SOCIAL BEHAVIOUR IN AN AFRICAN ELEPHANT HERD IN RELATION TO REPRODUCTIVE CYCLE PHASE

By

YVONNE DE VRIES
MSc Animal Biology and Welfare

September 2008
Abstract

This study investigated the relationship between behaviour and oestrus phase in a herd that consists of one pre-reproductive sub-adult female (Duna), one adult cycling female (Aja), and one sub-adult male (Tooth) housed in Ouwehand zoo, Rhenen the Netherlands over a period of 19 weeks. Behaviours were scored in six categories; distance, reproductive behaviour, active aggression, passive aggression, submissive behaviour and affiliative behaviour. Additionally, blood was collected and progestogen levels were determined from the two females weekly and during the oestrus phase biweekly. Frequency of the behaviours was compared between the three phases of the oestrous cycle; the anoestrus, oestrus and luteal phase. The male performed more smelling of urine and flehmen in the anovulatory and ovulatory phase than in the luteal phase and performed more genital inspections in the anovulatory phase than in the luteal phase. The cycling female showed more submissive behaviour in the anovulatory and ovulatory phase than in the luteal phase. However, because during this study only one complete cycle was observed, observations over a longer period of time could give more detailed results.

Other conclusions that could be made from this study are that this group lacks strong social bonds, especially between Aja and the other two elephants, which might have effected the results. During this study, Tooth had little interest in Aja. Two possible reasons for this are that Tooth does not perform his natural role in the group and is often acting as the matriarch and that Aja is starting to show signs that she is at a post reproductive age. This study also indicates that cyclicity of Duna might be suppressed by aggressive behaviour from Tooth and Aja. This study concludes that the best indicators of (impending) oestrus were rate of flehmen by the male and rate of submissive behaviour by the cycling female towards the male.

Recommendations for the herd in Ouwehand zoo are to separate Tooth and Duna temporarily to observe if cycling will start and to search for two additional, preferably related, sub-adult females.
# Contents

1 Introduction ................................................................................................................................. 1

2 Social situation in the wild ........................................................................................................ 3
   2.1 Female ................................................................................................................................ 3
   2.2 Male ................................................................................................................................. 4

3 Communication ......................................................................................................................... 5
   3.1 Olfactory communication ............................................................................................... 5
   3.2 Acoustic communication ................................................................................................. 5
   3.3 Visual communication ..................................................................................................... 7
   3.4 Tactile communication .................................................................................................... 7

4 Behaviour .................................................................................................................................. 8
   4.1 In the wild ....................................................................................................................... 8
      4.1.1 Social behaviour ...................................................................................................... 8
      4.1.2 Reproductive behaviour ....................................................................................... 8
   4.2 In captivity ...................................................................................................................... 11
      4.2.1 Social behaviour ................................................................................................... 11
      4.2.2 Reproductive behaviour ...................................................................................... 12

5 Hormone cycle ........................................................................................................................ 14
   5.1 Female ............................................................................................................................ 14
      5.1.1 Cycle ....................................................................................................................... 14
      5.1.2 Endocrine monitoring ......................................................................................... 16
   5.2 Male ................................................................................................................................ 17
      5.2.1 Cycle ....................................................................................................................... 17
      5.2.2 Endocrine monitoring ......................................................................................... 18

6 Reproductive problems ............................................................................................................ 19
   6.1 Female ............................................................................................................................ 19
      6.1.1 Anatomy ................................................................................................................. 20
      6.1.2 Behaviour ............................................................................................................... 22
   6.2 Male ................................................................................................................................ 23
      6.2.1 Anatomy ............................................................................................................... 23
      6.2.2 Behaviour ............................................................................................................... 24
      6.2.3 Other problems ..................................................................................................... 24
   6.3 Conclusions ..................................................................................................................... 24

7 Welfare of elephants in captivity ............................................................................................... 25
   7.1 Causes of impaired welfare ............................................................................................. 26
      7.1.1 Housing .................................................................................................................... 26
      7.1.2 Social grouping ....................................................................................................... 27
      7.1.3 Training ................................................................................................................... 28
   7.2 Effects of impaired welfare ............................................................................................. 29
      7.2.1 Stress ....................................................................................................................... 29
      7.2.2 Stereotypies and other abnormal behaviour ......................................................... 29
      7.2.3 Diseases .................................................................................................................. 30
      7.2.4 Longevity and life expectancy ................................................................................ 30
   7.3 Conclusions ..................................................................................................................... 31

8 Materials & Method ............................................................................................................... 33
Social behaviour in an African elephant herd in relation to reproductive cycle phase
By: Yvonne de Vries

8.1 Study animals ................................................................................................................. 33
8.2 Facility, housing and feeding ......................................................................................... 33
8.3 Behavioural data collection and analysis ....................................................................... 34
8.4 Sample collection and determination of oestrous state .................................................. 34
8.5 Statistical analysis .......................................................................................................... 35

10 Results ................................................................................................................................ 36
10.1 Social aspects ............................................................................................................... 36
10.2 Male – Female behaviour across the ovarian cycle...................................................... 38
10.3 Female – Male behaviour across the ovarian cycle...................................................... 40

11 Discussion ........................................................................................................................... 41
11.1 Methods ........................................................................................................................ 41
11.2 Results .......................................................................................................................... 43

12 Conclusions ........................................................................................................................ 46

13 Recommendations for this African elephant herd ......................................................... 47
13.1 The female herd............................................................................................................ 47
13.2 The male ....................................................................................................................... 48
13.3 Conclusions .................................................................................................................. 49

References ............................................................................................................................... 50
Appendix 1 Pregnancy ........................................................................................................... 55
Appendix 2 Artificial reproductive techniques ................................................................... 58
Appendix 3 Five freedoms .................................................................................................... 60
Appendix 4 Profiles of the three elephants of Ouwehand zoo............................................ 61
Appendix 5 Schematic drawing of the elephant facilities................................................... 64
Appendix 6 Ethogram ............................................................................................................ 66
Appendix 7 Barriers in the observation area ....................................................................... 70
Appendix 8 The immulite ..................................................................................................... 72
Appendix 9 Oestrous cycle Aja ............................................................................................. 73
Appendix 10 Article about Ouwehand zoo from ‘Elefanten in zoo und circus’ ............... 75
List of figures

Figure 1 Hormone cycle of the African elephant.
Figure 2 Progestogen profiles of a cycling and non cycling elephant
Figure 3 Time spend in proximity.
Figure 4 Submissive behaviour.
Figure 5 Trunk tip to mouth.
Figure 6 Flehmen Tooth to Aja.
Figure 7 Trunk tip to urine Tooth to Aja.
Figure 8 Genital inspections Tooth to Aja.
Figure 9 Submissive behaviour Aja to Tooth.
Acknowledgements

First and foremost I want to thank Jan Bos, Veterinarian at Ouwehand zoo and Con Mul, Jord de Meijer, Renate Wahlen and Olaf van Leeuwen, elephant keepers at Ouwehand zoo for their help in completing my research. Another word of thanks goes to José Kok, biologist at Ouwehand zoo, for giving me the opportunity to do my research at this facility and Gerard Meijer, curator at Ouwehand zoo, for providing me with additional information. Finally, I want to thank Lenny van Erp, my dissertation tutor, for her guidance during this project.
1 Introduction

In 2007, the numbers of wild African elephants were estimated to be 637,600 (Hermes et al., 2007) and in a lot of African countries, African elephants are thriving. In captivity, however, African elephant populations are not self-sustaining because birth rates are low (Brown, 2000; Olsen & Wiese, 2000; Hutchins & Keele, 2006). In the 1970’s and 1980’s enough animals were available from culling programs to add to the captive population (Olsen & Wiese, 2000). Because captive breeding was very expensive and expected was that not enough space was available for all the males, captive breeding was not performed much (Hutchins & Keele, 2006). Because of this, many elephants in captivity are currently post reproductive, making successful reproduction even harder. A study performed in 2000 about the future of the North American African elephant population shows that two thirds of the population will be post-reproductive around 2010 (Olsen & Wiese, 2000) and other captive populations suffer the same fate. Estimations are that six female offspring have to be produced each year to have a sustainable North American African elephant population (Brown et al., 2004b). Some authors believe captive African elephant populations might become self-sustaining without further importation (Olsen & Wiese, 2000), but others believe importation is inevitable (Brown et al., 2004b; Hutchins & Keele, 2006).

For successful captive reproduction it is important to know the exact moment of oestrus and ovulation. Determining progestogen concentrations in blood is a good method, but is not always possible in every elephant. Because behaviour is very important in African elephant societies, determining changes in behaviour can possibly be a good hands off method of determining oestrous phase of an elephant.

This study investigates the relationship between oestrous phase and social behaviour in the African elephant herd at Ouwehand zoo, Rhenen, the Netherlands. The herd consists of one pre-reproductive sub-adult female, one adult cycling female, and one sub-adult male. The aim of this study is to determine if social behaviour in this herd changes during oestrus and if this can predict onset and end of oestrus. The two hypothesis are:

*Social behaviour of the male can predict anoestrus and oestrus in a cycling female housed in the same exhibit.*
Social behaviour of the female can predict anoestrus and oestrus when she is housed with a male in the same exhibit.

First, the current literature about African elephant behaviour, reproduction and welfare will be discussed. Following this, the materials and methods for this study will be described, the results will be summed up, the results and study will be discussed and conclusions will be derived from this. Recommendations for the elephant herd of Ouwehand zoo will be given in the last chapter.
2 Social situation in the wild

Elephants are highly intelligent and social animals. They have the largest social network of any mammal studied to date other than humans (Clubb & Mason, 2002). One example of the social sophistication of elephants is that elephants, together with chimpanzees and dolphins have been described to be concerned with dying or dead members of their species, in contrast to most mammals (Douglas-Hamilton et al., 2006). They respond to distress calls of other elephants, regardless of their relationship with each other (Douglas-Hamilton et al., 2006). There are observations of apparently unrelated females guarding a recumbent animal or helping a threatened animal (Douglas-Hamilton et al., 2006). If an elephant is wounded and cannot stand up, other elephants gather round in an attempt to raise it (Schulte, 2000).

2.1 Female

African elephants live in matriarchal family units that consist of 4-12 elephants, usually closely related like mothers and daughters, sisters, or half-sisters with their offspring, led by the oldest and largest female, the matriarch (Rasmussen & Schulte, 1998; McComb et al., 2001, Vidya & Sukumar, 2005). Wittemyer et al. (2005) found that the size of the group was positively related to the age of the matriarch, herds with matriarchs older than 35 were larger than herds led by younger matriarchs. The group is very stable over time and will only split up when it becomes too large, although small sub-groups may leave the herd for short periods of time (Schulte, 2000; Clubb & Mason, 2002). Related groups may still associate with each other and form “bond groups” or “kinship groups” (Charif et al., 2005; Vidya & Sukumar, 2005) while unrelated groups rarely approach within 2 km of each other (Burks et al., 2004). Even under extreme circumstances, when the natural social system is disrupted, unrelated females will only interact with each other for a very short period (Burks et al., 2004).

African elephants live in fission-fusion societies which means that different related family groups join together to form herds of up to 100 elephants in the dry season when resources are limited (Charif et al., 2005; Vidya & Sukumar, 2005; Archie et al., 2006). In a Kenyan study population, a single family unit encountered on average 25 other family groups during one year, a total of around 175 adult females (McComb et al., 2001). The vocal calls of elephants are individually distinctive (Soltis et al., 2005a,b) and some matriarchs can recognise the vocal calls of around 100 individuals which is very important in these large groups (Clubb & Mason, 2002). Families with older matriarchs are better at this task, which was proven by
McComb et al. (2001). During this study, vocal playback calls of familiar and unfamiliar elephants were given to different herds. Herds with older matriarchs reacted more strongly to females with who they had a low association. They were not likely to react to calls of familiar elephants. Families with younger matriarchs reacted only slightly less to calls of familiar animals compared to unfamiliar animals. The average age of the adults in the herd had no significant effect indicating that it is the matriarch who signals if defensive behaviour is needed. Only reacting in situations where it is necessary can save time and energy which is probably why herds with older matriarchs have higher reproductive success. Additionally, herds with older matriarchs have more knowledge available which can be taught to other individuals, enhancing long-term reproductive fitness (McComb et al., 2001; Vidya & Sukumar, 2005). Matriarchs are important during periods of environmental or social stress to lead the group to safety (Freeman et al., 2004; Vidya & Sukumar, 2005). One good example of the importance of the knowledge of matriarchs for the survival of the herd was found in the Tarangire elephant population. During a period of draught of several years, herds with older matriarchs tended to move away from the river, where food was depleted, to find food, whereas herds with younger matriarchs stayed with the river. Consequently, the herd with older matriarchs had a lower death rate and calf mortality (Douglas-Hamilton et al., 2006).

2.2 Male

While young females stay with the herd for life, male offspring leave the group at an age of 9-18. Between the age of four to six years they already start to move between different bond groups (Clubb & Mason, 2002). Some authors say that the males are expelled by the matriarch, others say that they leave on their own (Clubb & Mason, 2002). Young adult males may form loose associations with two to four other males of the same age (Rasmussen & Schulte, 1998; Charif et al., 2005; Ortolani et al, 2005), but in general, no strong relationships are formed between these males (Charif et al., 2005). Adult males usually live solitary and visit female groups for mating (Schulte, 2000; Ganswindt et al., 2005; Ortolani et al, 2005). Male elephants do not defend territories, but they do have regular home ranges (Rasmussen & Schulte, 1998).
3 Communication

In highly social animals such as the elephant, communication is very important for several reasons including maintaining group cohesion and expressing physical and reproductive state. Elephants can use olfactory, acoustic, visual and tactile communication and most of the time, a combination of those is used.

3.1 Olfactory communication

Olfactory communication is used in individual recognition and advertisement of physiological and reproductive state. Chemical signals are of long duration and are therefore effective in the advertisement of hormone condition (Langbauer, 2000). Chemicals are carried out in urine, faeces, temporal gland secretion and saliva and are detected by sniffing or flehmen behaviour (Langbauer, 2000; Vidya & Sukumar, 2005), which is the transportation of odours with the trunk to the vomeronasal organ (VNO) which is located of the roof of the mouth (Rasmussen & Schulte, 1998). All adults and some juvenile African elephants are capable of temporal gland secretion, unlike Asian elephants, where only males in musth are capable of this (Vidya & Sukumar, 2005). In both males and females, temporal gland secretion is also associated with social excitement, stress or fright, but the composition of this fluid differs from that during sexually active periods (Rasmussen & Schulte, 1998). Urination and defecation are also seen in socially exciting events such as calving, mating and group defence (Vidya & Sukumar, 2005).

Chemical signals have proven to be very important in sexual communication (Rasmussen & Schulte, 1998). The chemical composition of urine varies significantly during the oestrous cycle of females and between non-musth and musth state of males (Rasmussen & Schulte, 1998). In African elephants, the exact compound responsible for this difference has not yet been identified (Vidya & Sukumar, 2005).

