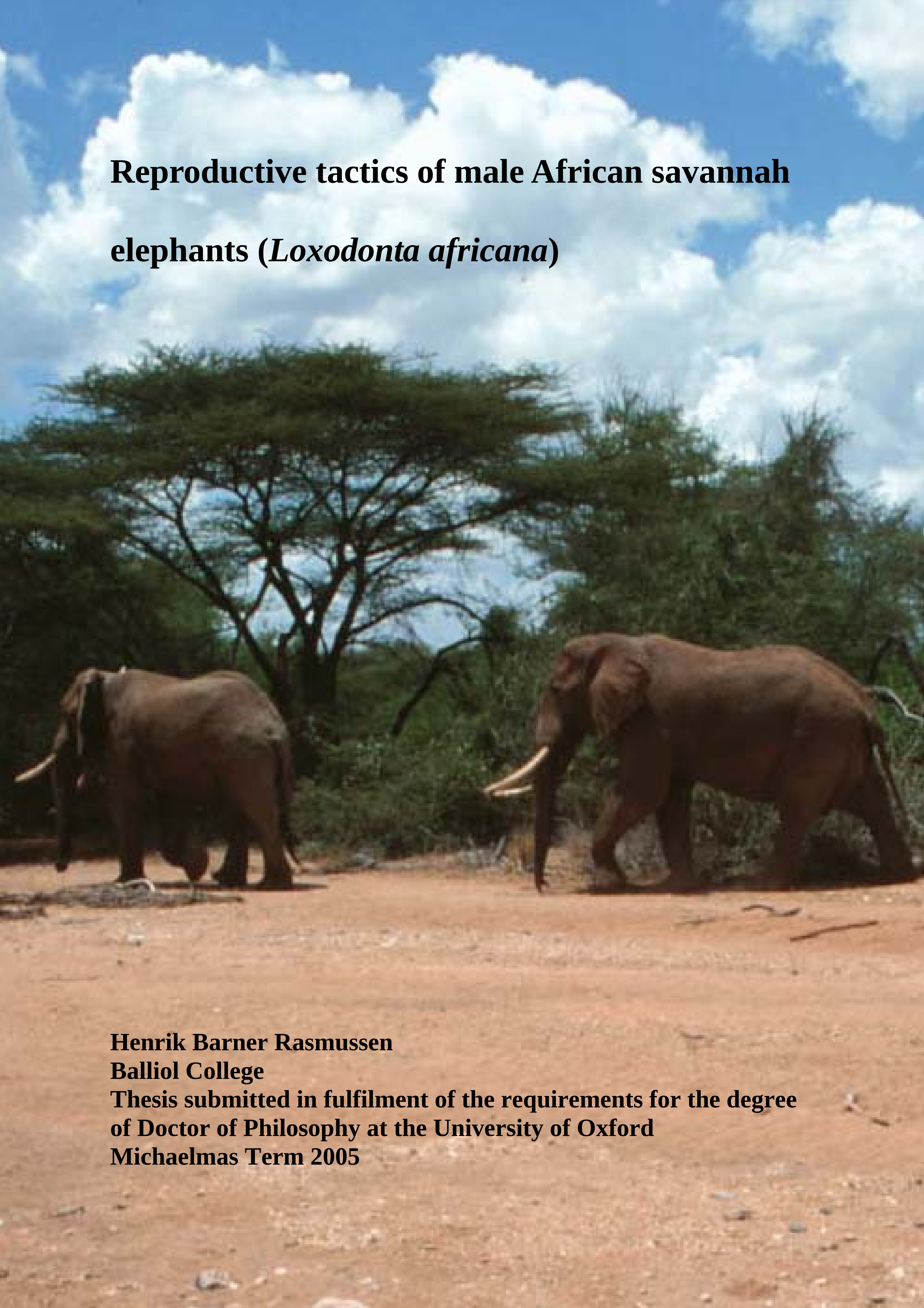

Reproductive tactics of male African savannah elephants (*Loxodonta africana*)

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Balliol College
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Michaelmas Term 2005

Abstract

The present thesis investigates aspects of the reproductive strategy of male African savannah elephants (*Loxodonata africana*). The existence of, and differences between alternative conditional dependent reproductive tactics are evaluated using a combination of behavioural, endocrinological and GPS tracking data and the age and tactic related success is measured using genetic paternity analysis.

Hidden Markov Models were used as a probabilistic framework for analysing temporal changes in reproductively active and inactive periods based on shifts in association preferences of individuals. Distinct shifts between active and inactive periods were evident well before the onset of the aggressive reproductive tactic of musth, seen in older dominant males, hence providing the first quantitative evidence for the previously suggested sexually active periods in non-musth males.

The link between hormones and reproductive status and tactics were investigated using a new technique for non-invasive faecal analysis of hormones. A combined analysis of androgens (Epiandrosterone) and glucocorticoid (3a,11-oxo-CM) hormones in relation to age, reproductive state and musth signals confirmed previously reported elevated levels of androgens during periods with temporal gland secretion and urine dribbling (Musth) but further showed that this increase is indeed linked to the presence of musth signals and not to the age of the individual. Androgen levels were generally increased during sexually active periods with a two-fold increase seen in active non-musth bulls and a four to six-fold increase in musth bulls. Contrary to expectations, increased glucocorticoids outputs were not seen in musth bulls but slightly elevated outputs were seen in active non-musth bulls. Brief peaks in glucocorticoids occurred immediately after physical fights and during periods of injuries. A general elevation of glucocorticoids was seen towards the end of the long dry season, a likely effect of ecological conditions. Behavioural changes and onset of musth signals occurred after an initial change in androgen levels suggesting that sex steroids may play an activiational role of sexually active periods as well as activation of the musth tactic within sexually active periods. Some evidence was found for increased androgen levels following encounters with receptive females, suggesting that such encounters may act as a “boosters vaccine” on androgen levels and hereby prolong active periods at times with high numbers of receptive females.

Both musth and sexually active non-musth (SAN) bulls reduced their foraging and walked more than sexually inactive bulls. However musth bulls had a higher time specific investment in reproduction compared to SAN bulls with musth bulls doubling their daily walking distance compared to a 50% increase in SAN bulls. The (younger) non-musth bulls spent a larger part of the year sexually active (70%) compared to musth bulls (20%) hence SAN bulls have a tactic of prolonged low investment compared to the short and high investment seen in musth bulls, emphasising the need for evaluating the duration of reproductive bouts when comparing overall investments between tactics.

The youngest (non-musth) bull to sire offspring was 21 years but older musth bulls above 35 years had a much higher age specific reproductive success compared to bulls below 30 years. However on a population level, bulls less than 30 years contributed 30% of the reproduction and 20-25% could be attributed to non-musth bulls.

Acknowledgements

This thesis would not have been written without the help an support of a great deal of people and for some of life's strange coincidences. Iain Douglas-Hamilton and my advisor Fritz Vollrath have supported me throughout the process and deserve a special thank together with Thiemo Krink. However without a casual remark from Morten Bjerrum drawing my attention to a small hand written note saying "Do you want to work with elephants" now many years ago at University of Aarhus I may have ended up above the arctic circle rather than studying elephants near equator.

Many others have helped me during the last years. The entire Save the Elephants team have provide immense help, especially Wainaina Kimani (WK), Juliet King and Njoki Kibanja for sorting out my permits and accounting mess, discussions and general help. In the field the sometime tedious hours would have been less fun without my assistant and great companion Lugard Lenaiyasa. I also owe much to George Wittemyer who has been a great friend, colleague and discussion partner in the field and during our collaborations on the genetic project. I am happy that one of my focal bulls probably saved you life when you "played" with one of the musth bull. Don't mess with the big guys. David Daballen also provided help in the field and has become one of my closest friends and family in the process. Two non-organic but potentially sentient beings "The Beast" and "The Lunch-box" provide transport in the field, don't know if I should thank or curse you!

Several collaborations have been an integrated part of this project. The physiological studies were conducted in collaboration with Dr. Andre Ganswindt and Prof. Keith Hodges from German Primate Centre in Göttingen, Germany. Genetic analysis of paternity was carried out in collaboration with John Okello, University of Makarere, Uganda and Dr. Hans Siegismund University of Copenhagen, Denmark and is part of a larger ongoing genetic study on African elephants conducted in collaboration between Save the Elephant and the Wildlife Genetic Project. Dr. Gerton Lunter enlightened and helped me in the use of hidden Markov models.

Without money this study would never have come to be. I am especially grateful for the support given by the International Elephant Foundation, by the funding provided by Iain Douglas-Hamilton's Dawkins price for animal conservation and welfare, Balliol College and the immense support provided by Save the Elephant and it's donors who believe that not all assets should be place on the stock exchange. However without emergency funding from my parents during the last leg of the project I would have been stranded before completion.

I would also like to thank The Office of the President of the Republic of Kenya the Kenya Wildlife Service and especially Samburu and Isiolo County council for permission to conduct this research as well as the warden and rangers of Samburu, Shaba and Buffalo Springs National Reserves.

Finally without David Mayntz and Cedric Dicko my time as a D.Phil. student here in Oxford would have been a great deal less joyful (although the completion of the thesis may have occurred earlier).

Oxford 28 September 2005

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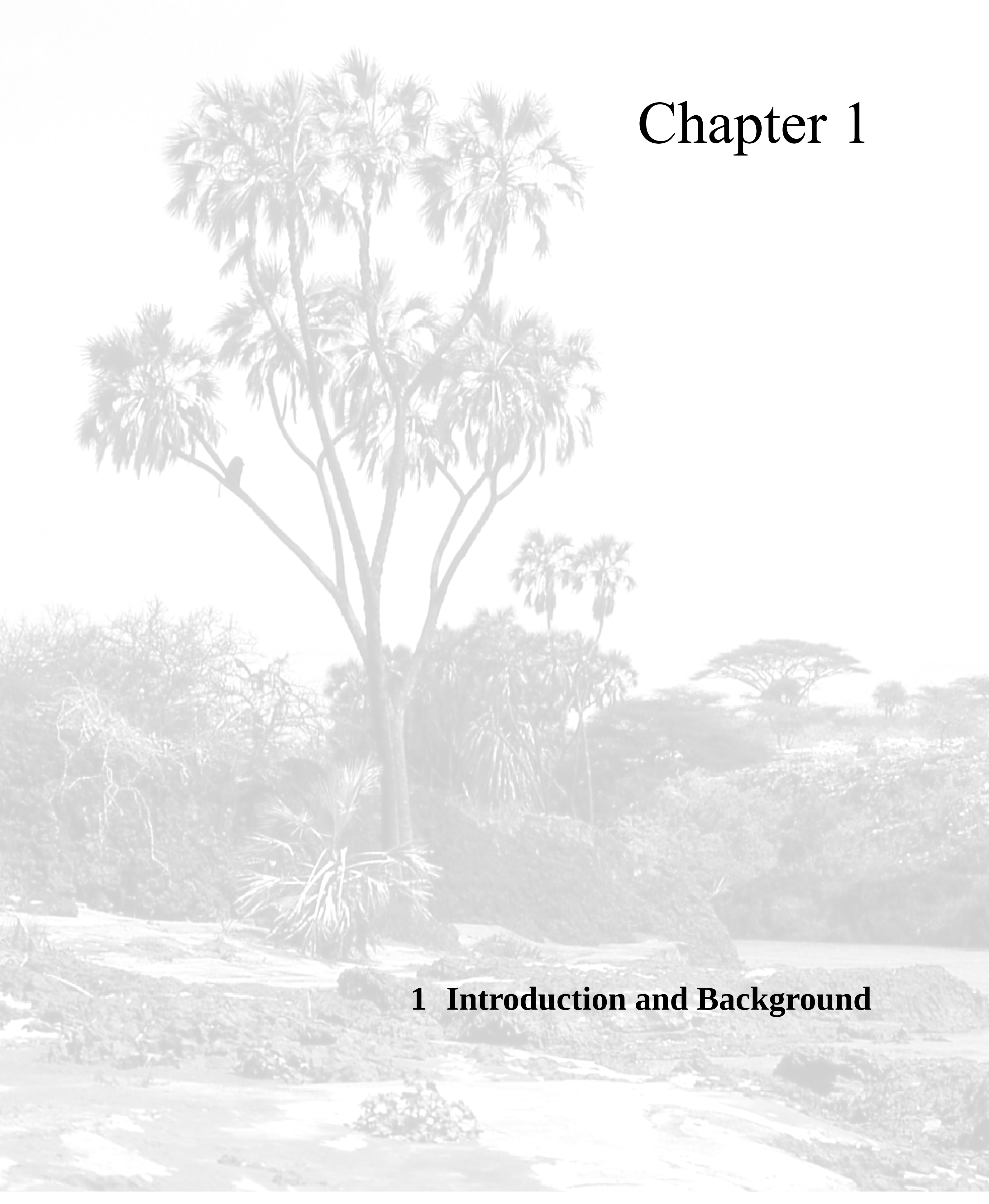


Chapter 1

1 Introduction and Background

1.1 Introduction

The African elephant is not only the largest terrestrial animal but also covers an amazing range of habitats. It can be observed from desert environments in Mali and Namibia to tropical rainforests in the Congo Basin and from the coast of the Atlantic and Indian Ocean to the alpine slopes of Mt. Kenya and Kilimanjaro (Blanc et al., 2003). This extreme ability to utilize such a wide diversity of habitats may partly be based on regional genetic adaptation with the forest elephant now considered a separate species (*Loxodonta cyclotis*) from the savannah elephant (*Loxodonta africana*) (Roca et al., 2001) and with a large degree of genetic differentiation among populations of the savannah elephant (Comstock et al., 2002; Eggert et al., 2002; Siegismund & Arctander, 1995; Georgiadis et al., 1994). However plasticity in behavioural phenotypes may also account for a large part of this ability to adapt to different environments. The influence of environmental, ecological and individual conditions on behaviour and decision-making is an interesting subject, which has attracted considerable attention over the last decades. In addition, recent advances in tracking technology has now made it possible to further investigate how these factors affect spatial use and movement patterns of individuals in addition to locating individuals for regular observations.

Many of the factors theoretically expected to influence both reproductive and foraging decisions in elephants are currently undergoing rapid changes on both local and continental scales. Many populations of African elephants have or will soon experience changes in spatial structure due to habitat fragmentation, increased fencing and changing climatic conditions. These changes are also likely to influence overall population densities, increasing in protected areas (as currently seen in Southern and East Africa) and decreasing outside parks and reserves (Blanc et al., 2003).

Figure 1.1 Current distribution of the African savannah and forest elephant. (Reproduced from (Blanc et al., 2003).

The increasing numbers within protected areas are a management concern with various measures of fertility control currently under investigation (Poole, 1994a; Fayrer-Hosken et al., 2000) with several of the considered solutions likely affecting the relative occurrence of receptive females and hence operational sex-ratio within such populations. Potential changes in the age structure, especially of males may also arise either via illegal hunting for ivory mainly affecting the older males or via legal sports hunting and problem animal control. Understanding how reproductive tactics and movements differ among male elephants and how these are influenced by individual conditions in conjunction with spatial, ecological and social factors is therefore both theoretically interesting and highly relevant for the conservation and management of the African elephant.

The topic of sexual selection and allocation of reproductive effort was first introduced by Charles Darwin (Darwin, 1871). Since the 1960's the theoretical framework and the number of field studies on optimal reproductive strategies under varying ecological and social conditions has undergone a rapid development and some general patterns are now emerging where male reproductive strategies to a large extent can be explained by the spatial and temporal distribution of females. (e.g. (Clutton-Brock & Harvey, 1978; Rubenstein, 1980; Clutton-Brock, 1989; Trivers, 1972; Emlen & Oring, 1977). Understanding the distribution and organisation of females and especially variations within these parameters are thus important in order to understand the reproductive decisions made by males. Furthermore, mating systems are now seen as the outcome of individual strategies rather than a species-specific characteristic (Clutton-Brock & Harvey, 1978; Emlen & Oring, 1977). Consequently, as individual conditions vary and to a large extent are conditional, we can expect to find intra species variation depending on the individual status as well as conditions of other individuals (Rubenstein, 1980).

1.1.2 Reproductive behaviour of male African elephants

Male African savannah elephants leave their natal family after puberty around the age of 14-17 years (Laws et al., 1975; Moss & Poole, 1983; Douglas-Hamilton, 1972) , giving a bull a potential reproductive lifespan of over 40 years. Post mature growth continues throughout most of their life, with bulls having almost doubled their size by the age of 45 years (Lee & Moss, 1995; Laws, 1966; Laws et al., 1975; Hanks, 1972b). Hence, during a bull's adult life it is faced with large changes in intrinsic factors like competitive ability, closely linked to size (Poole, 1989a) and expected future reproductive success, linked to age and mortality (Stearns, 1992). In addition local ecological and social conditions as well as competitive environment and density of receptive females often vary considerably. In this environment individual bulls most attempt to navigate a lifetime optimal reproductive decision path. In this process the individual is faced with both short and long-term reproductive decisions. Short term decisions influencing time-specific success such as when and where to search for females during a given year may not have long-term effects whereas decisions on general age specific investments in reproductions versus growth and level of competitiveness likely to influence mortality will have prolonged effects on future success.

Both sexes of elephants are promiscuous with males having no parental care (Sukumar, 2003). Males are generally regarded as having a rover mating strategy (Barnes, 1982). This strategy is probably due to the low frequency and unpredictable occurrence of receptive females, (Clutton-Brock, 1989) which in most populations can occur throughout the year (Laws et al., 1975; Hall-Martin, 1987; Poole & Moss, 1989b). Large sexual dimorphism and some degree of post mature growth, as seen in elephants are in other mammals like Elephant seals (*Mirounga angustirostris*) (Le Boeuf, 1974) Eastern grey kangaroos (*Macropus giganteus*) (Jarman & Southwell, 1986) and Polar bears (*Ursus maritimus*) (Ramsay &

Sterling, 1986) normally associated with a strong size dependent competition for mates (Whitehead, 1994; Gadgil, 1972). This is also the case in the African elephants with the outcome of antagonistic interactions strongly influenced by size (Poole, 1987; Poole, 1989a; Poole, 1994b).

Annually occurring periods of heightened sexual activity, known as musth has been known to occur among Asian elephants (*Elephas maximus*) since antiquity (Eisenberg et al., 1971). Earlier reports suggested that musth did not occur in the African species (Perry, 1953; Sikes, 1971) but has now also been shown to exist within the genus *Loxodonta* (Poole & Moss, 1981; Poole, 1987). The state of musth has been defined on the basis of conspicuous physical signals such as swelling and secretion from the temporal glands and urine dribbling (Poole, 1987) (Fig. 1.2) with an additional number of more subtle changes in posture and behaviours (Kahl & Armstrong, 2002).

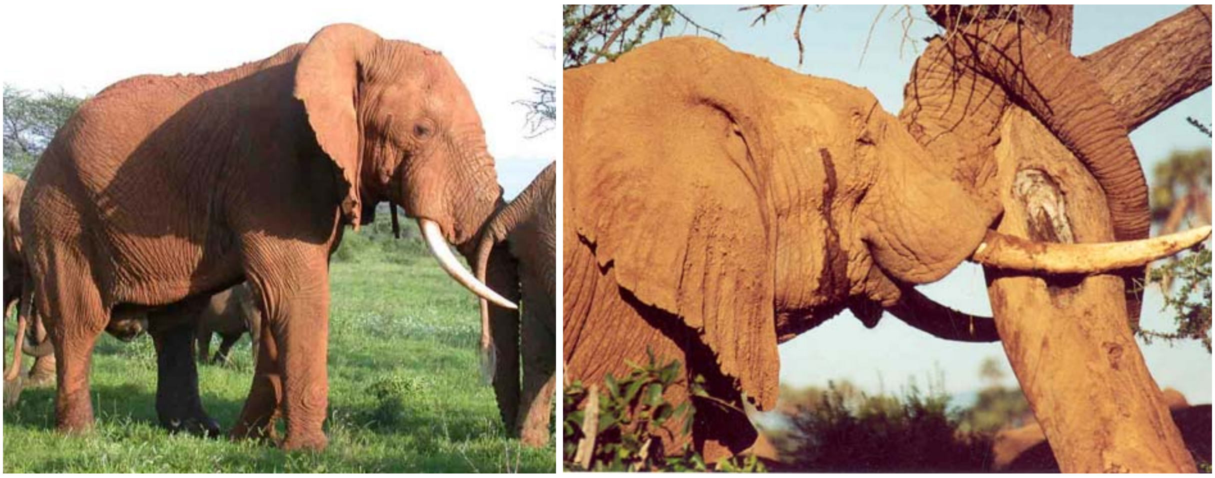


Figure 1.2 Bulls in musth: Left B1005-Abe (photo G. Wittemyer) and right B1009-PretiBomBom. Note swollen and secreting temporal gland, situated behind the eye, greenish coloration of the tip of the penis and urine dribbling evident as wet penis and hind legs.

Musth periods are synchronized between years in individual bulls but not in synchrony at a population level with musth bulls occurring year around (Poole, 1987; Hall-Martin, 1987). During periods of musth, the bulls decrease the time spent foraging and associate more frequently with females than when out of musth (Poole, 1989b). Musth has been likend to the rutting behaviour of ungulates (Eisenberg et al., 1971; Poole, 1987) and is associated with elevated levels of testosterone (Hall-Martin, 1984; Poole et al., 1984; Rasmussen et al., 1996). Musth bulls exhibit a high degree of aggression towards other bulls and even non-elephant objects (Fig 1.3) and the majority of escalated fights, occasionally associated with mortality occur between males where at least one of the contestants are in musth (Poole, 1989b; Hall-Martin, 1987).

Figure 1.3: Research vehicle after an encounter with the 35-year old bull B1172-Rommel in musth. During a fight between two musth bulls Rommel re-directed his aggression towards the researcher.

Bulls in musth can thus be regarded as being in a highly competitive reproductive mode. Musth is mainly seen among older 30+ year bulls with the occurrence and duration of musth periods being positively correlated with age (Poole, 1987). Theoretical studies have suggested that in a system with roving males and large size dependent competition for mates, males should delay competitive breeding until late in life where few males are larger than themselves and invest less (or not at all) in reproduction before reaching competitive age (Whitehead, 1994). Musth seems to be such an example of late occurrence of competitive breeding and provide support for Whitehead’s prediction.

Musth may however not be the exclusive reproductive tactic. During the 20-year period between puberty and musth becoming predominant around the age of 35, younger non-musth bulls are also known to obtain matings and have viable sperm (JoGayle et al., 1984; Hall-Martin, 1987; Barnes, 1982) but engage to a far lesser degree in aggressive interactions with competitors and seldom mate-guard receptive females (Poole, 1989c), hence are less competitive compared to musth bulls. Whether these younger bulls are opportunist breeders that only obtain matings by chance without any general investment in reproduction such as active search for receptive females or if they are engaged in a distinct alternative "non-musth” reproductive tactic is examined in this study.

Since musth was first recognized in African elephants in the early 1980’s (Poole & Moss, 1981) studies reporting on male elephant reproduction have been focused on this state and musth has often been used synonymous with sexual activity (Sukumar, 2003; Macdonald, 2001). The description of behavioural changes linked to reproductive activity have exclusively focused on bulls in musth and have been based on the a priori categorization of observations based on the occurrence of urine dribbling (UD) and temporal gland secretion (TGS). It has been suggested that younger non-musth bulls may have distinct sexually active periods (Poole, 1994b; Poole et al., 1984) However, the signals (UD and TGS) used for identifying musth do not by definition occur in non-musth bulls (whether sexually inactive or potentially in a sexually active state) and no other definitions or analysis has been presented in support of an alternative reproductive tactic among younger non-musth bulls.

Male elephants are polygynous and it is believed that reproductive success is highly skewed towards the relatively few older bulls and with bulls not siring offspring before engaging in periods of musth (Poole, 1994b; Poole et al., 1984). This has been based on their obvious competitive dominance and the large fraction of mate guarding and matings assigned to this state (Poole, 1987; Poole, 1989a). However genetic paternity analysis in other species often reveal large differences between behaviourally assessed reproductive success and actual reproductive success (Coltman et al., 1999; Pemberton et al., 1992; Amos et al., 1993; Comstock et al., 2002; Coltman et al., 2002). In the only genetic paternity study so far done on African elephants in Addo National Park (Whitehouse & Harley, 2002) the dominant musth bull was only assigned as the most likely father to 28% of the calves. However, low genetic variability of micro satellite markers due to a historic population bottleneck in this population (Whitehouse & Hall-Martin, 2000; Whitehouse & Harley, 2002) made the overall results somehow inconclusive. Despite this, it suggests that reproductive success may not be as heavily skewed towards older bulls as previously believed and that musth may not be the only reproductive tactic in elephants, exclusively responsible for reproduction. If indeed alternative reproductive tactics exist, questions arise as to how the tactic of these less competitive bulls compare to the tactics adopted by musth bulls, what governs the transitions between the different tactics as well as how variation in internal and external conditions influences the tactics adopted by the individual male.

The evolution and maintenance of systems with alternative reproductive phenotypes have generally been investigated within the framework of game theory and evolutionary stable strategies (ESS's) (Gross, 1996; Maynard Smith, 1976; Rubenstein, 1980). Reproductive *strategy* and *tactic* is sometimes used with slightly different meanings. In this thesis I follow the definitions by Gross (Gross, 1996) using reproductive *strategy* to refer to a combined genetically based reproductive rule-set controlling the allocation of reproductive effort in an organism whereas *tactic* is used to refer to a reproductive phenotype arising from a given strategy. Each tactic (e.g. *defend harem* or act as *satellite* or *sneak* male) is made up by a specific set of rules or behaviours. Two tactics may adopt the same rule for finding receptive females (search rule) but may have different rules once a receptive female is found (e.g. fight off other males and guard versus stay in perimeter and attempt mating without fighting). Hence two different tactics must deviate on at least one rule to be considered different but may also have similar rules.

Three categories of alternative intra specific strategies are generally considered, namely *alternative strategies* (AS), *mixed strategies* (MS) and *conditional dependent strategies* (CS) (Gross, 1996). A system with true alternative strategies require genetic polymorphism for the different strategies and can be maintained through frequency dependent selections where the two strategies have equal fitness at a given ESS frequency. In a system with a mixed strategy, a single genetic monomorphic strategy give rise to the probabilistic occurrence of alternative tactics with the probabilities for a given tactic maintained through frequency dependent selection with each tactic having equal fitness at the ESS frequency. Both alternative and mixed strategies have been extensively investigated theoretically but only few examples have been documented (Gross, 1996). Currently most variation in reproductive phenotypes is considered to arise from conditional dependent strategies (Gross, 1996). In such a system a single genetic monomorphic strategy exists which, depending on the status of the individual and it's social and ecological environment give rise to different tactics with unequal average fitness but with the existence of an ESS switch-point between tactics associated with the condition where the two tactics have equal fitness (Gross, 1996). If variation in external conditions influences the fitness of the different tactics equally this switch point may be stable (Fig 2 switch point S3, A1 to A2 and B1 to B2). However, if tactic specific fitness is influenced in the same direction but unequally (Fig.2A: A1 to A2 and B1 to B3) or in opposite directions (Fig.2A A2 to A1 and B1 to B2) the position of the status switch point will exhibit plasticity between different environments *i*) S2-S3 or *ii*) S1-S4 (Fig2a)

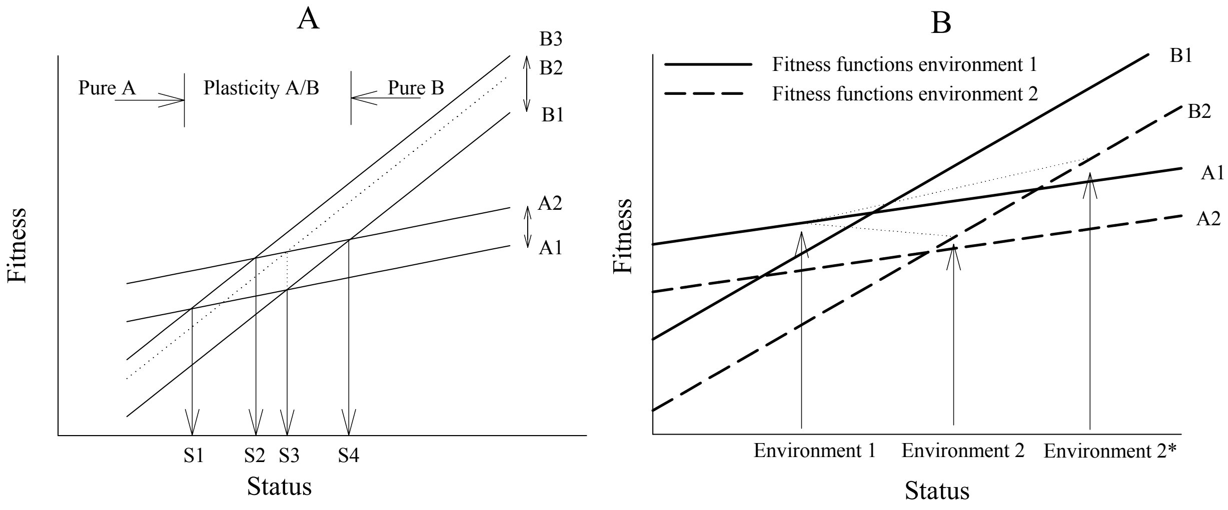


Figure 1.2: A: Conditional dependent strategies can arise when the different tactics (A&B) have crossing status dependent fitness functions. If the fitness functions are influenced unequally by external conditions (Condition 1-3) a region of tactic plasticity can occur where different ESS switch-points (S1-S4) exists between optimal tactics. (Adopted from Gross (Gross, 1996)). B: If spatio-temporal changes in environment are associated with changes in both fitness function and status an individual should chose the combination of environment and tactic with the highest fitness. Tactic A in environment 1 should be preferred over tactic B in environment 2. If the status increase associated with the shift to environment 2 is larger (2*) tactic B in environment 2 should be preferred.

The same *condition* of an individual may result in different *status* depending on the local competitive environment. Hence changes in the number or quality of competitors in an otherwise equal environment may result in shifting optimal tactic. If an individual can select between different environments either spatially or temporally and these environments affect both fitness functions and status the individual should select the combination of environment and tactic with the highest fitness (Fig1B). This optimum may or may not be associated with the highest quality environment or highest status. Hence this model offers a conceptual framework for investigating both the variation in observed tactics under varying conditions but also for when and where individuals choose to engage in reproduction and their selection of tactics.

The long duration between the age when musth is first observed (around 25 years) until males show regular prolonged periods of musth (at around 35-40 years) suggests that the optimal tactic may vary depending on local conditions during a period covering more than 10-15 years of the life of a bull. If this is true, even relative small changes in general ecological or demographic conditions could dramatically influence the observed reproductive behaviour of bulls including large shifts in occurrence of musth and frequency an intensity of antagonistic interaction among bulls.

1.2 Objectives and aims

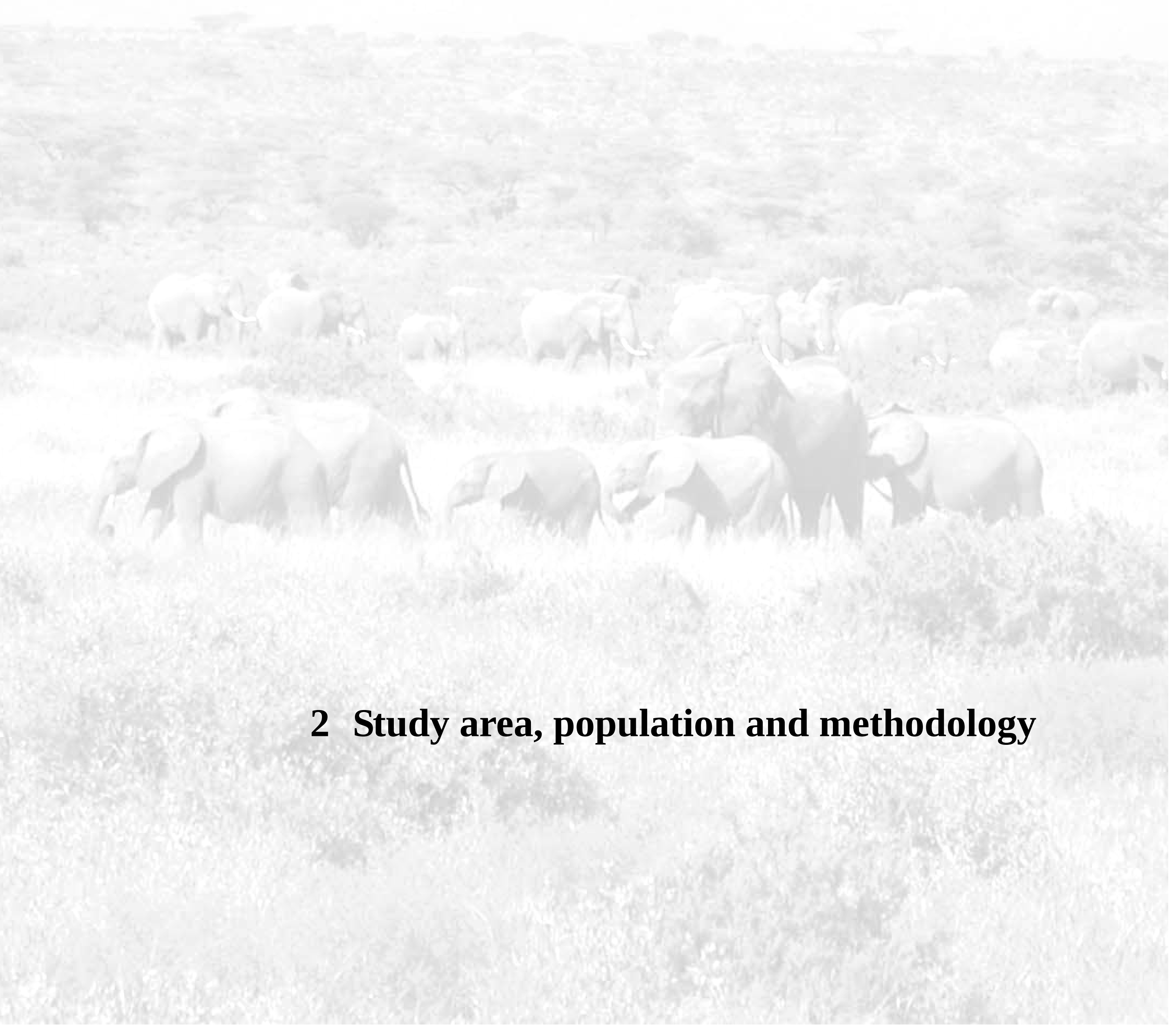
The objective of this thesis is to investigate the reproductive strategy of male African elephants and the potential age and status related shifts between alternative tactics as well as investigating the behavioural and endocrinological differences between alternative tactics.

More specifically the aims are to *i*) Establish if alternative reproductive tactics exists in male African elephants. *ii*) Evaluate if these tactics differ in sex steroid concentrations and if these steroids potentially play a proximate role in the activation of the different reproductive states and tactics. *iii*) Establish how these tactics differ in terms of behaviour and reproductive investment. *iiii*) Evaluate the reproductive payoff from the different tactics. These aims will be achieved by addressing the following four main components using a combination of behavioural, endocrinological and genetic data.

1) The occurrence of reproductive active and inactive periods in individual males.
2) The endocrinological correlates of reproductive states and the potential activational role of androgens on reproductive state and tactics.
3) Reproductive investment of different reproductive tactics.
4) The age and tactic specific reproductive success of bulls.

1.3 Thesis outline

In order to ease the reading and avoid any repetition the original manuscripts have been restructured into thesis chapters. Each chapter contains an introduction to and discussion of the specific issues. **Chapter two** contains a description of the study area and population as well as a summary of the data collection methods relevant for most of the chapters. Specific methods and procedures used for analysing e.g. hormones and DNA are presented in the relevant chapters. **Chapter three and four** describe and validate in detail the methods use for age estimation and for obtaining the temporal distribution of oestrus females within the study area, used for the analysis in the subsequent chapters. In **chapter five** a statistical framework is developed, based on shifts in individual associational preferences, to identify potential sexual active periods without using tactic specific signals such as urine dribbling and temporal gland secretion. The occurrence and temporal timing of the identified periods are compared to the presence of musth signals as well as the temporal distribution of oestrus females obtained in chapter three to evaluate if the periods with high preference for female association may present sexually active periods. In **chapter six** the endocrinological correlates of the reproductive states are analysed based on a combination of occurrence of musth signals, associational preferences and androgen levels. Furthermore, the longitudinal hormone profiles of individual males are investigated in relation of age, state and local conditions to evaluate the effect of external condition and the potential activational role of androgens on both sexually active periods and the shift between the alternative tactics. In **chapter seven** the investment in reproduction and the changes in daily movements is analysed in relation to shifting reproductive state and tactic. In **chapter eight** the age and tactic related reproductive success is evaluated based on paternity analysis using DNA micro-satellite markers. **Chapter nine** contains a combined discussion of the results.



Chapter 2

2 Study area, population and methodology

2.1 Study area

The study area is situated just north of the equator on longitude 37º, in and around Samburu and Buffalo Springs National Reserves, Kenya along the Ewaso Ngiro River, the only major permanent water source in the area (Fig 2.1). The area consists of a matrix of protected, unfenced national reserves, semi-protected wildlife conservancies and unprotected rangelands. The areas outside the national reserves are inhabited by a number of different tribes, mainly Samburu, Borana, Turcana and Somalis. Outside the few towns, people live in traditional small pastoral communities, mainly depending on cattle and other livestock.

The area consists of large, relatively flat regions, with isolated hills and small mountain ranges with a semi-arid savannah vegetation dominated by *Acacia tortilis* and *Commiphora* woody plant species and a large diversity of grasses and forbs, with patches of riparian woodland dominated by *Acacia elatior* and Doum palms, *Hyphaena coriacea* along the Ewaso Ngiro River (Barkham & Rainy, 1976).

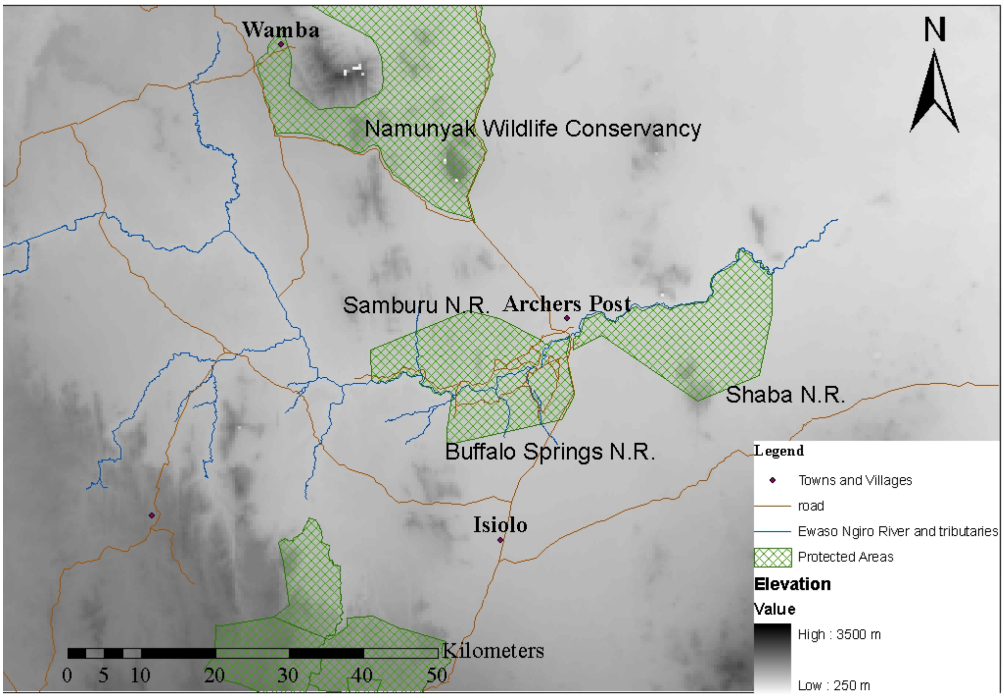


Figure 2.1: The core study area of Samburu (SNR) and Buffalo Springs (BSNR) National Reserves.

2.2 Climate

The climate is hot and dry with limited annual variation in the mean temperature of 30 ˚C. The annual rainfall pattern is bimodal but highly variable with the majority of the annual average precipitation of 380 mm (Range 143-847; year 1960-2002) occurring during Nov-Jan and April-May (Fig 2.2).

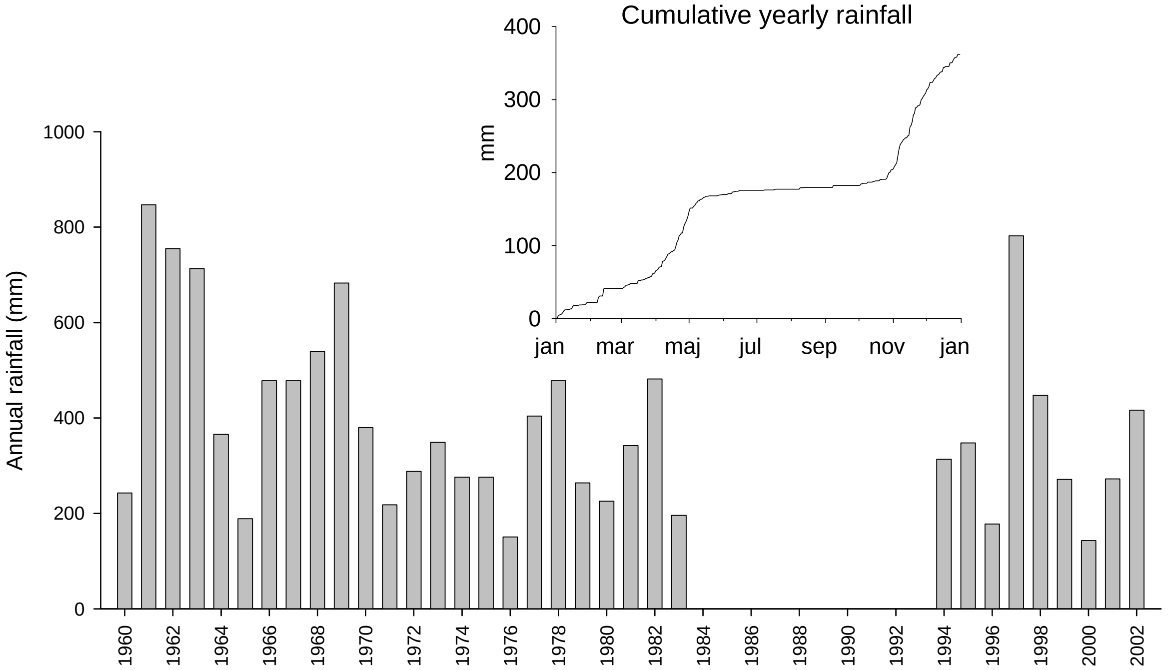


Figure 2.2: Yearly rainfall in Archer Post between 1960-2002, data between 1984-1993 not available. Insert: Average yearly cumulative rainfall (Data from 1996-2002).

2.3 Study population

The total elephant population within the Samburu/Laikipia ecosystem is estimated to number around 5000 individuals (Omondi et al., 2002) and is the largest free ranging elephant population in Kenya occurring mainly outside protected areas (Fig 2.3). Around 3000 occur on the Laikipia plateau (Altitude around 1800 m) and the remaining 2000 in the northern Samburu lowlands (Altitude around 900 meters) and along the Matthews Range. The study site is situated in the Samburu lowlands, centrally within the Samburu/Laikipia population.

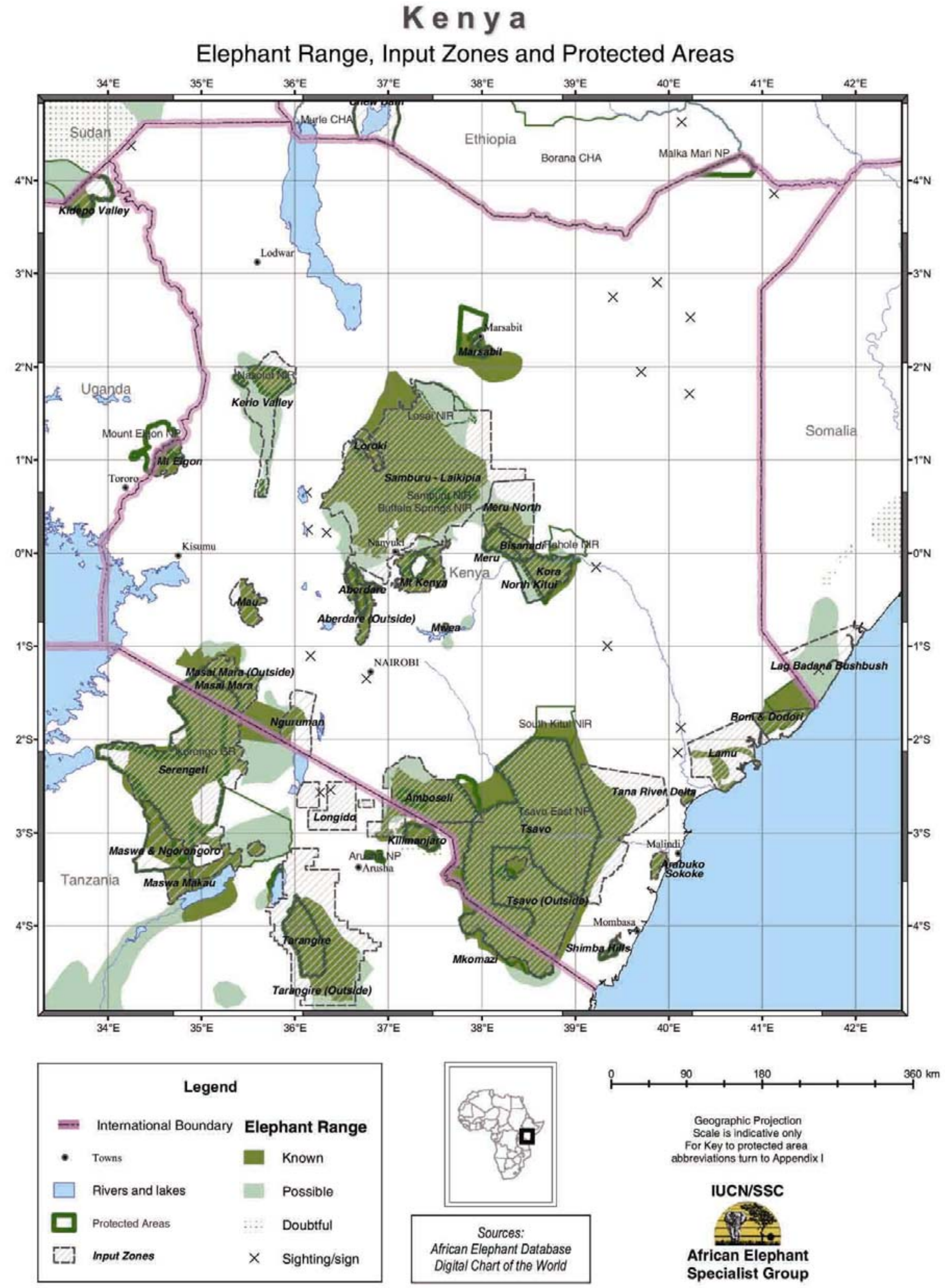


Figure 2.3 Current distributions of elephants in Kenya. (Reproduced with permission from (Blanc et al., 2003). The study site is situated within the large central Samburu/Laikipia population.

