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## Waterhole Dynamics and Chemical Signals of African Elephants ( *Loxodonta Africana* )

Stacie Marie Castelda

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WATERHOLE DYNAMICS AND CHEMICAL SIGNALS OF AFRICAN  
ELEPHANTS (*LOXODONTA AFRICANA*)

by

STACIE M. CASTELDA

(Under the Direction of Bruce A. Schulte)

ABSTRACT

The distribution of mammals in a savanna environment is influenced by abiotic and biotic factors. The African savanna experiences distinct wet and dry seasons. Species become spatially restricted around water resources during the dry season, resulting in increased intraspecies and interspecies resource-based aggregation. A dominant species, such as the African elephant (*Loxodonta africana*), may influence this aggregation. I examined the influence of wet and dry seasons and ambient temperature on the use of a waterhole by elephants as well as the co-occurrence of elephants and other mammals at the waterhole. Biodiversity scans (October 2004-June 2007) and were performed at a permanent waterhole on the Ndarakwai Ranch in northern Tanzania. More elephant and mammal species used the waterhole during the dry season and during higher ambient temperatures. Elephant-mammal co-occurrence was rare, suggesting the workings of interference competition by elephants. Intraspecific aggregation at waterholes can facilitate reproductive communication in elephants, often via chemical signals. This study also determined behaviorally via biological assays (June 2006-June 2007) whether four known insect pheromones identified in the ovulatory urine of African elephants serve as sex-specific pheromones. When tested singly, none of the four compounds evoked significant behavioral responses compared to control samples. Although this experiment was unable to confirm behaviorally an African elephant chemical signal, it provided insight into the next phase of study.

INDEX WORDS: Resource-based aggregation, Interference competition, Chemosensory, African savanna elephant

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ELEPHANTS (LOXODONTA AFRICANA)

by

STACIE M. CASTELDA  
B.A., LUTHER COLLEGE, 2005

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

MASTER OF SCIENCE

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2008

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## TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS.....	5
LIST OF TABLES.....	8
LIST OF FIGURES.....	10
PREFACE.....	12
CHAPTER I. ELEPHANT AGGREGATION AND ELEPHANT-MAMMAL CO- OCCURRENCE AT A PERMANENT WATERHOLE.....	13
ABSTRACT.....	13
INTRODUCTION.....	14
METHODS.....	17
RESULTS.....	21
DISCUSSION.....	25
REFERENCE.....	31
CHAPTER II. BIOASSAYS OF PUTATIVE CHEMICAL SIGNALS IN AFRICAN ( <i>LOXODONTA AFRICANA</i> ) FOR CONVERGENCE WITH INSECTS AND SIMILARITIES WITH ASIAN ( <i>ELEPHAS MAXIMUS</i> ) ELEPHANTS.....	53
ABSTRACT.....	53
INTRODUCTION.....	54
METHODS.....	58
RESULTS.....	62
DISCUSSION.....	64
REFERENCES.....	69
APPENDICES	
A. CROP-RAIDING INCIDENTS BY AFRICAN ELEPHANTS ( <i>LOXODONTA AFRICANA</i> ) IN TANZANIA.....	89
B. BIOLOGICAL ASSAYS OF AFRICAN ELEPHANT DUNG.....	100

C. DUNG SURVEYS AS MEANS TO ESTIMATE PRESENCE OF  
ELEPHANT.....104



## LIST OF TABLES

	Page
Table 1.1. The categorization of months as wet or dry, Ndarakwai Ranch (October 2004 - June 2007).....	36
Table 1.2. The average ( $\pm$ SE) mean daily temperature ( $^{\circ}$ C), cumulative rainfall and the number of wet and dry months of the three sampling years, Ndarakwai Ranch (October 2004 - June 2007).....	37
Table 1.3. Statistical values of the non-parametric two-way analysis of variance on the rank of monthly mean daily temperature ( $^{\circ}$ C) between the seasons and across the day, Ndarakwai Ranch (October 2004 - June 2007). ....	38
Table 1.4. Statistical values of chi-square goodness of fit test for differences in the frequency of occurrence across the day during the wet and dry seasons, Ndarakwai Ranch (October 2004 - June 2007).....	39
Table 1.5. List of mammalian species present and number of times sighted during the wet and dry seasons, Ndarakwai Ranch (October 2004-June 2007).....	40
Table 1.6. Summary of the scan counts under various parameters, Ndarakwai Ranch (October 2004-June 2007).....	41
Table 1.7 Summary of the distribution of elephants and other mammal species around a permanent waterhole during the wet and dry seasons, Ndarakwai Ranch (October 2004-June 2007).....	42
Table 2.1. Ethogram of behaviors performed by wild African male and female elephants to bioassay samples. ....	76
Table 2.2. The distribution of the number of times each sample was placed for biological assay, the number of times at least one African elephants was within one body length (proximity) of the sample and the total number of elephants that was within proximity of the sample, Ndarakwai Ranch, Tanzania (July 2006-June 2007).....	77
Table 2.3. Comparison of the time (s) spent by elephants within proximity (one body length) of the bioassayed samples at Ndarakwai Ranch, Tanzania (July 2006-June 2007).....	78
Table 2.4. Statistical details of the proportion of elephants that performed chemosensory behavior to the bioassayed samples while within one trunk length (near) at Ndarakwai Ranch, Tanzania (July 2006-June 2007).....	79

Table 2.5. Statistical details of the number of chemosensory behaviors per proximity (one body length) by elephants to the bioassayed samples at Ndarakwai Ranch, Tanzania (July 2006-June 2007).....80

Table 2.6. Statistical details of the rate (frequency/proximity) of chemosensory behaviors towards bioassayed samples by post-puberty male and female African elephants, Ndarakwai Ranch (July 2006-July 2007).....81

## LIST OF FIGURES

	Page
Figure 1.1. Map of the north-central border between Kenya and Tanzania.....	43
Figure 1.2. Overhead view of the waterhole showing the four sections, Ndarakwai Ranch, Tanzania. ....	44
Figure 1.3. The mean ( $\pm$ standard deviation) daily temperature ( $^{\circ}$ C) of the wet and dry seasons, Ndarakwai Ranch (October 2004 - June 2007).....	45
Figure 1.4. The hourly proportion of elephant and mammal sightings during the wet and dry seasons, Ndarakwai Ranch (October 2004-June 2007).....	46
Figure 1.5. The mean daily temperature ( $^{\circ}$ C) and the hourly proportion of elephant sightings during the wet and dry seasons, Ndarakwai Ranch (October 2004 - June 2007).....	47
Figure 1.6. The average number of elephant present per sighting during the wet and dry seasons, Ndarakwai Ranch (October 2004 - June 2007).....	48
Figure 1.7. The mean daily temperature ( $^{\circ}$ C) and average number of elephant present per sighting during the wet and dry seasons, Ndarakwai Ranch (October 2004 - June 2007).....	49
Figure 1.8. The cumulative co-occurrence of various mammal species with elephants during the wet and dry seasons at a permanent waterhole, Ndarakwai Ranch (October 2004 - June 2007).....	50
Figure 1.9. The monthly proportion of scans in which elephants co-occurred with other mammal species, elephants were alone, and other mammal species were alone, Ndarakwai Ranch (October 2004 - June 2007).....	51
Figure 1.10. The mean elephant group size per sighting and the proportion of elephant-mammal co-occurrence during the wet and dry seasons, Ndarakwai Ranch (October 2004 - June 2007).....	52
Figure 2.1. Map of the north-central border between Kenya and Tanzania.....	82
Figure 2.2. Response model of an African elephant female-to-male chemical signal in comparison to response levels of vanilla, luteal (or pre-puberty) urine and LH2 follicular urine.....	83
Figure 2.3. Overhead view of the waterhole showing six bioassay sites, Ndarakwai Ranch, Tanzania.....	84

Figure 2.4 The proportion of elephants that performed any chemosensory behaviors while within one body length (proximity) of sample over time once a sample was placed for a bioassay.....85

Figure 2.5. The proportion of elephants that performed any chemosensory behavior while within one body length (proximity) of placed samples, Ndarakwai Ranch (July 2006-June 2007).....86

Figure 2.6. The number of chemosensory responses per proximity (one body length) by elephants toward placed bioassayed samples, Ndarakwai Ranch (July 2006-June 2007).....87

Figure 2.7. The number of chemosensory responses per proximity (one body length) by elephants toward placed bioassayed samples by post-puberty male and female African elephants, Ndarakwai Ranch (July 2006-June 2007).....88

## PREFACE

With an exploding human population, the location and the rangeland of African elephant (*Loxodonta africana*) populations are becoming restricted to areas such as national parks and preserves and private game ranches (Kangwana, 1995; Hoare, 1999; Hoare & Du Toit, 1999; Osborn, 2002; Osborn, 2004). In return, landscapes are changed, standing biomass declines (Eckhardt et al. 2000; Augustine and McNaughton 2004), avian and herbivore diversity is reduced (Kerley and Landman 2006; Valeix et al. 2007; Ogada et al. 2008) and neighboring agricultural fields are raided (Kiiru, 1995; Hoare, 1999; Eckhardt et al., 2000; Sitati et al 2003; Augustine & McNaughton, 2004). Hence, the behavior of elephants plays an important role in their ecological community structure as well as in their management and conservation (Sutherland 1998; Festa-Bianchet and Apollonio 2003). The following thesis is composed of five different projects: evaluation of elephant aggregation patterns (Chapter I), biological assays of putative elephant chemical signals (Chapter II), assessment human-elephant conflicts via crop-raiding (Appendix A), biological assay of elephant dung (Appendix B) and estimating elephant population via dung counts (Appendix C).

CHAPTER I  
ELEPHANT AGGREGATION AND ELEPHANT-MAMMAL CO-OCCURRENCE AT  
A PERMANENT WATERHOLE

ABSTRACT

The distribution of mammals in an arid savanna environment is influenced by abiotic and biotic factors. During the nutritionally stressing dry season, species become spatially restricted around key resources resulting in increased intraspecies and interspecies aggregation. Keystone competitors, such as the African elephant (*Loxodonta africana*), may influence interspecies resource aggregation via interference competition. The first objective of the present study was to determine the relative influence of wet and dry season and ambient temperature on the intraspecies aggregation patterns of African elephants. The second objective was to evaluate the interspecies resource-aggregation (co-occurrence) of elephants with other mammals and determine the presence of spatial partitioning as a response to potential interference competition at a waterhole. Biodiversity scans were performed at a permanent waterhole on the Ndarakwai Ranch in northern Tanzania. Elephants were sighted at the waterhole more often in the dry season, whereas the number of elephants at the waterhole was greater in the wet season. The presence of elephants was related to ambient temperature regardless of season. As the dry season progressed, mammalian species aggregated around the limited water resource, however, other mammal species were more likely to be at the waterhole when elephants were not present and co-occurrence decreased as the number of elephants increased. The results of this study suggest spatial partitioning as one mechanism in which a species can reduce the effects of interference competition with elephants.

INDEX WORDS: Resource-based aggregation, African elephant, Interference competition

## INTRODUCTION

Abiotic factors such as ambient temperature and availability of water (Redfern et al. 2003, Valeix 2008), and biotic interactions like the presence of keystone species (Estes and Duggins 1995), the threat of predation (Short 1998), the extent of competition (Abramsky et al. 2001; Caro and Stoner 2003, Gehrt and Prange 2007) and the degree of mutualistic interactions (Janzen 1966) determine the population dynamics of mammals. The African savanna experiences distinctive wet and dry seasons (Western and Lindsay 1984). Water and high quality forage are plentiful in the wet season whereas in the dry season, forage quantity and quality decrease and water becomes scarce. During the nutritionally stressing dry season, many mammalian species become spatially restricted around a water resource (Western 1975; Owen-Smith 1982; Redfern et al. 2003). In addition, the location of water influences the dynamics and distribution of mammalian species (Redfern et. al. 2003), including African elephants (*Loxodonta africana*) (Bailey et al. 1996; Redfern et. al. 2003).

During the wet season when grasses become bountiful, elephant herd sizes increase as female family units aggregate into kinship groups and clans with males often joining them in search of sexually receptive females (Moss and Poole 1983; Western and Lindsay 1984, Wittemyer et al. 2005). During the dry season, resource availability and quality of forage decline and elephants gradually disperse into smaller family units (female) or become solitary (males), thereby decreasing herd size (Western and Lindsay 1984; Dublin 1996; Wittemyer et al. 2007). Although elephant herd size is reduced during the dry season, a reduction in water resource availability leads to intraspecific resource-based aggregation. The female family units tend to remain within proximity of

permanent water sources because calves are present (Stokke and du Toit 2002; Napora 2007) and elephant numbers at waterholes gradually increase and become more evenly distributed across waterholes as the dry season progresses (Chamaillé-Jammes et al. 2007; Chamaillé-Jammes et al. 2008).

Other mammal species also aggregate closer to a water source during the dry season; thereby increasing the likelihood of interspecies interaction (Trash et al. 1995). According to the competitive exclusion principle, two species that share an ecological niche and compete for the same resource cannot coexist because one of the two species will have some form of advantage that results in the complete displacement of the other species (Gause 1934; Hardin 1960). Hence, coexistence of species within a competitive biotic community is achieved through resource partitioning via interspecific competition (Lloyd 1967; Tokeshi 1999; Tsuruta and Gotom 2007; Chamaillé-Jammes et al. 2008).

Competitors can gain access to a resource by exploiting the resource (exploitation competition) or by behaviorally preventing others from having access to the resource (interference competition). For example, *Gerbillus pyramidum*, as the dominant gerbil species, reduces the access to food by the subordinate, sympatric *G. allenbyi* through aggressive interactions (Kotler et al. 1993; Ziv et al. 1993; Gehrt and Prange 2007). Keystone competitors, typically large species that can dominate a resource because of their size (Kotler et al. 1993; Ziv et al. 1993), play an important role in structuring communities (Persson 1985; Valeix et al. 2007). As a result of interference competition, keystone competitors can cause temporal or spatial partitioning in resource use (Abramsky et al. 2001; Albrecht and Gotelli 2001; Harris et al. 2006). In savannas, African elephants are considered one of the predominant keystone competitors as they



can maintain primary access to a water source via interference competition (Kerley and Landman 2006; Valeix et al. 2007; Ogada et al. 2008; Valeix et al. 2008). In a study in Zimbabwe, as the number of elephants at waterholes increased, the probability of giraffes drinking from the waterhole decreased and giraffes, along with warthog and zebra, spent more time accessing water (Valeix et al. 2008). Four other species (impala, kudu, roan and sable) exhibited temporal partitioning in response to elephant by altering the time of day they visited waterholes when elephants were present (Valeix et al. 2007). Interestingly, these four species were most likely to suffer from costly interference from elephants (Valeix et al. 2007). Spatial partitioning as response to interference competition, however, has yet to be examined.

The present study examined intraspecies and interspecies resource-based aggregation dynamics of elephants and other mammalian species at a permanent waterhole in Tanzania. The first objective was to describe the influence of wet and dry seasons and ambient temperature on the intraspecies aggregation patterns of African elephants. Specifically, I examined whether wet and dry seasons and temperature would dictate the frequency, time of day and the number of elephants present at the waterhole. I predicted that elephants would be present at the waterhole more and in greater numbers during the dry season when water was limited and that they would use the waterhole most often during daily times of peak temperature. As keystone competitors, elephants have the potential to influence resource-based aggregation of mammalian species around a limited water sources (Valeix et al. 2007; Valeix et al. 2008). The second objective was to evaluate the interspecies resource aggregation (co-occurrence) of elephants with other mammals at a permanent waterhole. I investigated whether seasonality influenced the

frequency that elephants and other mammalian species co-occurred. I also determined whether the spatial distribution (proximity to a water source) of co-occurrence around a waterhole fit with a pattern explained by interference competition. Elephant-mammal co-occurrence events were predicted to be greater in the dry than the wet season. Therefore, during the dry season under an interference competition scenario, spatial partitioning between elephants and other mammal species should be present.

## **METHODS**

### **Study Site and Population**

All observations occurred at Ndarakwai Ranch (Figure 1.1). Ndarakwai Ranch is a ca. 4300 ha, privately owned, semi-protected ranch in the Sihia District of northern Tanzania (S03°00.663' E037°00.133') in the greater West Kilimanjaro Area (WKA). Ndarakwai Ranch is located between three national parks, Kilimanjaro National Park to the east (ca. 20 km), Arusha National Park to the south (ca. 30 km) and Amboseli National Park (Kenya) to the north (ca. 40km) and is used as a corridor when elephants are migrating among parks (Kikoti 2002). No fences are present but the area is patrolled by a ranger staff.

Mixed acacia woodland (mainly *Acacia mellifera*, *A. tortilis* and *Commiphora africana*) dominate the western section of the ranch (Napora 2007). The remaining area is predominantly grassland (*Spike sporibolous*). The Ngare Nairobi River (North River) demarcates the western and southwestern borders of the property. Water is diverted from the Ngare Nairobi River by smaller streams into a 4300 m<sup>2</sup>, man-made, permanent waterhole. A six-meter high observation platform is adjacent to the waterhole.

## Data Collection

Observations occurred from the observation platform from October 2004 through June 2007. Data from October 2004 - June 2005 were collected by D. Vyas (2006) and data from July 2005 - June 2006 by E. Napora (2007). Data were collected on an average of  $13.12 \pm 1.15$  days/month and were not available for February 2005 and May 2006. For approximately one-month, researchers sequentially overlapped at the study site (Vyas and Napora in 2005, Napora and Castelda in 2006). During these times, simultaneous observations were made by the pair of researchers. The new researcher was trained in this manner until interobserver reliability was between 95-100%. Vyas (2006) accounted for 50.0 % (1553/3106 scans), Napora (2007) 25.5% (795/3106) and Castelda 24.4% (758/3106) of the field effort. Sunrise varied between 600 h and 700 h and sunset between 1100 h and 1900 h. Most visits to waterholes by mammals occur during the daytime hours (Valeix et al. 2007; Valeix et al. 2008). All observers used instantaneous scan sampling (Martin and Bateson 1993) with on the hour recordings from 900-1700 h. Ambient temperature was recorded on the hour from the observation tower under full sun exposure, and used to represent the air temperature around the waterhole.

The waterhole was divided into two spatial zones (Figure 1.2). Zone 1 encompassed the water and the area within 25 m of the bank's edge and zone 2 extended from 26 m to 100 m from the bank's edge. Species in zone 1 were considered within proximity to the waterhole with the potential to use (drink, mud or wallow) the waterhole whereas species located in zone 2 were near the waterhole but may not necessarily use the waterhole. To facilitate data collection, the waterhole was partitioned into four sections based on cardinal points (Figure 1.2).

