phases, the luteal phase (nonreceptive, 8-12 weeks) in which there are elevated progesterone levels, and the follicular phase (terminating with ovulation) lasting 4-6 weeks (Asian: Hess et al. 1983, African: Plotka et al. 1988). During the follicular phase of the cycle two surges of luteinizing hormone (LH) occur approximately three weeks apart (Kapustin et al. 1996, Brown et al. 1999). The first LH surge occurs about three weeks after the decline of progestins and is referred to as anovulatory. The second surge takes place 20 days later and corresponds with ovulation (Brown et al.,

1991, 1999, Czekala 2003). The function of the first LH surge is unknown but it has been speculated that it acts as an early advertisement to attract appropriate males in time for ovulation (Brown 2000). In Asian elephants, the pre-ovulatory pheromone is found in urine from the mid-follicular phase (ca. time of first LH surge) of the estrous cycle and is not detectable in luteal phase urine (Rasmussen 1999).

The Asian elephant (*Elephas maximus*) female pheromone has been identified as (Z)-7-dodecenyl acetate (Z7-12:Ac). It is a single compound, although it may be associated with a carrier protein for full activity (Rasmussen et al. 1997, Rasmussen 2001). Z7-12:Ac peaks in concentration at ovulation, acting as a female to male specific signal that advertises reproductive-readiness to attract males (Rasmussen et al. 1997). The concentration of Z7-12:Ac and the chemosensory responses of sexually mature male elephants are positively correlated. Male Asian elephants perform the highest rates of flehmen to preovulatory urine (Rasmussen et al. 1997). Female elephants show no response beyond novel compound interest to this compound (Rasmussen & Schulte 1998).

Z7-12:Ac is a compound used as “part of a pheromone blend” by females to attract potential mates in over one hundred species of insects, primarily lepidoptera (Rasmussen et al. 1996). African male elephants do not respond to the Asian elephant estrous pheromone. However, African male elephants respond to conspecific, follicular phase urine in a similar manner as Asian male elephants to conspecific preovulatory urine (Bagley 2004). The occurrence of a similar estrous pheromone in African female elephants is suspected but currently not known.

Thus, I hypothesize that African female elephants also release an estrous pheromone in their urine. As part of the search for this pheromone, male African elephants were presented with several compounds identified in follicular phase urine acquired from female African elephants. The chemical compounds used are also found in other natural products. Three compounds, alpha-Damascone, beta-Damascone and Damascenone, fit these criteria. When possible, compounds that serve as pheromones in insects were selected. Specifically, Verbenone and Verbenol are part of the pheromonal repertoire in bark beetles (Birch 1984). Each of the compounds tested were available commercially and did not have to be synthesized. Therefore, we examined the potential of these five compounds as pre-ovulatory pheromone candidates in female African elephant urine.

METHODS

Captive Study

Bioassays with captive elephants were conducted at Riddle's Elephant Sanctuary in Greenbrier, Arkansas. This facility houses six African male elephants and is located 30 miles from Hendrix College where Dr. Goodwin, of the Chemistry Department, identified compounds found in female African elephant urine. For the purpose of this study only four males were assayed (Willie: 23yrs old; Toby: 22yrs old; Solomon: 19yrs old; Artie: 19yrs old). The captive study was conducted in May-June 2002. On each trial, six compounds were assayed, one of which was water. Five of the compounds are known to occur in follicular urine collected from African elephants. Water was used as the control because it was the solvent for the other compounds. Five compounds were of

liquid form (alpha Damascone, beta Damascone, Damascenone, (1S)-(-)-Verbenone, Water) and one was a solid ((S)-cis-Verbenol). A couple of drops of each compound and a pinch of the solid form were each separately diluted in 100 ml of water. Actual concentrations of compounds in natural urine are not known. The concentrations used for our study are similar concentrations used in previous assays by Dr. Goodwin. The six compounds were presented simultaneously to the male elephants on three separate days and their responses were recorded on paper and video. The compounds were poured directly on the ground and stone plates were used as markers. The elephants were observed from a distance of 10m in a position outside the enclosure. Behavioral responses included trunk tip responses such as sniffs, checks, places and flehmen (Fig. 1). The number of approaches (an elephant being within one body length to a sample) and the number of nears (an elephant's trunk being within a trunk length to a sample) were also recorded. Other behaviors such as body posture, erections, vocalizations, and the investigation of nearby elephants also were noted.

Wild Study

The wild study was conducted in Addo Elephant National Park, located approximately 70 km from Port Elizabeth, South Africa. The assays were conducted between April and September 2003. This is a wild population enclosed in an area of 103 km². The same compounds were prepared and assayed as in the captive study, but a control consisting of vanillin (a mixture of 1ml vanilla extract in 100ml water) was used. Vanillin is a compound that naturally occurs in Asian elephant secretions, but elicits low chemosensory responses from African elephants as well. This was done to keep all

compounds presented novel. Once a male elephant was located, the samples were presented to the elephant in one of three ways: 1) pouring the sample from the window of the vehicle and backing up to a point of clear view of elephant and sample, 2) predicting the path of travel of the elephant and placing samples in the elephants path, or 3) the samples were placed at a waterhole (Marion Barea) known to be frequented by male elephants. This was the only waterhole out of sight of tourists and easily accessible at a close distance with a vehicle. The third process proved to be the most successful of the three. Chemosensory behaviors were not recorded as rate per time as determining a start/stop time was difficult. Many males walked passed the compounds on their way to the waterhole and this was recorded as an approach, but they did not revisit the compounds, therefore making it difficult to determine a time frame.

The compounds were prepared and then poured into containers prefilled with dirt collected from outside the Game area. This was done for two reasons, 1) to eliminate the possibility of presenting dirt contaminated with elephant odors and 2) to identify where the compounds were situated once placed on the ground. The same behavioral data were collected as in the captive study.

Analysis

The data were first tested for normal distribution and equal variances of the dependent variables using the JMP 4 statistical program. A Friedman's ANOVA on ranks was used to determine difference in response behaviors to the different compounds, between individual animals and over the three days of assays. Specifically, the average number of times per hour an elephant approached a sample was calculated. Similarly, the

average number of times the elephant's trunk neared a sample was calculated. The total chemosensory responses for the captive males were sniff and check, as these were the only behaviors exhibited and were analyzed as rate per hour. The total chemosensory behaviors for the wild population include sniff, check, place and flehmen and were calculated as a frequency. In order to compare the responses by captive and wild elephants, the rate of chemosensory responses also were calculated on a per approach basis.

Two-way ANOVA's (age * sample) were used to statistically analyze the assays on the wild population as the same individual was only tested once, and not on a repeated basis as with the captive study. A total of 33 males were assayed with the compounds and they ranged in ages from one to forty-nine. Only three individuals were assayed twice therefore an average of their responses was calculated and t-tests were used to determine if their responses changed by trials.

T-tests were also used to determine differences in the total chemosensory behaviors per approach between the wild and the captive populations.

RESULTS

Captive Study

A single compound that serves as a pheromone should elicit high levels of response, including flehmen, relative to non-pheromonal compounds. In this study, none of the compounds were approached significantly more than the control (Friedman's: $\chi^2 = 10.54$, $df = 5$, $P > 0.05$). Over the three days, responses to the compounds decreased, indicating the novel nature of the compounds was fading (Friedman's: $\chi^2 = 8.00$, $df = 2$,

P<0.05). Variation among the elephants was evident (Friedman's: $\chi^2 = 12.75$, $df = 3$, P<0.05, Fig. 18). The dominant male, Willie, showed low levels of responses to all the compounds. However, one of the subordinate males, Solomon, a 19-year old male, approached all the samples consistently more than the other males. The same pattern was seen for the average number of trunk near compounds with Solomon averaging more than the other males (Solomon, nears per hour: 1.25-3.37, Toby: 0-2.50, Artie: 0-0.65, Willie: 0.37-1.05) (Fig. 19). For the total check responses, there were significant differences for elephant and day (elephant $\chi^2 = 29.40$, $df = 3$, P<0.05; day $\chi^2 = 7.13$, $df = 2$, P<0.05). There was no significant difference in compound ($\chi^2 = 9.18$, $df = 5$, P>0.05). Solomon demonstrated a greater average of check responses to alpha-damascone (3.87 ± 2.95 checks per hour) and beta-damascone (3.63 ± 0) compared to the other samples, where the next highest responder, Toby averaged 1.29 ± 1.29 and 1.21 ± 0.61 checks per hour, respectively (Fig. 20). The only chemosensory behaviors exhibited by the four males were either sniffs and/or checks (S+C: total chemosensory). There were significant differences between elephants, compounds and the day of assay for total chemosensory behaviors ($\chi^2 = 12.65$, $df = 3$, P<0.05; $\chi^2 = 13.50$, $df = 5$, P<0.05; and $\chi^2 = 7.13$, $df = 2$, P<0.05, respectively)(Fig. 21).

Wild Study

Responses to the compounds from the wild population did not elicit high rates of chemosensory behaviors. There was a nearly significant difference in the average number of approaches to the samples by age class (ANOVA: $F_{3,192} = 2.53$, P=0.058). No significant difference to a particular sample approached was seen ($F_{5,192} = 0.13$,

$P=0.99$)(Fig. 22). Once the elephants had approached a sample, pubescent males' trunks neared the samples significantly more than the other age classes ($F_{3,192}=4.76$, $P=0.003$)(Fig. 23). The elephants in Addo exhibited several different types of chemosensory behaviors (sniffs, checks, places, flehmens and blows) to the six samples presented. There were only significant differences between age classes for check behaviors ($F_{3,192}=2.87$, $P=0.04$)(Fig. 24 shows the total of all the aforementioned behaviors) but no differences in age class, sample or the interaction between the two for the other behaviors exhibited.

Of the three males that were assayed on two different days, only one showed an interest on the first day followed by a significant decline in approaches ($t=3.79$, $df=5$, $P=0.01$), but no difference in the number of near behaviors ($t=0.54$, $df=5$, $P=0.61$). No chemosensory behaviors were demonstrated on either day. The remaining two elephants did not approach or demonstrate chemosensory behaviors to the samples.

Wild vs. Captive

The one-way ANOVA did not indicate a significant difference between the number of chemosensory behaviors per approach of a sample between wild and captive elephants ($F=1.27$, $df=4$, $P=0.30$, Fig. 25). The means for the wild population range from 0.60 (± 0.40) to 6.14 (± 2.20), and the mean for the captive population was 0.53 (± 0.11) chemosensory behaviors per approach.

DISCUSSION

The goal of this study was to determine whether one of five chemical compounds found in the follicular urine of female African elephants would elicit similar behaviors in male African elephants as those evoked by the Asian estrous pheromone, Z7-12:Ac, in male Asian elephants. Unfortunately, none of the compounds elicited interest beyond that of a novel compound. Hence, the African elephant estrous pheromone remains undiscovered.

The male elephants of Riddle's Elephant Sanctuary participate in assays on a regular basis; hence, they are familiar with the procedures of such experiments. This however, did not affect the results of this study because all the compounds that were presented were novel substances to the elephants. Overall, the dominant bull (23 years old at time of assays) did not exhibit many responses. Solomon, a 19 year old male, showed a higher rate of responses than the other males. Another sub-dominant male, Toby, 22-years-old, also exhibited fairly high rates of responses to the compounds. The dominant bull, a successful breeder, did not show interest in the compounds. This permitted the younger, non-sexually experienced males the opportunity to spend more time investigating these novel compounds. Similar observations were made for Asian elephant males. Subordinate males only expressed interest in the estrous pheromone when the dominant male was absent (Rasmussen et al. 1996). In the current study, all males showed a slight increase in approaches and checks responses on the second assay day, but by the third trial the rate of responses declined dramatically to practically no responses at all.