The monitoring program, carried out by Save the Elephants within the core study area of Samburu and Buffalo Springs National Reserves has since 1997, observed and identified around 900 individual elephants and compiled photo id files enabling identification based on individual recognisable features such as ear patterns and tusk size and shape (Douglas-Hamilton, 1972; Moss, 1996) . Hence, a large proportion of the northern Laikipia/Samburu population utilises this area along the Ewaso Ngiro River to a greater or lesser extent. This individually identified study population consists of approximately 220 breeding females, 540 calves and 160 adult males (Wittemyer, 2001; Wittemyer et al., 2005) and has on average increased with 4.6% per year since 1997 (not including any immigration) (Wittemyer et al., 2005).

Very few un-identified males and females have been seen and identified since 2001 with the majority of newly identified bulls being between 15 and 25 years old. This indicates, that the vast majority of the elephants utilising this area has been recorded. However, the study “population” is unconfined and to some degree continuous with unknown elephants occurring in adjacent areas and the currently identified “population” of especially males does not represent a static, homogenous group of individuals. For example some of the more recently identified and now frequently observed males especially younger individuals around 20-25 years may be recent immigrants to the study area. Likewise some males may have been frequently around during the first couple of years but have since either left or died. In addition different males and female family groups spends varying amount of time within the study area, evident both from the GPS tracking data and from a large variation in the frequency of observations of specific individuals with some predominately or exclusively seen within the Shaba NR, east of the core study area (Figure 2.1).

The age distribution in 1998 of all 174 currently (2004) identified independent males (including very infrequently observed individuals and individuals now dead) is shown in figure 2.4. Not all individuals between 5-10 years (in 1998) are yet recorded (2004) as independent hence this age group does not represent all individuals additionally some 15-20 year old individuals may still not being identified whereas no unknown older bulls are expected still to occur in the study population. Some of the individuals including 3 of the oldest have died or are presumed dead since 1998. The oldest age groups seems to be slightly under represented in the study population assuming a constant natural adult mortality of 3-5 percent per year and a maximum longevity of app. 60 years as reported from other populations (Laws et al., 1975; Moss, 2001). This may be a remnant effect of the severe ivory poaching occurring in the area between the mid seventies to the late eighties.

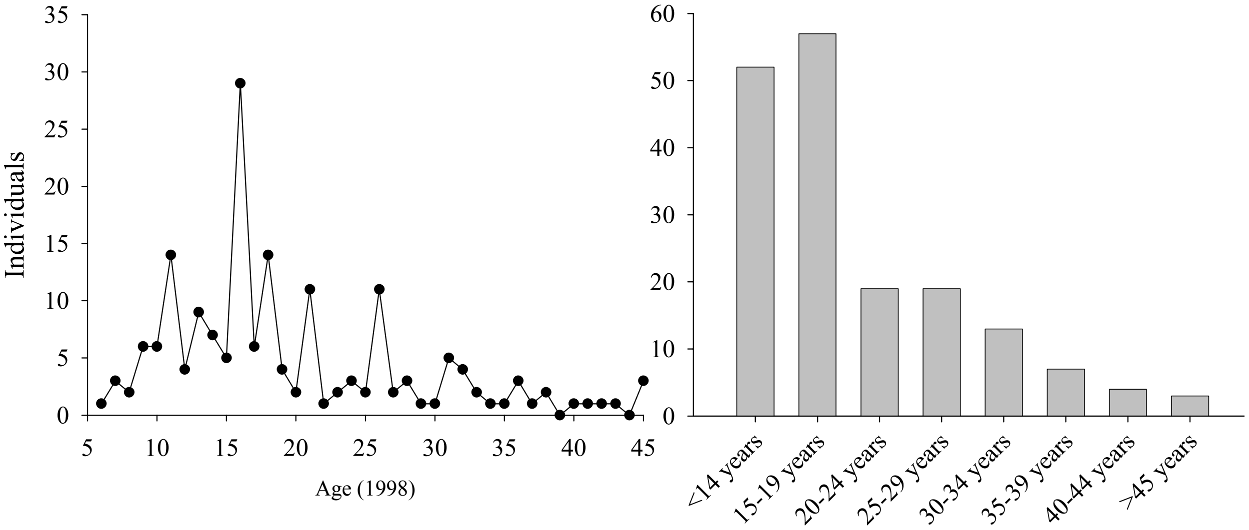


Figure 2.4: Age-structure (in 1998) of all 174 males identified since the start of the monitoring program in 1997. Age technique described in chapter 3.

2.4 Data collection

This study was conducted as an integrated part of Save the Elephant's ongoing study program in Samburu. Analysis in this thesis relies on data collected during a four-year study period between October 1999 and November 2003. The data consists of a combination of spot observations of individually known elephants; activity recordings and tracking of GPS collared individuals as well as non-invasively collected faecal samples for DNA and hormone analysis. In addition remote sensing data from the AVHRR and SPOT programs, provided by the Earth Observing System Data and Information System (EOSDIS) and the VEGETATION program are used for the analysis of effects of seasonal changes in vegetation productivity and was obtained from http://free.vgt.vito.be (SPOT data) and ftp://disc1.gsfc.nasa.gov/data/avhrr/ (AVHRR data).

2.4.2 Spot observations of individuals

Spot observation of individually known males (and females) were collected by the author between October 1999-May 2000; December 2000- May 2001; January 2002- May 2003 and October -November 2003. In addition similar data were collected by Save the Elephants ongoing monitoring program as well as by G. Wittemyer's project on female social organisation. These spot observations consisted of individual ID and ID of associating adult individuals, time, position and general activity (Browsing, Grazing, Drinking, Standing, Resting, Walking or Comfort). For the males the degree of temporal gland swelling, temporal gland secretion and urine dribbling were additionally recorded following the definitions by J. Poole (Poole, 1987) (Figure 2.5). All data were compiled onto identical data sheets and combined within a general database. In case of multiple observations of the same individual on the same day only the first were used.

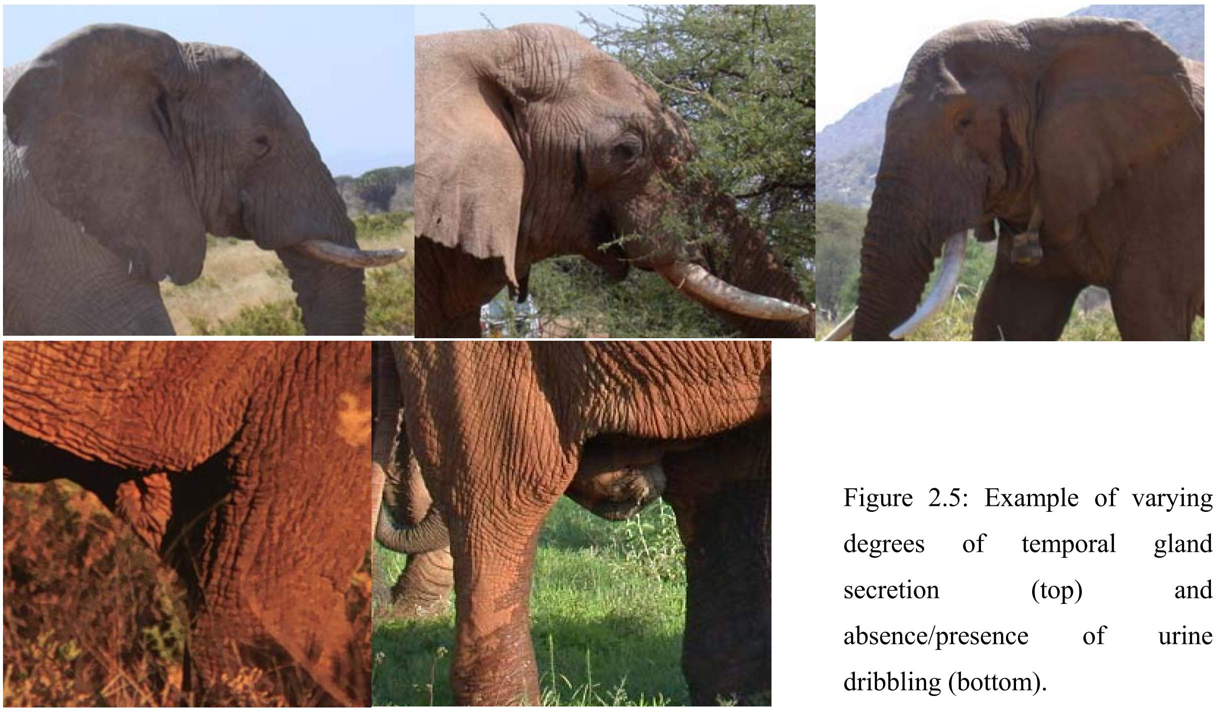


Figure 2.5: Example of varying degrees of temporal gland secretion (top) and absence/presence of urine dribbling (bottom).

When an individual was encountered where no DNA sample had yet been collected, this individual would be followed until defecating and fresh mucus lining from the dropping would be collected and stored in DMSO (Amos & Hoezel, 1991) for later analysis.

During the study period a total of 5496 single observations of males were collected with 3720 of those collected by the author (68%). To obtain longitudinal time records of associations, musth signals and hormone levels of individuals the GPS or radio-collared males (see next section) were attempted located on a regular basis every 7-10 days between January 2002 and November 2003 using normal telemetry tracking of the beacon signal using a directional four-cell yagi antenna. When located the normal spot observation data were collected and the individual was followed until it defecated and faecal samples for hormone analysis where collected and stored in ethanol for later analysis. A median of 91 bull observations was made per month (range 21-393) with a median of 33 different individuals being observed per month (range 12-60) (Fig.2.6). The large variation in total observations, especially the high number of observations during mid 2002 is due to varying number of observers in the field (effort).

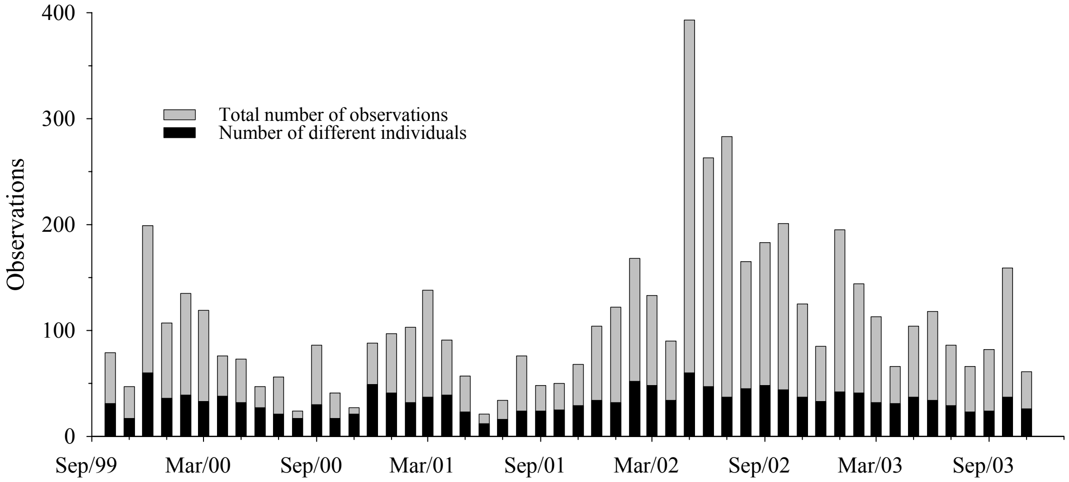


Figure 2.6: Monthly number of bull observations during the study period. The higher number of observations during the last half of the study period is due to increased field effort and more observers.

2.4.3 GPS tracking data

Data from Save the Elephants (STE) tracking program in the Samburu/Laikipia area, conducted by Dr. Iain Douglas-Hamilton, was made available for this study and made it possible to locate individuals on a regular basis to obtain longitudinal time series of behavioural and associational recordings as well as samples for hormone analysis and detailed information on movements. In addition to STE’s tracking of females, 20 bulls were fitted with either GPS or radio beacon collars between 2000 and 2004. These individuals were chosen to cover the age spectrum between 22-48 years and to cover individuals not yet observed in musth, having short highly sporadic periods of musth as well as older individual known to have regular recurrent annual periods of musth. In order to secure data on bulls exhibiting regular musth the number of collared individuals were slightly biased towards older individuals. The GPS tracking study conducted by Save the Elephants is carried out in collaboration with The Kenyan Wildlife Service (KWS). KWS is the official regulatory body for wildlife research, conservation and park management in Kenya.

2.4.4 Collar types and recording periods

A combination of different collar types was used concurrently during the study period. The GPS collars were manufactured by Lotek Wireless Inc. Canada (type GPS1000, GPS2000), Televilt AB Sweden (type Simplex) and African Wildlife Tracking Ltd., South Africa. The radio collars were from Telonics Inc. USA. All GPS collars were scheduled to record positions every hour on the hour. In addition the six Simplex collars were scheduled to record every 15min on two consecutive days each week (Monday & Tuesday). An overview of the age of the collared individual and operational periods is shown in figure 2.7 with detailed periods, collar types and performance records shown in appendix B.

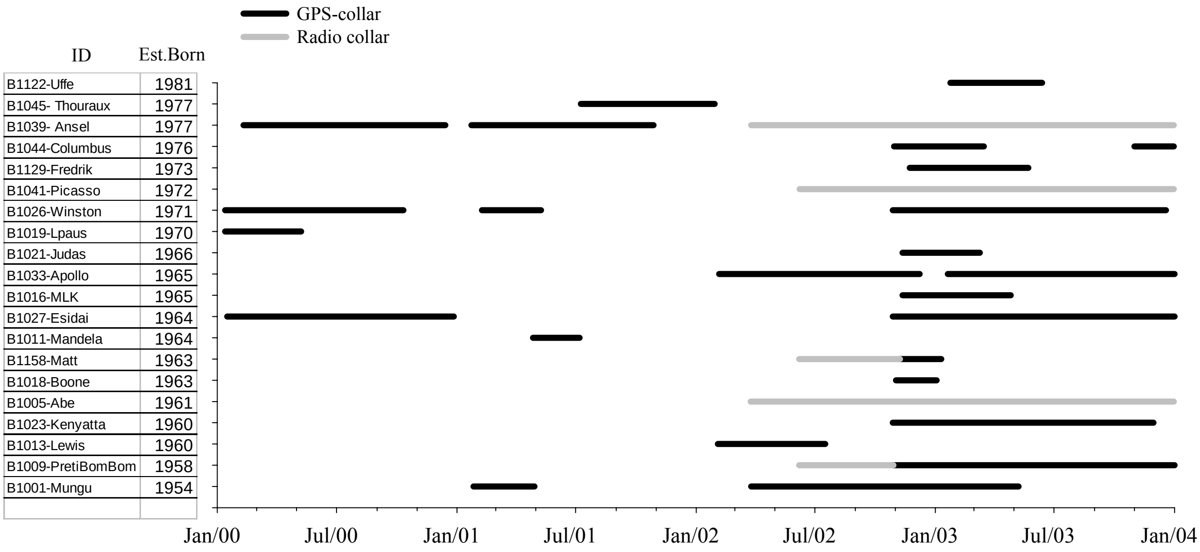
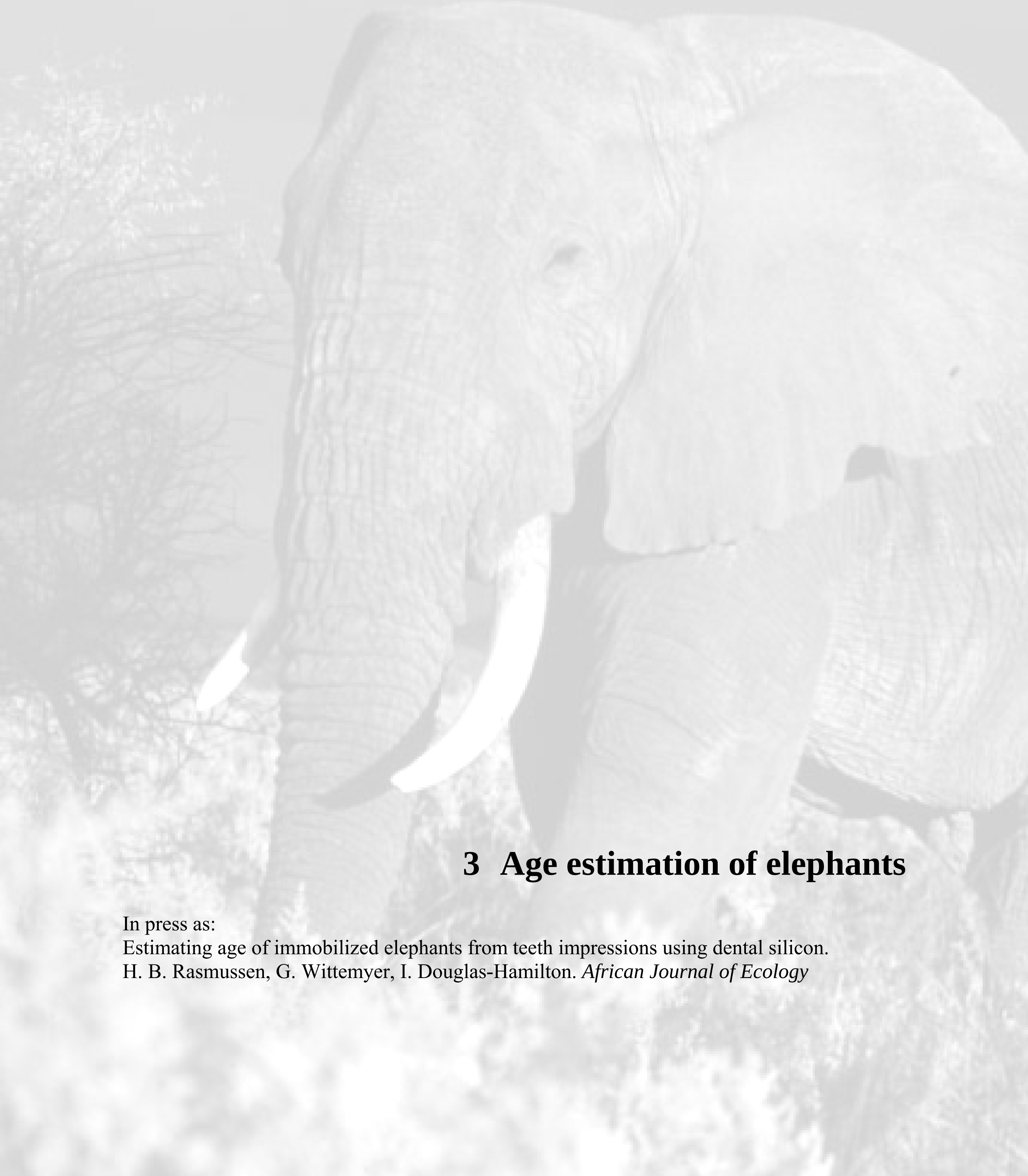


Figure. 2.7: ID and estimated age of the 20 bulls fitted with radio and GPS collars. Lines indicate the functional period of the collars (Grey = radio collar, black = GPS collar)

2.4.5 Immobilizations

Save the Elephants carried out the immobilisations and collaring operations on the request from Kenyan Wildlife Service (KWS). All personnel responsible for stalking, darting and subsequent collar attachment had experience from previous operations. A KWS certified veterinarian carried out the Immobilisations using a Daninject dart-gun with M99 (Etorphin) used as tranquilliser and M50 (Diprenorphine) for revival. Depending on the size of the target animal between 15 and 18 mg of M99 was used for males (and 12-15 mg for females) and 30-60 mg of M50 for revival. Initially between 50-100 mg of xylacine (sedative) was added to the tranquilliser. However, during “fast” operations where the revival was done less than 10 min after the elephant went down the effects of the sedative had not worn off making the elephant “sluggish” and slow in getting back on the feet. This was considered undesirable and the use of a sedative was discontinued.

Darting were mainly carried out from a vehicle but occasionally done on foot or helicopter when the terrain prohibited the use of a car. After the dart hit the target individual, the darting team stayed still for approximately 5 min. in order not to further disturb the elephants. As long as visual contact was kept with the target, no action was taken. At all time, the maximum possible distance was kept to the target in order to keep both the target and other associating individuals calm. This generally resulted in a quick return to normal with the elephants typically running for <50 meters before slowing down after the dart was fired. A spotter plane was used on occasions when the darting occurred in closed terrain to keep the overall picture and avoid unnecessary close contact between the ground team and the elephants before the target went down. When the target went down, the darting team would approach by car or occasionally on foot. Upon arrival, the first person would straighten the trunk for easier breathing and pour water on the ear for cooling. The veterinarian would tend to animal welfare, one would take biopsy samples and other measurement, and one would remove the collar while one kept a lookout for other elephants. Revival drug was on average injected less than 20 min. after the elephant went down. After injecting revival drug the team would pull back 30-40 meters while still having visual contact. The target elephant would typically get up after 3-4 min and slowly walk away.



Chapter 3

3 Age estimation of elephants

In press as:
Estimating age of immobilized elephants from teeth impressions using dental silicon.
H. B. Rasmussen, G. Wittemyer, I. Douglas-Hamilton. *African Journal of Ecology*

Abstract

Obtaining reliable age estimates was important for most of the analysis in this thesis. High precision condensation dental silicon, Zetalabor^{TM}, was used to create moulds of the lower jaw molars from 22 of the immobilized elephants during radio collaring operations. These moulds were used to determine the elephant's age using Laws' and Jachmann's molar aging criteria. The technique proved easy and fast and produced useful imprints in 90% of the cases. The age estimates, based on physical appearance, and made prior to immobilizations were relatively accurate, with 75% within +/- 3 years and 95% within +/- 5 years from the age indicated from molar evaluation. When re-collaring the same individuals in 2-3 years, new moulds will be made to compare a known time period with the degree of tooth wear. This will provide verification of Laws age estimates from free-ranging elephants.

3.1 Introduction

Estimating the age of live elephants is important for assessing population age structures and was crucial for the analysis of age related effects in this study. Criteria for the use of physical appearances, such as facial shape and tusk development, to estimate age have been developed (Moss, 1996). This method is assumed accurate to within +/- 3 years but has not been verified against known aged individuals and may be subject to large inter observer variability. Elephants grow throughout most of their life and shoulder height or hind foot length has been used as correlates of age (Hanks, 1972b; Lindeque, 1993; Lee & Moss, 1995). However, the low annual increment in size of older individuals (25+ years) and inter individual variance creates large confidence limits of age estimates for older age groups. Furthermore, due to possible inter population variation in growth rates resulting from genetic or environmental effects, this technique requires population specific validation before being applied.

Age can be estimated more accurately using tooth development and wear. Two techniques have been developed; one based on the stage of molar progression (Laws, 1966) and the other based on the number of disappeared molar lamellae (Sikes, 1967). The original categories made by Laws tend to overestimate ages between 10-30 years and a correction has been applied (Jachmann, 1988). The method by Sikes may be problematic due to varying number of molar lamellae in each of the six molars (Jachmann, 1988; Hanks, 1972a). Both methods suffer from being verified against relatively few known aged elephants in captive settings (Short, 1969; Jachmann, 1985).

For obvious reasons methods involving the measurement of tooth development is difficult to apply to live, free-ranging elephants, thus the less accurate methods involving height and physical appearance are generally used. Due to the technical advances in radio and

GPS tracking of animals, increasing numbers of elephant studies involve immobilizing and collaring numerous individuals. This offers an opportunity to apply tooth-ageing techniques to live, immobilized elephants. These more accurately aged individuals can then potentially serve as standards from which the accuracy of other age estimates using physical appearance can be increased. The direct assessment of molar progression by hand during immobilizations has been applied (I. Whyte personal comments.) but cannot be verified by independent observers.

In this study a new technique was investigated based on tooth imprints taken from immobilized elephants using a fast acting dental condensation silicon. This material provides a high precision mould of the molars within minutes that can be used to assign the age of an individual. If moulds are taken from the same individuals across multiple year intervals (for example during collar removal), tooth wear can be linked to a known time period and a revision of the current tooth aging technique can be made, helping to improve future aging techniques.

3.2 Material and moulds:

For the moulds we used Zetalabor™ high precision condensation silicon from Zhermark® with Indurent Gel® catalyst. After adding the catalyst to the silicon and mixing for 2 minutes, this silicon hardens (within 6 minutes) without heating up, producing a hard but elastic shape/dimensional stable mould. After immobilization, the distance between upper and lower jaw was investigated by hand to assess if enough working space existed for tooth moulding, which was the case in most situations. Any grass or branches between the jaws were removed by hand to provide a clean molar surface for the mould. Thereafter the catalyst was added to 400 grams of pre-measured silicon. After mixing, the putty was inserted into the mouth by hand and pressed onto the molar surface in the lower jaw. During the hardening process the substance was continuously worked around the tooth to improve the final mould (Fig 3.1). The mould was then used to assign age categories according to Laws (Laws, 1966) incorporating the adjustments suggested by Jachmann (Jachmann, 1988) (see Appendix A)

Figure 3.1: The dental silicon is applied to the lower jaw molar of an immobilized elephant.

3.3 Results

Dental moulds were made on 10 adult bulls and 12 females. An example of one of the moulds can be seen in figure 3.2. All but 2 moulds (females) proved accurate enough to assign age categories. The immobilized individuals ranged from an estimated age of 21-46 years. In figure 3.3, eight moulds from bulls representing the age range are shown. In approximately half the cases the material could not be pressed all the way to the back of the last molar and thus gave an incomplete mould (see mould 1 and 4 in fig 3.3). However, enough information was preserved in the mould to assign an age.

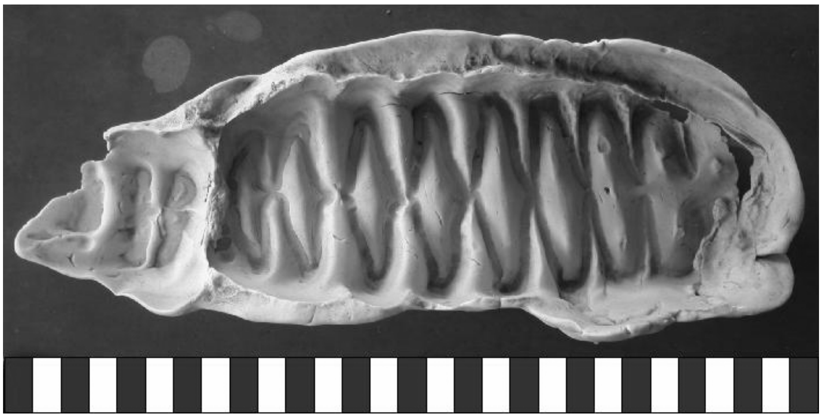


Figure 3.2: Mould of the lower jaw molars of a bull. Anterior end right, posterior end left. 5th molar in full use, 6th molar starting to wear on first 2 enamel loops. Age-group XVIII, 30 +/- 2 year old (Jachmann, 1988; Laws, 1966). 1-cm between measurement marking.

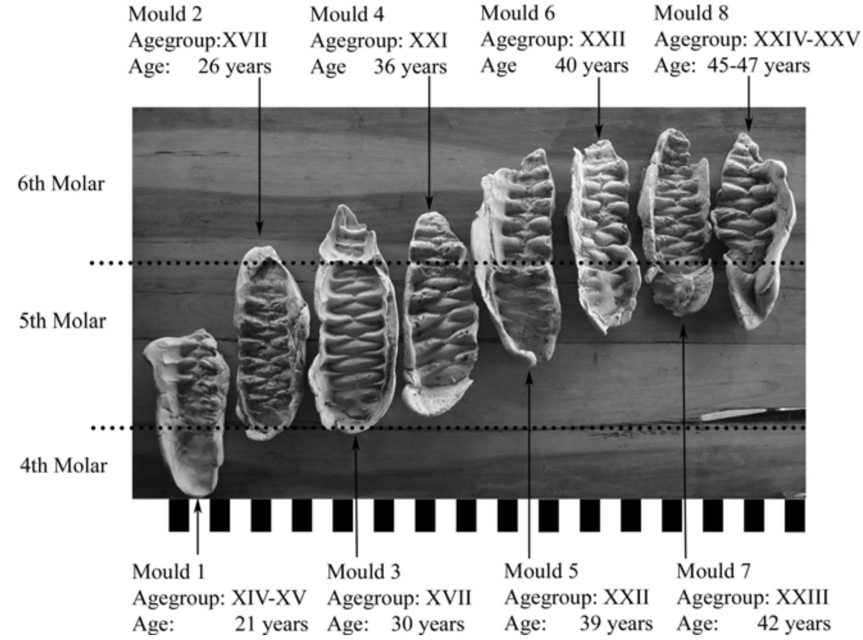


Figure 3.3: Example of 8 moulds of lower jaw molars from bulls arranged according to age. Note that some moulds are incomplete, missing the posterior part. However enough information is preserved in the mould to assign age group. Age groups according to Laws (Laws, 1966) and Jachmann's revision. (Jachmann, 1988).1-cm between measurement marking.

To evaluate the accuracy of the age estimates based only on physical appearance the 20 estimates based on moulds where combined with the 11 estimates based on hand assessment of molar progress and 6 molar estimates from the jaws of known dead individuals. Of these 37 individuals, 28 (75 %) of the age estimates based on physical appearance where within +/- 3 years from the molar age and only 2 (5%) deviated more than 5 years (Fig. 3.4) with a tendency for slightly underestimating the age of bulls and overestimating females.

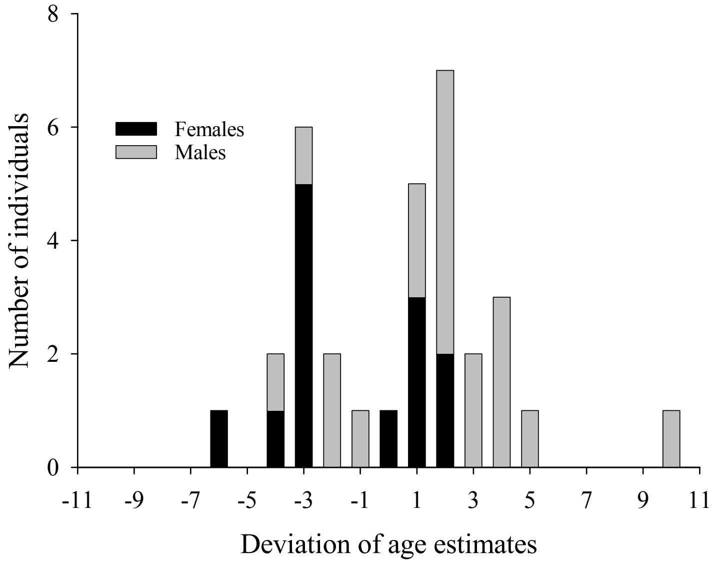


Figure 3.4: Deviation of age estimate based on physical appearance from estimated based on molar state. 75% of estimates based on physical appearance were within +/- 3 years and 95% within +/- 5 years from molar age.

3.4 Discussion

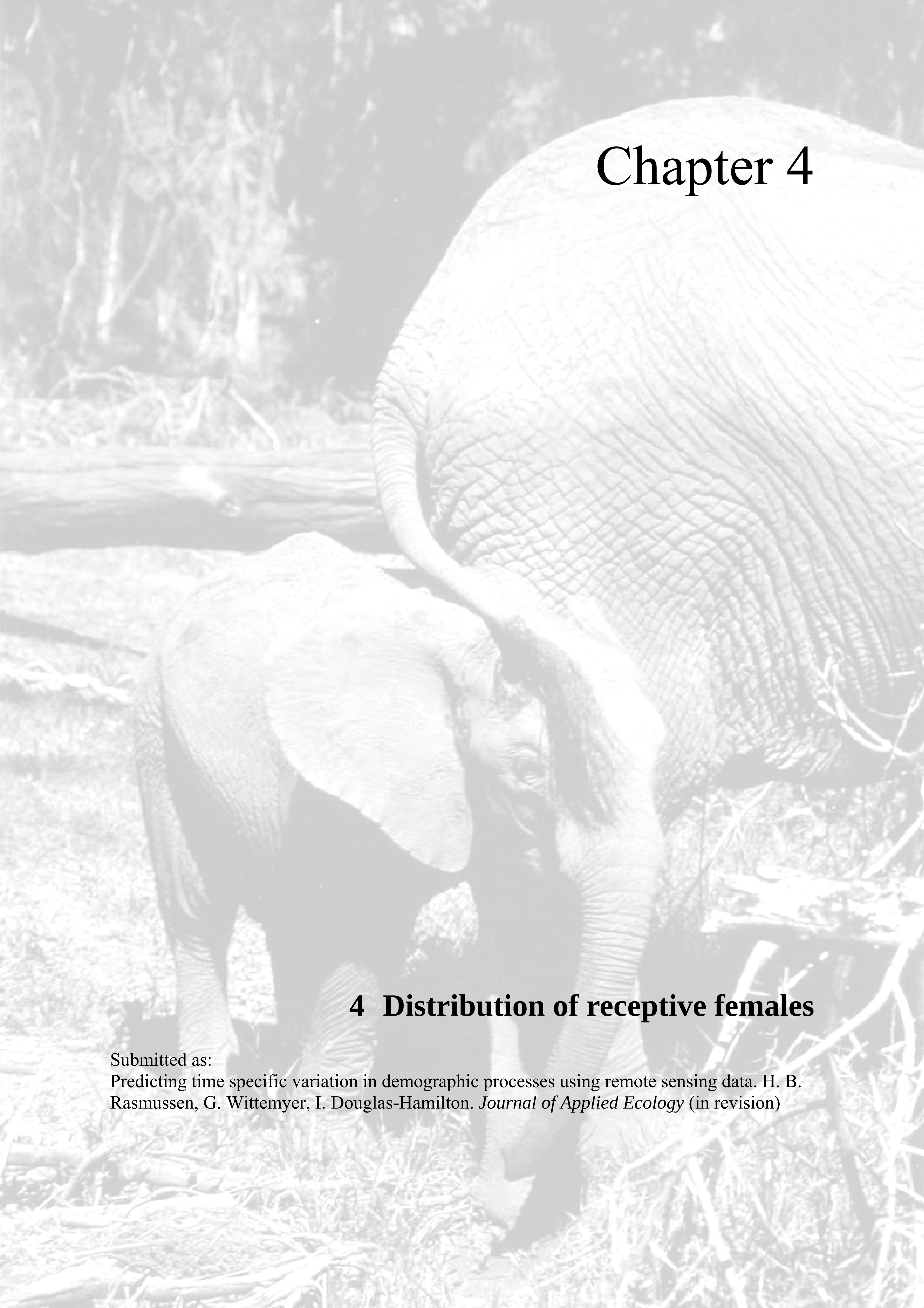
The Zetalabor^{TM} condensation silicon proved highly suitable for obtaining good imprints of the lower jaw molars in the majority of cases. Obtaining a good mould in females turned out to be slightly more difficult than males, probably due to the generally smaller size of females resulting in a smaller working space between the upper and lower jaws. The two cases where the moulds failed completely was caused by adding an un-precise amount of Indurent Gel catalyst; resulting in the material hardening too fast to shape it properly in one incident and the material not hardening enough in the other, and thereby breaking apart when pulled from the mouth.

The assignment of the molar number (M1-M6) proved straightforward. Measurement of length and width of the molar imprints where done to assign molar number. However, this was un-necessary since the age difference between the same molar state of consecutive molars are large, especially in 15 + year old individuals. For example mould #5 in fig 3.3 would give either age-group XIV (20 years) if the mould represented molars M4 and M5, or age-group XXII (39 years) if the mould represent M5 and M6, a difference large enough to tell apart having seen the live animal.

A complete mould was not obtained in all cases, with the missing part being the extreme posterior end. In the event where imprints of two molars could be seen, an age group could easily be assigned even with a missing posterior part as the necessary information for age group assignment exists in the degree of wear of the for-most (oldest) molar (fig 3.3 mould 1,4,5,6). In case no evidence of two molars could be seen in an incomplete mould (fig 3.3 mould 8), an attempt to feel the posterior part by hand was made during the immobilization. Even without the use of this undocumented information, imprints of a large enough part of the molar was present in all cases to assign an approximate age based on the possible 2-3 consecutive age groups. Comparing the age estimates based on physical appearance with that obtained from molar state showed that 75% were within +/- 3 years and only a single individual (Bull, molar age 40 years) was severely overestimated by 10 years. This supports the previous assumption that elephants can be relatively accurately aged by their physical appearance.

The age groups and associated ages follow the age groups described by Laws (Laws, 1966) incorporating the changes proposed by Jachmann (Jachmann, 1988). The age associated with a given molar stage may not be as close to the actual age as described by Laws, especially for 30 + year old individuals. However, the relative age difference between individuals is likely to reflect the actual difference. By taking multiple moulds of a group of individuals in various age groups, separated by 2-3 years it will be possible to assign duration to a specific amount of wear and development. Hence this technique could provide a “ground truthing” of Laws aging technique on free-ranging elephants.

The technique described here provided a number of known aged individuals that was used as reference points for aging the remaining bulls in the Samburu population. The use of “reference” individuals is believed to have increased the accuracy of the age estimates of the remaining individuals in the population, based on height and physical appearance to below +/- 3 years.



Chapter 4

4 Distribution of receptive females

Submitted as:
Predicting time specific variation in demographic processes using remote sensing data. H. B. Rasmussen, G. Wittemyer, I. Douglas-Hamilton. *Journal of Applied Ecology* (in revision)

Abstract

The time specific number of receptive females in the study population is investigated using observations on calf birth in combination with a model predicting conception rates in females based on seasonal specific vegetation quality.

Levels of abortions were estimated to be low making the number and distribution of calf birth a good indicator of oestrus events occurring 656 days prior (average gestation period). A model based on maximum seasonal NDVI value was used to estimate the occurrence of oestrus during the later part of the study period. This model was able to trace the large variation in observed seasonal specific reproductive rates of breeding females, with a median deviation from observed values of 0.06. By combining the model of seasonal specific reproductive rates with the average seasonal distribution of conception dates, the monthly number of conceptions (range 0-22) could be predicted within +/-3 with 80% confidence corresponding to low medium and high numbers. The strong predictive power of NDVI on time specific variation in reproductive rates is likely to be generally applicable to resource limited ungulate species occurring in ecologically variable ecosystems and can potentially be a powerful factor in demographic population modelling.

4.1 Introduction

The number of females coming into oestrus at a given time of year is one of the main factors expected to influence bull reproductive decisions. Hence it was important to obtain a measure of the number of receptive females during the study period. Female elephants show signs of oestrus for only a short period of 4 – 6 days (Moss & Poole, 1983). This short duration of an oestrus event compared with the frequency of observations on specific females in the study area, results in only a small fraction of the actual oestrus events being detected. The number of observed oestrus events is thus not expected to be a good indicator of actual occurrence of oestrus. However an estimate of when a female has conceived and thus been in oestrus can be obtained by subtracting the average gestation period of 656 days (Moss 2001) from the time of birth. Accurate estimates of calf age (+/-1 week) can be done until 1 month after birth (Moss, 1996). Out of the approximately 220 breeding females within the total identified population, around 170 are seen on a regular basis and constitute the core female study population. Of the 265 births that have occurred in this group between 1998 and 2003, 95% have been observed less than one month after birth (Last observation of female without calf in combination with physical appearance of calf) providing an estimate of the frequency of oestrus events resulting in calf birth during a period of 7 years (13 rainy seasons).

The number of oestrus events will be higher than the number of calves born since some females do not conceive and others miscarry. Hence before using data on calf birth as an estimate for the frequency of oestrus the fraction of oestrus females failing to conceive or aborting during pregnancy must be assessed as well as if this fraction varies between seasons. This approach cannot provide information about oestrus for the last 2 years before present since one have to wait until the calves are born. Hence this method could not be used to obtain information on oestrus covering the last part of the study period.

Although oestrus can occur year around (Poole et al. 1989) the peak numbers are often seasonal especially in regions with distinct rainy seasons with oestrus occurring during and right after the rain (Laws et al. 1975; Hall-Martin 1987). In addition inter annual fluctuations in ecological quality have been shown to influence reproductive rates in numerous species (Jarman 1979, Sinclair, Dublin, & Borner 1985) thus suggesting that a model could be developed to predict the number of oestrus females based on time specific seasonal ecological quality.

Models for predicting time specific population dynamics in which reproductive rates are a core factor have been widely applied (Ogutu & Owen-Smith, 2003; Georgiadis et al., 2003) and are of great management and economic importance. Species that inhabit ecosystems with known limiting factors are often modelled by identifying these key factors on which to base population changes. For example, in tropical and sub tropical areas with relatively low annual variation in temperatures and day-length, precipitation is the main factor determining seasons (Huntley 1982). In these areas, rainfall has generally been used as an indirect measure of the seasonal changes in green biomass of the ecosystem (Sinclair et al., 2000) and harvesting models of ungulates in grasslands have been based on rainfall as a predictor of time specific carrying capacity and population growth rate (Georgiadis et al., 2003). However, unless limited directly by available drinking water most ungulates are only indirectly affected by rainfall via its effect on vegetation growth.

Records of precipitation, temperature, or day length may not reflect the quantitative changes in vegetation growth if other factors have a compounding influence. For example, in savannah ecosystems variability in rainfall pattern (du Plessis, 2001), soil type, and the degree of habitat degradation in an area (Wessels et al., 2004) can have significant effects on the response of vegetation to rain. This indirect and often complex connection between the employed variable and actual factors influencing population dynamics may result in low explanatory power of such demographic models. To be more reliable, such models should preferably rely on more direct measures of underlying ecological processes regulating the focal population rather than proxy factors (Brashares 2002). Such a more direct measure of time specific vegetative productivity may be available from high-resolution remote sensing data.

Normalized Differential Vegetation Index (NDVI), available from the NOAA AVHRR and SPOT programs, is an index value calculated as the ratio between remote sensing red and near infrared reflection. As such, it is a measure of an area's greenness and a direct measure of spatially explicit vegetative productivity (Goward & Prince, 1995) as well as a quantitative measure of longitudinal variability in productivity (Scanlon et al., 2002). Hence, NDVI potentially provides a better, more direct measure for predicting time specific rates of reproduction and mortality in resource limited ungulates by circumventing some of the problems of methods based on indirect measurements.

In this chapter it is investigated if the number and distribution of calf birth can be used as an estimate of the occurrence of oestrus 656 days prior. Further the utility of NDVI and rainfall for predicting time specific variation in reproductive rates in the study population is investigated by evaluating the explanatory power of the two ecological correlates, rain and NDVI on seasonal specific reproductive rates. Based on these results a model on seasonal specific reproductive rates is developed and the temporal resolution at which the model can reliably predict numbers of receptive females is investigate. The results indicate that remote sensing data sources offers an improvement to previous approaches for accurately determining conditional dependent demographic processes. Hence, apart from providing specific predictions on occurrence of receptive females for this study the method has general implication for behavioural studies of reproductive strategies in other herbivores and may provide better insight to the potential effects of climatic change or direct human induced changes in ecological conditions on the demographic dynamics of a population.

4.2 Methods

4.2.2 Demographic Data

Demographic data from the core study group of 170 regularly observed females was used for analysis. Of the 265 calves born between 1998 and 2004, 95% were observed within one month after their estimated date of birth and the remaining were known to within the season of birth. The date of conception can be obtained by subtracting the average gestation period of 656 days (Moss, 1983) from the date of birth, giving corresponding dates of conceptions for these calves between mid 1996 and mid 2002. This highly accurate data, including pregnant and non-pregnant periods of females, was used to investigate the demographic processes in the study population covering a period of 7 years (13 wet seasons).

4.2.3 Measures of ecological quality

Daily rainfall measures were obtained from the only long-term weather station in the area at Archers Post situated adjacent to the core study area (Fig 4.1). Wet seasons were defined as beginning on the first 10-day period with 10 mm or greater rainfall (the approximate amount of precipitation required to get a vegetative response) and ending on the last 10-day period with rain followed by 30 days of 0 mm rainfall. Single day showers between two-week periods of 0 mm rainfall were not considered to illicit a seasonal change

Remote sensing NDVI data (10 day composite S10 products) were obtained from the SPOT (1998 -) and AVHRR (1996 - 2000) programs (data currently available free at http://free.vgt.vito.be and ftp://disc1.gsfc.nasa.gov/data/avhrr/). To cover the entire study period (1996-2002), data from the AVHRR and SPOT programs were combined. The two-year overlap between AVHRR and SPOT data (1998-2000) revealed a shifted baseline for the two data sets and the AVHRR data was subsequently calibrated to SPOT level by adding 0.06 to all values. The mean 10-day NDVI values were extracted from an area defined by a 10-km buffer around permanent rivers in a 40-km radius from the centre of the core study area, in which hills and mountains were excluded (1771 km^2) (Fig. 4.1). This area was chosen since the study population seldom ventures further then 10 km away from permanent water or on to steep slopes (unpublished radio tracking data).

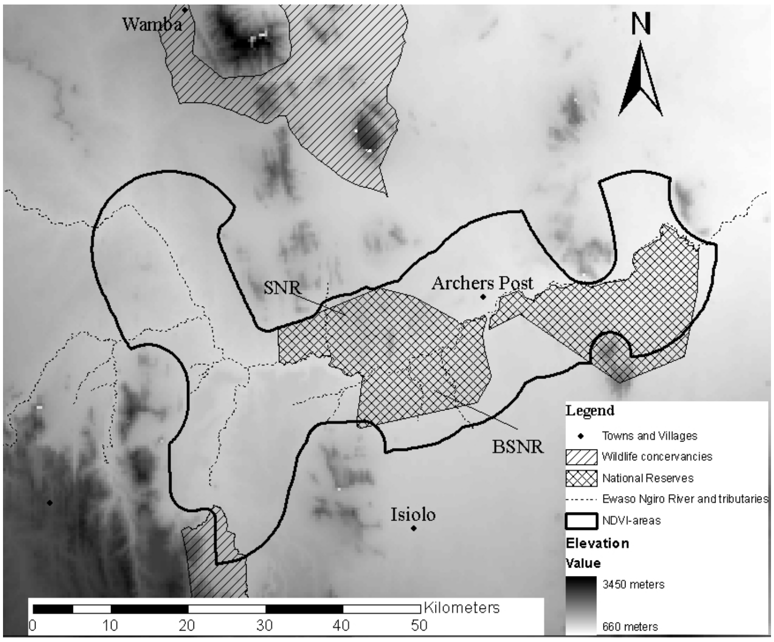


Figure 4.1:Core study area of Samburu and Buffalo Springs National Reserves (SNR& BSNR) and area used for NDVI extraction.

The NDVI data was extracted using ArcView3.2© and WinDisp 5.12. Using NDVI data, wet seasons were defined as occurring when values exceeded two standard deviation above dry season baseline, except during the 1998 El Ninõ event when two seasons where delineated by the lowest NDVI value recorded during the 8 month period of elevated NDVI values. A breeding season was defined as a combined wet/dry cycle starting with the onset of a wet season and lasting until the subsequent wet season. Using the two quality measures did not result in different identified breeding seasons.

4.2.3.1 Ecological quality indices

Different indices were investigated for each of the two measures of ecological quality. For rainfall the three indices investigated were 1) the duration of the rainfall defined wet seasons ($Rain_{dur}$) 2) the total amount of rainfall per season ($Rain_{tot}$) and 3) the greatest 10-day amount of rainfall per season ($Rain_{max}$). For NDVI the indices were 1) the duration of the NDVI defined wet seasons ($NDVI_{dur}$) 2) the sum of NDVI values above the baseline level per wet season ($NDVI_{tot}$) and 3) the maximum recorded NDVI value during a season ($NDVI_{max}$).

4.3 Results

4.3.2 Conceptions between 1996-2002

The temporal distribution of females conceptions, based on calf birth within the group of regularly observed females was obtained from 1996-2002. The estimated time of conception from calf birth is only likely to be within +/- 2 weeks due to the +/- 7 day error on estimated time of birth and some expected variation in gestation period hence data was summed on a monthly running basis (+/- 15 days of date) and is shown in fig 4.2 together with 10-day rainfall and NDVI records.