Scans of elephants and other mammals were conducted on the hour following a 15-min habituation period if the observer arrived within 15-min of the hour. Scans started in the southeastern section and then moved clockwise. During each scan the species, the number of individuals of that species and location (zone) were recorded. When the number of individuals could not be accurately counted (greater than 30 individuals) an estimate based on units of 10 individuals was used. Identification of individuals and new and revisits were not differentiated for any species, including elephants. A high density of trees in the southwest section reduced visibility to approximately 25 m. A total of 3106 scans were taken over the course of the study. A scan in which both an elephant and another mammal species were present within 100 m of the waterhole, regardless of zone, was considered a co-occurrence event. Otherwise, the scan was classified as elephant alone, other mammal alone, or all species absent.

The 31-month sampling period was divided into two seasons based on the occurrence of rain. Months with rainfall on more than 10% of the days were classified as wet (October - December 2004, March - May 2005, November - December 2005, March - April 2006 and October - January 2007). The seasonal classification was based on methodology used by researchers at Amboseli National Park (Moss 2001). Daily rainfall (mm/day) records for the course of this study were study kindly provided by the owners of Simba farm located 5 km north of Ndarakwai Ranch. Rainfall data were collected via water gauge and checked daily by personnel at Simba Farm. Wet months averaged  $9.81 \pm 1.50$  days of rainfall/month while dry months average  $2.73 \pm 0.89$  days of rainfall/month (mean  $\pm$  SE). A total of 1496 scans (48%) occurred during the wet season and 1610 scans (52%) occurred during the dry season. The instantaneous scanning

events were grouped into three time periods: morning (900 h-1100 h; n = 1095), mid-afternoon (1200 h - 1400 h; n = 968) and late-afternoon (1500 h - 1700 h; n = 1043).

### **Data Analysis**

A one-way ANOVA was used to examine whether daily ambient temperature differed across the three sampling years and a two-way ANOVA was performed to test the effects of year and season. A paired t-test was used to examine any difference in the mean daily temperature between the seasons. Mean maximum daily temperature did not meet normality (Bartlett's test for homogeneity of variances and Kolmogorov-Smirnov test for goodness of fit) after undergoing transformation, thus a Mann-Whitney U test was used to test differences in mean daily maximum temperature between the seasons. The mean daily and maximum temperatures within each season were calculated using monthly averages. A non-parametric Scheirer-Ray-Hare two-way design for ranked data was used to compare season, time of day (morning, mid-afternoon and late-afternoon) and mean daily temperature (Sokal and Rohlf 1995). The proportion of elephants sightings in relation to time and ambient temperature were analyzed using linear or curvilinear (quadratic) regression. The strength of the relationship (weak, moderate or strong) was based on guidelines used by Martin and Bates (1993).

A chi-square test for independence was used to test for differences in the frequency of co-occurrence between seasons with a sequential Bonferroni adjusted p-value as *a posteriori* pair-wise test (Sokal and Rohlf 1995; Ruxton and Beauchamp 2008). Spatial distribution was assessed by comparing the location of elephants and mammals when alone (not co-occurring) relative to location when co-occurring. A chi-square goodness of fit was used to test difference in spatial distribution of co-occurrence.

Only 5.8% (64/1096) of the scans occurred consecutively with the same species, thus the assumption of independence for all chi-square analysis was considered to have been met.

All analyses were conducted using JMP IM 4.0.4 (SAS Institute 2000) for Windows operating systems. Descriptive statistics are presented as mean  $\pm$  standard error unless noted.

## RESULTS

### Sampling Variation

The sampling effort significantly differed among sampling years ( $\chi^2 = 28.47$ ,  $df = 2$ ,  $p < 0.001$ ) with twice as much effort in 2004-2005 compared to 2005-2006 (post-hoc test:  $\chi^2 = 17.19$ ,  $df = 1$ ,  $p < 0.001$ ) and 2006-2007 (post-hoc test:  $\chi^2 = 20.89$ ,  $df = 1$ ,  $p < 0.001$ ). Sampling effort was equally distributed across the day in 2004-2005 ( $\chi^2 = 4.42$ ,  $df = 2$ ,  $p = 0.11$ ) and 2005-2006 ( $\chi^2 = 4.08$ ,  $df = 2$ ,  $p = 0.13$ ), but not in 2006-2007 ( $\chi^2 = 46.04$ ,  $df = 2$ ,  $p < 0.001$ ); for 2006-2007 sampling was significantly less in the morning (9:00 h – 11:00 h) than in the mid-afternoon (post-hoc:  $\chi^2 = 43.35$ ,  $df = 1$ ,  $p < 0.001$ ) and late-afternoon (post-hoc:  $\chi^2 = 31.70$ ,  $df = 1$ ,  $p < 0.001$ ). All three years had an equal proportion of wet and dry months ( $\chi^2 = 0.74$ ,  $df = 2$ ,  $p = 0.70$ ), although the categorization of any particular month as part of the wet or dry season may have differed across years (Table 1.1). Collectively, the number of scans performed throughout the day differed in both the wet and dry seasons with more scans performed in the morning in both seasons (wet season post hoc test: morning-mid-afternoon:  $\chi^2 = 6.89$ ,  $df = 1$ ,  $p = 0.009$ ; morning-late-afternoon:  $\chi^2 = 7.87$ ,  $df = 2$ ,  $p = 0.019$ ; dry season post hoc test: morning-mid-afternoon:  $\chi^2 = 25.23$ ,  $df = 1$ ,  $p < 0.0001$ ; morning-late-afternoon:  $\chi^2 = 17.01$ ,  $df = 1$ ,  $p < 0.0001$ ). Thus, when applicable, the proceeding statistical analyses were adjusted for the

sampling differences by weighing the morning sample effort relative to that of mid and late-afternoon.

### **Seasonal Differences**

The daily average temperature among the three sampling years did not differ ( $F_{2,26} = 0.53$ ,  $p = 0.59$ ; Table 1.2) nor did it differ by season (year:  $F_{2,23} = 0.97$ , season:  $F_{1,23} = 0.12$ ,  $p = 0.73$ ; interaction:  $F_{2,23} = 0.22$ ,  $p = 0.80$ ). Since the years had similar ambient temperature patterns, they were combined for analysis. The wet and dry seasons did not significantly differ in mean daily temperature ( $t = -0.44$ ,  $df = 27$ ,  $p = 0.66$ ) nor in mean daily maximum temperature ( $U = 80$ ,  $df = 1$ ,  $p = 0.29$ ). The mean temperature varied across the day (morning, mid-afternoon and late-afternoon) (Figure 1.3), but no interaction between seasonal mean temperature and time of day was evident (Scheiner-Ray-Hare: season:  $H_{1,82} = 0.44$ ,  $p = 0.50$ ; time of day:  $H_{2,82} = 3.18$ ,  $p = 0.046$ ; interaction:  $H_{2,82} = 0.31$ ,  $p = 0.74$ ). In both the wet and dry seasons, the morning ( $24.09 \pm 0.59$  °C) experienced a lower average temperature in comparison to the mid-afternoon ( $26.28 \pm 0.60$  °C) and late-afternoon ( $25.91 \pm 0.60$  °C, see Table 1.3); however, mean morning temperature did not significantly differ between season ( $t = 0.90$ ,  $df = 28$ ,  $p = 0.37$ ). Peak temperature occurred at 12:00 h in the wet season and at 15:00 h in the dry season.

Using the quantitative data acquired from Simba farm, cumulative rainfall was shown to differ among the three sampling years with 2004-2005 being the driest year (Table 1.1). Rainfall averaged  $113.8 \pm 22.47$  mm /month during wet months and  $24.56 \pm 8.89$  mm/month during dry months, where wet and dry were determined by the 10% days of rain per month method as used by researchers in nearby Amboseli National Park,

Kenya (Moss 2001). Thus, difference between wet and dry season is due to the presence of rain and not temperature.

### **Elephant Occurrence**

Of the total number of scans taken during the wet and dry seasons, the proportion of elephant sightings was greater in the dry season than the wet season ( $\chi^2 = 15.67$ ,  $df = 1$ ,  $p < 0.0001$ ). The proportion of scans in which elephants were present at the waterhole (elephant sightings) had a strong, curvilinear relationship with the time of day in both the wet and dry seasons (Figure 1.4). During the wet season, elephants were present more in the mid-afternoon scans than in the morning and late-afternoon scans (Table 1.4), whereas in the dry season, elephants were present more often in both the mid-afternoon and late-afternoon scans than the morning scans (Figure 1.4). A strong positive, linear relationship between hourly proportion of elephant sightings and temperature was evident during both the wet and dry seasons (Figure 1.5).

Of the scans in which elephants were present, more elephants were observed in the wet season,  $20.5 \pm 2.13$  per scan, compared to the dry season  $12.72 \pm 0.78$  per scan ( $U = 753$ ,  $df = 1$ ,  $p = 0.046$ ). In the wet season, the number of elephant present at the waterhole demonstrated a moderate, significant, curvilinear relationship with time of day (Figure 1.6) with significantly more elephants occurring between 13:00 and 15:00 h ( $U = 977$ ,  $df = 1$ ,  $p = 0.047$ ). Ambient temperature also showed a moderate, significant, positive linear relationship with average number of elephants present (Figure 1.7). In the dry season however, no association between the number of elephants and time of day ( $H = 2.74$ ,  $df = 2$ ,  $p = 0.25$ , Figure 1.6), nor with ambient temperature was evident ( $R^2 = 0.002$ ,  $p = 0.84$ ).



## Elephant-mammal Co-occurrence

During the 31-month field season, 12 different mammal species were present at the waterhole (Table 1.5). The number of scans that included any mammal species other than elephants was greater in the dry season than in the wet season ( $\chi^2 = 31.05$ ,  $df = 1$ ,  $p < 0.001$ ). Out of the total number of scans taken, elephants co-occurred with other mammal species 29.6% of the time (Table 1.6). Of the 12 mammal species that co-occurred with elephants, zebra accounted for 30% of the co-occurrence events during the wet season and 40% in the dry season (Figure 1.8). The second most common species to co-occur with elephants was impala (22%) in the wet season and warthog (20%) in the dry season.

The distribution of co-occurrence between an elephant and another mammal species was clustered around the dry months (Figure 1.9) with the dry season experiencing a greater number of co-occurrences than the wet season ( $\chi^2 = 39.72$ ,  $df = 1$ ,  $p < 0.0001$ ). 68.6% (72/105) of the co-occurrence events occurred during between June 2005-October 2005; this was significantly greater than expected regardless of differences in field effort over the three dry seasons ( $\chi^2 = 45.45$ ,  $df = 2$ ,  $p < 0.0001$ ). The overall frequency of co-occurrence relative to the frequency that elephants and other mammal species were not co-occurring was only significant in the dry season (wet season:  $\chi^2 = 0.12$ ,  $df = 1$ ,  $p = 0.73$ ; dry season:  $\chi^2 = 6.46$ ,  $df = 1$ ,  $p = 0.01$ ). Other mammal species were more likely to be alone at the waterhole in comparison to co-occurring with elephants (post-hoc:  $\chi^2 = 12.30$ ,  $df = 1$ ,  $p = 0.0005$ ). Elephant-mammal co-occurrence was not associated with the number of elephants present at the waterhole during the wet

season. However, during the dry season, a moderate, negative significant linear relationship was demonstrated ( $R^2 = 0.62$ ,  $p < 0.0001$ , Figure 1.10).

### **Spatial Distribution**

The spatial distribution of elephants and other mammal species while within 100 m of the waterhole differed between the wet and dry season (Table 1.7). When only elephants were present, elephants were likely to be in zone 2 during the wet season and ( $\chi^2 = 4.94$ ,  $df = 1$ ,  $p = 0.02$ ) in zone 1 during the dry season ( $\chi^2 = 7.69$ ,  $df = 1$ ,  $p = 0.006$ ). Under a mammals alone condition (no elephants present with other mammal species), there was an equal probability that mammalian species would be in either zone 1 or zone 2 in both the wet and dry season (wet:  $\chi^2 = 0.23$ ,  $df = 1$ ,  $p = 0.63$ ; dry:  $\chi^2 = 1.23$ ,  $df = 1$ ,  $p = 0.27$ ). When specifically looking at co-occurrence spatial distribution, elephants had an equal chance of being in the same zone as the other mammal species ( $\chi^2 = 3.24$ ,  $df = 1$ ,  $p = 0.07$ ) in the wet season. However, in the dry season, elephants and other mammal species were more likely to co-occur in different zones within 100 m of the waterhole ( $\chi^2 = 5.88$ ,  $df = 1$ ,  $p = 0.02$ ). Elephants were in zone 1 while other mammal species were in zone 2 (post hoc test:  $\chi^2 = 14.86$ ,  $df = 1$ ,  $p = 0.0001$ ).

### **DISCUSSION**

The first objective of this study was to describe the influence of wet and dry seasons and ambient temperature on the intraspecies aggregation patterns of African elephants. Both seasonality and temperature influenced the distribution of elephants at the Ndarakwai Ranch permanent waterhole. The number of African elephant sightings was greater in the dry season while the number of elephants per sighting was greater in

the wet season. Elephant occurrence at the waterhole also was associated with higher ambient temperature during both the wet and dry seasons.

In African savanna ecosystems, the wet season is marked by an abundance of high quality forage and water, whereas in the dry season, forage quantity and quality and water availability are reduced (Owen-Smith, 1982). Elephant herds, especially female family groups, increase their proximity and frequency of visitation to permanent water during the dry season (Western and Lindsay 1984; Stokke and du Toit 2002; Leggett et al. 2004; Napora 2007). Calves cannot travel long distances without water because their smaller surface area to volume ratio limits their area available for heat transfer (Williams 1990); therefore, female herds are restricted in their movements during the dry season (Stokke and du Toit 2002). Elephants will use free standing water not only for drinking but also to cool themselves by mudding and wallowing (Ayeni 1977; Wright and Luck 1984; Vyas 2006). The arrival time of African elephants at a water source also varies between seasons. In the wet season, elephants arrive at a waterhole in the early-afternoon, but this shifts to late-afternoon in the dry season (Napora 2007). Elephant occurrence at the waterhole was greater in the dry season and coincided with the hottest part of the day at the Tanzanian study site (Napora 2007; this study), likely reflecting the reduced availability of surface water regionally and the greater thermal stress in the dry season.

A recent study in Zimbabwe reported that elephant numbers at waterholes increased as the dry season progressed (Chamaillé-Jammes et al. 2007; Chamaillé-Jammes et al. 2008), but the present study in Tanzania showed no such trend. The number of elephants present per a scan was greater in the wet season than in the dry season. During the wet season herd size increases temporarily as males join cow-calf

herds in order to gain access to estrous females (Moss 1983; Moss and Poole 1983; Western and Lindsay 1984); whereas, in the dry season, large herds gradually break up because of the decline in food and water resources (Western and Lindsay 1984). Ndarakwai Ranch functions as a corridor for elephants migrating among parks (Kikotei 2002). Thus, the lower number of elephants at the waterhole during the dry season is reflective of the fission-fusion (joining and separation) dynamics of elephant herds (Moss & Poole 1983; Archie et al. 2006).

Seasonality also influences the use of a water source. In the dry season, the number of elephants present in each scan was relatively constant regardless of temperature or time of day, and elephants were likely to be in zone 1 ( $\leq 25$  m) to the waterhole. However, in the wet season, group size increased with ambient temperature and peaked at mid-afternoon when most elephant sightings occurred and elephants were not common within 25 m of the waterhole. In the wet season, the presence of male groups at a waterhole are correlated to the presence of female cow/calve groups and mixed groups (males with female groups) (Napora 2007). As previously mentioned, during the wet season males join cow-calf herds in order to gain access to estrous females (Moss 1983; Moss and Poole 1983; Western and Lindsay 1984). These findings suggest that in the wet season, the waterhole serves not only a water source but also as an aggregation point, possibly facilitating male-female encounters for reproduction.

Water scarcity results in high levels of interspecies aggregation (Trash et al. 1995). The distribution of co-occurrence between elephants and other mammal species at the permanent waterhole was clustered around the dry months and occurred more frequently in the driest sampling year. However, in the dry season other mammal species

were more likely to be at the waterhole when elephants were not present and co-occurrence decreased as the number of elephants increased. When elephants and other mammal species did co-occur within 100 m of the waterhole, the heterospecifics were spatially segregated. These patterns may be explained by resource partitioning brought about by interspecies resource competition, particularly interference competition.

As a keystone competitor (Kerley and Landman 2006), elephants can gain access to a water source by exploitation or by behaviorally preventing others from having access to the water. In the present study, complete exploitation of water was not possible because water was present all year round. Interference competition, on the other hand, can occur directly via costly, aggressive interactions or indirectly through a reduction in intake rate caused by less time using the resource as a result of greater vigilance or reduced access (Goss-Custard 1980; Valeix et al. 2008). The effects of interference competition may be reduced by temporal or spatial partitioning (Abramsky et al. 2001). When species (e.g., impala, kudu, roan and sable) suffer from costly interference from elephants, they exhibit temporal partitioning in response to elephant by altering the time of day they visit waterholes when elephants are present (Valeix et al. 2007). The present study demonstrated that when elephants and other mammal species were within 100 m of a waterhole, spatial partitioning also may be used as a mechanism to reduce the effects of interference competition with elephants. While elephants were within proximity to the water source ( $\leq 25$  m), typically other mammal species were further away.

African elephants can have a tremendous impact on their habitat as an ecological engineer (Laws 1970; Jones et al. 1994; Osborn 2002; Western and Maitumo 2004). Landscapes are changed, standing biomass declines (Guy 1981; Eckhardt et al. 2000;

Augustine and McNaughton 2004) and avian and herbivore diversity is reduced with the increase in the number of elephants (Kerley and Landman 2006; Valeix et al. 2007; Ogada et al. 2008). Hence, the behavior of individual species plays an important role in ecological community structure and conservation (Sutherland 1998; Festa-Bianchet and Apollonio 2003). Typically, wildlife species avoid areas with high human and domestic livestock activity (Leggett et al. 2004). Ndarakwai Ranch, as a semi-protected conservation area, functions as an oasis for elephants and other mammalian species (Napora 2007). During the dry season when the riverbeds are dry, the only available water within a 15 km radius is the Ndarakwai Ranch permanent waterhole (Napora 2007). Elephants have the potential to influence the biodiversity around water sources as a result of interference competition (Valeix et al. 2007). Therefore, Ndarakwai Ranch is vulnerable to the effects of elephants. As elephant densities increase, the managers may have to choose between conserving elephant populations and conserving biodiversity.

The present study examined intraspecies and interspecies resource-based aggregation dynamics of elephants and other mammalian species at a permanent waterhole. The frequency, time of day and the number of elephants present at the waterhole were reflective of surface water availability and ambient temperature. Elephants were sighted at the waterhole more in the dry season, whereas the number of elephants at the waterhole was greater in the wet season. Moreover, the waterhole functioned not only as a water source but also as an aggregation point in the wet season. As the dry season progresses, mammalian species aggregate around a limited water resource, thereby intensifying competition for the shared resource (Trash et al. 1995). As a keystone competitor elephants can gain access to water via interference competition.