In the wild, the males assayed ranged from ages of one to forty-nine. The younger age classes from 0-9 year old exhibited high numbers of chemosensory behaviors, whereas the adult males (>19 yrs old) exhibited little or no behaviors to the compounds. The pubescent males (10-19 yrs old) exhibited fewer behaviors than the younger male, but more than the adult males. The younger males also demonstrated a greater array of types of chemosensory behaviors than the older males. No difference was seen in the number of chemosensory behaviors per approach to a sample between the wild and the captive population. The captive males ranged from ages 19 to 23 years old. This again supports the idea of the less sexually experienced males showing interest to novel compounds, but the older males not responding.

. The compounds used in this study did not elicit responses high enough by either captive or wild elephants to conclude that they are part of the African female pheromone. This was the initial attempt to try and identify the African estrous pheromone. This is an ongoing project; thus, other pheromone candidates will be bioassayed in the future.

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Table 2. Total number of types of chemosensory behaviors within age classes.

| | Sniff | Check | Place | Flehmen | N |
|-----------------------------------|-------|-------|-------|---------|----|
| A. To General Surroundings | | | | | |
| Males | | | | | |
| Calves | 5 | 51 | 0 | 1 | 10 |
| Juveniles | 21 | 58 | 2 | 1 | 11 |
| Pubescents | 38 | 153 | 7 | 4 | 18 |
| Adults | 59 | 157 | 1 | 3 | 15 |
| Females | | | | | |
| Calves | 19 | 45 | 5 | 1 | 7 |
| Juveniles | 6 | 51 | 2 | 0 | 10 |
| Pubescents | 4 | 70 | 3 | 0 | 6 |
| Adults | 21 | 174 | 4 | 0 | 21 |
| B. To Urine/Feces | | | | | |
| Males | | | | | |
| Calves | 1 | 1 | 0 | 0 | 1 |
| Juveniles | 10 | 39 | 4 | 0 | 6 |
| Pubescents | 2 | 47 | 10 | 3 | 5 |
| Adults | 14 | 60 | 19 | 2 | 9 |
| Females | | | | | |
| Calves | 14 | 55 | 33 | 0 | 3 |
| Juveniles | 0 | 4 | 0 | 4 | 1 |
| Pubescents | 0 | 0 | 0 | 0 | 0 |
| Adults | 2 | 10 | 0 | 0 | 4 |

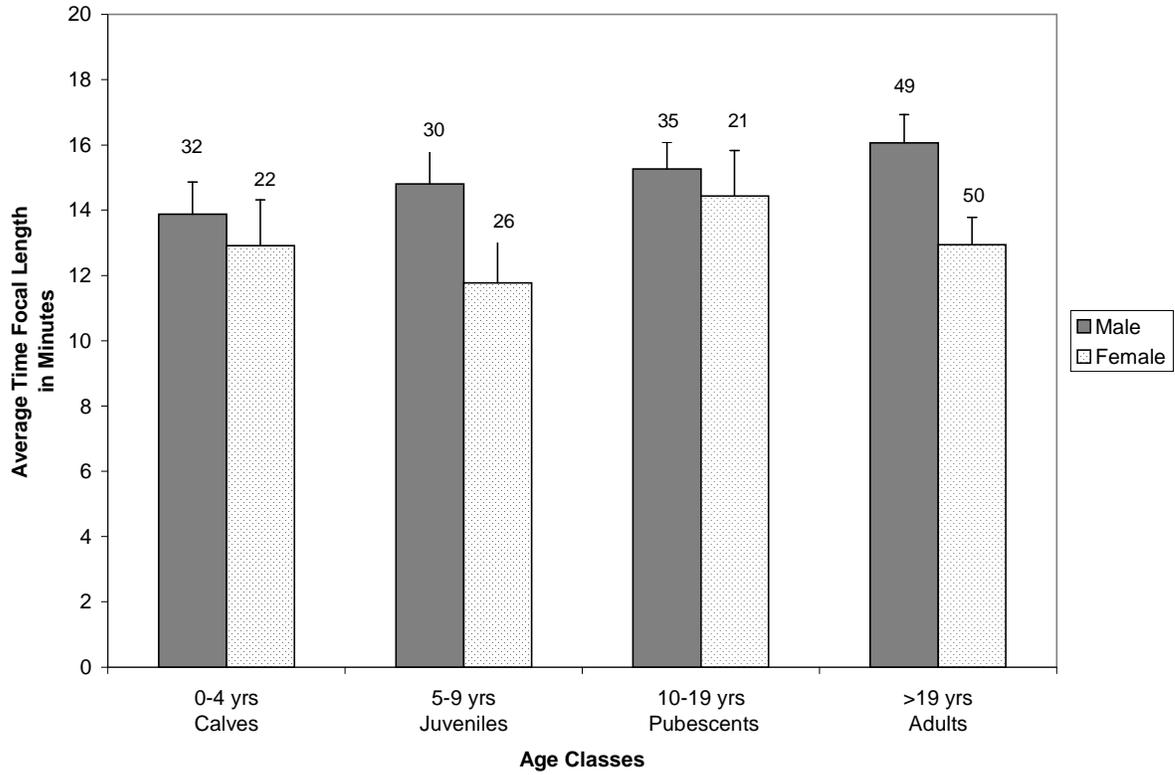


Figure 2. Average focal length per age class for males and females at AENP. Sample size is shown above each bar.

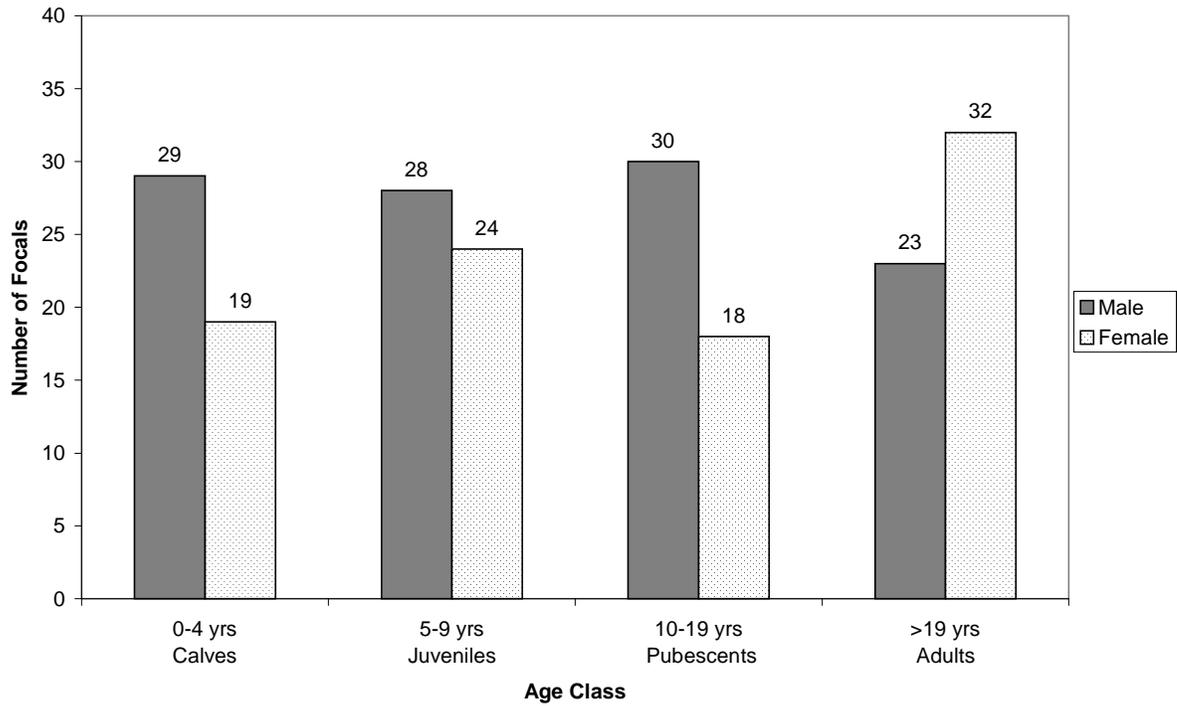


Figure 3. Number of individual focal animals for each sex and age class at AENP. Sample size shown above bars.

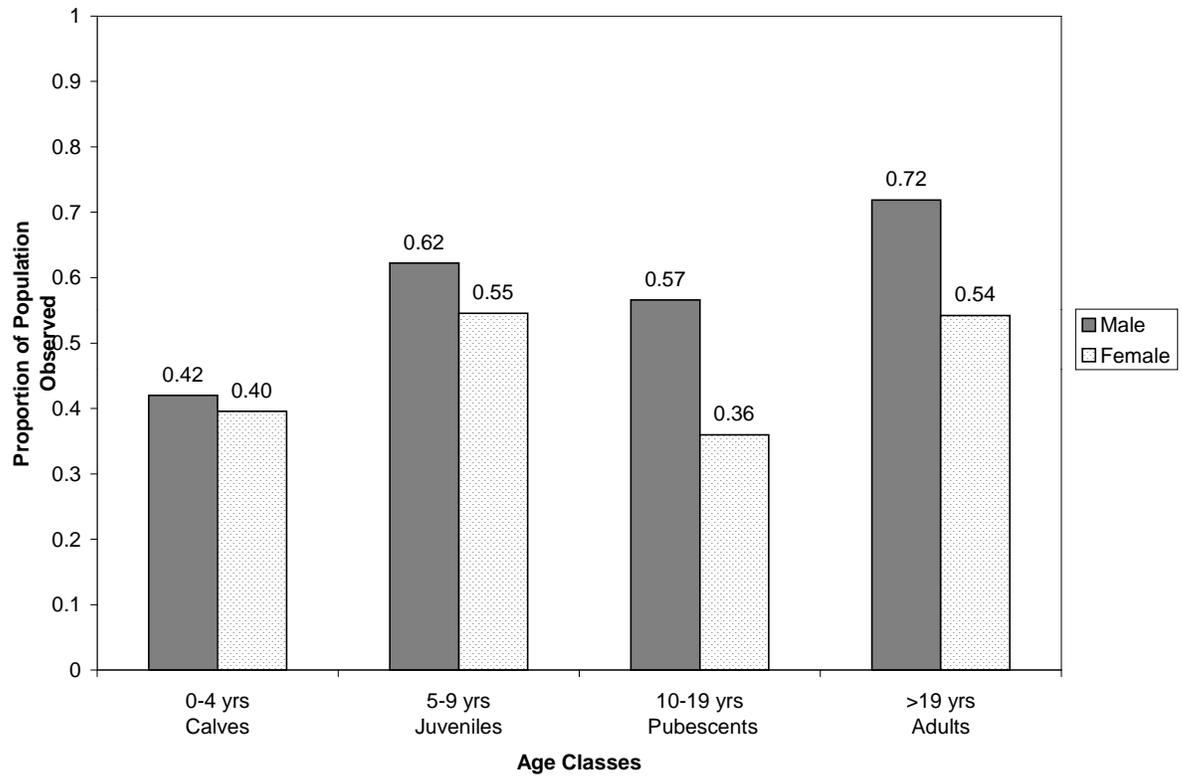


Figure 4. Proportion of total population per age class sampled at AENP.