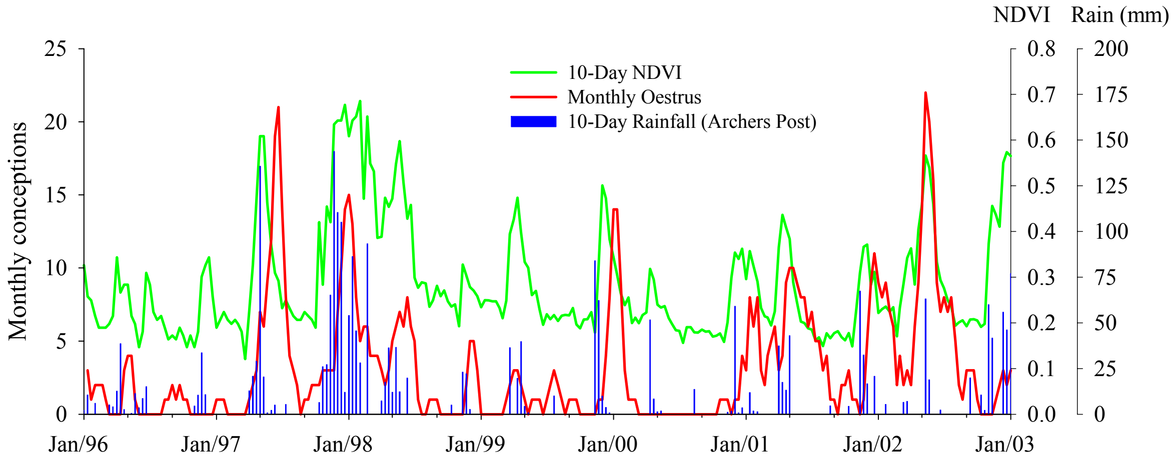


Figure 4.2: Monthly number of conception (based on calf birth) and 10-day rainfall (Archers Post) and median NDVI values for the core 700-km^2 area.

The mean monthly number of conceptions leading to calf birth was clearly bimodal and linked to the two annual seasons (Fig 4.3) Both seasons had on average the same number of conceptions but showed a very large variation between years. This variation was both linked to a large variation of non-pregnant breeding females between seasons (range 25 - 106) but also to a large variation in the fraction of these non-pregnant breeding females conceiving and carrying to term between seasons (Range 0 - 0.63). The conceptions had a time lack in relation to season start with peaks in conceptions occurring a median number of 68 days after seasonal onset (inter-quartile range: 47–94 days).

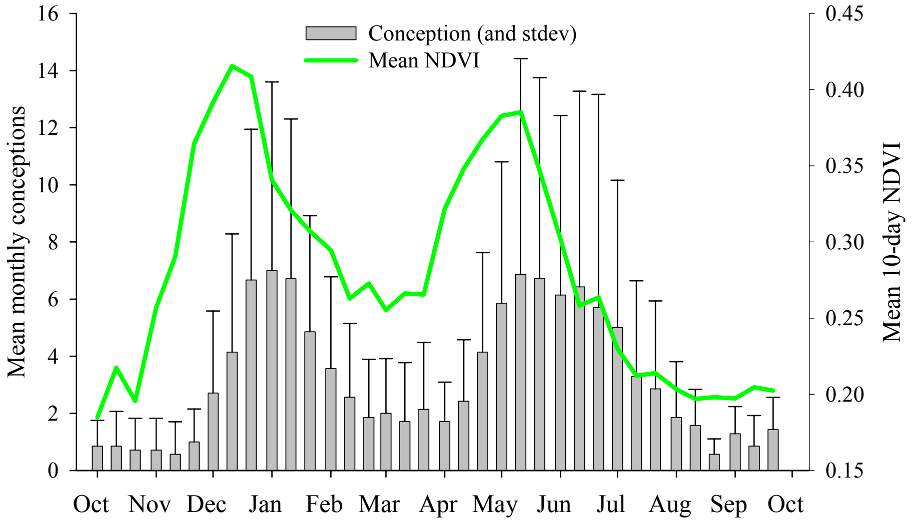


Figure 4.3 Mean number of conceptions per month (10 day running sum) and mean 10-day NDVI (1995-2002).

To evaluate if specific conditions during each season affected the likelihood of conception among females the fraction of known non-pregnant breeding females conceiving per season were correlated to the two measures of ecological quality, namely rainfall and NDVI. This would further reveal if season specific conditions could be used to model the inter-seasonal number of receptive females and which of the measures (Rain,NDVI) had the best explanatory power.

4.3.3 Effect of ecological quality indices on reproductive rates

The effects of the three quality indices on the variation in seasonal reproductive rates (conceptions per available female R_{avail}) were investigated for both rainfall and NDVI. The residuals of the correlation between conceptions per available females during a season (R_{avail}) and these indices were investigated. Where residuals were significantly correlated with observed values or showed signs of non-linearity, the data was transformed to obtain a linear relation between index values and reproductive rates. Of the indices, $Rain_{tot}$ was inverse transformed and $Rain_{max}$ and $NDVI_{tot}$ was natural log transformed. A degree of collinearity existed between indices of the same quality measure. However, instead of comparing the explanatory power of the indices via separate regressions the indices were compared in a multiple regression analysis using backwards stepwise removal of indices with $p > 0.10$. This was done to evaluate whether they combined explained a larger part of the variance. To evaluate the relative explanatory power of the two quality measures the regression models were compared. Finally, a similar approach was used to compare significant indices from both quality measures. Again a level of collinearity exited between indices of rainfall and NDVI and a multiple regression analysis was chosen over direct comparison of the separate regression models to assess to what degree the explanatory power of the model based on one measure was embedded in the other. All proportional data were normalized by arc-sin transformation. Statistical analysis was carried out in Statistica©.

In the rainfall regression model only the total seasonal amount ($Rain_{tot}^{-1}$) had a significant effect (Table 4.1) and in the NDVI regression model only $NDVI_{max}$ had a significant effect (Table 4.2). The NDVI based model explained a greater proportion of the variance in reproductive rates (R^2=0.79) as compared to the rainfall-based model (R^2=0.33).

Table 4.1: Multiple regression model of effect of rainfall indices on seasonal conception rates. Stepwise removal of factors with P>0.1

Factors	Steps	Degr. of	F	P	MultipleR^2	AdjustedR^2	Effect
$Rain_{tot}^{-1}$	Step 1	1	2.562	0.144			In
LN($Rain_{max}$)		1	0.026	0.875			Removed
$Rain_{dur}$		1	0.454	0.5174			In
Whole Model-Step 1		**3**	**2.144**	**0.165**	**0.417**	**0.222**	
$Rain_{tot}^{-1}$	Step 2	1	5.749	0.037			In
$Rain_{dur}$		1	0.501	0.495			Removed
LN($Rain_{max}$)		1					Out
Whole Model-Step 2		**2**	**3.549**	**0.068**	**0.415**	**0.298**	
$Rain_{tot}^{-1}$	Step 3	1	6.911	0.023			In
$Rain_{dur}$		1					Out
LN($Rain_{max}$)		1					Out
Whole Model-Step 3		**1**	**6.911**	**0.023**	**0.386**	**0.330**	

Table 4.2: Multiple regression model of effect of NDVI indices on seasonal conception rates. Stepwise removal of factors with P>0.1

Factors	Steps	Degr. of	F	P	MultipleR^2	AdjustedR^2	Effect
LN(NDVItot)	Step 1	1	0.104	0.755			Removed
NDVImax		1	0.442	0.523			In
NDVIdur		1	0.684	0.430			In
Whole Model-Step 1		**3**	**15.478**	**0.00067**	**0.838**	**0.784**	
NDVIdur	Step 2	1	1.791	0.210			Removed
NDVImax		1	2.405	0.152			In
LN(NDVItot)		1					Out
Whole Model-Step 2		**2**	**25.445**	**0.00012**	**0.836**	**0.803**	
NDVImax	Step 3	1	45.802	0.000031			In
NDVIdur		1					Out
LN(NDVItot)		1					Out
Whole Model-Step 3		**1**	**45.801**	**0.000031**	**0.806**	**0.789**	

The correlation between the proportion of available females conceiving and the significant quality indices from rain and NDVI are shown in fig 4.4 A & B. For rainfall the “El Ninõ” season appeared as an outlier in the relation between rainfall and conception rates with a very high rainfall of 802 mm. (Figure 4.4 A). However the same season was not an outlier in the relation between NDVI and conceptions. Removal of this season in the rainfall model did not alter the predictive power of rainfall.

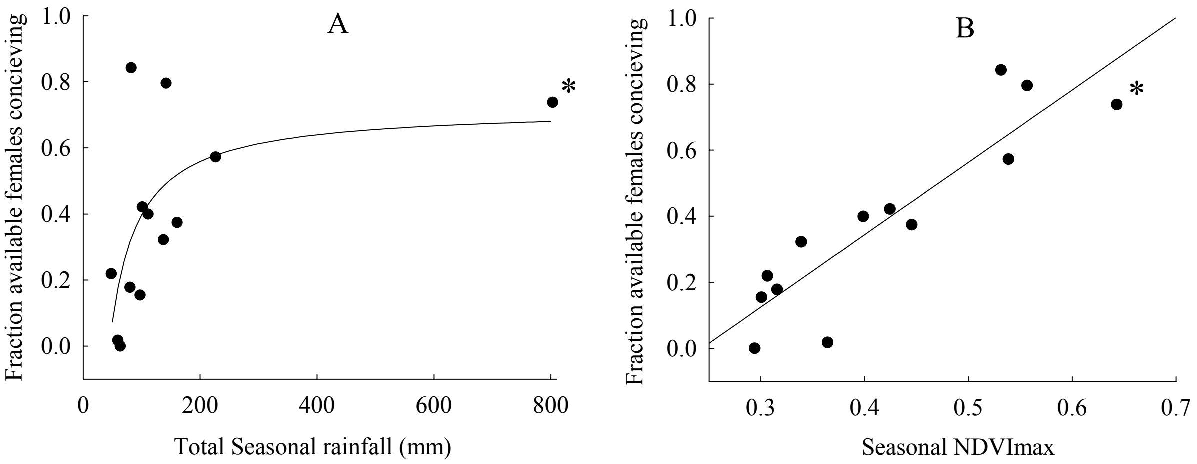


Figure 4.4: Total seasonal rainfall ($Rain_{tot}$) and maximum recorded seasonal NDVI ($NDVI_{max}$) in relation to the fraction of known non-pregnant females conceiving during each season (Arcsin transformed) (Season N= 13). (*) El Ninõ season.

The two significant indices from the rainfall and NDVI based regressions were then run together in a third multiple regression model to see whether additional information could be obtained using both measures. Here, only $NDVI_{max}$ came out significant (Table 4.3). Hence all the power of the model based on rainfall is embedded in the NDVI model. Again, removal of the "outlying" El Ninõ season did not alter the results. Based on these results only $NDVI_{max}$ was used as factor in our predictive model.

Table 4.3: Multiple regression model of effect of significant rainfall and NDVI indices on seasonal conception rates. Stepwise removal of factors with P>0.1

Factors	Steps	Degr. of	F	P	MultipleR^2	AdjustedR^2	Effect
$Rain_{tot}^{-1}$	Step 1	1	0.0188	0.894			Removed
NDVImax		1	21.77	0.000886			In
Whole Model-Step 1		**2**	**20.87**	**0.000270**	**0.807**	**0.768**	
NDVImax	Step 2	1	45.80	0.000031			In
$Rain_{tot}^{-1}$		1					Out
Whole Model-Step 2		**1**	**45.80**	**0.000031**	**0.806**	**0.789**	

The proportion of female conceptions carried to term is thus clearly related to ecological conditions during the season of conception with $NDVI_{max}$ best explaining the observed variation. This effect could result from either high rates of foetal abortions during poor seasons or condition dependent oestrus. If this effect is linked to high levels of abortion the number of calf birth will not reflect the actual number receptive females to which the bulls are expected to respond.

4.3.3.1 Oestrus and calf birth

To distinguish between the two potential effects of abortion and conditional dependent oestrus, the proportion of observed oestrus events that resulted in observed births was assessed from 31 observed oestrus events between 1999 and 2002. If females were aborting foetuses in response to poor seasonal conditions, we would expect the mean number of oestrus/mate-guarding events to be equal across seasons, or at least correlated with the number of non-pregnant breeding females. Oestrus events were not correlated to the number of available females ($R^2 = 0.059$, $y = 0.019x + 2.720$, $p = 0.527$). However, observed oestrus events were strongly correlated with the number of conceptions carried to term ($R^2 = 0.689$, $y = 0.158x + 1.011$, $p < 0.006$) and positively but not significantly correlated with the maximum seasonal NDVI ($R^2 = 0.363$, $y = 11.719x + 1.303$, $p = 0.086$). Of the 31 events, three were definitively erroneous as they occurred during mid pregnancy when the female could not have ovulated. Of the remaining 28 events, six (21.4%) did not result in calf birth 21-22 months later. Of these six, four females gave birth approximately 16 weeks after the estimated birth date from the observed mate-guarding event. It appears these females did not abort but rather failed to conceive during the mate-guarding event, experienced their full, 16 week ovarian cycle (Hodges, 1998a), and subsequently ovulated and conceived. In 3 of the 4 cases this missed oestrus event and subsequent conception occurred within the same breeding season (thus not affecting the results in our analysis). The last two cases were potentially conceptions carried to term where the calf was lost before it was observed, as these two females subsequently gave birth > 4 years after the observed mate-guarding event. Hence, no apparent abortions linked to poor season quality were observed and the rate of female abortions appears to be lower than detectable in a sample size of 28 oestrus events. This shows that the number of calves born can be used as measure of the number of oestrus events occurring 22- month prior.

4.3.4 Model of reproductive rate

4.3.4.1 Demographic data for models

Estimating the number of available females or reproductive population size is essential for predictive models. However, highly accurate demographic data are only available for closely studied populations in which demographic changes are often explicitly known. Thus, useful predictive models must be based on coarser information that can be easily obtained from populations with little to no prior information. Due to the long, 22-month, gestation period of elephants, a proportion of breeding females in a population is pregnant at any given time. The proportion of breeding females known to be pregnant (giving birth less than 22 month later) and thus unable to conceive varied between 18-77% per season, demonstrating that the total number of breeding females is a poor estimate of the number of reproductively available females. Elephants have an average inter-calving period of 4.5 years (Moss, 2001), and generally conceive when their youngest calf is just over two years old. Of the 265 recorded births, only one female was found to conceive with a calf less than 6 months of age. Thus, the age class of a female’s youngest calf may serve as a useful measure of the number of reproductively available females in the population. Calves under the age of 10 years can be aged accurately to within 1 year, and under the age of 1 year to within a month (Moss, 1996) making such data easy to obtain from field observations. The suitability of estimating the proportion of available females from the age of their youngest calf was assessed by comparing the actual fraction of known available females (F_{avail}) (non-pregnant females with calves older than 6 month per breeding female), calculated from complete demographic records of births, with the fraction estimated from females with calves between (a) 0.5 -2.5 years ($F_{(0-2.5)}$) and (b) 0.5 –3.0 years ($F_{(0-3.0)}$).

4.3.4.2 Modeling steps and design

A simple empirical approach was employed correlating the previously determined significant measures of seasonal-specific quality with the coarse demographic information. The relationship was then used to develop a regression-based model to estimate reproductive rates. In the model, seasonal specific reproductive rates refer to the season of conception, where the resulting birth occurs 22 month later. Data on seasonal-specific reproductive rates (conceptions resulting in birth per breeding female per season) were available for 13 seasons (7 years). The first 6 seasons was used for model building and parameter estimation and the remaining 7 seasons for model validation.

The model was expanded to predict time-specific variation in the number of receptive females by combining the intra-seasonal temporal distribution of conceptions with the predicted seasonal population reproductive rate. The temporal distribution of receptive females was estimated from our known dataset of conception events (based on calf birth) occurring within the first 6 seasons. Females have been shown to time conceptions according to average seasonal onset rather than specific onset (Wittemyer et al., submitted) allowing a function describing the temporal distribution of seasonal specific conception events to be incorporated into the model. The first 6 seasons (97 conception events) were used to obtain the distribution of time delay between season onset and conceptions The accuracy of the modeled distribution was evaluated by distributing the known number of seasonal conceptions during the last 7 seasons (43 month). The modeled distribution was then applied to the estimated number of females conceiving per season based on our regression model. The real and predicted numbers of estrus events were calculated as monthly sums (+/- 15 days) to account for error introduced during calf age estimation.

4.3.4.3 Model

The fraction of females with 0.5-2.5 year old calves ($F_{0.5\text{-}2.5}$) demonstrated the best correlation with the actual number of available females ($\mathrm{Asin}(F_{avail}) = 0.9117*\mathrm{Asin}(F_{0.5\text{-}2.5})-0.0069$; $R^2 = 0.815$; N=13; $P<0.0001$). $F_{0.5\text{-}2.5}$ was therefore used as the measure of available females.

The model was developed using data from the first 6 seasons of the study, the minimum number of season required to cover the full spectrum of NDVI values during the seven-year study. The seasonal specific reproductive rate of estimated available females (R_{avail} = conceptions per estimated available female) was regressed with the seasonal specific $NDVI_{max}$ ($\mathrm{Asin}(R_{avail}) =1.684* NDVI_{max} - 0.437$; $R^2 = 0.878$; N=6; P=0.0021). Using parameters from this regression, the seasonal specific population reproductive rate for season i ($R_{pop\ i}$) was calculated as:

$$Rpop_{i} = F_{0.5\text{-}2.5\ i} * R_{avail\ i} = F_{0.5\text{-}2.5\ i} * \sin(1.684 \times NDVImax_{i} - 0.437) \qquad \text{(Eqn. A)}$$

$F_{0.5\text{-}2.5}$ = Fraction of females with calves between 0.5-2.5 years.

$NDVImax_i$ = Maximum 10-day mean NDVI value within season i.

$Rpop_{i}$ = Population reproductive rate during season i. (resulting in birth 22 month later)

$R_{avail\ i}$ = Conceptions per available female during season I

The observed seasonal specific reproductive rate ($R_{pop\ i}$) varied between 0 and 0.4 (Fig 3.5) with the model having a median deviation from observed values of 0.067. The model had the highest deviations during the last 3 seasons. This deviation from observed rates was negatively correlated, though not significantly, to the fraction of known available females with calves older than 2 years (F_{2+}) (model deviation = -0.277 * Asin(F_{2+}) + 0.0222; R^2 = 0.4359; N=7; P=0.106).

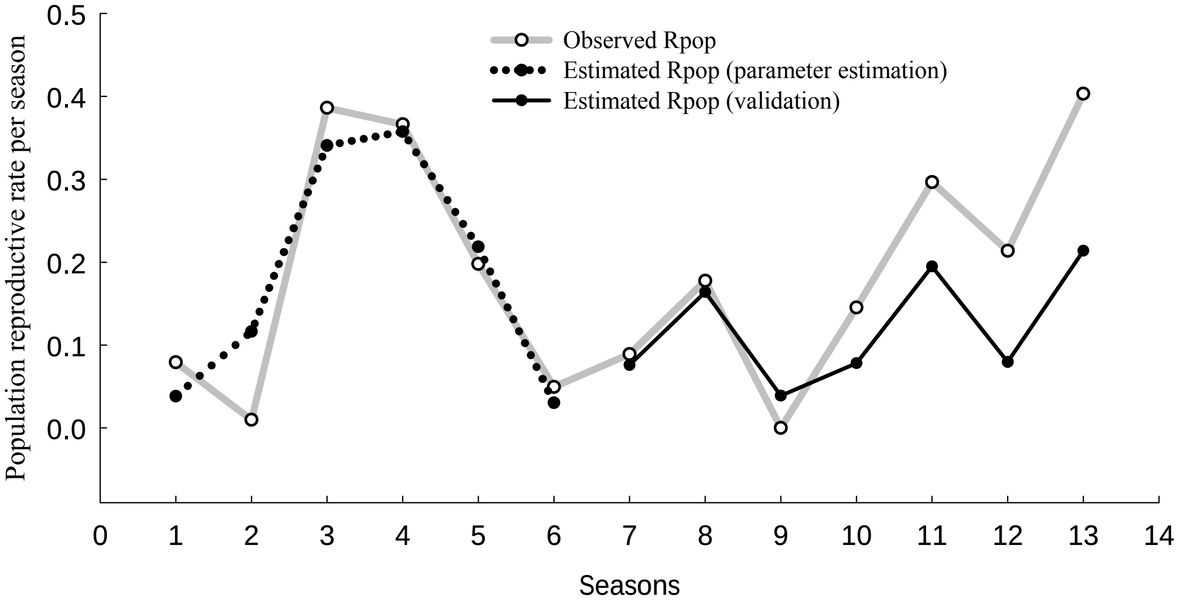


Figure 4.5:Observed and predicted seasonal reproductive rates. The first 6 seasons were used for model building and parameter estimation and the last 7 seasons for validation.

4.3.4.4 Temporal distribution model

The model of seasonal specific reproductive rates was expanded to predict time-specific number of receptive females using an approach based on combining predicted population reproductive rates with the intra-seasonal distribution of conceptions. The timing of conceptions in relation to seasonal onset was evaluated in order to see if the females time their oestrus according to actual or average seasonal onset

The mean durations between specific seasonal starts and conception events for a season were negatively correlated to that seasons relative start date (R^2 = 0.62; p = 0.02; y = 42.9 - 0.57 * x; Fig. 3.6). Hence, during relatively early seasons, females wait longer after the seasonal start date before conceiving compared to late starting seasons. This shows that the overall timing of conceptions is linked to average season start and is likely due to female elephants aiming to time parturition, enabling elephant to generally coincide birth events with the initial greening of the ecosystem. Due to the fixed gestation period, such timing of parturition must be regulated at the time of conception. This does not contradict the response to seasonal specific NDVImax since conceptions occurred a median of 34 days (IQR range: 5–52 days) after the seasonal NDVI peak (maximum breeding season NDVI value) with 78% percent of all conceptions occurring after the peak during the period when the greenness index of the study ecosystem is decreasing

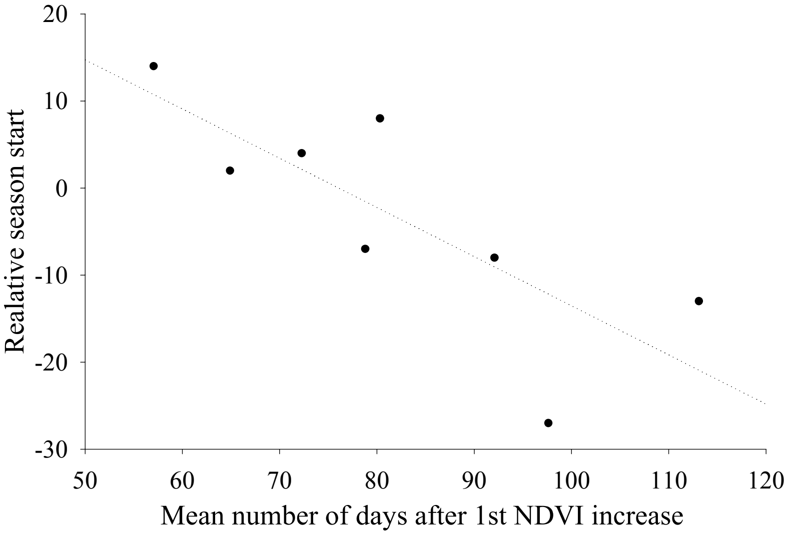


Figure 4.6: Mean delay of conceptions from season start versus relative season start. Seasons with less than 10 births excluded (N= 8).

The intra seasonal distribution of receptive females was estimated from our known dataset of conception events occurring within the first 7-seasons. The frequency distribution of the time between estimated conception dates and the average date of seasonal onset was plotted in order to find a function describing the temporal distribution of seasonal specific conception events. The distribution did not deviate significantly from a normal distribution (Chi square: Df=4 p=0.47) (Fig 4.7).

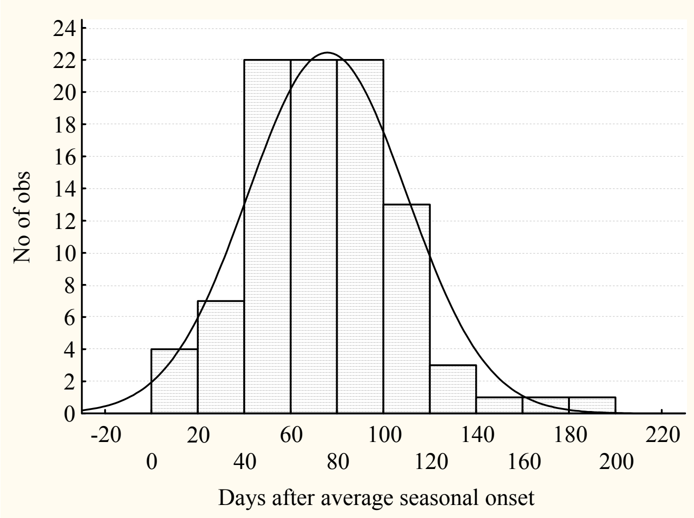


Figure 3.7: Distribution of time lag between average seasonal onset and estimated time of estrus (N=96) (Normal(x, μ=75.29, σ=34.11) ; Chi p=0.468).

Combining the model predicting seasonal specific reproductive rates with numbers of breeding females in the population ($Npop_i$), the seasonal number of conceptions ($N_{conception}$) was estimated by:

$N_{conception} = Rpop_i * Npop_i$ (Eqn. B)

Combining equation B with the temporal distribution of intra-seasonal conception events (Fig 3.7), a model of the monthly number of conceptions (receptive females) was obtained. The model starts at the average seasonal onset (day i = 1) until the 200th day of the season, thereby distributing 99% of the estimated conceptions:

$$\text{Conceptions day i} = \int_{i-15}^{i+15} Normal(i;75.29;34.11) * N_{conception} \quad i = [1 \text{ to } 200] \qquad \text{(Eqn. C)}$$

The distributions were slightly overlapping during late March-April, Hence the total number of predicted receptive females was calculated as the sum of the two overlapping distributions at each time step.

The distribution model (eqn. C) was first evaluated by distributing the known number of seasonal conceptions and comparing that distribution to the known monthly numbers of conceptions during the 43 months not used in the estimation of the distribution parameter.

The predicted monthly number of conceptions (range 0-16) was significantly correlated to the observed (range 0-22) (Observed = 0.9109* predicted+ 0.336; R^2 = 0.666; N=43; p< 0.0001) with 80% of months being within +/- 2.8 and 90% within +/- 3.5 conceptions.

The same steps were applied to the predicted seasonal number of conceptions calculated using equation B. Here the monthly numbers of conceptions were also significantly correlated with the observed number of conceptions (Observed = 1.55* predicted + 0.105; R^2 = 0.606; N=43; P< 0.0001) with 80% being within +/- 3.1 and 90% within +/- 4.8 conceptions. The known monthly numbers of conceptions are shown in Figure 3.8 together with the modelled distribution of known and predicted seasonal conceptions. The distribution of the known seasonal conceptions generally follow the observed both in terms of timing and peak height whereas the distribution of predicted generally underestimates values as a result of the underestimation of seasonal number during the last three seasons.

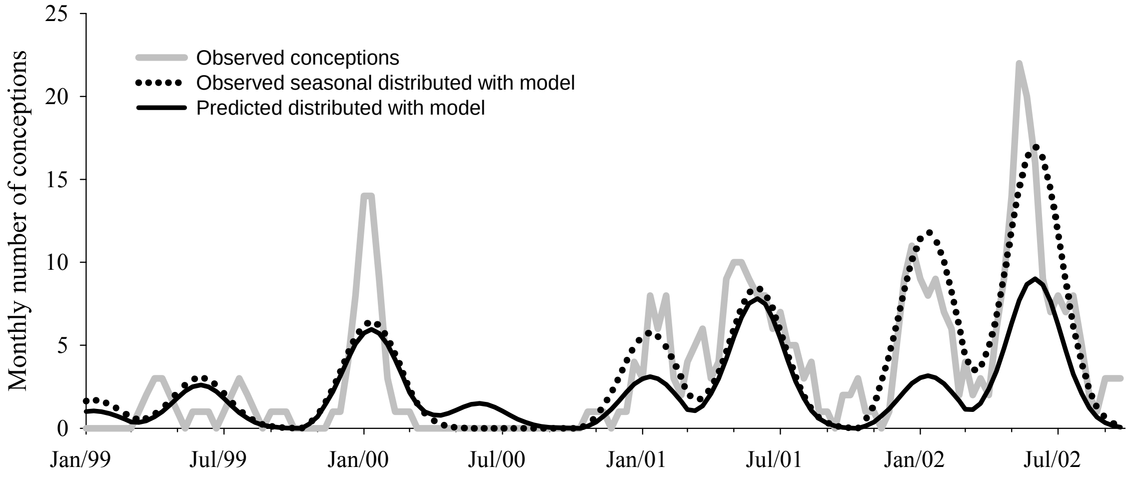


Figure 4.8: The temporal distribution of conception events obtained from calf births (grey line) and the distribution of known seasonal number of conception based on distribution model (dotted line) and distribution of estimated conceptions based on available females and 10-day NDVI values of the core study area (Black line).

4.3.5 Longitudinal number of receptive females during study period

By combining the number of receptive females based on observed calf birth between 1999 and 2002 with numbers predicted from the oestrus model the estimated monthly number of receptive females (Oestrus females) was obtained and is shown in fig. 4.9.

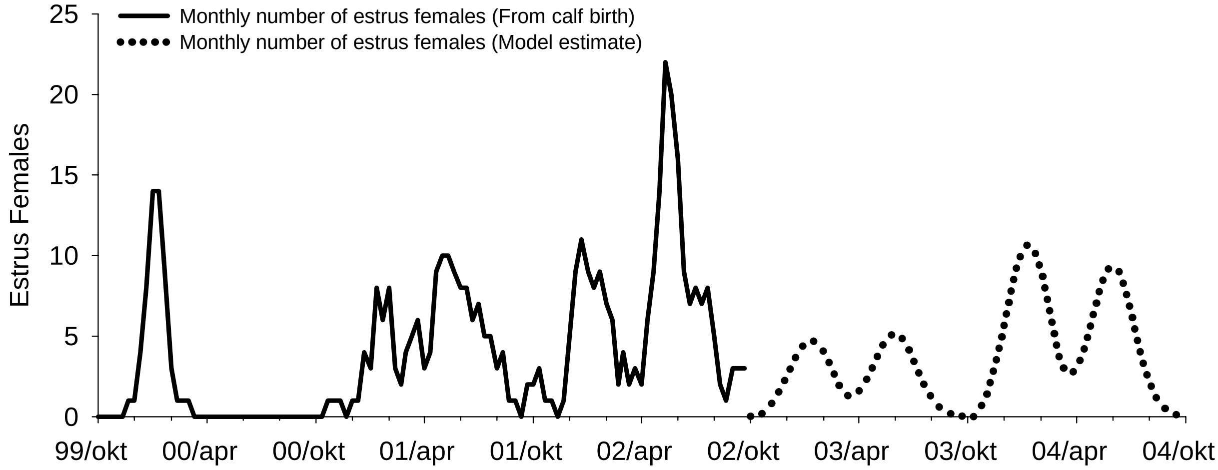


Figure 4.9: Monthly running sum (+/- 15 days) of estrus females between October 1999 and October 2004 based on observed calf birth (solid line) and model predictions (dotted line).

4.4 Discussion

The analysis of observed oestrus events shows that very low frequencies of abortions occur in the study population. Furthermore, the inter-seasonal variation in birth numbers is due to variation in receptive females rather than variation in fraction of conceptions carried to term. Hence calve birth can provide a good estimate of the number of receptive females occurring 22 month prior. In the sample of 29 oestrus events 4 (14%) conceived 16 weeks later than predicted from the observed oestrus event indicating that a few females fail to conceive and experience their ovarian cycle of 16 weeks before conceiving. This will result in the number of oestrus events being slightly higher than that predicted from calf birth. The long term monitoring of the study population provided accurate data on the temporal occurrence of calf birth in the 75 % most frequently observed breeding females. The remaining females are likely to exhibit the same patterns in conceptions hence the total number oestrus females in the overall population will be higher than that obtained from the most frequently observed but have the same relative distribution. Females are not present in the core study area throughout the year. Hence, some of the regularly observed females may have been absent from the core study area at the time of conception and some of the infrequent visitors may have been present making the total number of conceptions and over estimate of what occurred in the study area. Despite these various effects the estimated longitudinal distribution of oestrus females is expected to be a good relative measure of actual numbers.

Since data on oestrus could not be obtained directly from the last seasons an attempt was made to develop a predictive model of seasonal specific number of receptive females as well as the distribution with each season.

Both rainfall and NDVI were correlated with the proportion of known non-pregnant females conceiving per season, indicating that elephant reproduction is highly influenced by inter seasonal fluctuations in ecological conditions. However, the total seasonal rainfall explained far less of the variation (33%) compared to the maximum seasonal NDVI value (79%), demonstrating that remotely sensed NDVI data is a better indicator of the conditions influencing reproduction than the traditionally used measure of rainfall. Indeed, the combined model of both rainfall and NDVI demonstrated that rainfall did not add to the overall explanatory power. Hence the effect seen from rainfall is likely due to the correlation with NDVI.

The first 6 seasons covered the complete variation in observed vegetative productivity, measured by NDVI and a subsequent rerun of parameter estimation using the complete dataset only marginally changed correlation parameters. However, six seasons may be few for accurate estimation of parameters in some systems. The model based on NDVImax was able to trace the variation in the observed seasonal specific population reproductive rate (range 0-0.4) and had a median deviation from observed values of only 0.06. The model generally underestimated the rates during seasons with large fractions of available females whose youngest calf was between 2 and 2.5 years old, as such females are probably more likely to conceive than females with calves between 0.5 and 2 years. This especially occurred during the 2001 and 2002 seasons in which many females had 2-3 year old calves as a result of the 1998 El Niño event, which had the greatest rainfall and NDVI values recorded and associated high conception rates. This indicates that a stage specific model with age-class specific parameters for females with different aged calves could out perform our model. Such a model will require a larger, longer-term data set to accurately parameterize each age class and is, therefore, beyond the scope of our current seven-year data set. The specific parameter values estimated in the current study may be partially population/area specific hence parameter validation to local conditions is needed before application of these model steps to other populations.

The high predictive power of the NDVI-based model suggested that it could be further developed to estimate actual numbers of receptive females in the population at a higher temporal resolution. The temporal distribution of intra-seasonal conception events around the average seasonal onset date was used to model time-specific reproduction in the study population. A simple normal distribution of this temporal delay predicted the distribution of known number of conception during the 43 month used for validation to within +/- 2.8 events in 80% of the month with both the timing and peak height corresponding to the observed distribution. This indicates that conceptions can be accurately distributed over the season when an accurate input of seasonal number of conceptions is available. Using the temporal distribution-parameter in combination with predicted numbers of receptive females, derived from the NDVI model of reproductive rates per season, estimated the monthly number of conceptions within +/- 3.1 events with 80% confidence. The occurrence and duration of peaks in oestrus females were generally well predicted by the model (Fig 4.8), but the actual peak sizes were not. This is due to the underestimation of seasonal conceptions during the last seasons, which appear to be an effect of the extreme ecological conditions associated with the 1998 El Niño event. The level of accuracy attained in this model is useful for assigning the seasonal timing of peaks in receptive females as well as categorical levels (low, medium or high) of receptive females at a one-month resolution.

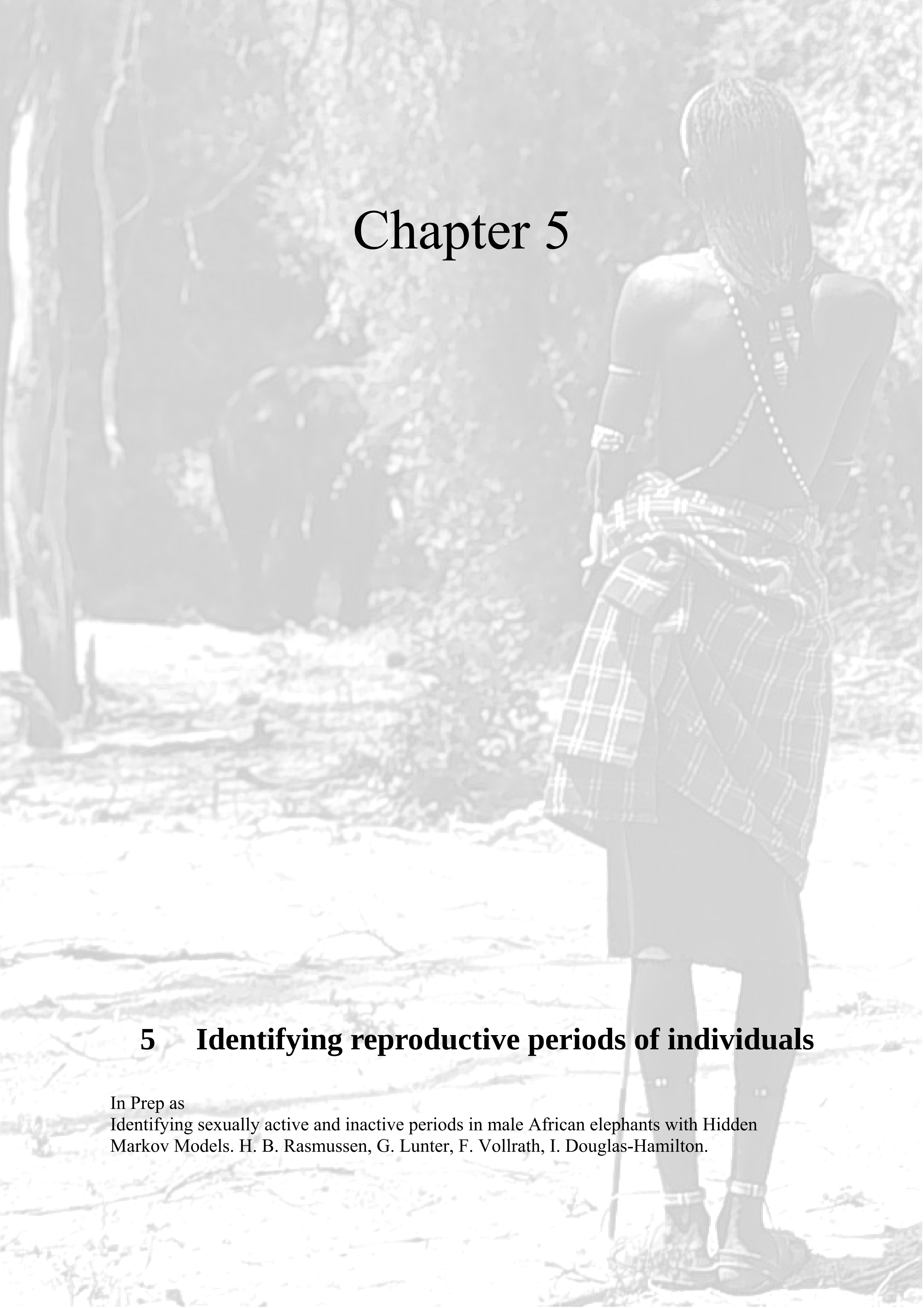
Elephants are long-lived animals with prolonged gestation and post-partum lactation periods resulting in an average inter-calving interval of 4.5 years (Moss, 2001) and a variable proportion of females being pregnant at any given time. These life history traits necessitated the estimation of the time-specific proportion of available females in order to model the population demographic processes. In species with shorter gestation periods where individual reproductive bouts occur seasonally or annually, such variability may not need to be incorporated in a demographic model. For the study elephant population, our model of seasonal specific reproductive rates was developed based on the fraction of breeding females with calves between 0.5 and 2.5 years rather than on the total number of reproductive females in the population. This coarser demographic data is available at the time of conception, two years before the actual birth of a calf, and obtainable from populations not under intense monitoring through relatively simple collection protocols like sample counts of female groups.

The focus was only on reproductive rates as this factor is affecting bull reproductive decisions. However, observational evidence from the study system suggests the same ecological variation governing reproductive rates affects mortality, especially among juveniles (Wittemyer et al., In Press-a). Thus, it is likely a NDVI based model could be used to predict mortality as well as conception rates. Such a model may be highly applicable to systems in which mortality is the driving factor for population growth rates. Postponing reproduction for a single season due to poor vegetative productivity may have less effect on the lifetime reproductive success of elephants than that of shorter-lived ungulates with short-interval breeding cycles. In the later system, females may attempt breeding regardless of specific seasonal conditions but with these conditions having effects on offspring survival and general mortality. Despite these likely species-specific differences, the better predictive power of NDVI as compared to rainfall demonstrated in our study system shows that NDVI is a more accurate metric for the analysis of links between ecological variability and demographic parameters such as mortality, reproductive rates, and carrying capacity.

Time-specific information on the number of conceptions (receptive females) is important for population management as well as behavioural studies of reproductive strategies and individual decision-making processes. Getting data on the density and distribution of receptive females are often difficult under field conditions in free ranging populations, despite being core factors affecting female social organization and male reproductive decisions (Emlen & Oring, 1977b). The results obtained here show that relatively accurate information on time specific number of conceptions can be acquired by combining coarse demographic information with measures of seasonal specific quality and intra season distribution of conceptions. The resolution at which this can be predicted in other systems will depend on the relationship between seasonal specific quality and conception probabilities in combination with the degree of variability in intra-seasonal breeding phenology (Sinclair et al., 2000).

Prior to its employment, the characteristics and limitations of NDVI in relation to the ecology of the system to be modeled should be considered. An ecosystem wide measure such as NDVI (or rainfall) may be better for predicting the population processes of generalist herbivores as compared to a specialist or frugivorous species dependent on specific plants or fruiting events. If population processes are regulated by factors unrelated to general vegetative productivity, such as predation or disease (Sinclair et al., 1985; Gasaway et al., 1996), NDVI is not likely to be a good factor with which to compare ecologically related changes. NDVI only measures actively growing vegetation therefore dry-season or winter standing biomass cannot be directly measured using this index. In addition, NDVI cannot directly differentiate between vegetation types, so grazers and browsers may respond differently to the same NDVI values depending on which type of vegetation is responsible for the remotely sensed photosynthetic activity. In predominantly wooded areas and forests, the inter-annual fluctuation in NDVI values are limited and weakly correlated with relative vegetation growth (Scanlon et al., 2002), particularly in respect to sub-canopy growth which can not be evaluated by remote sensing. With these limitations in mind, which are also generally applicable to using rainfall as a proxy, NDVI can provide a better more direct measurement of the ecological quality influencing population dynamics of herbivore species. For practical purposes NDVI may also be more easily accessible in areas where few rainfall stations exist or detailed rainfall records are not available.

In conclusion, the presented results based on demographic data on a free-ranging elephant population demonstrate the utility of NDVI in predicting seasonal specific reproductive rates. Our results show that remotely sensed NDVI data has much greater predictive power than the widely used rainfall proxy in this system. These results are likely to be generally applicable to ungulates occurring in rainfall-dependent ecosystems and are promising for the use of remote sensing data in demographic population models. NDVI data may offer an improvement to previous data sources for the estimation of harvesting and conservation protocols. The method has further implications for behavioural studies of reproductive strategies and may provide better insight to the potential effects of climatic or direct human induced changes in ecological conditions on the demographic dynamics of a population.



Chapter 5

5 Identifying reproductive periods of individuals

In Prep as
Identifying sexually active and inactive periods in male African elephants with Hidden Markov Models. H. B. Rasmussen, G. Lunter, F. Vollrath, I. Douglas-Hamilton.

Abstract

Analysing and comparing how individuals differ in behavioural solutions to identical goals requires a framework for identifying the underlying motivation behind their behaviour. To avoid any a priori categorisation such framework must be based on proper statistical tools relying on data unrelated to tactic specific behaviours.

Hidden Markov Models (HMM) proved highly successful in identifying changing associational preferences over time. In older bulls above 35 years the periods with predominant female associations were closely linked to musth periods known to be associated with reproduction. This indicates that the associational shifts are linked to reproductive decisions. However, already by the age of 20 years well before the onset of the competitive reproductive state of musth, bulls showed repeated shifts in associational states alternating between periods of high and low female affiliations. The signals associated with musth (urine dribbling and temporal gland secretion) started gradually to occur in bulls at the age of 25-30 years within the periods of high female association. Musth did not become synonymous with these periods before the age of 40 years. Periods with high female association occurred year around among different bulls but for both musth and non-musth bulls the majority coincided with peak numbers of receptive females but not with seasonal changes in vegetative productivity. This strongly suggests that these periods are also linked to reproduction in the younger bulls. This provides the first quantitative evidence of “sexually active non-musth” (SAN) periods in young bulls. Bulls engaging in this alternative reproductive tactic of SAN have well established, regular occurring period of SAN 5-10 years before the onset of the competitive reproductive state of musth. In addition these results show that Hidden Markov Models are highly suited for identifying patterns in time structured behavioral data and for this system can provide a framework for analyzing differences in reproductive tactics.

5.1 Introduction

The description of behavioural changes linked to reproductive decisions in musth bulls has been based on an a priori categorization of observations based on the occurrence of urine dribbling (UD) and temporal gland secretion (TGS). Although the state of musth is clearly linked to reproduction, the period with musth signals may not reflect the exact duration of the sexually active period. Indeed older musth bulls are known to start showing signals several weeks before starting to associate with any females (Poole, 1987). In addition, younger musth bulls are known to switch "musth" signals on and off within hours depending on the presence of older, more dominant musth bulls (Poole, 1989a) but may not change reproductive mode. In order to investigate under which conditions males emits musth signals and how the timing of these signals are correlated with their reproductive mode and for example endocrinological changes such as changing androgen levels, a method for identifying reproductive state, unlinked to the presence of signals is needed.

The state of musth may also not be the exclusive reproductive tactic. Musth is mainly seen among older 35+ year old bulls with the occurrence and duration of musth periods being positively correlated with age (Poole, 1987). During the 20-year period between puberty and musth becoming predominant, younger non-musth bulls are also known to obtain matings and have viable sperm (JoGayle et al., 1984; Hall-Martin, 1987; Barnes, 1982) but engage to a far lesser degree in aggressive interactions with competitors and seldom mate guard receptive females, hence are less competitive compared to musth bulls. These bulls may rely on chance encounters with females or engage in a distinct alternative reproductive tactic. Since the signals associated with musth by definition do not occur in non-musth bulls, investigations of potentially alternative reproductive tactics will suffer from a lack of a common definition of sexually active periods. In fact the existence of an alternative "non-musth" reproductive tactic is still uncertain due to the difficulty in distinguishing non-musth bulls potentially focused on reproduction from bulls not in reproductive mode. This in turn makes it difficult to interpret whether the observed behavioural changes seen in musth bulls is linked to the competitive state of musth, generally linked to reproductively active periods, or linked to external conditions such as number of receptive females, operational sex ratio or the local competitive environment.

In summary a proper statistical framework is needed to identify when an individual is focused on reproduction, compared to for example foraging before we can hope to understand how individual bulls navigate a life-time reproductive decision path by altering the spatio-temporal timing and level of investment in reproduction as well as degree of competitiveness and spatial search patterns. Such a framework could potentially be based on shifting associational preferences. Bulls in musth have been shown to spend more time in association with females than when out of musth strongly suggesting that the preference for female company is linked to reproductive decisions.