3.2 Acoustic communication

Acoustic signals are used in conflict, threat display, nervousness, communicating reproductive state and in social contact and greeting between bond group members, and are used for short and long distance contact (Vidya & Sukumar, 2005). Because acoustic signals are short-lived, they give information about the immediate state of the elephant and are less suited for advertising of a constant state (Langbauer, 2000). Two exceptions are the musth
and oestrous rumble which will be described later in this paper. Long distance communication, which is usually used to keep in contact with bond groups, occurs through the use of infrasound (low-frequency sound) which can be transmitted seismically through the ground. Elephants can hear infrasonic calls over distances of 4 to 10 km under optimal conditions (Langbauer, 2000) but they might not be able to communicate over these distances. McComb et al. (2003) showed that individual recognition can only be achieved over distances of 2.5 km or less and is usually achieved over distances of 1-1.5 km. This might be because the frequency that is used for individual recognition is well above the infrasonic range and is lost or distorted at longer distances (McComb et al., 2003).

Male elephants give fewer different calls than females. This may be because females live in groups and need to communicate with different female groups for group coordination and infant care while males only need to communicate for breeding (Langbauer, 2000). The meaning of elephant calls have been studied with the use of playback experiments, during which different calls are given and the reaction of the elephants is recorded. However, these experiments can only work if the elephant who made the recorded call is out of sight, the loudspeaker is hidden in a location where the caller could be and the area is open enough for observations (Langbauer, 2000). Reactions are also different between calls of familiar and unfamiliar elephants (Langbauer, 2000). Because these conditions are hard to meet, these experiments are quite difficult.

Experiments in captive elephants indicate that females are twice as likely to rumble shortly after the rumble of another group member (Soltis et al., 2005a). Elephants are most likely to rumble in response to their most preferred partner (Soltis et al., 2005a). In a study by Soltis et al. (2005a), rumbles occurred in different situations such as when animals were out of contact, during reunions, when animals are in close proximity and when they are approached by more dominant group members. This suggest that the rumble has several functions such as reinforcement of social bonds, a short-distance contact call and a signal of submission (Soltis et al., 2005a). Another study by Soltis et al. (2005b) indicate that these rumbles may not be acoustically distinct, but because some contexts for rumbling do not exist in captivity as they do in the wild, future studies in wild animals may contradict this finding.

As mentioned, subordinate animals sometimes use rumbles when a dominant animal approaches them aggressively (Soltis et al., 2005a). This could be to show submission and reduce aggression in the dominant animal (Soltis et al., 2005a). Another possibility of the function of this behaviour is to recruit allies (Soltis et al., 2005a).
3.3 Visual communication

Visual communication is only effective over short distances and with no obstacles. Elephants can distinguish between different combinations of head, ear, eye, trunk, tail, body, feet and postures (Vidya & Sukumar, 2005) for example ear flapping during aggressive or defensive behaviour. Visual signs are usually used in combination with other communications in the advertisement of hormonal or emotional state, group cohesion and coordination and aggression (Langbauer, 2000).

3.4 Tactile communication

Tactile communication is important in elephant social contact and is used to exhibit reassurance, affection, affiliation, exploration, aggression and play (Vidya & Sukumar, 2005). The trunk is the most sensitive part of the elephants body. It is placed in another elephants mouth for reassurance and on the ears, mouth, eyes, tail and body during greeting (Langbauer, 2000; Leong et al., 2005; Vidya & Sukumar, 2005). During play behaviour, young calves climb onto each other, play mounting, trunk wrestle and push and shove which prepares them for their future roles (Vidya & Sukumar, 2005). Young males tend to play with other males of similar age, sometimes from another family, while young females play with younger calves (Vidya & Sukumar, 2005).
4 Behaviour

In elephants societies, behaviour is a very important part of live. Understanding the behaviour and behavioural needs can seriously improve animal welfare and reproduction in captivity. This chapter describes the normal behaviour in the wild and compares this to behaviour recorded in captivity.

4.1 In the wild

4.1.1 Social behaviour

Social contact is very important in African elephants. The group will spend 80% of the time together and coordinate cooperation in group defence, acquisition of resources and care for offspring (Vidya & Sukumar, 2005). Bonds are renewed through frequent contact and greeting ceremonies, which include placing the trunk tip in the mouth, on the temporal gland or between the legs of the other (Clubb & Mason, 2002). The elephant herd is centred around raising and caring for calves (Schulte, 2000). Females, usually nulliparous females, frequently show mother-like behaviour to calves that are not their own, which is called allomothering (Rasmussen & Schulte, 1998; Clubb & Mason, 2002). This is not only beneficial to the calf and its mother, but also for the young female. The first benefit is that caring for the young of a high-ranking female may provide her with high quality resources (Schulte, 2000). They also gain experience in rearing or they receive help with their own offspring in return for their help (Clubb & Mason, 2002; Schulte, 2000). Allomothering may also strengthen social bonds and reduce aggression in the group (Schulte, 2000). One indication for this is that aggression in captive groups where allomothering takes place is lower than aggression in groups without allomothering (Schulte, 2000). Finally, allomothers enhance their genetic fitness by caring for the young of related elephants, which is a form of kin selection, which is suggested to be the primary goal of allomothering (Schulte, 2000). Calves are weaned at an age of six and even after that the bond between mother and calf stays strong (Clubb & Mason, 2002).

4.1.2 Reproductive behaviour

Elephants are polygynous animals which means that both males and females mate with more individuals during a receptive period (Rasmussen & Schulte, 1998). Age of sexual maturity varies between 9 and 22 (average 11-14) years in females and is on average 24 years in males
(Vidya & Sukumar, 2005), although males are capable of sperm production at an age of 10-13 (Rasmussen & Schulte, 1998).

**Female reproductive behaviour**

The oestrous cycle of the African elephant is 12-18 weeks long and consists of a 8-14 week luteal phase and a 4-6 week follicular phase (Vidya & Sukumar, 2005). In the elephants, two successive waves of follicular development take place. Only the second wave results in a Graafian follicle that ovulates (Ortolani *et al.*, 2005; Vidya & Sukumar, 2005). It has been suggested that the first wave has two uses; that enough corpora lutea are formed to enable pregnancy and to start early advertisement of oestrus which results in the availability of more males during oestrus (Ortolani *et al.*, 2005; Vidya & Sukumar, 2005; Hildebrandt *et al.*, 2006).

The reproductive cycle is further explained in chapter 5. Because females are only in oestrus for a very short period, around two to six days (Clubb & Mason, 2002; Leong *et al.*, 2005), signalling oestrus is very important. Females may signal their oestrus through auditory, chemical and visual signs (Ortolani *et al.*, 2005; Vidya & Sukumar, 2005). During an early stage of oestrus, females will perform the oestrous rumble to attract males (Langbauer, 2000; Soltis *et al.*, 2005a,b) and the rate of rumbling will increase before ovulation (Soltis *et al.*, 2005a). If more females in one herd are in oestrus, rumbling of the whole group increases, increasing the chance of attracting males (Soltis *et al.*, 2005a). When males approach the herd, females in oestrus may perform the oestrus walk during which the female walks away from her group with her head held high looking back over her shoulder before she returns back to her group (Vidya & Sukumar, 2005). If she is pursued by a male, the female may run a circle before returning back (Vidya & Sukumar, 2005). Females are very aware of male presence during this period. She can lure a male by tail-flicking, in which she will slap the tip of her tail against her urogenital region and holds it up for a few moments. This is a visual sign as well as a chemical sign (Vidya & Sukumar, 2005). When a male encounters a herd he will usually check out every female with its trunk to see if a female is in oestrus (Rasmussen & Schulte, 1998; Ortolani *et al.*, 2005; Vidya & Sukumar, 2005). If a female is receptive, the male will respond with flehmen behaviour which will increase as ovulation approaches (Rasmussen & Schulte, 1998). Not only males (Bagley *et al.*, 2006), but also females (Vidya & Sukumar, 2005) can detect if a female is in oestrus through chemicals in the urine.
Male reproductive behaviour

Although adult males are fertile the whole year, in the wild they will only mate with females when they are in musth. Musth is a state of increased testosterone levels in the blood which causes increased bouts of travel, greater interest in females and heightened aggressiveness (Schulte, 2000; Charif et al., 2005; Hollister-Smith et al., 2007). Indicators of musth are enlargement of and secretions from the temporal gland, dribbling of urine, increased aggression towards elephants and other objects, increased association with female herds and increased sexual activity (Rasmussen & Schulte, 1998; Ganswindt et al., 2005; Vidya & Sukumar, 2005). Other behaviours seen during musth are ear wave during interaction between males and during musth rumbling, rubbing the temporal gland with the trunk, marking trees with the temporal gland area (which will release pressure as well as serve as a sign for other elephants (Rasmussen & Schulte, 1998)), urination with the penis inside the sheath, musth walk, in which the head is held high, the ears spread out and the head swung from side to side, head oscillation in younger males, tusking the ground and hurling vegetation and mud, the musth rumble (Vidya & Sukumar, 2005), restlessness and reduced feeding activity (Ganswindt et al., 2005). The musth rumble happens more often when the male is alone and appears to cause a response from females (Vidya & Sukumar, 2005).

In Amboseli national park, average age at first musth was 29 (Vidya & Sukumar, 2005) and is usually not lower than 25, unless poaching of males has caused a skewed sex ratio (Rasmussen & Schulte, 1998). Males experience on average one musth each year (Schulte, 2000). Higher ranking males come into musth more regularly and stay in musth longer than low ranking males (Langbauer, 2000; Hollister-Smith et al., 2007), especially when more females are in oestrus (Vidya & Sukumar, 2005). Males in musth are dominant to all other males, regardless of their size (Rasmussen & Schulte, 1998; Langbauer, 2000; Hollister-Smith et al., 2007). Other males can make a choice if they want to fight or retreat based on the asymmetry of fighting capability (Vidya & Sukumar, 2005). Non-musth males will always retreat and will avoid fighting (Rasmussen & Schulte, 1998; Langbauer, 2000). Musth males may retreat if they are smaller or may fight, often to the death (Rasmussen & Schulte, 1998, Langbauer, 2000). Younger males will sometimes decide not to exhibit musth or drop out of musth to avoid fights with high ranking males (Rasmussen & Schulte, 1998; Vidya & Sukumar, 2005), although males that drop out of musth still experience elevated levels of testosterone (Clubb & Mason, 2002). Because musth is a physiologically stressful state, exhibiting musth is a sign of health and healthy males are more likely to enter musth and
remain in musth longer (Rasmussen & Schulte, 1998; Schulte, 2000; Vidya & Sukumar, 2005).

**Mate choice**

Females have an indirect and a direct method of mate choice. A direct choice is when the female runs from one male, but stands for copulation by another (Rasmussen & Schulte, 1998). Females can express indirect mate choice by attracting multiple males, for instance through post-copulatory vocalization, which can lead to male to male competition (Rasmussen & Schulte, 1998; Ortolani *et al.*, 2005; Vidya & Sukumar, 2005). Females can mate with several males during an oestrus if the male does not perform mate guarding or if more dominant males arrive. Females can distinguish between musth and non-musth urine and prefer musth males over non-musth males (Langbauer, 2000; Schulte, 2000; Hollister-Smith *et al.*, 2007). Females might be induced into receptivity by the presence of males in musth (Rasmussen & Schulte, 1998) resulting in the guaranteed availability of a musth male. Because of these methods, most matings are by musth males over the age of 35 (Hollister-Smith *et al.*, 2007).

**4.2 In captivity**

**4.2.1 Social behaviour**

In general, social structure in captivity resembles the social structure in the wild. Females are usually held in a group while males are housed solitary (Schulte, 2000). In most cases there is one dominant female, which might be important for maintaining social harmony (Freeman *et al.*, 2004), but because knowledge of the environment is not critical for survival of the group, a true matriarch is not needed (Schulte, 2000). Dominance hierarchy of captive females also represents the wild. Dominant females are larger and spend more time overseeing behaviour and disciplining herd mates (Freeman *et al.*, 2004). A difference is that in captivity most females do not reproduce (Hermes *et al.*, 2007) and therefore in most groups calves are not a part of the herd. A consequence of this is that in captivity, most elephant herds consist of unrelated females which can lead to a higher incidence of aggression (Clubb & Mason, 2002) and a lack of social bonds. The effect that these differences have on welfare of the elephants is discussed in more detail in chapter 7.
Because male elephants in the wild show less social behaviour, male elephants in captivity are more independent and aggressive than females and might show less cooperative behaviour (Schulte, 2000).

4.2.2 Reproductive behaviour

Advertising reproductive state in captivity is not very important, because usually no males have to be gathered to fight for the female. However, it might be a useful signal for keepers to determine the right moment of Artificial Insemination. Some studies have been performed on oestrus behaviour with conflicting results. Some researchers saw no visual indicators of oestrus in the female and one research indicated that oestrus could be detected by closely monitoring the behaviour of the female in the presence of a male (Ortolani et al., 2005).

Ortolani et al. (2005) studied the reproductive behaviour of a group of four females and one male in captivity. The male followed more and carried out more genital inspections, flehmen and trunk to mouth behaviours towards cycling females during their ovulatory phase compared to the luteal and anovulatory phase and compared to non-cycling females. Females did not display more tactile behaviour towards the male but did show a greater variety of behaviours. For example, trunk to eye, trunk to ear and trunk to temporal gland were only seen in females during the ovulatory phase. This study also indicated that behaviours that have been associated with reproduction such as driving the female with the trunk, mounting, pushing rump against the bull, standing with legs wide apart and intromission were almost only seen on the day of the ovulatory LH surge suggesting that these behaviours are not part of courtship but are copulatory behaviours. This study concluded that there is a clear pattern between male behaviour and the females reproductive state and that tactile and olfactory behaviours are important correlates of female oestrus in a captive environment.

Although in the wild females advertise their reproductive state to males using low-frequency acoustic communication (Ortolani et al., 2005). Ortolani et al. (2005) and Leong et al. (2003) observed no vocalisations during oestrus in captive females, but observed an increased the rate of vocalisations during the anovulatory phase when the male was absent from the herd. This may indicate that females do not signal oestrus through vocalisations but rather that oestrus is impending (Leong et al., 2003; Ortolani et al., 2005). When the male was present in the herd, no differences in vocalisation were observed through the different phases (Ortolani et al, 2005). Leong et al., (2005) studied vocalizations in relation to oestrous cycle in a captive female herd. Conclusions from this second study were that the rate of production of
low-frequency vocalizations was connected to the females rank in the hierarchy and may be a result of dynamics in the group and therefore not a signal to distant males. In this study, females in oestrus received significantly more aggressive interactions than when they were not in oestrus. Low ranking females in oestrus used less vocalizations when they received aggression than high-ranking females. Therefore, vocalization when receiving aggression may signal that a high ranking female is willing and has the ability to assert her status whereas the low ranking females silence prevents escalation into a conflict.