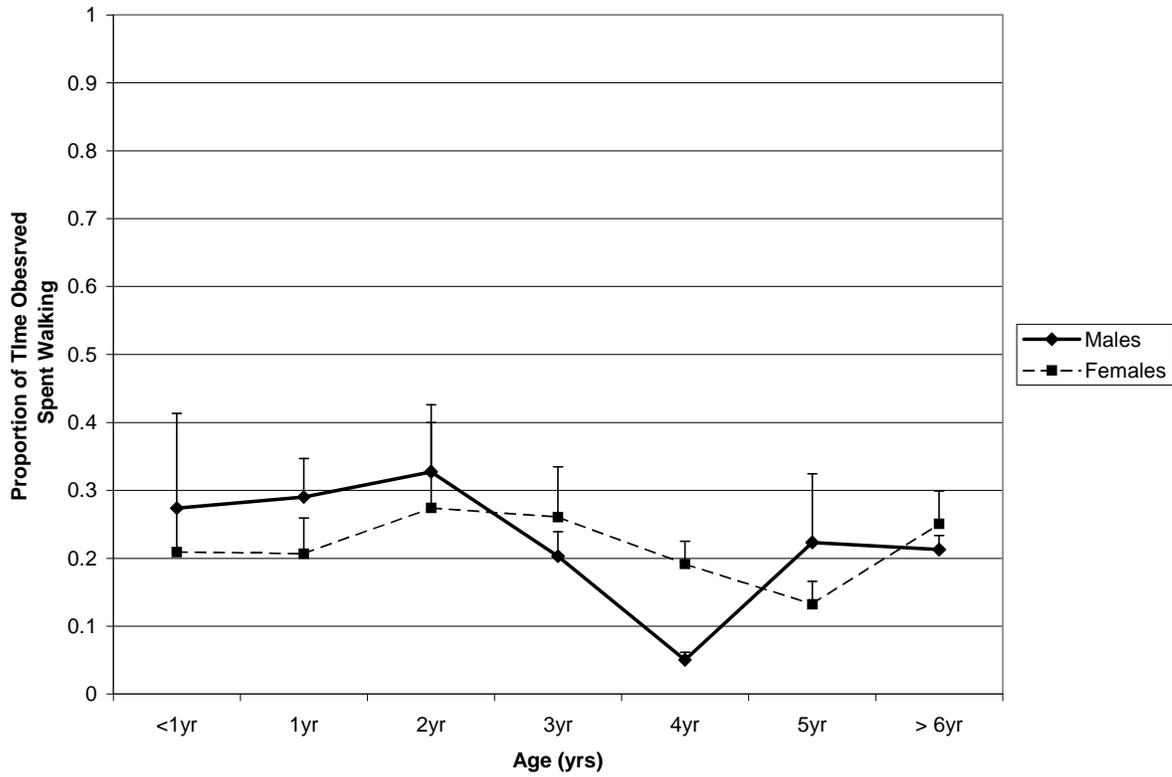


Figure 5. Mean (+ SE) proportion of time spent walking during an observation time for males (N=54, range per category 0.27 to 0.21) and females (N=40, range per category 0.21 to 0.25) up to nine years old.

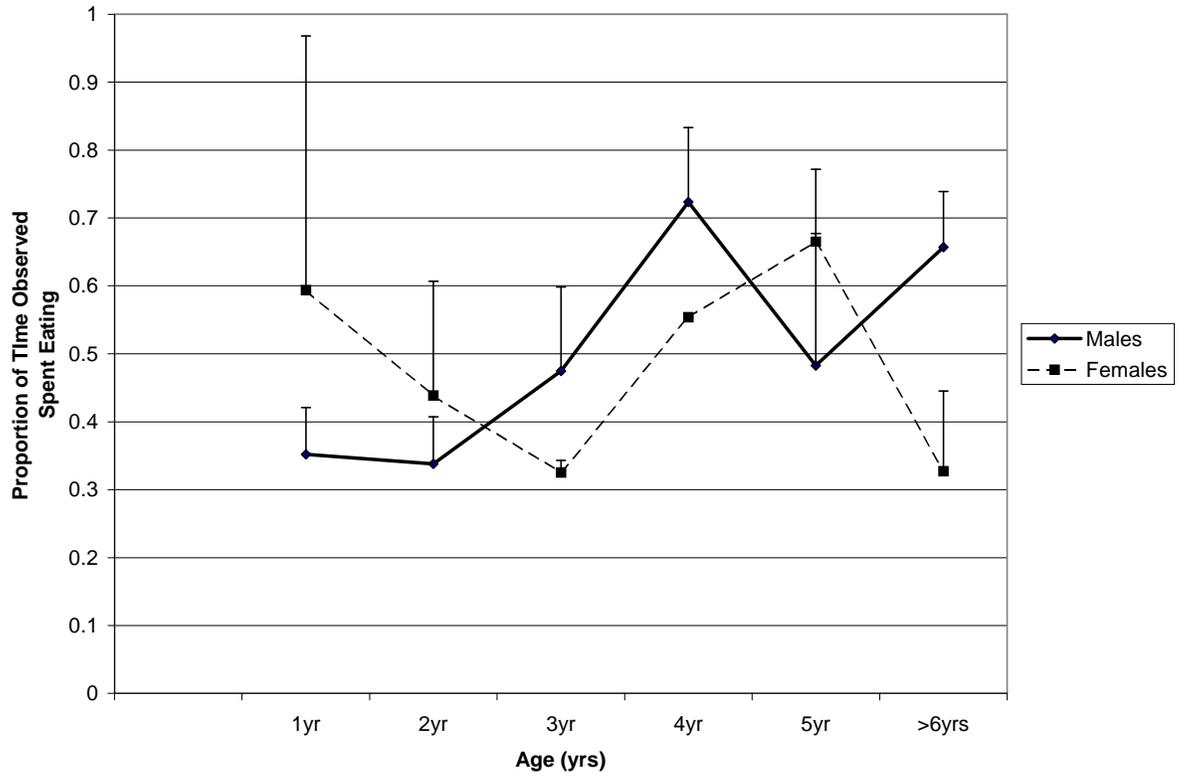


Figure 6. Mean (+ SE) proportion of time spent eating during an observation time for males (N=39, range per category 0.35 to 0.66) and females (N=20, range per category 0.59 to 0.33) up to nine years old. No data were available for females younger than 1 year old.

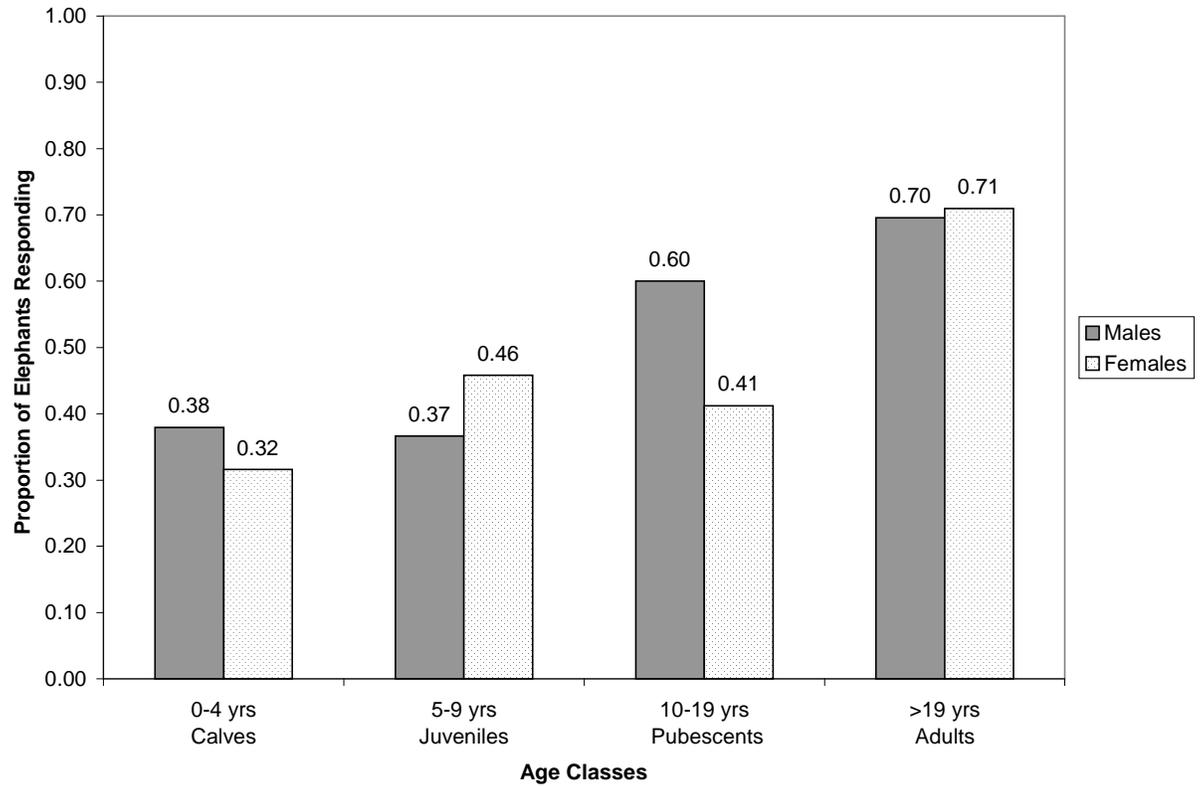


Figure 7. Proportion of elephants per age class that exhibited a response to general surroundings.

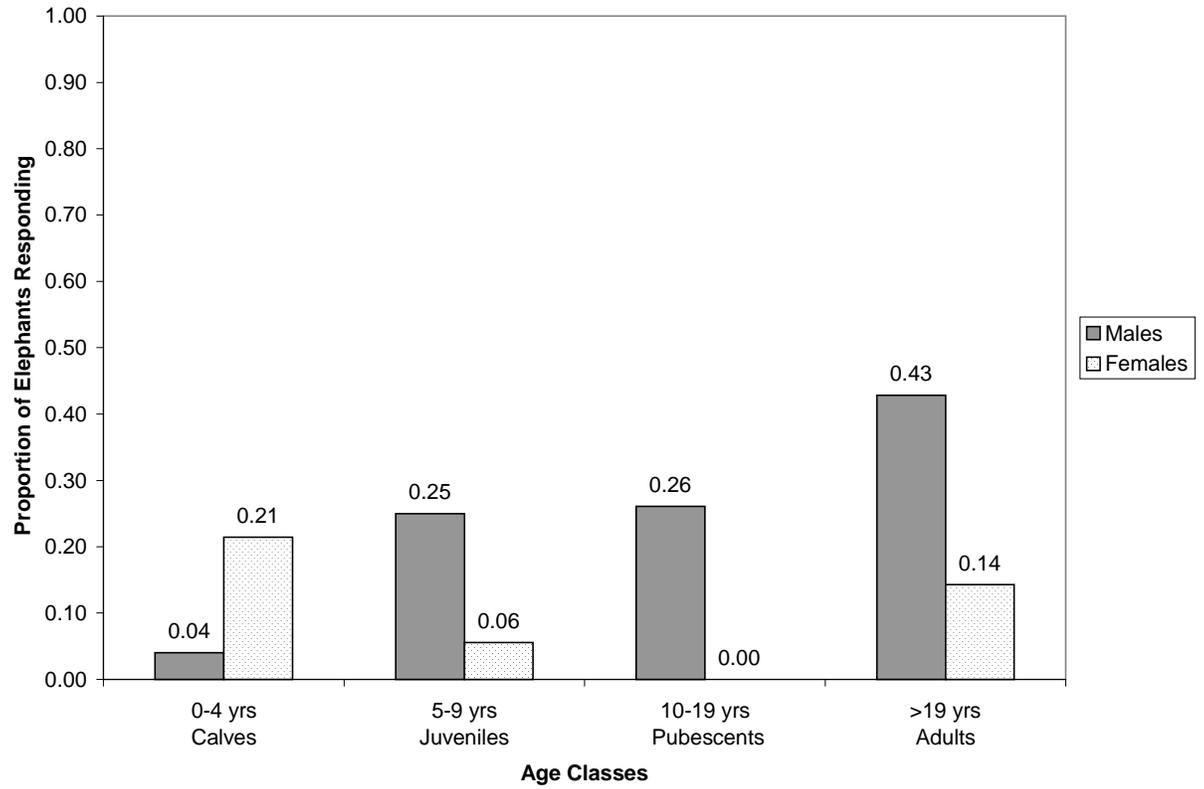


Figure 8. Proportion of elephants per age class that exhibited a response to urine and feces.