Hidden Markov models (HMMs) were initially developed in the early 1970's to analyse sequential data in the field of speech recognition, and are currently widely used for analysis of DNA and protein sequences (for general introduction to HMMs see for example: (Rabiner, 1989,Durbin, 1998 #95; Durbin et al., 1998). HMMs model systems that can be in a number of unobservable (hidden) states. In each state the system emits an observable symbol with varying state-specific probabilities. These properties resembles a situation where the frequency of different types of association (with bulls, alone or with females) depend on the underlying motivational state of the individual but where the motivational state is unknown and only the associations can be recorded. The HMM framework allows to infer model parameters, as well as the sequence of unobserved states, from the observed sequence of associations. Hence, hidden Markov models could provide a tool for identifying potential patterns in associational preferences linked to changing reproductive motivations in time-structured data.

5.2 Material and methods

5.2.2 Data collection and categorization:

Spot observations on bulls (and females) have continuously been collected within the study area since 1998. These spot observations include recording of identity of all associating individuals, position, using GPS and for the bulls, the degree of Temporal Gland Secretion (TGS), Temporal Gland Swelling (TG), and Urine Dribbling (UD) using Poole's definitions (Poole, 1987). During spot observations, individuals are considered to be associating when occurring clustered and moving in a coordinated way and/or interacting with other individuals.

Each observation of an individual bull was assigned a group type depending on association. Three group types were differentiated: 1 = in association with other bulls and not with females; 2 = Alone, no signs of association with other individuals; 3 = in association with mature females, regardless of the presence of other bulls.

All bulls within the study population leave the core study area for prolonged periods of time every year, creating gaps in the observational record of individuals. Since 2000 a varying number of bulls have been collared with either radio or GPS collars. These bulls have been tracked and located on a regular basis hereby obtaining continued longitudinal records of associations. Analysis in this paper is based on four years of data (approx 6000 single observations of individuals) collected between December 1999 and December 2003 where the most complete records on bulls exist and includes both collared and non-collared individuals from which good longitudinal data sets where available.

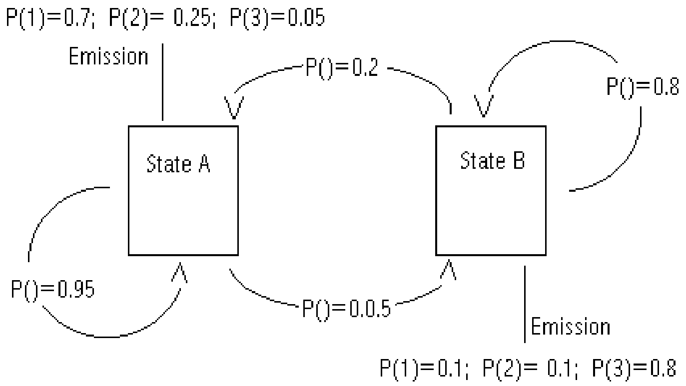
Longitudinal observation sequences lasting 2-4 year with a minimum of 30 obs/year were available from 38 individuals, (est. age range 18-49 years). Of these, 12 belonged to 35+ year old bulls (at beginning of sequence) known to have regularly occurring musth periods and 21 belonged to younger bulls less than 30 years (at end of sequence) which had never been seen in musth or with highly sporadic musth periods of less than 7-day duration. The remaining 5 sequences were from bulls between 30 and 35 years showing some level of recurring musth periods.

5.2.3 Analysis of associations

The aim was to investigate whether bulls have changing associational patterns, and if so whether these are linked to reproductive mode and can be used to identify sexual active and inactive periods. The analysis was therefore initially focused on bulls older than 35 years known to have regular musth periods. These periods are known to be linked to reproductively active periods offering a guide to evaluate the model results.

5.2.3.1 Model background:

The longitudinal association sequences of individual bulls were analysed using a hidden Markov model (HMM). HMMs model systems with a number of unobservable (hidden) states. At each instant in time, the system is in one particular state, from which a single observable symbol is emitted with state-specific probabilities. The system then transitions to a subsequent (possibly the same) state with certain state-dependent transition probabilities. An example of a simple HMM consisting of two states each emitting three possible symbols is shown in fig. 5.1, together with a possible emission sequence (with the "hidden" state path shown for illustration).



Emissions
(Observed) 1 2 1 2 2 1 1 1 3 1 1 2 2 1 1 3 2 3 3 3 3 3 1 3 3 3 3 3 1 1 1 1 1 1 1 1 2 1 2 1
State
(Hidden) A A A A A A A A A A A A A A A B B B B B B B B B B B B B A A A A A A A A A A A A

Figure 5.1: Example of simple HMM consisting of two states and three emissions with a possible path of observable emissions and underlying hidden states.

An HMM, λ, thus consist of a triplet parameter set λ =(Π, A, B) where Π is the initial state probability vector Π= (π_1...,π_S) specifying the probabilities of starting in each of the S states; **A** is the transition parameter matrix **A** = (A_{ij}) consisting of the probabilities of chancing to state *j* given that the current state is *i* (for example, the probability of a bull changing from an inactive to an active state); and finally **B** is the emission parameter matrix **B** = (B_{ie}) consisting of the probabilities of emitting symbol *e* given that the system is in state *i*. (for example, the probability of an active bull associating with females). Often, Π has its probability mass concentrated in a single state termed the Start state.

In the above description the parameters of the HMM are constant with time. However, since the time interval between observations of a specific individual varied, having constant probabilities for transitions between states is inappropriate. We therefore used the framework of time-inhomogeneous HMMs, which allow time-dependent rates in the state transition (and emission) matrix, which allowed the probability of a shift occurring between states to depend on the time between observations.

In an HMM, the underlying sequence of hidden states is memoryless (a Markov chain) since the system state at time t only depends on the previous state and the transition probabilities. The implication is that the time spent in each state has an exponential distribution (or geometric, to be precise), comparable to the exponential distribution of survivorship given a fixed mortality rate. This may not be biological realistic. For example a bull that has just become sexually active is unlikely to change state immediately but instead is likely to stay active for a period of time, for example until energy reserves are depleted or until the season changes.

An initial indication of the shape of state duration distribution could be obtained by looking at the raw association data. Associations with bulls, alone and with females were assigned values from 1 to 3 and the running average of the previous, present and subsequent association was calculated and the duration of periods with predominately female association (average above 2) could be obtained. 34 periods from the 12 bulls above 35 years were identified in this way. The distribution of these periods was not exponential but did not deviate significantly from a gamma distribution (Chi-Square = 2.41, df = 2 (adjusted), p = 0.30) (fig 5.2).

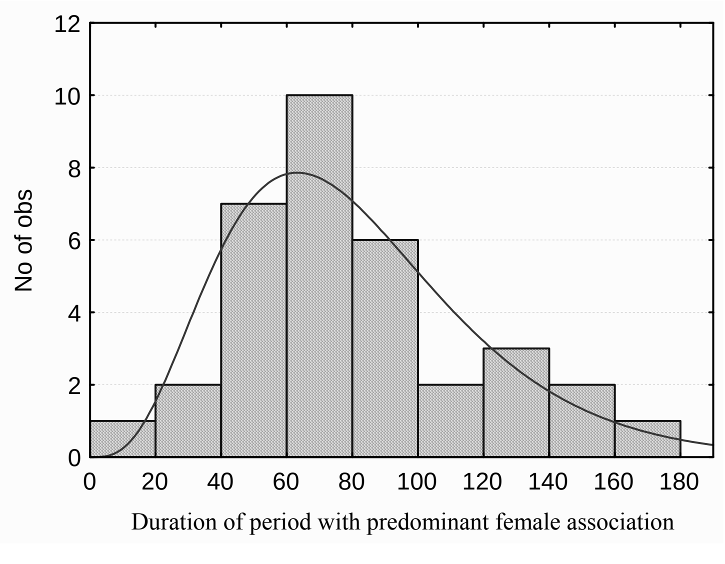


Figure 5.2: Distribution of periods with predominant female association fitted with gamma distribution curve (34 periods from 12 different bulls).

The state duration distribution of a HMM can be changed by the introduction of pseudo states, mimicking a memory without losing the simplicity of a memoryless model. Pseudo states are created by partitioning a given state s into N sub-states $s_1,...,s_N$, with transitions linking the sub-states consecutively, and with all sub-states having the same parameters (identical emission and transition probabilities). Each pseudo state (sub-state) has an exponential state duration distribution that combined creates a "discrete" gamma distribution (Erlang distribution, or more precisely its discrete counterpart, the negative binomial distribution) with the number of sub-states being equivalent to the shape parameter. Technically, pseudo states behave no different from ordinary states, and we distinguish them because of their modelling role in approximating the desired waiting time distribution instead of having a direct biological interpretation, since all pseudo states have the same parameters.

5.2.3.2 Modelling steps:

The number and type of emissions was known, namely group type 1,2,3 corresponding to being with other bulls, alone or with females. However to evaluate if bulls shifts between states with changing associational preferences the number of states was varied and embedded models with increasing number of states were compared, with the simplest one-state model corresponding to no shift in associational preference. For the models with more than one state the numbers of pseudo states where varied between one and four to optimise the state duration distribution

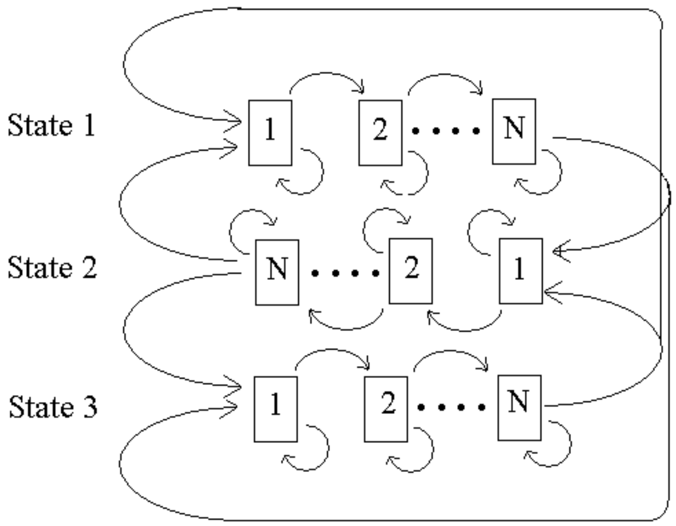


Figure 5.3: Example of final model design with 3 states. The number of states and pseudo states where varied between one and four keeping all states connected but only allowing the n pseudo state to change to the n+1 pseudo state.

5.2.3.3 *Model optimisation and parameter estimation*

The emission or transition probabilities of an HMM are seldom known *a priori*. In this situation these parameters correspond to the probability of different associations in a given state or the probability of shifting from one state to another within a given time period. These probabilities are traditionally obtained by applying the maximum likelihood criterion on Pr(observations|model) using the Baum-Welch algorithm, i.e. solving for the maximum likelihood parameters given the observed sequence (Baum & Petrie, 1966) (see Parameter estimation, Appendix C). This algorithm can converge to local optima depending on the initial parameter combination. To evaluate this we used a combination of initial parameter settings. To avoid model over-fitting, all parameter optimisation was done simultaneously on all sequences combined.

5.2.3.4 *Model likelihood*

For the multiple state models the optimal number of pseudo states was evaluated by comparing the likelihood estimate for the different numbers of pseudo-states. Since the addition of pseudo states does not add parameters to the model, the likelihood estimates can be directly compared. To evaluate the addition of extra states to the HMM, the likelihood of the N-pseudo-state model with the highest likelihood was compared between the S and S+1 state models. Adding parameters will always increase the explanatory power of a model (increase the likelihood estimate). Therefore the significance of the likelihood increase was evaluated using the likelihood ratio test with the degrees of freedom being the difference in parameters between the two consecutive models (number of parameters = transition parameters, S^2-S, plus emission parameters, 2S).

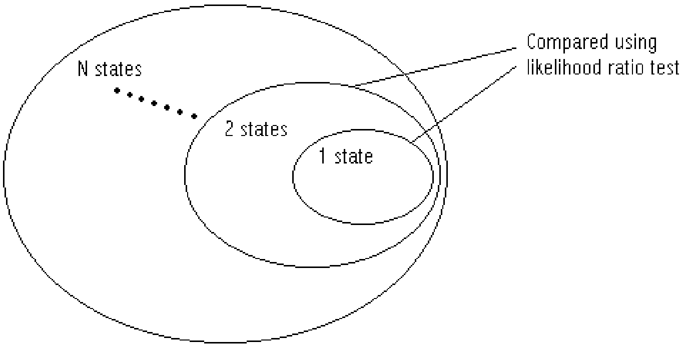


Figure 5.4 The models with increasing number of states where compared by likelihood ratio test

5.2.3.5 State sequences

After obtaining the optimal parameters for the model, λ, the Viterbi algorithm is commonly used to decode the most likely state sequence, *p*, behind each observed sequence, *x*, by maximizing the probability of observed sequence given model parameters $\Pr(x, p \mid \lambda)$ (Forney, 1973) (see Recovering the hidden path, Appendix C). We applied the same procedure adapting the Viterbi algorithm to continuous time (see Time-inhomogenous hidden Markov models, Appendix C). All analysis of HMMs was carried out in *Mathematica*.

5.2.4 Ecological conditions and receptive females

To evaluate if the association shifts where linked to reproductive decisions or general ecological conditions the fraction of the investigated bulls in periods of high female association where compared to the temporal changes both in NDVI and occurrence of receptive females. The temporal distribution of conceptions are linked to periods with increased NDVI values (Chapter 3). However, conceptions occur a median of 71 days after the first increase in NDVI thus potentially making it possible to separate these effects despite being partly synchronous in time.

5.3 Results

5.3.2 Analysis of bulls above 35 years

5.3.2.1 Number of states and optimal state duration distribution:

Association and state transition parameters where optimised on the 12 sequences from bulls older than 35 years using models with 1-4 states and 1-4 pseudo states. The optimisations were carried out on all 12 sequences combined. Using different initial parameter guesses for the Baum-Welch algorithm did not change the optimal parameter combination in any situation indicating that the identified parameter combinations where global optima. Three pseudo states had the highest likelihood in all models (Fig. 5.5). Consequently, the models with 3 pseudo-states were used for further evaluations.

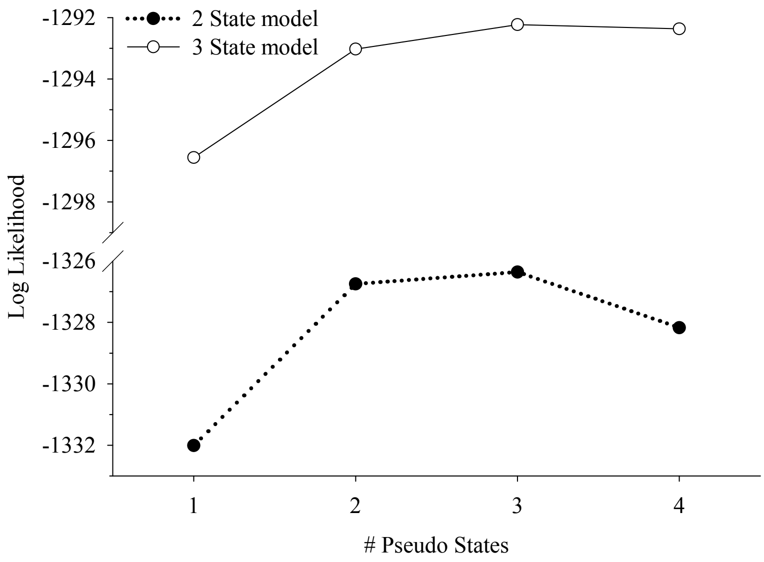


Figure 5.5: Comparison of likelihood between increasing number of pseudo states in the 2 and 3 state models

A very large, significant increase in likelihood was found between the one and two state models (table 5.1). This shows that the introduction of shifts in underlying states highly improved the model's ability to describe the observed data sequences. Therefore the one-state model (no change in associations probabilities) was not considered further. The increase in likelihood was smaller but still significant between the 2 and 3 state model. Introducing a fourth state did not significantly increase the likelihood and this model was therefore disregarded and no higher state models considered.

Table 5.1: Likelihood of 1-4 state models optimised on bulls older than 30 years with regular musth periods

States (pseudo states)	Parameters	Log likelihood	Log likelihood ratio w.r.t. previous model	Likelihood ratio test p value (d.f.)
1 (n/a)	2	-1649.26	n/a	n/a
2 (3)	6	-1326.36	322.90	<0.0001 (4)
3 (3)	12	-1292.25	34.13	<0.0001 (6)
4 (3)	20	-1284.74	7.49	0.0595 (8)

In the two state model (2SM), the states differed in emission parameters with state one (2SM-S1) having high probabilities of bull associations and state two (2SM-S2) having high probabilities of female associations. In the three state model (3SM) the parameters of state one (3SM-S1) and state two (3SM-S2) were almost identical to 2SM-S1 and 2SM-S2 but with a third state emerging (3SM-S3) predominately modelling bulls being solitary (Table 5.2).

Table 5.2: Emission parameters of 2 and 3 state models

2 and 3 state models optimised on 35+ year bulls having regular musth periods			
2 state model (2SM)			
State	P(bulls)	P(alone)	P(females)
1	0.819	0.100	0.081
2	0.067	0.315	0.618
3 state model (3SM)			
State	P(bulls)	P(alone)	P(females)
1	0.86	0.06	0.08
2	0.05	0.22	0.73
3	0.29	0.55	0.16

The transition rates (combined rate of all pseudo states) corresponded in the 2SM to bulls spending much time in state 1 and little time in state 2, and similarly in the 3SM to much time in state 1 and little time in state2 and 3 (Table 5.3).

Table 5.3: Transition rates (combined rates of n pseudo states) and average time spent in states.

Model	Transition	Rate/day	Average time in state
2 State model	S1 to S2	0.0062	S-1 161 days
	S2 to S1	0.0104	S-2 96 days
3 State model	S1 to S2	0.0029	S-1 139 days
	S1 to S3	0.0043	
	S2 to S3	0.0105	S-2 65 days
	S2 to S1	0.0050	
	S3 to S1	0.0055	S-3 56 days
	S3 to S2	0.0125	

Using the time-inhomogeneous Viterbi algorithm, the maximum likelihood state path for each sequence used in the optimisation was obtained for the two and three state models. The transition between states could only be assigned as occurring between two subsequent observations. Since the time between such two observations varied and for some transitions were more than 4 weeks, the actual time spent in a given period could not always be assigned accurately. Therefore the comparison of state periods was based on the number of observations within a given state period rather than actual time spent in the period.

Table 5.4: Percent of observations in state 1&2 in the 2SM assigned to state 1-3 in the 3SM

2SM-S1			2SM-S2		
3SM-S1	3SM-S2	3SM-S3	3SM-S1	3SM-S2	3SM-S3
87.0	0.4	12.6	0.5	77.1	22.4

Periods assigned to state 1 (high bull association) in the 2SM was on average preserved 87% in the 3 state model with 5 of the bulls having a 100% match. Periods assigned to state 2 (High female association) in the 2SM was in the 3SM split between state 2 and state 3, but almost never assigned to state 1 (high bull association) (Table 5.4). The fraction of 2SM-S2 assigned to 3SM-S3 had a very large variation between individuals (mean= 0.22 range 0 - 0.63) with some individuals having their 2SM-S2 exclusively assigned in the 3SM to state 2 and others predominately to State 3.

5.3.2.2 Associational state and musth

To evaluate how the identified state periods corresponded to musth periods, known to be associated with reproduction, the frequency of observations with urine dribbling within periods assigned to each state was compared (Fig. 5.6). Urine dribbling (musth) was almost never observed within state 1 in both models, but occurred frequently both in 2SM-S2 and 3SM-S2&3. Since observations where not evenly distributed results shown in figure 5.4 cannot be directly translated to amount of time within each state spend in musth, for examples on individual periods see fig. 5.5.

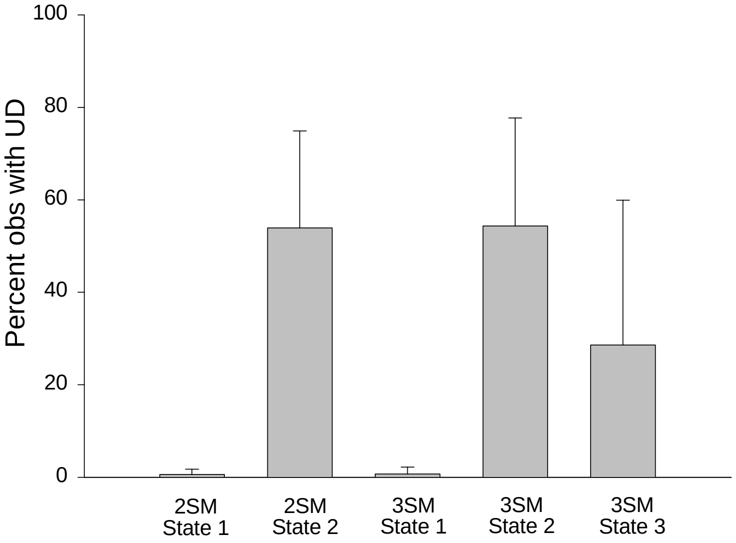


Figure 5.6: Occurrence of urine dribbling (musth) within state periods identified using the 2 (2SM) and 3 (3SM) state model (sequence n=12; number of obs= 1585).

5.3.2.3 Individual profiles in older bulls

In figure 5.7 the individual observation sequences of 5 bulls are shown to illustrate the temporal synchrony between the associational states and musth periods. In addition, the distribution of conceptions in females is shown to indicate the temporal quality from a reproductive point of view. A high overlap exist between musth signals and state 2 in the 2SM and state 2 and 3 in 3SM with a tendency for the state 3 in the 3SM to occur just prior to a state 2 (and musth) period.

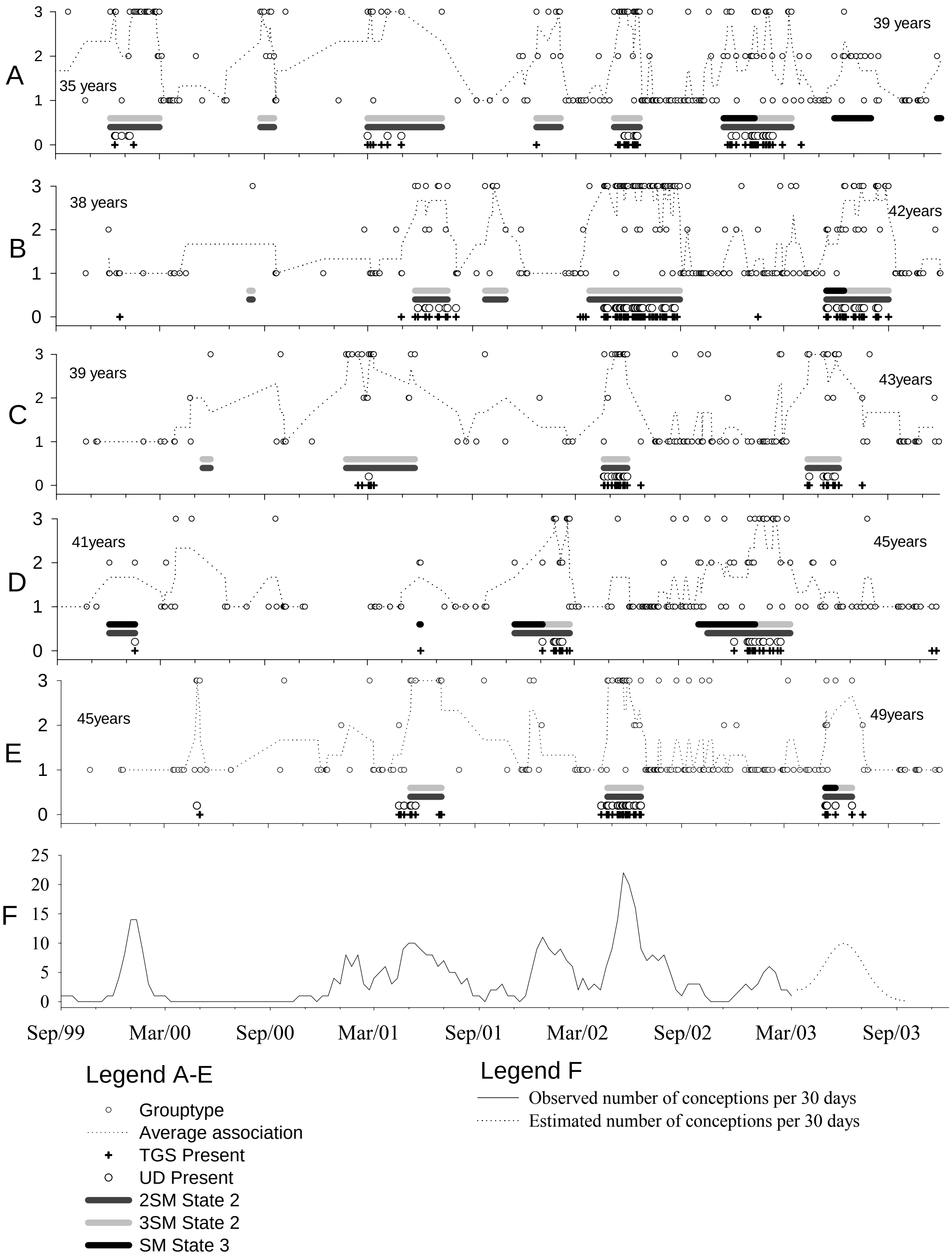


Figure 5.7: A-E: Longitudinal time sequences of associations from 5 bulls between 35-49 years. State path from 2 and 3 state models optimised on bulls older than 35 years. The presence of TGS and UD are shown as well as number of oestrus females within study area (monthly running average). Estimated age of individual indicated at start and end of sequence.

The state sequences based on the 2SM show that the presence of musth signals is always closely associated with state 2 periods. Interestingly, several bulls, especially in the age-group just older than 35 years, showed extensive periods in state 2 without having musth signals (fig 5.5 A&B) or shifting between showing or not showing musth signals without changing associational state (Fig 5.8). If state-2 periods are synonymous with reproductive activity this suggests active periods without musth signals. Based on these results the associational sequences belonging to younger bulls less than 30 years (at end of sequence), which had never been seen in musth or with highly sporadic musth periods of less than 7-day duration were investigated to see if similar patterns of associational shift occurred in this group.

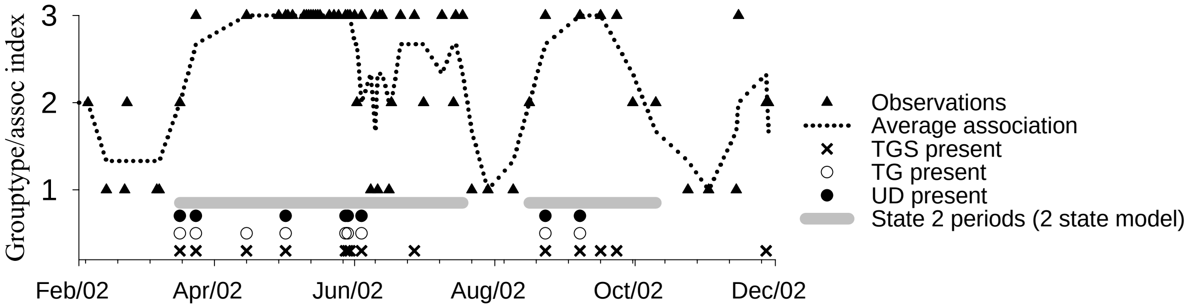


Figure 5.8 Example of shifting musth signals within same period of high female association (B1033-Apollo; est. born 1965; 37 years at time of observation sequence)

5.3.3 Analysis of younger "non-musth" bulls

Modelling steps identical to the once on the sequences from older bulls (old bull models) were carried out on the 21 sequences belonging to young bulls (Young bull models). Like in the old bull models different initial parameter guesses did not change the optimal parameter combination. However in the young bull models two pseudo states had the highest likelihood both in the 2, 3 and 4 state models (compared to three in the old bulls). Consequently, the two pseudo-state models were used for further evaluations in this group. Similar likelihood changes between the 1-4 states models were seen in the younger bulls compared to the old bulls (Table 5.5) and the 1 and 4 state models where not considered further.

Table 5.5: Likelihood of 1-4 state models optimised on bulls less than 30year old without regular musth periods

States (pseudo states)	Parameters	Log likelihood	Log likelihood ratio w.r.t. previous model	Likelihood ratio test p value (d.f.)
1 (n/a)	2	-1913.10	n/a	n/a
2 (2)	6	-1737.83	175.27	<0.0001 (4)
3 (2)	12	-1700.90	36.93	<0.0001 (6)
4 (2)	20	-1699.20	1.70	0.907 (8)

Table 5.6: Emission parameters of 2 and 3 state models

2 and 3 state models optimised on bulls less than 30year old without regular musth periods			
2 state model (2SM)			
State	P(bulls)	P(alone)	P(females)
1	0.70	0.14	0.16
2	0.10	0.14	0.76
3 state model (3SM)			
State	P(bulls)	P(alone)	P(females)
1	0.77	0.07	0.16
2	0.10	0.08	0.82
3	0.17	0.43	0.40

Association parameters in the young bulls models were comparable to the old bulls (Table 5.6). However the transition rates were quite different between the young and old bulls resulting in young bulls spending little time in state 1 and much time in state 2 in the 2SM, and similarly, little time in state 1 and much time in state 2 and 3 in the 3SM (table 5.7). The changes in the state paths between the 2 and 3 state models were also comparable to that seen in older bulls. Periods assigned to state 1 (high bull association) in the 2SM was on average preserved 88% in the 3 state model with 9 of the young bulls having a 100% match. Periods assigned to state 2 (High female association) in the 2SM was in the 3SM split between state 2 and state 3, but almost never assigned to state 1 (high bull association) (Table

8). The fraction of 2SM-S2 assigned to 3SM-S3 had again a very large variation between individuals (mean= 0.14 range 0– 0.91) with some individuals exclusively being assigned to state 2 and others predominately to State 3.

Table 5.7: Transition rates (combined rates of n pseudo states) and average time spent in states.

Model	Transition	Rate/day	Average time in state
2 State model	S1 to S2	0.015	S-1 68 days
	S2 to S1	0.0088	S-2 114 days
3 State model	S1 to S2	0.012	S-1 60 days
	S1 to S3	0.0043	
	S2 to S3	0.0090	S-2 101 days
	S2 to S1	0.00093	
	S3 to S1	0.0031	S-3 110 days
	S3 to S2	0.0061	

Table 5.8: Percent of observations in state 1&2 in the 2SM assigned to state 1-3 in the 3SM

2SM-S1			2SM-S2		
3SM-S1 88.3	3SM-S2 1.9	3SM-S3 9.7	3SM-S1 0.7	3SM-S2 85.1	3SM-S3 14.2

5.3.3.1 *Individual profiles in younger bulls*

The state paths using the old bull 2SM model were compared to the state paths using the young bull 2SM model for all bulls younger than 35 years, including the 5 sequences from bulls between 30 and 35 years (sequences not used for optimisation). State-1 periods (high bull association) were on average preserved 92.1% whereas state-2 periods (high female association) were preserved 96.4%. This shows that despite some variation in emission and transition parameters between the old and young bull model the resulting state paths are relatively robust. In figure 5.9 individual association sequences of 4 bulls younger than 30 and one intermediate aged are shown together with the state sequences generated using both the old and young bull model as well as the sporadic occurring musth signals.

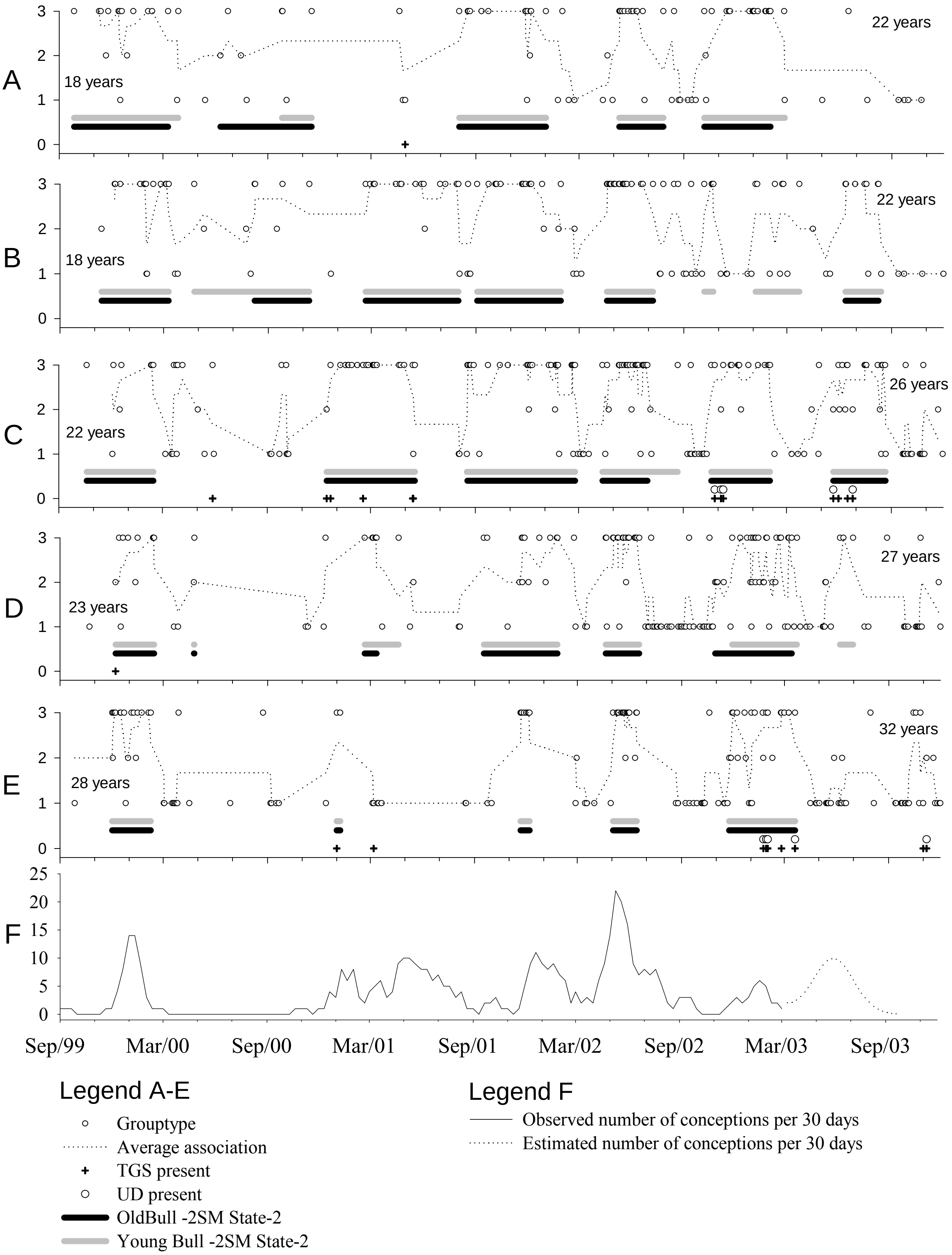


Figure 5.9: A-E: Longitudinal time sequences of associations from 5 bulls between 18-32 years with state path from Young (Grey line) and old Bull model models (Black line) as well as the presence of TGS and UD. F: Number of oestrus females, monthly running average. Age Estimated age of individual indicated at start and end of sequence.

5.3.4 Combined model

The close similarities in associational parameters between the models optimised on young and old bulls plus the robustness of state sequences seen when applying the old bull model to younger bulls suggested that a combined model could be developed. Only a 2 state model was optimised on all 38 sequences following similar steps as previously described. For the combined model the optimal number of pseudo states was one. The preservation of state periods between the old and young bull model compared to the combined model was for old bulls for state-1: 95% (Range 81-100) and state-2: 96% (Range 76-100) and for young bulls state-1: 91% (Range 64-100) and state-2: 99% (Range 96-100) indicating that this combined model identified almost the same periods as the models optimised on specific age groups.

5.3.5 Occurrence of state two periods, ecological conditions and receptive females

The occurrence of state two periods both in older musth bulls and younger non-musth bulls, using the combined 2SM were compared to ecological conditions and the occurrence of receptive females. Of the 38 bulls used in the analysis of associational states, the transition date between state 1 and state 2 periods could be assigned with +/- 15 days in 25 bulls (12 bulls above 35 years and 13 bulls less than 35 years). All sequences did not cover the full 4-year study period. In addition a few sequences had data gaps where the associational state could not be assessed due to lack of observations (e.g. fig 7 D and E). Therefore the fraction of bulls in state 2 was calculated on the basis of available sequences at any given time treating young and old bulls as separate groups. The median number of used sequences in old bulls was 8 (range 5-11) and in young bulls 9 (range 5-13). Due to the +/- 15 days uncertainty of assigning some of the state periods and comparable uncertainty on the timing of conceptions both number of oestrus and fraction of bulls in stat two was calculated as the running 30-day average value. The fraction of older musth bulls and younger non-musth bulls in state 2 is shown in fig. 8 together with the temporal distribution of receptive females as well as NDVI values. The seven main peaks in oestrus numbers occurred a median of 30 days after the peak in NDVI. The corresponding peaks in state 2 periods of older and younger bulls occurred a median of 31 and 33 days after the peak in NDVI. The time delay between NDVI peak and state 2 peaks were positively correlated with time delay between NDVI peak and oestrus peak although only significant in younger bulls (Spearman rank correlation: Younger bulls R=0.90; t= 4.64 p=0.0056 N=7 Older bulls: R=0.59; t= 1.65 p=0.16 N=7) (Fig 5.10).

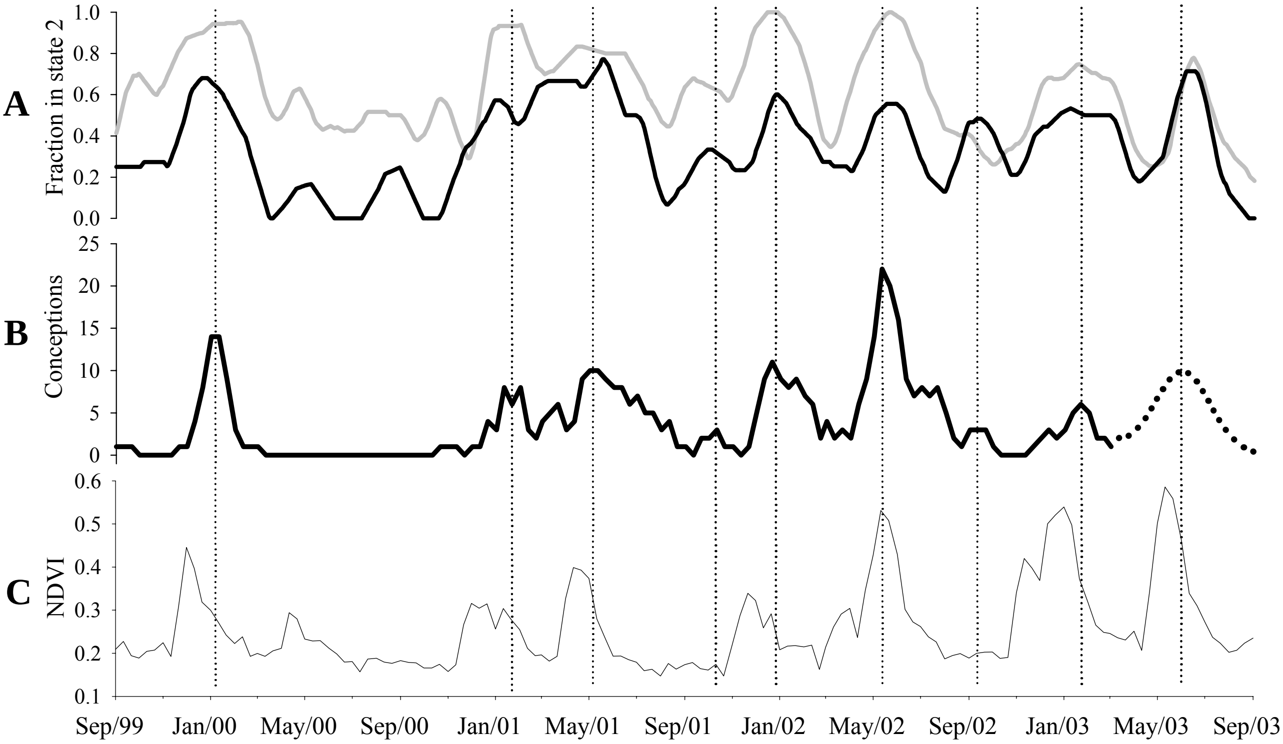


Figure 5.10: A: Fraction of bulls in state two based on combined model (values calculated as running 30 day average). Older “musth” bulls (black line N=12) and younger non-musth bulls (grey line N=13). B: Number of conceptions (calculated as running 30-day sum) last season based on model prediction (Rasmussen et al., Submitted). C: 10-day mean NDVI values of study area (measure of vegetative productivity). Vertical dotted lines indicating peaks in conception number.

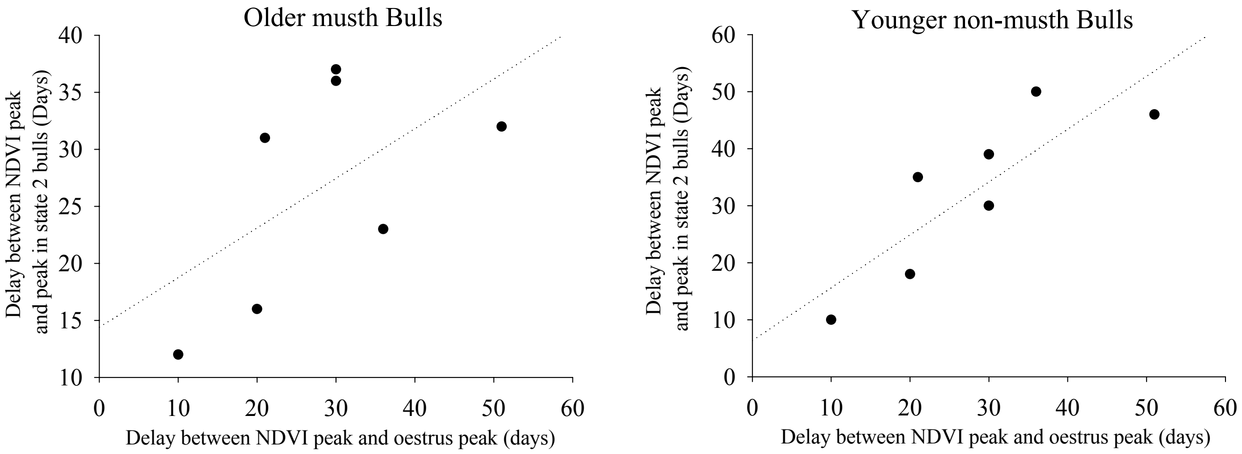


Figure 5.11: Delay of peak in state 2 periods versus delay in oestrus peak both relative to peak in NDVI for old and young bulls (N=7)

5.4 Discussion

Hidden Markov models proved highly successful for analysing time structured associational sequences. The overall aim was to evaluate if shifts in associational preferences occurred over time and if these reflected changing reproductive status. Therefore the analysis initially focused on older bulls above 35 years with regular occurring musth periods. Based on the results obtained from this group the analysis was expanded to younger bulls not having regular musth periods. However, to simplify the discussion the results obtained from the older and younger bulls are discussed concurrently.

5.4.2.1 The use of pseudo states to optimise state duration distribution

A preliminary investigation of the associational sequences of musth bulls revealed that the duration of periods with high female association was very different from the exponential distribution of state durations generated by simple HMMs with direct transitions between states. This is likely to be the case in many biological systems where the duration of time spend on a given task may depend on intrinsic energy availability or extrinsic factors like season or resource availability, rather than being a simple memoryless process.

We investigated if a more realistic state duration distribution of the model could be obtained by partitioning each state into multiple pseudo states. Only the shape of the distribution is affected by the introduction of pseudo states, whereas the mean state duration is governed by the transition parameters. Our results show that within the constrains of the resulting gamma distribution this approach could indeed be used to optimise the state duration distribution without changing general model structure or affecting biological relevant parameters. The difference of two and three optimal pseudo states in young and old bulls has only a small effect on the shape of the distribution.

5.4.2.2 Number of states and association parameters

For the models optimised on both old and young bulls a very high and significant increase in likelihood was seen between the one and two state models. This shows that the observed association sequences are much better explained with a model allowing for several behavioural modes, with different probabilities of association in each mode.

A smaller but still significant increase in likelihood was seen between the 2 and 3-state models, adding an additional fourth state did not significantly increase the likelihood compared to the 3 state model. Therefore no additional states were investigated and the one and four state model was not considered further. Despite the significant increase in likelihood between the 2 and 3 state models both were investigated further to evaluate results from both models in a biological context.

The association parameters for both the 2 and 3 state models were comparable between the young and old bulls (table 2&6) indicating similar associational preferences in the two age groups. The transition rates between states were however different (table 3&7) reflecting differences in the time spend in different states between older and younger bulls. A time-inhomogeneous Viterbi algorithm was used to identify the state periods for the individual sequences using both the 2SM and 3SM optimised on the relevant group. Periods assigned to state 1 in the 2SM were almost identical to periods assigned to state 1 in the 3SM whereas periods assigned to state 2 in the 2SM were split between state 2 and 3 in the 3SM (Table 5.4 & 5.8 and fig 5.7). The preservation between the two models of state-1 periods was seen in all bulls both old and young. On the contrary, a large individual variation was observed in whether periods in state 2 based on the 2SM were assigned to state 2 or 3 in the 3SM with an additional tendency for state 3 to occur just prior to state 2. This suggests that the increased likelihood of the 3SM and the splitting of the 2SM state 2 when adding an extra state and is partly linked to variation between individuals rather than representing two separate states within individuals. The tendency for 3SM-S3 to occur just prior to 3SM-S2 in some bulls could however suggest that this state is linked in some bulls to short transition phases between high bull and high female association predominantly spend alone. The periods identified as state 3 in the 3SM (mainly alone) was predominately categorized as state 2 in the 2SM indicating that these periods are more closely linked to periods of high female associations than with periods of bull associations.

5.4.2.3 Musth signals and associational states

In the group of older bulls, musth signals almost never occurred in state 1 in both the 2SM and 3SM. On the contrary musth signals were seen in the majority of observations occurring in periods assigned to state 2 and occurred frequently in state 3 (Fig 5.6). However, due to the variation in the frequency of observations over time the percent of observations with musth signals cannot directly be translated to the amount of time bulls exhibit musth signals in each state. Looking at the individual sequences (Fig. 5.7) the high level of synchrony between musth signals and associational states strongly suggest that these associational periods are linked to reproductive modes with state 2 in the 2SM and both state

2 and 3 in the 3SM being linked to sexually active periods. This further supports that state 2 and 3 in the 3SM represent similar underlying motivation. This variation between individuals could be linked to rank, where lower ranking bulls tend to stay in areas with lower densities of females and hence encounter fewer females and spend more time alone. The higher likelihood of the 3-state model is therefore viewed as partly resulting from “over fitting” of individual differences and the two-state model considered to better represent the general underlying biological system.

5.4.2.4 Combined model

The parameters were very similar between the model optimised on young and old bulls and the state-paths generated by applying the old bull model to the young bulls were almost identical to the state paths generated by the model optimised on this group, indicating that model results on state paths are relative robust to smaller individual or age related differences in association probabilities. Therefore a combined model was optimised on all bulls to see if such a model could be used for identifying the state-path of all bulls. Surprisingly the optimal number of pseudo states dropped to one (exponential distribution) when combining all bulls. This is likely due to the large differences in the average time spent in each state between young and old bulls, reflected in the differences in state transition rates between the old and young bull models. Hence by combining short and long durations spent in each state by young and old bulls the distribution becomes “drawn-out” and thus best approximated by an exponential distribution. Hence, the transition rates and associated state duration distribution estimated in the combined model is probably a biological unrealistic compromise between the two groups. Despite this the combined model still identified individual state paths with a high level of agreement to the state paths identified using the models optimised on the separate subgroups. When comparing bulls of different ages a combined model, with common parameters is an advantage and the high agreement between the age specific models and the combined model show that this simplification does not affect the overall results.