Temporal partitioning is one mechanism in which a species can reduce the effects of interference competition (Valeix et al. 2007); however, the results of the present study also suggest the workings of spatial partitioning as means to counter the effects of interference competition with elephants.

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Table 1.1. The categorization of months as wet or dry, Ndarakwai Ranch (October 2004 - June 2007).

<b>Month</b>	<b>2004</b>	<b>2005</b>	<b>2006</b>	<b>2007</b>
January		Dry	Dry	Wet
February		-	Dry	Dry
March		Wet	Wet	Dry
April		Wet	Wet	Dry
May		Wet	-	Dry
June		Dry	Dry	Dry
July		Dry	Dry	
August		Dry	Dry	
September		Dry	Dry	
October	Wet	Dry	Wet	
November	Wet	Wet	Wet	
December	Wet	Wet	Wet	

\*Data for February 2005 and May 2006 was not available.

Table 1.2. The average ( $\pm$  SE) mean daily temperature ( $^{\circ}$ C), cumulative rainfall and the number of wet and dry months of the three sampling years, Ndarakwai Ranch (October 2004 - June 2007).

Year	Mean Temperature	Rainfall (mm)	Wet Months (N)	Dry Months (N)
October 2004-September 2005	25.6 $\pm$ 0.99	604.5	6	5
October 2005-September 2006	25.7 $\pm$ 1.10	822.5	4	7
October 2006-June 2007	26.6 $\pm$ 1.10	761.5	4	5

Table 1.3. Statistical values of the non-parametric two-way analysis of variance on the rank of monthly mean daily temperature ( $^{\circ}\text{C}$ ) between the seasons and across the day, Ndarakwai Ranch (October 2004 - June 2007).

Parameter	Statistic	df	p-value
a. Season (Wet/Dry)	H = 0.44	1, 82	p = 0.50
b. Time	H = 3.18	2, 82	p = 0.046
<i>a posteriori</i>			
Morning vs Mid-afternoon	U = 591	1	p = 0.02
Morning vs Late-afternoon	U = 567.5	1	p = 0.045
Mid-afternoon vs Late-afternoon	U = 445.5	1	p = 0.70
c. Season/Time interaction	H = 0.31	2, 82	p = 0.74

\* Scheirer-Ray-Hare two-way design for ranked data was performed with *a posteriori* Mann-Whitney U test with a sequential Bonferroni adjusted p-values

Table 1.4. Statistical values of chi-square goodness of fit test for differences in the frequency of occurrence across the day during the wet and dry seasons, Ndarakwai Ranch (October 2004 - June 2007).

<b>Parameter</b>	<b><math>\chi^2</math></b>	<b>df</b>	<b>p-value</b>
<b>Scans with elephants</b>			
a. Wet Season	7.19	2	p = 0.03
<i>a posteriori</i>			
Morning vs Mid-afternoon	7.05	1	p = 0.007
Morning vs Late-afternoon	1.60	1	p = 0.20
Mid-afternoon vs Late-afternoon	4.05	1	p = 0.04
b. Dry Season	24.28	2	p < 0.001
<i>a posteriori</i>			
Morning vs Mid-afternoon	10.09	1	p = 0.01
Morning vs Late-afternoon	23.14	1	p < 0.001
Mid-afternoon vs Late-afternoon	2.50	1	p = 0.11

\*Sequential Bonferroni adjusted p-value for pair-wise test.



Table 1.5. List of mammalian species present and number of times sighted during the wet and dry seasons, Ndarakwai Ranch (October 2004-June 2007).

Common Name	Species Name	Total sightings	
		Wet	Dry
Impala	<i>Aepyceros melampus</i>	84	169
Vervet monkey	<i>Cercopithecus aethiops</i>	49	99
Blue monkey	<i>Cercopithecus mitis</i>	2	-
Wildebeest	<i>Connochaetes taurinus</i>	-	13
Zebra	<i>Equus burchellii</i>	103	236
Giraffe	<i>Giraffa camelopardalis</i>	-	6
Egyptian mongoose	<i>Herpestes ichneumon</i>	1	2
Slender mongoose	<i>Herpestes sanguineus</i>	1	-
Common waterbuck	<i>Kobus e. ellipsiprymnus</i>	25	23
African elephant	<i>Loxodonta africana</i>	102	182
Kirk's dikdik	<i>Madoqua kirkii</i>	26	30
Banded mongoose	<i>Mungos mungo</i>	13	6
Olive baboon	<i>Paplo cynocephalus</i>	55	84
Warthog	<i>Phacochoerus africanus</i>	52	97
Eland	<i>Tragelaphus oryx</i>	7	20

Table 1.6. Summary of the scan counts under various parameters, Ndarakwai Ranch (October 2004-June 2007).

<b>Parameter</b>	<b>Total</b>	<b>Wet season</b>	<b>Dry Season</b>
Scan performed	3106	1496	1610
No mammals present	2010	1073	937
Any mammal present	1096	423	673
Elephants present	284	102	182
Elephants absent	2822	1394	1428
Elephant-mammal co-occurred	105	25	80
Elephants alone	179	77	102
Mammals without elephants	917	321	491

Table 1.7. Summary of the distribution of elephants and other mammal species around a permanent waterhole during the wet and dry seasons, Ndarakwai Ranch (October 2004-June 2007). Zone 1 encompassed the waterhole and area within 25 m of the waterhole edge and zone 2 extend from 26 m to 100 m from the waterhole edge.

	<b>Wet Season</b>	<b>Dry Season</b>
Elephant alone *	Zone 2	Zone 1
Other mammal alone	Zone 1 & Zone 2	Zone 1 & Zone 2
Co-occurrence *		
Elephant	Zone 1 & Zone 2	Zone 1
Other mammal	Zone 1 & Zone 2	Zone 2

\* Results are issued from chi-squared test,  $p < 0.05$ .

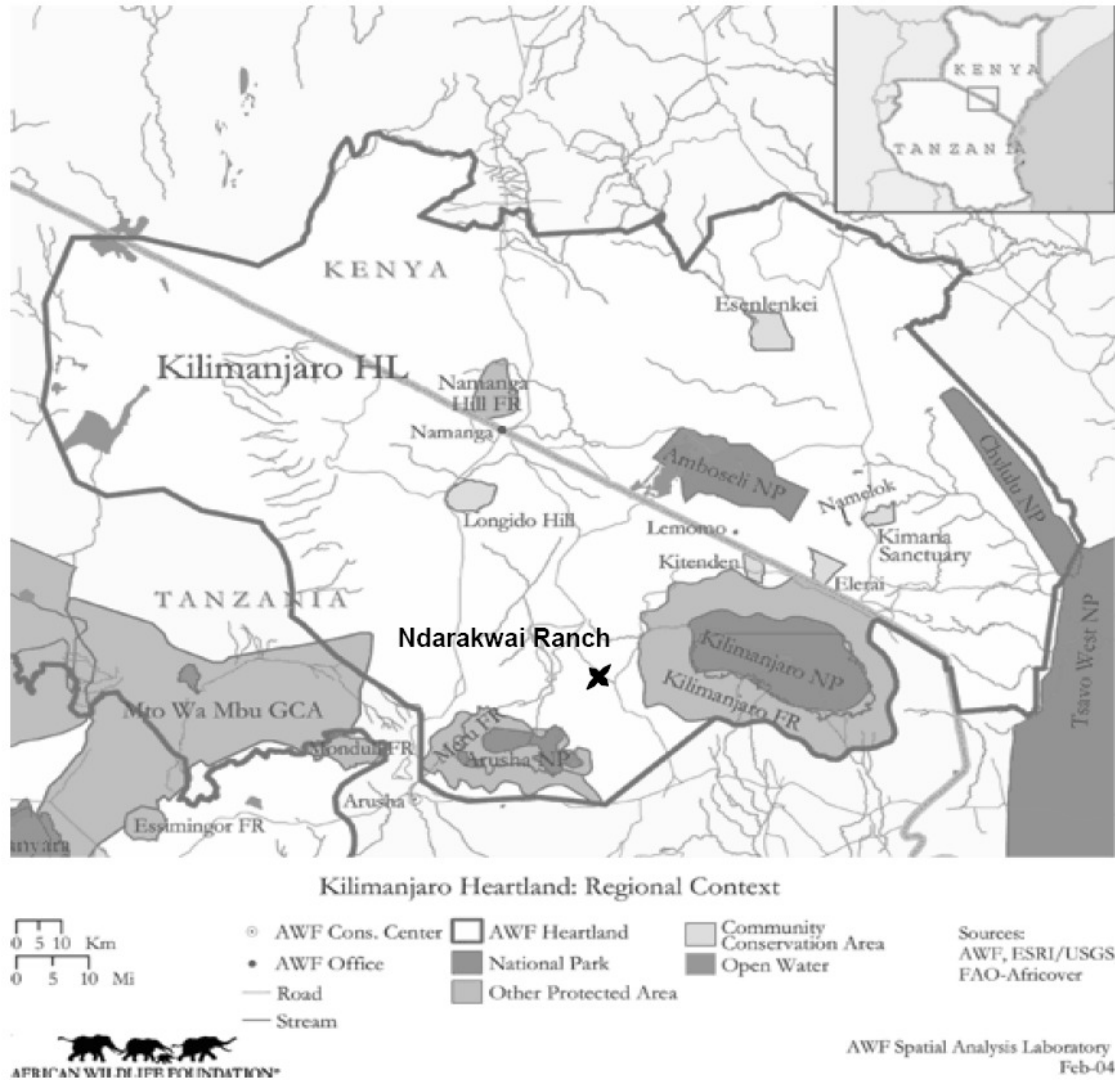


Figure 1.1. Map of the north-central border between Kenya and Tanzania. Ndarakwai Ranch is marked with an “X” (AWF Spatial Analysis Laboratory 2004).

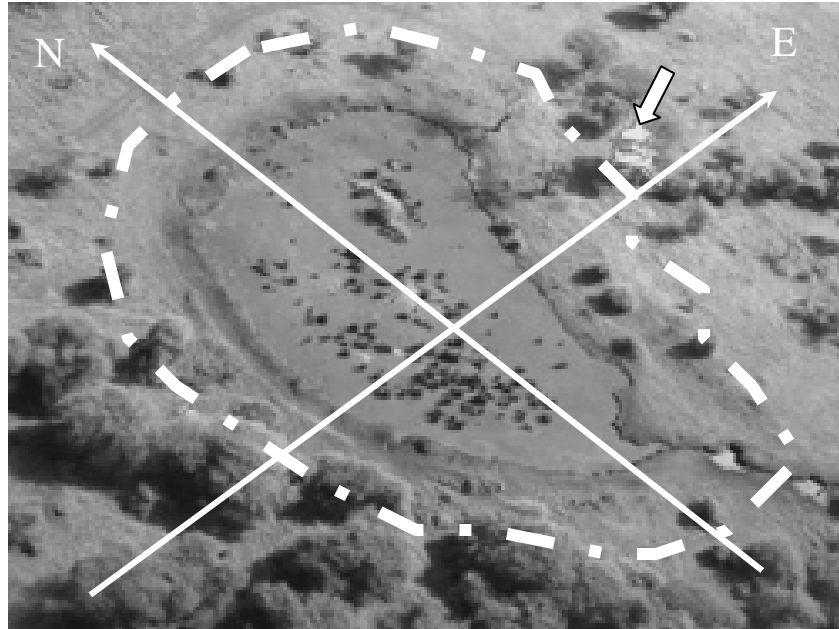


Figure 1.2. Overhead view of the waterhole showing zone 1 and four cardinal sampling sections, Ndarakwai Ranch, Tanzania. The dash line denotes 25 m from the edge of the waterhole and the observation platform is marked by the arrow in the NE section. (Vyas 2006).

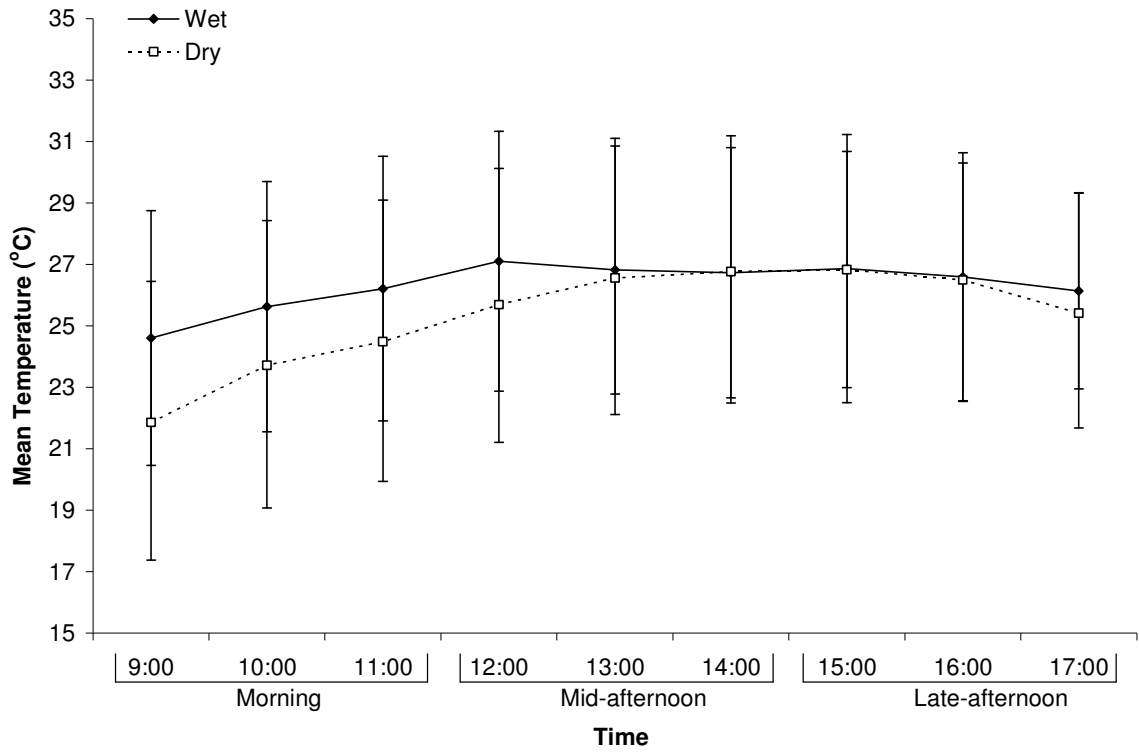


Figure 1.3. The mean ( $\pm$  standard deviation) daily temperature ( $^{\circ}$ C) of the wet and dry seasons, Ndarakwai Ranch (October 2004 - June 2007).

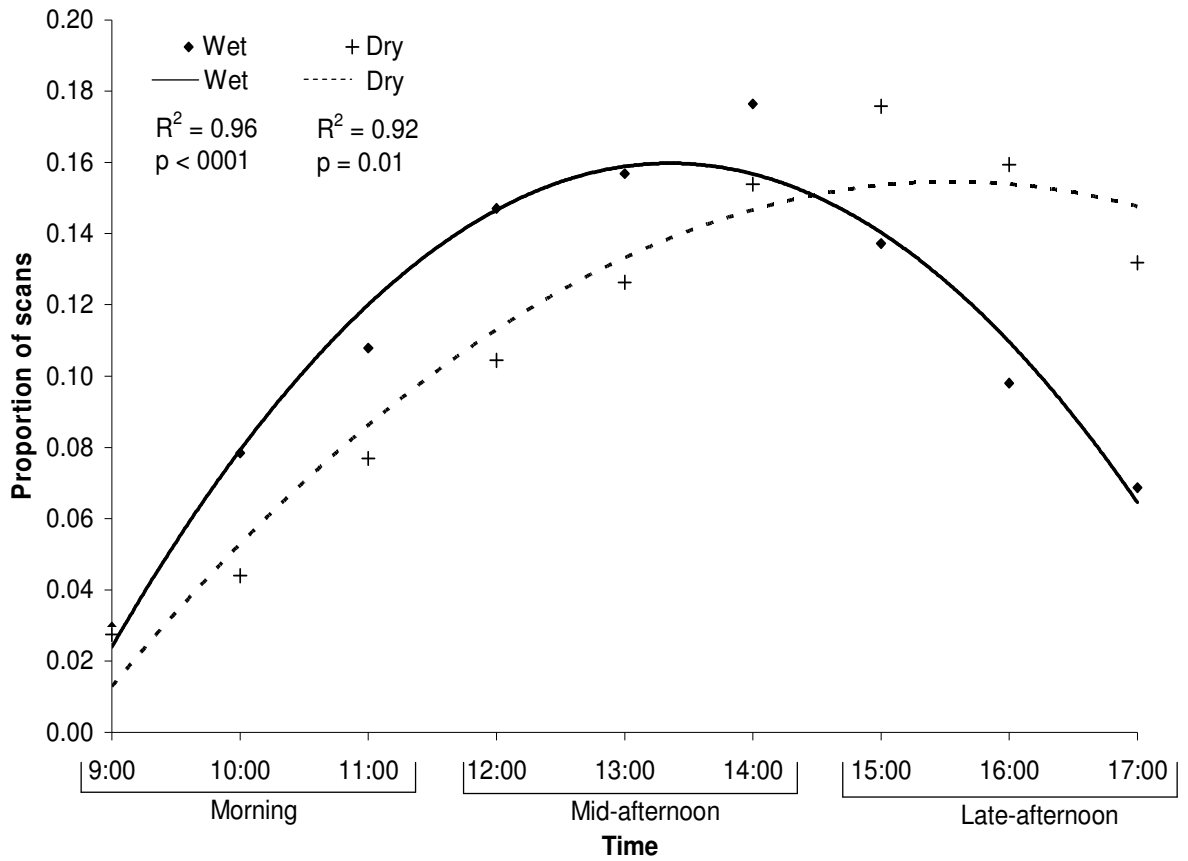


Figure 1.4. The hourly proportion of elephant sightings during the wet and dry seasons, Ndarakwai Ranch (October 2004-June 2007).