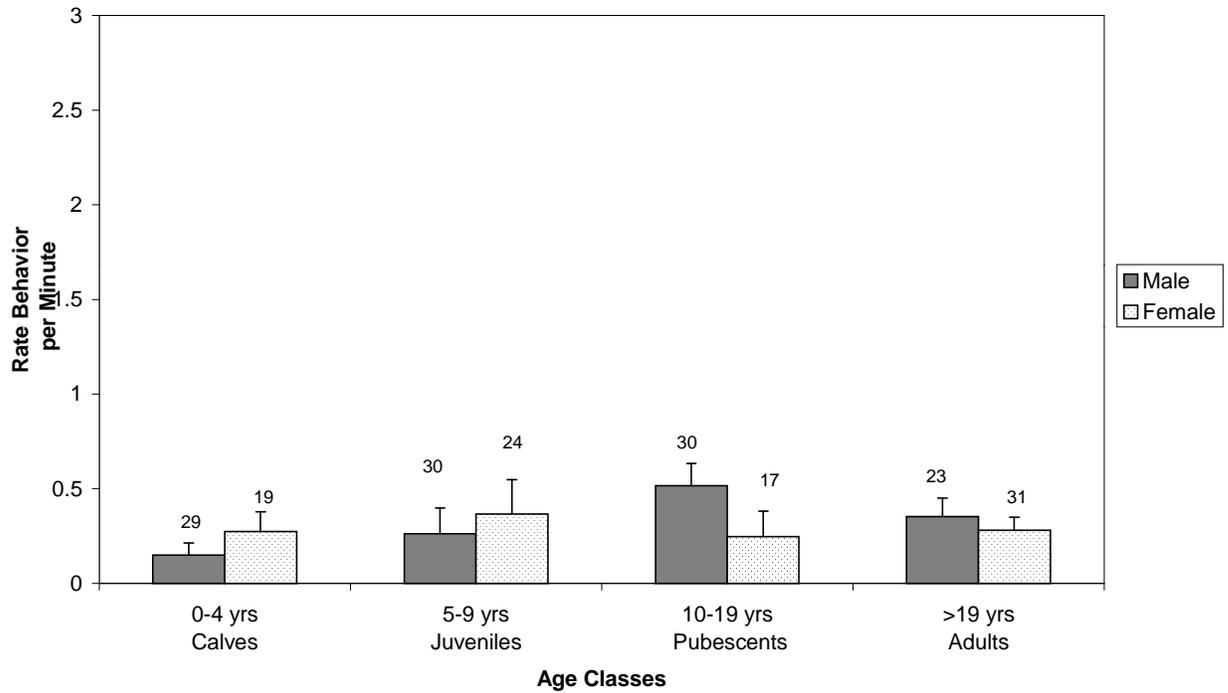


Figure 9. Mean (\pm SE) rate of chemosensory behaviors to general surroundings (including no responses) for males and females at different age classes. Sample sizes are shown above bars. (2 way ANOVA age class: $F=0.64$, $df=3,195$, $P=0.59$; sex: $F=0.12$, $df=1,195$, $P=0.73$; interaction: $F=1.15$, $df=3,195$, $P=0.33$).

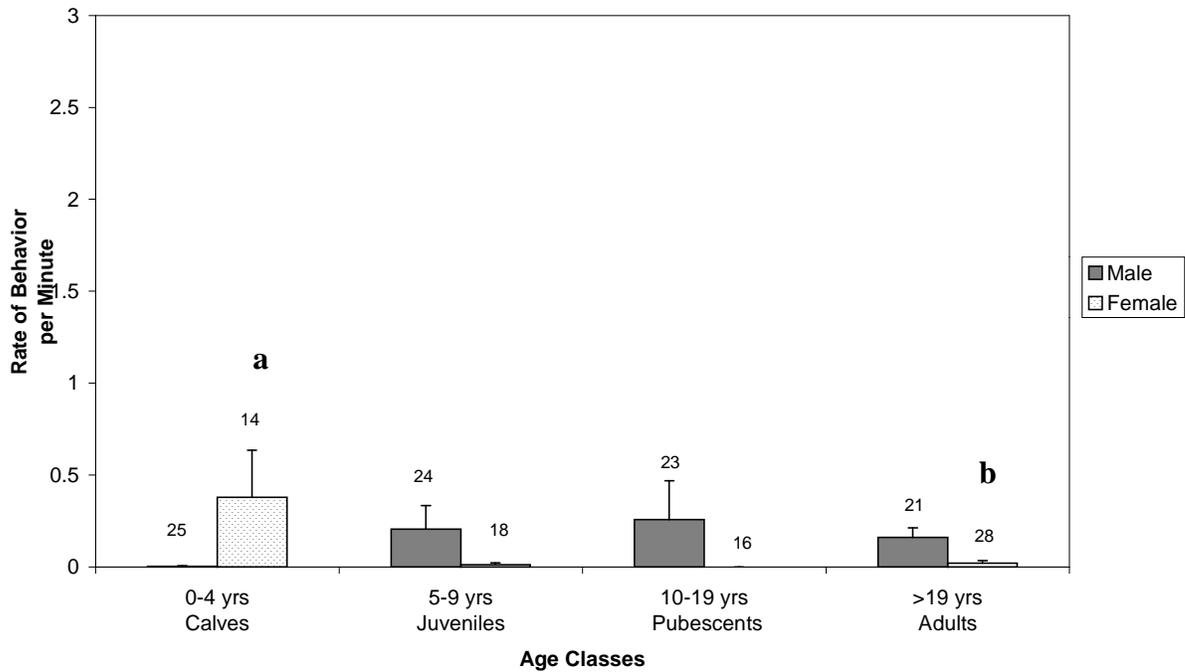


Figure 10. Mean (\pm SE) rate of chemosensory behaviors (including no responses) for males and females within the age classes to urine and/or feces. Sample sizes are shown above bars. (2 way ANOVA age class: $F=0.27$, $df=3,161$, $P=0.84$; sex: $F=0.42$, $df=1,161$, $P=0.52$; interaction: $F=2.79$, $df=3,161$, $P=0.04$). *A posteriori* tests showed significant differences between the female calves (a) and adult females (b) for responses to urine and feces (Tukey-Kramer, $P<0.05$).

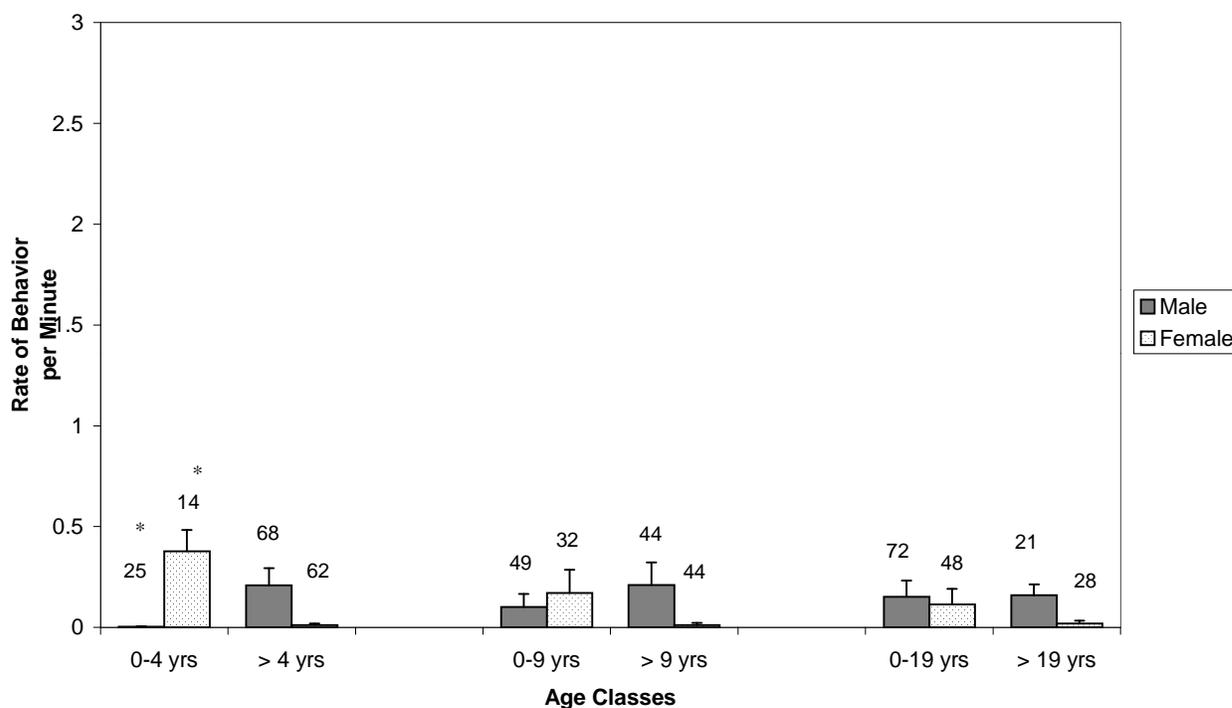


Figure 11. Mean (\pm SE) rate of chemosensory behaviors to urine and/or feces comparing developmental age classes (including no responses). Sample sizes are shown above bars. * indicates a significant interaction between age and sex. (see Table 3 below).