5.4.2.5 State periods and sexual activity:

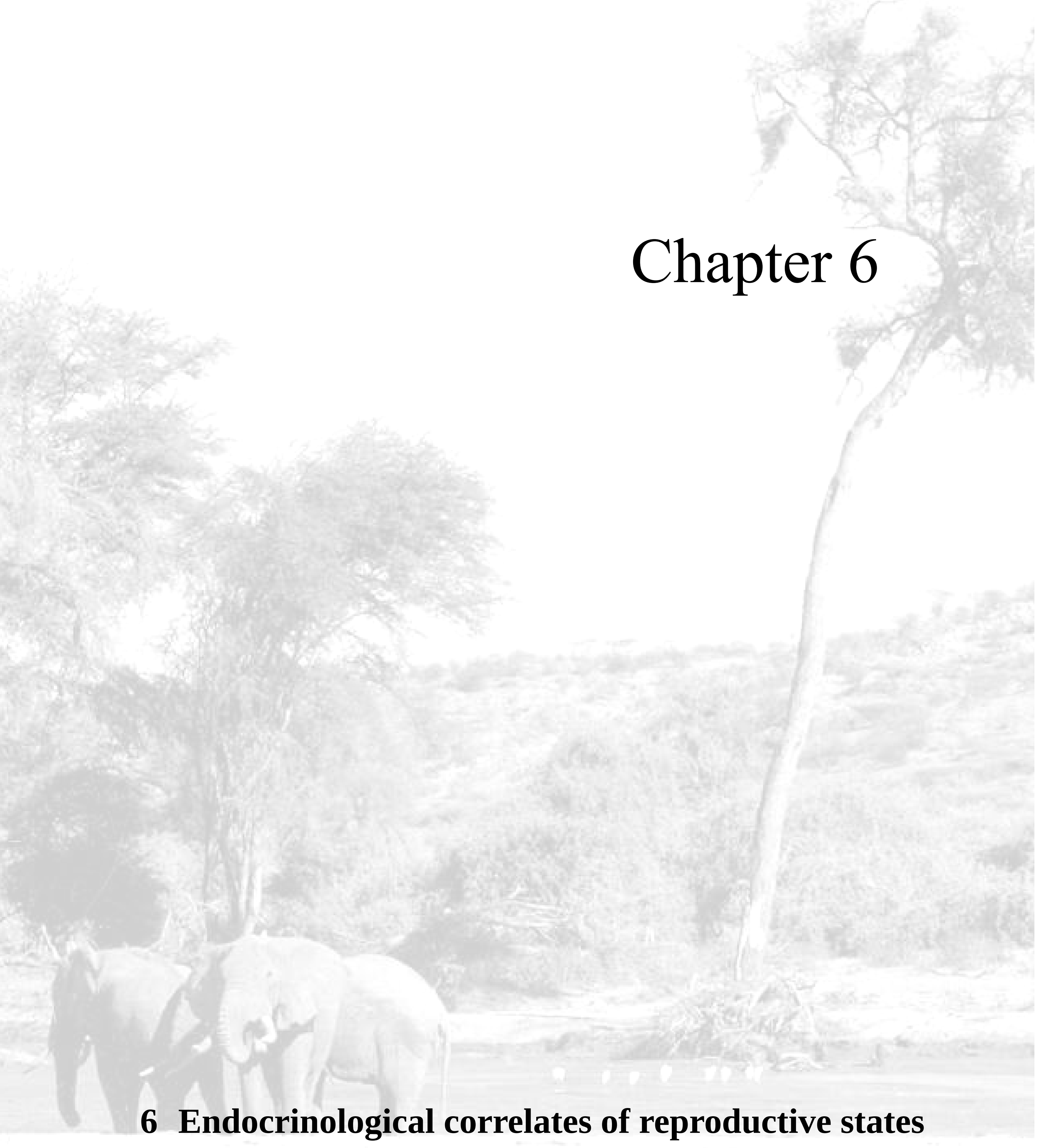
The high overlap between the identified state-2 periods and the occurrence of musth in older bulls (Fig 5.7 & 5.8) combined with the gradual occurrence of musth signals in already established annual occurring state-2 periods in young bulls (see for example fig. 5.9 C&E) strongly suggests that the state-2 periods with high female associations are representing reproductively active periods regardless of musth signals being present or not. However, high association with females could at least for the younger non-musth bulls also be linked to ecological conditions where periods of abundant forage during the rainy seasons allow closer proximity to females without adverse effects from inter-individual competition.

Individual bulls may not focus on the periods with the highest numbers of receptive females since the competition are likely to be higher during these periods or some individuals may associate with female despite poor ecological conditions. However the timing of large numbers of bulls, on a population level, associating with females are likely to reflect the temporal occurrence of the factor to which they respond. Hence, either coincides with peak ecological conditions or peak numbers of receptive females.

The fraction of older musth bulls in state two and fraction of younger bulls in state 2 were generally in synchrony apart from the first being generally lower (Fig.5.10). They both had an annual tri-modal pattern with peaks in Dec/Jan/Feb April/May/June and August/Sept. The NDVI values, representing vegetative productivity and thus availability of forage had annual bimodal peaks corresponding to the two annual rainy seasons in Nov/Dec and March/April. The number of oestrus females have, like the NDVI generally annually bimodal peaks but slightly delayed compared to the NDVI apart from 2000 when no females conceived during May due to poor rainfall and low vegetation productivity. However, two smaller peaks occurred during Aug/Sept in 2001 and 2002. These smaller peaks have since 1996 also been observed in 1996, 1998 and 1999. The most likely explanation for this smaller "third season" is females failing to conceive during first ovulation and subsequently experience their 16-week ovarian cycle (Hodges, 1998b) corresponding temporally with conceptions in Aug/Sept. A similar effect is not seen from the January season, as these female will coincide with the April/May season. The peaks in the fraction of both older musth bulls and younger non-musth bulls in state 2 periods generally occur slightly delayed compared to peak NDVI values but concurrently with peaks of receptive females, including the smaller peaks in conceptions occurring in Aug/Sept which are unrelated to forage availability. Moreover the variation in time delay between NDVI peaks and conceptions and NDVI peaks and state 2 periods where positively correlated indicating that the peaks in state 2 periods are in synchrony with conceptions rather than NDVI. This further supports that the associational shifts are linked to reproductive decisions not just in musth bulls but also in young non-musth bulls.

This provides the first quantitative evidence for the existence of distinct sexually active non-musth (SAN) periods in male African elephants as previously suggested by Poole (Poole, 1987; Poole et al., 1984). Figure 5.7 and 5.9 represent examples of individual association sequences from bulls between 18 and 49 years, hence providing a "composite" view on the occurrence of SAN and musth over a complete bull lifetime. This shows that bulls have established periods of sexual activity (SA) already at the age of 20. The first short periods of musth are seen around the age of 25 which is comparable to that reported from Amboseli National Park (Poole, 1987). Not before the age of 35 is the state of musth predominant during periods of SA, and musth as an exclusive state during SA periods is not seen before around 40 years of age. Interestingly, the oldest bull (43 years) with longer periods in state 2 without musth signals (Fig 5.7 D) is tuskless and relatively low ranking compared to his age. Younger bulls (<30 years) have generally two SA periods per year mainly overlapping with the peaks in oestrus numbers whereas older bulls only have one recurrent period a year. In addition the overall duration of the SA periods decreases with age.

In conclusion, HMMs proved highly suited for identifying patterns in time-structured association data. Musth signals occurred almost exclusively during periods with high female association and for both musth and non-musth bulls these periods where in synchrony with the number of receptive females but not general ecological conditions. This indicates that the shifts between associational states are representing shifts between reproductively active and inactive states. A single combined model accurately identified the state periods for bulls of all ages. This provides the ability to use a common definition to identify reproductively active periods based on associations and gives the ability to compare different mating tactics without using tactic-specific signals, such as urine dribbling for categorization. Based on these results specific analysis of individuals can be used to evaluate variation in movements and search patterns for females, androgen levels and levels of energy or time investment in reproduction potentially linked to individual rank, age, competitive state and or spatio-temporal variation in density of females or competitive environment. In general HMMs may provide a powerful tool for analysing time structured behavioural data sets by identifying patterns linked to underlying motivational states.



Chapter 6

6 Endocrinological correlates of reproductive states

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And in prep as: Longitudinal changes in hormones of male African elephants linked to activation of sexual active periods and shifts between reproductive tactics. H. B Rasmussen, A. Ganswindt, F. Vollrath, I. Douglas-Hamilton.

Abstract

In this chapter androgen and glucocorticoid levels are investigated in relation to reproductive state, identified using the results from chapter 5 as well as presence and degree of musth signals and age to provide a more detailed picture about the endocrinological changes, characteristics and timing associated with male reproductively active periods.

Androgen (epiandrosterone) levels were slightly elevated during sexually active non-musth (SAN) periods with an approximately two-fold increase observed in individuals. The presence of TGS and/or UD was positively linked to epiandrosterone (EA) levels with a five to six-fold increase seen in individuals exhibiting both signals. No effect of age was seen showing that the changes in EA levels associated with SAN and musth are similar in both younger and older bulls. Musth/SAN within SA periods was generally on/off states rather than a gradual shift with urine-dribbling being the best indicator of very high androgen levels and competitive status. The initial increase in EA levels occurred approximately 10-20 days prior to changes in associational preferences. Furthermore the occurrence of shorter musth periods (presence of TGS and UD) within SA periods where temporally closely associated with additionally increased EA levels. The downstream occurrence of behavioural changes relative to hormonal changes suggests a potential activational role of androgens on the shifts between active and inactive periods as well as between the musth and SAN states.

Glucocorticoid levels (3a,11oxo-CM) were generally elevated during the late dry season and in individuals having physical injuries and for brief periods following physical fights. A slight increase in 3a,11oxo-CM was associated with SAN. However no physiological stress response linked to musth was evident This endocrinological work was done in collaboration with the German Primate Centre in Göttingen Germany and Dr. Andre’ Ganswindt.

6.1 Introduction

Musth in the African elephant has been defined by the occurrence of urine dribbling (UD) and secretion from the temporal glands (TGS) (Poole, 1987). The presence of these musth signals have been linked to elevated levels of androgens in both in African (Poole et al., 1984; Hall-Martin, 1984; Brannian et al., 1989; Rasmussen et al., 1996) and Asian elephants (Cooper et al., 1990; Rasmussen & Perrin, 1999; Jainudeen et al., 1972). However, the majority of physiological studies have included relatively few individuals and many have been conducted in captive settings (both Asian and African elephants). Several factors in captive settings deviate from the natural settings in free ranging animals. The individuals are fed large quantities of high quality food making them energetically in very good conditions. On the contrary the captive settings and housing conditions results in stressful situations resulting in for example high mortality (Clubb & Mason, 2002). Males are often housed separately from females and the low number of females in any given zoo may result in long time durations between the occurrences of oestrus females. The age of captive bulls are generally low with the majority being less than 25 years (http://www.elephant.se/elephant_database.php) the age when musth is starting to occur in free ranging individual thus making it difficult under captive settings to evaluate effects of social conditions, energy constrains and age/status on the endocrinological status. In addition, the existence of sexually active non-musth (SAN) periods have not been considered or studied in captive settings and are unlikely to be carried out in zoos since due to the difficulty of recognising this state under non-free ranging conditions.

In free ranging African elephants, the occurrence and duration of periods with musth signals are positively correlated with age (Poole, 1987). This age-relatedness of the signals used to define musth, however, raises the possibility that behavioural and/or endocrine changes commonly associated with these signals, could in part be related to the age of the individual rather than exclusively to the occurrence of musth itself. Indeed, in one of the few physiological studies of free ranging male African elephants high levels of androgens, comparable to those showed by animals in musth, were found in some of the sampled individuals assumed to be sexually active (SA) but not showing musth signals (Poole et al., 1984). Data from this group were however limited (five individuals, single samples) and values obtained showed the full range from those in sexually inactive (SI) bulls to those in musth. Thus, in terms of androgen levels, it remains unclear if SAN bulls are physiologically different from individuals in musth. Within the context of sexual activity, bulls exhibiting the full range and intensity of musth signals and SAN not exhibiting any signals could represent end points in a continuum with a graduate onset of signal presence and intensity linked to age, rather than being distinct states. On the contrary although the occurrence and duration of musth is positively correlated with age, large variation in the age of musth onset exists. In captive bulls where little or no intra sexual competition exist musth signals, including urine dribbling have been observed in individuals as young as 13 years (Ganswindt, 2004). Likewise, in a population of African elephants in Pilanesberg, prolonged periods of musth were observed in young males (<19 years) during a period where no older males where present. After the introduction of older males to the park musth in the younger males where suppressed (Slotow et al., 2000). Younger musth males have also been reported to be forced out of musth by older more dominant bulls (Poole, 1989b; Poole, 1987). This suggests that the shift between the SAN and musth tactic is not directly linked to age but rather linked to the relative status/rank of the individual, which to a large extent is determined by the relative but not absolute age of the individuals.

In the relative plasticity hypothesis, shifts between different reproductive phenotypes (tactics) in systems with conditional dependent reproductive tactics have been suggested to be under proximate control of changing androgen levels (Moore, 1991; Moore et al., 1998). Different androgen levels have been shown to exist between males engaging in different alternative reproductive tactics in a large number of species including fish (Oliveira et al., 2001; Uglem et al., 2002) reptiles (Moore et al., 1998) and birds (Wingfield, 1984) and partial shift in tactics can be obtained in marine iguanas by administering testosterone to individuals (Wikelski et al., 2004). Although the majority of studies have been conducted on non-mammalian vertebrates this suggests that the changing reproductive tactics of elephants could at least partially, be controlled by changing hormonal concentrations. A combined analysis of the occurrence of the various physical musth signals, age, behaviour and androgen levels is needed to evaluate this. Apart from the potential differences in average androgen levels linked to different combinations of signal presence and age, the temporal timing between changes in reproductive mode, onset and termination of musth signals and endocrinological changes needs to be investigated to evaluate the underlying cause and effect behind the onset and termination of active periods and what triggers the occurrence of musth. For example, do changes in associational preferences occur first and subsequently triggers a hormonal response or is the behavioural changes a downstream effect of hormonal changes? Currently only a few longitudinal hormone profiles are available from individual male Asian (Lincoln & Ratnasooriya, 1996; Rasmussen & Perrin, 1999) and African elephants (Ganswindt et al., 2002), all resulting from captive studies. One of these studies of a male Asian elephant revealed that musth signals occurred after the peak in serum testosterone levels (Lincoln & Ratnasooriya, 1996) and that elevated levels of aggression was temporally linked to receding levels of testosterone. Since no study of longitudinal hormone changes has been done on free-range individuals the relative timing of hormonal changes and shifts in behaviour such as associational preferences an the onset of search for receptive females is unknown. In addition, the captive setting of most studies has largely prevented analysis of the effect of extrinsic events like the presence or absence of oestrus females or local competitive environment.

Another point of interest, which remains to be addressed in free-ranging animals, is whether the reproductively active states are associated with increased adrenal activity. For female African elephants, it has been shown that limited access to food and water, which leads to a decline in body condition, is associated with an increase in adrenal endocrine function resulting in elevated glucocorticoid levels (Foley et al., 2001). The competitive condition of musth is known to be associated with increased restlessness and reduced feeding activities, which often leads to a progressive loss of condition and significant weight reduction (Poole, 1989b; Barnes, 1982). Physiologically it has been shown in a captive Asian elephant that musth with the associated elevation in testosterone levels was linked to increased lipase activity and elevated levels of triglyceride concentrations and excretion of ketones indicating that musth bulls mobilize stored fat reserves and may be in a state of starvation during musth (Rasmussen & Perrin, 1999). Based on this it would be reasonable to assume that musth may be associated with an elevation in glucocorticoid output as a result of this energetically expensive event.

6.2 Methods

6.2.2 Observations and sample collection

In addition to the general monitoring program, the selective monitoring of bulls has been continuously performed within the study area since 1999. During these observations, the identity of all associating individuals (males and females) was recorded. Furthermore, for each bull, the presence and degree of urine dribbling (UD), temporal gland swelling (TG) and temporal gland secretion (TGS) (all common visible signs assigned to musth) was rated on an increasing scale as described by Poole (Poole, 1987). Between January 2002 and December 2003 756 faecal samples were collected for hormone analysis from 64 individual bulls (mean: 11 samples per individual, range: 1–68), ranging in age from 18 to 49 years. Of the 756 samples, observations on signal levels/presence were incomplete in 26 samples, these samples were excluded from the analysis of signal presence/level but included in the longitudinal time profiles of individuals. Samples were collected from fresh droppings of identified individuals. The faecal bolus was homogenized by hand (using rubber gloves), and an approximately 10–15 g aliquot was stored in a polyethylene tube, filled with 25 ml of 99% ethanol (Wasser et al., 1988). The samples were refrigerated until transport to the German Primate Centre.

6.2.3 Fecal extraction and hormone assays.

The extraction and hormone analysis was conducted by Andre' Ganswindt at the German Primate Centre. Faecal samples were homogenized in the original ethanolic solvent according to the procedure described by (Ziegler et al., 2000). The ethanolic faecal suspension of each sample (including a 3-5 ml 80% methanolic rinse of the original sample tube) was then transferred into two 50 ml tubes, and steroids were extracted by shaking for 15 min on a multi-tube vortex. Following centrifugation at 3000 rpm for 15 min, both supernatants were combined, the total volume recorded, and the extracts finally stored at 48 °C until hormone analysis. The remaining faecal pellets were dried in a vacuum oven at 50 °C, and the dry weight of individual samples was determined. Extraction efficiency, determined in a subset of 20 randomly selected faecal samples by monitoring the recovery of (3H) estradiol added to the samples prior to homogenisation, was 72.0% +/- 5.3% (mean +/- SD). Faecal extracts were measured for immunoreactive epiandrosterone (EA), a major secretory steroid of the mature boar testes (Raeside et al., 1992), which have been shown to be potentially useful for monitoring male gonadal function in different mammalian species (Palm & Möstl, 1993; Möstl et al., 2002) including African elephants (Ganswindt et al., 2002). In addition, in all faecal samples collected, glucocorticoid concentrations were measured using a validated enzyme immunoassay (EIA) for 3a,11oxo-cortisol metabolites (3a,11oxo-CM) which have previously been shown to provide reliable information on adrenal function in the male African elephant (Ganswindt et al., 2003). The epiandrosterone EIA used an antibody raised in a rabbit against 5a-androstane-3a-ol-17-one-HS and 5a-androstane-3,17-dione-thioether conjugated with biotin as a label (Palm & Möstl, 1993). The 3a,11oxo-CM EIA used an antibody raised in a rabbit against 5ß-androstane-3a-ol- 11-one-17-CMO and 5ß-androstane-3a-ol-11-one-17-CMO conjugated with LC-biotin as a label (Möstl et al., 2002). Cross-reactivities of the two antibodies are described in (Ganswindt et al., 2002) for EA and (Möstl et al., 2002) and (Huber et al., 2003) for 3a,11oxo-CM. Serial dilutions of faecal extracts gave displacement curves which were parallel to the respective standard curve in both assays. Sensitivity of the assays at 90% binding was 3.0 pg/well for both EA and 3a,11oxo-CM. Intra- and inter-assay coefficients of variation, determined by repeated measurements of high and low value quality controls ranged between 2.4% and 16.9% for the EA, and 2.6% and 17.5% for the 3a,11oxo-CM measurements.

6.3 Cross sectional analysis of age, signal level and sexual states

6.3.2 Sample classification with respect to sexual state

The initial categorization of the samples as belonging to either sexually active (SA) or sexually inactive (SI) bulls was based on the quantitative shifts in individual bull association patterns. The sexual states of 37 individuals (673 samples) were identified using the combined Hidden Markov Model for all age groups (described in chapter 5). The remaining 27 individuals (85 samples) did not have sufficient longitudinal observation profiles for states to be identified in this way. For those individuals an association index on the day of sampling was calculated by averaging the association type of the previous, present, and following observation. This was done by assigning values from 1 to 3 to the three different types of association: 1 = in association with other bulls only; 2 = alone, no signs of association with other individuals and 3 = in association with mature females, regardless of the presence of other bulls. An index value of < 2 (mainly in association with other bulls or alone) were considered to represent periods in which the bull was sexually inactive (SI), whereas bulls in periods with values > 2 (mainly with females or alone) were considered to be in a sexually active (SA) state. If the observation on the day of sampling was separated from other observations by more than 2 weeks, the association index was equalled to the observation group type at day of sample collection. Of the 430 samples classified as belonging to sexually inactive individuals, 25 showed some combination of UD and/or TGS. These samples occurred around the transition period between inactive and active periods and were excluded in the analysis of inactive individuals. Samples coming from SA bulls were further subdivided depending on the degree and presence of TGS, TG, and UD, initially scored according to the definitions described by Poole (1987). However, this resulted in too many different categories (N>100), reducing the sample size in each to below a reasonable level. All samples were therefore re-assigned and the occurrence of temporal gland swelling was considered as an automatic effect of changes in temporal gland secretion (TGS) and not considered for sample sub-grouping. Samples were then assigned if TGS was absent (-), present low (1), medium (2), or high (3) in combination with the absence (-) or presence (+) of urine dribbling (UD), giving a total of 8 potential subcategories for sexually active bulls (SA) plus one category for sexual inactivity (SI). Since urine dribbling at no point was observed without any temporal gland secretion, the final number of categories was 7 SA groups and 1 SI group (Table 6.1).

Table 6.1: Categories of study animals according to the state of sexual activity, presence, and degree of temporal gland secretion (TGS) and urine dribbling (UD) and age.

Category	Presence SA	Presence UD	Degree of TGS	Abbreviation	Number of individuals	Age Median (Range)
1	-	-	-	SI	49	27 (18-49)
2	+	-	-	SA--	43	25 (18-44)
3	+	-	1	SA-1	8	33 (18-44)
4	+	-	2	SA-2	7	38 (24-42)
5	+	-	3	SA-3	7	38 (24-48)
6	+	+	1	SA+1	7	37 (25-48)
7	+	+	2	SA+2	10	39 (27-48)
8	+	+	3	SA+3	17	37 (25-48)

6.3.3 Data analysis of cross sectional data

When multiple samples from the same individual in the same category existed, the median value was used for analysis. Differences in hormone concentrations between categories were examined by Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks followed by all pair-wise post hoc analysis. All tests were two-tailed, with the a-level of significance set at 0.05. In cases of all pair-wise multiple comparison procedures, the a-level was adjusted using sequential Bonferroni adjustment. Nonparametric Spearman Rank Order correlation coefficients were calculated for the relationships between age and median hormone levels within separate conditions (as per Table 6.1).

6.3.4 Endocrinological correlates of age and signal levels

During the 24-month study period, 77% (n = 49) of all bulls were observed to be sexually active on at least one occasion and of those, 86% (n = 42) were at least partially observed in a sexually active condition without showing any TGS or UD (SA--). Moreover, 29% (n = 14) of all SA bulls were observed with an additional occurrence of TGS (SA-/+), and 41% (n = 20) showed both, TGS and UD (SA+/+).

6.3.4.1 *Endocrinological changes in relation to age*

The age distributions of bulls within each of the 7 subcategories of SA were significantly different (Kruskal–Wallis ANOVA; $H_{6,99} = 33.07$, $P< 0.0001$); in particular, the median age of bulls showing TGS and/or UD was higher than that of SA individuals showing no additional signs (Table 1). The non-parametric nature of the data prevented an overall full factorial analysis for age and signal effects. Each of the seven subgroups were therefore analyzed separately for age effects on EA and 3a,11oxo-CM levels (using Spearman Rank order correlation). Neither EA nor 3a,11oxo-CM levels were significantly correlated with age in any of the 7 SA subgroups even before a-level adjustment ($t =-1.4$ to 2.53; $P = 0.939$–0.052). Age was therefore excluded as a factor in subsequent analyses.

6.3.4.2 *Endocrinological changes in relation to the degree of TGS*

The effect of increasing levels of TGS on androgen and glucocorticoid levels was investigated in the subgroups with urine dribbling (SA+1, SA+2, SA+3) and without urine dribbling (SA-1, SA-2, SA-3). No significant differences could be found between the respective categories (Kruskal–Wallis one-way ANOVA on ranks, $H_{2,22/34} = 4.03$–0.123, $P = 0.94$–0.133). Since the hormone levels had no significant relationship with the intensity of TGS, the samples were re-assigned according to whether TGS was absent (-) or present (+) in combination with the absence (-) or presence (+) of UD, thus giving a total of three potential sub-categories for sexually active bulls (since UD did not occur without TGS), with one additional category for sexually inactive animals (SI) (see Table 6.2).

Table 6.2: Final established categories with respect to the state of sexual activity and the presence/absence of temporal gland secretion (TGS) and urine dribbling (UD)

Category	Presence SA	Presence UD	Presence TGS	Abbreviation	Number of individuals
1	-	-	-	SI	49
2	+	-	-	SA--	43
3	+	-	+	SA-+	16
4	+	+	+	SA++	20

6.3.4.3 *Endocrinological changes in relation to sexual state and signal categories*

In Fig. 6.1, EA and 3a,11oxo-CM levels representing samples from all 64 bulls categorized according to the four established groups (1 inactive and 3 active) are shown. Within the group of sexually inactive bulls (SI) and sexually active animals showing no physical signs (SA--), the variation in the individual androgen levels was relatively low compared to both the SA-+ and SA++ subgroups.

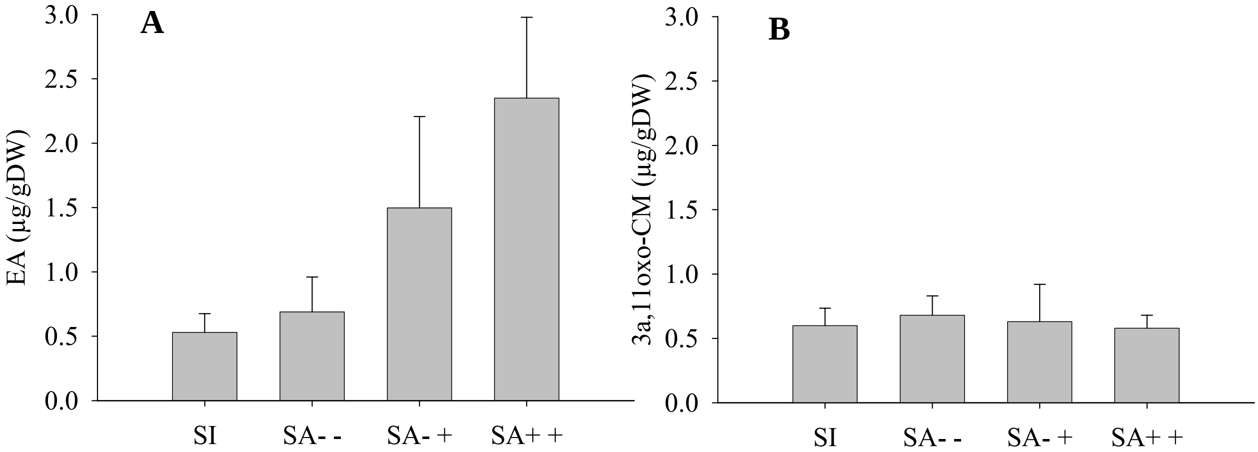


Figure 6.1: Median concentrations of EA (A) and3a,11oxo-CM (B) N = 64 bulls. Error bars represent 25-75% percentile. Group SI includes all sexually inactive individuals. The SA-- group includes all bulls in sexual activity state but not showing UD or TGS. The SA -+ includes all SA bulls which showed TGS but not UD. SA++ includes all bulls showing both TGS and UD. All groups in A are significantly different non of the groups in B are significantly different.

Overall median EA levels were different from each other (Kruskal–Wallis one-way ANOVA on ranks, $H_{3,128} = 62.66$, $P < 0.0001$, with all groups being different (post hoc analysis using Kruskal–Wallis one-way ANOVA, a-level adjusted to 0.008). In contrast to the data for EA, no significant differences were found in 3a,11oxo-CM levels between the four categories (Kruskal–Wallis one-way ANOVA on ranks; $H_{3,128} = 2.578$, $P = 0.461$). To verify that the non-significant effect of age on EA levels found in the initial eight subcategories was not a result of low sample size the age effects were re-analysed within the final four groups.

Again, no significant effects of age were found in any of the groups (Spearman Rank order correlation, t = -1.4 to 1.589, P = 0.798 – 0.168).

To verify that the 3a,11oxo-CM levels do reflect physiological stress and that the assay can be use to assess changes in 3a,11oxo-CM levels, a longitudinal profile of a bull with a badly injured hind leg was investigated as a form of biological validation. The bull started to show signs of injury with a badly swollen foot and severe walking difficulties at the end of Jan 2002. During late February early March the conditions appeared to improve but on the 28th of March the bull was found lying down with open multiple bone fractures in the foot and was shot, as recovery seemed impossible. The longitudinal profile of this bull is shown in figure 6.2

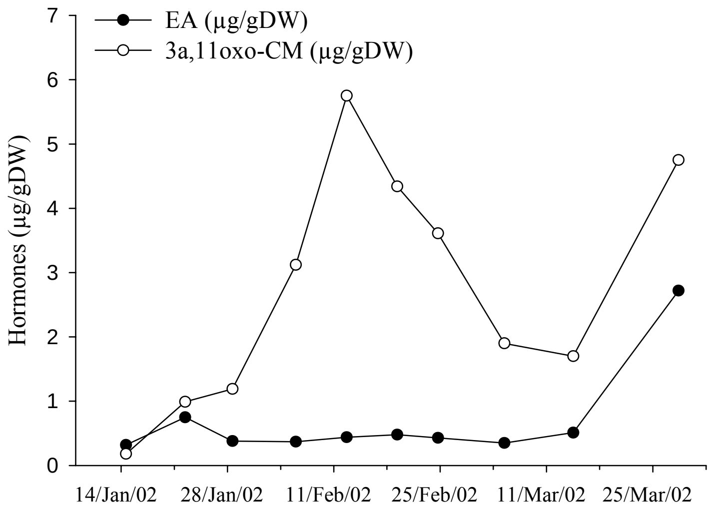


Figure 6.2: Longitudinal hormone profile of a 43 year old bull (B1011-Mandela) with a severely injured hind foot. (The bulls was shot on the 28th of March after recovery was assessed to be impossible)

A very large increase in 3a,11oxo-CM is evident with a further increase in the EA levels in the last sample. Hence, this biological validation shows the 3a,11oxo-CM levels do increase dramatically during severe stress and that this elevation is detected by the assay.

6.3.4.4 Occurrence of different signal groups in sexually active bulls

To get an indication if the three different categories of sexually active bulls (SA--, SA-+, and SA++) are clearly distinct, or whether the SA-+ condition represents a temporarily more limited transition-state between SA-- and SA++, each observation of the complete observational data set on sexually active individuals between 1999 and 2003 (n =1662) was categorized as belonging to either SA--, SA-+ or SA++ (see Fig. 6.3). In figure 6.4 the same data is shown including the signal intensity for both temporal gland secretion and swelling.

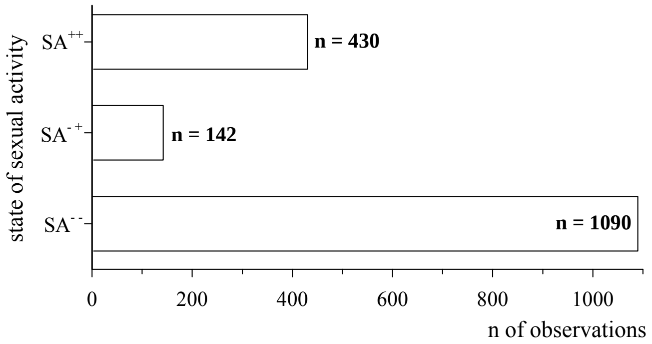


Figure 6.3: Horizontal bar plot of the complete observational data set categorized to the number of observations within each of the three different states (SA--, SA-+, and SA++) of sexual activity.

The data clearly show that most observations within the group of sexually active individuals were done on bulls showing either no signs (SA -), or both TGS and UD (SA++). Least recorded was SA bulls showing additionally TGS only (SA-/+). Although some of the samples in the SA-/+ group came from bulls just in pre or post SA++ conditions and could thus be viewed as transition, some bulls had longer periods in SA-/+ without showing UD and some without showing UD at any point within that SA period.

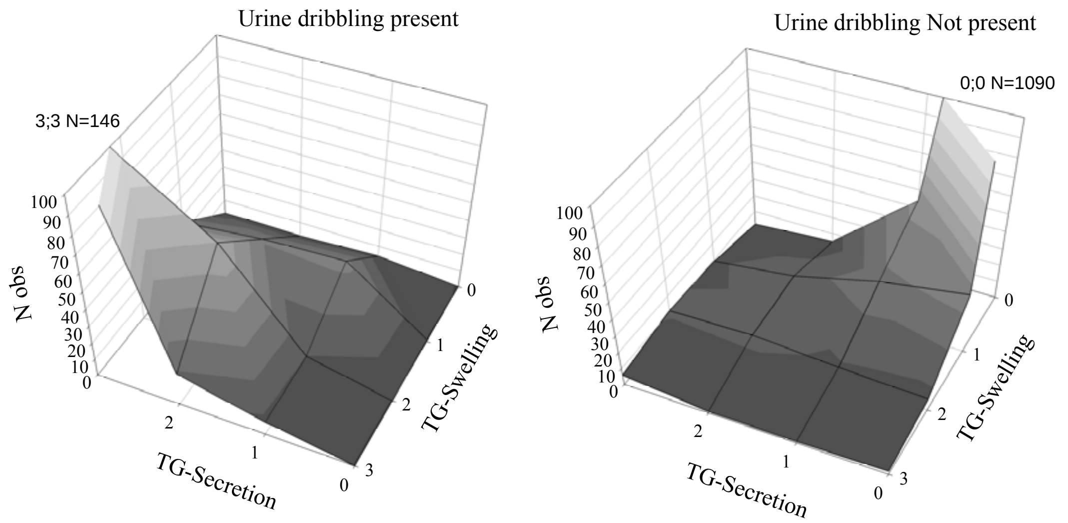


Fig 6.4: The number of observations of SA bulls with different signal combinations and intensity (N=1662).

6.4 Analysis of individuals and longitudinal profiles

Fourteen of the sampled individuals had a sample frequency allowing for the creation of longitudinal profiles with 9 covering a full two-year period. In addition, in some individuals with fewer samples, the temporal occurrence of the available hormone samples in respect to the timing of active and inactive periods could be identified. Hormone concentrations were analysed non-invasively via faecal samples. Hence due to the food passage time a time delay of approximately 24-36 hours exist between actual hormonal changes and until the "signal" can be detected in the faeces (Ganswindt et al., 2003). This effect must be considered when comparing individual profiles to specific events such as antagonistic interaction with competitors or association with a specific receptive female since a sample collected on the day of the event will reflect the hormonal status of the individual the previous day. Furthermore, although a relative high frequency of observations exist on these 14 individuals many events likely to influence the endocrinological status will have gone unnoticed.

6.4.2 Data analysis

Before the analysis of individual temporal profiles, the population "average" was investigated for general temporal variation in EA and 3a,11oxo-CM levels. This was obtained using samples from all individuals recorded as sexually inactive and in addition showing no signs of TGS or UD (SI). The median values of these SI bulls where obtained within each non-overlapping 15-day period between Jan 2002 and Dec 2003 hereby creating a "composite" temporal profile of population level EA and 3a,11oxo-CM in SI individuals. To evaluate if individuals differed in the baseline levels of EA and 3a,11oxo-CM the median hormone levels of the 14 individuals with high sample frequency were compared by Kruskall-Wallis ANOVA on ranks during their inactive periods.

6.4.2.1 Longitudinal data

The longitudinal profiles were analysed for intra individual variation in 3a,11oxo-CM and EA levels with respect to individuals reproductive state using Mann-Whitney U-test. In addition the timing between active periods and signal onset and termination and between known physical fights and associations with oestrus females were investigated.

To evaluate if any general trends occurred over the course of an active period including the immediate pre and post active period, profiles from several individuals were combined to average out any individual "noise" potentially linked to specific events. In order to evaluate such temporal changes the transition date between SI and SA periods (identified by the hidden Markov model procedure based on associations) needed to be known within 5-10 days, hence only profiles where a high temporal resolution of observation existed during the transition periods and where hormone data was available could be used. Individuals that showed mixed periods of musth and SAN was not considered. 23 such SA periods including the 2-month periods occurring before and after the onset and termination of the active period were identified and selected from 15 individuals. Of those, 14 periods from 9 bulls belonged to individuals that consistently showed musth signals during their recurrent sexual active periods. The remaining 9 periods belonged to 6 individuals not showing periods of musth.

For the musth bulls, samples belonging to the pre and post active periods were grouped into 10-day intervals calculated from the day of period onset and termination respectively. The duration of the SA periods varied between individuals (median 98 days, range 53-162). In order to assess any potential changes occurring over the course of an SA period the temporal position of samples within active periods were calculated as relative to the duration of the period i.e. for example as occurring after 20% or 45% of the active period had elapsed. The samples within the SA periods were grouped into 10% intervals. In addition all observation of the selected individuals including observation without hormone samples were categorized in a similar way and the fraction of observations with TGS and urine dribbling was obtained. The SA periods were defined based on changes in associations (via the HMM). However to assess if the associational changes occurred gradually or if smaller changes occurred prior, during or after the defined active period, the fraction of observations in bull, solitary or female association were calculated in the same categories. The SAN profile was analysed in the same way as the average musth profile. However since fewer active periods with generally fewer hormone samples were available from this group of bulls the categories were enlarged to cover 20-day periods in the pre and post active periods and 20% sections during the active period.

6.4.3 General temporal variation

Median values for both hormones generally showed low variation around an overall median value for EA of 0.52 µg/gDW and 0.58 µg/gDW for 3a,11oxo-CM, except towards the end of the long dry season in Sept.- Nov. 2002 where a three-fold increase of median values of both EA and 3a,11oxo-CM was seen (fig. 6.5). Shortly after the onset of vegetation growth in Nov. 2002 the values return to normal. This increase, especially in the 3a,11oxo-CM levels is most likely linked to environmental conditions where poor forage quality may not be adequate to meet energetic requirements resulting mobilization of stored resources. A similar increase was not seen following the short dry seasons in March 2002 and March 2003. However, the duration of the short dry seasons is shorter than the 5-month delay between peak conditions in May 2002 and the peaks in hormones seen in October 2002 hence may be too short to trigger an energetic stressful situation.

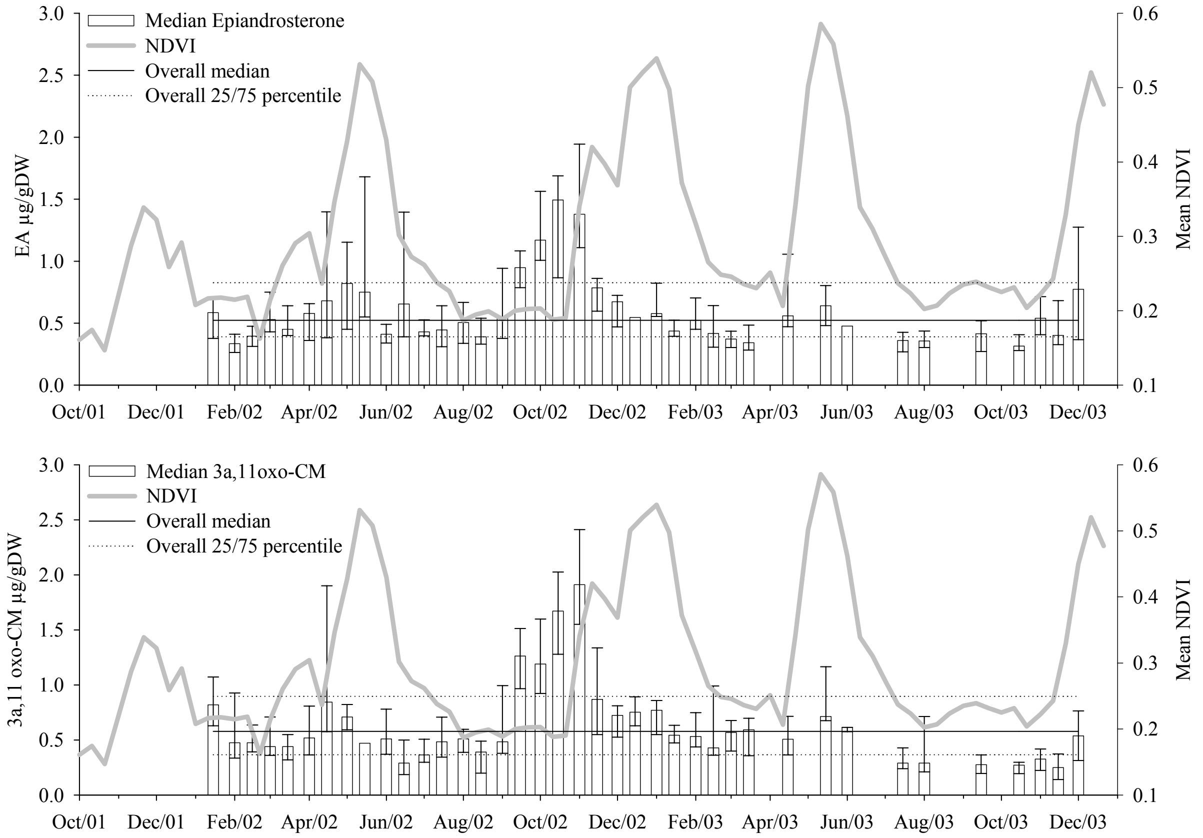


Figure 6.5 Temporal variation in median androgen (Epiandrosterone (EA)) and glucocorticoid (3a,11oxo-CM (Oxo)) levels in sexually inactive individuals and mean NDVI between Jan 2002 and Dec 2003. Medians and 75% percentiles of hormones calculated in non-overlapping 15 day intervals. Some 15-day "blocks" missing due to low sample size (N= 425 samples).

Fewer samples were available towards the end of the long dry season in 2003. However no evidence of an increase in neither 3a,11oxo-CM nor EA was seen. This absence of any hormonal response towards the end of the long dry season in 2003 could be due to this season occurring after three consecutive rainy seasons well above normal, hence the elephants were likely in very good conditions at the beginning of this dry season. On the contrary the rainy seasons of 2000 and 2001 were all well below norm with only one good season in May 2002.

6.4.4 Inter individual variation in inactive baseline levels

Samples from the period between 1 Sept-30 Nov 2002, where general elevated levels of both EA and Oxo were likely linked to environmental conditions were excluded from the analysis of inter individual differences in hormone levels during inactive periods. The 14 bulls with high sample frequency had an overall median EA level of 0.46 µg/gDW (Range 0.35-0.6) with no significant individual differences (Kruskal-Wallis ANOVA by Ranks: H $_{13,229}$ =15.24 p =0.29) (Fig 6.6).

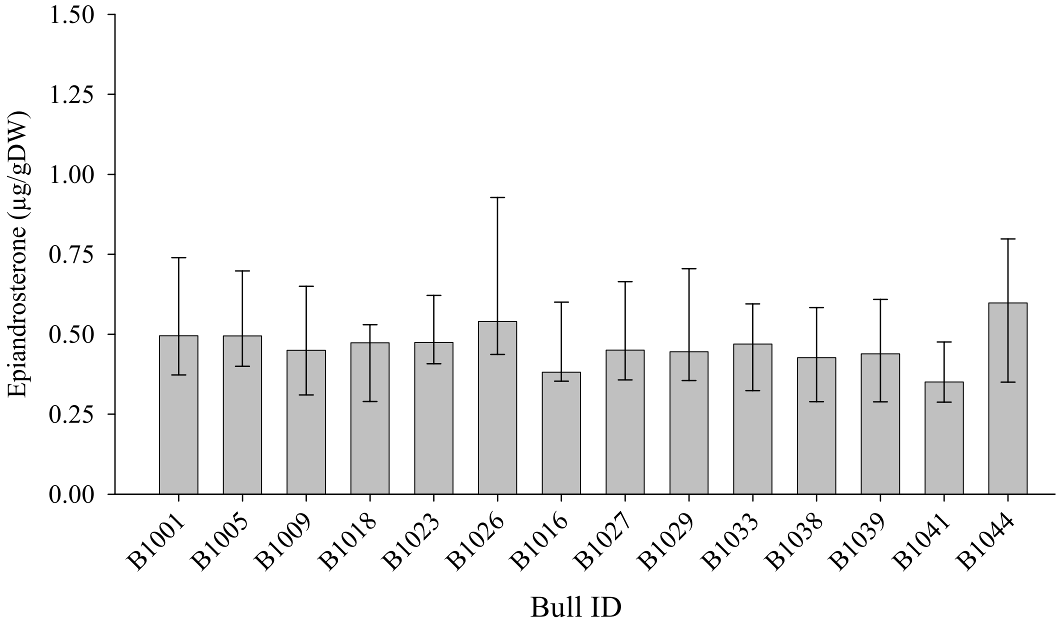


Figure: 6.6: Median individual EA levels (with 25-75 percentile) during inactive periods (excluding end of long dry season 2002) (Kruskal-Wallis ANOVA by Ranks: H $_{13,229}$ =15.24534 p =0.29) (N=14 individuals, Sample per individuals 7-32)

The overall median level of 3a,11oxo-CM was 0.51 µg/gDW (Range 0.36-0.70) did vary significantly between individuals (Kruskal-Wallis ANOVA by Ranks: H $_{13,229}$ =26.38 p =0.015) (Fig 6.7). However the differences were small and unlinked to age (Spearman rank correlation t_{12}=0.68 p= 0.51)

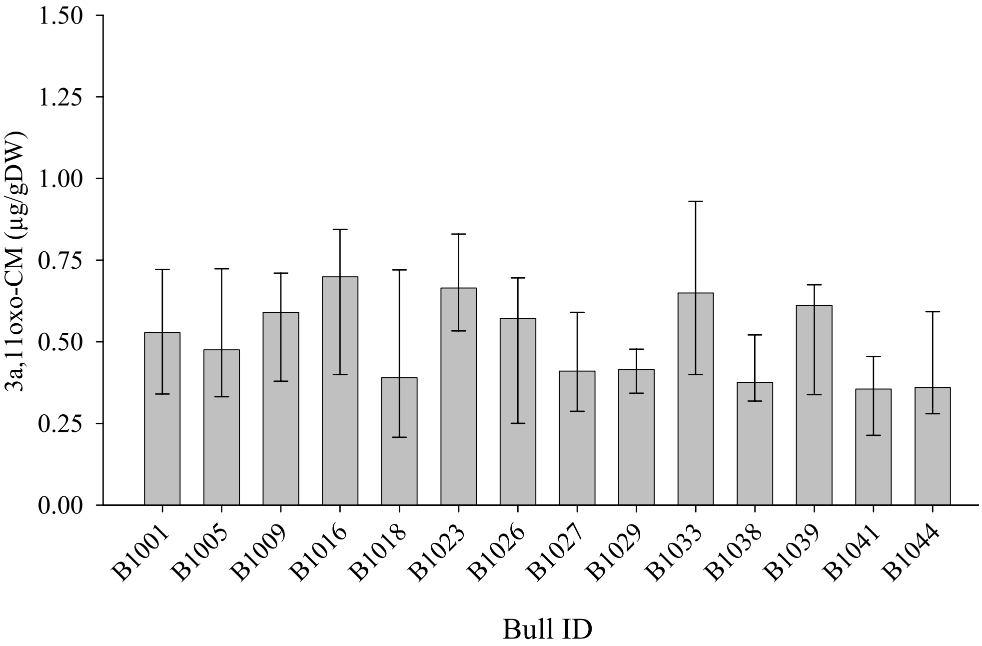


Figure: 6.7 Median individual oxo levels (with 25-75 percentile) during inactive periods (excluding end of long dry season 2002) (Kruskal-Wallis ANOVA by Ranks: H $_{13,229}$ =26.38 p =0.015) (N=14 individuals, Sample per individuals 7-32)

6.4.5 Individual longitudinal profiles

Ten of the longitudinal hormone profiles are shown in figure 6.8, 6.9 and 6.10 arranged according to age. Epiandrosterone and 3a,11oxo-CM (Glucocorticoids) are shown together with the presence or absence of musth signals, known associations with oestrus females and the general temporal distribution of oestrus in the population.