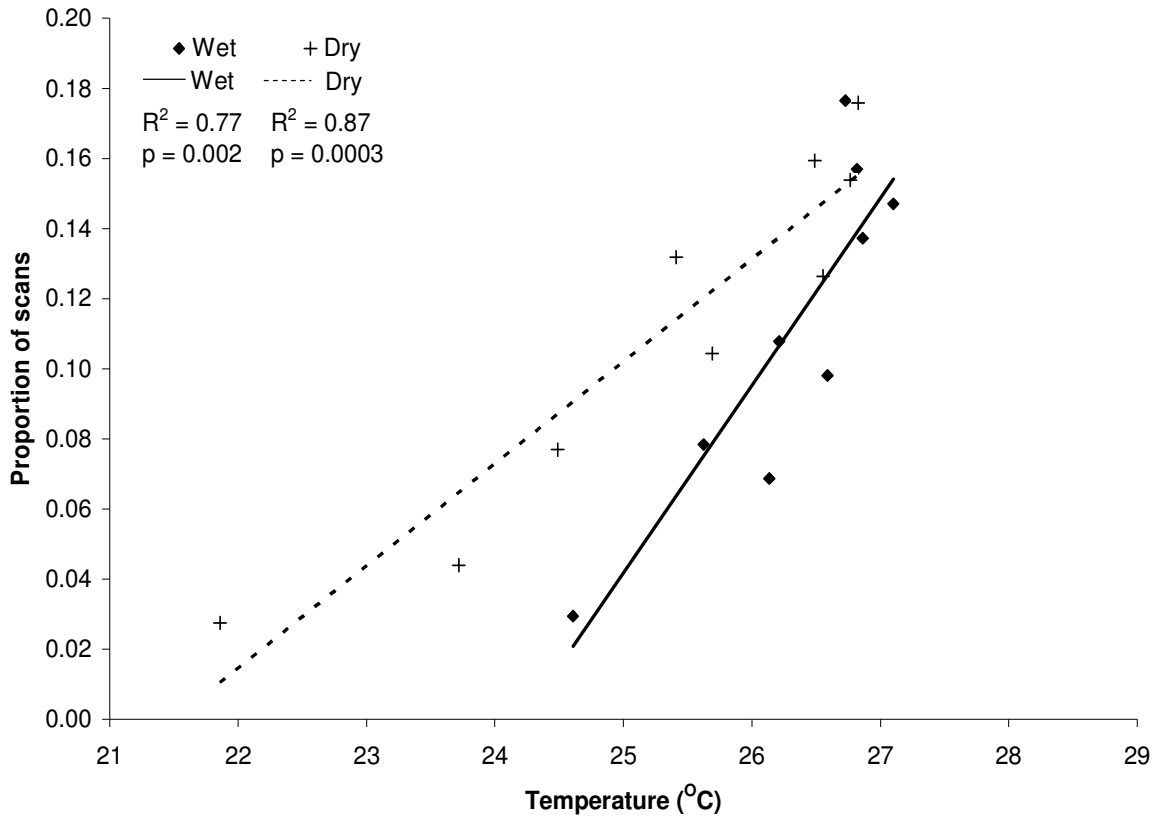


Figure 1.5. The mean daily temperature (°C) and the hourly proportion of elephant sightings during the wet and dry seasons, Ndarakwai Ranch (October 2004 - June 2007).



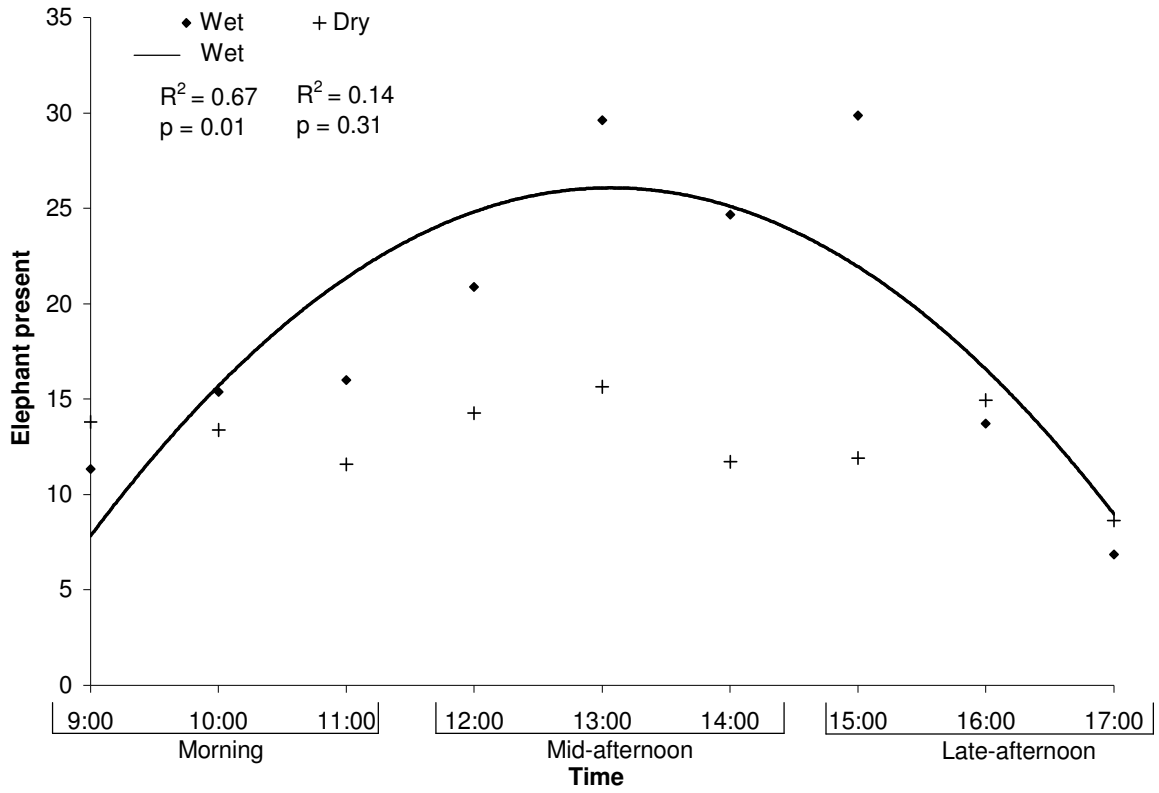


Figure 1.6. Average number of elephant present per sighting during the wet and dry seasons, Ndarakwai Ranch (October 2004 - June 2007).

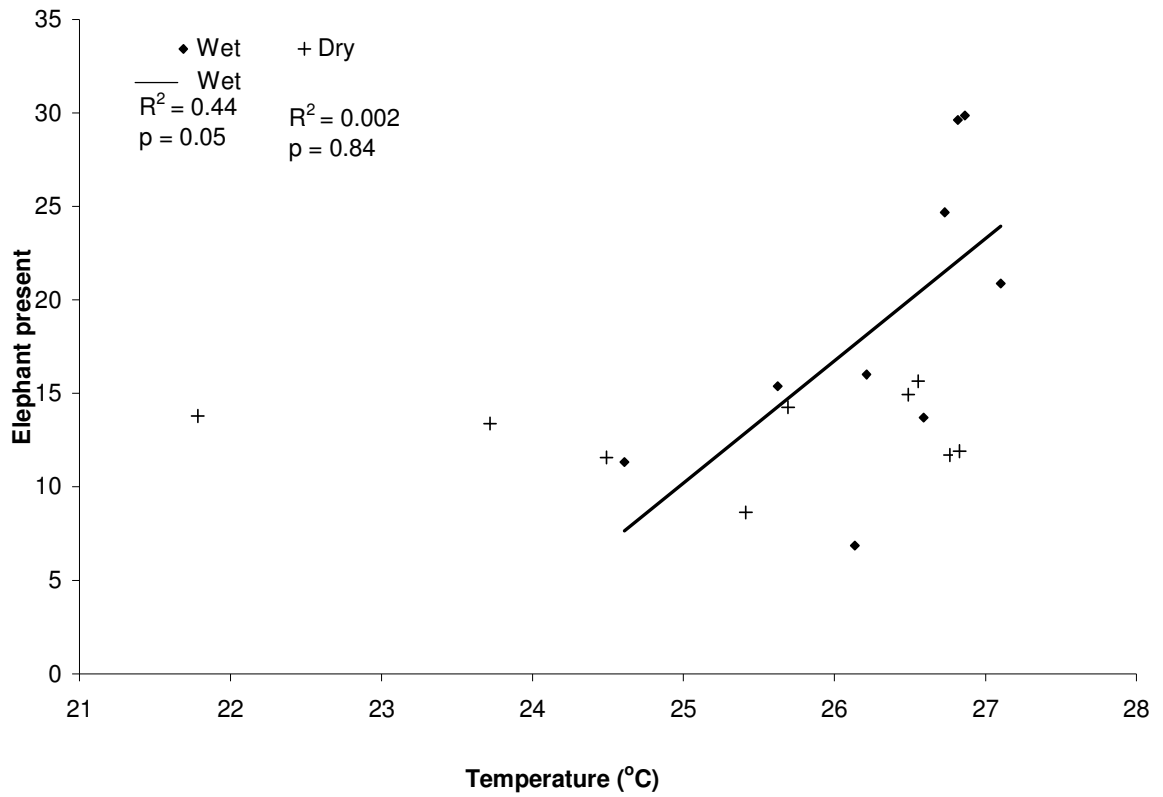


Figure 1.7. The mean daily temperature (°C) and average number of elephant present per sighting during the wet and dry seasons, Ndarakwai Ranch (October 2004 - June 2007).

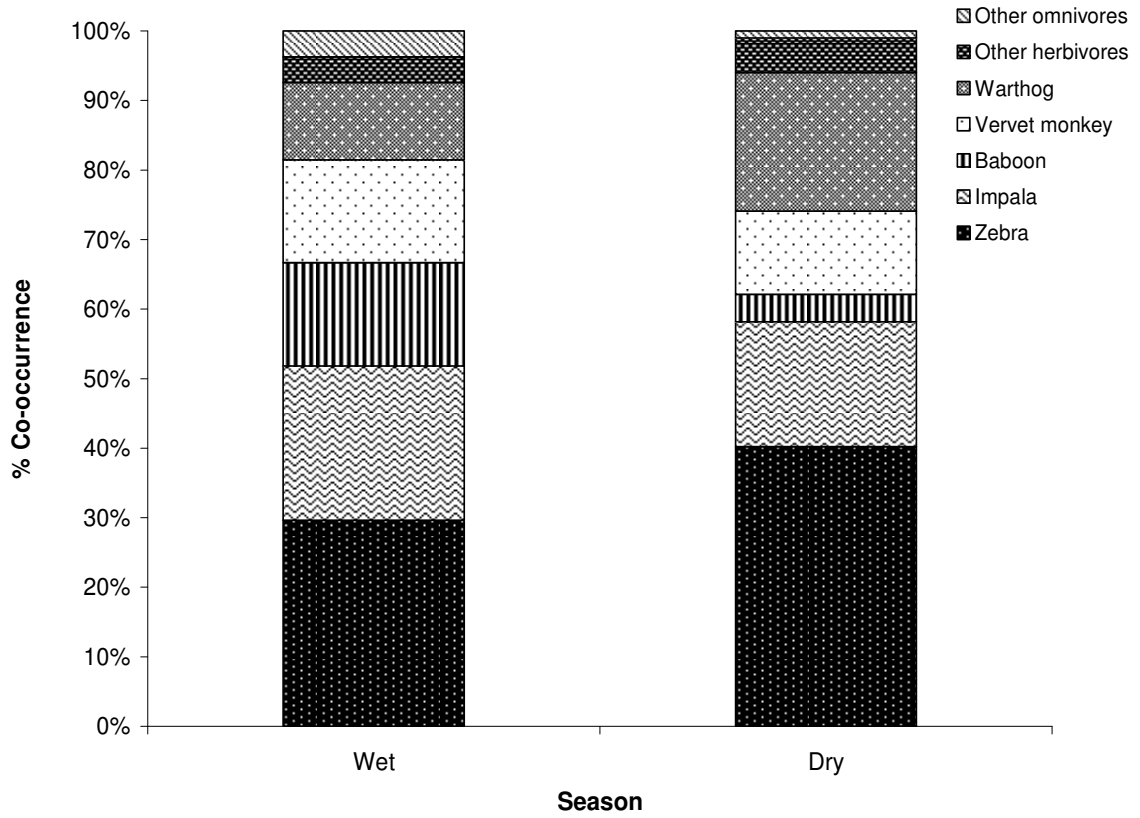


Figure 1.8. The cumulative co-occurrence of various mammal species with elephants during the wet and dry seasons at a permanent waterhole, Ndarakwai Ranch (October 2004 - June 2007).

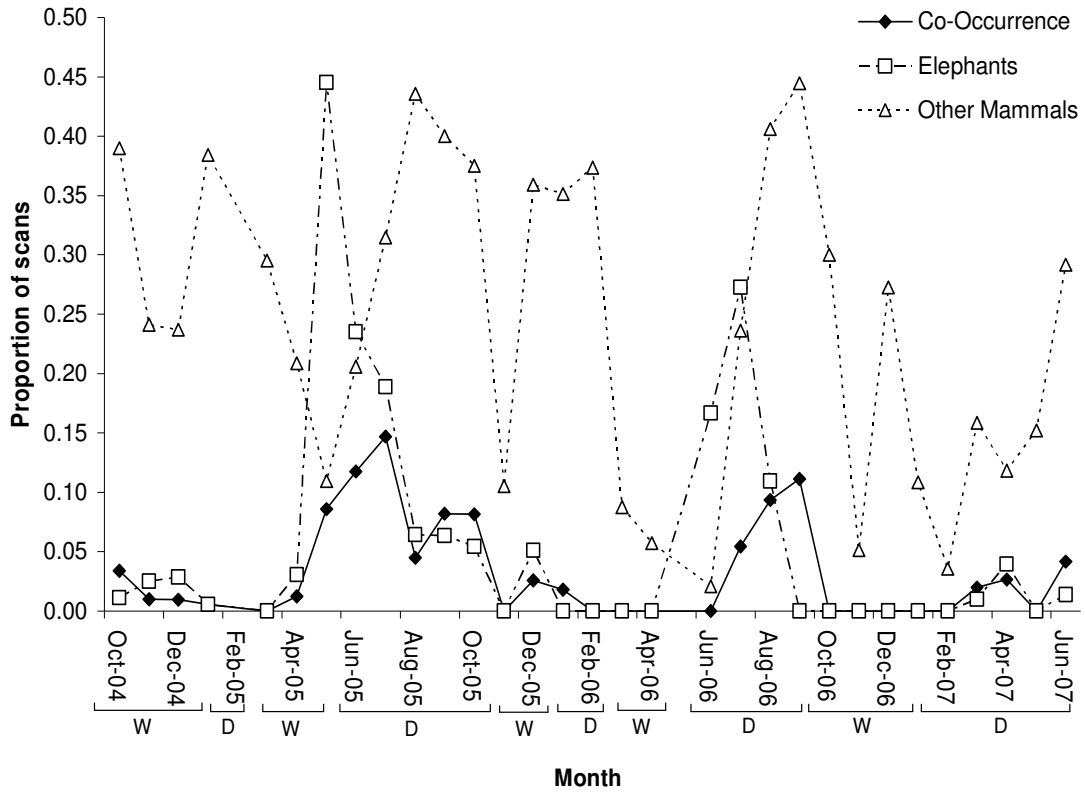


Figure 1.9. The monthly proportion of scans in which elephants co-occurred with other mammal species, elephants were alone, and other mammal species were alone, Ndarakwai Ranch (October 2004 - June 2007). W demotes wet months and D indicates dry months.

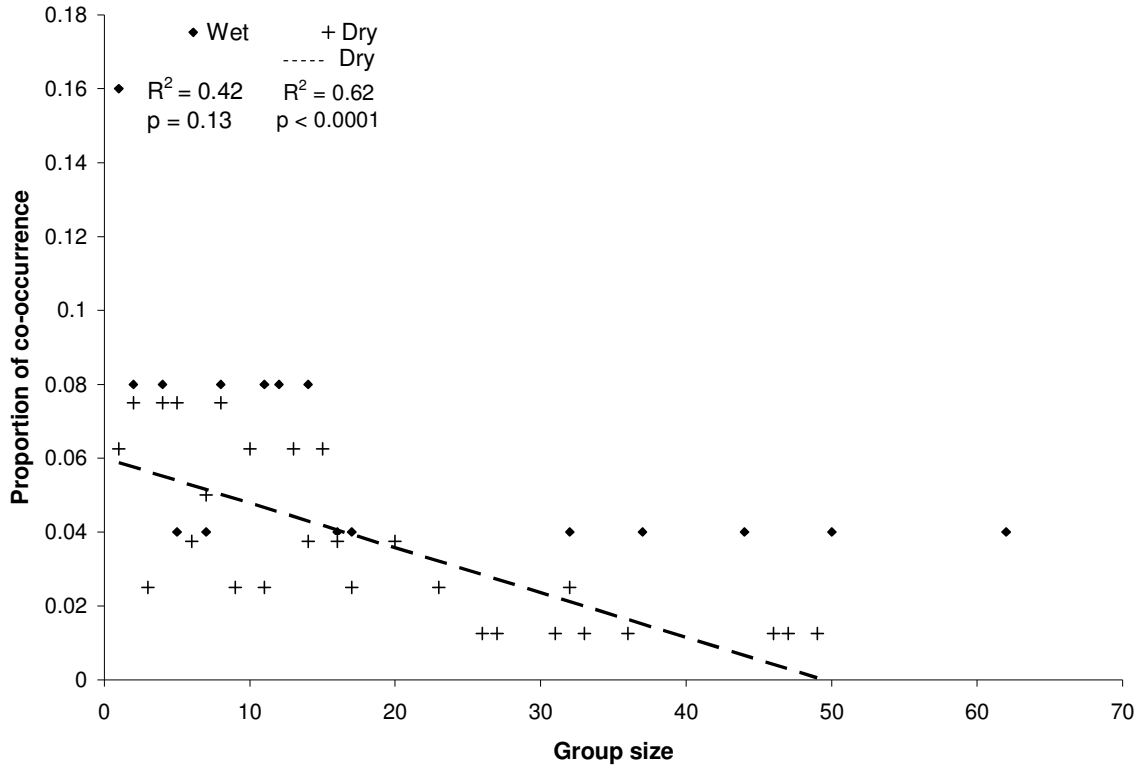


Figure 1.10. The mean elephant group size per sighting and the proportion of elephant-mammal co-occurrence during the wet and dry seasons, Ndarakwai Ranch (October 2004 - June 2007).

## CHAPTER II

# BIOASSAYS OF PUTATIVE CHEMICAL SIGNALS IN AFRICAN (*LOXODONTA AFRICANA*) FOR CONVERGENCE WITH INSECTS AND SIMILARITIES WITH ASIAN ELEPHANTS

### ABSTRACT

Intersexual and intrasexual communication in African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants is mediated in part by chemical signals. Chemical signals released in secretions and excretions aid in locating mates and identifying their sexual state across broad spatial and temporal scales. *Z*-7-dodecenyl acetate, a component of the sex pheromone in many insects, has been identified as the preovulatory pheromone in Asian elephants. While an estrous pheromone in African elephants has not been identified, its existence is supported by recent research with elephants in captivity. The objectives of this study were to determine behaviorally whether four known insect pheromones (*endo*-brevicommin, *exo*-brevicommin, *E,E*- $\alpha$ -farnesene and frontalin) identified in the ovulatory urine of African elephants elicited responses from wild elephants that are indicative of a meaningful chemical signal, and to discern if one or more compounds might serve as sex-specific pheromones. Biological assays were performed at a waterhole on the Ndarakwai Ranch in northern Tanzania. The proportion of elephants responding and the rate of response from elephants toward *endo*-brevicommin, *exo*-brevicommin, *E,E*- $\alpha$ -farnesene and frontalin were statistically similar to vanilla and pre-puberty female urine controls. The results suggest that when tested singly, none of these compounds serves as an African elephant chemical signal. Although this study was unable to confirm behaviorally an African elephant chemical signal, I would suggest performing additional assays with *E,E*- $\alpha$ -farnesene and frontalin in anestrus urine before concluding that these compounds have no signal value as single compounds.