Table 3. The F-ratios and P-values for analysis of variance for comparing differences in age classes and sex (DF = 1, 164).

| <u>Age Classes</u> | 0-4yrs, >4yrs | | 0-9yrs, > 9yrs | | 0-19yrs, >19yrs | |
|--------------------|-------------------------|----------|--------------------------|----------|---------------------------|----------|
| | F | P | F | P | F | P |
| Age Class | 0.66 | 0.42 | 0.09 | 0.76 | 0.21 | 0.65 |
| Sex | 0.83 | 0.36 | 0.60 | 0.44 | 0.94 | 0.33 |
| Interaction | 8.44 | 0.004* | 2.62 | 0.11 | 0.29 | 0.59 |

*Significant at the 0.05 level

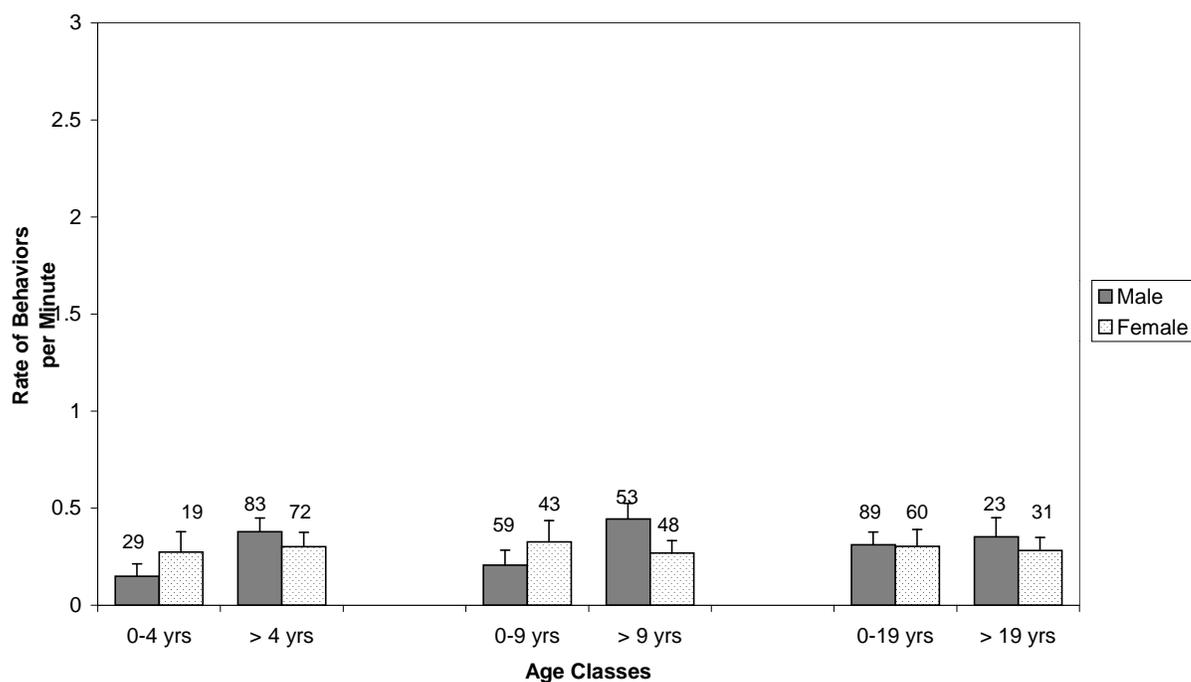


Figure 12. Mean (\pm SE) rate of chemosensory behaviors to general surroundings (including no responses) comparing developmental age classes. Sample sizes are shown above bars.

Statistical values for the ANOVA are shown in Table 4 below.

Table 4. The F-ratios and P-values for analysis of variance for comparing different age classes and sex (DF = 1, 164).

| <u>Age Classes</u> | 0-4yrs, >4yrs | | 0-9yrs, > 9yrs | | 0-19yrs, >19yrs | |
|--------------------|-------------------------|------|--------------------------|------|---------------------------|------|
| | F | P | F | P | F | P |
| Age Class | 1.69 | 0.19 | 1.21 | 0.27 | 0.01 | 0.91 |
| Sex | 0.06 | 0.81 | 0.11 | 0.74 | 0.16 | 0.69 |
| Interaction | 1.02 | 0.31 | 3.11 | 0.08 | 0.10 | 0.75 |

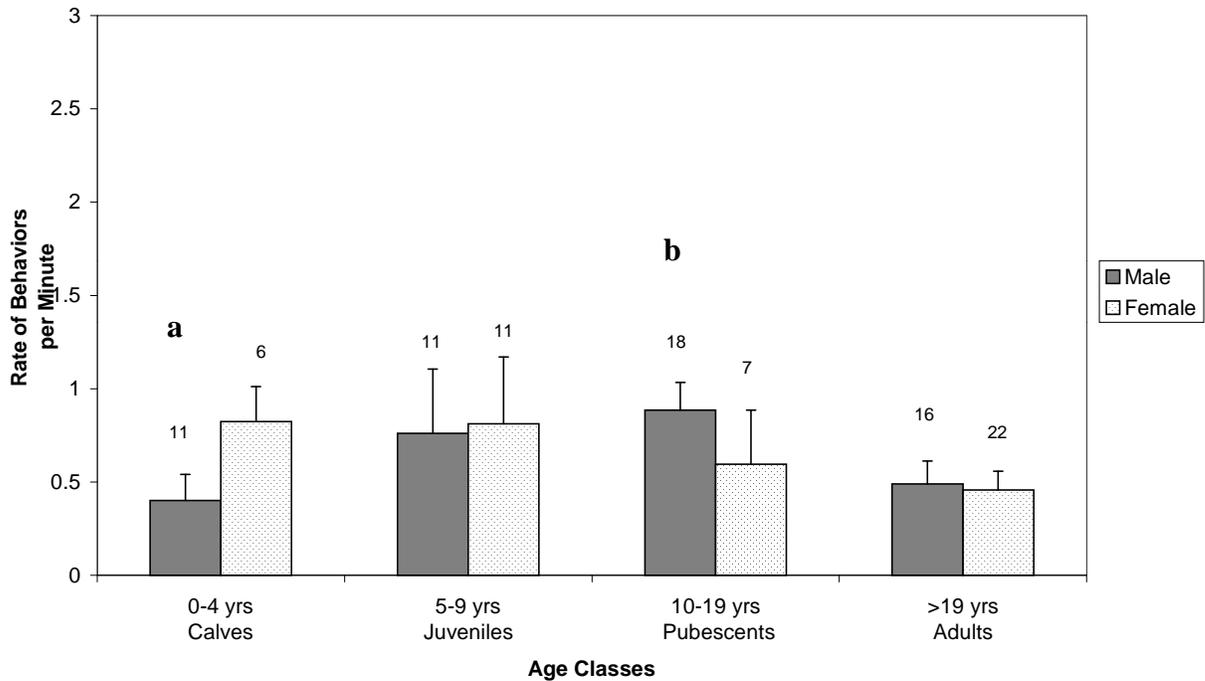


Figure 13. Mean (\pm SE) rate of chemosensory behaviors of individuals that exhibited responses to general surroundings only. Sample sizes are shown above bars. (2 way ANOVA age class: $F=0.40$, $df=3,94$, $P=0.75$; sex: $F=0.001$, $df=1,94$, $P=0.97$; interaction: $F=3.06$, $df=3,94$, $P=0.03$). *A posteriori* tests showed differences only between male calves (a) and pubescent males (b) for chemosensory responses to general surroundings (Tukey-Kramer, $P<0.05$).

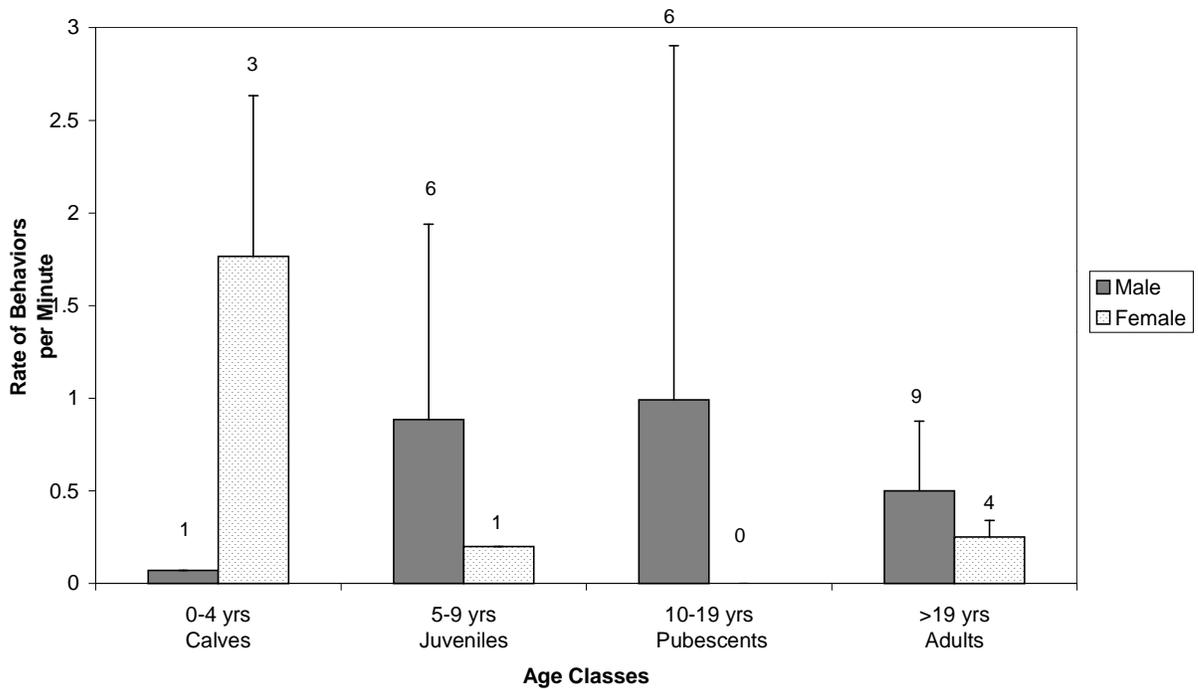


Figure 14. Mean (\pm SE) rate of chemosensory behaviors of individuals that exhibited responses only to urine and/or feces. Sample sizes are shown above bars. (2 way ANOVA age class: $F=0.14$, $df=2,18$, $P=0.87$; sex: $F=0.33$, $df=1,18$, $P=0.57$; interaction: $F=3.04$, $df=2,18$, $P=0.07$). The pubescent age class was excluded from the analysis because no data were available for females of this age class.

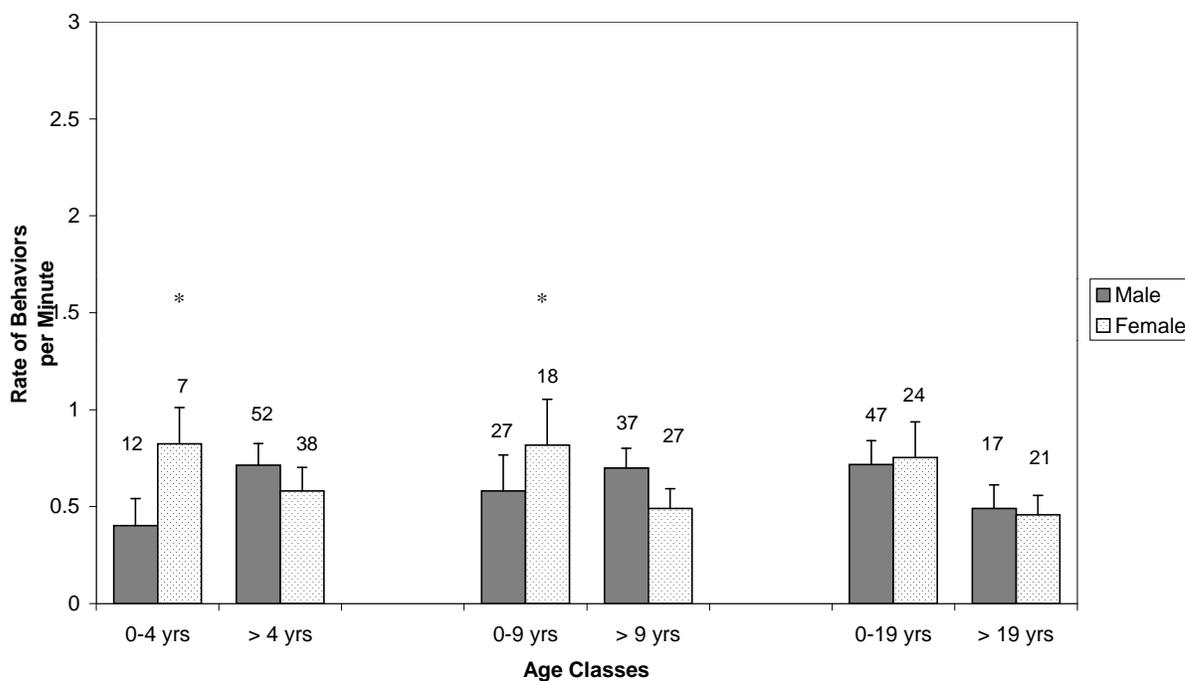


Figure 15. Mean (\pm SE) rate of chemosensory behaviors to general surroundings only for individuals who demonstrated a chemosensory behavior comparing developmental age classes. Sample sizes are shown above bars. * indicates a significant interaction between age and sex. (see Table 5 below).