Males still experience musth in captivity, but this has no benefits for the animal (Schulte, 2000). In fact, bulls in musth can become highly aggressive and difficult to handle. This is especially a problem because musth in captivity is very frequent and prolonged because there are no factors that force a bull out of musth (Rasmussen & Schulte, 1998; Brown, 2000; Clubb & Mason, 2002). Elephants in musth in captivity seem to have poor quality sperm (<5% motility) and low libido (see also chapter 6) (Hildebrandt et al., 2006). Hildebrandt et al. (2000) found another reproductive state in the male African elephants, characterised by mild musth-like symptoms experienced in the presence of cows in oestrus in captivity and suggests the term breeding excitement for this state. During this time (max of 5 days) semen motility is superior (around 90%) and libido is high. During breeding excitement urine dribbling and temporal gland secretions are less severe than during musth (Hildebrandt et al., 2006). During musth, aggression is undirected while aggression during breeding excitement is directed towards occupying the females in oestrus (mate guarding) which can prevent keepers from engaging with the females normally (Hildebrandt et al., 2006).
5 Hormone cycle

Knowledge of the reproductive cycle of elephants is necessary for the improvement of reproduction in captive elephants. This chapter describes the normal cycle of male and female elephants and gives different methods of monitoring hormone concentrations, which is important in successful reproduction in captivity.

5.1 Female
Breeding can occur during the whole year (Leong et al., 2005) but in some herds there is a higher birth rate just before or during the wet season (Clubb & Mason, 2002). Some authors state that it is common for elephants to synchronise births which is achieved by synchronising oestrus or adjusting gestation length (Rasmussen & Schulte, 1998). In captivity, females housed at the same facility often show some degree of synchronisation (Rasmussen & Schulte, 1998; Brown, 2000), but differences in oestrous state are also common (Rasmussen & Schulte, 1998). Elephants in captivity that are housed inside for more than 3 months due to extreme cold weather have also been described to experience a prolonged non-luteal phase after which they start cycling again in the spring (Hildebrandt et al., 2006).

5.1.1 Cycle
As mentioned earlier, the oestrous cycle of the African elephant is within the 12-18 week range consisting of a 8-14 week luteal phase and a 4-6 week follicular phase (Hodges, 1998, Brown, 2000; Vidya & Sukumar, 2005).

The luteal phase is characterised by high levels of progestogens. The major circulating progestogen in elephants is not progesterone as it is in most mammals, but 5α-reduced pregnanes (Hildebrandt et al., 2006). As shown in figure 1, progestogens inhibit follicular development.

FSH initiates follicular development and is secreted in the Pituitary Gland. The concentration of FSH is highest at the end of the luteal phase and decrease during the beginning of the non-luteal phase (Brown, 2000; Brown et al., 2004a). In the elephant, two successive waves of follicular development occur during the follicular phase under the influence of FSH. During the first wave, several follicles develop which do not reach Graafian size or ovulate (Hermes et al., 2000). Unique for the elephant hormone cycle is the double LH peak that takes place
after each follicular wave (Brown, 2000; Brown et al., 2004a). Only the second peak initiates ovulation and development of the graafian follicle, which is comparable to the function of the LH peak in other mammals (Ortolani et al, 2005; Vidya & Sukumar, 2005; Hildebrandt et al., 2006). The first peak, also called anovulatory LH or anLH peak, occurs 12 to 21 days after progestogen concentrations decline to baseline, the second peak, the ovulatory LH or ovLH peak, occurs 19 to 22 days later (Brown, 2000; Hildebrandt et al., 2006; Hermes et al., 2007).

As mentioned in chapter 4, the first wave is suggested to have two uses; that enough corpora lutea are formed to enable ovulation and pregnancy and to start early advertisement of oestrus which results in the availability of more males during oestrus (Vidya & Sukumar, 2005; Hildebrandt et al., 2006; Hermes et al., 2007).

Figure 1 Hormone cycle of the African elephant. Profiles of serum LH and progestogens (top), and FSH and inhibin (bottom) throughout the oestrous cycle of the elephant (Brown, 2000). Horizontal lines (top) represent estimated time course of follicular waves.
Inhibin is produced in the follicular cells of the ovaries and is inversely related to FSH (Brown, 2000).

Prolactin may be involved in follicular development in African elephants (as seen in some other species) because concentrations are elevated during the follicular phase (Brown et al., 2004a). This is in contrast to Asian elephants where prolactin remains stable throughout the oestrous cycle (Brown et al., 2004a).

Oestradiol concentrations do not reflect the two follicular waves and circulating concentrations in the elephant are low (<10 pg/mL) (Hodges, 1998; Hildebrandt et al., 2006, Hermes et al., 2007). It is not exactly clear why no pattern exists but this is possibly due to the small follicular size (20±2 mm) compared to body mass (Hildebrandt et al., 2006) which provides physiological changes that are too small for current tests to measure (Brown, 2000). Alternatively, it might be possible that elephants produce another form of oestrogen as has been shown for progestogens (Brown, 2000). The third possibility is that oestradiol works as a conjugated, rather than a free steroid form (Brown, 2000). More research needs to be performed on this topic for this to be clarified.

5.1.2 Endocrine monitoring

Monitoring of hormone cyclicity is important for the timing of AI or introduction for natural mating. Recent advances in the detection of reproductive hormones in blood and urine greatly improved monitoring of the oestrous cycle (Clubb & Mason, 2002; Hermes et al., 2007). Hormone concentrations can also be used to monitor pregnancy and predict parturition, which is described in more detail in appendix 1.

Serum

The oestrous cycle can be monitored by measuring serum progestogen concentrations. Blood collection should start as soon as an animal will tolerate blood collection to monitor age of sexual maturity and avoid unexpected pregnancies in very young animals (Brown, 2000). Recommended is monthly sampling which is increased to weekly sampling when the female reaches the age of 4 to 5 (Brown, 2000).

There are two methods to estimate exact timing of ovulation, which is especially important for artificial insemination (more information about AI and other ART’s in elephants can be found in appendix 2) but is also necessary for natural breeding when males and females are
not housed together (Brown, 2000). First, serum LH can be measured. Ovulation takes place 3 weeks after the first LH surge (Brown, 2000; Hermes et al., 2007). The second method is to measure the increase in progestogen at the end of the follicular phase, 3-4 days before ovulation (Hodges, 1998; Brown, 2000). Both methods require daily blood collection. Disadvantages of the first method are that LH analysis is only performed in a few laboratories, and it takes 2-3 days to get results (Brown, 2000). However, this method provides nearly 3 weeks of preparation time, which is needed if bulls, cows and insemination equipment are not present at the same facility (Brown, 2000). In contrast, progesterone is measured in several facilities and results can be available in 1 day (Brown, 2000). Unfortunately, there is little time between the progesterone drop and time of insemination (Brown, 2000). Making LH analysis available in commercial kits is essential if AI is going to be used more in the future (Brown, 2000).

**Urine & faeces**

Hormone levels can also be determined in urine or faeces (Brown, 2000). These methods are safer to perform and hormone profiles are comparable to that found in serum (Brown, 2000). However, urine samples can be difficult to obtain and require additional processing steps in the analysis of creatinine levels to account for differences in fluid intake (Brown, 2000). Faecal samples are easier to collect but require a more expensive process, lack a suitable index (such as creatinine in urine) to standardise results and suffer from a relatively long excretion lag time, which can be a big disadvantage in the prediction of specific events (Brown, 2000). Additionally, steroid concentrations in faeces are not evenly distributed, which means that appropriate sample collection is very important (Brown, 2000).

### 5.2 Male

Less is known about male endocrinology than of the female hormone cycle (Brown, 2000). As described in chapter 4, males experience musth which are periods of heightened aggressiveness and sexual behaviour characterised by increased temporal gland secretion, urine dribbling and increased testosterone secretion (Brown, 2000; Ganswindt et al., 2005).

#### 5.2.1 Cycle

Male elephants have intra-abdominal testicles that can reach a mass of 2 kg each in an adult bull (Hildebrandt et al., 2006). During musth, the volume of these testicles may reach 4 times
the non musth volume (Hildebrandt et al., 2006). Testosterone production is age dependent and is correlated with testicular weight (Brown, 2000). It can also be related to social rank where dominant males have higher concentrations of testosterone than subordinate males (Brown, 2000). Concentrations of testosterone in blood are <2 ng/mL during non musth (in pre-pubertal males <0.5 ng/mL) with occasional spikes up to 10 ng/mL (Brown, 2000). During pre- and post-musth circulating concentrations are 10-20 mg/mL and during musth levels can sometimes exceed 50 ng/mL (Brown, 2000). Androgen secretion is under control of LH with LH pulses occurring at around one pulse every 3 hours (Brown, 2000). There is not much difference in these pulses during musth and non-musth, however, mean concentrations of LH increase about 4 weeks before the onset of musth and decline to baseline soon thereafter (Brown, 2000). Although in the wild only musth males mate, it is not necessary for successful reproduction. Non-musth males have known to sire offspring and have comparable sperm quality (Ganswindt et al., 2005).

In captivity, age of sexual maturation in males is much earlier than in the wild (Clubb & Mason, 2002), there are reports of 8 year old African elephants that successfully sired a young (Hildebrandt et al., 2006). Age of first musth, which can be as early as 10-15 years in captivity, is also lower than in the wild where musth is rarely observed before the age of 25 (Brown, 2000). This early maturation might be due to high quality food or the lack of suppression by older males (Hildebrandt et al., 2006).

### 5.2.2 Endocrine monitoring

Long term monitoring of bulls is important in the determination of reproductive problems (see also chapter 6). Testosterone concentrations can be measured in serum, urine and faeces. Because bulls, especially bulls in musth, are often too dangerous for blood collection, urine and faeces analysis is preferred (Ganswindt et al., 2002; Brown, 2000), but because even urine collection proves to be difficult in wild and some captive bulls, measurement of androgens in faeces is a reliable and safe method of monitoring reproductive health of bulls (Ganswindt et al., 2002).
6 Reproductive problems

Captive African elephant populations are not self-sustaining because of a lack of reproduction, poor calf rearing, a shortened reproductive life span, problems at birth, a small percentage of males that have produced offspring and an aging population (Brown, 2000; Olsen & Wiese, 2000; Hutchins & Keele, 2006). Insight in the factors that cause reproductive problems are important in order to overcome them and increase reproduction rates. This chapter will describe the most important causes of infertility in African elephants. Problems that occur during gestation and birth are described in more detail in appendix 1.

6.1 Female

Reproductive problems are a large cause of the low birth rate of elephants in captivity. Around 29% of females African elephants are not cycling or are cycling irregularly based on progestogen profiles (Brown et al., 2004a; Brown et al., 2004b; Freeman et al., 2004) and less than 10% of females of reproductive age have produced offspring (Brown et al., 2004a). In non-cycling elephants, serum progestogen concentration remain at baseline, which is why these animals are often called “flatliners” (progestogen profiles of a cycling and non cycling elephant can be seen in figure 2) (Brown, 2000; Schulte et al., 2000; Brown et al., 2004a). FSH values stay at baseline and no LH surges are observed (Brown et al., 2004a). There are many causes for ovarian inactivity such as reproductive tract pathologies, hormone receptor dysfunction, metabolic or nutritional deficiencies, stress and other social factors, hypothalamic-pituitary disruptions (Brown et al., 2004a), climate and season (Schulte et al., 2000).
6.1.1 Anatomy

Olson & Wiese (2000) performed a study on the North American African elephant population and found that first time mothers were between the age of 10-22 with 70% of the females under the age of 15. In captivity, older first time mothers (>15 years) have a higher chance to develop dystocia because of reproductive tract pathologies (Hildebrandt et al., 2006). 30% of calves born from first time mothers in this age group die before or during birth (Hildebrandt et al., 2006).

In the wild, females are either pregnant or lactating and experience very few cycles in their lifetime (Brown et al., 2004b; Hermes et al., 2004; Hildebrandt et al., 2006). Additionally, age of the first hormone cycle in captivity is a lot earlier than in the wild, possibly due to the high quality feed in captivity (Clubb & Mason, 2002; Hildebrandt et al., 2006). While females
in the wild start cycling at an age of 10-12, some African elephants in captivity start at an age as early as 7 (Hildebrandt et al., 2006). Because the current recommended age of first conception is 10-12 years, females will have had many non-fertile cycles before first conception (Hildebrandt et al., 2006) and may have ovulated as many eggs by the age of 12 as a wild female does in her entire lifetime (Freeman et al., 2004). This accumulation of non-fertile cycles may lead to premature alterations in the reproductive tract, also called asymmetric reproductive aging (Hermes et al., 2004; Hildebrandt et al., 2006; Hermes et al., 2007). On average there seems to be a 10-15 year window between the start of cyclicity and the decline in reproductive fitness in nulliparous elephants (Clubb & Mason, 2002; Brown et al., 2004; Hermes et al., 2004). Generally, nulliparous females in captivity are post reproductive at an age of 35 because of the greater risk of dystocia and stillbirth and the higher incidence of reproductive tract pathologies (Hildebrandt et al., 2006). In contrast, cows in the wild can reproduce into their fifties (Hildebrandt et al., 2006).

Reproductive pathologies reported in African elephants are cysts, polyps and tumours of the uterus, vagina, vestibule and ovary (Brown et al., 2004b). Vestibular polyps occur in 70% of all nulliparous females over 30 years of ages (Hildebrandt et al., 2006; Hermes et al., 2007). In these nulliparous cows over 30 years, vaginal cysts and neoplastic formations can be so extensive that they fill the vaginal lumen and consequently block the semen flow (Brown et al., 2004b; Hildebrandt et al., 2006; Hermes et al., 2007). These cows can experience discomfort during oestrus and mating and can have periodic discharge of mucous and clotted blood (Hildebrandt et al., 2006; Hermes et al., 2007). Around 15% of African elephants with limiting breeding opportunities suffer from ovarian cysts (Brown et al., 2004b; Hildebrandt et al., 2006). In comparison, less than 1% of free-ranging females suffer from this (Brown et al., 2004b; Hildebrandt et al., 2006). Besides the lack of breeding opportunities, excess weight has also been associated with the high incidence of ovarian cysts in captivity (Clubb & Mason, 2002). It was not determined if the animals in this study were also acyclic or infertile (Brown, 2000; Brown et al., 2004), however, some other studies showed a lack of cyclicity in the presence of ovarian cysts (Brown et al., 2004b). The birth of a calf from an Asian elephant that showed endometrial cysts indicate that not all pathologies lead to infertility (Brown et al., 2004b). Brown et al. (2004b) studied the reproductive cyclicity of the North American elephant population. This study showed that there were more non-cycling females that exhibited reproductive-tract pathologies than cycling females, however, there were also some

21
cycling females that showed reproductive-tract pathologies and acyclic females with normal urogenital tracts (Brown et al., 2004b).