INDEX WORDS: Elephants, sex pheromones, estrous, chemosensory

## INTRODUCTION

In sexually segregated invertebrates and vertebrates, chemical signals assist in locating mates and facilitating mate selection. Virgin female emperor moths (*Saturina pavonia*) attract mates and female tobacco moths (*Ephesia elutia*) are able to select larger males to mate with through the use of sex pheromones (Fabr  1911; Phelan and Baker 1986). Mohor gazelle (*Gazella dama mhorr*) use chemical signals found in urine to determine which female is the most fertile (Pickard et al. 2003). Chemical signals are also a common form of intra- and intersexual communication in African (*Loxodonta africana*) and Asian elephant (*Elephas maximus*) societies. Female elephants remain with their natal herd throughout their life, but males disperse during early puberty (around 14 years of age) and travel in bachelor herds or alone (Laws 1969; Jainudeen et al. 1972; Poole 1994). As males search of mates, they acquire reproductive information about conspecifics through chemical signals (Rasmussen and Schulte 1998; Langbauer 2000; Schulte et al. 2005).

Chemical signal investigations by elephants are performed by the trunk and are directed toward the body of the elephant, especially the genital region, mouth and temporal gland, or to excretions and secretions of conspecifics (Poole and Moss 1989; Rasmussen and Schulte 1998; Langbauer 2000; Rasmussen and Krishnamurthy 2000). The major chemosensory behaviors that elephants use to evaluate excretions and secretions are termed sniff, check, place and flehmen (Schulte and Rasmussen 1999). However, a number of other accessory trunk behaviors may also be involved (Bagley et al. 2006; Vyas 2006; Schulte et al. 2007; Meyer et al. 2008).

Elephants exhibit sexual dimorphism in chemosensory behaviors. Work at Addo Elephant National Park, South Africa, indicated that males were more likely than females

to investigate elephant urine and feces (Loizi et al. 2009) and upon entering a region around a waterhole, males had the highest rates of sniff behavior towards feces (Merte 2006). Similar trends were found at Ndarakwai Ranch, Tanzania, via biological assays of urine and feces from a juvenile female elephant. Adult males showed the highest rate of chemosensory behaviors for any age and sex class (Vyas 2006).

Chemical signals released by female elephants reveal their receptivity. Females have a 12-16 week estrous cycle composed of luteal (8-12 weeks) and follicular (4-8 weeks) phases (Hess 1983; Plotka et al. 1988; Kapustin et al. 1996). Within follicular phase, females experience two luteinizing hormone (LH) peaks during the. The first LH peak (LH1) does not induce ovulation; ovulation is stimulated three weeks later by a second LH (LH2) peak (Brown et al. 1999; Hermes et al. 2000; Czekala et al. 2003). The estrous pheromone (*Z*-7-dodecenyl acetate (*Z*7-12:Ac) was isolated from the preovulatory urine of female Asian elephants (Rasmussen et al. 1997; Rasmussen et al. 2005). The concentration of *Z*7-12:Ac in urine becomes detectable by chemical analyses around the first luteinizing hormone peak and increases to a maximum at ovulation (Rasmussen 2001). Flehmen responses from males increase in frequency as a female approaches ovulation and are linked to the female's high *Z*7-12:Ac concentrations (Rasmussen et al. 1997). Such behavior provides evidence that *Z*7-12:Ac functions as female-to-male estrous signal in Asian elephants.

Annually, adult male elephants enter an asynchronous rut-like condition known as musth (Eisenberg et al. 1971; Pool and Moss 1981). The state of musth can be recognized by a continuous discharge of urine, copious secretion from the swollen temporal gland and heightened interest in females (Jainudeen et al. 1972; Poole 1989;



Rasmussen et al. 1990). Older males experience longer and more predictable musth cycles than younger males (Poole and Moss 1989). Females mate significantly more often with older males, but also exhibit preferences for older males in musth (Moss 1983; Hollister-Smith et al. 2007; Rasmussen et al. 2007). In mature male Asian elephants, frontalin (found in temporal gland secretions) serves to signal musth to conspecifics (Rasmussen 1998; Rasmussen and Greenwood 2003).

African and Asian elephants share a common elephantid ancestor dating back to the Miocene (Maglio 1973); however, Z7-12:Ac has never been detected in female African elephant urine collected at the time of LH1 or LH2 (Rasmussen 1998; Rasmussen and Schulte 1998; Goodwin et al. 2005). Male African elephants do not show heightened chemosensory behaviors toward Z7-12:Ac (Rasmussen 1998). Yet, a recent study with captive male African elephants revealed that adult African males respond more to preovulatory urine than luteal urine and a control solution of vanilla extract in water, indicating the presence of a female-to-male chemical signal (Bagley et al. 2006). The two elephant species were once sympatric in Africa (Maglio 1973; Shoshani and Tassy 1996); hence, divergence in their estrous signals might be expected.

Over 200 different compounds have been identified in urine of African elephants (Goodwin et al. 2005). Interestingly, a handful of these compounds are known insect pheromones and their precursors (Goodwin et al. 2006). Z7-12:Ac, the Asian elephant estrous pheromone, is used by over 126 species of Lepidoptera as a component of their sex pheromone (Rasmussen et al. 1996; Rasmussen 1998). Frontalin, which functions as a musth pheromone in Asian elephants, is also a bark beetle aggregation pheromone (Rasmussen 1998). Thus, two compounds that play a role in Asian elephant

communication are also insect pheromones, illustrating convergent evolution (Rasmussen et al. 1996; Rasmussen 1998; Rasmussen and Greenwood 2003). Because of the convergence between insect and Asian elephant chemical signals, compounds identified in female African elephant urine that are known insect pheromones are good candidates as African elephants pheromones.

*Endo*-brevicomin, *exo*-brevicomin, *E,E*- $\alpha$ -farnesene and frontalin are four insect pheromones that have been identified in ovulatory urine of African elephants (Goodwin et al. 2006). *Endo*-brevicomin, *exo*-brevicomin and frontalin are known bark beetle aggregation pheromones (Francke et al. 1995, Wyatt 2003). *E,E*- $\alpha$ -farnesene serves as an alarm pheromone termites (*Prorethia* *canalifrons*) (Šovotnik et al. 2008) and is a component of a primer pheromone blend in mice (Novotny et al. 1999). Although none of the above compounds are specifically sex pheromones, it is not uncommon for a compound to have multiple functions. For example, (*E*)- $\beta$ -farnesene functions as an alarm pheromone in aphids but is also a component of a primer pheromone in mice (Wyatt 2003). Therefore, I hypothesized that any of these four compounds might act singly as an important chemical signal to African elephants. The first objective of this study was to determine the bioactivity of *endo*-brevicomin, *exo*-brevicomin, *E,E*- $\alpha$ -farnesene and frontalin. If one or more putative pheromones functioned as a chemical signal, I predicted the behavioral response elicited would be greater than that elicited by a low response (vanilla) control and urine from a female in the luteal phase (controls based on Bagley et al. 2006 and references therein). Chemical signals also may be sex-specific. The second objective of this study was to examine differences in behavioral responses between post-puberty males and females toward each compound. If any of these

compounds act specifically as a female-to-male signal, then I predicted that responses would be greater by post-puberty males than females.

## **METHODS**

### **Study Site**

From July 2006 to June 2007, the study was conducted at the 4300 ha Ndarakwai Ranch, a privately owned, semi-protected property in the greater West Kilimanjaro Area (WKA) of northern Tanzania (Figure 2.1). The ranch has no fences, but a ranger staff patrols the area. Mixed acacia woodland (mainly *Acacia mellifera*, *A. tortilis* and *Commiphora africana*) dominates the western section of the ranch (Napora 2007). The remaining area is predominantly grassland (*Spike sporibolous*). The Ngare Nairobi River (North River) demarcates the western and southwestern borders of the property. Water is diverted from the Ngare Nairobi River by smaller streams into a 4300 m<sup>2</sup>, man-made, permanent waterhole.

### **Identification of Elephants**

Over 280 elephants have been identified on Ndarakwai Ranch (Vyas 2006; Napora 2007) using ear morphology (e.g. vein patterns, holes, and tears), tusk characteristics (e.g. broken/missing tusk and curvature), and other noticeable physical characteristics (Moss 1996). The ages of the elephants were estimated based on the size of the elephant at the shoulder and by tusk circumference and length (Moss 1996). Elephants were demarcated into two age classes: Pre-puberty (0-9 yrs) and Post-puberty ( $\geq 10$  yrs). The age classes coincide with major developmental stages. Pre-puberty elephants remain closely associated with the natal herd, whereas post-puberty elephants

are either with their natal herd and mating (females) or dispersing and traveling with same sex conspecifics (males).

### **Bioassay Samples**

Vanilla solution, pre-puberty female (PPF) urine and four insect pheromones that were identified in female African elephant urine (*endo*-brevicommin, *exo*-brevicommin, *E,E*- $\alpha$ -farnesene and frontalin) were behaviorally bioassayed. Individual compounds were presented by using 100  $\mu$ l of putative pheromone to 500 ml water. The presence of a compound in the water was confirmed by the smell of the solution; the odors of the four compounds could not be differentiated in solution. The vanilla extract water solution and PPF urine served as controls. Vanillin is a natural component of Asian elephant urine and elicits a low but regular level of interest in both African and Asian elephants (Schulte and Rasmussen 1999; Bagley et al. 2006). Imitation vanilla extract, which contains synthetic vanillin, has successfully been used as a control in bioassays (e.g., Loizi 2004; Bagley et al. 2006) and is a mean measure response to novel substance. Due to the lack of response toward the vanilla extract solution during previous biological assays at the Ndarakwai waterhole (Vyas 2006), the concentration of vanilla was increased ten-fold by combining 25 ml of Tone's® synthetic vanilla extract with 500 ml water. Analogous to luteal urine, PPF urine functioned as a higher response control that was of African elephant origin. In a study with captive, adult male African elephants, responses were highest to follicular urine, lowest to the vanilla control and in-between to luteal urine (Bagley et al. 2006) (see Figure 2.2 for response model).

In the present study, urine was not available from adult female elephants in known parts of their estrous cycle. Therefore, we obtained urine from an eight-year-old

habituated juvenile female African elephant that had been used in earlier biological studies (Vyas 2006; Napora 2007). During the latter part of the present study, behavioral observations made by her caretakers suggested that she may have begun her estrous cycle. All urine used in the bioassays was collected before the supposed onset of puberty

Samples were placed when elephants were approaching the water and left out for the remainder of the day. Because they may dissipate over time, it is possible that they may drop below threshold level during the course of the day. Hence, I checked to see if the likelihood of responding decreased with the time since the sample was placed.

### **Bioassay Protocol**

A double-blind procedure was implemented for bioassays. Vanilla, PPF urine (when available) and one compound were used per trial. Bioassay samples were placed within 10 m of the waterhole in locations that elephants were likely to visit (Figure 2.3). The bioassay site was randomized and determined one day before the bioassay. Only one of the four compounds was placed per location per day. The ground for each bioassay was leveled with the shoe of the observer to minimize the spreading of the solution. The location was then rinsed with water from the waterhole in order to provide a “clean” area to place the samples. After preparation, the sites were marked with branches arranged in the shape of an “X” to assist visual identification from the platform; however, samples were not placed on the prepared sites until elephants were within 300 m of the waterhole. Due to the porous nature of the ground, the sites were dry by the time a sample was placed on a prepared site. Samples were not replenished between approaching groups (Vyas 2006). At the end of the day, each location was rinsed with water from the

waterhole to dilute any of the remaining samples. If it rained after the samples were placed, the bioassay was discontinued for the day.

### **Focal Location Observations**

Observations were made from a six-meter high observation platform that was adjacent to the waterhole. The sex, age class and identity were noted for each elephant. Trunk-related chemosensory behaviors directed toward samples and accessory trunk behaviors, as well as other non-trunk behaviors were recorded using continuous focal sampling (Altmann 1974). These were based on published ethograms of chemosensory behaviors (Table 2.1, see also Bagley et al. 2006; Vyas 2006; Meyer et al. 2008). The observation of a particular individual was terminated once the elephant was greater than one body length from the sample or was out of the observer's sight for three minutes. Duration of the time within one body length of a sample was recorded.

### **Statistical Analysis**

To examine if the likelihood of response by elephants decreased with the time since the sample was placed, I used a Spearman Rank test. I correlated the time since placement with the proportion of elephants that performed any chemosensory behaviors while within one body length (proximity) of sample.

The response of elephants to each putative pheromone was compared to the response of elephants toward vanilla (low response control) and PPF urine (elephant origin control). Interest in the bioassay sample was determined by measuring the duration, proportion and rate of responding. Duration was recorded as time spent within one body length of a sample. Proportions were calculated based on the number of elephants that came within one trunk length (near) the samples and performed some

behavioral response. The proportion of elephants responding was analyzed using chi-square goodness of fit test. If a significant difference among the vanilla, PPF urine and putative pheromone occurred, an *a posteriori* pair-wise comparison test with a Bonferroni correction was used (Sokal and Rohlf 1995; Ruxton and Beauchamp 2008). Rate was based on the number of times the elephant performed chemosensory responses while within one body length. Because the rate and duration data were not normally distributed, I used the non-parametric Mann-Whitney U-test or a Kruskal-Wallis test with a sequential Bonferroni adjustment for post hoc comparisons. The Scheirer-Ray-Hare two-way design for ranked data was used to examine the relationship between sex, age and duration for each compound to determine if a compound served as a sex-specific chemical signal. Power test were used to determine the level of power of analysis as well as an appropriate sample size a power level of 90% (Sokal and Rohlf 1995)

Values are reported as means and one standard error measurements (SEM). All statistical analyses were conducted using JMP IN 4.0.4 (SAS Institute 2000).

## **RESULTS**

Bioassays samples were placed around the waterhole 31 of the 158 days spent at the waterhole. The other days lacked elephants. A total of 115 bioassays (Table 2.2) were performed by elephants; however, only a power  $(1-\beta) \leq 0.30$  (30%) was reached for each compound. A sample size of at least 20 bioassays per a compound is required to reach a power level of 90%. The response to the PPF urine control did not vary over the study ( $R_s = 0.26$ ,  $N = 17$ ,  $p = 0.31$ ). On any single day, the ability of elephants to detect a sample did not appear to be affected by the length of time since the sample was placed. Specifically, the proportion of elephants that performed any chemosensory behavior

while within proximity of the samples did not change with time since placement (Figure 2.4).

African elephants spent 1-55 s performing chemosensory responses while within proximity to the samples. Elephants did not differ significantly in the duration spent within proximity of vanilla ( $8.67 \pm 2.70$  s) and PPF urine ( $11.00 \pm 2.35$  s) ( $U = 218$ ,  $df = 1$ ,  $p = 0.25$ ). The time elephants spent within proximity of all three of the putative pheromones did not differ significantly in comparison to vanilla and PPF urine control (*endo/exo*-brevicommin;  $13.44 \pm 3.46$  s; *E,E*- $\alpha$ -farnesene;  $15.00 \pm 3.47$  s. and frontalin;  $11.31 \pm 3.24$  s; Table 2.3). The duration that elephants spent within proximity of the sample did not vary by sex and age (stimulus:  $H_{4,65} = 1.57$ ,  $p = 0.19$ ; sex:  $H_{1,65} = 3.63$ ,  $p = 0.06$ ; interaction:  $H_{4,65} = 0.92$ ,  $p = 0.46$ ).

The proportion of elephants that performed chemosensory behaviors while within one trunk length (near) of vanilla was similar to that of PPF urine (Table 2.4). A significantly lower proportion of elephants performed any chemosensory behavior while near *endo/exo*-brevicommin in comparison to PPF urine but not in comparison to vanilla (Figure 2.5). The proportion of elephants that were near *E,E*- $\alpha$ -farnesene and frontalin and that performed any chemosensory responses did not significantly differ from either vanilla or PPF urine controls (Table 2.3).

The rate of chemosensory behavior (number of any chemosensory events performed while within one body length of stimulus) of elephants followed similar patterns to that of the proportion of elephants responding. The rate of chemosensory behavior (from elephants directed towards vanilla ( $1.76 \pm 0.72$ /proximity) and PPF ( $2.65 \pm 0.56$ /proximity) were similar ( $U = 240$ ,  $df = 1$ ,  $p = 0.06$ ). *Endo/exo*-brevicommin ( $0.9 \pm$



0.42/proximity) elicited a significantly lower rate of chemosensory responses in elephants in comparison to PPF urine but did not differ significantly from vanilla (Figure 2.5). *E,E*- $\alpha$ -farnesene ( $1.65 \pm 0.76$ /proximity) and frontalin ( $3.45 \pm 0.97$ /proximity) did not elicit significantly different rates of chemosensory responses from elephants in comparison to the vanilla extract in water solution or the PPF urine (Table 2.5, I). The rate of chemosensory behavior directed towards *endo/exo*-brevicommin was significantly lower in comparison to urine in post-puberty males ( $U = 64$ ,  $df = 1$ ,  $p = 0.02$ ), but not females ( $H = 7.70$ ,  $df = 4$ ,  $p = 0.10$ ; Figure 2.6). Each single chemical sample elicited a similar rate of chemosensory behavior from male and female elephants (Table 2.6).

A total of 133 chemosensory events were performed by the 59 responding elephants. Sniff, check, place and flehmen response (SCPF) made up 66.9% of the chemosensory repertoires of elephants while raised sniffs and accessory trunk behavior represented 8.3% and 24.8% of the repertoire, respectively. The rate of SCPF, raised sniffs or accessory trunk responses of elephants did not differ significantly between vanilla and PPF urine (Table 2.5, II). *Endo/exo*-brevicommin was the only putative compound to elicit a differing rate of SCPF response in comparison to the controls; *endo/exo*-brevicommin elicited significantly lower rate of SCPF than PPF urine ( $U = 390$ ,  $df = 1$ ,  $p = 0.0006$ ). The rate of raised sniffs and accessory trunk responses of elephants did not differ significantly between the two controls, or when comparing each putative pheromone to the controls (Table 2.5, III and IV).