Table 5. The F-ratios and P-values for the analysis of variance for comparing different age classes and sex (DF=1,105).

| <u>Age Classes</u> | 0-4yrs, >4yrs | | 0-9yrs, > 9yrs | | 0-19yrs, >19yrs | |
|--------------------|-------------------------|-------|--------------------------|--------|---------------------------|------|
| | F | P | F | P | F | P |
| Age Class | 0.04 | 0.85 | 0.09 | 0.75 | 1.49 | 0.22 |
| Sex | 1.63 | 0.21 | <0.01 | 0.97 | 0.11 | 0.74 |
| Interaction | 6.43 | 0.01* | 4.56 | 0.035* | <0.01 | 0.99 |

*Significant at the 0.05 level

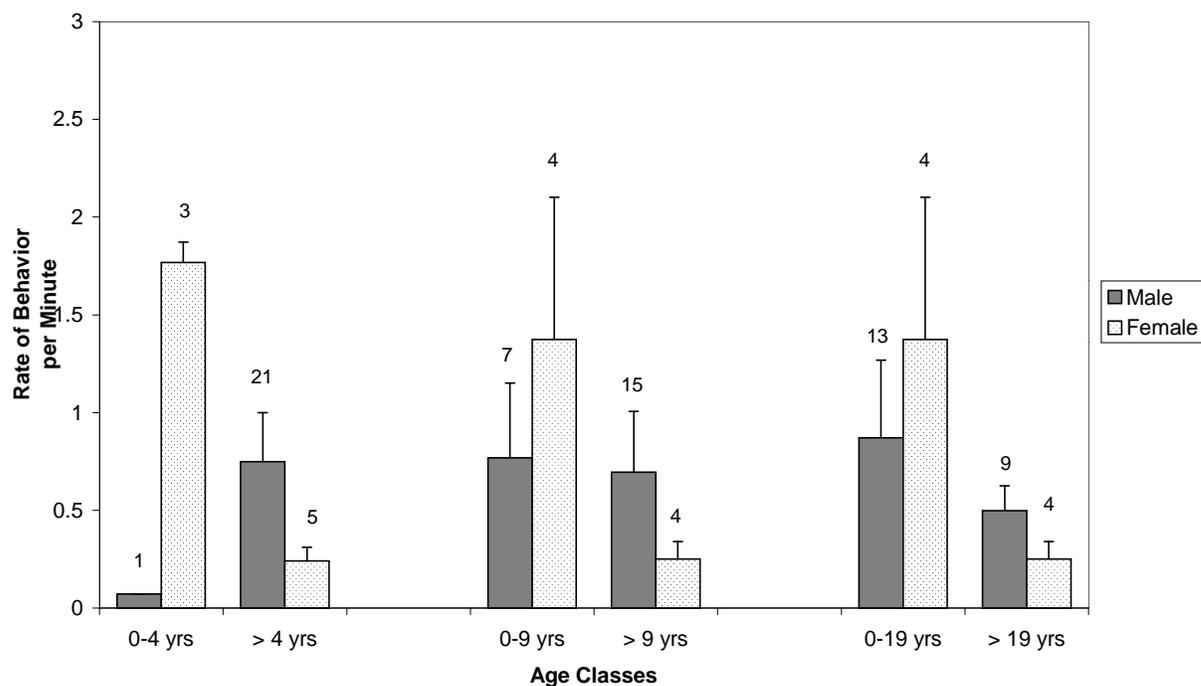


Figure 16. Mean (\pm SE) rate of chemosensory behaviors by age class only for males and females who demonstrated a chemosensory behavior to urine and/or feces. Sample sizes are shown above bars. * indicates a significant interaction between age and sex. (see Table 6 below).

Table 6. The F-ratios and P-values for the analysis of variance for comparing different age classes and sex (DF= 1, 26).

| <u>Age Classes</u> | 0-4yrs, >4yrs | | 0-9yrs, > 9yrs | | 0-19yrs, >19yrs | |
|--------------------|-------------------------|--------|--------------------------|------|---------------------------|------|
| | F | P | F | P | F | P |
| Age Class | 0.03 | 0.86 | 1.49 | 0.23 | 2.44 | 0.13 |
| Sex | 1.73 | 0.199 | 0.05 | 0.82 | 0.06 | 0.80 |
| Interaction | 4.41 | 0.045* | 1.58 | 0.22 | 1.02 | 0.32 |

*Significant at the 0.05 level

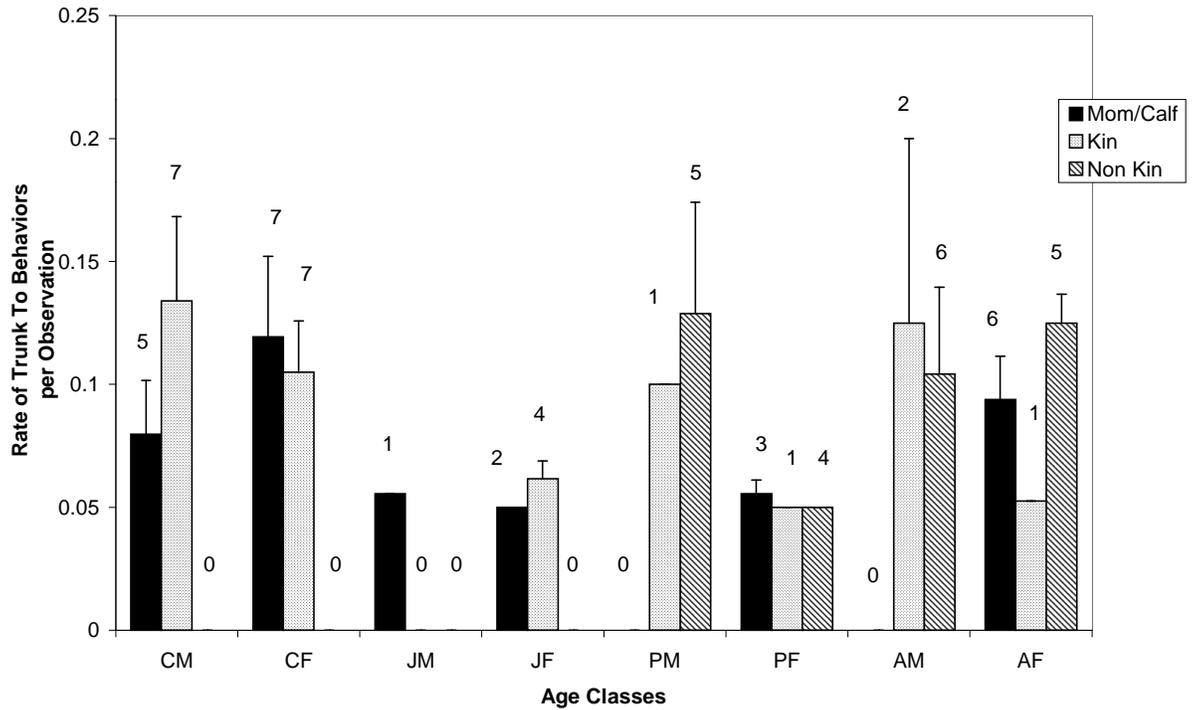


Figure 17. Mean (\pm SE) trunk tip touches to other elephants and the focal elephant's relationship to those elephants. N's are shown above each bar. 44% of all trunk tip touch relationships could not be determined (2-way ANOVA: **mom/calf**- age class: $F=1.66$, $df=3,18$, $P=0.21$; sex: $F=2.17$, $df=1,18$, $P=0.15$; interaction: $F=0.37$, $df=2,18$, $P=0.78$; **kin**-age class: $F=1.49$, $df=3,16$, $P=0.26$; sex: $F=0.32$, $df=1,16$, $P=0.58$; interaction: $F=0.53$, $df=3,16$, $P=0.67$; **non-kin**-age class: $F=2.20$, $df=3,15$, $P=0.13$; sex: $F=0.60$, $df=1,15$, $P=0.45$; interaction: $F=0.31$, $df=3,15$, $P=0.82$).

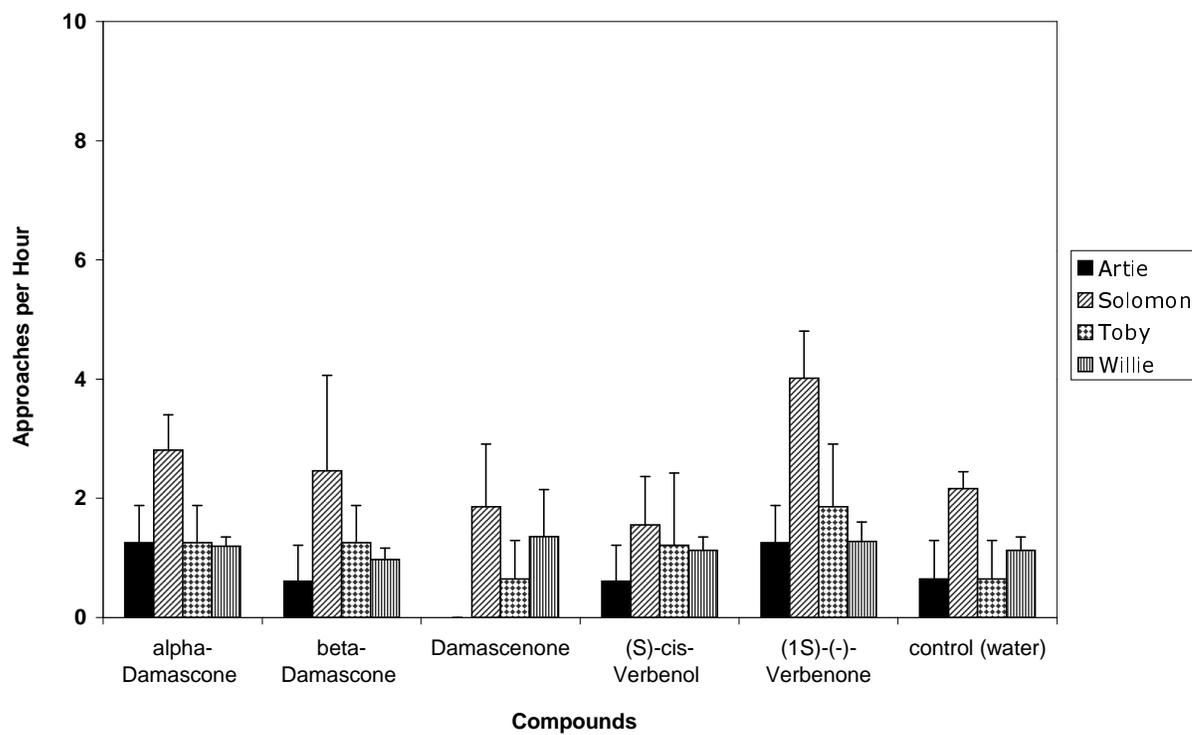


Figure 18. Mean (\pm SE) number of approaches to compounds by each individual elephant over three trials.