The solution to the problem of asymmetric reproductive aging is not to lower the recommended age at first pregnancy, because pregnancy in young cows can have negative consequences for both cow and calf, but rather to delay the onset of puberty (Hildebrandt et al., 2006). This can be accomplished by changes in social grouping, modifications to diet and/or medical treatments (Hildebrandt et al., 2006).

### 6.1.2 Behaviour

Cessation of cyclicity can not only be the result of asymmetric reproductive aging, but might also be a normal method for elephants in the wild to prevent pregnancy under sub-optimal conditions (Schulte et al., 2000). In the wild, dominant elephants sometimes suppress cycling in subordinate animals when resources are limiting to increase their own reproductive success (Schulte et al., 2000; Hermes et al., 2004; Soltis et al., 2005a). This can be done either by the use of chemosignals or the induction of stress by dominance (Brown et al., 2004b; Freeman et al., 2004). Although suppression of oestrus is a normal mechanism in the wild, the occurrence of this in captivity can be a sign of sub-optimal conditions (Freeman et al., 2004). Schulte et al. (2000) found that temporarily acyclic periods in captive elephants occurred more during the winter, when the elephants spend less time outside. During this study, the most subordinate female experienced more and longer period of acyclic activity, indicating that this could be a result of social suppression. The relatively small, unrelated and frequently changing groups in captivity may negatively impact reproduction (Clubb & Mason, 2002; Freeman et al., 2004). In the wild, the matriarch is constantly on guard, securing the safety of the herd (Freeman et al., 2004). Without this security, elephants may spend more energy on group protection and competition for rank and less on reproduction (Clubb & Mason, 2002; Freeman et al., 2004). Additionally, large herds provide a stimulus for breeding because they ensure that enough elephants are available to assist in rearing of the young (Freeman et al., 2004). However, if space is limited, large or even small groups can increase stress and inhibition of reproduction (Freeman et al., 2004).

In a study by Freeman et al. (2004) several behavioural factors were compared with cyclicity to see if there was any correlation. Dominance status proved to be positively correlated with ovarian activity where non-cycling females usually ranked higher in the dominancy hierarchy,
which is in contrast with the wild (Freeman et al., 2004). Although older females experience more reproductive tract pathologies, and are therefore more likely to cease cycling because of this, age does not appear to be the primary cause of reproductive acyclicity (Freeman et al., 2004).

6.2 Male

Infertility in bull elephants has a larger impact on the lack of reproductive success than that of females because of the low numbers of males in captivity (Hildebrandt et al., 2000; Clubb & Mason, 2002; Hildebrandt et al., 2006). For example, in North America bull elephants comprise only 10% of the Species Survival Plan (SSP) population and only a few of these bulls have sired offspring (Hildebrandt et al., 2006).

In a study by Hildebrandt et al., 9.1% of African males had reproductive tract pathologies (Clubb & Mason, 2002). The same study revealed that 32% of apparently healthy bulls were infertile, indicated by low sperm motility, due to other causes such as diet that lacks in some nutrients, excessive body weight or behavioural difficulties (Clubb & Mason, 2002). 76% of the bulls studied produced low amount of ejaculate (under 100 ml), and experienced lack of libido or unsuccessful copulations (Clubb & Mason, 2002). The low quality sperm of these bulls also rules out use of their sperm in artificial insemination (Clubb & Mason, 2002).

6.2.1 Anatomy

There appear to be less reproductive tract pathologies in males than in females in captivity, indicating that lack of opportunity to breed does not have a significant impact on male sexual organs (Hermes et al., 2004; Hildebrandt et al., 2006).

Conditions attributed to aging in the male elephant are reduced testis volume, diminished sperm motility and increased percentages of abnormal sperm (Hermes et al., 2004). A valuable tool in the assessment of bull sexual maturation is the use of ultrasonographic and spermatological evaluations (Hildebrandt et al., 2000; Hildebrandt et al., 2006) and androgen evaluations (Brown, 2000). Consistently low circulating testosterone indicate compromised fertility because testosterone is needed for spermatogenesis, normal function of the epididymis and accessory glands and stimulation of sexual interest (Brown, 2000).
6.2.2 Behaviour

Although males do not experience much reproductive tract pathologies, many bulls fail to reproduce because of lack of libido or sub optimal sperm quality (Hermes et al., 2004; Hildebrandt et al., 2006). An important cause of infertility in bulls is suppression and induction of stress by dominant bulls, cows or handlers (Clubb & Mason, 2002; Hildebrandt et al., 2006). The lack of contact with females can also be a problem. Contact with females can stimulate the production of testosterone, and in the wild, males often come into musth after the have associated with a group of females for several weeks (Clubb & Mason, 2002). Musth in captivity is another factor associated with low libido, which is described in more detail in chapter 4. Because musth is dependent on nutritional status, water and food depravation is a common method of suppressing musth (Brown, 2000). Compounds such as GnRH agonists and antiandrogens can reduce testosterone during musth, however, long time effect on fertility and behaviour has not been tested (Brown, 2000).

6.2.3 Other problems

Mounting involves a lot of physical effort and weight on the joints (Clubb & Mason, 2002). Because foot and joint problems are often a problem in zoo elephants, some males are physically not able to impregnate a female (Clubb & Mason, 2002).

6.3 Conclusions

Active monitoring of hormone profiles and reproductive tract pathologies is needed for both males and females to identify the causes of reproductive dysfunction. It is recommended to perform routine hormone and ultrasound assessment on all males and females, even if there are no plans for breeding or the female is considered to be post-reproductive. Because not all acyclicity can be attributed to reproductive-tract pathologies, long term research is needed to identify the causes and develop treatment or solutions for reproductive problems which can be a great step in the improvement of reproductive success in captive African elephants.
Welfare of zoo animals has become increasingly important in the last decades, which is indicated by the increase of animal rights groups dealing with this topic. Especially the ethics of keeping large, intelligent, social animals such as elephants in captivity are currently on debate because keeping these animals in zoos has proven to be a challenge (Veasey, 2006).

Welfare refers to the emotional state of an animal; what an animal feels. Welfare is very important and sub-optimal welfare of animals can have serious consequences such as reproductive problems, occurrence of stereotypic behaviour, higher incidences of aggression and diseases and a higher death rate. Measuring welfare is possible by looking at behavioural indicators such as successful reproduction, activity patterns, lack of stereotypic behaviour and animal health and mortality (Hutchins, 2006; Veasey, 2006), physiological indicators such as elevated corticosteroids and analysing the animals environment with the help of the five freedoms (more information on the five freedoms can be found in appendix 3) (Veasey, 2006).

Meeting welfare needs of elephants in captivity is a challenge for two reasons; elephants are highly intelligent and social animals and therefore require more stimulation and adequate social grouping to meet behavioural needs and elephants are large animals and therefore require more space, especially if they are kept in the appropriate social grouping of a minimum of five to six animals (Veasey, 2006). Although conditions of exhibits and zoo animal care have improved greatly in the past decades due to increased knowledge of their needs, zoo elephant programs are still criticized (Hutchins, 2006). Critic say that elephants live miserable lives in captivity and should therefore not be kept in zoos (Hutchins, 2006). They compare the captive situation with the wild and conclude that no captive facility can give the animals what they need (Hutchins, 2006). However, comparing captive situations with the wild is difficult because there is a great deal of variation between natural situations (Hutchins, 2006). Therefore, many wild animals can tolerate a wide range of social and environmental conditions, especially widely distributed species such as the elephant (Hutchins, 2006).
In this chapter, all factors that negatively or positively affect welfare of elephants in captivity are discussed, the current state of the welfare of elephants in captivity is examined and possible points of improvement are listed. This chapter also includes arguments for and against holding elephants in captivity and discusses their value.

7.1 Causes of impaired welfare

7.1.1 Housing

In the wild, elephants travel distances of up to 80 km each day (Hutchins, 2006). Some critics including some zoo professionals state that because of this, elephants could not be given enough space in zoos and should not be held in captivity (Hutchins, 2006). However, in the wild the amount that elephants travel is dependent on the availability of food, water and mates and differs considerably between herds and seasons (Hutchins, 2006). Although large habitats are still necessary, they do not have to be as large as home ranges in the wild and Hutchins (2006) concludes that enclosures for elephants should be 3 to 7 acres (12 to 28 hectare). Not only enclosure size, but also the complexity of the enclosure is important (Hutchins, 2006) which includes toys or objects the elephants can manipulate, alteration of feeding regime and situations in which the animal has to work for its food, an increased number of social partners, training and a view of the surrounding environment (Clubb & Mason, 2002). In a compatible herd, herd mates are a highly effective form of enrichment and therefore very important (Hutchins, 2006, Veasey, 2006), as will be discussed in the next paragraph. Failure to provide adequate room or enrichment can result in increases in aggression and the formation of stereotypies (Clubb & Mason, 2002), which is further explained in paragraph 7.2.2. Close examination for stereotypic behaviour, foot problems and activity levels can give a better insight in the effect of the enclosure on the welfare of the elephants (Hutchins, 2006).

Critics also argue that keeping elephants in zoos in colder climates is bad for welfare (Hutchins, 2006). Although Asian elephants are not subjected to colder climates in the wild, African elephants live in desert habitats where temperatures can fall below freezing at night, which is why they can tolerate a wider range of temperatures than expected (Hutchins, 2006, Veasey, 2006). Housing elephants inside for longer periods of time however, may cause a lowered welfare because of reduced activity and behavioural opportunities and an increase in exposure to potentially dangerous compounds from faeces and urine (Veasey, 2006).
7.1.2 Social grouping

Many problems that zoos have with management of elephants can be attributed to inadequate social grouping (Veasey, 2006). Isolation or social incompatibility in social animals can have serious consequences such as increased heart rate and locomotion, elevated plasma corticosteroids, reduced immune function and an increase in stereotypic behaviour (Clubb & Mason, 2002). The AZA recommends that at least three females are housed together, but some experts state that five or six is the minimum (Clubb & Mason, 2002; Hutchins, 2006). The AZA also states that males may be housed singly from the age of six, but they must be able to see, smell and touch other elephants (Clubb & Mason, 2002). This is still very different from the wild, where males do not leave the herd until they are 9 to 18 years of age, but the effect that solitary housing has on males has not been researched (Clubb & Mason, 2002).

Group compatibility might even be more important than group size (Hutchins, 2006), however, getting compatible groups can be a challenge in captivity. Only 17.7% of facilities that keep elephants house the mother of at least one other member of the group (Clubb & Mason, 2002). Therefore, captive elephant groups usually consist of unrelated females. Because unrelated females are unlikely to form bonds, aggression is more likely to occur (Clubb & Mason, 2002). On occasion, it has been seen in the wild that unrelated females form functional herds, studies about this subjects can possibly help in the improvement of captive herds (Schulte, 2000; Charif et al., 2005). Although captive herds usually consist of unrelated females, stable groups have been formed (Hutchins, 2006). Unrelated females of the same age are more likely to form special relationships (Clubb & Mason, 2002).

Elephants are often transported between zoos for breeding purposes which causes increased aggression (Burks et al., 2004), the breaking of bonds (Clubb & Mason, 2002) and often cessation of the reproductive cycle due to stress (Hermes et al., 2004). Separating animals that have formed bonds can cause increased searching behaviour, vocalisation, altered feeding and sleep patterns, cessation of play, elevated corticosteroid levels, increase in stereotypic behaviours and changes in heart rate and body temperature, all indications of stress (Clubb & Mason, 2002). Especially the bond between mother and calf is important and the AZA therefore recommends that calves are not separated form their mother before the age of three and the EAZA recommends not earlier that four to five years of age (Clubb & Mason, 2002, Hutchins, 2006). This might even be too early because in the wild calves suckle until the age of six to eight (Clubb & Mason, 2002). Early separation from the mother can have serious
consequences throughout the entire lifetime of the calf such as showing of abnormal and stereotypic behaviours, impaired reproductive performance, impaired maternal behaviour and the way in which an animal can adapt to adverse events in the future (Clubb & Mason, 2002). To be on the safe side, females can be kept with their mother indefinitely and males can be separated from the mother at an age of 5 to 8 (Hutchins, 2006). When herds must be split up, matrilines (female with her female offspring) can be transported together (Hutchins, 2006).

In the wild, elephant herds are centred around calves, and calves unify an elephant group (Hutchins, 2006). Younger elephants are taught a variety of essential behaviour from older elephants such as how to forage, where to find resources and how to take care of calves (see also paragraph 4.1) (Clubb & Mason, 2002; Veasey, 2006). In Captivity, infants were only held by 27% of all facilities that held African elephants in Europe, which was higher than the percent of infants in North America (Clubb & Mason, 2002). Because calves are rare in captivity, many older elephants do not have sufficient maternal skills to pass on to younger generations or assist in the birth and further parental care of calves from primiparous cows which is a large factor in the high incidence of infanticide and lack of overall reproductive success in captivity (Hutchins, 2006; Hutchins & Keele, 2006, Veasey, 2006). Recommended is to let female elephants in zoos grow up with at least one experienced female to teach them maternal skills and assist during birth (Clubb & Mason, 2002).

Behaviours that can be assessed to evaluate if the social needs are met are the absence of agitation, aggression, lethargy and stereotypic behaviours (Hutchins, 2006).

7.1.3 Training
Training elephants can have both positive and negative effects on welfare (Veasey, 2006). In the free contact system, handlers walk amongst the elephants and training is usually performed with the use of an ankus or elephant hook and the trainer is dominant over the animals (Veasey, 2006). In protected contact, elephants are trained through a barrier with positive reinforcements, and elephants can choose if they want to cooperate with training or not (Veasey, 2006). Training provides exercise and enrichment to the elephants, gives the opportunity to develop positive relationships between elephants and handlers and allows for veterinary and health procedures without chemical immobilization (Veasey, 2006). Potential negative aspects of training are that the animals are not in control (Veasey, 2006), however
this is less so with protected contact training because the elephant can choose if it wants to cooperate. Training also asks for temporary separation of elephants with close bonds, which can have welfare consequences (Veasey, 2006). Further, the punishment used in free contact systems has a significant negative effect on animal welfare (Veasey, 2006).

7.2 Effects of impaired welfare

7.2.1 Stress
Impaired welfare almost always leads to stress. Negative effects of stress include an increase in abnormal behaviours, impaired immune function and increased susceptibility to diseases and reduced reproduction (Brown, 2000). Glucocorticoids are an indicator of stress. Because serum glucocorticoid concentrations can fluctuate, analysis of excreted glucocorticoids in urine, faeces and saliva may be a better indicator of adrenal activity (Brown, 2000). Standardised timing of urine collection is important because glucocorticoid levels in elephants are greater in the morning than in the afternoon (Brown, 2000).