## **DISCUSSION**

The wild African elephants of this study did not behaviorally confirm that one or more of the four bioassayed compounds function as meaningful chemical or sex-specific

signal. These pheromones did not evoke behavioral responses greater than the vanilla extract solution or the pre-puberty female urine. African elephants spent an equal amount of time within proximity of the controls and putative pheromone sample. The proportion of elephants responding and the rate of response toward *endo*-brevicommin, *exo*-brevicommin, *E,E-α*-farnesene and frontalin were statistically similar to vanilla, indicating that these four compounds elicited a low level of bioactivity from African elephants. However, a lower proportion of elephants responded to *endo*-brevicommin and *exo*-brevicommin in comparison to PPF urine and elephants differed in their rate of responses.

The duration between the time of placement and when the bioassay occurred did not affect the bioactivity of these sample. Sample size, on the other hand, may have been a factor. A power test indicates that the lack of significance in the rate of chemosensory behavior among the controls and each putative pheromone is due to a low level of power (Sokal and Rohlf 1995). A sample size of at least 20 bioassays per a compound is required in order to reach a power level of 90%. *E,E-α*-farnesene and frontalin elicited a somewhat elevated rate chemosensory investigation in post-puberty elephants however, sample size was particularly low for post-puberty males and females.

The similarities in response level between vanilla and the PPF urine in this study could be explained in two ways. First, a ten-fold higher concentration of vanilla extract was used in this study because no responses were elicited by our standard concentration in a previous study at this field site (Vyas 2006). Second, the pre-puberty urine (PPF) may not have been as interesting to elephants as luteal urine from a cycling female. However, a meaningful chemical signal would evoke a response level from elephants that is greater than that of both PPF or luteal urine and vanilla solution. Since *endo*-

brevicommin, *exo*-brevicommin, *E,E*- $\alpha$ -farnesene and frontalin did not elicit behavioral responses greater than vanilla or PPF urine, the results suggest that when tested singly, none of these compounds serves as an African elephant chemical signal.

The matrix (media) in which a chemical signal is presented may enhance or inhibit its chemoresponse activity. Chemosensory responses, especially flehmen events, in male Asian elephants increased when Z7-12:Ac was presented in anestrus urine in comparison to acetate buffer (pH 6.0) and a water media (Rasmussen et al. 1997). Proteins also play an important role among vertebrate and invertebrate pheromone systems. Odorant-binding proteins (OBP) and major urinary proteins (MUP) are used by both insects and mammals to extend the lifespan of otherwise very volatile compounds (Wyatt 2003). In Asian elephants, the volatility of Z7-12:Ac is lowered by binding to a urinary protein (Rasmussen et al. 2003). The bioactivity of the four putative pheromones bioassayed in this study may be enhanced by presenting them in a media other than water, especially anestrus urine.

Species and sex specificity of chemical signals can be acquired by configurational restrictions. For example, male Asian elephants respond only to the Z isomeric form of 7-dodecenyl acetate (Rasmussen et al. 1997). The enantiomeric ratio of frontalin in the TGS of male Asian elephants changes with age. Pubescent Asian males release a greater proportion of (+) than (-) frontalin, but older elephants release a racemic (1:1) mixture (Greenwood et al. 2005). The racemic mixture is repugnant to males as well as pregnant and lactating females but attracts estrus female Asian elephants (Rasmussen and Greenwood 2003). Both the *endo*- and *exo*- isomer of brevicomin were

bioassayed in this study but no differences in chemosensory behavioral responses between the two were found.

One of the challenges in identifying chemical signals is the diversity of pheromone compounds. Asian elephants demonstrated that a single compound can serve as a pheromone; often however, a blend of compounds is required. Silverstein (1977) found that the male pheromone in bark beetle *Ips paraconfusus* consists of three compounds, none of which is active by itself. In the cabbage looper (*Trichoplusia ni*), a precise blend of compounds that includes Z7-12:Ac is needed for biological activity (Bjostad et al. 1984). It is possible that African elephant chemical signals require a specific substrate matrix, are experiencing configuration restrictions, and/or a precise blend of compounds is needed in order to obtain full bioactivity.

The low bioactivity level of frontalin was one of the more intriguing results of the present study. Of the four putative pheromones bioassayed, frontalin is the only compound identified in the urine of male Asian and female African elephants (Rasmussen 1998; Goodwin et al. 2006), suggesting common biosynthetic pathways. Similarities in compounds are also found in the musth urine in male Asian and African elephants (Rasmussen and Wittemyer 2002). Chemical signals are subjected to ecological and sexual pressures (Greenfield 2002; Wyatt 2003). *Mammuthus*, *Elephas*, and *Loxodonta* were present in Africa during the Miocene period about 7 million years ago (Maglio 1973) and the formation of distinct chemical signals may have allowed species recognition and the prevention of interbreeding among the three genera (Kelly 1996; Tillman 1999). The low behavioral response of African elephant towards frontalin may be indicative of the differentiation of chemical signals in sympatric species,

especially if low response levels are maintained over multiple exposures. In many instances, both closely related and divergent species demonstrate convergent evolution in pheromone biosynthesis (Rasmussen 1998; Kelly 1996; Goodwin et al. 2006; Symonds and Elgar 2007). Although the biosynthetic pathway of frontalin has been conserved between the two elephant species, its function as chemical signal may not have been.

Chemical signals emanate from secretions and excretions and provide a comprehensive and honest mode of communication in many animals (Thornhill and Alcock 1983; Wyatt 2003), including elephants (Rasmussen and Krishnamurthy 2000; Schulte et al. 2007). Two decades of research has allowed us to gain an understanding of the importance of chemical signals in conveying reproductive condition in African and Asian elephant societies (Rasmussen et al. 1982; Rasmussen 1999; Rasmussen 2006). Asian elephants display convergent evolution with insects in estrous and musth pheromones, and known insect pheromones have been isolated in preovulatory urine of African elephants.

Although this study was unable to behaviorally confirm an African elephant chemical signal, it provided insight into the next phase of biological assays. The relatively small sample size per compound, especially post-puberty males and females, limits the ability to draw definitive conclusions. Thus, more bioassays, particularly for *E,E*- $\alpha$ -farnesene and frontalin are recommended. I also suggest that these compounds be presented in anestrus urine to see if the matrix has an important influence. The discovery of the estrous pheromone in Asian elephants required 15 years of exploration (Rasmussen et al. 1982; Rasmussen et al. 1996). While the knowledge gained from the work with Asian elephants and new technologies in chemical analyses should facilitate

the discovery of an African estrous pheromone, only through repeated bioassays and field studies will the existence of such a chemical signal be confirmed.

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Table 2.1. Ethogram of behaviors performed by wild African male and female elephants to bioassay sample. Specific behaviors were categorized into Approach, SCPF response, Raised Sniff, Accessory Trunk and Other (Bagley et al. 2006; Meyer 2006; Vyas 2006).

<b>Behavior categories and defined behaviors</b>	<b>Definition</b>
<b>Approach</b>	
Proximity	Elephant within one body length of sample
Near	Elephant within one trunk length of sample
<b>Chemosensory Behaviors</b>	
<b>SCPF Response</b>	
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
<b>Raised Sniffs</b>	
Horizontal sniff	Either the distal end or the entire trunk is elevated above and parallel to the ground and pointed away from the elephant
Periscope sniff	Trunk is raised to air above head level and held for at least 2 seconds
<b>Accessory Trunk</b>	
Blow	Performed after inspecting a sample. Air is expelled quickly from nasal openings of trunk; usually audible and mucus expelled usually visible
Dig	Elephant used trunk tip or foot to displace ground at sample area
Pinch	The two fingers of trunk pick up dirt around the sample
Rub	After placing entire trunk tip on a stimulus, the trunk is moved in a circular motion
Trunk Flick	Performed after inspecting a sample. Bottom ¼ of trunk moves up and down rapidly
Wriggle	Performed after inspecting a sample. Trunk twists and then untwists once at a moderate pace (slower than trunk flick)
<b>Other</b>	
Dust	Elephant throws dirt from sample area on body using trunk
Ear Wave	Ears extend out and rapidly brought back to the body
Motionless	Elephant exhibits no behavior for at least 5 s
Other	Behaviors exhibited that are not defined in ethogram
Vocalize	Elephant vocalizes after investigating sample

Table 2.2 The distribution of the number of times each sample was placed for biological assay, the number of times at least one African elephants was within one body length (proximity) of the sample and the total number of elephants that was within proximity of the stimulus, Ndarakwai Ranch, Tanzania (July 2006-June 2007). PPF = pre-puberty female.

<b>Sample</b>	<b>Placed</b>	<b>Visited</b>	<b>Elephants</b>
Vanilla	23	12	21
<i>Endo-brevicomin</i>	4	3	14
<i>Exo-brevicomin</i>	6	4	16
<i>E,E-<math>\alpha</math>-farnesene</i>	6	4	17
Frontalin	7	7	30
PPF urine	8	5	17

Table 2.3 Comparison of the time (s) spent by elephants within proximity (one body length) of the bioassayed samples at Ndarakwai Ranch, Tanzania (July 2006-June 2007). PPF = pre-puberty female.

	<b>U/H</b>	<b>df</b>	<b>P-value (<math>\alpha = 0.05</math>)</b>
<b>All chemosensory responses</b>			
a) Vanilla vs PPF urine	U = 218	1	0.25
b) Vanilla vs Endo/Exo-Brevicomin vs PPF urine	H = 1.30	2	0.52
c) Vanilla vs <i>E,E</i> - $\alpha$ -farnesene vs PPF urine	H = 2.10	2	0.35
d) Vanilla vs Frontalin vs PPF urine	H = 1.42	2	0.49

Table 2.4 Statistical details of the proportion of elephants that performed chemosensory behavior to the bioassayed samples while within one trunk length (near) at Ndarakwai Ranch, Tanzania (July 2006-June 2007). PPF = pre-puberty female.

	$\chi^2$	df	P*-value ( $\alpha = 0.05$ )
<b>Any chemosensory responses</b>			
a) Vanilla vs PPF urine	3.62	1	0.057
b) Vanilla vs <i>Endo/Exo</i> -Brevicomin vs PPF urine	12.19	2	0.0022
Vanilla vs <i>Endo/Exo</i> -Brevicomin	2.80	1	0.094
PPF urine vs <i>Endo/Exo</i> -Brevicomin	12.18	1	0.0005
c) Vanilla vs <i>E,E-<math>\alpha</math>-farnesene</i> vs PPF urine	3.84	2	0.147
d) Vanilla vs Frontalin vs PPF urine	4.85	2	0.088

\*Sequential Bonferroni adjusted p-value for pair-wise test.



Table 2.5 Statistical details of the number of chemosensory behaviors per proximity (one body length) by elephants to the bioassayed samples at Ndarakwai Ranch, Tanzania (July 2006-June 2007). PPF = pre-puberty female.

Parameter	H/U	df	P*-value ( $\alpha = 0.05$ )
<b>I All chemosensory responses</b>			
a) Vanilla vs PPF urine	U = 240	1	0.06
b) Vanilla vs <i>Endo/exo</i> -brevicomin vs PPF urine	H = 12.70	2	0.002
Vanilla vs <i>Endo/exo</i> -brevicomin	U = 390.5	1	0.07
PPF urine vs <i>Endo/exo</i> -brevicomin	U = 393	1	0.0005
c) Vanilla vs <i>E,E-<math>\alpha</math>-farnesene</i> vs PPF urine	H = 4.63	2	0.10
d) Vanilla vs Frontalin vs PPF urine	H = 3.94	2	0.14
<b>II SCPF responses</b>			
a) Vanilla vs PPF I urine	U = 237	1	0.07
b) Vanilla vs <i>Endo/exo</i> -brevicomin vs PPF urine	H = 12.03	2	0.002
Vanilla vs <i>Endo/exo</i> -brevicomin	U = 241	1	0.08
PPF urine vs <i>Endo/exo</i> -brevicomin	U = 390	1	0.0006
c) Vanilla vs <i>E,E-<math>\alpha</math>-farnesene</i> vs PPF urine	H = 4.95	2	0.08
d) Vanilla vs Frontalin vs PPF urine	H = 2/96	2	0.23
<b>III Raised Sniffs</b>			
a) Vanilla vs PPF urine	U = 192.5	2	0.50
b) Vanilla vs <i>Endo/exo</i> -brevicomin vs PPF urine	H = 2.78	2	0.25
c) Vanilla vs <i>E,E-<math>\alpha</math>-farnesene</i> vs PPF urine	H = 0.57	2	0.79
d) Vanilla vs Frontalin vs PPF urine	H = 1.60	2	0.45
<b>IV Accessory responses</b>			
a) Vanilla vs PPF urine	U = 201	2	0.37
b) Vanilla vs <i>Endo/exo</i> -brevicomin vs PPF urine	H = 4.61	2	0.10
c) Vanilla vs <i>E,E-<math>\alpha</math>-farnesene</i> vs PPF urine	H = 1.10	2	0.56
d) Vanilla vs Frontalin vs PPF urine	H = 0.70	2	0.70

\* $\alpha$  for all non-parametric post hoc comparisons was modified using a sequential Bonferroni method.

Table 2.6 Statistical details of the rate (frequency/proximity) of chemosensory behaviors towards bioassayed samples of post-puberty male and female African elephants, Ndarakwai Ranch (July 2006-July 2007). PPF = pre-puberty female.

<b>Rate of chemosensory responses</b>	<b>H/U</b>	<b>df</b>	<b>P-value (<math>\alpha = 0.05</math>)</b>
a) Sample: Males*	H = 9.67	4	0.046
b) Sample: Females	H = 7.70	4	0.10
d) Vanilla: male-female	U = 39	1	0.79
e) <i>Endo/exo</i> -brevicomin: male-female	U = 77.5	1	0.57
f) <i>E,E-<math>\alpha</math>-farnesene</i> : male-female	U = 20.5	1	0.24
g) Frontalin: male-female	U = 57	1	0.77
h) PPF urine: male-female	U = 22	1	0.34

\**a posteriori* Mann-Whitney U test showed that post-puberty males had a significantly greater rate of chemosensory response towards urine than *endo/exo*-brevicomin,  $p = 0.02$ .

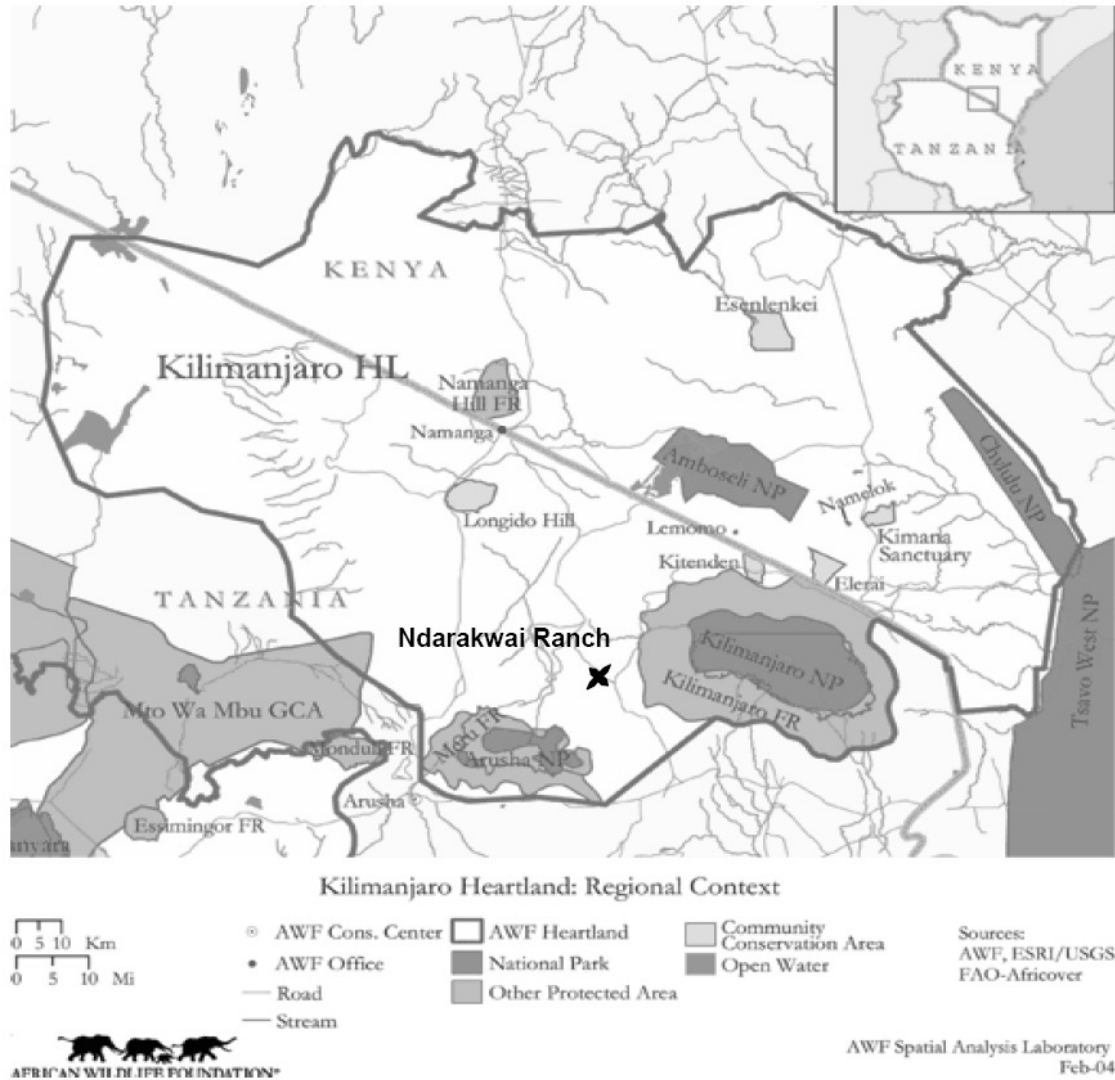


Figure 2.1. Map of the north-central border between Kenya and Tanzania. Ndarakwai Ranch is marked with an “X” (AWF Spatial Analysis Laboratory 2004).