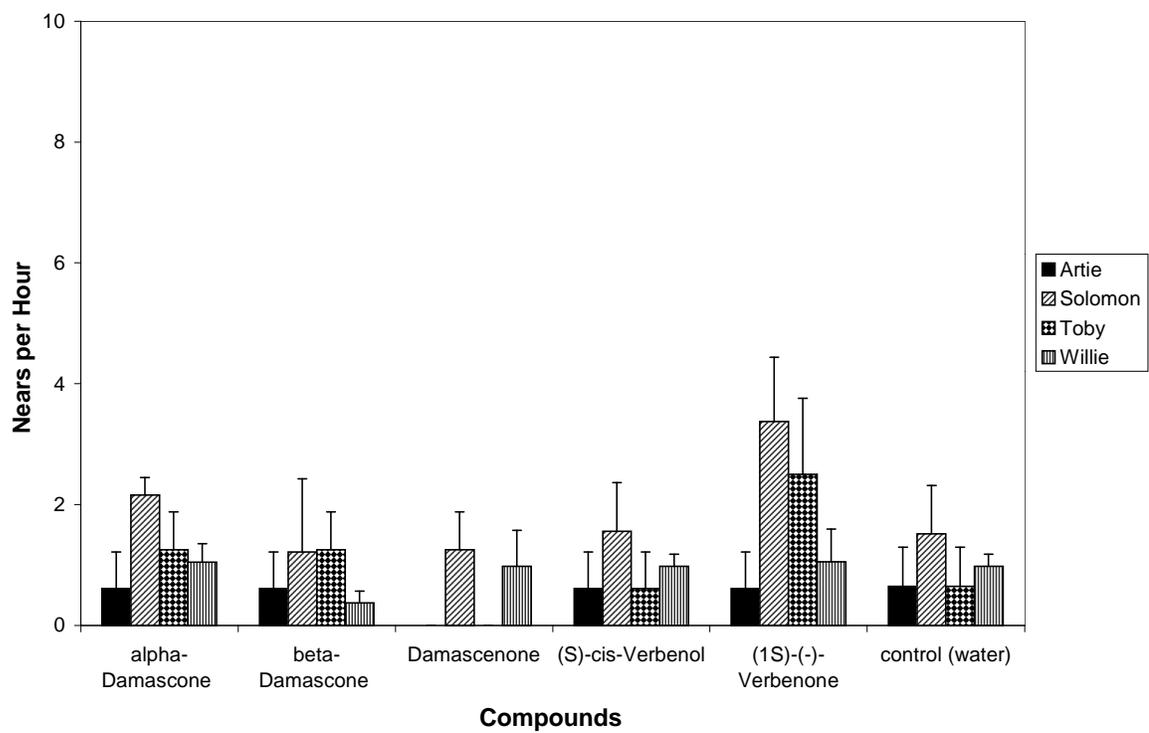


Figure 19. Mean (\pm SE) number of trunk near compounds by each individual elephant over three trials.

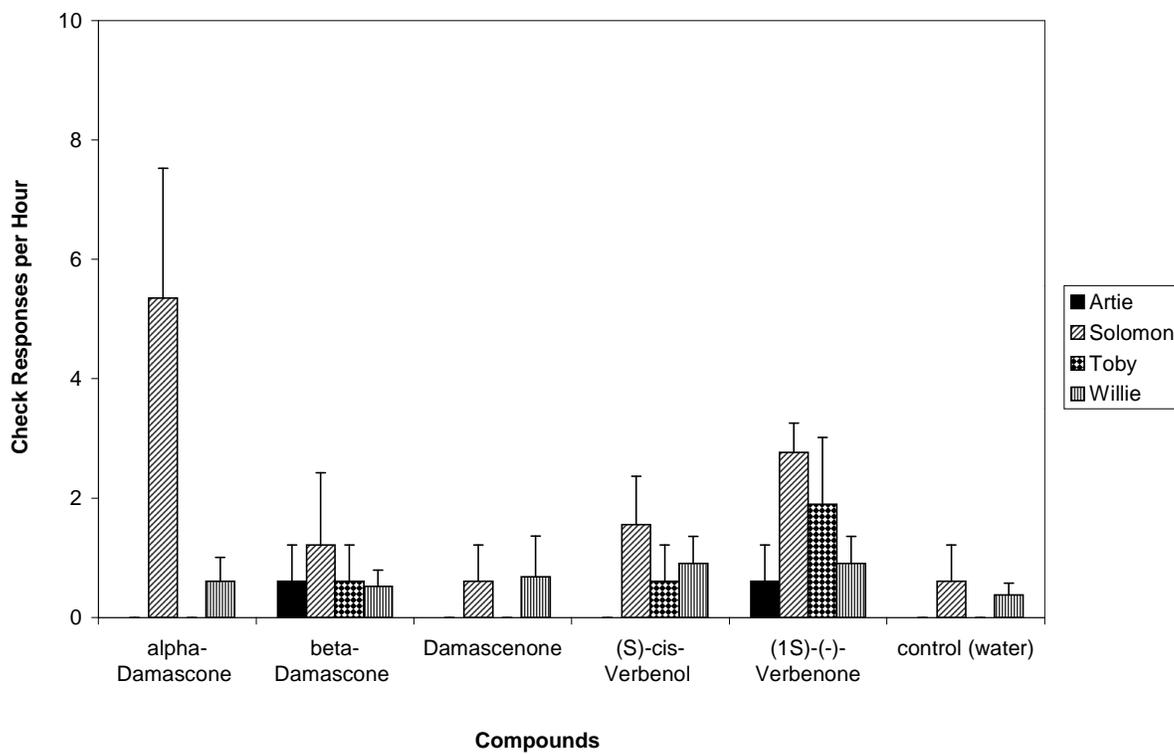


Figure 20. Mean (\pm SE) number of check responses to compounds by each individual elephant over three trials.

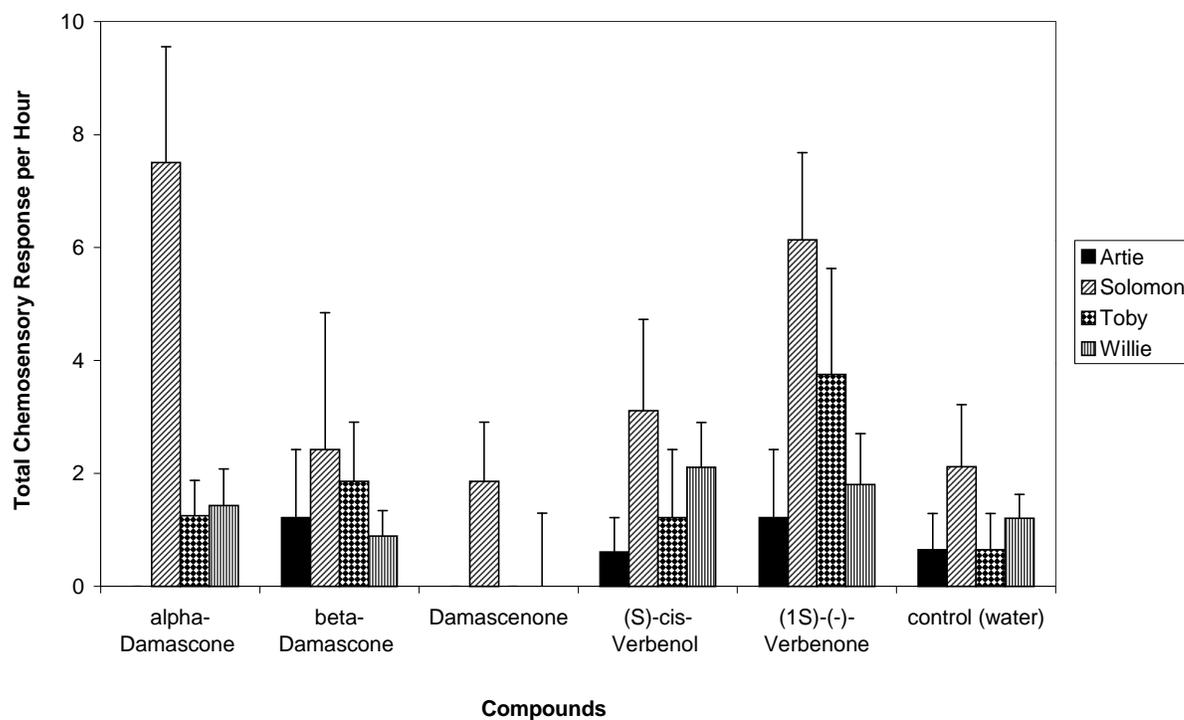


Figure 21. Mean (\pm SE) number of total chemosensory responses to compounds by each individual elephant over three trials.

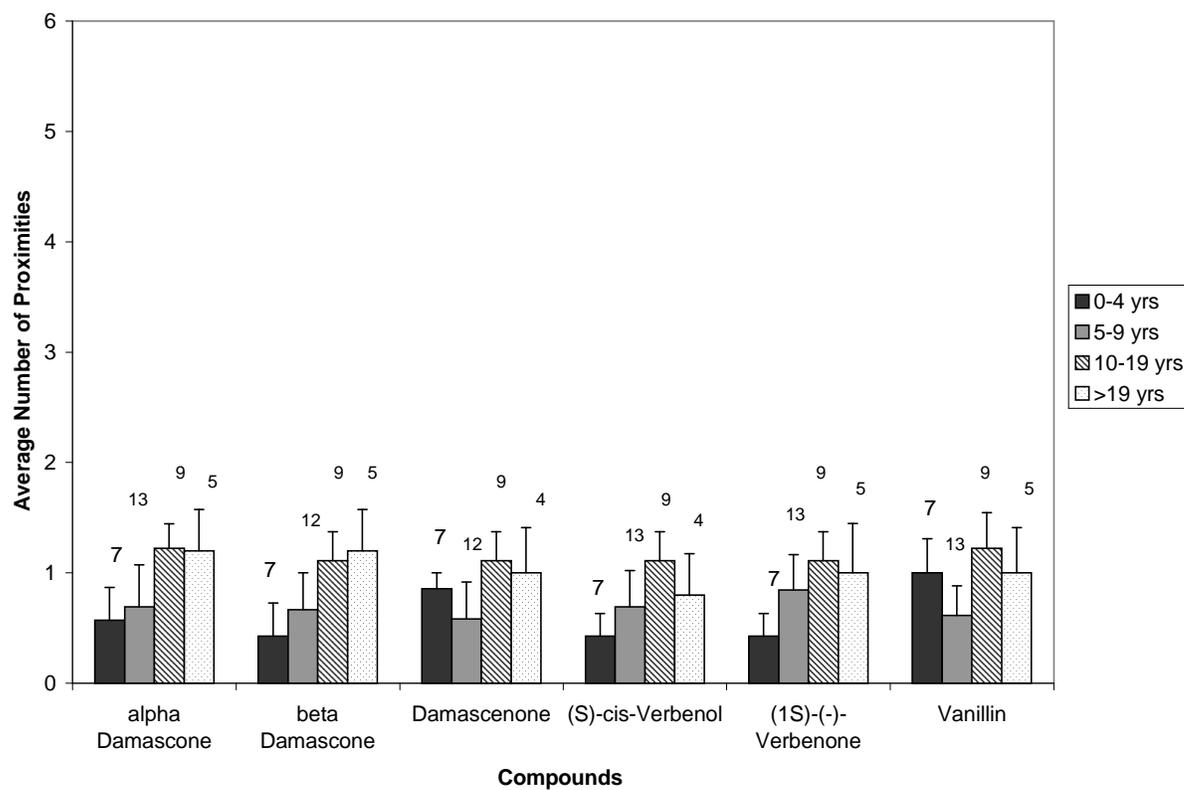


Figure 22. Mean (\pm SE) number of approaches to compounds by age classes of male elephants. Sample size for each age group is shown above bar.