7.2.2 Stereotypies and other abnormal behaviour
Stereotypic behaviours are repetitive unvarying and apparently pointless behaviours that have been linked to poor welfare because they develop in animals that live in small environments or suffer from pain or distress (Clubb & Mason, 2002; Veasey, 2006). Stereotypic behaviours help the animal cope with unpleasant stimulation or boredom and animals performing these behaviours therefore have lower heart rates and reduced cortisol levels than not-stereotypic animals under the same conditions (Clubb & Mason, 2002; Veasey, 2006). Therefore, stereotyping animals may not be suffering from impaired welfare, but presence of stereotypic behaviours indicate the presence of a factor that lowers welfare (Veasey, 2006).
Stereotypic behaviours can also develop if the behavioural needs of the animal are not met (Veasey, 2006). Behavioural needs are behaviours that an animal needs to perform in order to have a satisfied welfare, but they may not be essential for survival at this point (Veasey, 2006). One example that often arises in captive elephant keeping is the need to forage. In the wild, elephants forage for 14-20 hours each day. In captivity, elephants may be satisfied in terms of nutrition requirements in 2 hours because food is readily available, but they still have a need to forage more extensively (Clubb & Mason, 2002; Veasey, 2006). Because the gap in
time budget is so large in this case, this has to be compensated in other ways such as enrichment or a different food dispensation to avoid stereotypic behaviours.

Factors that affect the chance of stereotypic behaviour are size of the enclosure, complexity of the environment, time spend feeding, social isolation, group size and separation from social companions (see also earlier in this chapter) (Clubb & Mason, 2002). However, stereotypic behaviours can also occur in excited animals and should therefore be considered together with other measures of welfare (Veasey, 2006). Additionally, animals can persist in their stereotypies, even though conditions have been improved, which is why the occurrence of stereotypies does not always reflect on the current management system (Clubb & Mason, 2002; Veasey, 2006).

Not only stereotypic behaviour, but also other abnormal behaviours have been linked to lowered welfare. These may include signs of distress (vocalizing, extreme timidity or aggression, escape behaviours), self mutilation and a reduction in grooming, mating and foraging behaviour (Veasey, 2006).

### 7.2.3 Diseases

Critics argue that diseases are more common in zoo elephants than in wild elephants, which is a sign of impaired welfare (Hutchins, 2006). However, wild elephants can suffer and die from many of the same diseases as captive elephants, such as herpes viruses, tuberculosis and arterial disease (Hutchins, 2006). The incidence of these diseases varies between habitat and season. 60% of deaths of captive adult elephants can be attributed to a disease, which is expected because every non-accidental death is caused by a disease of some sort (Veasey, 2006). The most significant cause of death in zoo elephants appear to be circulatory problems which are caused by chronic stress and obesity and could therefore be caused by inadequate management and facilities (Veasey, 2006). The second largest health problems are foot problems, which can be caused by lack of exercise, excess body mass, unhygienic or excessively moist substrate, climate and performance of stereotypic behaviours (Veasey, 2006). Inside facilities with sand floors reduce the chance of food problems because it spreads the load over the entire foot, is well drained and increases lying behaviour (Veasey, 2006).

### 7.2.4 Longevity and life expectancy

Longevity (longest lived animal) and life expectancy (average age at death) are frequently used indicators of welfare of a population (Wiese & Willis, 2004). Longevity is easily
Life expectancy can be measured by many different methods that can give very different answers and care should be taken by choosing the right method (Wiese & Willis, 2004). In the beginning of this century, life expectancy of zoo elephants was calculated with the use of only dead animals (Clubb & Mason, 2002), which gives misleading results (Wiese & Willis, 2004). For the North American African elephant population, mean age at death of the dead animals was 16.9 years and mean age with the living animals included was 24.6 years which shows that the mean age at death was not representative of the entire population (Wiese & Willis, 2004). Wiese & Willis (2004) calculated life expectancy with the use of a predicted dead for the living animals and concluded an current average life expectancy of 33.0 years. However, because elephants have not been kept in zoos for very long, and most animals have not had the chance to die at an old age, estimated life expectancy of the population is likely to rise (Wiese & Willis, 2004). Additionally, because elephants live very long, the longest lived animal has lived through a period when management was different from today and animals born in times with modern husbandry standards have not had the chance to live a full life (Wiese & Willis, 2004). Therefore, impact of improved husbandry will only be detectible after an entire generation has lived under these circumstances (Wiese & Willis, 2004).

It is very difficult to compare these results with those of wild populations because data availability and analysis are very different (Wiese & Willis, 2004). However, Clubb & Mason (2002) give an estimate of life expectancy for wild African elephants of 34 years, which is comparable with these results (Wiese & Willis, 2004).

7.3 Conclusions
Contrary to the opinion of the critics, welfare of captive elephants can be higher than that of wild elephants because wild elephants suffer from predation, disease, parasitism and starvation. Welfare gain on these factors however, can not compensate a decrease in stimulation and behavioural opportunities, because those are more likely to cause chronic decreases in welfare. All these factors have to be of high standard before welfare of elephants in captivity is acceptable. Habitats should be large enough and provide enough enrichment. Groups should be large enough and compatible, and the goal should be all family herds, where mothers and offspring will be kept together and in the need of transport, transported together. If herds are not compatible, elephants need to be transferred. Transferring single
elephants should be avoided and elephants should ideally be moved in groups of three or more compatible animals. Bulls are transferred between zoos to keep genetic diversity while females stay in the herd. Because elephants in the wild spend most of their time feeding, feeding in captivity should take up most of the animals time without causing obesity. Because of this, low quality bulk feed should be provided both during the day and night, preferably in a manner in which elephants have to work for their food.
8 Materials & Method

8.1 Study animals
Ouwehands zoo currently houses 3 African elephants, one male, Tooth, 15 years of age and two females, Aja, 37 and Duna, 11 years of age (in appendix 4, management profiles of the three elephants are included). Tooth and Aja are born in the wild, but kept in captivity from an early age. Duna is captive born. Both females are nulliparous. Aja showed cycles at the time of the study, Duna did not show cycles yet but was at an age at which these could start at any moment. All animals are kept in a protected contact situation (for more information on training, see paragraph 7.1.3) and trained once or twice each day for procedures such as cleaning or blood collection. Tooth and Aja have showed matings in the past. In this report, the names of the three elephants will be used directly.

8.2 Facility, housing and feeding
The elephant exhibit in Ouwehand zoo was build in 2007 and contains separate inside facilities for a male and a group of up to six females and two outside areas. The outside area of the females is 3,670 m² and that of the bull is 1,200 m². The inside facility of the females consist of 6 stalls of approximately 24 m² each with concrete flooring and one central area of 72 m² covered with sand. The females have access to all area’s when they are inside, except during training. The inside facility of the male consists of two area’s of 80 m², one covered with sand, the other concrete. A schematic drawing of the facilities is added in appendix 5.
During the day when the animals are outside, the male is usually housed together with the females, during which they have access to both the male and the female outside area. If some aggression between the elephants has taken place and the elephant keepers feel it is not appropriate to house them together, the male will be housed separately outside, but will still be able to have tactile contact with the females. The male is housed separately from the females during the night. If temperature allows it (night temperature above 15˚C) the animals will have access to both their inside and outside facilities during this time.
The animals are fed with hay and branches during the day and night. In the morning the elephants are offered a mixture of pellets and linseed. A mixture of bread, carrots, apples, pears and onions is used as reinforcement during training and as an addition during the afternoon feeding.
8.3 Behavioural data collection and analysis

The three elephants were observed 3 to 4 days each week during a period of 19 weeks (from April 2008 to August 2008). Each session lasted preferably 2 hours but at least 1 hour on days where changes in husbandry routine prevented longer observations. During the observations the elephants were recorded with a sony mini DV video camera (DCR-HC14E) by one observer (see appendix 5 for the area of observation). Only social behaviour was analysed, therefore two or three elephants that were in proximity of each other were taped. Behavioural data will be collected in six categories: distance, reproductive behaviour, active aggression (which involves rapid body movement and/or contact with another individual), passive aggression (no rapid body movement or physical aggression), submissive behaviour and affiliative behaviour (an ethogram can be found in appendix 6). Active and passive aggression are two separate categories in order to distinguish between behaviour that could lead to injury and aggression that does not involve physical contact. The initiator and recipient of the behaviours was scored. All behaviours, except for time spend in proximity, were measured in frequency per hour. Time spend in proximity of each other has not been recorded for all observations but has started at the beginning of the fifth week and was measured in percentages.

8.4 Sample collection and determination of oestrous state

Blood samples were collected from the two females weekly and during the end of the follicular phase biweekly in order to determine the end of this phase more accurately. Samples were collected from a vein on the caudal side of the ear by either an elephant keeper or the zoo’s veterinarian. Samples were then kept under room temperature and sent to the veterinary department of Rotterdam zoo to be analysed for the level of progestogens on the same day. Samples were analysed with the use of a chemiluminescent immuno-assay (immulite) (for a full description of this method, see appendix 8).

Determination of oestrous state was calculated from progestogen concentrations. There are several methods described in the literature for the determination of oestrous state. The method most frequently used was described by Leong et al. (2003) and Ortolani et al. (2005). They calculate baseline values by taking the average concentration of all samples. All samples which were greater than the average plus 1.5 standard deviations were considered elevated. These values were temporarily excluded form the data set the average was calculated again.
This removal process was repeated until no values exceeded the mean plus 1.5 SD. The remaining values were considered baseline. Because no clear pattern could be found with the described method of calculating baseline values (see also appendix 9) and because different people agree that Aja is cycling (Mul, C., personal communication, 2 September 2008, Boer, M. de, personal communication, 10 September 2008) baseline values in this study were defined as being lower than or equal to 0.26, because this did reveal a cycling pattern.

Leong et al. (2003) and Ortolani et al. (2005) defined oestrus with the help of the two LH peaks. LH analysis was not possible in this study, therefore end of oestrus was the last baseline value before the increase in progestogen and the beginning of the luteal phase was defined as the first elevated value. Behavioural measurements between these two values were not included in any phase (one exception was the transition between the last oestrus and luteal phase. Because blood samples could only be collected once a week during this time, the first value after the last baseline value was also considered to be oestrus and the last value before the first value that was elevated was also considered to be luteal phase). Oestrus was defined as the three weeks before the end of oestrus and anoestrus was defined as the two weeks before oestrus. The end of the luteal phase was 0.5 week before the beginning of the anoestrus to account for variations in the length of the anoestrus phase. The observations made during this 0.5 week were not included in any phase.

8.5 Statistical analysis
All statistical tests were conducted with SPSS 14.0. A Mann-Whitney test was used to compare the behaviours between the different cycle stages and for the comparison of behaviours between elephants for the determination of dominance etc.. The behaviours of the two females are not compared directly for conclusions about the difference between cycling and non-cycling females. This is because the elephants differ too much in age and behaviour that the differences would probably have more to do with differences in character. This study concentrates on the behaviours between Aja and Tooth, but some interactions with Duna will be mentioned.
10 Results

A total of 66 observations were performed with an average length of 110 minutes between 7 April 2008 and 12 August 2008. During this period, 22 blood sample were collected from Aja.

10.1 Social aspects

Aja and Tooth spend significantly more time in proximity of each other (within 8 m) than each of them with Duna (both P<0.01) (see also figure 3).

Duna performed significantly more submissive behaviour to Tooth and Aja than Tooth or Aja to Duna (both P<0.01), Aja showed significantly more submissive behaviour to Tooth than Tooth to Aja (P<0.01) (Figure 4).

Aja and Duna showed significantly less trunk to mouth behaviour with each other than with Tooth (P<0.01). Tooth and Duna show more trunk tip to mouth behaviour with each other than with Aja (P<0.01) (figure 5).

![Time spend in proximity](image)

Figure 3 Time spend in proximity. Average time spend in proximity (for all observations except the first 14) for all three elephant combinations. Different letters indicate a significant difference: Aja and Tooth spend significantly more time together than Aja and Duna (P<0.01) and Duna and Tooth (P<0.01).
Figure 4 Submissive behaviour. Average rate of submissive behaviour per hour for all observations. Different letters indicate a significant difference: Duna performed significantly more submissive behaviour to Tooth than Tooth to Duna (P<0.01), Aja performed significantly more submissive behaviour to Tooth than Tooth to Aja (P<0.01) and Duna performed significantly more submissive behaviour to Aja than Aja to Duna (P<0.01).

Figure 5 Trunk tip to mouth. Average trunk tip to mouth per hour for all observations. Different letters indicate a significant difference: Duna and Tooth performed significantly more trunk tip to mouth with each other than with Aja (P<0.01), Aja performed significantly more trunk tip to mouth to Tooth than Tooth to Aja (P<0.01), Aja to Duna (P<0.01) and Duna to Aja (P<0.01) and Tooth performed significantly more trunk to mouth to Aja than Duna to Aja (P<0.01) or Aja to Duna (P<0.01).
10.2 Male – Female behaviour across the ovarian cycle

Tooth showed more flehmen behaviour during the anovulatory phase than during the ovulatory (P<0.05) or luteal (P<0.01) phase, and more during the ovulatory phase than during the luteal phase (P<0.05) (figure 6). Tooth also showed more trunk tip to urine during the anoestrus and oestrus than during the luteal phase (P<0.01 and P<0.01) (figure 7). Tooth performed more genital inspections of Aja during the anoestrus period than during the luteal phase (P<0.01) (see also figure 8) or of the non cycling female (P<0.05) (averages of the whole observation period compared).

On average, Tooth and Aja spend more time in proximity off each other during the anoestrus and oestrus phases of the oestrous cycle. However, because time spend in proximity differed greatly between observation days, there is no significant difference. Approach, leave, follow and pass by from Tooth in relation to Aja did not differ between cycle stages. Tooth showed almost no trunk to mouth behaviour to Aja (see also figure 5).

Trunk tip to temporal gland was very rare, it happened throughout the entire cycle and there was no pattern between oestrous periods. Other behaviours often associated with reproduction; trunk tip to eye, trunk tip to ear, trunk tip to head, trunk to head, trunk over back resting, trunk over back driving, trunk tip to base of tail, resting chin over back, trunk to tail and trunk tip to hind leg/foot were to rare to analyse. The behaviours tail grab, leg grab, mounting, attempted mount, intromission and attempted intromission were not observed at all.

![Flehmen Tooth to Aja](image-url)

*Figure 6 Flehmen Tooth to Aja. Average flehmen per hour from Tooth to Aja during the different phases of the reproductive cycle. Different letters indicate a significant difference: Tooth performed more flehmen to Aja in the anoestrus than in the oestrus (P<0.05) or luteal (P<0.01) phase and Tooth performed more flehmen in the oestrus than the luteal phase (P<0.05).*
Figure 7 Trunk tip to urine Tooth to Aja. Average trunk tip to urine from Tooth to Aja during the different phases of the reproductive cycle. Different letters indicate a significant difference: Tooth performed more trunk tip to urine behaviour in the anoestrus (P<0.01) and oestrus (P<0.01) than in the luteal phase.