Figure 6.8 and Figure 6.9A shows bulls with both TGS and UD predominately present during their SA periods (SA marked with grey) and with absence of signals linked to the onset or termination of the SA period. Figure 6.9B&C and Fig 6.10A shows bulls with varying occurrence of musth signals within SA periods and with the absence of signals occurring through their SA period. Fig 6.10C shows a bull not exhibiting musth signals. All bulls showed a very similar patterns of elevated levels of 3a,11oxo-CM during Sept 2002- Nov 2002, the end of the long dry season. This pattern is identical to the patterns seen in the overall median values of all inactive individuals.

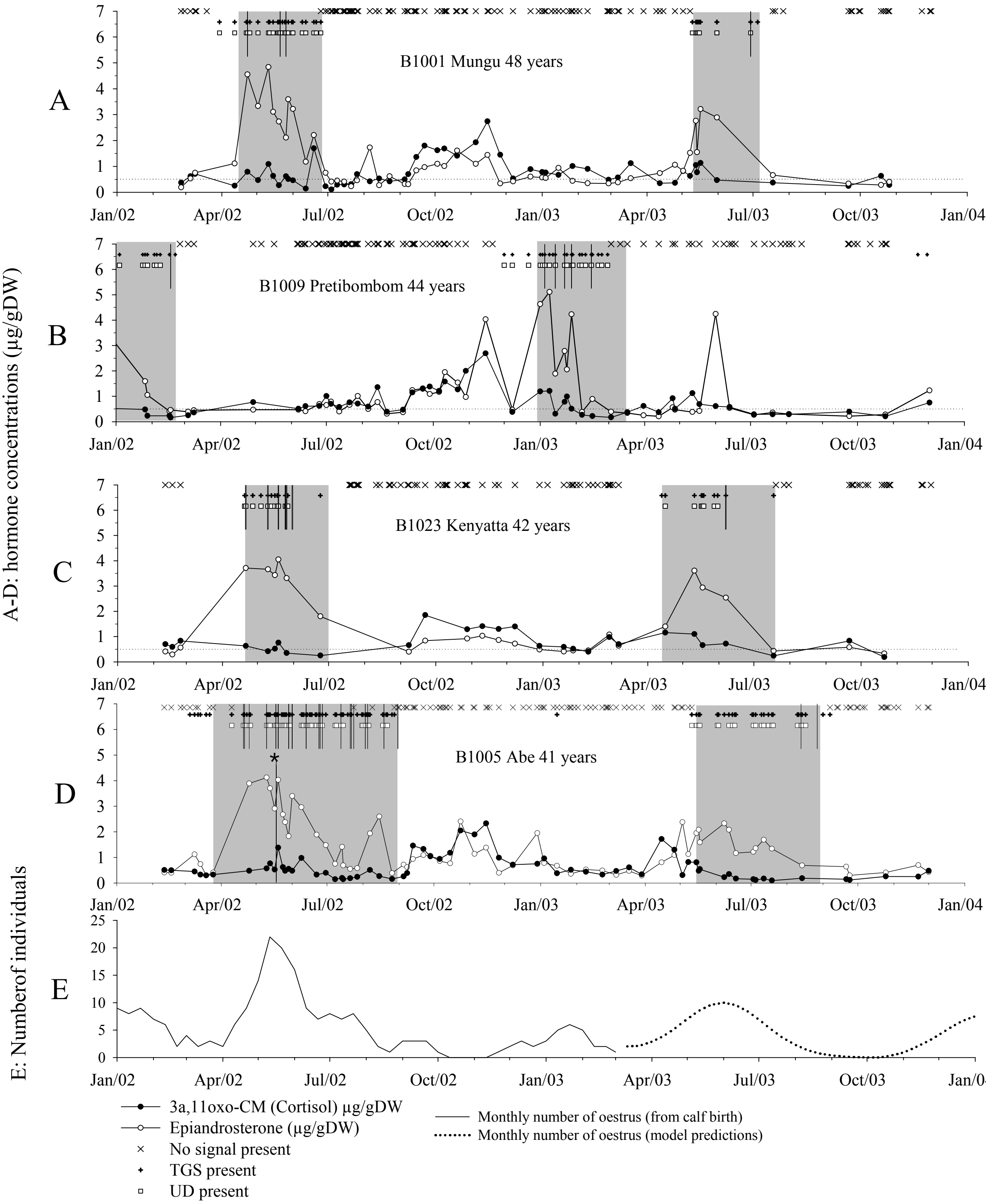


Figure 6.8:Longitudinal time profiles of epiandrosterone and 3a,11oxo-CM in four bulls consistently showing musth signals in their active periods (Grey) Age refers to est. age in 2002. Top vertical lines indicate observations in association with oestrus females. Vertical line with * indicate fight. Last graph show overall number of oestrus females in population.

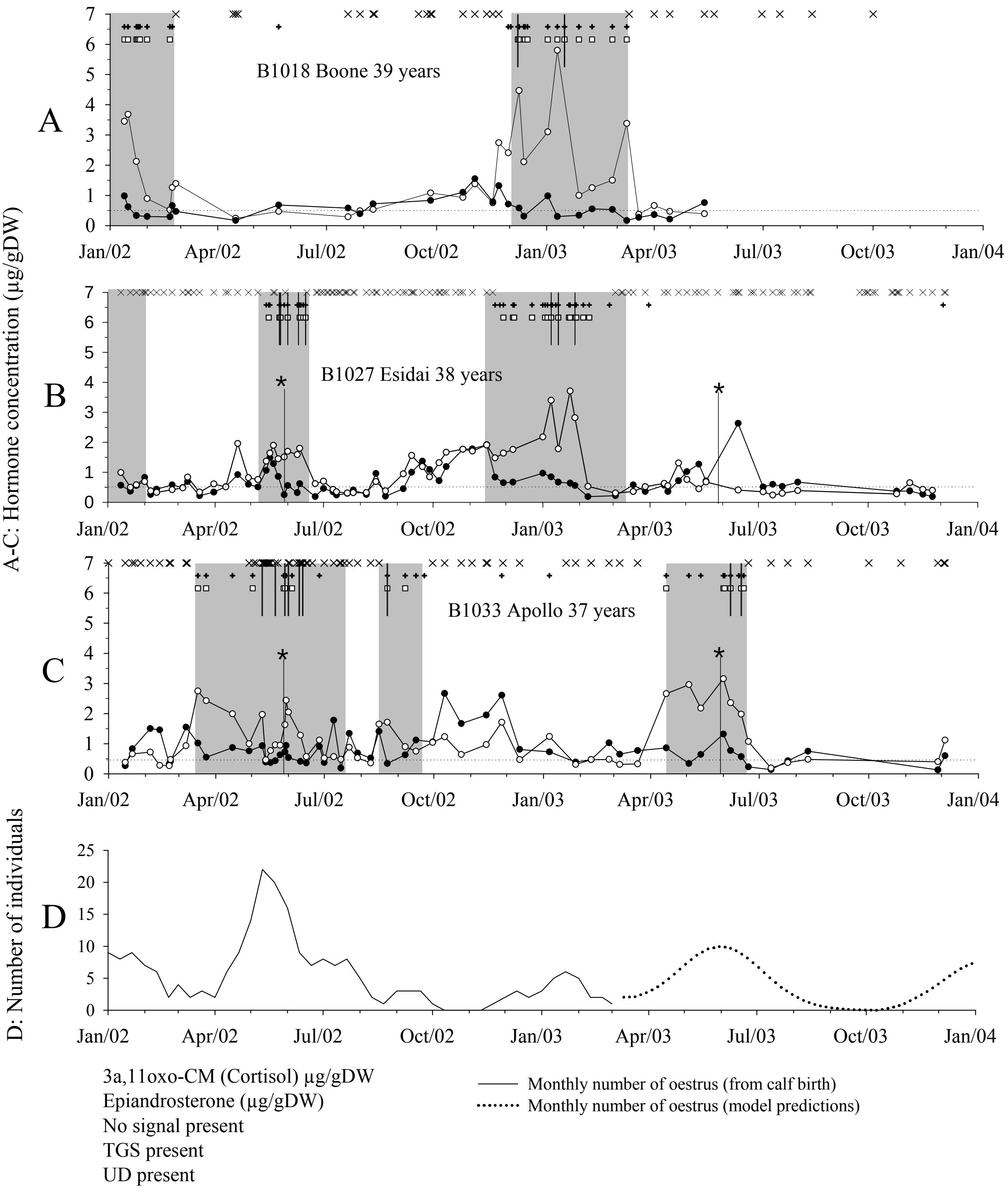


Figure 6.9:Longitudinal time profiles of epiandrosterone and 3a,11oxo-CM in three bulls. A consistently showing musth signals, B&C shifting between SA--, SA-+ and SA++ during active periods (Grey) Age refers to est. age in 2002. Top vertical lines indicate observations in association with oestrus females. Vertical line with * indicate fight.. Last graph show overall number of oestrus females in population.

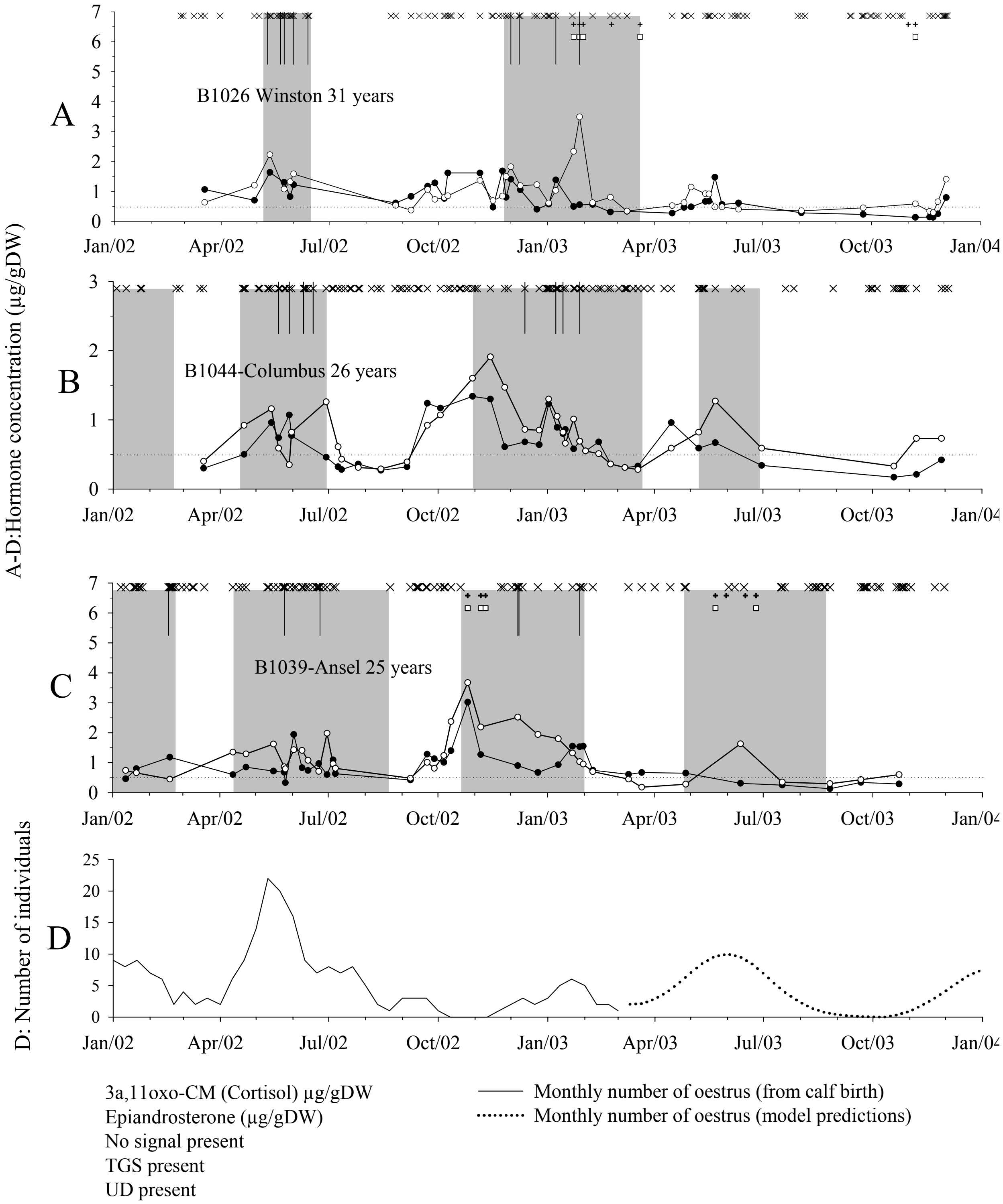


Figure 6.10: Longitudinal time profiles of epiandrosterone and 3a,11oxo-CM in three bulls only showing sporadic musth signals (A&C) or not showing signals (B, note different scale) in their active periods (Grey) Age refers to est. age in 2002. Top vertical lines indicate observations in association with oestrus females. Last graph show overall number of oestrus females in population.

6.4.5.1 Musth profiles

In the ten profiles of bulls predominantly showing musth signals during the active periods (3 profiles not shown) no general elevation of 3a,11oxo-CM occurred linked to active periods. Excluding all samples from Sept 2002-Nov2002 where elevated levels were likely due to environmental conditions no overall significantly difference existed between SA and SI periods of individuals (Mann-Whitney U test, 8 individuals p>0.30, 2 individual p<0.05) (Fig 6.11 A) hence giving the same results as the cross-sectional analysis of hormone levels.

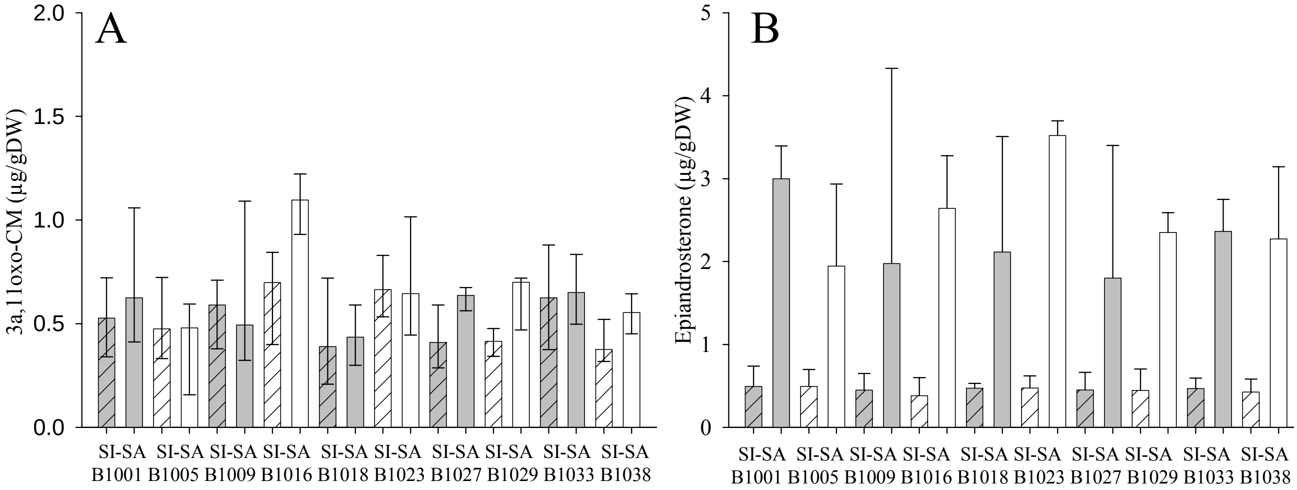


Figure 6.11: Median hormone levels (error bar 25-75 percentile) during SI and SA++ periods in the 10 bulls showing consistent musth periods A: 3a,11oxo-CM, SI and SA++ periods only significantly different in B1027 and B1016 (Mann-Whitney U test p=0.029-0.05) all other bulls not different (Mann-Whitney U test p>0.30). B: Epiandrosterone, SI and SA++ periods significantly different in all bulls (Mann-Whitney U test p<0.0001)

Epiandrosterone levels in these bulls also showed very similar patterns with clear elevated EA levels closely linked to the SA period with an additional period of increase linked to the late dry season 2002. All 10 musth individuals had significantly elevated levels during SA periods with TGS and UD compared to SI periods (Mann-Whitney U test 10 individuals p<0.0001) (Fig 6.11B). Seven of the musth bulls generally showed consistent TGS and UD throughout the active period except towards the end (see e.g. Fig 6.8A,B,C; Fig

6.9A) The remaining three (Fig 6.8D Aug 2002 and Fig 6.9B&C June/July 2002) shifted to some degree between having both TGS & UD (SA++), TGS only (SA+-) or no signals (SA--). Only one of these bulls (B1033 fig 6.9C) had sufficient samples in each category for analysis. In this bull EA levels were significantly elevated between SI and SA -- (Mann-Whitney U-test p = 0.0005) and between SA-- and SA+- (Mann-Whitney U-test p = 0.007) but not between SA+- and SA++ (Mann-Whitney U-test p = 0.93) indicating that the switching on and off of UD did not reduce EA levels but periods without signals was associated with intermediate levels of EA (Fig 6.12)

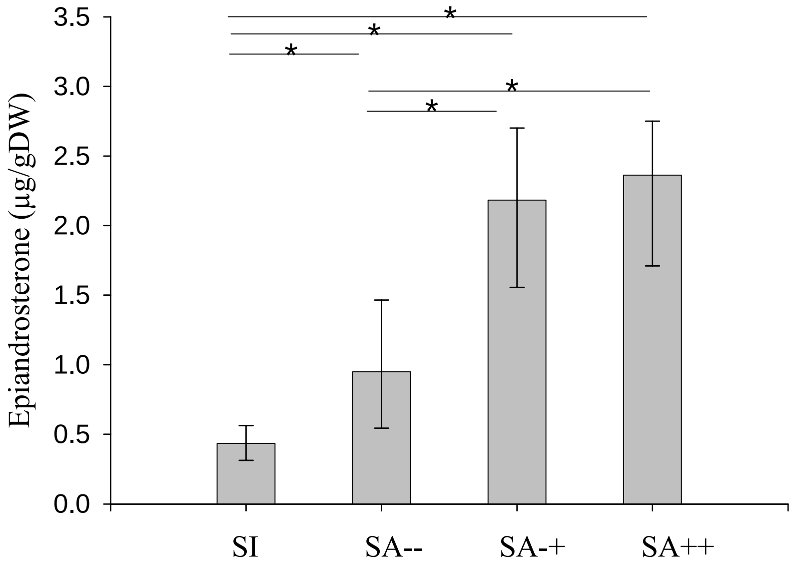


Figure 6.12: Median EA levels (error bar 25-75 percentiles) of B1033 Apollo during inactive and the three different active categories. Asterisks indicate significant differences (Mann-Whitney U-test p < 0.007)

Some evidence could be seen for elevated 3a,11oxo-CM levels linked to individual events. A number of physical fights with other bulls showed up as single peak values but quickly returned to baseline levels (e.g. fig 6.8D). An example of the potential costs (and gains) of such non-lethal fights can be seen in fig 9 B&C. B1027-Esidai (Fig 6.9B) normally has two SA periods per year overlapping with the peak number of females. Early May 2003 he started shifting associational preference indicating the beginning of an active period (See Fig 5.7A Chapter 5) however after a fight in late May 2003 with the similar aged bull B1033-

Apollo (Fig 6.9 C) (same bulls as he fought with in May 2002) he got injured and lost one of his tusks. Both bulls showed elevated glucocorticoid levels after the event with Esidai's levels increasing four fold. After a period spent exclusively alone Esidai returned to his "bull area" in association with other inactive individuals thus missing a full breeding season due to this encounter. For Apollo this encounter however resulted in a full breeding season with one closely matched competitor less and may have established their relative rank in the future.

Generally musth bulls showed very large variation in EA levels during active periods sometimes doubling levels within a few days. These peaks did for some bulls tend to occur during or just following associations with oestrus females however due to the temporal resolution of the data and the delay between actual increase in hormone level and the detection of the change in the faeces combined with the error rate on the hormone measurement and the temporal resolution of the samples makes it difficult to assess such fine scale changes.

6.4.5.2 Sexually active Non musth bulls

In the three bulls with extensive or exclusive active periods without musth signals the SAN periods were associated with a significant 1.5-2 time increase in median 3a,11oxo-CM levels (Mann-Whitney U test 3 bulls, $p<0.05$) (Fig 6.10A). Likewise a significantly two-fold increase in median EA levels was seen in the three bulls (Mann-Whitney U test 3 bulls, $p<0.03$) (Fig 6.10B). Two of the three bulls (B1026 and B1039) had very short occurrences of TGS and UD during their SA periods the 4 samples collected during these two incidences were associated with EA levels comparable to bulls exclusively showing musth signals (B1026: 2.34 & 3.49 µg EA /gDW; B1039 3.67 & 2.19µg EA /gDW).

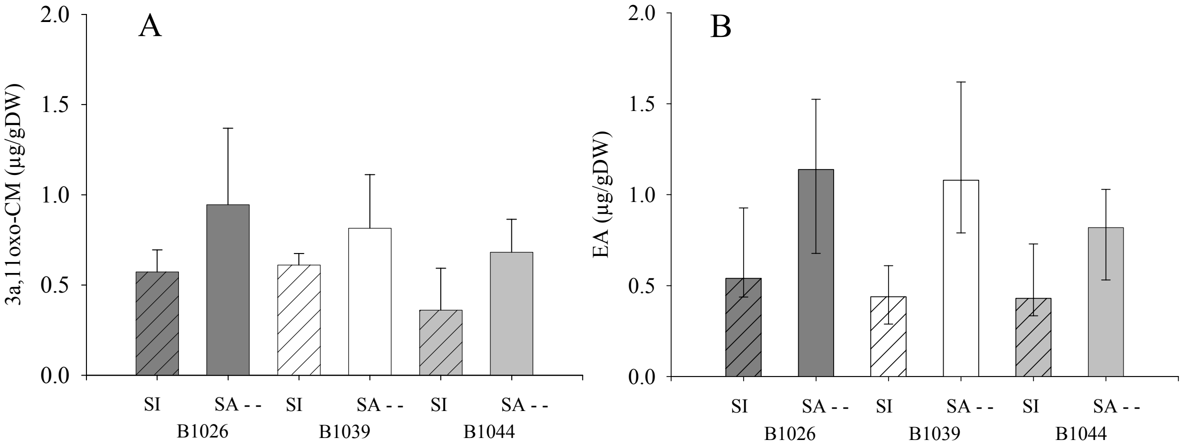


Figure 6.10 Median hormone levels (error bar 25-75 percentile) during SI and SA-- periods in the 3 bulls showing prolonged active periods without musth signals A: 3a,11oxo-CM, SI and SA-- periods significantly different in all bulls (Mann-Whitney U test $p<0.05$). B: Epiandrosterone, SI and SA-- periods significantly different in all bulls (Mann-Whitney U test $p<0.03$)

6.4.5.3 Standard musth profile

To avoid any effect from the general increase in EA and 3a,11oxo-CM levels during the late dry season of 2002 any samples in the 2-month pre and post SA periods belonging to this period were disregarded from the profiles. The median EA and 3a,11oxo-CM levels prior, during and after musth periods as well as the fraction of observations with TGS and UD plus fraction of observation in bull, solitary of female groups are shown in figure 6.11. Up to 30 days prior to the onset of the sexually active periods EA levels were identical to SI individuals and no musth signals were observed (Fig 6.11-1 period A). A month prior to the onset of the SA period the EA levels started to rise reaching approximately double the level of SI individuals. Concurrently musth signals started to appear but no gradual change in association could be seen (Fig 6.11-1 period B). At the onset of the SA period the EA levels rose sharply and peaked during the first 10-20% of the active period. At the same time both TGS and UD was observed in over 90% of the observations. The shift in associations, identified by the HMM was abrupt shifting from 95% bull association to 2% bull associations within 10 days (in Fig 6.11-1 period C).

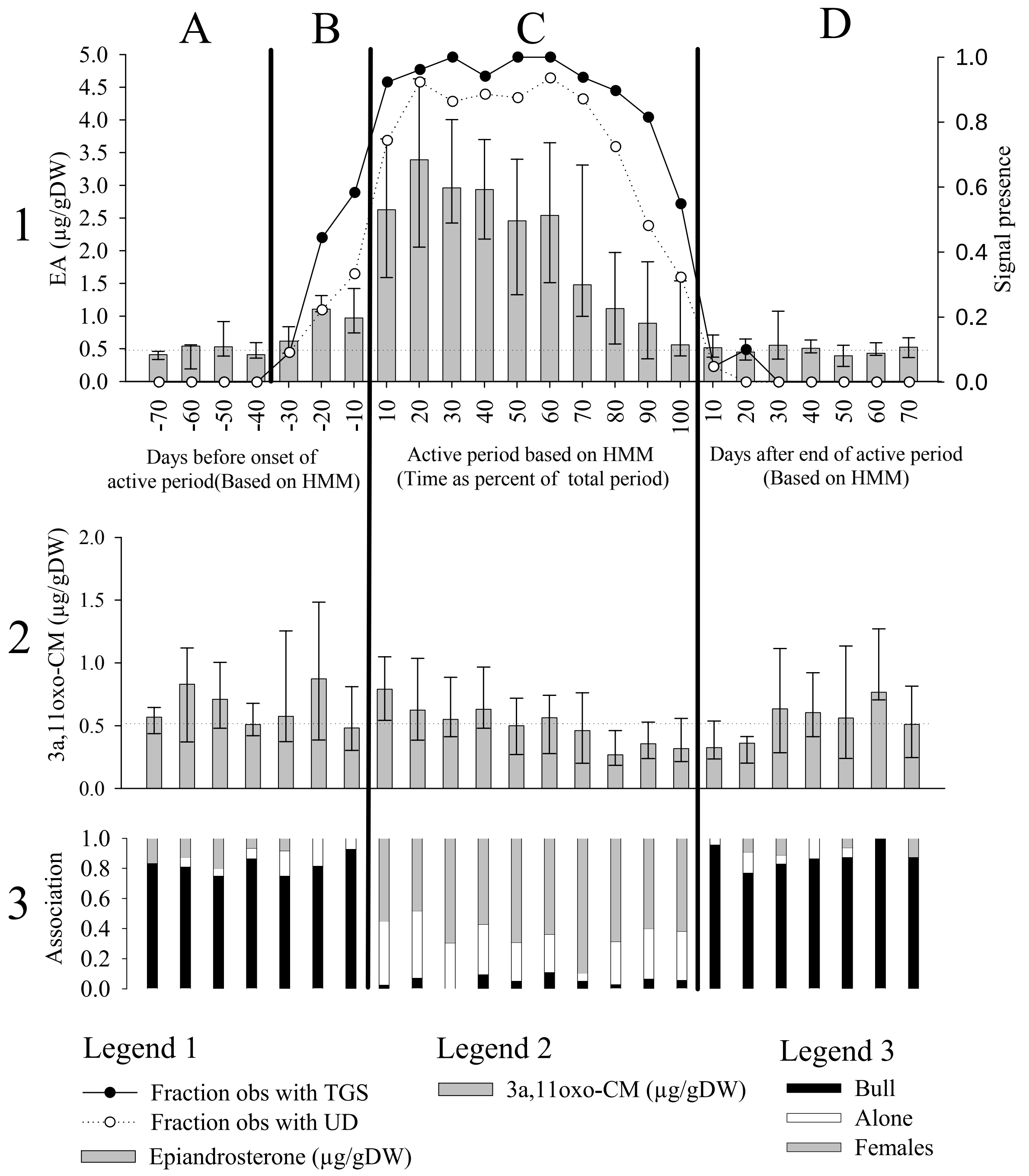


Figure 6.11 Median androgen levels (1) and median 3a,11oxo-CM levels (2) (N= 214 samples) before, during and after sexually active periods in "pure" musth bulls (N= 16 period in 9 bulls) and fraction of observations with TGS and urine dribbling (1) (N= 575 observations). Time before and after active period shown in days. The time during active period calculated as percent of total active period to normalising varying period durations. 3: Fraction of observations in association with bulls, alone or with females (data used by the HMM to assess sexual state).

After the initial peak in EA the levels slowly dropped through the course of the SA period reaching inactive levels at the point of termination of the SA period with musth signals slowly starting to disappear during the last 20% of the SA period. However the associations stayed unchanged until termination. After the termination of the SA period EA levels were identical to inactive levels with only a few occurrences of musth signals during the first 10-20 days after termination and with the associations shifting abruptly back to pre active levels at the point identified by the HMM

6.4.5.4 Standard active non-musth profile

The standard active non-musth profile was created similarly to the musth profile, also disregarding samples from the late dry season of 2002. More than 20 days prior to the onset of the SA period EA levels were identical to SI levels (Fig 6.12-1 period A). Only 2 samples were available for the 20 days prior to the onset of the SA period but the increased levels seen in those (Fig 12-1 period A) suggest an initial increase in EA levels prior to the SA period comparable that seen in musth bulls. At the onset of the SA period the EA levels was doubled compared to SI periods. The peak EA levels occurred during the first 20% of the active period and thereafter steadily declined during the SA period until inactive levels were reached at the end of the SA period. Hence the temporal timing of EA changes in SAN bulls was similar to the musth bulls except that EA levels only doubled during the early peak EA part of the SA period compared to a 6 or 7 fold increase seen in the musth bulls. The maximum level reached by SAN bulls is this comparable to the level reached by musth bulls just prior to the onset of their active period (and musth).

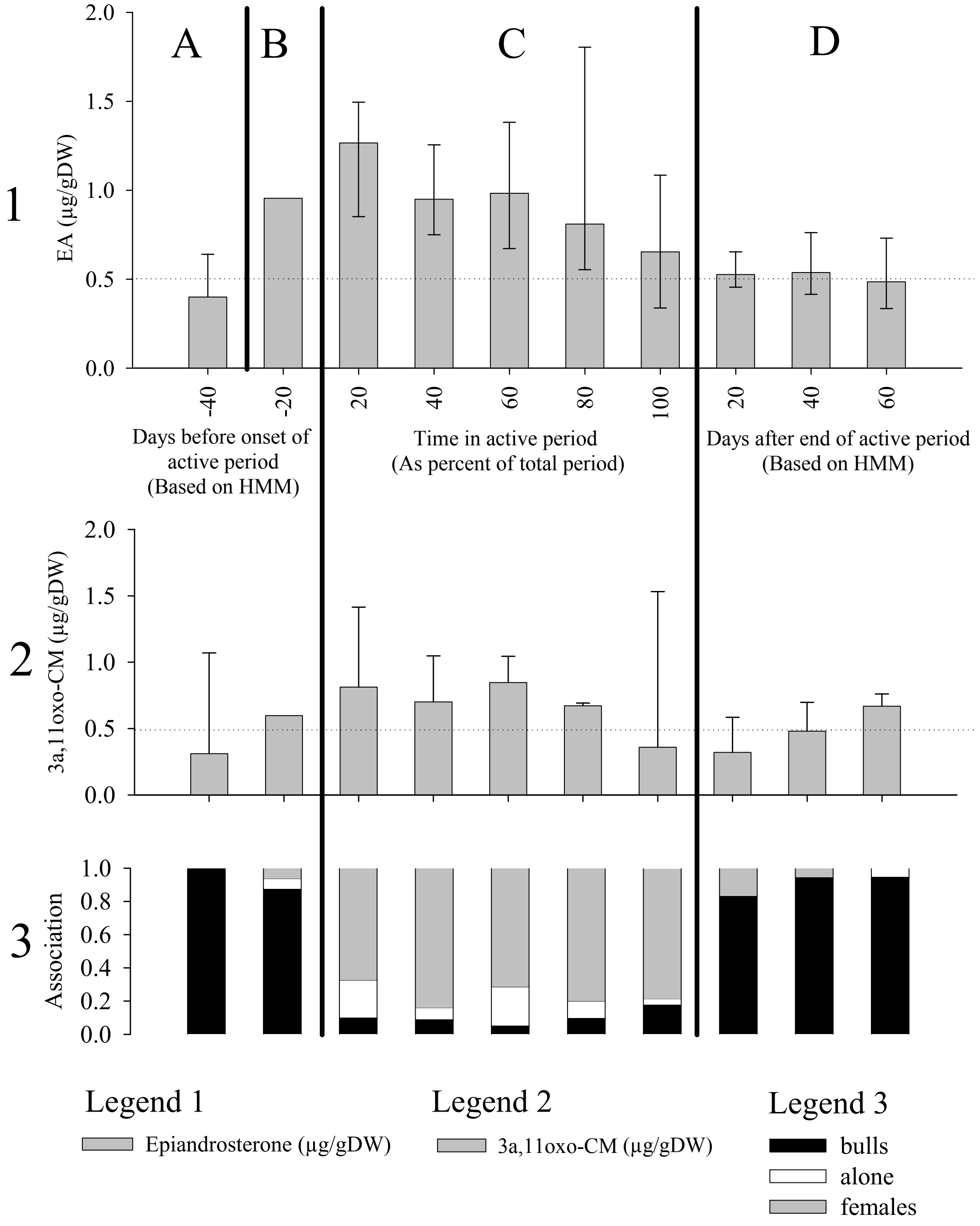


Figure 6.12 Median androgen levels (1) and median 3a,11oxo-CM levels (2) (N= 84 samples) before, during and after sexually active periods in bulls not exhibiting musth signals (N= 9 period in 6 bulls). Time before and after active period shown in days. The time during active period calculated as percent of total active period to normalising varying period durations. 3: Fraction of observations (N= 315) in association with bulls, alone or with females (data used by the HMM to assess sexual state).

6.5 Discussion

The presented results for the first time examines endocrinological differences between qualitatively defined categories of sexual states and present longitudinal profiles in free ranging African elephants. The results show that measurements of faecal epiandrosterone (EA) and 3a,11oxo-cortisol metabolites (3a,11oxo-CM) generate useful information on androgen and glucocorticoid status in elephant bulls under free-ranging conditions, as recently described for animals in captivity (Ganswindt et al., 2002; Ganswindt et al., 2003).

Androgen levels were generally elevated during SA periods with periods of TGS and/or UD associated with the highest levels. The positive correlation between age and the occurrence of musth signals (Poole, 1987; Poole, 1989b) and the elevated levels of testosterone seen in bulls assumed to be sexually active but not in musth in the study by Poole (Poole et al., 1984) made it unclear if the elevated androgen levels generally reported in studies of musth bulls were linked to age of the sexually active bull rather than the presence of musth signals. The present study demonstrates that the elevation in androgen levels seen in bulls showing either sign was unrelated to age, indicating that the presence of the signal, rather than age, is the main predictor of increased androgens. Thus, by showing that younger musth bulls, at least with respect to EA levels are not false signalling, our results support the earlier predictions of (Poole, 1989b) that musth is an honest signal.

Although EA concentrations were significantly higher in bulls showing TGS compared to bulls without TGS, the signal strength was not related to androgen level, irrespective of whether or not UD was present. On the basis of the cross sectional analysis of data, four reproductive categories of adult bull elephant emerged; three sexually active (SA only, SA plus TGS, and SA plus TGS and UD) and one sexually inactive. Among the SA bulls, the

TGS only group occurred at a relatively low frequency and had only a 2-fold increase in EA levels compared to a 4-fold increase in the group with UD. Although bulls do occasionally show TGS within an SA period without having UD at any point (see e.g. Chapter 5 Fig 5.9C) the majority of observations on TGS-only were associated with bulls that at one point during the period showed the full combination of both TGS and UD and most often occurred towards the end of an SA period. Thus the TGS only group represent in the majority of cases a transitional condition linked to the termination of a sexually active SA++ period rather than a separate and distinct state. Thus, the two sexually active states, musth (SA++) and SAN (SA--), seem to be on/off states rather than a continuous change. A clear example of this can be seen in the profile of B1026 (Fig 6.10A) where the short SA++ period within the SA-- period was linked to a short rapid increase/decrease in EA levels.

The only other study describing quantitative differences in androgen levels according to reproductive state (Poole et al., 1984), also demonstrated an elevation of testosterone levels in SAN bulls compared to the group of SI individuals, but since sample size was low and no clear definition of the state of SAN was given, direct comparison of results is difficult. Nevertheless, the findings of both studies indicate that within the reproductive context of male African elephants, two distinct states of sexual activity exist linked to differences in the occurrence of the musth signs (TGS and UD). The present study now show that these two states also differ in terms of androgen levels. Based on the comparison of SI; SA-- and SA++ periods in individuals the SA-- state is linked to an approximate doubling of EA levels compared to a five fold increase in SA++.

The very high androgen levels in the SA++ state provide a likely physiological explanation for the observation that this category of bulls also exhibited the highest rates of aggression (Poole, 1989b), since androgenic steroid hormones are known to mediate aggressive behaviour in diverse mammalian species (Brain, 1979; Leshner & Moyer, 1975; Ogawa et al., 1996; Rose et al., 1974). The simultaneous occurrence of elevated androgens, high levels of aggression and therefore increased likelihood of engaging in physical fights in SA bulls showing TGS and UD (Hall-Martin, 1984; Poole, 1989b) indicates that this state should be viewed as a competitive strategy. On the basis of this, only this competitive part of African bull sexual activity should be called musth and not the whole complex of sexual activity, hence supporting Pooles (Poole, 1987) original definitions.

A few bulls did shift frequently between SA--,SA+- and SA++ during their active period. In one of those bulls sufficient samples in each category was available for analysis and showed that EA levels did not differ between SA+- and SA++ whereas SA-- were significantly lower (and comparable to levels in SA—bulls not exhibiting signals at any point) (Fig 6.12). Hence the bulls in the TGS only group (SA-+) where the lack of UD was not associated with the last terminal phase of a SA++ period may represent bulls primed for a competitive mode including high androgen levels but with the local social and competitive environment determining if they proceed to signal dominance by UD. For example observations showed that bulls can switch urine dribbling on within hours of finding an oestrus female and switch it off within minutes of arrival of a much higher ranking musth bull, whereas TGS continued to be seen for several days after UD had ceased. This has been reported from other studies (Poole, 1989b). Likewise on several occasions TGS only bulls switched on UD for brief periods linked to the approach of the research car and SA++ bulls increased rate of dribbling during encounters with other SA++ bulls. Hence showing the rapid response of this signal to changing local social conditions and the direct link between the presence of UD and the show of dominance both as a male/male and a male/female directed signal.

Variation in androgen levels among bulls showing physical signs was considerably higher than in the other groups. This may be an indication that other factors could influence androgen status in these individuals. Encounter of an oestrus have been observed to trigger the onset of UD so association with an oestrus female could potentially be such an external factor affecting androgen levels. The link between encounters of oestrus females and EA levels were somewhat inconclusive. Due to the uncertainty of the duration of association with the females prior to the observation and sample combined with the time delay of hormone signal in the faeces due to the food passage time and the relative low frequency of samples in some situation makes detailed analysis difficult. If the encounter of oestrus females results in increased androgen levels such events may act as “booster vaccines” on androgen levels and result in longer musth periods when many oestrus females are encountered and contrary shorter periods when few females are encountered offering a mechanism of measuring time specific resource quality. Further studies using higher temporal resolution of observations and samples are needed to reveal if such a mechanism do indeed exist.

By combining several individual profiles the general course of an SA period was investigated for both musth and SAN bulls. For musth bulls the sequence of events is an initial medium increase in androgens approximately 2-3 weeks prior to the associational shift. This increase is followed by first TGS and shortly after UD with both signals being present at the abrupt shift in associations. At the same time androgen levels increase dramatically reaching approximately 5-6 times baseline levels. During the last half of the SA period (base on associations) the androgen levels slowly drops with first UD and then TGS signals starting to disappear during the last 20-30% of the active period. Hence the initial increase in androgens are not triggered by female company but contrary both signals and associations occur downstream from the initial increase in androgens. The course of an SAN period is somewhat similar except for the lack of the dramatic increase in androgens and the per definition lack of signals. Which factor that triggers the initial androgen increase and hence the decision to become sexually active is not clear. A high level of synchrony of individual active periods especially active musth periods are seen, with older bulls focusing on only one of two equally good seasons per year but consistently choosing the same. This high level of synchrony is also seen in Amboseli (Poole & Moss, 1981; Poole, 1987; Poole, 1989b) where some bulls repeatedly have focused on the same time of year for more than 20 years. If active periods were simply triggered by internal energy stores the high levels of inter seasonal variation in forage availability would be expected to quickly disrupt any cyclic pattern. Likewise general seasonal changes in ecological conditions are unlikely to be responsible as some bulls consistently choose one of two wet seasons, other consistently the same of two dry seasons. Apparently bulls have an internal annual clock announcing when their time slot has come up.

6.5.2.1 Physiological stress

Several different types of stressors were found to result in increased glucocorticoid levels. An overall 3-4-fold increase was found in all individuals in Sept-Nov 2002. This period was also associated with a general increase in EA levels. This increase occurring towards the end of the long dry season is likely linked to low energy availability in the forage and may represent a situation where individuals shift from a net energy intake to net energy expenditure and start metabolising stored fat reserves. Such ecological factors have previously been shown to increased adrenal endocrine function in female elephants (Foley et al., 2001). In addition, severe physical trauma resulted in a more than 10 time increase in 3a,11oxo-CM and prolonged elevated levels in one bull (Fig 6.2). Shorter physical antagonistic interaction with competitors without any visible injuries also showed up as single "outlier" points (e.g. Fig 6.8D) but quickly returned to baseline levels. However, in the cross sectional analysis no general increase in 3a,11oxo-CM was seen linked to SA periods. Likewise in the analysis of individuals no overall increase could be seen between SI and SA periods in bulls showing both TGS and UD during their active periods with only 2 out of 10 bulls having a significant but small increase (Fig 6.11A). On an individual basis, any elevation in glucocorticoids that may occur would likely to be seen towards the end of the SA period when loss of condition and energetic stress may be highest, however such trend was not evident in the individual profiles and also did not emerge from the analysis of the combined "musth" profiles (Fig 6.12). These results conform to those of a recent study in captive elephants (Ganswindt et al., 2003), which also revealed no elevation of 3a,11oxo-CM during the competitive state of musth. On the contrary all three bulls not showing TGS or UD during the majority of their active periods all had a significant increase, approximately doubling their 3a,11oxo-CM levels during active periods. This could suggest that the lower ranking SA bulls not exhibiting musth signals do experience increase physiological stress during active periods.

Thus, based on faecal glucocorticoid profiles in both captive (Ganswindt et al., 2003) and wild animals (present study), available information provides no indication that musth in the African elephant represents a physiological stress associated with activation of the hypothalamic–pituitary– adrenal axis (HPA axis). But that this may occur in lower ranking SAN bulls. Stress-related factors independent of enhanced adrenal activity, such as increased secretion of endogenous opiod peptides (e.g. (Abbott et al., 1997) is also known to be involved in stress response in mammals. Therefore further studies are needed to explore other metabolic pathways that could respond to the potential stressful condition of musth. Further, in contrast to bulls in captivity (Ganswindt et al., 2005 (in press)) the possibility of a suppressive effect of elevated androgen levels on adrenocortical activity in mammals (Kenagy et al., 1999) through mechanisms involving enhanced glucocorticoid feedback regulation(Viau & Meaney, 2004) may have obscured any stress-related adrenal response. However the only bull having a musth period overlapping with the long dry season of 2002 also showed signs of elevated 3a,11oxo-CM suggesting that the high EA levels associated with musth do not suppress adrenocortical activity.

In conclusion, it has been shown that general elevated levels of androgens exists during SA periods with SAN periods being associated with a moderate doubling of androgen levels and musth periods associated with very high 5-6 fold increase. These elevated levels appear to be irrespective of the age of the individual, hence supporting that these signals are honest signals. Based on this, the term "musth" should be exclusively linked to the competitive state in SA male elephants and that the presence of urine dribbling should be the physical signal used for defining this state. Further, the presence of oestrus females may positively influence androgen levels in individuals, however further more detailed studies are needed before reaching any conclusive results. In addition it has been shown that the initial increase in androgen levels occur prior to onset of musth signals and 2-3 weeks prior to the shift in associational preferences. Hence endocrinological changing occurs prior to behavioural changes. The results of glucocorticoid levels represent the first recorded levels of adrenal activity in free ranging elephant bulls and indicate that the state of musth do not seem to stimulate HPA axis activity. SAN generally seen in younger lower ranking individual may be associated with a moderately increased glucocorticoid output.

Chapter 7

7 Reproductive investments of alternative tactics

In Prep as: Reproductive investment by alternative reproductive tactics in male African elephants H. B. Rasmussen; F. Vollrath ; I. Douglas-Hamilton)

Abstract

The investment in reproduction is compared between the two observed reproductive tactics by analysing time budgets in combination with daily travelling distance and duration of sexually active periods in males of different age engaging in different tactics.

Sexually active non-musth bulls (SAN) foraged for significantly less time and spent more time walking compared sexually inactive bulls (SI) but had intermediate changed compared to that observed in musth bulls. No differences were found between SAN and musth bulls in terms of numbers of females encountered per hour or the number of shifts between female herds. GPS tracking data showed a similar pattern in daily walking distance, with SAN bulls increasing the daily walking distances by approximately 50 % while musth bulls doubling the daily distances compared to SI bulls. The largest increases in hourly walking distances were seen during morning and afternoon indicating that female searching is predominately done during these periods. Both active states were also associated with an increase in daily displacements.

A shift occurred in the proportion of time spent in the SAN or musth state during sexually active (SA) periods from younger bulls (<25years) only in SAN to older (40+ year) bulls almost exclusively in musth during sexually active periods. This shift was associated with a decrease in the total proportion of time spent sexually active from around 55% in younger bulls to around 25% in older bulls. Hence the shift from SAN to musth is associated with an increase in time specific investment but a reduction in the overall duration of active periods

7.1 Introduction

Within female defence polygynous mating systems, a continuum exists between "stayer" and "rover" behaviour in males. An extreme "stay and defend" strategy are e.g. seen in red deer *Cervus elaphus* (Clutton-Brock et al., 1982) where males, once they have located a group of females attempt to continuously monopolize this harem group. An intermediate strategy is seen in muskoxen (*Ovibos moschatus*) (Forchhammer & Boomsma, 1998) where the males sequentially defend female groups with varying degrees of exploration time. In other systems only individual females are defended during their short receptive phase and almost all the time is allocated to searching for a receptive female by moving (roving) between female groups as e.g. seen in the Indian chital *Axis axis* (Shchaller, 1967).

The degree to which males adopt either stayer or rover tactics are largely determined by factors like spatial distribution of females, frequency and duration of female receptive events, female choice, operational sex ratio and male fighting ability (Emlen & Oring, 1977a; Clutton-Brock, 1989; Clutton-Brock & Harvey, 1978; Forchhammer & Boomsma, 1998). Within this stayer/rover continuum, two alternatives reproductive tactics (ART) are often observed and can be broadly categorized as i) the "bourgeois males" which engage in high intra sexual competition and defence of reproductive resource (either harem holding or guarding of individual females) or ii) the "satellite males" associated with low investment in competition and limited defence of females (Gross, 1996). These two tactics are often status dependent with less competitive able individuals (often younger) adopting the satellite tactics and more dominate (often older) individual adopting the bourgeois tactic.