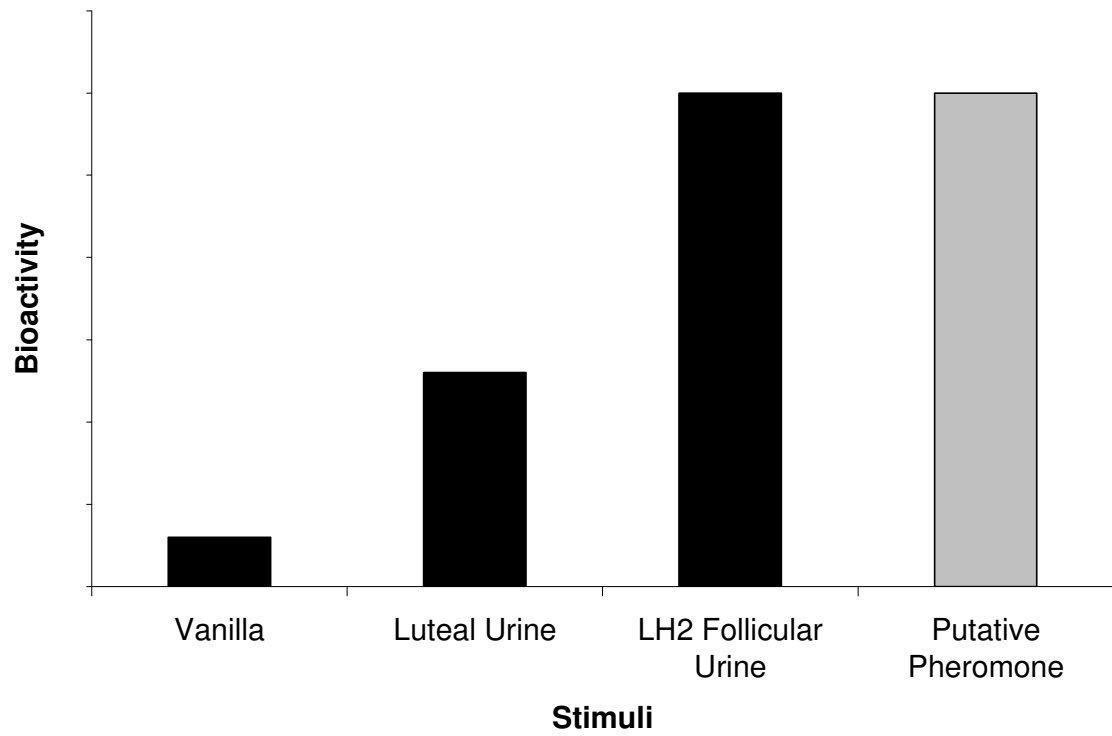


Figure 2.2. Response model of an African elephant female-to-male chemical signal in comparison to response levels of vanilla, luteal (or pre-puberty) urine and LH2 follicular urine. Adapted from Bagley et al. (2006).



Figure 2.3. Overhead view of the waterhole showing six bioassay sites, Ndarakwai Ranch, Tanzania. The observation platform is marked by the arrow and the filled circles indicate the six bioassay sites.

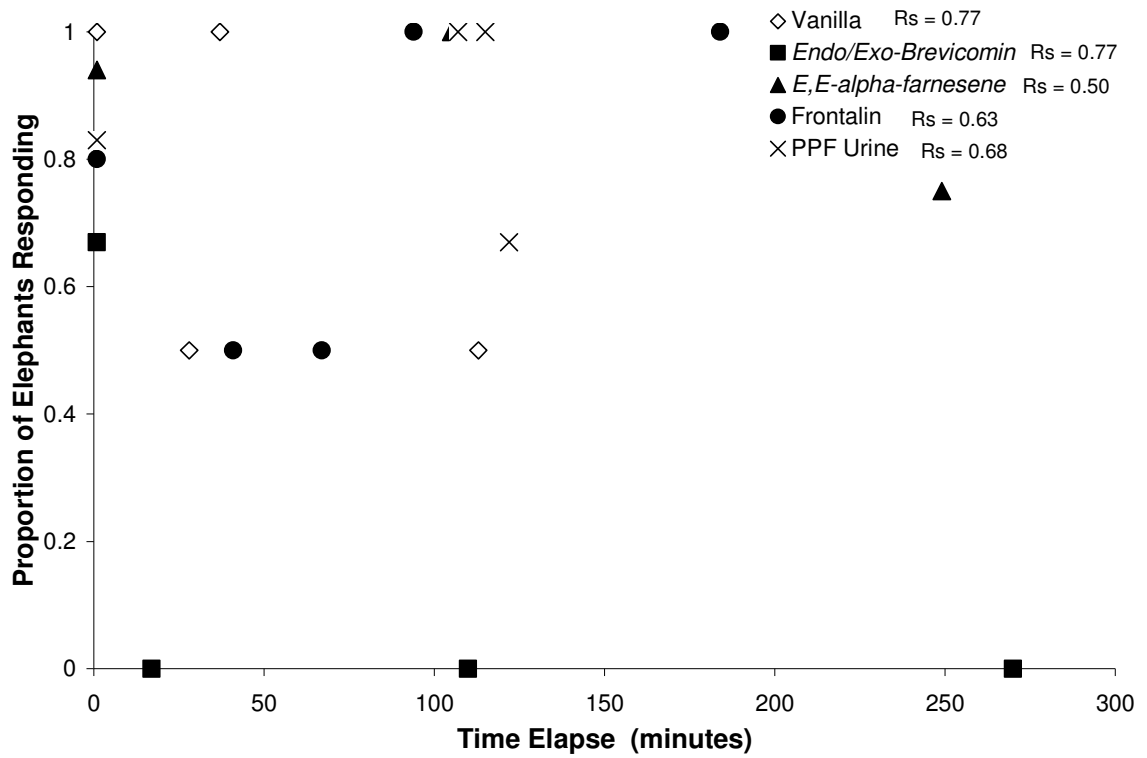


Figure 2.4 The proportion of elephants that performed any chemosensory behaviors while within one body length (proximity) of sample over time once a sample was placed for a bioassay. All Spearman rank correlations were not significant,  $p > 0.05$ .

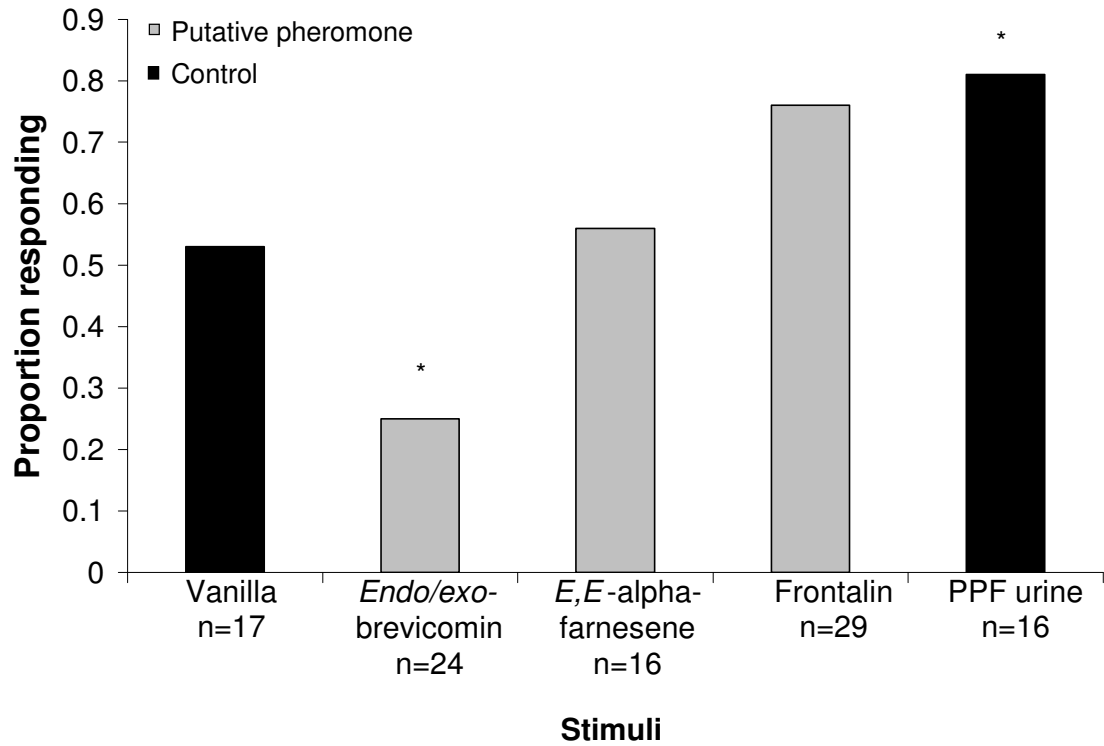


Figure 2.5. The proportion of elephants that performed any chemosensory behavior while within one body length (proximity) of bioassayed samples at Ndarakwai Ranch, Tanzania (July 2006-June 2007). Each putative pheromone was compared to the vanilla and luteal urine control. \* $p < 0.05$ . PPF = pre-puberty female.

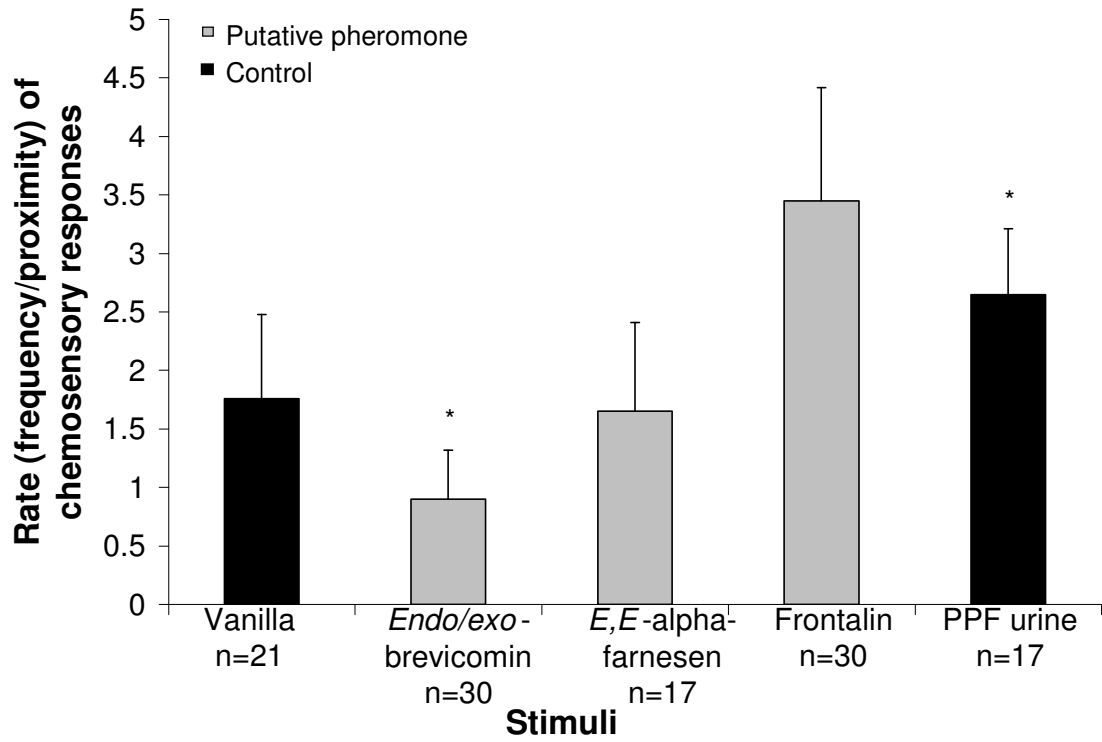


Fig 2.6. The number of chemosensory responses per proximity (one body length) by elephants towards the placed bioassayed samples, Ndarakwai Ranch, Tanzania (July 2006-June 2007). Each putative pheromone was compared to the vanilla and luteal urine control. \* $p < 0.05$ . PPF = pre-puberty female.



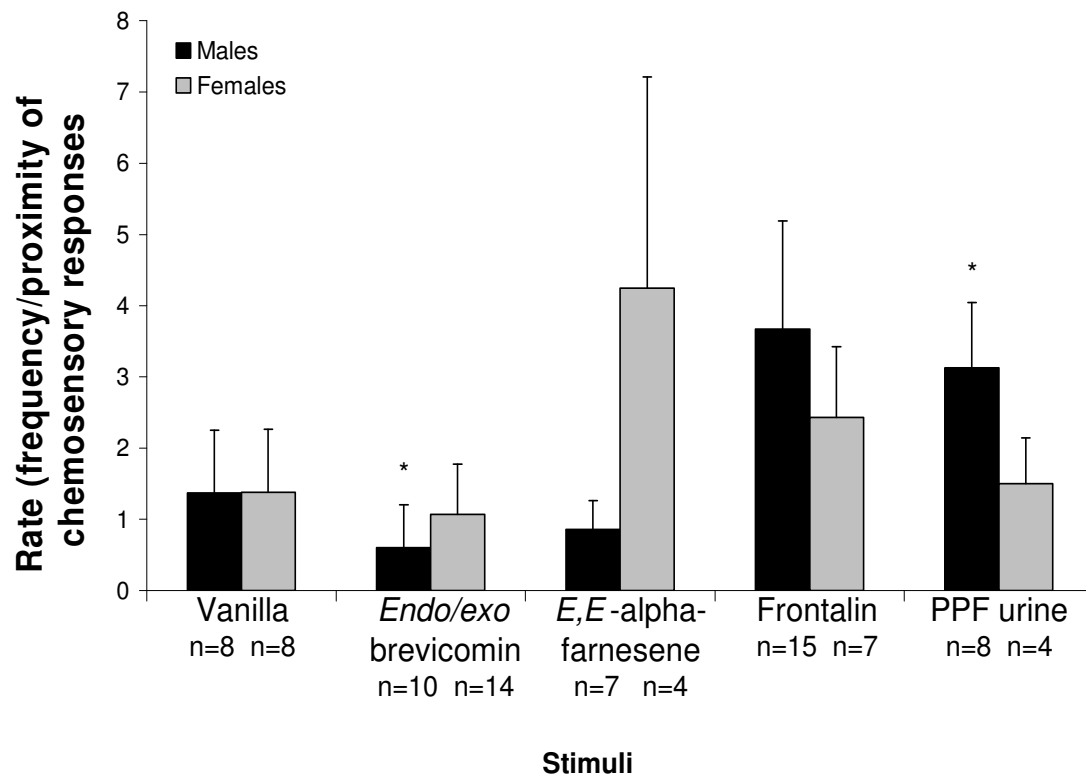


Figure 2.7. The number of chemosensory responses per proximity (one body length) by elephants towards placed bioassayed samples of post-puberty male and female African elephants at Ndarakwai Ranch, Tanzania (July 2006-June 2007). \* $p < 0.05$ . PPF = pre-puberty female.

APPENDIX A  
CROP-RAIDING INCIDENTS BY AFRICAN ELEPHANTS (*LOXODONTA  
AFRICANA*) IN TANZANIA

**OBJECTIVES**

In recent years, human-elephant conflicts have become a major conservation issue (Kangwana 1995; Parker & Osborn 2001). Conflicts between humans and elephants arise when elephants raid crops, alter habitat, damage buildings and property, cause death to humans and livestock, and compete for land and resources (Damiba & Ables 1994; Kiiru 1995; Hoare 1999; Eckhardt et al. 2000; Sitati et al. 2003; Augustine & McNaughton 2004). Of these various types of human-elephant conflicts, the current study focused on crop-raiding activities.

Crop-raiding occurs when elephants expand their range into human settlements in order to feed on crops (Hoare 1999). With an exploding human population, elephant populations are becoming increasingly restricted to areas such as national parks and preserves, and private game ranches (Kangwana 1995; Hoare 1999; Hoare & Du Toit 1999; Osborn 2002; Osborn 2004). Elephants naturally expand their range in search of other food sources when food quality declines (Osborn 2004) and the proximity of farms provides additional sources of nutrition for elephants.

Grasses are at their highest nutritional quality during the rainy season but decline with the onset of the dry season. Agricultural crops reach their nutritional peak as the nutritional value of grasses declines and as a result, elephants began to raid mature crops (Osborn 2004). Thus, crop-raiding activities increase during the transition between grazing and browsing when mature crops are at their peak nutritional value (Osborn

2004). Although elephants are not the only crop raiders, their actions are the most severe per incident because they are capable of destroying an entire farm's production in one raid (Naughton-Treves 1997). The objective of this study was to assess crop-raiding incidence at farms near Ndarakwai Ranch. I examined what type of crop was raided more frequently as well as quantified how much of the crop was destroyed.

## **METHODS**

### **Study Area**

A total of 46 crop fields were observed between June 2006-June 2007 in the pastoral communities of Miti Mirefu and Tanzania Livestock Research Organization (TALIRO) in Si Hai district, West Kilimanjaro, Tanzania. Miti Mirefu was classified as a Kitongogi (subvillage) until April of 2007; at which time Miti Mirefu received the official title of village and received a village number. Current census reports 250 plus residential homes in Miti Mirefu (pers. comm. Ndarakwai Ranch Manger Koisiana 2007). During the German and British colonial rule Miti Mirefu was originally the Two Rivers Farm, which was a livestock, dairy and agriculture production. After the Arusha Declaration in 1964, the owner of the Two River Farms abandoned the area and the Tanzanian government leased the land to a group of farmers from Lawate. Due to Rinderpest disease and theft of their cattle by the Maasai, most of the farmers returned to Lawate (pers. comm. Koisiana 2007). Some of the Lawate farmers remained in the area and established Miti Mirefu. The Engare Nairobi (South) River separates Miti Mirefu and TALIRO. TALIRO was established by the government as a livestock and veterinary care research facility. Both villages are adjacent to the south and southeast boundaries of Ndarakwai Ranch and are separated from the ranch by the Engari Nairobi (North) River.

Only crops fields located within 3 km of the Ndarakwai's permanent waterhole were used.

### **Crop Field Determinate**

Fields differed in crop type and/or time of planting. The perimeter of each field was demarcated through GPS coordinates. A quadrat system was used to assess crop damage for each field. The quadrats ran along the perimeter of the field and were 1m x 1m in size (Figure A.1a). If the perimeter quadrats did not account for 10% of the field's area, a mid-field transect running parallel with the longest side was established with the 1m x 1m quadrats on each side of the transect (Figure A.1.b).

Once the crop field was planted, the field was visited once every two weeks. The date of planting was noted upon initial visitation as well as the type of crop and the perimeter of the field. Once the crop reached seedling stage, an estimated crop density was obtained. The number of stalks per quadrat was counted in 10 quadrats and then averaged. The following was observed: tomatoes-10 stalks/quadrat; beans-15 stalks/quadrat, green pepper-6 stalks/quadrat, and onion-80 stalks/quadrat. For corn, the number of stalk per quadrat varied so much that no average was obtained; every stalk in all perimeter quadrant was counted.

### **Elephant Damage Assessment**

Upon each visitation, my assistant and I walked the quadrats along the perimeter of the fields and interior transect, if applicable. My assistant was a local farmer who lived in Miti Mirefu. Together, all quadrats were assessed for damage. A stalk was classified as damaged when a stalk had been broken, trampled, or uprooted and was not able to bear or sustain fruit. When damage was spotted, the quadrat location (number of

steps it took to reach the location, 1.5 steps/meter), the GPS coordinates, the number of stalks damaged and the source of damage were recorded. Source of damaged was discerned by examining feeding patterns and/or prints found in the soil. Damage does not take into account yield lost.

When elephants raid, they do enter the crop field. However, there were some crop fields in which the owner of the field was not comfortable with me walking through the field. Therefore, assessment of crop damage was restricted to perimeter and interior transects (when applicable).