Figure 8 Genital inspections Tooth to Aja. Average genital inspections from Tooth to Aja during the different phases of the reproductive cycle. Different letters indicate a significant difference: Tooth performed more genital inspections in the anoestrus than in the luteal phase (P<0.01).
10.3 Female – Male behaviour across the ovarian cycle

Aja showed more submissive behaviour, primarily consisting of backing up, to Tooth during the anovulatory (P<0.05) and ovulatory (P<0.01) phase than during the luteal phase (see also figure 9).

Aja followed Tooth more in the anoestrus (P<0.05) and the oestrus (P<0.01) than in the luteal phase. Aja also approached (P<0.01), left (P<0.01) and passed by (P<0.01) Tooth more in the oestrus than the luteal phase. She also showed some of these behaviours more in relation to Duna (Approach, P<0.05, pass by, P<0.01). Trunk to mouth behaviour from Aja to Tooth happened regularly (figure 5) (unlike trunk to mouth behaviour from Tooth to Aja) but did not differ between cycles. Genital inspections from Aja to Tooth also happened regularly, and averages were higher in the anoestrus and oestrus, but this was not significant.

![Submissive behaviour Aja to Tooth](image)

Figure 9 Submissive behaviour Aja to Tooth. Average submissive behaviour from Aja to Tooth during the different phases of the reproductive cycle. Different letters indicate a significant difference: Aja showed more submissive behaviour during the anoestrus (P<0.05) and oestrus (P<0.01) than during the luteal phase.
11 Discussion

This study shows that even with the absence of mating and copulatory behaviours, a pattern of other behaviours can be used to determine the stages of the reproductive cycle in a herd existing of one male, one cycling female and one acyclic female. However, the exact moment of ovulation could not be determined by looking at behaviour alone.

This chapter discusses first the methods and secondly the results of this study. Assumptions are made and explained in this chapter.

11.1 Methods

Because the cycle of African elephants is 12 to 18 weeks, only one complete cycle could be monitored during this study. For more reliable results it would have been better to observe more cycles. In that case, a paired test (such as the Wilcoxon signed ranks test) could be used for statistical analysis between the averages of the phases over the different cycles which could give better results. It would also have been more likely that conclusions could be drawn from the frequency of the more rare behaviours mentioned in the results.

It was not possible to use LH determination, which would have given a more accurate end of the anovulatory and ovulatory phase. Because biweekly progestogen analysis was used for the determination of the end of the oestrous cycle, the exact moment could have been a few days earlier or later than the first elevated value of progestogen.

There was only one person to observe three elephants. It is possible that some behaviours such as smelling of urine or flehmen are missed because these behaviours can occur without another elephant in proximity. It is therefore possible that another elephant was taped at that moment. Additionally, in two cases of smelling of urine and faeces the originating elephant was unknown, therefore these behaviours were not scored at this time. Because of the complexity of the outside exhibit, some area’s are blocked from sight or difficult to see from the observation spot which is why it is possible that some behaviours are not seen and scored.

However, the amount of time the elephants spend out of sight was believed to be less than 5% and when two or more animals were out of sight in the same area, and it was possible that they could perform social behaviours, the observation was paused.
Auditory signals are not scored because these often happens when the elephant is alone which means that there is a chance that the originating elephant is not taped at that moment. Even when the elephant is taped it is sometimes difficult to see on video which elephant made the sound. Because scoring these results would be too unreliable in the eyes of the author, auditory behaviours have not been scored. During observations, rumbling from the cycling female in particular increased during oestrus but as said, this has not been quantified. Additionally, because the rumble has several functions (Soltis et al., 2005a) (as discussed in chapter 3) it is very difficult to distinguish between the different signals and their meaning which would have made the results of this study less reliable. However, this is an interesting topic for future study in this elephant herd.

The animals are not often distracted by the observer but they are distracted by visitors, the elephant keepers or other people that work behind the outside exhibit. Especially the vehicle that brings branches is a large distraction. When the branches are offloaded next to the exhibit, Tooth will wait next to the fence until everyone has gone, which inhibits the performance of other behaviours. Also management, especially the way in which the animals are fed, differs between observation days and can have influenced the results. Some days a lot of hay is placed in the outside exhibit and other days it is placed only in a concrete enrichment feeder. Most days the animals are given branches during the observations, but this does not happen every time. These changes can affect the time the animals spend in proximity of each other and the behaviours they perform.

For determination of dominance, the incidence of submissive behaviour is used. If for example the behaviour displacement was also scored with the more dominant animal as ‘passive aggressive’, the results would have been more detailed (see also an ethogram in appendix 6). This is because during the study it became clear that Duna would show submissive behaviour even when Tooth of Aja just passed by and did not perform any form of aggressive behaviour. Separating these behaviours could give insight in the level of dominant behaviour in addition to submissive behaviour. Clearly not all incidences of submissive behaviour are proceeded by passive or active aggression from the more dominant elephant.
11.2 Results

This study shows an increase in smelling of Aja’s urine by Tooth, followed by flehmen during the anoestrus and oestrus compared to the luteal phase and an increase of genital inspections during the anoestrus compared to the luteal phase. This supports the literature, which states that olfactory cues are very important in elephant reproduction (Rasmussen & Schulte, 1998). However, flehmen behaviour usually increases when ovulation approaches (Rasmussen & Schulte, 1998), which is supported by a study by Ortolani et al. (2005) where the male followed more and carried out more genital inspections and flehmen behaviours towards cycling females during their ovulatory phase compared to the luteal and anovulatory phase and compared to non-cycling females. This was not the case in this study where frequency of flehmen and smelling of urine and genitalia was higher in the anovulatory than in the ovulatory phase. Because only 8 observations were made during the anoestrus, observations during more cycles can determine if this is the case in general in this herd or if this is only characteristic for this cycle.

In the study by Ortolani et al. (2005), cycling females did not display more tactile behaviour towards the male but they did show a greater variety of behaviours during the ovulatory phase, for example, trunk to eye, trunk to ear and trunk to temporal gland. In this study, frequency of these behaviours was too rare to make conclusions because only one cycle was observed and there was limited contact between Tooth and Aja. The observation of more cycles could possibly give more answers on this topic.

Aja did show an increase of approaching, leaving, passing by and following in relation to both Tooth and Duna during the oestrus which indicates an increased movement of Aja during this phase. This correlates with the increase in behaviours during the oestrus phase often described in the literature (Vidya & Sukumar, 2005).

In the study by Ortolani et al. (2005), contact was usually initiated by the male while the female terminated this contact by moving away or allowed contact by staying. In the current study, contact was almost always initiated by the female, which was usually backing up against the male. This was rarely followed by a reaction of the male, indicating a lack of interest from the male. During the observation period no matings or other copulatory behaviours (driving the female with the trunk, mounting, pushing rump against the bull, standing with legs wide apart and intromission (Ortolani et al., 2005)) were observed. Except
for smelling of urine and genitalia, no other behaviours indicate any sign of interest from Tooth to Aja. The results also show that Duna and Tooth regularly perform trunk to mouth behaviour suggesting a bond between the elephants. Trunk to mouth behaviour is often described in the literature as the most important affiliative behaviour (Leong et al., 2005) and absence, which is apparent between Aja and Duna can be seen as lack of a bond. Ortolani et al. (2005) also found that trunk to mouth behaviour increases when ovulation approaches. Trunk to mouth behaviour between Aja and Tooth is only one way, again suggesting lack of interest from Tooth to Aja, which may be part of the reason for the low amount of reproductive behaviours scored.

Another reason for relatively low amount of reproductive behaviours demonstrated might be that Tooth is often acting as the matriarch. In the wild, the matriarch indicates when defensive behaviours are needed (McComb et al., 2001). During this study, all incidences of bunching were initiated by a call from Tooth after which the females would run to Tooth. From the incidences of bunching that have been observed during this study, Tooth was always on the front, the place that the matriarch has in the wild (Freeman et al., 2004). Studies in captive elephants show that the most dominant females are more likely to be acyclic than more subordinate females (Freeman et al., 2004). One reason that is given for this difference is that dominant females spend more energy on disciplining and group defence (Freeman et al., 2004). The fact that Tooth takes over the role of matriarch and therefore spends more energy on this task and the fact that Tooth does not perform his natural role in the group, may lead to lowered libido or lowered sexual interest, which is a large cause of infertility in captive bulls (Hermes et al., 2004; Hildebrandt et al., 2006). This might be the reason why Tooth showed mating during the first two oestrus’s, when he was new in the herd, but not during the last three.

It is also possible that Aja is at the end of her reproductive life. Her cycles during the past year were irregular and the method often described for calculating baseline described in materials & method did not reveal a cycling pattern (see also appendix 9). The values of progestogen in her blood were also much lower than described in cycling females in the literature (Hodges, 1998, Brown, 2000), however, this difference could be attributed to a different method of progestogen analysis. From communication with W. Schaftenaar (9 September 2008), veterinarian at Rotterdam zoo, the Netherlands, can be concluded that
progestogen values of Aja are low and she is most likely at the end of her reproductive lifetime although a cycle is still recognisable. This is not rare for a female of her age (37 years). Generally, nulliparous females in captivity are post reproductive at an age of 35 (Hildebrandt et al., 2006) although older females may still have regular cycles (Brown et al., 2004).

Another thing that became apparent during this study was that Duna performed a high frequency of submissive behaviour, and the author noticed that Duna was almost constantly alert to approaches of Tooth or Aja, which were usually followed by avoiding. The amount of submissive behaviour that the elephants performed shows that Tooth is the most dominant and Duna the most submissive elephant in the herd. This was expected because males are usually dominant over females and older females are dominant over younger females. Aja and Tooth spend more time in proximity of each other because they often eat together while Duna only eats away from the others.

Around week 5 of the observations there was some trouble between Duna and Tooth where Tooth would chase or follow Duna around the exhibit, sometimes together with Aja. This happened a couple of times and Duna had to be separated from Aja and Tooth. Afterwards, Duna was very cautious around both Aja and Tooth the rest of the observation period, which has affected the behaviours scored. This is especially obvious in play behaviour between Duna and Tooth which happened regularly in the beginning but did not happen much the rest of the period after these incidences. In the months after this incident, Duna was chased by Tooth five more times during the hours of observation.

In the wild, unrelated females are usually chased away by the matriarch, and bonds are rarely formed between unrelated animals (Burks et al., 2004). In captivity, groups of unrelated females show a higher incidence of aggression which can induce stress which may have serious consequences including reduced reproductive function (Brown, 2000; Clubb & Mason, 2002; Freeman et al., 2004). Duna is currently 11 and has not started cycling yet. This might be normal, because elephants in the wild start cycling around the age of 10-12 (Hildebrandt et al., 2006). However, in captivity cycling usually starts at an earlier age (Hildebrandt et al., 2006). It might be possible that cycling in Duna is suppressed by the existence of stress through social factors (see also chapter 6).
12 Conclusions

For this group of captive African elephants, the different stages of the oestrous cycle of one cycling female can be determined by looking at the behaviour of the male and especially the female in proximity of each other. In this herd, the best indicators of (impending) oestrus were rate of flehmen by the male and rate of submissive behaviour by the cycling female towards the male. However, exact moment of ovulation could not be predicted by looking at behaviour without the occurrence of mating.

It might be that Aja is at the end of her reproductive life and is therefore starting to show more irregular cycles. It is possible that because of this, Tooth does not show enough interest and therefore no mating takes place. It might also be that Tooth has low libido from abnormal social behaviour because the group is lacking strong social bonds.

It is possible that Duna has not started cycling yet because of social suppression.
13 Recommendations for this African elephant herd

This chapter will describe recommendations for management and future study for the African elephant herd in Ouwehand zoo derived from this study.

13.1 The female herd

It is very rare for unrelated females in the wild to form a bond and unrelated herds rarely approach within 2 km of each other (Burks et al., 2004). In captivity, many herds consist of unrelated females which can lead to a higher incidence of aggression (Clubb & Mason, 2002) and a lack of social bonds. Many problems that zoos have with management of elephants can therefore be attributed to inadequate social grouping (Veasey, 2006). Consequences of inadequate grouping are stress, higher susceptibility to diseases and reduced reproduction or cessation of cyclicity. Because Duna is already 11 years old and elephants in captivity usually start cycling at an earlier age and the fact that it became clear during observations that Duna is constantly alert to approaches from Tooth or Aja, it is possible that Duna has not started cycling yet due to suppression of cyclicity by social factors.

Separating Tooth and Duna might be a good temporary method for stimulation of cycling in Duna. They will still have contact with each other through a barrier, which may be positive for the onset of cycling because in the wild females sometimes start cycling in the presence of a male (Rasmussen & Schulte, 1998). Once Duna has started cycling regularly, and is in the oestrus period, Tooth can be introduced again. It might be interesting to study if and how behaviour of Duna changes when Tooth is no longer housed with the herd during the day, but in his own outside exhibit.

Although it is less likely for unrelated females to form bonds as it is for related females it is possible and stable herds consisting of unrelated females have been formed (Hutchins, 2006). Females of around the same age are more likely to form a bond (Clubb & Mason, 2002). It is therefore recommended to add two more young females of around Duna’s age, 10-12 years. This will give Duna a chance to build a bond and have social contact. This will also mean that there are more elephants on the same dominance level as Duna, which can mean less attention from Tooth and less social suppression. If two new females are found and will be introduced to the herd, it might be interesting to study behaviour between the herd mates before
Social behaviour in an African elephant herd in relation to reproductive cycle phase
By: Yvonne de Vries

Introduction, during the introduction period and after introduction, to monitor changes in social behaviour, but also stereotypic and other stress related behaviours.

Two more elephants can also help in the formation of a stable female herd, especially if they are related to each other. Ideally, these two elephants have spend their lives with their mother in a stable herd, which ensures they have been around calves and experienced mothers and therefore have a higher chance of successfully raising calves than elephants without this experience (Hutchins, 2006; Hutchins & Keele, 2006, Veasey, 2006). This is in this herd very important because the existing females have no experience around calves.

Especially the bond between mothers and female calves is important. In the future, females can be kept in the herd while males are transported to other facilities at the age of 5 to 8 (Hutchins, 2006). Breeding bulls can be exchanged between zoos to ensure genetic diversity and avoid inbreeding. A herd consisting of at least 3 adult females with their immature offspring is recommended by the AZA (Clubb & Mason, 2002). If the herd becomes to large, whole matrilines (females with their female offspring) should be transported together to start a stable herd at another facility.

Although rumbles have been heard in this herd, the amount of rumbles throughout the cycle have not been quantified. Studies in other herds indicate an increase in rumbling in the anovulatory phase when the male was absent from the herd. This might be an interesting topic for future studies in this herd. In addition to information about reproductive cyclicity, rumbling can also give insight in social relationships. Many rumbles observed during this study were answered by another elephant, quantifying the initiating and answering elephant can give more information on this topic.

13.2 The male

In the wild, adult males usually live solitary and only visit a female group for mating (Schulte, 2000, Ganswindt et al., 2005, Ortolani et al, 2005). They will travel with the herd for several weeks and then move on and therefore do not form bonds with the females.