Female elephants live in a social fission/fussion system but with a small relatively stable core family unit consisting of 1-5 breeding females (Moss & Poole, 1983; Wittemyer et al., In Press-b). They have a long inter-calving interval of around 4 years in most populations (Laws et al., 1975; Moss, 2001) combined with a short 4-6 day period of behavioural oestrus (Moss, 1983) which can occur throughout the year although clumped during and just following the rainy seasons (This thesis chapter 4; (Laws et al., 1975; Moss, 2001). Hence, the frequency of oestrus within any given group of females is extremely low favouring a roving tactic in males with very short exploration time of each encountered females group (Whitehead, 1990). Theoretical predictions have suggested (Barnes, 1982)that only under extremely low elephant densities will a herd bull encounter more oestrus females than a roving male and hence be favoured. Currently it also seems that males belong to the extreme roving end of the continuum, only staying with females during periods of guarding/following of individual females in oestrus (Barnes, 1982; Poole, 1987; Poole & Moss, 1989b) with no evidence of the herd bull tactic.

The high level of aggression and increased likelihood of physical fights seen in musth bulls (Poole, 1989a; Hall-Martin, 1987) combined with the much higher occurrence of mate guarding by musth bulls (Poole, 1989c) indicates that musth can be viewed as a high dominance and competitive bourgeois tactic compared to the a satellite tactic adopted by SAN males when following an oestrus female.

Figure 7.1 Example of the SAN tactic. During a standoff between two musth bulls a young bull chase the disputed oestrus female (photo 1) and successfully mount her (not shown) before the two musth bulls rush in and chase him off (photo 2) The much younger and smaller SAN bull wisely makes no attempt to defend but immediately stops and backs off.

Younger bulls are also rarely seen to engage in the musth tactic, hereby avoiding the costs associated with fighting. Musth bulls have been shown to invest in reproduction by reducing the amount of time spend foraging during musth periods and spending more time walking, probably linked to searching for receptive females (Poole, 1989a). In addition to these “visible” investments the higher levels of androgens may also cause an increase in basal metabolic rate and have suppressive effect on the immune system (Roberts et al., 2004). Furthermore due to the high level of aggression and likelihood of physical fights, musth bulls are likely to incur costs in terms of increased mortality or physical injuries. Contrary to musth, no studies have yet investigated the potential investment by SAN bulls or compared the level of investment between the two reproductive tactics.

Natural selection will favour individuals that maximise the sum of current and expected future reproductive success. Since the investment of resources in any of the two is complementary, the payoff from any investment in current reproduction should be larger or equal to the reduction in future payoff resulting from that investment. Apart from a reduction in the amount of available resources for future reproduction, either as stored energy or invested in growth, investment in current reproduction may also reduce future reproduction by increasing mortality or injuries if risks are associated with reproduction. Hence optimisation of this long-term trade-off between current and future reproduction and the degree of risk taken (level of competitiveness) is expected to determine the amount of resources (X) invested in present reproduction (current season/year). In a system with post mature growth and large size-dependent competition for mates, like elephants, a competitive strategy associated with increased mortality is not expected to pay before late in life (Whitehead, 1994). Thus bulls below competitive age are predicted to invest overall less in reproduction than are bulls of competitive age. Hence younger SAN bulls are expected to have an overall lower investment in current reproduction.

How to invest the resources allocated to current reproduction (i.e. the time specific level of investment $dX/dt = \Delta X$) is expected to be determined from a similar but independent short-term optimisation process maximising the payoff per unit resource. As the short-term optimisation is acting on payoff per unit energy it is independent of the total amount of energy/resources available (X). Hence variation in X will not be expected directly to affect ΔX but only to affect the duration of the reproductively active period ($X/\Delta X= t$). Whether SAN bulls should invest less, equal or more energy per unit time will depend on the relative relation between investment and payoff between the two tactics.

Successful reproduction in males generally requires both the location of the receptive female (reproductive resource) and, once located, access to this resource. The search for and location of the resource is in most cases not associated with direct competition between males (non-competitive phase), as no resource is yet available to contest. Once located the individual male must acquire successful access to this resource. This phase is in many situations associated with some level of intra sexual competition and inter sexual acceptance (female choice) and can broadly be viewed as the competitive phase of male reproduction.

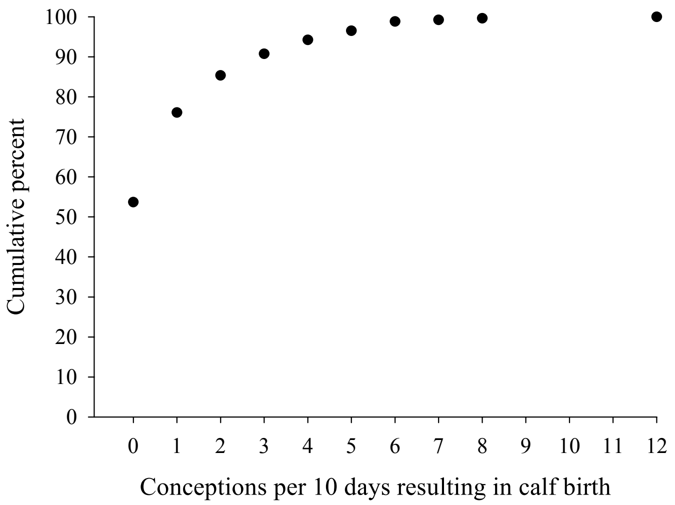


Fig 7.2: Cumulative percent of 10-day periods versus number of conceptions (N= 259 periods, 277 conceptions)

In elephants, oestrus females have a low density and infrequent occurrence. During the 7-year period between 1996 and 2002, the core group of 170 breeding females had a frequency of nil or one conception in 80% of all 10-day intervals (figure 7.2) hence the majority of time allocated to reproduction in males are likely linked to the non-competitive search phase rather than the competitive phase of gaining access or defending an oestrus female. Since the search phase is largely non-competitive the success of locating females is unlikely to be linked to relative status (or difference in competitive mode). The relation between investment in search (reduced feeding increased walking) and success in locating receptive females is thus likely identical between musth and SAN bulls. The relation between the amount of energy allocated to searching and the number of receptive females located is unknown but likely to be a decreasing function, as the rate of "new" females encountered within a finite group of females will decrease with increasing effort. With a similar basic metabolic cost and similar success in locating a female with a given investment the optimal investment in search is unlinked to the probability of gaining successful access to a located female (Figure 7.3). Under such conditions different tactics during the competitive phase or a level of female choice is not expected to affect the optimal investment in search.

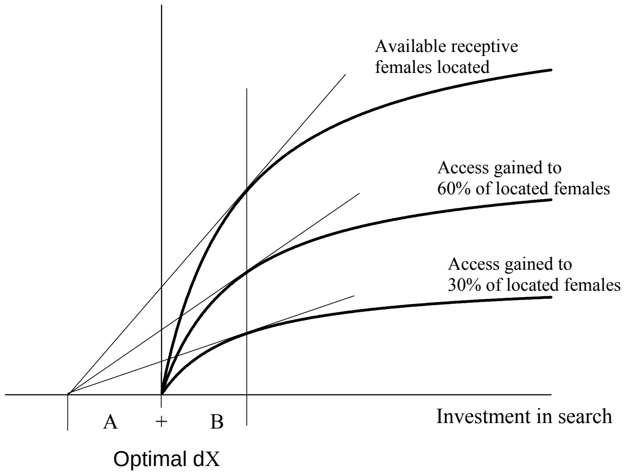


Figure 7.3: Theoretical relation between investment and success of locating and gaining access to oestrus females. A = basic metabolic costs B= "Visible" costs associated with search such as walking and decreased foraging. The optimal investment in search (vertical line) is not affected by a reduced probability of gaining access to the located female.

However musth is likely associated with an increased basic cost due to the increased androgen levels, maintenance of urine dribbling etc. which is also incurred during the non-competitive search phase. Such a larger baseline cost will increase the point of optimal investment (Figure 7.4). Hence musth bulls are expected to have a larger time specific investment compared to SAN bulls.

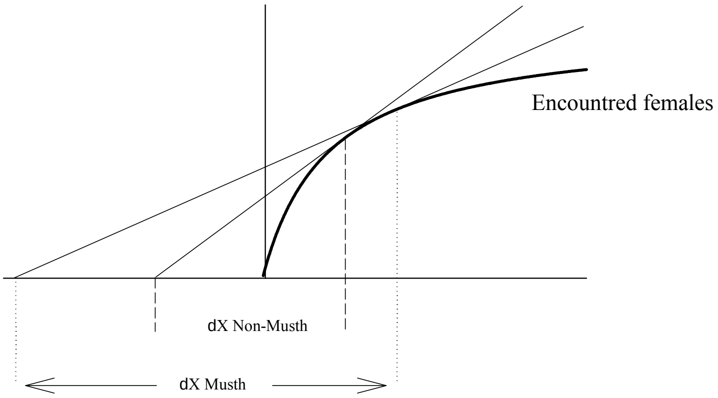


Figure 7.4 If a larger basic cost is incurred by musth bulls the optimal time specific investment in searching should be higher during musth compared to non-must

Whether an individual bull should choose i) a competitive musth tactic with high time specific investment or ii) a non-competitive SAN tactic with low time specific investment will depend on how much a shift of tactic will affect the access success to a female once located relative to the change in investment level. In addition there are potential adverse effects on future success linked to increased probability of injuries if a competitive tactic is adopted. Younger males with low fighting ability are not likely to obtain a large increase in success (Figure 7.5 musth payoff A) resulting in the SAN tactic having a higher payoff per unit investment,

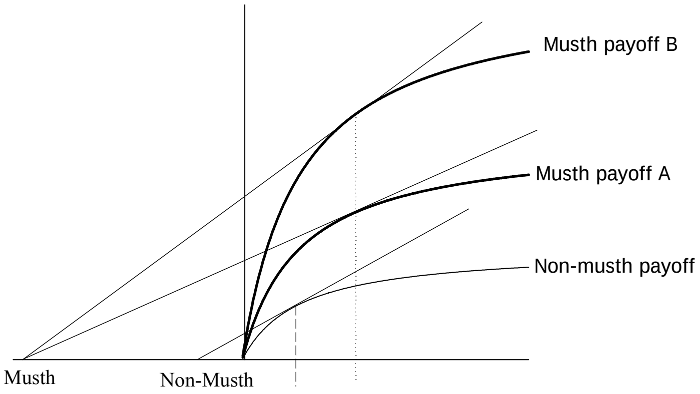


Figure 7.5: In addition to the effect on future reproduction the choice of tactic will depend on how much the probability of successful access to a located female can be increased by shifting to a musth tactic. An individual bull should choose non-musth if it can only increase it's payoff to level A by shifting to musth, but choose musth if ableto shift to level B.

whereas older individual with higher competitive ability will increase the payoff per unit investment by a musth tactic (Figure 7.5 musth payoff B). Since the time specific investment is expected to be lower in SAN bulls the total duration of reproductively active periods may be longer. However the optimal amount of resources allocated to current reproduction is also expected to be lower in younger bulls (Whitehead, 1994) making it difficult to predict whether the resources allocated to present reproduction are used up faster in bulls engaging in the musth or SAN tactic. In the following sections the level of investment in reproduction is compared between the two tactics and the effects of tactic and age on the duration of sexually active periods are investigated.

7.2 Methods

The time specific level of investment in reproductive activities in the two reproductive tactics, musth and SAN was investigated using proxy measures of time spend foraging and walking as well as daily walking distances relative to the levels of observed during sexually inactive periods. Furthermore the proportion of time spent sexually active was analysed in relation to age and reproductive tactic to evaluate if changes in tactics affects the amount of time spent active.

7.2.2 Daily activity patterns

The daily allocation of time to various activities in the three sexual states was investigated based on 380 hours of focal observations on 23 different bulls carried out between 21st of January and 4th of July 2000. Based on a combination of the results from the HMM (Chapter 5) and the presence or absence of urine dribbling (Chapter 6) the bulls were categorised into three states, one sexually inactive state (SI) and two sexual active states, (SAN and musth). On observation days, a bull was located and followed for 4-8 hours depending on when it was located. During focal observations ‘on the minute’ observations on activity was done every 15 min. Additional, the number of breeding females within an estimated 200m around the focal individual was recorded as well as herd shifts and the presence or absence of oestrus females, identified using behavioural signs described by C. Moss (Moss, 1983).

7.2.3 Daily Movements

Since 2000, 18 bulls have been tracked for various periods with GPS collars set to collect positions every hour on the hour (for collar type, periods and immobilisation procedures see chapter 2). These GPS tracking data were used to obtain information on daily movements and to compare changes in these between the three different sexual stats. For each individual the period with GPS tracking data was assigned as belonging to either sexually inactive (SI) or sexually active (SA) periods based on results from Chapter 5. SA periods were then subdivided into SAN and musth periods based on the presence or absence of TGS and UD. Brief (<1week) periods without UD and or TGS within longer musth periods or gradually disappearing signals towards the end of a sexually active period exclusively spent in musth were considered as being part of a musth periods. Similarly single observations of TGS, without UD within SAN periods without signals was not considered to constitute a change from SAN to musth. If gaps of more than 4 weeks existed in the associational observations the sexual state were considered unknown and the GPS data were not included in the analysis.

Distances between consecutive hourly positions were calculated in ArcView3.2 / ArcGIS 9.0. If any of the consecutive hourly positions were missing, distances between the available consecutive positions were calculated but these distances were excluded in any analysis of hourly distances. Daily walking distances were calculated as the 24-hour sum of the distances between positions. If more than 3 consecutive positions were missing or more than 6 position missing within any given 24-hour period the daily distance was not calculated as this would lead to an un-comparable underestimation of the distance covered. Daily displacements were calculated as the distance between consecutive 12:00 positions. If any of the 12:00 positions were missing this value was not obtained.

7.2.4 Duration of sexually active periods

The effects of bull age and reproductive tactic on the time spent sexually active was investigated from individual associational profiles with the reproductive states identified using the HMM approach (see chapter 5). In order to insure equal coverage of active and inactive periods of the individual bull, only full year profiles were included. The date of transition between states was taken as the mid point between the last observation assigned to one state and the first observation assigned to another state. Only full years where the date of state transition could be assigned within +/- 2 weeks (observation gab< 4 weeks) were used. Duration of musth and SAN periods within sexually active periods was based on the presence/absence of urine dribbling.

7.3 Results

7.3.2 Daily activity patterns

The 380 hours of focal observations on the 23 different bulls were distributed with 180 hours on 18 SI bulls (sexually inactive), 100 hours on 10 musth bulls and 100 hours on 7 SAN bulls. The daily activity budgets for the three different sexual states are shown in Figure 7.6.

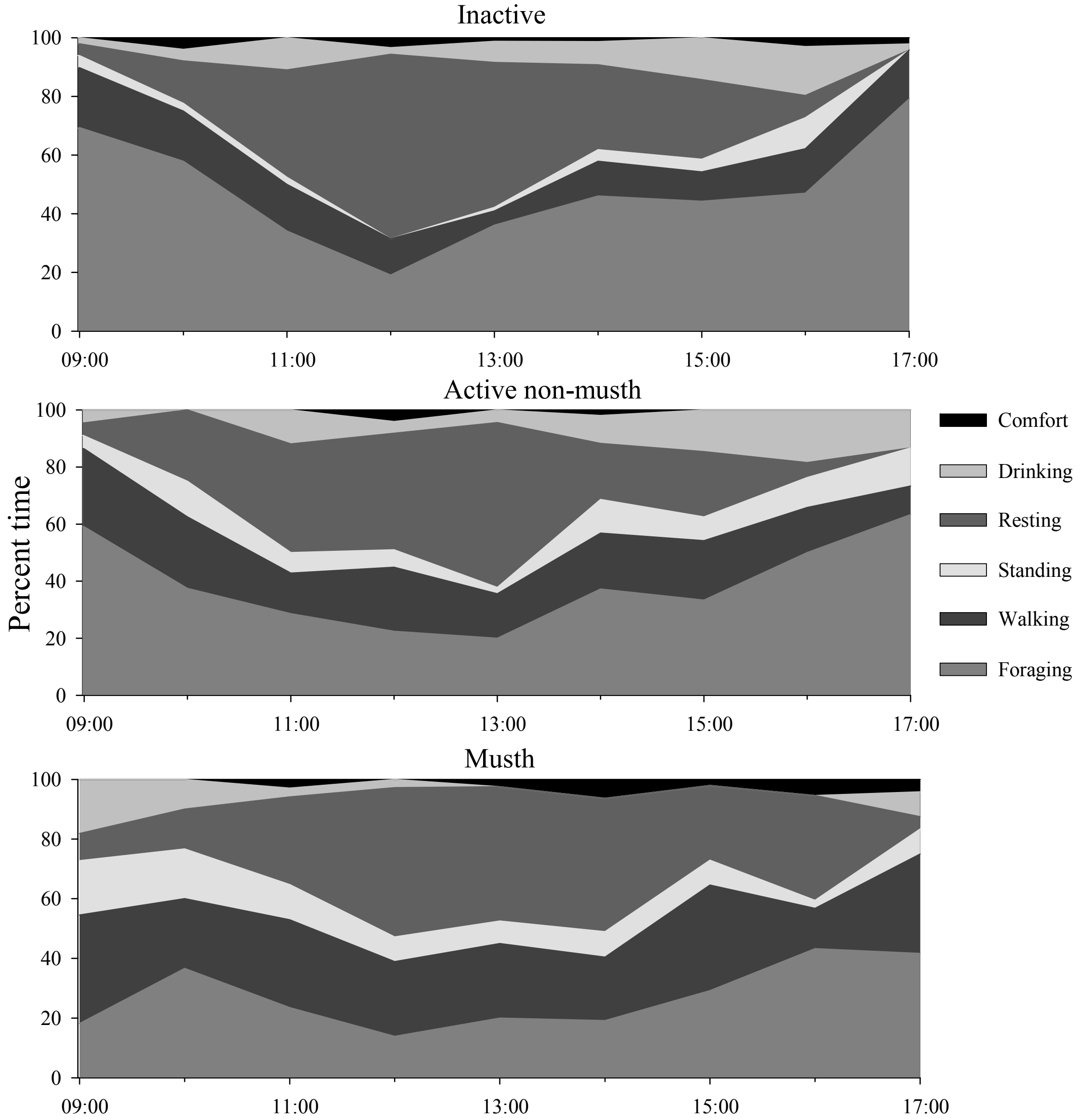


Figure 7.6 Daily allocation of time to the seven general activities by bulls identified as sexually inactive, sexually active non-musth and musth periods (Observation N inactive= 720; active non-musth= 400; musth= 400).

The allocation of time to the different activities was dependent on the time of day but the pattern did not change between the sexual states. Morning and afternoon were predominantly spent foraging or walking and midday spent on resting with comfort and drinking often occurring just before and or after the midday resting period. The four main activities, Standing, Walking, Resting and Foraging are shown separately in figure 7.7.

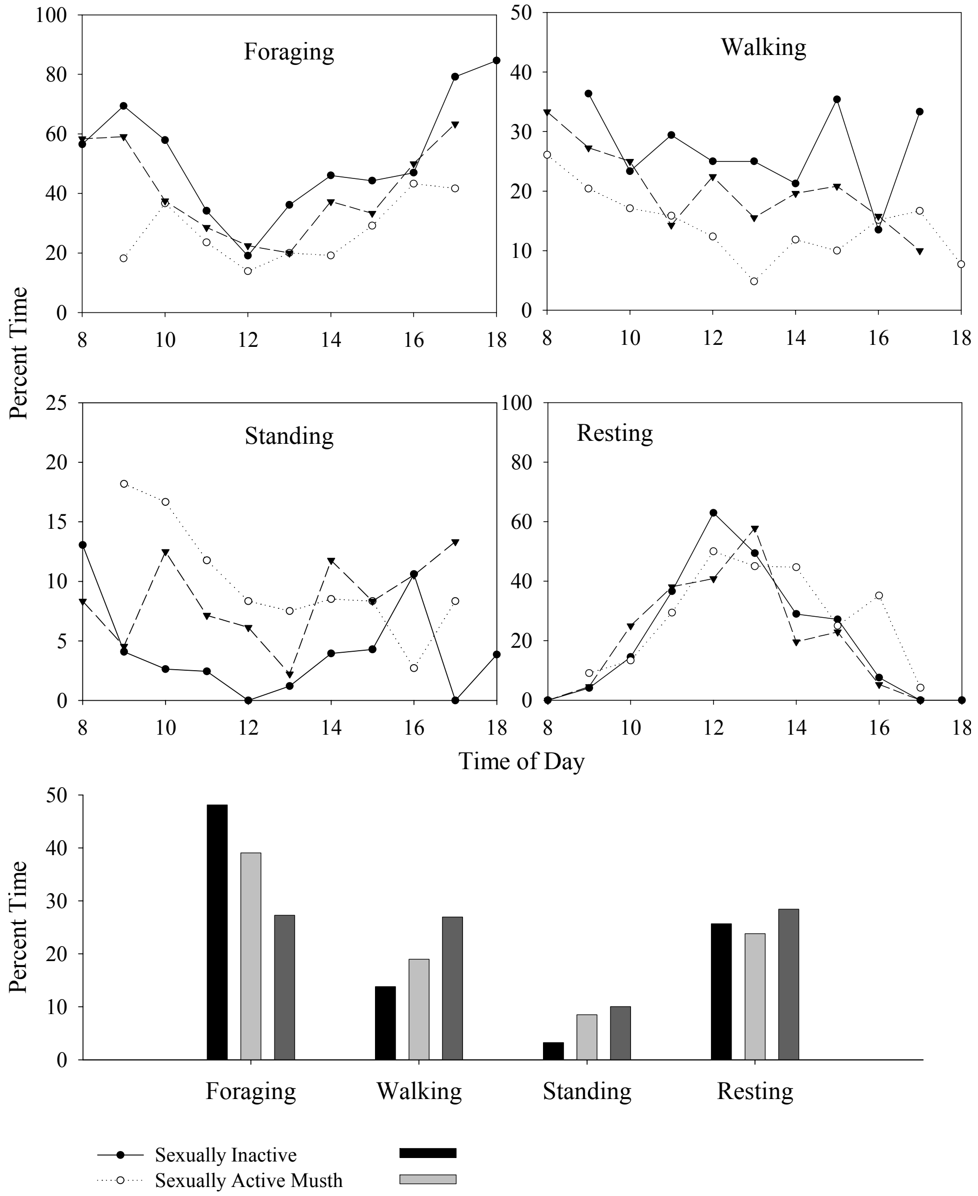


Figure 7.7 A: Daily distribution of the four the main activities recorded (foraging, walking standing and resting) during sexually inactive, sexually active non-musth and musth periods. B: Average daily (08:00-18:00) time allocation to the four main activities

Table 7.1: Proportion of time allocated to the 4 main activities in the three sexual states.

Activity	SI	SAN	Musth	d.f.	ANOVA P
Foraging	0.48	0.39	0.27	2	= 0.023
Walking	0.14	0.19	0.27	2	= 0.003
Standing	0.03	0.09	0.10	2	= 0.010
Resting	0.26	0.24	0.28	2	= 0.94

A significant overall effect of sexual state existed on time spent walking standing and foraging but not on resting (Table 7.1). SAN bulls foraged less and were standing more compared to SI bulls but foraged more than musth bulls (Table 7.2). Thus, SAN bulls were intermediate between musth and SI bulls.

Table 7.2: Daily allocation of time to the four main activities compared between the states using paired t-test of the mean hourly time allocations from 8:00 to 18:00.

State and activity	d.f	Paired t-test p	State and activity	d.f	Paired t-test p
Foraging			**Standing**		
SI vs. SAN	9	0.02	SI vs. SAN	9	0.03
SI vs. Musth	9	0.004	SI vs. Musth	9	0.02
SAN vs. Musth	8	0.03	SAN vs. Musth	8	0.51
Walking			**Resting**		
SI vs. SAN	9	0.02	SI vs. SAN	9	0.57
SI vs. Musth	9	0.001	SI vs. Musth	9	0.52
SAN vs. Musth	8	0.03	SAN vs. Musth	8	0.38

7.3.3 Herd shifts and female encounter rate

The frequency of herd shifts was calculated as the number of times a bull left or joined a group of females divided by the observation period. Observation periods, where the focal bull guarded and/or followed an oestrus female were excluded from the analysis of both herd shifts; female encounter rate and time spent in association with females, since bulls are expected to cease searching for other receptive females during the period they follow one.

The frequency of herd shifts was not significantly different between SAN and musth bulls, with SAN and musth bulls leaving or joining a group of females at a rate of 0.33 and 0.37 shifts per hour respectively (t test: DF= 24; t=0.44; P=0.66). The female encounter rate was calculated as the total number of different breeding females observed less than 200 meters from the focal bull during an observation period divided by the observation period. The number of different females encountered per hour was not significantly different with SAN bulls and musth bulls encountering on average 2.3 and 1.7 females per hour (t-test: DF=24; t = - 1.27; P=0.22). However, SAN bulls spent more time in association with females (69.5%) than musth bulls (46.4%) (t-test: DF= 24; t= - 2.24 P=0.035).

7.3.4 Daily and movements

Of the 18 GPS tracked individuals five were not used for analysis due to i) short and poor performance of the collar ii) range shift to very different forest habitat near Mt Kenya or iii) lack of auxiliary associational data to determine sexual state. The remaining 13 individuals had tracking data from periods where the sexual state was known based on associational data. Of those, three only had data from one sexual state with the remaining 10 having data from two or three states. The median daily distance travelled and median daily displacements during different sexual states are shown for each individual in table 7.2

Table 7.2 Median daily walking distance and daily displacements during SI, SAN and musth periods for 13 individually tracked bulls. Number of hourly positions used for analysis (sexual state known), with the total number of recorded positions in (). N.O = State not observed in this individual during study period. N.A. = Not Available, State observed but no tracking data available from these periods. Pair wise comparison Mann Whitney U test.

ID and Age (2002)	# Pos. used for analysis. Total rec. in ( )	Daily median distance (km) N days in ( )				Daily median displacement N days in ( )			
		SI (a)	**SAN (b)**	**Musth (c)**	**Pairwise p**	**SI (d)**	**SAN (e)**	**Musth (f)**	**Signif.**
B1001 48y Mungu	12054 (12054)	6.45 (437)	N.O.	13.92 (64)	a,c<0.00001	1.59 (432)	N.O.	2.89 (64)	a,c<0.00001
B1009 44y P.BomBom	11519 (11519)	6.49 (339)	N.O.	12.13 (138)	a,c<0.00001	1.98 (332)	N.O.	2.19 (137)	a,c=0.41
B1023 42y Kenyatta	5585 (7913)	7.60 (146)	N.A.	17.61 (60)	a,c<0.00001	2.01 (137)	N.A.	5.82 (42)	a,c<0.00001
B1018 39y Boone	1468 (1468)	9.32 (18)	N.O.	9.21 (41)	a,c=0.59	0.34 (19)	N.O.	0.14 (41)	a,c=0.09
B1011 38y Mandela	1706 (1706)	7.89 (71)	N.O.	N.A.		2.3 (68)	N.O.	N.A.	
B1027 38y Esidai	14264 (17578)	4.98 (35)	9.13 (120)	12.23 (120)	a,b<0.00001 a,c<0.00001 b,c<0.00004	1.35 (336)	2.02 (119)	3.05 (120)	a,b=0.0061 a,c<0.00001 b,c=0.015
B1016 37y MLK	3937 (3937)	7.09 (35)	N.O.	10.48 (106)	a,c<0.00001	2.00 (35)	N.O.	3.79 (97)	a,c=0.004
B1033 37y Apollo	15497 (15497)	11.75 (392)	14.24 (91)	15.37 (168)	a,b=0.00003 a,c<0.00001 b,c=0.12	2.88 (384)	2.87 (92)	4.02 (156)	a,b=0.75 a,c=0.007 b,c=0.014
B1026 31y Winston	8641 (18306)	7.21 (306)	13.20 (138)	16.94 (24)	a,b<0.00001 a,c<0.00001 b,c=0.013	1.64 (285)	2.73 (131)	1.63 (24)	a,b<0.00001 a,c=0.95 b,c=0.0022
B1044 26y Colombus	3213 (3213)	N.A.	9.86 (136)	N.O.		N.A.	0.34 (135)	N.O.	
B1039 25y Ansel	8641 (14021)	5.61 (87)	8.85 (265)	N.O.	a,b<0.00001	1.63 (93)	3.26 (261)	N.O.	a,b=0.02
B1045 25y Thouraux	1430 (4365)	N.A.	10.95 (57)	N.A.		N.A.	2.19 (34)	N.A.	
B1122 21y Uffe	2852 (2852)	8.43 (78)	11.99 (20)	N.O.	a,b=0.032	0.25 (81)	0.52 (21)	N.O.	a,b=0.14
Overall Median		7.31	10.95	13.11	a,b=0.0024 a,c=0.0007 b,c=0.13	1.76	2.30	3.33	a,b=0.19 a,c=0.038 b,c=0.25

Four of the five bulls exclusively observed in musth during their SA periods covered significantly longer daily distances during musth periods compared to SI periods (with the bull not increasing movements relying on a short 18 day pre active period for data on SI movements) and three had a significant increase in daily displacement distance. The five bulls observed in SAN all had a significant increase in daily distance during SAN periods with three having significant increase in daily displacement. Three of the five bulls observed in SAN were also observed in musth. Of those three all had an additional increase in daily distances, during musth although only significant in two. Two of the three had additional increase in daily displacements. Examples of the longitudinal time profiles of daily travelling distance, sexually active periods and occurrence of must signals are shown for 6 bulls in figure 7.8-7.13.

Box 7.1

Legend for Figure 7.8-7.13

A: Daily walking distance and occurrence of musth signals Grey shading indicate sexually active periods (assigned based on HMM analysis of associations).
B: Median hourly distances travelled during different reproductive states
C: Median daily walking distance during sexual states
D: Median daily displacement (distance between 12:00 positions on consecutive days) during sexual states

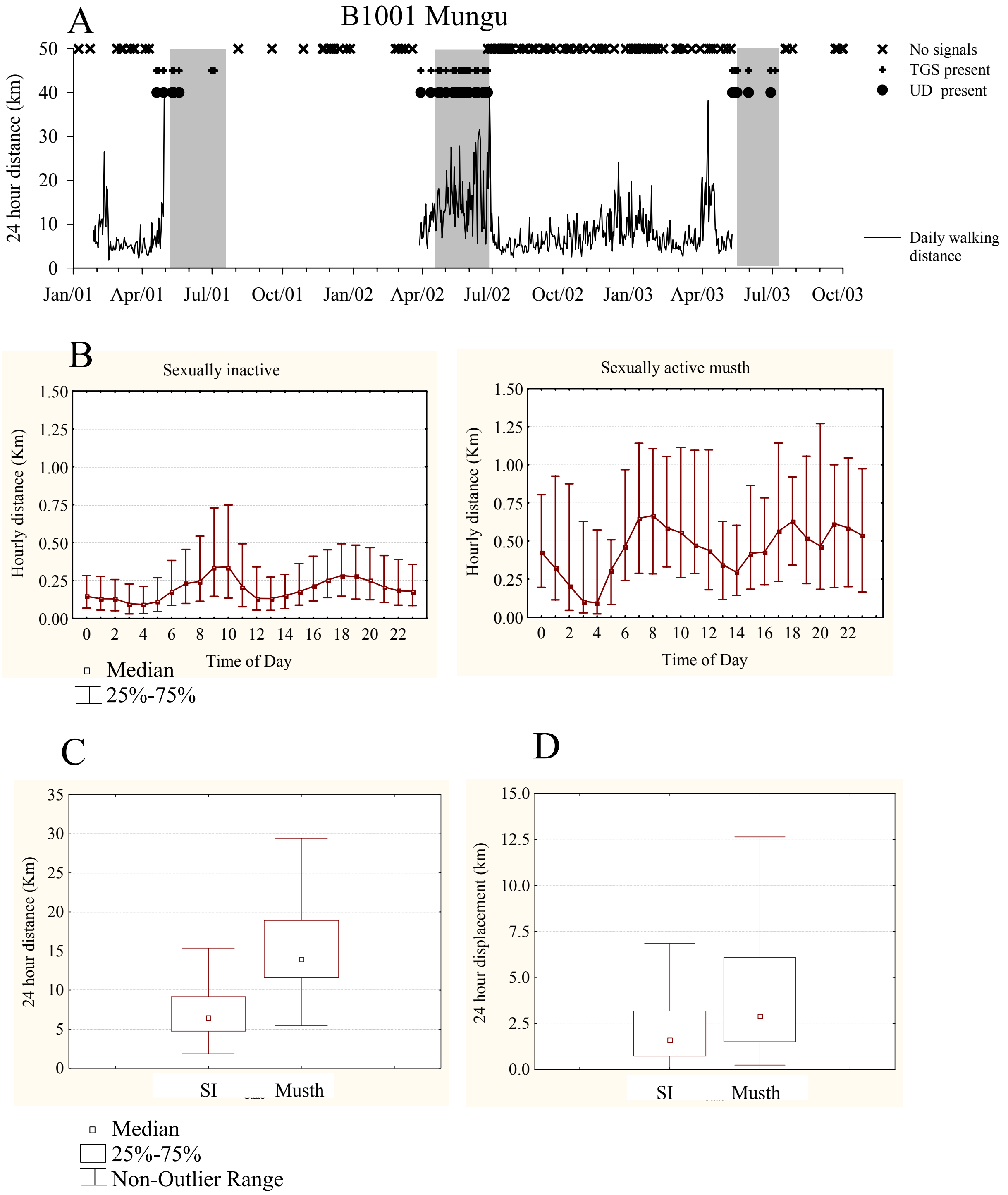


Figure 7.8: B1001-Mungu est. born 1954. (Legend see box 7.1 page 139)

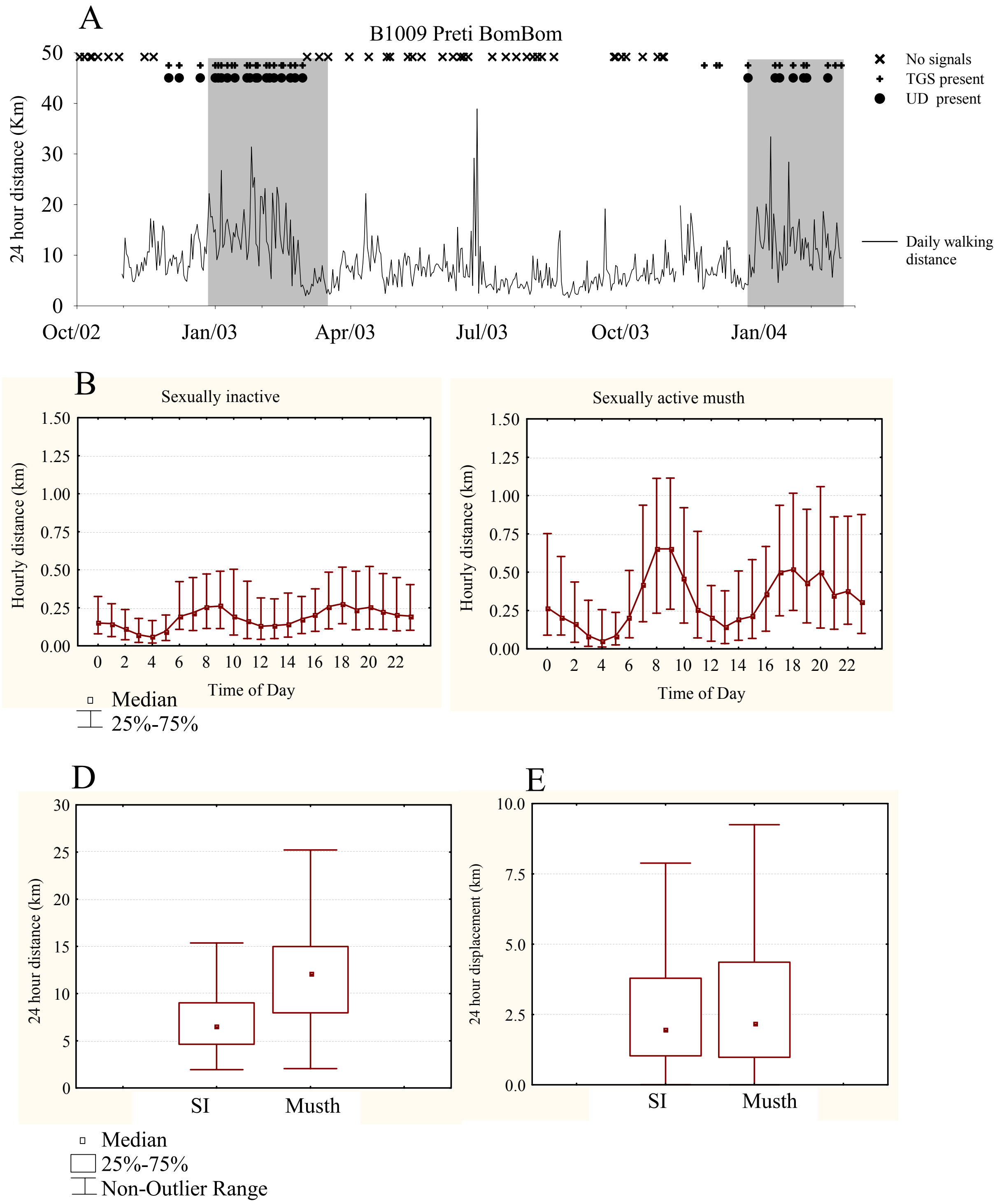


Figure 7.9: B1009-PretiBomBom est. born 1958

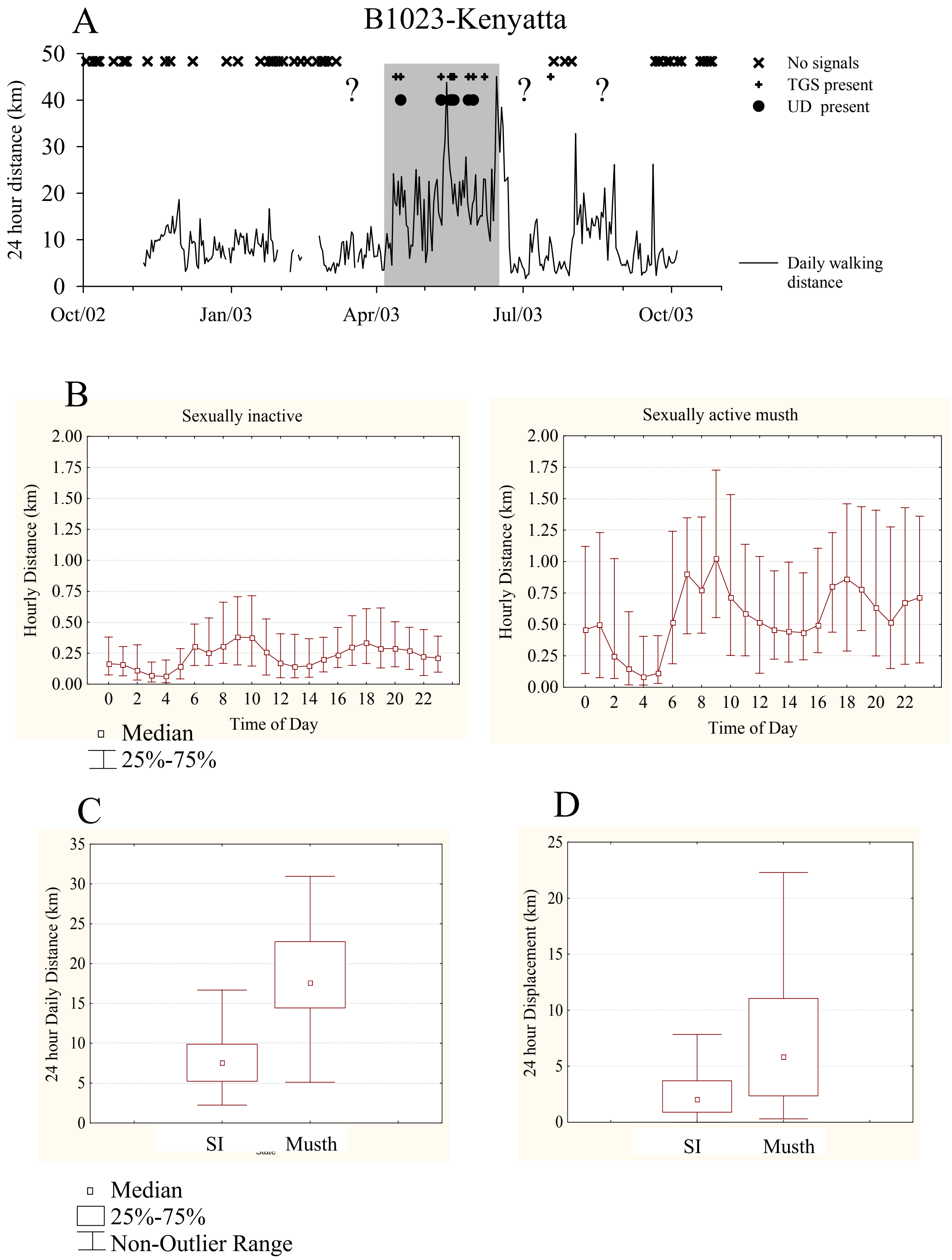


Figure 7.10: A: B1023-Kenyatta est. born 1960.

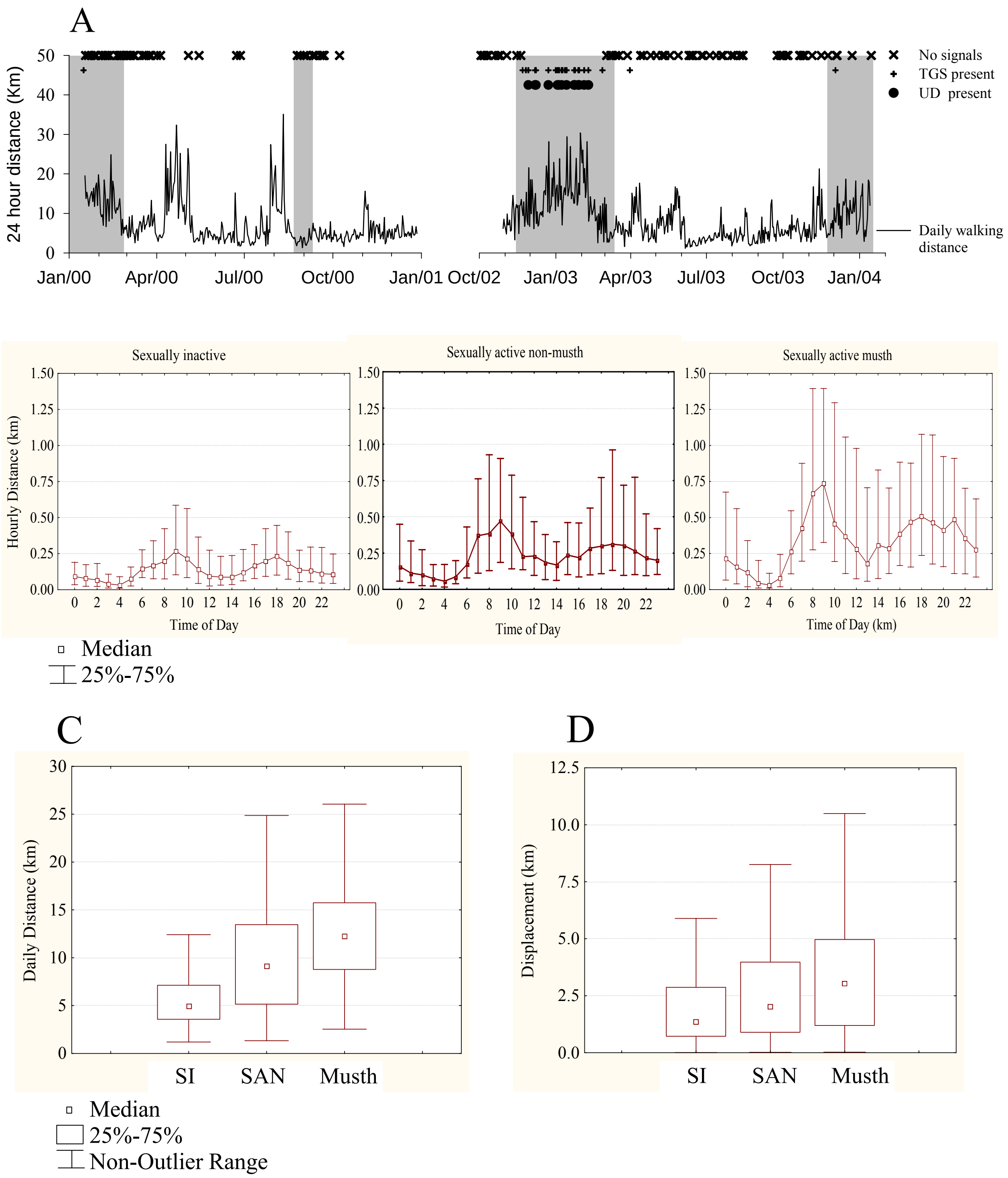


Figure 7.11: B1027-Esidai est. born 1964.

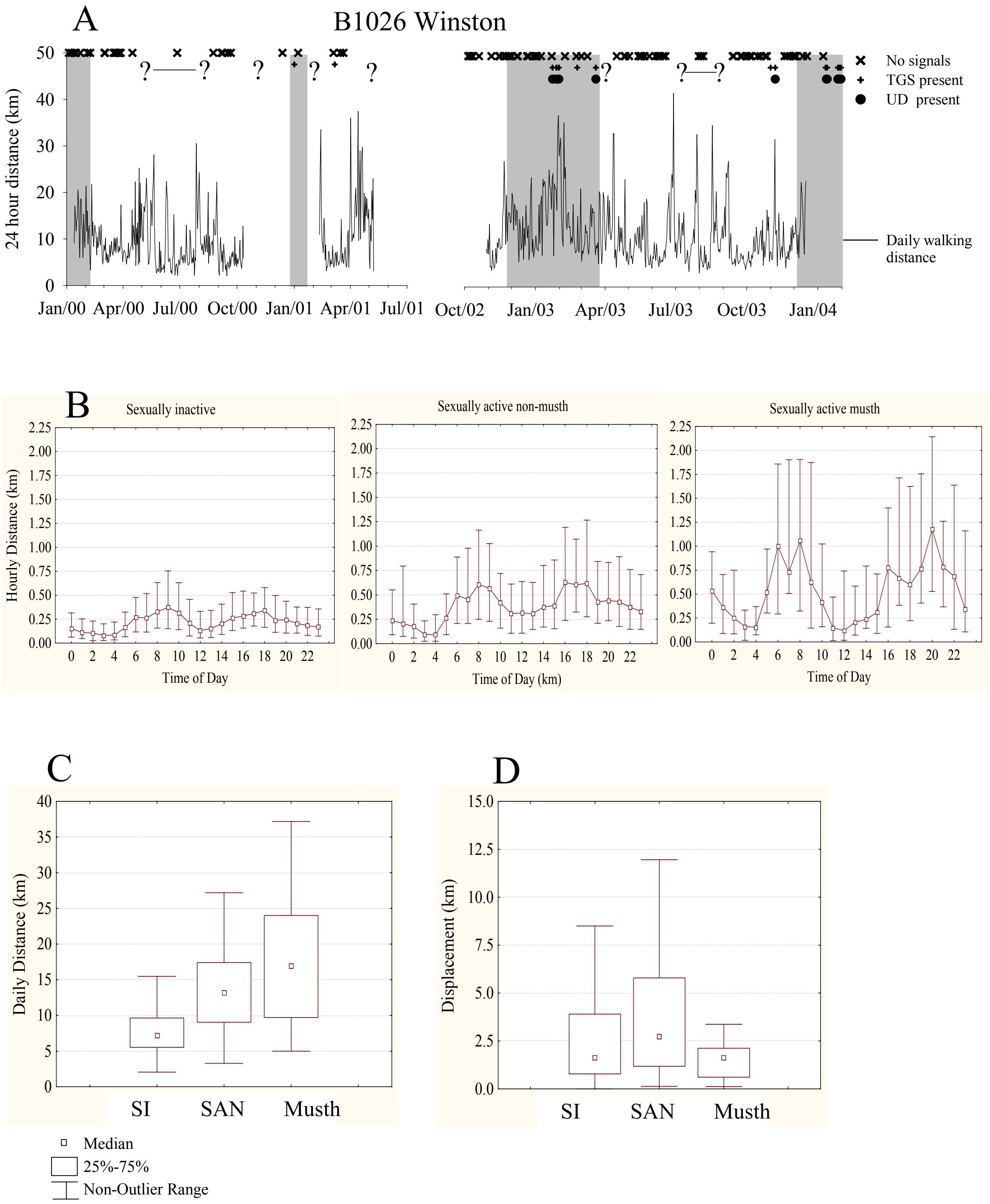


Figure 7.12: B1026-Winston est. born 1971.