## **RESULTS**

Three distinct growing seasons were present: Long 2006, Short 2006 and Long 2007 (Table A.1). Collectively, tomatoes were the predominant crop among all crop fields (Figure A.2), especially during the Long 2007 planting season (Table A.2). Damage to crops occurred during the months with no rain (Table A.1). Only 10 of the 46 (21.7%) of the fields were raided and the number of quadrats that contained damaged stalk ranged between 0-38% of the area surveyed (Table A.3). Tomato, corn and bean received some damage, while pea, green pepper and onion received no damage (Table A.2).

## **DISSCUSION**

The amount of crop loss due to elephant raids was minimal and followed “typical” crop raiding trends with the majority of the crop-raiding incidents occurred during dry months. The dry season quantity and nutritional quality of grasses decline which is when grasses are poor in quality and crops are in nutritional (Osborn 2004).

Elephants tend to be a generalist (Naughton-Treves 1997; Osborn 2004). In this study, elephants fed mainly on tomato; however, tomato also was the predominant crop planted.

One of the limitations of this study was the reliance of perimeter transects as the main way to assess the quantity of elephant damage. As previously mentioned, elephants are not limited to feeding on the exterior (first meter) of a field. Although percent damaged was assessed relative to the area surveyed, it is likely that this calculation underestimates the amount of a field that was damaged by elephants. This study did not take into consideration actual yield loss due to elephant activities. The number of stalks damaged should reflect the loss in yield; however, potential yield was never measured.

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Table A.1. Summary of the number of fields planted, harvested and damaged by month and the presences of rain during the Long 2006, Short 2006 and Long 2007 growing season, Miti Mirefu and Tanzania Livestock Research Organization (June 2006-June 2007).

<b>Season</b>	<b>Month</b>	<b># Planted</b>	<b># Harvested</b>	<b># Damaged</b>	<b>Rain</b>
Long 2006	March 2006	7	-	-	Yes
	April 2006	0	-	-	Yes
	May 2006	0	-	-	Yes
	June 2006	1	2	2	No
	July 2006	0	6	3	No
Short 2006	August 2006	7	0	0	No
	September 2006	2	0	2	No
	October 2006	14	0	0	Yes
	November 2006	0	0	0	Yes
	December 2006	0	12	0	Yes
	January 2007	0	8	0	Yes
Long 2007	February 2007	6	0	0	No
	March 2007	7	4	0	No
	April 2007	1	0	0	No
	May 2007	1	5	2	No
	June 2007	0	5	3	No
	July 2007	0	4	0	No



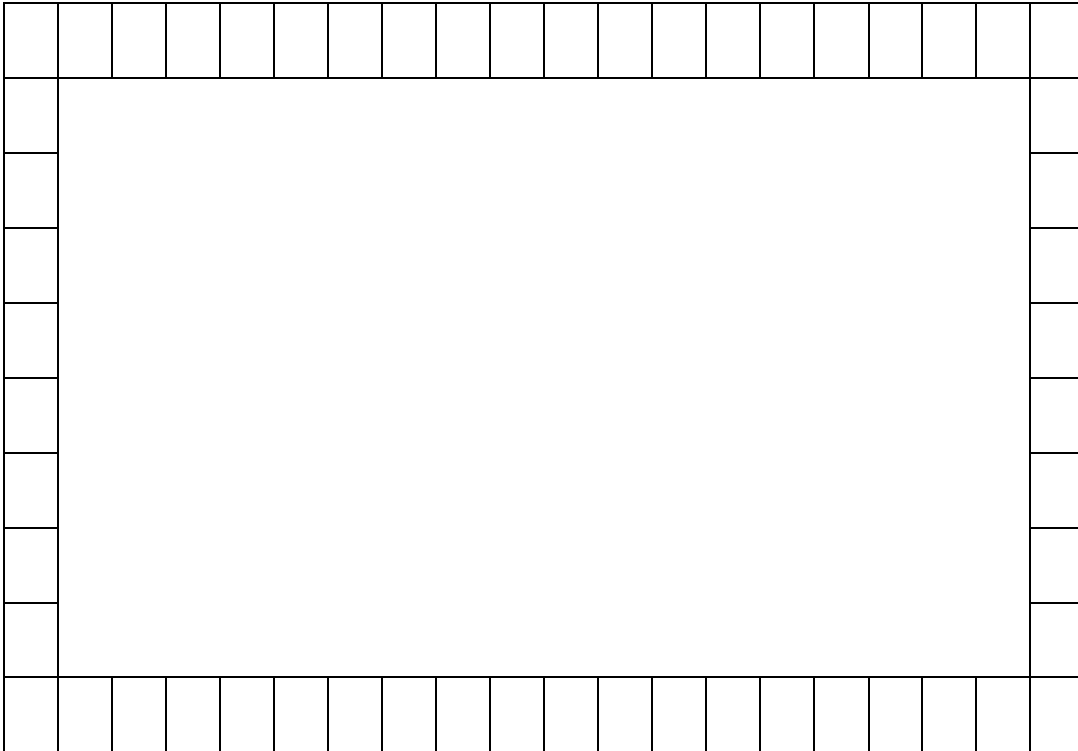
Table A.2. Summary of the type of fields, number of fields and number of damaged fields surveyed, Miti Mirefu and Tanzania Livestock Research Organization (June 2006-June 2007).

<b>Season</b>	<b>Crop</b>	<b>Number of fields</b>	<b>Number of Fields with Damage</b>
Long 2006	Bean	2	0
	Corn	2	2
	Green pepper	1	0
	Tomato	3	2
Short 2006	Bean	7	0
	Onion	1	0
	Pea	6	0
	Tomato	9	2
Long 2007	Bean	3	1
	Green pepper	1	0
	Tomato	11	3

Table A.3. Percent of surveyed quadrats that received damaged due to elephant crop-raiding incidents in damaged fields, Miti Mirefu and Tanzania Livestock Research Organization (June 2006-June 2007).

<b>Season</b>	<b>Damaged field</b>	<b>Crop Type</b>	<b>% Damage</b>
Long 2006	AA1	Tomato	12.7
	AA2	Tomato	5.4
	AD	Corn	3.0
	AF	Corn	5.9
Short 2006	BA1	Tomato	9.4
	BE1	Tomato	17.0
Long 2007	CD1	Tomato	8.8
	CE1	Tomato	3.9
	CH1	Bean	38.1
	CK1	Tomato	21.0

a.



b.

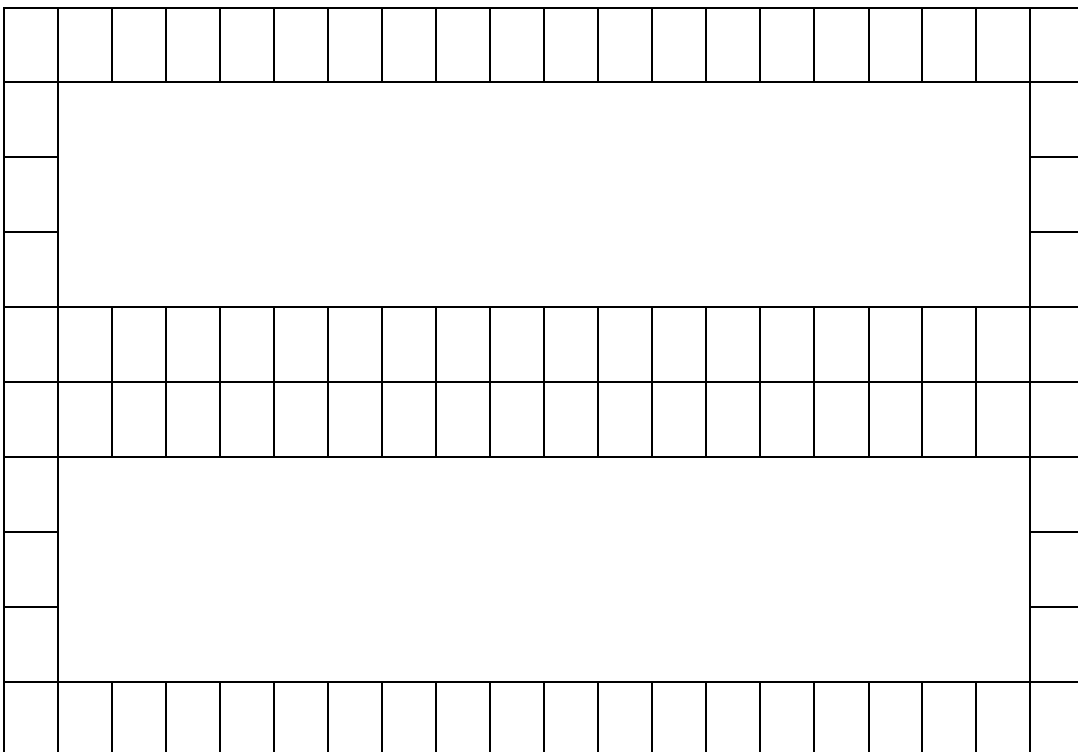


Figure A.1. Crop field 1m x 1m quadrat (a.) and mid-field transect (b.) system, Miti Mirefu and Tanzania Livestock Research Organization (June 2006-June 2007).

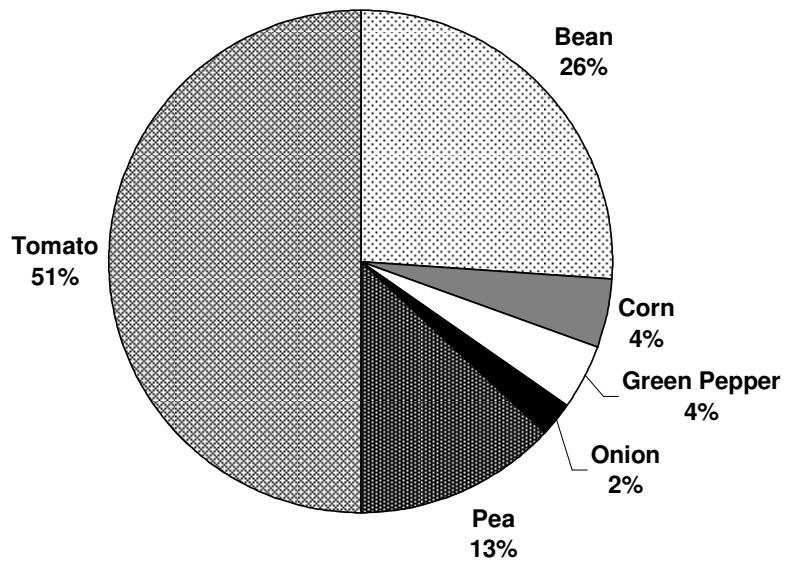


Figure A.2. Percent crop type (n = 46), Miti Mirefu and Tanzania Livestock Research Organization (June 2006-June 2007).

## APPENDIX B

### BIOLOGICAL ASSAYS OF AFRICAN ELEPHANT DUNG

#### **OBJECTIVES**

Chemical signals are present in the secretions and urine of elephants (Rasmussen and Schulte 1999; Langbauer 2000; Rasmussen and Krishnamurthy 2000). However, the lifespan of potential chemical signals in dung from the time of defecation is unknown. The object of this study was monitor dung pile in order to determine length of bioactivity.

#### **METHODS**

##### **Dung pile monitoring**

Dung piles were mapped and monitored at the permanent waterhole on Ndarakwai Ranch. Upon arrival, I walked around the waterhole and mapped the location of dung within 10 m from waterhole edge. Each dung pile was given an ID number and the age category, defecation composition, and diameter of the boluses were recorded (Table B.1). If the defecation was witnessed the age and sex of the elephant that defecated was noted. The waterhole was surveyed after elephant(s) vacated the area to record new deposits. Each dung pile was monitored until it was no longer identifiable.

##### **Bioassay**

Observations were made from a six-meter high observation platform adjacent to the waterhole. The sex, age class and identity of the elephants investigating the dung as well as the ID of the dung pile were noted. Trunk-related chemosensory behaviors directed toward dung were recorded using an established ethogram (Table 2.2). The observations on a particular individual were terminated once the elephant was greater

than one body length from the dung pile or was out of the observer's sight for three minutes.

## **RESULTS**

A total of 226 dung piles were monitored from June 2006-June 2007. Only three dung pile bioassays (investigations) were witnessed. Two of the dung piles were categorized as fresh and one as aged. All investigating elephants were adult males. The sex, age or identity of defecating elephant (source of the dung) was not known for any of the three dung piles. All three dung piles elicited at least one chemosensory behavior (Table B.2).

## **DISCUSSION**

Because of the very small sample size, further investigation is needed in order to determine the lifespan of chemical signals in African elephant dung.

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Table B. 1. Categorical definitions of the age and defecation composition of African elephant dung.

<b>Age</b>	
Fresh	Bolus intact, moist inside, may be dry on the outside but moves when pressure is applied. Typical less than 48 hours old.
Aged	Dry and hard on the outside, does not change shape when pressure is applied but is semi moist inside
Decay	Dry, not moist inside, some of the boli has disintegrated
Grass	Bolus is completely disintegrated, pile forms a flat mass. No bolus intact
End	Pile of flat mass is no longer identifiable
<b>Defecation composition</b>	
Pile	More than one bolus within two bolus lengths
Line	Boluses are distributed unevenly in a line and are more than two bolus lengths apart
Single	Single bolus present

\* Age category was modified from Barnes & Jensen 1987

Table B.2. Summary of chemosensory response to dung pile at Ndarakwai Ranch, Tanzania (July 2006-June 2007).

<b>Elephant performing chemosensory behavior</b>	<b>Dung pile age category</b>	<b>Chemosensory behavior (quantity)</b>
Adult Male	Aged	Sniff
Adult Male	Fresh	Sniff, Check (2), Place, Flehmen
Adult Male	Fresh	Sniff



## APPENDIX C

### DUNG SURVEYS AS MEANS TO ESTIMATE PRESENCE OF ELEPHANT

#### **OBJECTIVES**

Dung surveys are one method commonly used when conducting an elephant census. Dung counts not only provide information about the relative distribution of the elephant population, but also have the ability to be translated into an estimate of population size (Barnes and Barnes 1992; Barnes 1993; 1996). The number of elephants in a study population (E) can be calculated from the dung-pile density (Y), the rate of dung-pile decay (r) and the rate of defecation (D);  $E = (Yr/D)$  (Barnes and Jensen, 1987; Barnes et al. 2001). The objective of this study was to determine seasonal abundance by estimating the presence of elephants via dung counts.

#### **METHODS**

##### **Dung-Pile Density**

Pervious work by Napora (2007) established 40 permanent vegetation belt transects within two vegetation sampling plots on Ndarakwai Ranch property. For the current study, a fecal line transects was placed within each vegetation belt transect (Plumpter 2000). Each fecal line transect was 50 meters in length and ran from East to West (Figure C.1) and was within 1 km from a permanent waterhole. Transects were walked once a month from July 2006-June 2007 and elephant boluses (dung-pile) on and within 1 m north and 1 m south of the transect were assessed. Each bolus was marked with paint in order to distinguished new from old and the age category, defecation composition and diameter were recoded (Table B.1). Monthly density of dung-pile for

each transect was calculated by dividing the number of boluses in each transect by the total area of the transect ( $D = n/\text{area}$ ).

### **Rate of Dung-Pile Decay**

The rate of dung-pile decay was monitored at the permanent waterhole on Ndarakwai Ranch. The data gathered in Appendix B was used to determine the rate of decay. Rate of decay was calculated from the average number of days it took a dung pile to pass from the fresh category to the end category (Table B.1).

### **Rate of Defecation**

The rate of defecation was determined by recording the defecation events of a habituated eight-year-old, female African elephant that lives on the Ndarakwai property and by reviewing previous literature (Table C.1).

## **RESULTS**

Upon the initial dung count, the number of dung piles per transect did not differ between vegetation plot types ( $t = 1.67$ ,  $df = 38$ ,  $p = 0.12$ ), with an average of  $3.6 \pm 0.49$  piles per a transect or 36 dung pile per 1000  $\text{m}^2$ . On average,  $2.35 \pm 0.6$  new dung piles were present in every month per transect from July 2006-Nov 2006 & June 2007. Over 60% of the transects had no new piles of dung from Dec 2006-April 2006. The average decay rate of dung pile around the waterhole was  $23.00 \pm 3.4$  days. No new dung piles were recorded at the waterhole from November 2006-March 2007 and during this time  $18 \pm 1.7$  ( $\pm$  SEM) of the transects did not contain new dung piles. The defecation rate of the habituated African elephant was one defecation every  $97.7 \pm 9.62$  minutes or roughly 14.74 defecations per day. The population of elephants on Ndarakwai Ranch was estimated at 56 elephants / 1000  $\text{m}^2$ .

## **DISCUSSION**

The estimation of 56 elephants / 1000 m<sup>2</sup> is quite high in comparison to other surveys of elephant populations (Barnes 2001). More than likely, the proximity of the transects to a permanent waterhole and a river accounts for the high elephant density. All transects are within 200 m – 1000 m of a water source and elephants tend to defecate while at or near a water sources (Barnes 1996); thereby, over calculating elephant density. The rate of decay also was much faster ( $23.00 \pm 3.4$  days vs  $88.65 \pm 24.89$  days) in this study in comparison to work conducted at Banyang-Mbo Wildlife Sanctuary in south-western Cameroon (Nchanji and Plumptre 2001). Again, the proximity of the piles to a water source may account for a rapid rate of decay. Elephants and other mammals trample dung piles (per. observation) when accessing water from the waterhole. The rate of defecation is slightly lower than that found in the literature (Table C.1.). The caretakers of the habituated eight-year-old, female may not have witnessed every defecation event, especially in the evening.

Although this technique was inaccurate in estimating the density of elephants, dung counts assisted in determining the presence and absences of elephants. Elephants were not seen at the waterhole from the end of November 2006 in till March of 2007, concurrently, no new dung piles were recorded at the waterhole nor in roughly half of the fecal line transects.

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Table C. 1. Literature review of rate of defecation

<b>Rate of Defecation (times / day)</b>	<b>Citation</b>
16.0	Wing & Bass 1970
19.8	Tchamba 1991
15.9	Nchanji et al. 2001

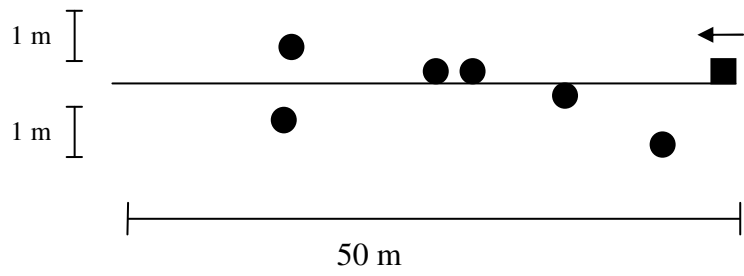


Figure C.1. 50 meter fecal transect. Filled circles denote fecal piles and filled square indicates starting point. Every bolus within 1 m of transect was assessed.