This is very different in the herd in Ouwehands zoo where the male spends around 6 hours each day with the females in the same exhibit. Because of this he is able to form relationships and take in a place in the herd. This does not have to be a problem because the oldest female in captivity will often still act as the matriarch, even though this is not as crucial as in the
wild. In this herd however, the oldest female is not acting as a matriarch and it might be that because of this, Tooth takes over some of her tasks. For instance, group defence against an outside source during this study was always initiated by Tooth. After his call, the females approached and the herd started bunching. The role of matriarch can take up a lot of energy which is why Freeman et al. (2004) concluded that the matriarch in captivity had a higher chance of being acyclic than the other elephants. If this is the case in this herd, taking up this role can be the reason for the lowered interest from Tooth to Aja. Temporarily separating Tooth from the herd (for instance during periods when none of the females are in oestrus) may present a more natural social situation. Once a stable herd is created, Tooth might not feel the need to act as a matriarch anymore if he is kept with the herd during the day, which might increase reproductive interest.

13.3 Conclusions
- Keep Tooth separated from Duna for a while to see if Duna will show less stress related behaviours and start cycling.
- Introduce two more related females of around 10-12 years of age to the herd.
- Work towards a stable, all related female herd of at least 3 adult females with their immature offspring.
References


Boer, A.M. de, Schaftenaar, W. ‘Monitoring of the oestrous cycle of Asian elephants (*Elephas Maximus*) as an important tool in reproduction and parturition management using a chemiluminescent immunoassay, immulite, to measure blood progesterone’, Proceedings of the 24th Annual Meeting of the Association of Zoo Veterinary Technicians.


Social behaviour in an African elephant herd in relation to reproductive cycle phase
By: Yvonne de Vries


Social behaviour in an African elephant herd in relation to reproductive cycle phase
By: Yvonne de Vries


Appendix 1 Pregnancy

Pregnancy detection and prediction of parturition

The gestation period of elephants is 20 to 23 months ranging from 640 to 673 days (Hildebrandt et al., 2006). The corpora lutea are the most active at 3-15 months of gestation (Hodges, 1998; Hildebrandt et al., 2006). Progestogen concentrations are on average higher than during the non-pregnant luteal phase (Brown, 2000; Hildebrandt et al., 2006). During the gestation period there is a drop in progestogen concentrations after c. 2.5 months and concentrations start to decline gradually after c. 13.5 months (Hildebrandt et al., 2006). An ultrasound study in wild elephants did show new follicles and fresh corpora lutea on both ovaries 4-5 months after conception which persisted through at least 12 months of gestation (Hildebrandt et al., 2006). After 20 weeks of gestation, pregnancy can be diagnosed by measuring prolactin in the blood, which is increased after this time (Hodges, 1998, Brown, 2000). Ultrasonographic detection of embryonic structures before 8 weeks is impossible (Hildebrandt et al., 2006; Hermes et al., 2007). After the progestogen drop around 2.5 months after gestation an embryonic vesicle of 10 mm diameter is detectable (Hildebrandt et al., 2006; Hermes et al., 2007). The use of three-dimensional ultrasonography can give even more informations on foetal development (Hildebrandt et al., 2006). With the use of ultrasonographic techniques, sex determination is possible at 8-12 months of gestation (Hildebrandt et al., 2006). In Asian elephants, foetal sex can also be determined by measuring serum testosterone concentrations after 60 weeks of gestation, which is higher in the presence of male calves (Brown, 2000; Hermes et al., 2007). If this is possible in African elephants is not yet studied (Brown, 2000).

Neonate mortality is zoo’s is high compared to semi-natural and free-ranging populations, in large part due to lack of appropriate maternal behaviour (see also chapter 7) (Szdzuy et al., 2006). Knowing exact time of parturition is important in improving calf survival (Brown, 2000; Szdzuy et al., 2006). Because gestation length can vary between individuals, precise prediction of parturition is necessary (Hildebrandt et al., 2006). Daily monitoring of progestogen concentrations during the last month of pregnancy is recommended (Brown, 2000). One method is the detection of a drop in progestogens to baseline concentrations 2-5 days before parturition which can be measured in serum and urine (Brown, 2000; Hildebrandt et al., 2006; Hermes et al., 2007). Measurement in urine is not used widely because frequent urination, which is common just before parturition, can dilute urine and can result in unreliable
measurements (Hildebrandt et al., 2006). Additionally, parturition can be predicted by daily transrectal ultrasound assessment of the caudal genital tract (Hildebrandt et al., 2006). In this case, the cow needs to be familiar with the procedure and used to strangers and technical equipment at least 6 months before parturition (Hildebrandt et al., 2006).

Physiological signs associated with impending parturition are loss of the mucous plug and start of milk production 2-5 days prior to birth (Szdzuy et al., 2006). An increase in stereotypic behaviour, increased disquietness, an increased rate of urination and defecation and tail beating against the vulva are behaviours associated with impending parturition (Szdzuy et al., 2006).

In most mammals, including the elephant, lactation inhibits cyclicity. On average, this postpartum lactational period, which is characterised by low progestogen concentrations, is 46 weeks in elephants but can be reduced to as short as 8 weeks when problems such as retained placenta, reduced milk production, death of the calf or premature weaning take place (Brown, 2000).

**Problems during gestation and birth**

Even if elephants become pregnant, problems such as early embryonic loss, dystocia, maternal aggression and inexperience in infant care can still limit reproductive success (Hermes et al., 2004).

In addition to some diseases, stress is an important cause of early embryonic loss (Clubb & Mason, 2002). This can be the reason for the poor conception rate seen in elephants that have been transferred to other zoos for mating (Clubb & Mason, 2002).

Excessive body weight, older cows, stress, hypocalcaemia and diseases such as the endotheliotropic elephant herpes virus (EEHV) are some known causes of stillbirth in Asian elephants (Clubb & Mason, 2002). Because not much data is available on stillbirths in African elephants, the exact amount of stillbirths is unknown, but this appears to be less than in the Asian elephant (Clubb & Mason, 2002). Stillbirth can not only result in death of the calf, but in 5% of the cases also in death of the cow (Hermes et al., 2007). Dystocia is more likely to arise in primiparous females older than 20 years (Hermes et al., 2007). Other risk factors of dystocia are a prenatal herpes infection, lack of physical fitness, excessive body weight and an inability to adapt to the birthing process (Hermes et al., 2004).
It is necessary that all pregnant elephants are monitored carefully and put on a diet to avoid these complications (Hermes et al., 2004; Hermes et al., 2007). Before gestation, the position of the foetus can be determined with the use of ultrasound (presence, anterior position, or absence, posterior position, of foetal toe nail) (Hildebrandt et al., 2006). This is important because more dystocia complications can arise with the anterior position (Hildebrandt et al., 2006). Caesarean section is described as a surgical intervention of dystocia, however, all seven caesarean sections that are reported resulted in death of the female and calf (Hermes et al., 2007). Other methods to use in case of dystocia are transrectal massage in combination with application of oestrogens, episiotomy and if all else fails, foetotomy to save the cows live (Hermes et al., 2007). Care must be taken with the administration of oxytocin, a frequently used method in dystocia in other animals, because the elephants uterus reacts strongly to this hormone, and fast exhaustion and uterus rupture are common complications (Hermes et al., 2007).
Appendix 2 Artificial reproductive techniques

In many endangered species, assisted reproductive techniques (ART) such as artificial insemination (AI), in vitro fertilization/embryo transfer and gamete cryopreservation can assist in conservation management programs (Brown, 2000). The use of these ART’s can help in the improvement of the reproduction rate of captive elephant populations and the maintenance of genetic diversity (Brown, 2000, Hildebrandt et al., 2006). Especially in populations where males and females are held at separate locations and few males are held, these are very valuable (Brown, 2000; Olsen & Wiese, 2000). In addition, because ART’s eliminates the need for transportation of elephants, and semen is pre-screened for diseases, the risk of disease transmission is greatly reduced (Hildebrandt et al., 2006).

Currently, only AI is used in elephants. However, because of the size of the reproductive tract of the elephant, 3 m from vestibule to ovary, the difficult anatomy, vaginal os of 4 mm x 2 mm in diameter with two blind pouches an each side and difficulties determining exact timing of ovulation, AI in the elephant is very difficult (Brown, 2000; Hildebrandt et al., 2006; Hermes et al., 2007). Recently developed transrectal ultrasound techniques (Hildebrandt et al., 2006; Hermes et al., 2007) and better endocrine monitoring (Brown, 2000; Hermes et al., 2007) lead to better results in the past few years.

An alternative method of AI is surgical AI in which a small incision is made, the opening of the vagina is visualised and semen is deposited (Hermes et al., 2007). Although this method is technically not as demanding as non-surgical AI, it requires a 4-6 week recovery time, which is not ideal for repeated inseminations (Hermes et al., 2007).

Another technique that might be useful, but is not yet performed in elephants is sperm sexing. If reproduction increases, the same amount of male and female calves will be born which means there will be more bull calves than zoos can handle. With sperm sexing combined with AI, the number of males can be controlled, and more females and less males will be born. Hermes et al. (2007) determined that the use of non-sexed sperm combined with the use of female biased sperm after 10 years proved to have the best results for the viability of the population.
In species with very low numbers, the use of IVF, ICSI and Embryo Transfer (ET) is necessary for the preservation of all genetic material and reduce the inbreeding factor (Hermes et al., 2007). In elephants, these techniques have only been performed with material collected from death animals. In livestock, follicles have been collected successfully from living animals, however, in large animals such as elephants, their anatomical features and poor skin healing abilities are a challenge. Some successes have been made with transrectal ultrasound-guided follicle aspiration in a rhinoceros, which provides good prospects for the future (Hermes et al., 2007). Embryo transfer has not yet been performed in elephants, but recent advances in endocrine monitoring provide good conditions for this method (Hermes et al., 2007). More research is needed in order to develop techniques to harvest from live donors and use embryo transfer in elephants.

One problem for the development of these techniques is that large amounts of material is needed, while not enough animals are available (Hermes et al., 2007). Additionally, although elephant sperm can be cryopreserved, all successful inseminations were from freshly collected sperm (Hermes et al., 2007).
Appendix 3 Five freedoms

The five freedoms give a framework in which to assess an animal's environment in order to improve welfare. Although they were developed for agricultural animals, they are also applicable to zoo animals.

The five freedoms:
1. Freedom from injury and disease
2. Freedom from hunger, thirst and malnutrition
3. Freedom from discomfort
4. Freedom to express “natural behaviour”
5. Freedom from fear and distress
Appendix 4 Profiles of the three elephants of Ouwehand zoo
(translations from keepers handbook)

Elephant profile Aja

1 Statistics

Name: Aja  
Breed: Loxodonta Africana  
Gender: Female  
Date of birth: 1971 in Zimbabwe  
Origin: 2005-7th April 2006 Sevilla (Spain)  
1974-2005 Zoo Erfurt (Germany)  
Species ID: Arks 1639

2 Character of the animal

Dominant, nice, lots of life experience. Is sometimes aggressive while eating and can react aggressively to Duna at this time. The elephants should therefore be separated while eating. Stable in training over time.

3 Relation

Dominant cow or matriarch.

4 Management system

Protected contact

5 Remaining aspects

Still cycling regularly, determined by weekly blood draw. Performs more stereotypic behaviour in the beginning of the anoestrus.
Elephant profile Duna

1 Statistics

Name: Duna  
Breed: Loxodonta Africana  
Gender: Female  
Date of birth: 18.12.1996  
Origin: 2001-7th April 2006 Sevilla (Spain)  
Species ID: Arks 1638  

2 Character of the animal

Studious, can react very strongly, especially to new situations, nice, most subordinate, investigative, learns new thing very fast during training.

3 Relation

Most subordinate, has to endure a lot from both Aja and Tooth…

4 Management system

Protected contact

5 Remaining aspects

Weekly brood draw since march 2007 to determine possible onset of cycling.
Elephant profile Tooth

1 Statistics

<table>
<thead>
<tr>
<th>Name:</th>
<th>Tooth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed:</td>
<td>Loxodonta Africana</td>
</tr>
<tr>
<td>Gender:</td>
<td>Male</td>
</tr>
<tr>
<td>Date of birth:</td>
<td>1993, Kruger park</td>
</tr>
<tr>
<td>Species ID:</td>
<td>Arks 2013</td>
</tr>
</tbody>
</table>

2 Character of the animal

Studious, can react very strongly, especially to new people, undertakes a lot, temperamental, can react aggressively if irritated.

3 Relation

Is the intended breeding bull, has no offspring to date.

4 Management system

Protected contact.

5 Remaining aspects

Is A.I. donor.
Appendix 5 Schematic drawing of the elephant facilities

Figure 10 Elephant exhibit. 1; trainings area. 2; outside area of the bull. 3; outside area of the females. Red cross; position of observer. During observation all elephants has access to area’s 2 and 3.
Appendix 6 Ethogram

Behaviours of the first category (distance) do not count if there is a barrier between the elephants (eg: fencing between the male and female exhibit, a large tree trunk, see also appendix 7) because during observations it became clear that the most subordinate animal would approach the most dominant animal if there was a barrier in between where she normally would avoid him. Counting this as proximity therefore would give unreliable results. All other behaviours are also scored if the elephants show this with a barrier between them.