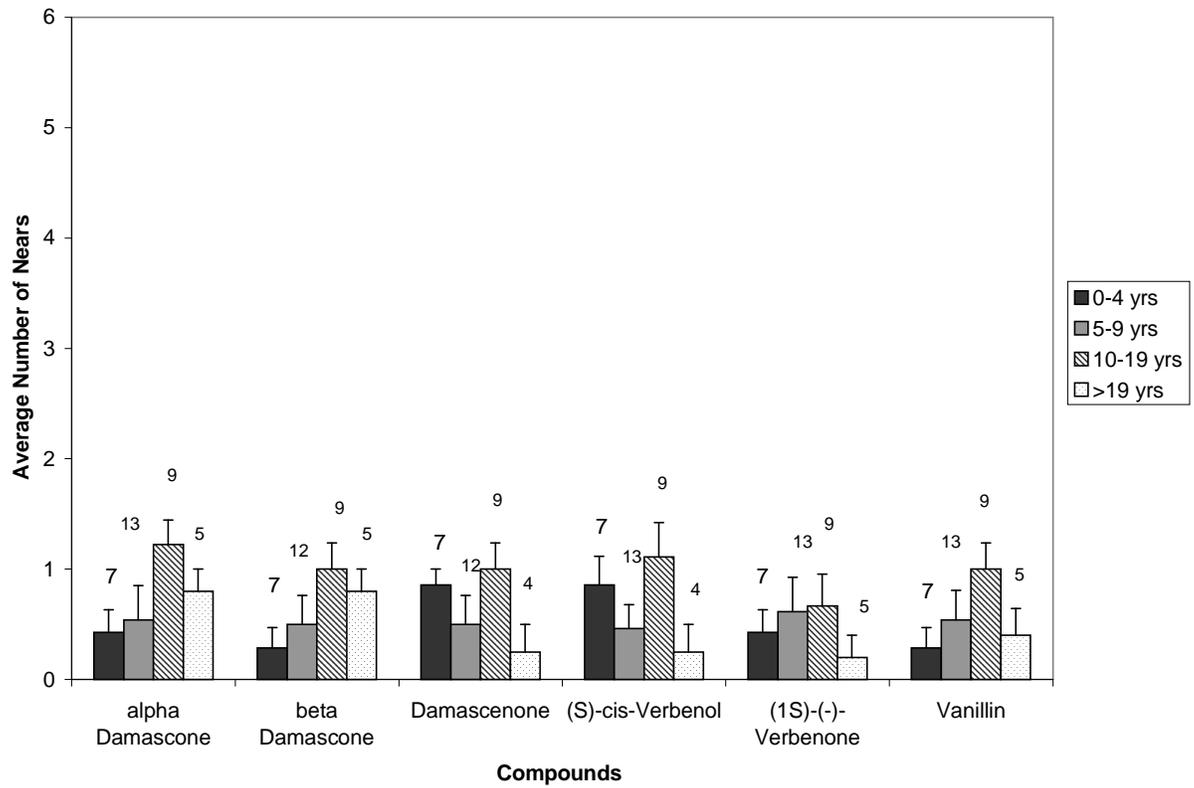


Figure 23. Mean (\pm SE) number of trunk tip near compounds by age classes of male elephants. Sample size for each age group is shown above bar.

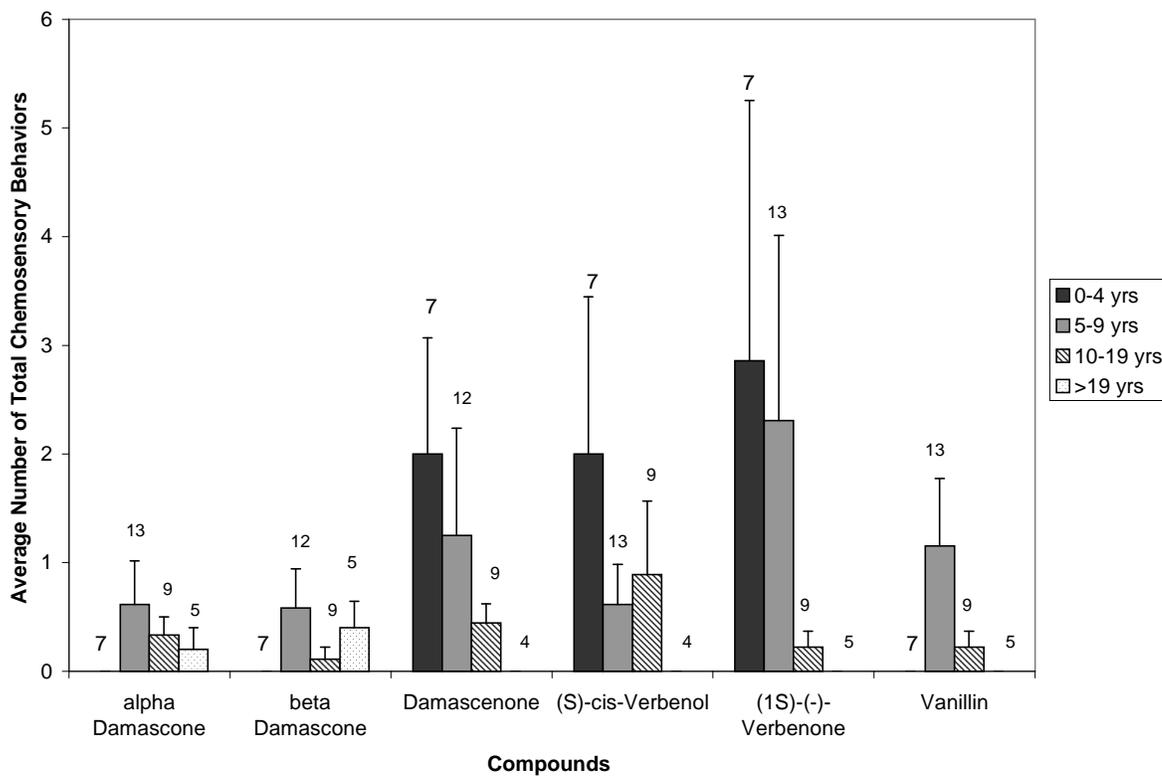


Figure 24. Mean (\pm SE) number of total chemosensory responses to the compounds by age classes of male elephants. Sample size for each age group is shown above bar.

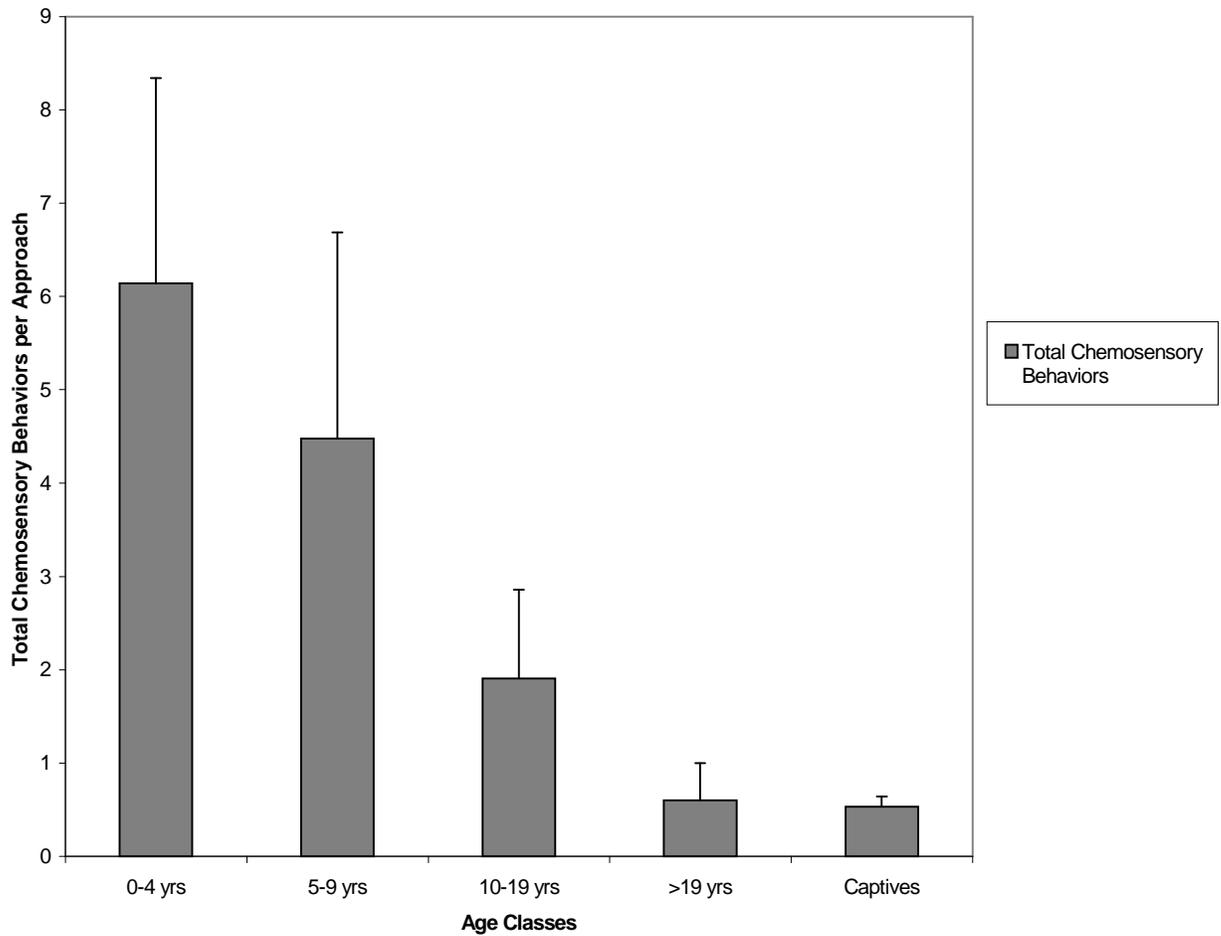


Figure 25. Mean (\pm SE) for the total chemosensory behaviors (wild: sniff + check + place + flehmen; captive: sniff + check) per approach to a sample for the different age classes in the wild population and compared to the captive population.