<table>
<thead>
<tr>
<th>Category</th>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance</td>
<td>Proximity</td>
<td>One elephant standing within two body lengths (approx 8 m) of one or more elephants, both must be stationary.</td>
</tr>
<tr>
<td></td>
<td>Time in proximity</td>
<td>The time that both elephants are in proximity (approx 8 m) of each other.</td>
</tr>
<tr>
<td></td>
<td>Approach</td>
<td>One elephant decreases its distance to one or more stationary elephants within a radius of two body lengths, must end stationary.</td>
</tr>
<tr>
<td></td>
<td>Leave</td>
<td>One elephant increases its distance to one or more stationary elephants within a radius of two body lengths, must be stationary at start.</td>
</tr>
<tr>
<td>Reproductive</td>
<td>Trunk tip to mouth</td>
<td>Trunk tip touches mouth region</td>
</tr>
<tr>
<td>behaviour</td>
<td>Trunk tip to eye</td>
<td>Trunk tip touches eye region</td>
</tr>
<tr>
<td></td>
<td>Trunk tip to head</td>
<td>Trunk tip touches frontal-superior area of head</td>
</tr>
<tr>
<td></td>
<td>Trunk to head</td>
<td>Entire trunk rests onto superior region of head</td>
</tr>
<tr>
<td></td>
<td>Trunk tip to</td>
<td>Trunk tip touches temporal gland</td>
</tr>
<tr>
<td><strong>temporal gland</strong></td>
<td><strong>Trunk tip to ear</strong></td>
<td><strong>Trunk tip touches ear region</strong></td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td><strong>Trunk over back resting</strong></td>
<td>Male places trunk over Female’s back and rests it there for at least 2 sec while stationary (1–2 steps allowed) Placing at least two thirds of length of the trunk over the back, head or neck of another. Does not include sweeping hay, sand or other material from another. This behaviour may be confused with the affiliative variations. See Sweeping listed in Affiliative Behaviours for distinctions.</td>
<td></td>
</tr>
<tr>
<td><strong>Trunk over back driving</strong></td>
<td>Male places trunk over Female’s back and pushes her forward (Male and Female are moving for more than 2 steps).</td>
<td></td>
</tr>
<tr>
<td><strong>Resting chin over back</strong></td>
<td>Male rests lower jaw over Female’s buttocks, stationary.</td>
<td></td>
</tr>
<tr>
<td><strong>Trunk tip to base of tail</strong></td>
<td>Trunk tip touches area around anus and base of tail.</td>
<td></td>
</tr>
<tr>
<td><strong>Trunk to tail</strong></td>
<td>Trunk tip touches the tail.</td>
<td></td>
</tr>
<tr>
<td><strong>Tail grab</strong></td>
<td>Trunk wrapped around tail, pulling.</td>
<td></td>
</tr>
<tr>
<td><strong>Trunk tip to hind leg/foot</strong></td>
<td>Trunk tip touches inferior region of leg or foot area.</td>
<td></td>
</tr>
<tr>
<td><strong>Leg grab</strong></td>
<td>Trunk wrapped around other individual’s leg, exerting force.</td>
<td></td>
</tr>
<tr>
<td><strong>Trunk tip to genitalia</strong></td>
<td>Trunk tip touches genital area (opening of urogenital canal for Female, the penis for Male).</td>
<td></td>
</tr>
<tr>
<td><strong>Trunk tip to urine</strong></td>
<td>Trunk tip comes in contact with another individual’s urine.</td>
<td></td>
</tr>
<tr>
<td><strong>Trunk tip to faeces</strong></td>
<td>Trunk tip comes in contact with another individual’s faeces.</td>
<td></td>
</tr>
<tr>
<td><strong>Flehmen</strong></td>
<td>Following olfactory investigation of another Elephant’s genitals/urine/faeces, the trunk tip is placed in the mouth.</td>
<td></td>
</tr>
<tr>
<td><strong>Mounting</strong></td>
<td>Male places front legs over Female’s hips standing on back legs only for at least 2 sec; may be stationary or moving.</td>
<td></td>
</tr>
<tr>
<td>Attempted mount</td>
<td>When mounting lasts 2 sec.</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------</td>
<td></td>
</tr>
<tr>
<td>Attempted intromission</td>
<td>Male attempts to intromit penis into vagina but fails.</td>
<td></td>
</tr>
<tr>
<td>Intromission</td>
<td>Male inserts entire penis (or part of) into Female’s vagina.</td>
<td></td>
</tr>
<tr>
<td>Active aggression</td>
<td>头对头，抬起脖子，相互拉扯和推挤，用鼻甲相互接触。另外，也可以看到它们在玩耍时的差异。</td>
<td></td>
</tr>
<tr>
<td>Head butt</td>
<td>Butting forehead or base of trunk against body or forehead of another.</td>
<td></td>
</tr>
<tr>
<td>Tusk</td>
<td>Contact of tusk(s) with another elephant or object accompanied by a forward, lunging motion. Head of the initiator is usually held up.</td>
<td></td>
</tr>
<tr>
<td>Charge</td>
<td>Rapid, forward lunging motion or rapid gait toward another with head held above shoulders, ears held perpendicular to the body and trunk held up. No physical contact.</td>
<td></td>
</tr>
<tr>
<td>Push</td>
<td>Contacts another with the body, head or base of trunk causing other to move.</td>
<td></td>
</tr>
<tr>
<td>Drive</td>
<td>Placing head or tusks to rear of another resulting in a continual displacement of at least two body lengths.</td>
<td></td>
</tr>
<tr>
<td>Trunk hit</td>
<td>Quick sharp contact to another with the dorsal side of the distal end of the trunk.</td>
<td></td>
</tr>
<tr>
<td>Spar</td>
<td>Individuals face each other head-to-head with raised chins, pulling and pushing with intertwined trunks. Includes mouth wrestling with trunks thrown back over the head. Head is held above shoulders. Also seen in play situations. See distinctions under Affiliative Behaviours.</td>
<td></td>
</tr>
<tr>
<td>Push down on head</td>
<td>Pushing down on another’s head with the base of trunk, tusks or open mouth.</td>
<td></td>
</tr>
<tr>
<td>Kick</td>
<td>Kicking with forefoot or hind foot. May be observed in play. Distinction: in play it will be directed at objects.</td>
<td></td>
</tr>
<tr>
<td>Throw object</td>
<td>Throwing an object toward a conspecific utilizing the trunk.</td>
<td></td>
</tr>
<tr>
<td>Kick object</td>
<td>Kicking an object toward a conspecific.</td>
<td></td>
</tr>
<tr>
<td>Chase</td>
<td>Rapid pursuit of a conspecific actively moving in a direction away from the initiator.</td>
<td></td>
</tr>
<tr>
<td>Other active</td>
<td>Any other active aggressive behaviour.</td>
<td></td>
</tr>
</tbody>
</table>
### Social behaviour in an African elephant herd in relation to reproductive cycle phase

By: Yvonne de Vries

<table>
<thead>
<tr>
<th>Aggression</th>
<th>Description</th>
</tr>
</thead>
</table>
| **Passive aggression**            | **Head shake**  
Head is held above shoulders and shaken vigorously from side to side causing trunk to move vigorously. May be observed in play situations. Distinction: in play, it will be directed at objects. |
| **Head up/ears out**              | Head is held up above shoulders with trunk up and ears out perpendicular to the body. Ears may be waved. May be observed with Head shake. May be observed in play situations. Distinction: in play, it will be directed at objects. |
| **Water spray**                   | Expulsion of water previously drawn into the trunk in the direction of a conspecific.                                                        |
| **Other passive aggression**      | Any other passive aggressive behaviour.                                                                                                     |
| **Submissive behaviour**          | **Avoid**  
Moving/orienting away from another animal that is either approaching or within trunk length.                                                    |
| **Back up**                       | Walking backward into another animal.                                                                                                       |
| **Scream**                        | High frequency vocalization emitted from the opened mouth (not from the trunk).                                                              |
| **Affiliative behaviour**         | **Trunk intertwine**  
Animals stand head-to-head, intertwine trunks. This behaviour may be confused with trunk wrestling/sparring. Distinctions: less muscle tension in trunk, heads are not held above shoulders. |
| **Play spar**                     | Individuals face each other head-to-head with raised chins, pulling and pushing with intertwined trunks. Includes mouth wrestling with trunks thrown back over the head. This behaviour is also seen in aggressive situations. Distinctions: overall intensity level is less during play, heads may/may not be held above shoulders. |
| **Sweeping**                      | Placing two-thirds of the trunk over the back or head of another, with sweeping motions. This behaviour may be confused with Trunk over back. Distinction: The aggressive version includes a higher degree of tension in the trunk tip. |
| **Head rubbing**                  | Rubbing head, face or body against another.                                                                                                  |
Appendix 7 Barriers in the observation area

Figure 12 Barrier 1, tree trunk.

Figure 13 Barrier 2, water pool.
Figure 14 Barrier 3, iron fencing between the two outside areas.
Appendix 8 The immulite

The Immulite is a random access chemiluminescent immunoassay analyser. The system is built around a proprietary test unit that provides for rapid and efficient washing of a captive antibody-coated bead. The test unit serves as the reaction vessel for the immune reaction, incubation, wash and signal development. Light emission from the chemiluminescent substrate reacting with enzyme conjugate bound to the bead is proportional to the amount of analyte originally present in the elephant sample. The progesterone assay is a two-step competitive immunoassay with an incubation time of 2x 30 minutes. The bead is coated with polyclonal rabbit anti-progesterone. The first reagent is a ligand-labeled synthetic progesterone. The second reagent is an alkaline phosphatase (bovine calf intestine) conjugated to anti-ligand in buffer. The measured signal is inversely proportional with the concentration of the progesterone. (Boer & Schaftenaar)
Appendix 9 Oestrous cycle Aja

Figure 15 Progestogen values during the oestrous cycle of Aja over the past 6 months. The pink line (at 0.15) indicates the line between baseline and elevated values calculated with the described method from Leong et al. (2003) and Ortolani et al. (2005). This method did not reveal a cycling pattern.
Figure 16 Progestogen values during the oestrous cycle of Aja over the past 6 months. The pink line (at 0.26) indicates the line between baseline and elevated values used in this study. The dark blue lines indicate the luteal phase, the green lines indicate the anovulatory phase and the red lines indicate the ovulatory phase. The light blue lines indicate the values that are not classified in any phase.
Appendix 10 Article about Ouwehand zoo from ‘Elefanten in zoo und circus’.

The following article about the elephants in Ouwehands zoo Rhenen was published in a German magazine (Elefanten in zoo und circus number 12, November 2007) about elephants.
Die neue Elefantenanlage im Zoo Rhenen

Bearbeitung und Fotos:
Joachim Endres, Olaf Paterok und
Jürgen Schillfarth


Elefantenkuh „Ajá“, 35 Jahre alt.


Blick auf die große Elefantenanlage im Zoo Rhenen vom Bullengehege aus (rechts).

Die 11-jährige Elefantenkuh „Duna“.

Der 14-jährige Bulle „Tooth“.
Zur Historie der Elefantenhaltung und -anlagen in Rhenen


Nach einem erneuten Wechsel in der Leitung des Zoos standen wieder die Elefanten auf dem Programm und man plantete eine komplett neue Elefantenanlage, zunächst in Richtung asiatische Elefanten. Die Verhandlungen mit einem italienischen Circus über den Erwerb von 3 älteren Asiatenkühen zerschlugen sich jedoch, weil die niederländischen Behörden betriebsdienstliche Gesundheitszeugnisse für die Tiere verlangten, welche vom Circus nicht beigebracht werden konnten.

Die neue Elefantenanlage im Zoo Rhenen

Elefanten in Rhenen seit 1935

<table>
<thead>
<tr>
<th>Art</th>
<th>Name</th>
<th>Geburt</th>
<th>Tod</th>
<th>Stationen</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.m.</td>
<td>0.1 Berga</td>
<td>~1930</td>
<td>wild</td>
<td>tot</td>
</tr>
<tr>
<td>E.m.</td>
<td>0.1 Nelly</td>
<td>~1934</td>
<td>wild</td>
<td>tot</td>
</tr>
<tr>
<td>E.m.</td>
<td>1.0 Robbie</td>
<td>~1937</td>
<td>wild</td>
<td>?</td>
</tr>
<tr>
<td>E.m.</td>
<td>0.1 Elma</td>
<td>?</td>
<td>wild</td>
<td>?</td>
</tr>
<tr>
<td>E.m.</td>
<td>0.1 Sonja</td>
<td>?</td>
<td>wild</td>
<td>?</td>
</tr>
<tr>
<td>E.m.</td>
<td>0.1 Rhena</td>
<td>?</td>
<td>wild</td>
<td>?</td>
</tr>
<tr>
<td>E.m.</td>
<td>0.1 Anka</td>
<td>1965</td>
<td>wild</td>
<td>lebt</td>
</tr>
<tr>
<td>E.m.</td>
<td>0.1 Raja</td>
<td>1965</td>
<td>wild</td>
<td>lebt</td>
</tr>
<tr>
<td>L.a.</td>
<td>0.1 Rhena</td>
<td>?</td>
<td>wild</td>
<td>tot</td>
</tr>
<tr>
<td>E.m.</td>
<td>0.1 Khaing Nwe</td>
<td>1980</td>
<td>wild</td>
<td>tot</td>
</tr>
<tr>
<td>E.m.</td>
<td>0.1 Khaing Soe Soe</td>
<td>1982</td>
<td>wild</td>
<td>lebt</td>
</tr>
<tr>
<td>E.m.</td>
<td>0.1 Khaing Phyoe Phyoe</td>
<td>1981</td>
<td>wild</td>
<td>lebt</td>
</tr>
<tr>
<td>E.m.</td>
<td>0.1 Yu Yu Yin</td>
<td>1979</td>
<td>wild</td>
<td>lebt</td>
</tr>
<tr>
<td>E.m.</td>
<td>0.1 Tin Tin Htoo</td>
<td>1983</td>
<td>wild</td>
<td>tot</td>
</tr>
<tr>
<td>E.m.</td>
<td>0.1 Khaing Hnin Hnin</td>
<td>1982</td>
<td>wild</td>
<td>lebt</td>
</tr>
<tr>
<td>L.a.</td>
<td>0.1 Aja</td>
<td>1972</td>
<td>lebt</td>
<td></td>
</tr>
<tr>
<td>L.a.</td>
<td>1.0 Tooth</td>
<td>1993</td>
<td>lebt</td>
<td></td>
</tr>
</tbody>
</table>

Die neue Elefantenanlage im Zoo Rhenen

Im Frühjahr 2007 konnten „Aja” und „Duna” in ihre endgültige Unterkunft ziehen und somit war der Platz im Bullenhaus frei für die Ankunft von „Tooth“.

„Tooth” ist erst der zweite nachgewiesene Elefantenbulle in der Haltungsgeschichte von Ouwehands Dierenpark, jedoch der erste männliche Afrikaner auf dem Grebbeberg in Rhenen.

Details der neuen Elefantenanlage im Zoo Rhenen
(vgl. auch Grundrisse)

Blick auf die beiden Elefantenhäuser: links das für Kühe, rechts die Bullenstellungen.

Außenkral
(Vorgehege sowie Trainingsareal + Verbindungsfunction zwischen Häusern und Außengehegen): 360 m²

Außenanlage für Bulle: 1.200 m²
Außenanlage für Kühe: 3.670 m²
Gesamtfläche außen: 5.230 m²
Gesamtfläche innen: 376 m²
Gesamtfläche innen und außen für Elefanten: 5.606 m²

Abb. rechts: Grundriss der Elefantenanlage:
1 = Kral, 2 = Bullenanlage, 3 = Kuhanlage, 4 = Bullenhaus, 5 = Haus für Kühe
Die neue Elefantenanlage im Zoo Rhenen

Haltungssystem: Geschützter Kontakt


Im Außengehege der Kühe befindet sich ein Badebecken, daneben wurde noch zusätzlich eine Dusche installiert. Als Komfortstellen zur Körperpflege sowie als fixe Punkte zum Umrunden und Ausweichen dienen Baumstämme und Felsen. Zur Beschäftigung ist auch ein Bierfass mit Löchern vorgesehen, welches von den Elefanten gedroht werden kann, um Nüsse und Pellets aus den Löchern zu schleudern – was sich bei den Elefanten großer Beliebtheit erfreut.

Danksagung

Für die Überlassung der Grundrisszeichnungen und bautechnischer Einzelheiten bedanken wir uns bei Ouwehands Dierenpark recht herzlich!