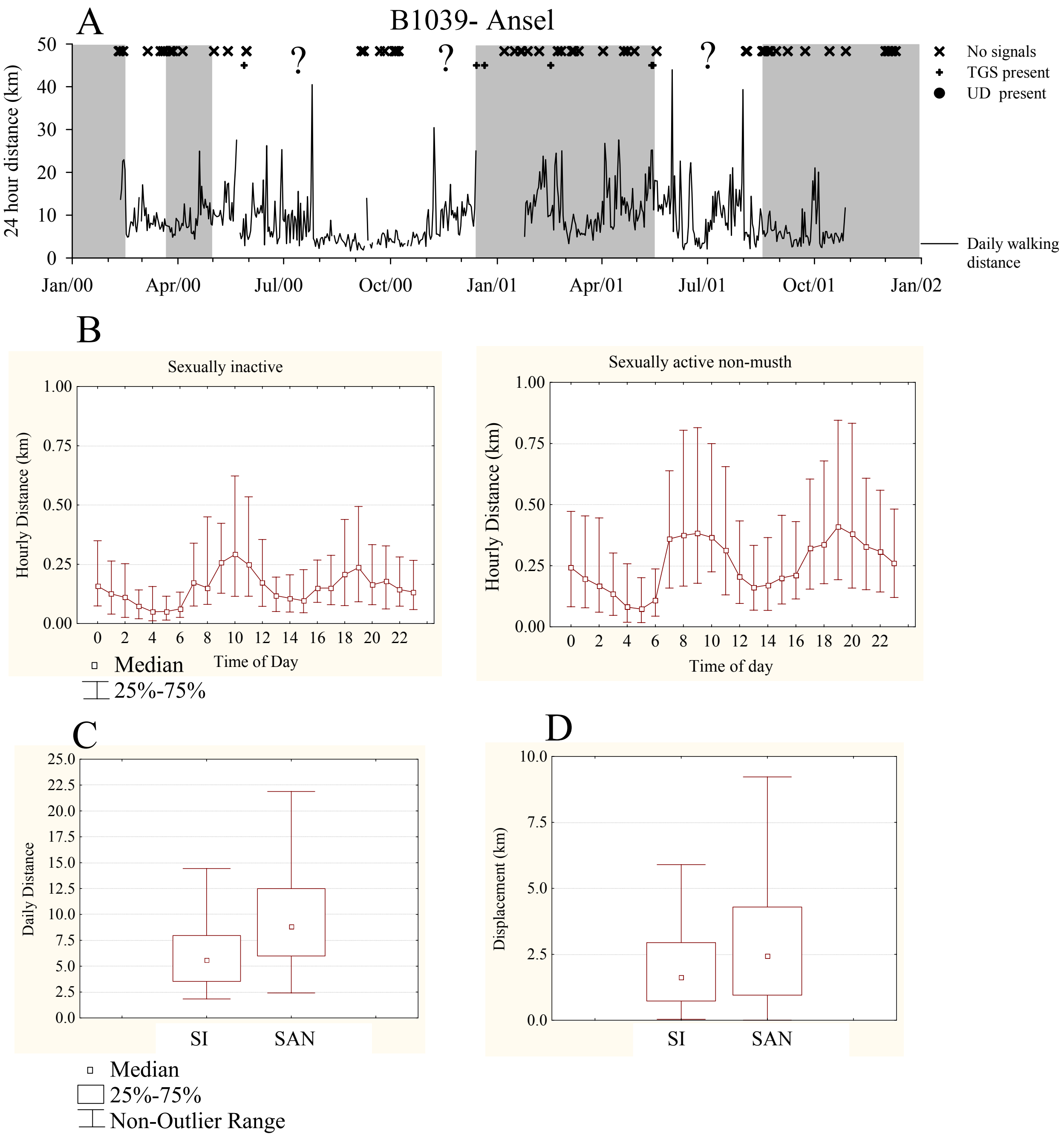


Figure 7.13: B1039-Ansel est. born 1977.

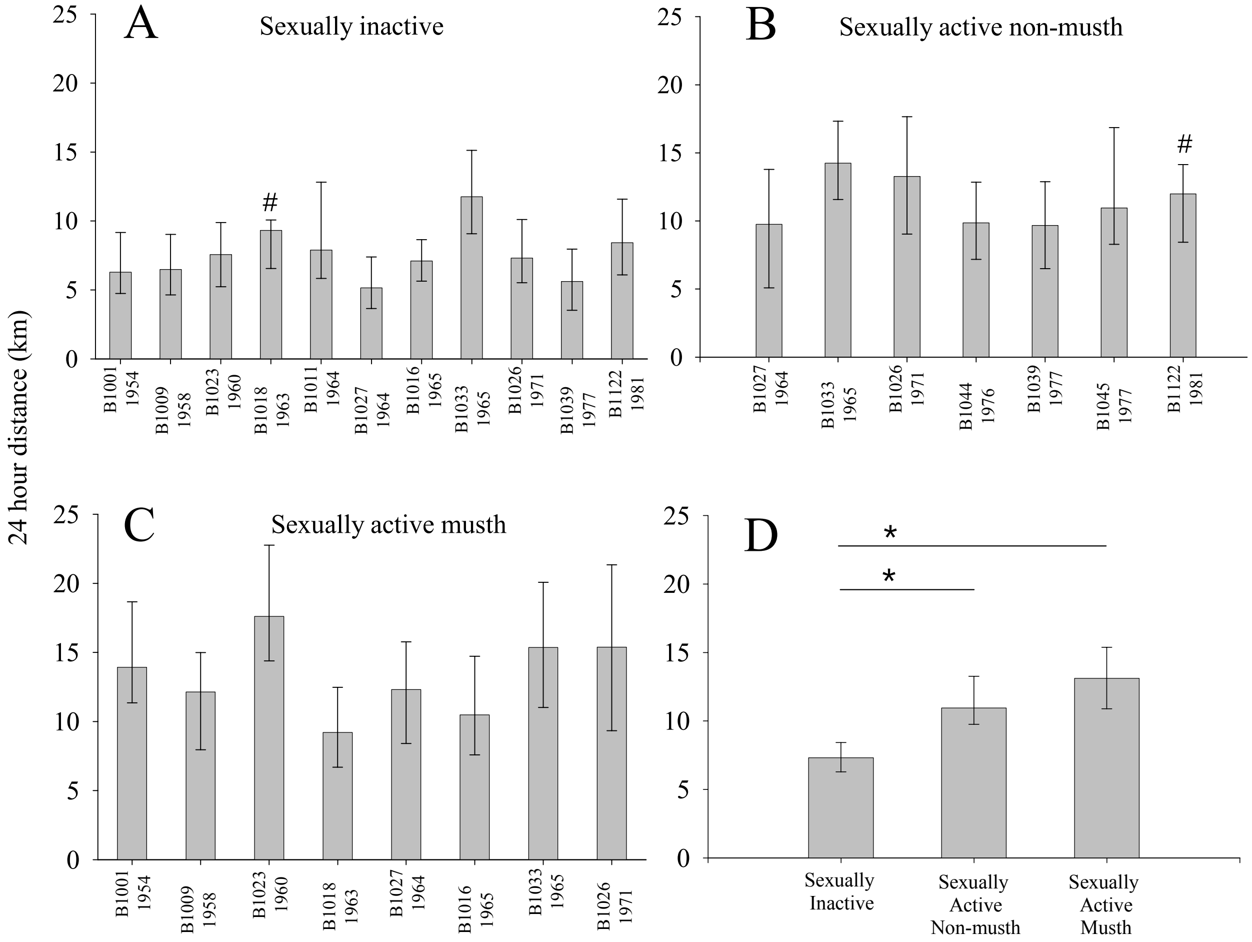


Figure 7.14: A-C: Median distance walked per day for individual bulls during SI, SAN and musth periods (# = sample size less than 30 day), error bar = 25%-75%. D: Overall median daily distances of all bulls, marked differences pair wise significantly different (Mann-Whitney U-test).

Table 7.3 Comparison of walking distance and displacement between individuals and states.

Daily walking distance	States	Median km (Range)	d.f	Test	P
Difference between individuals	SI	7.31 (4.98-9.32)	10; 2433	Kruskal Wallis, 564.39	<0.0001
	SAN	10.95 (8.85-14.24)	6; 793	Kruskal Wallis 96.15	<0.0001
	Musth	13.11 (9.21-17.61)	7; 738	Kruskal Wallis 94.32	<0.0001
Difference between states	Overall		2; 26	Kruskal Wallis 15.88	<0.0004
	SI vs. SAN		11;7	MW-U-test Z=-3.03	<0.001
	SI vs. Musth		11;8	MW-U-test Z=-3.38	<0.001
	SAN vs. Musth		7; 8	MW-U-test Z=-1.50	=0.15

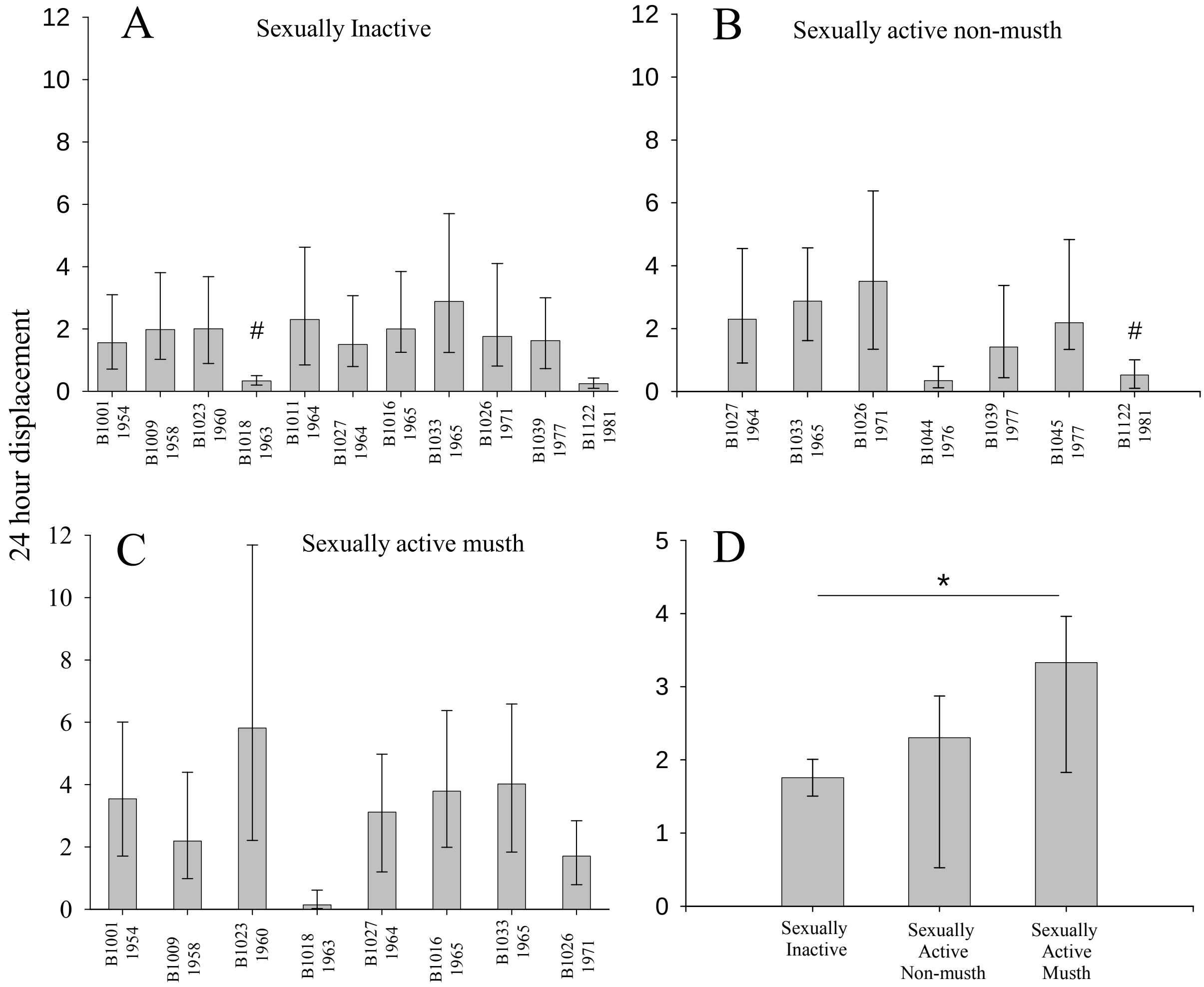


Figure 7.15: A-C: Median distances between subsequent 12:00 positions (Displacement) for individual bulls during SI, SAN and musth periods (# = sample size less than 30 day), error bar = 25%-75%. D: Overall median displacement distances of all bulls, marked differences pair wise significantly different (Mann-Whitney U-test).

Table 7.4 Comparison of displacement between individuals and states.

Daily Displacement	States	Median km (Range)	d.f	Test	Kruskall-Wallis P
Difference between individuals	SI periods	1.76 (0.34-2.88)	10; 2374	Kruskal Wallis, H=251.72	<0.001
	SAN periods	2.30 (0.34-2.87)	6; 759	Kruskal Wallis, H=196.38	<0.001
	Musth periods	3.33 (0.14-5.82)	7; 698	Kruskal Wallis, H=141.67	<0.001
Difference between states	Overall		2; 26	Kruskal Wallis, H=5.09	=0.078
	SI vs. SAN		11;7	M-Whitney-U-test Z=-1.31	=0.19
	SI vs. Musth		11;8	M-Whitney-U-test Z=-2.06	=0.04
	SAN vs. Musth		7; 8	M-Whitney-U-test Z=-1.15	=0.24

The daily walking distances and displacements were significantly different between individual bulls both during SI, SAN and Musth (Table 7.3&4, Figure 7.14 A-C & Figure 7.15A-C). This variation was unlinked to the age of the individual for both walking distance and displacement in all states (Daily distance: Spearman rank N=7 - 11, R=-0.17-0.13, p>0.60, Daily Displacement: Spearman rank N=7 - 11, R=-0.04 - 0.13, p>0.70). Overall bulls walked longer per day and displaced more between days during SAN and musth periods compared to SI periods with SAN bulls being intermediate between SI and Musth (Table 7.4, Figure 7.14 D & 7.15 D)

7.3.5 Duration of reproductive periods

Longitudinal time sequences where the transitions between active and inactive periods were known within +/- 2 weeks were available from 23 bulls (20 - 46 year of age). These profiles with durations of 1, 2 or 3 year were used to assess the duration of sexually active periods and the proportion of sexually active periods spent in SAN and musth. Since subsequent years from individual bulls cannot be assumed to be independent, the proportion of time spent active and time spent in different states (tactics) was calculated on the basis of the full profile (1-3 years). In case of 2 or 3-year periods the estimated age of the bull at the middle of the period was used. The proportion of time spent sexually active (combined SAN and musth) was negatively correlated to the fraction of active time spent in musth (Spearman Rank N=28, R=-0.78, P<0.00001). Bulls exclusively in SAN spend on average 55% of their time active compared to bulls exclusively in musth spending 25% of their time active. The proportion of time spent sexually active was negatively correlated with age (Spearman Rank N=28, R=-0.83, P<0.00001) whereas the proportion of time spent in musth was positively correlated with age (Spearman Rank N=28, R= 0.85, P<0.0001) (figure 7.16)

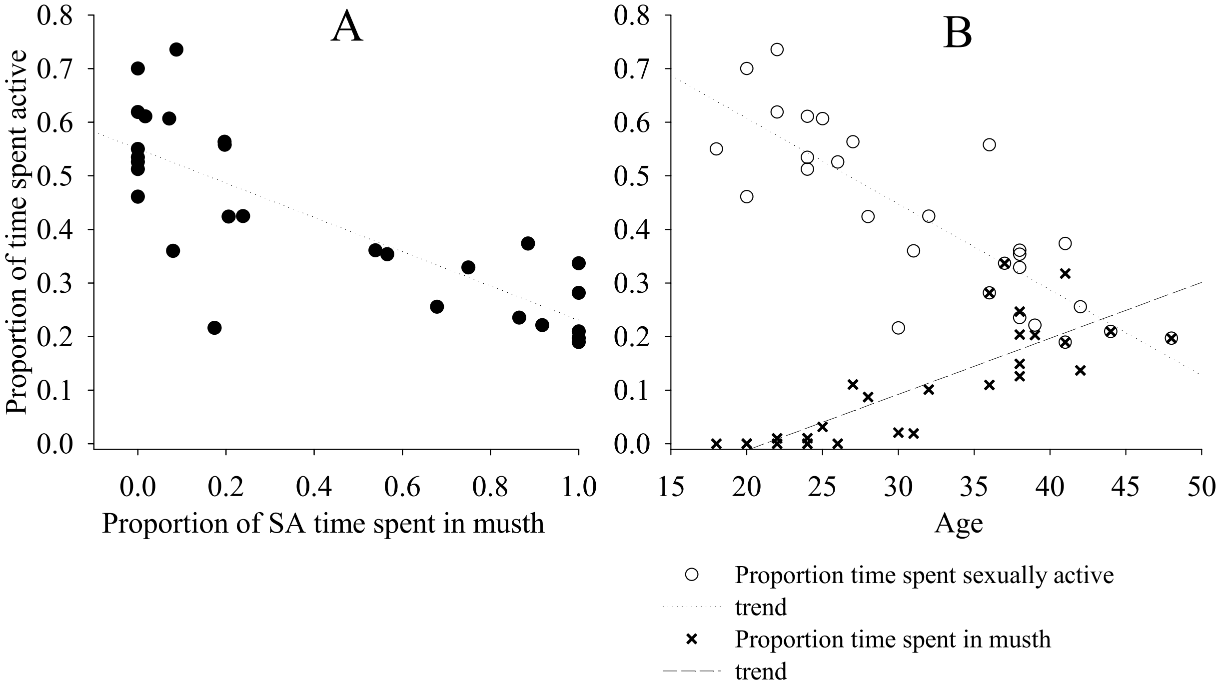


Figure 7.16 A: Proportion of time spent sexually active versus the proportion of sexually active time spent in musth (Spearman Rank N=28, R=-0.78, P<0.00001). B Proportion of time spent active (o) versus age of individual (Spearman Rank N=28, R=-0.83, P<0.00001) and proportion of time spent in musth (x) versus age of individual (Spearman Rank N=28, R= 0.85, P<0.00001).

The proportion of SA time spent in musth appeared to be non-linearly related to age with a relatively rapid transition from predominantly SAN to predominantly musth occurring around the age of 35 years (figure 7.17).

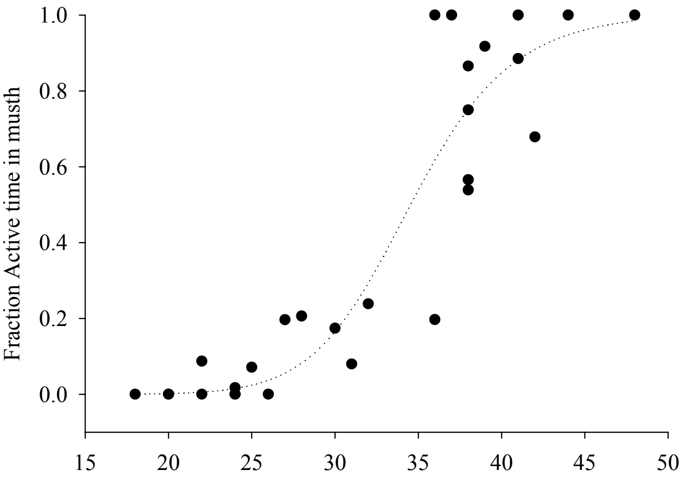


Figure 7.17: Fraction of sexually active time spent in musth versus age of individual, sigmoid curve fitted as trend line

7.4 Discussion

The reduction in foraging and increase in walking seen in bulls in musth is comparable to that reported from males in the Amboseli population (Poole, 1987). The intermediate reduction in forage time and increase in walking and standing seen in sexually active non-musth bulls is in accordance with predictions that this tactic should have a lower time specific investment compared to musth.

For the first time, high resolution GPS tracking made it possible to analyse actual daily distances covered by bulls. During SI periods, all bulls showed a consistent very similar daily pattern walking very short distances between 2:00-5:00, a period used for sleeping and often associated with lying down (Fritz Vollrath personal comments, personal observations, (Douglas-Hamilton, 1998) and a second period of reduced hourly distances between 11:00 and 14:00, associated with the mid day resting period (figure 7.6 & 7.7). The morning period between 5:00-11:00 and afternoon between 14:00- 20:00 was associated with larger distances covered per hour (around 250m/hour) and occurred during the periods predominantly used for foraging (Figure7.6&7.7). After 21:00 the distances covered gradually decreased until the early hours of the morning.

Active search for oestrus females is expected to result in i) increase in shifts between areas utilised ii) increase in the daily distances covered with the daily distances linked to search intensity. During both SAN and musth periods similar short distances were covered during the early morning probably associated with a similar sleep period as seen in SI individuals. The general daily pattern was identical between SI and SA periods. However SA periods were associated with a general increase in distances covered, especially during the morning and afternoon period. SAN bulls were intermediate between SI and musth, which was also reflected in the overall daily distances.

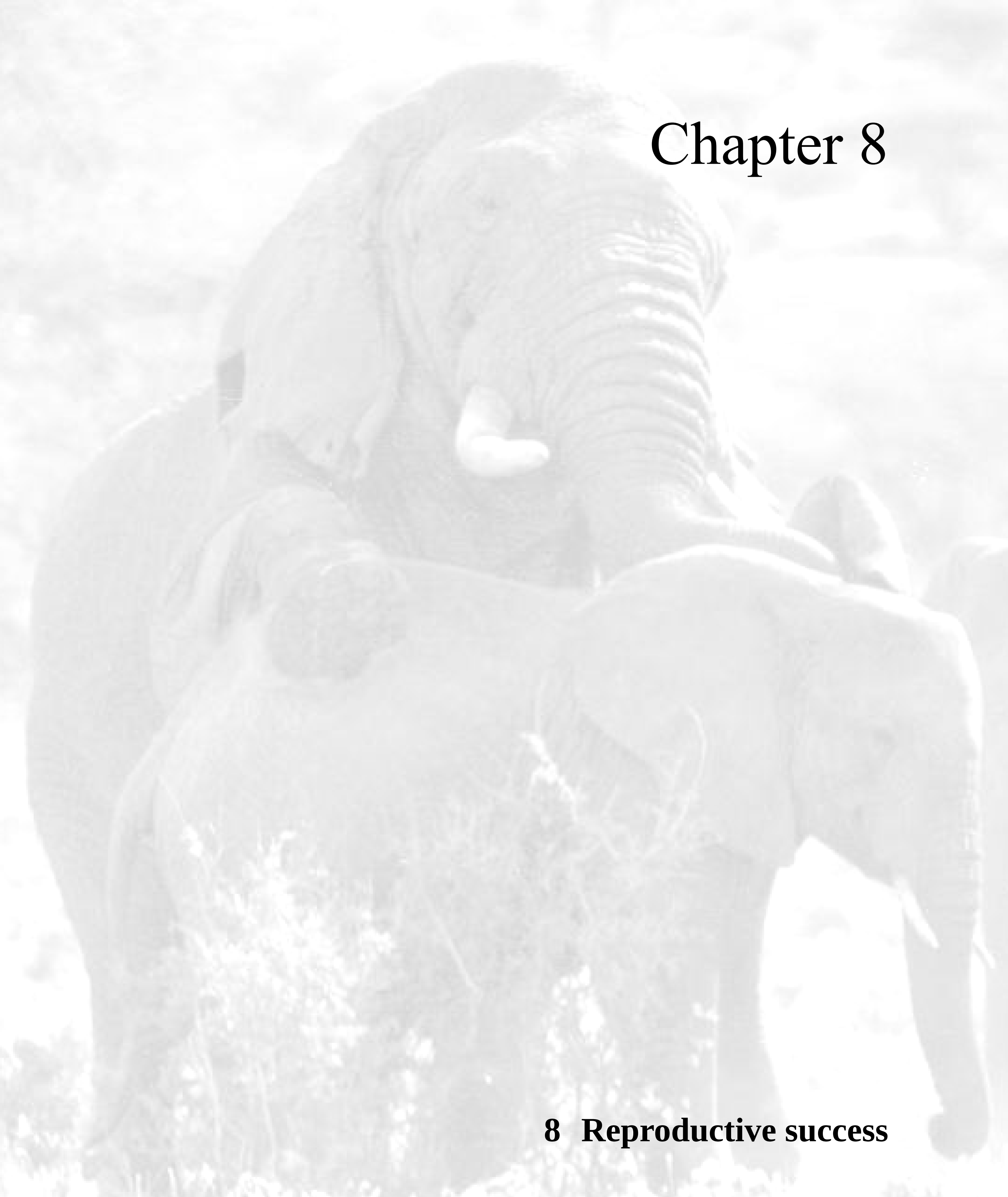
These results show as predicted that SA bulls shift area and walk longer than SI bulls. Overall SAN bulls tended to increase daily walking distances by 50%, whereas musth bulls doubled their daily distances. The walking distance varied significantly between individuals in the same state, however this variation was not linked to the age of the individual and pair wise analysis of different states in individual bulls observed in all three states showed intermediate level of daily walking distance during SAN periods. This indicates that the level of daily movements is linked to reproductive tactic rather than age and hence further support the intermediate level of investment seen in SAN bulls.

The increase in occurrence and duration of musth periods with age found in this study is in accordance with the observations made by Poole (Poole, 1989a; Poole & Moss, 1981; Poole, 1987). However, analysing time spent in both sexual tactics revealed that this increase in time spent in musth was associated with a decrease in total time spent sexual active with younger bulls exclusively in SAN spending approximately 55% of the year active compared to older bulls exclusively in musth spending only around 25% of their time active. Hence the lower level of time-specific investment seen in SAN bulls seems (at least partly) to be used to extend the duration of SA periods. This suggests that the lower time-specific level of investment is a result of optimising short-term reproductive success rather than a result of a long-term optimisation of the trade off between current and future reproduction.

Whether overall younger bulls invest less in reproduction (i.e. invest in expected future success by reducing current investment) could not be analysed as the relative small size of the data set combined with the high correlation between the proportions of time spent in musth and the age prevented a combined multi factorial analysis of age, tactics and duration of active periods.

The proportion of sexually active time spent in SAN or musth appeared to be non-linearly related to the age of the individual rapidly chancing from predominately SAN in bulls below 30 years to predominately musth in bulls older than 40 years. As there was only data available on a few 30-35 year old individuals, the age of "intermediate" time spent in musth, it was difficult to assess whether predominately musth or SAN is separate clusters or a continuum with a rapid transition.

In conclusion the reproductive tactic of SAN, seen in younger bulls, is associated with lower but more prolonged investment compared to the musth tactic of older individuals. This emphasise the need for considering the duration of reproductive periods when comparing investments between tactics.



Chapter 8

8 Reproductive success

Submitted as: Musth and Reproductive Success in African elephants, H. B. Rasmussen; J. Okello; G. Wittemyer; H.Siegismund; P. Arctander; F.Vollrath; I. Douglas-Hamilton.

Abstract

The age and tactic specific reproductive success was studied based on nuclear DNA micro satellite markers. Due to the long gestation period and difficulties in sampling young calves the reproductive success of males could not be assessed for the period forming the database for analysis in the previous chapters. Therefore the analysis in this chapter relies on calves sired during the first years of the monitoring program between 1996 and 2001where some information on bull behaviour was available. Almost all of the sampled calves (79) with resolved paternity (46%) were sired by regularly occurring males and not by sampled irregular occurring males, suggesting that calves with un-resolved paternities were sired by males that have disappeared or died rather than from males from surrounding areas.

The youngest male to sire offspring was 21 years. However the age specific reproductive success increased late in life with old bulls (40+ year) siring approximately 4 times more offspring than 25-30 year old males. It was estimated that males less than 30 years of age combined contributed approximately 30% to reproduction with males above 35 years contributing 60%. Around 25% of the reproduction was estimated to arise from sexually active non-musth (SAN) bulls. A reduction in the age specific reproductive success occurred around the age of 32 years, the time of transition between SAN and regular occurring musth. This could reflect a transient cost associated with switching tactics.

8.1 Introduction

Since musth was recognised in the African elephant (Poole & Moss, 1981) research on male reproduction has mainly focused on the easily recognisable and conspicuous state of musth. Mate guarding is predominantly done by musth males and a large fraction of the successful mountings observed in a study of the Amboseli elephant population could be attributed to older males mainly in musth (Poole, 1987; Poole, 1989a; Poole, 1989c). This has lead to the assumption based on behaviour that males do not sire their first calf before the age of 30, and that musth bulls are responsible for almost all reproduction. However, the existence of distinct periods of strong association with females in younger non-musth males (Chapter 5) associated with changing androgen levels (Chapter 6) and reduced foraging and increased walking distances (Chapter 7) show that these males also have distinct periods focus on reproduction which seems to be associated with some level of investment. Whether these younger males obtain any reproductive payoff from this behaviour is currently unknown.

Genetic paternity analysis in other systems have revealed that proxy measure of reproductive success (such as number of matings, mate guarding and dominance) may not always be an accurate measure of reproductive success. Both under and overestimates have been reported in e.g. red deer (*Cervus elaphus*) (Pemberton et al., 1992) and grey seals (*Halichoerus grypus*) (Amos et al., 1993). In bighorn sheep (*Ovis canadensis*) (Coltman et al., 2002) a relatively large fraction of the offspring is sired by an alternative less competitive tactic.

To date there has been only one genetic paternity study on African elephants in the Addo National Park (Whitehouse & Harley, 2002). The relatively small population (320 individuals) was at the time of the study confined by fences to a small area (103km^2) (Whitehouse & Schoeman, 2003) and thus prevented from natural socio-spatial structure.

Such a situation with few females in a small confined area may well favour monopolisation of breeding by the dominant bull(s). Based on samples from 61 calves and all males above 25 years (N=13) this study could exclude the most dominant musth bull from siring at least 30% of the offspring and with this male being the most likely father of only 28% of the offspring. Further only 7 % of the calves could be assigned with 75% confidence to any of the males above 25 years with all older males excluded from siring several of the calves. However, due to low genetic variability of micro satellite markers as a result of a historic population bottleneck (Whitehouse & Hall-Martin, 2000; Whitehouse & Harley, 2001) the overall results were not conclusive. Nevertheless this study suggests that reproductive success may not be as heavily skewed towards older bulls as previously believed and that musth bulls may not be exclusively responsible for reproduction.

Genetic paternity analysis using DNA micro satellite markers were carried out in order to evaluate the age and tactic specific reproductive success. To minimize the disturbance of the study animals, genetic material for DNA micro-satellite analysis was collected non-invasively from faecal samples. Due to the very low defecation rate in calves younger than 1 year, very few samples were obtained from calves sired during the first half of the study period. Additionally due to the long gestation period none of the calves sired during the second half of the study period was born before completion of the fieldwork. Hence paternity analysis in this chapter relies on calves' predominantly sired prior to the collection of the detailed bull data between end 1999 and 2003. This prevented the analysis of the reproductive success resulting from the period used for analysis in the previous chapters. However the general monitoring program has collected data on male presence and the occurrence of musth since 1997, although at a lower frequency. These data in conjunction with individual age estimates enabled an analysis of general age and tactic related reproductive success within the Samburu population.

The genetic paternity analysis is part of a larger genetic research initiative on African elephants carried out in collaboration between Save the Elephants, University of Oxford, the Wildlife Genetic Project at University of Copenhagen, Denmark and the University of Makerere, Uganda. This collaboration aims to investigate the genetic components of social and reproductive strategies in both males and females and on a wider scale to gather information on genetic diversity in African elephants from regional to continental levels. Field collection of samples was conducted as part of this larger genetic project by the author as well as G. Wittemyer and the Save the Elephant monitoring team. John Okello conducting the lab analysis at the genetic labs at University of Makerere, Uganda and University of Copenhagen, Denmark.

8.2 Methods

Identified individuals were followed until they defecated and the mucus lining of the fresh droppings where collected using sterilized equipment. Samples were collected in vials containing 25% Dimethylsulphoxide-saturated (DMSO) with 5M NaCl (Amos & Hoezel, 1991) or 70% Ethanol, and stored at ambient temperature during day of collection, 0°C at the field station and -80°C after shipment to in the laboratory.

8.2.2 Sampled individuals

Calves sired one year prior to the start of the monitoring program were included in the analysis of male age and tactic related reproductive success with the assumption that only minor changes in age structure and musth patterns of bulls occurred between 1996 and 1997. Of the 322 calves sired between 1996 and 2001, samples where obtained from 79 (25%) with samples from the behaviourally determined mothers obtained from 66 of those (83%). To analyse genetically related questions other than paternity, including the analysis of overall data quality (see below) samples from calves (and their mothers) sired prior to 1996 were also collected. Of the 385 known calves sired between 1985 and 1995, 120 were sampled (31%) and samples obtained from the mothers of 72 of those (Fig 8.1).

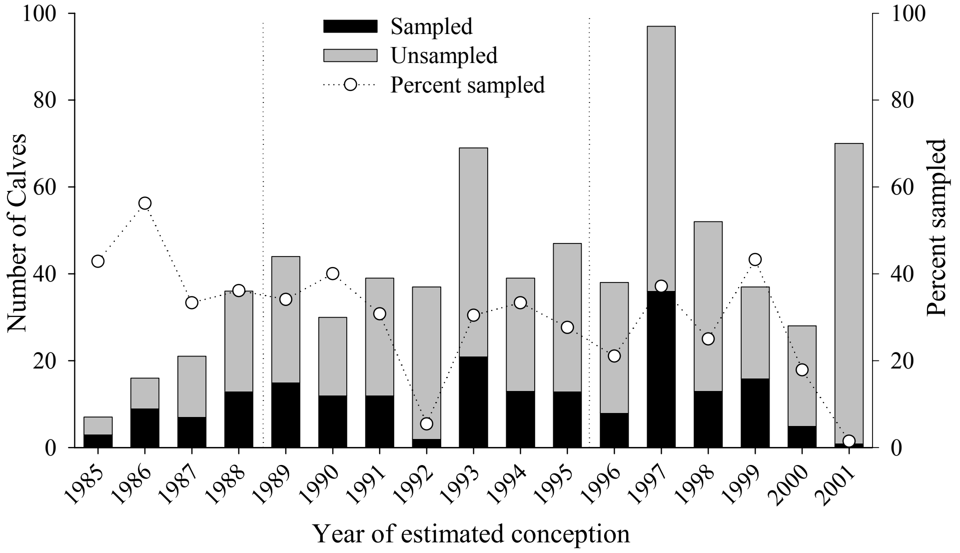


Figure 8.1: The number of sampled and un-sampled individuals conceived after 1985 and observed since 1997. Individuals conceived before 1989 (born before 1990, 7 + years at onset of project) does not represent all individuals of that age as these have not all been assigned an estimated year of birth. Calves conceived from 1996 onwards (n=79) were used for paternity analysis.

Samples from 88 independent adult males (candidate fathers) were collected representing 51% of the total number of currently identified independent males (N=174). More older males (30+ year) than younger males (<30 year old) were samples (Figure 8.2). Since paternity analysis was based on calves sired up to 7 years prior to sample collection, some of the sampled males had been young and not mature (<15 years) at the time the calves were sired. In addition some males known to have been around during the time the calves were sired have died (disappeared and presumed dead) before genetic sampling was initiated including three of the older individuals above 40 years.

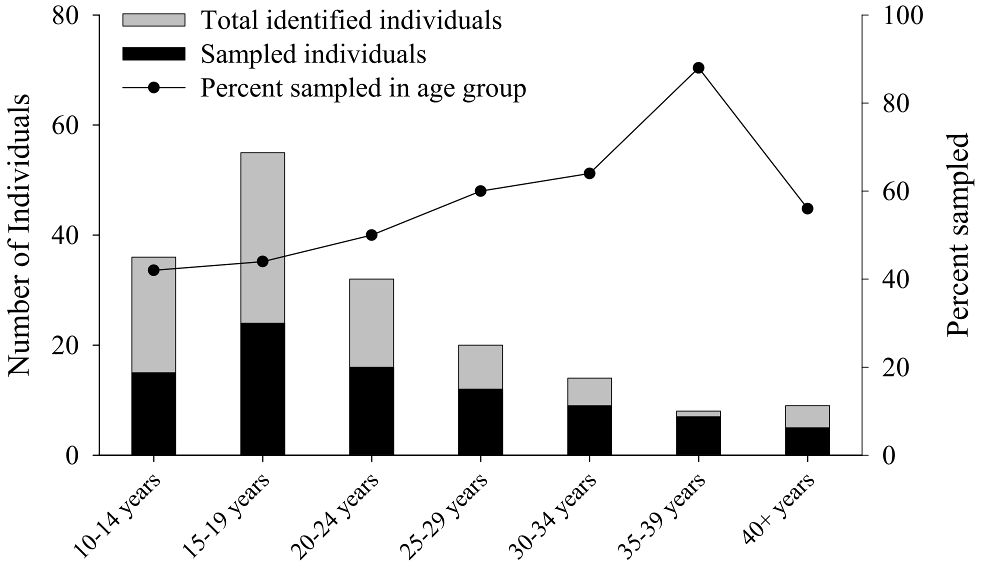


Figure 8.2: Age distribution (in year 1998) of all identified bulls and proportion of those sampled for DNA. Three un-sampled individuals in the oldest age group have not been observed since 1997, 1998 and 1999 respectively and are presumed dead. The total number of individuals in the youngest age groups does not represent all individuals, as some of these males have not been individually identified yet or are not considered independent. (Total identified =174, total sampled =88)

8.2.3 Age and tactic specific success

All males with genetic data (N=88) were included in the paternity assignment. However, the relative age and tactic related reproductive success could not be estimated directly from the number of calves assigned to this group. The study population is not confined, hence males may obtain breeding opportunities with unknown females outside the core study area and known females may breed with unknown males when away from the core area. The level of this reproductive interaction with elephants in surrounding areas is unknown. In addition, the group of known identified males spends a varying amount of time within the core study area. Some males including older 40+ year males are predominantly seen during their sexually inactive periods in association with other males but not (or seldom) during their presumed sexually active periods including periods in musth. Hence the reproductive success of sampled males, as reflected from the sampled group of calves, could not be directly compared due to their varying "effort" directed towards the study females.

Males that were observed on a regularly basis within the core study area during 1997-2000 was therefore selected as the group reflecting males with equal access to the study females within the study area during the period when the calves were sired. A total of 83 males were selected including 48 of the 88 sampled males (Figure 8.3)

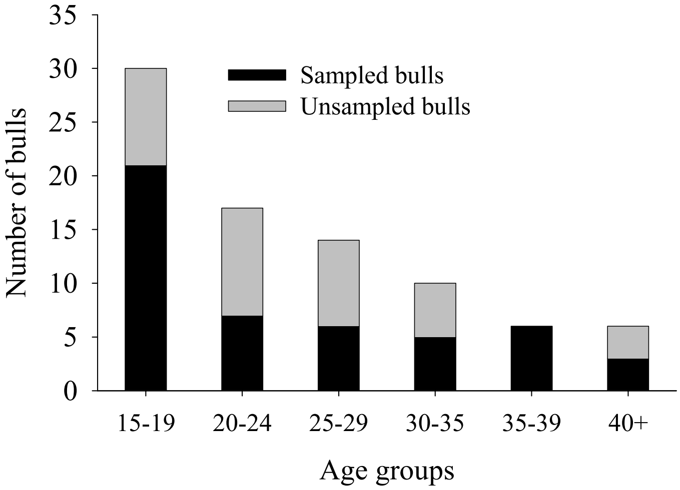


Figure 8.3: The age distribution and number of sampled males in the group of regularly occurring males

The selection was done together with G. Wittemyer, the principal scientist responsible for the collection of monitoring data during 1997-1999. The selection of these males were done prior to the actual paternity analysis to avoid biasing towards males know to have sired off spring. The age specific reproductive success (m_x) was calculated within this group of selected bulls as the number of calves assigned to bulls of a given age divided by the number of males having had that age during the period the calves were sired. To account for the +/-3 year error on bull age estimates mx was calculated based on bulls and calves +/- 3 years of a given age as:

$$\text{Age specific success at age i } (m_x) = \sum_{i-3}^{i+3} \text{calves with father of age i} \Big/ \sum_{i-3}^{i+3} \text{males with age i}$$

The relative age specific contribution to reproduction was calculated within 6 age groups (15-19y; 20-24y; 25-29y; 30-34y; 35-39y; 40+y) by multiplying the average age specific success within these groups (based on the 48 selected males) with the total number of regularly observed males (N=83) within each age group (using the age of bulls in 1998). Likewise the tactic specific contribution was calculated as the proportion of calves sired by males known to be either in SAN or musth within each age group multiplied by the contribution of that age group.

8.2.4 DNA extraction, Micro-satellite Screening and Optimisation

Following a pilot screening of 40 available micro satellite primers on 15 individuals, 20 primers (eleven tetra-nucleotide and nine di-nucleotide) were selected for the analysis based on their high level of polymorphism and clear banding patterns (For primer list see appendix C). Ph.D. student John Okello at University of Kampala, Uganda and at University of Copenhagen, Denmark carried out the lab work including DNA extraction; micro-satellite optimisation and screening. Lab procedures and rules for obtaining consensus genotypes are described in details in appendix C.

8.2.5 Data quality

All genetic analysis will contain errors, either due to mistakes during handling or due to genotyping errors (Broquet & Petit, 2004). Further, null alleles resulting from poor primer annealing due to mutations in the flanking regions or large allelic dropout (Wattier et al., 1998; Kwok et al., 1990) are also likely to occur and result in incorrect genotypes. Non-invasively collected samples often contain lower concentrations of genetic material that may prevent DNA amplification during the PCR (Gagneux et al., 1997). Hence, concerns still exist over the accuracy of nuclear DNA markers from such samples (Bayes et al., 2000; Broquet & Petit, 2004; Roon et al., 2005). Genotyping errors and null allele can compromise conclusions drawn from such genetic data sets if occurring at high frequencies (Hoffman & Amos, 2005; Dakin & Avise, 2004). Therefore a detailed study was conducted to evaluate the data quality affecting all subsequent analysis including the paternity analysis presented in this chapter. One of the most robust ways of investigating such error rates is by analysing pedigree data (Hoffman & Amos, 2005; Marshall et al., 1998). To investigate the data quality and assess if valid conclusion could be drawn from the genetic data the rate of genotyping errors and null alleles frequency as well as potential large allelic dropouts were assessed based on mother/calf pairs.

8.2.6 Paternity assignment

Even true combination of father/offspring/mother combinations may contain incompatibilities (mismatching genotypes) due to genotyping errors and or null alleles. The estimated level of genotyping errors and null alleles were low (see results), however even these low rates would result in the majority of true fathers being excluded when comparing 20 loci across a triplet of individuals (mother/calf/father). For these reasons paternity assignment purely based on exclusion are seldom applied and likelihood based approaches are generally used (Marshall et al., 1998). Paternity assignment in this study was based on a combination of a likelihood based approach, exclusion based on number of incompatibilities and finally a calculation of relatedness between the assigned father and mother of the calf to evaluate if the candidate father/calf match could be due to shared maternal inheritance.

8.2.6.1 Likelihood

The program Cervus2.0© (Marshall et al., 1998) was used to assign the two most likely fathers and calculate the confidence of the most likely candidate being the true father. This program calculates the log likelihood ratio (LOD) between the likelihood of a candidate father siring the offspring and that of an average random male from a "population" of simulated genotypes. This likelihood is calculated based on shared alleles incorporating maternal information (if available) as well as population level allele frequencies i.e. shared alleles that are rare at a population level weigh relatively more than shared common alleles. It further incorporates the possibility of genotyping errors by allowing mismatching loci to arise from true genotypes being replaced with a randomly draw allele from the observed alleles within the population (again incorporating the actual allele frequency). This error rate (probability of replacing a true allele with a randomly drawn allele) is user defined and hence (if set to zero) will result in exclusion if any mismatch exists between candidate father and calf. Although the statistically properties behind null alleles are slightly different this error rate also counters exclusion due to null alleles. The significance of the ratio of LOD scores between the two most likely candidates (Δ score= LOD_1 –LOD_2) cannot be obtained analytically but is based on the results of a simulation of a large number of parent/offspring combinations using the population level allele frequencies. The distribution of Δ score of the most likely candidate being the true or non-true father is then obtained and the delta score corresponding to a given confidence (typically 80 and 95%) is obtained. The following parameters were used in the simulation required to calculate LOD scores obtain critical delta values:

<u>Proportion of loci typed:</u> The average percentage of loci genotyped was 95% and this value was used in the simulations.

<u>Proportion of loci mistyped:</u> Using Cervus the estimated mistyping rate from mother calf pairs averaged 1.7 % (Range 0-5%) whereas results from repeated typing genotyping gave an average of 2%. A value of 1.5 % was used for the simulation.

<u>Cycles (simulated number of offspring)</u>: 50000 cycles were used for all simulations

<u>Confidence</u>: Strict confidence and relaxed confidence levels were set to 95 and 80%

Number of candidate parents: Should ideally match the number of breeding males in the population. As the number of breeding males was a priori unknown this value was set to 80, equivalent of the number of observed males older than 20 years in 2002.

Proportion of Candidate fathers sampled: This proportion was also unknown. Decreasing the estimated proportion of fathers sampled makes the Cervus predictions more conservative. A crude guideline to whether this proportion is set correctly can be obtained by comparing the predicted fraction of calves with resolved paternities with the actual fraction resolved. Four simulations were run with values of 0.20; 0.40; 0.60 and 0.80.

Changes in all simulation parameters except error rate will only affect the confidence level of the most likely father but not change the ranking between the candidates with positive LOD scores (more likely than average of being father). E.g. an increase of the estimated fraction of candidate fathers sampled will increase the predicted confidence, whereas an increase in total number of candidate fathers (breeding males) will decrease the predicted confidence. Contrary, changing the error rate may shift the ranking of likely candidates as the balance of likelihood between shared alleles (determined by allele frequencies) and incompatibles (determined by error rate) will changes. However changing the error rate within it’s likely range of 1-3 % will only have minor effects (Marshall et al., 1998).

8.2.6.2 Exclusion

A true “non-father” may be assigned a high LOD score even with a relative high number of genotype incompatibilities to the calf (CF/C-mismatches) and across the candidate father/calf/mother triplet (CF/C/M-mismatches) in situations where, by chance the shared alleles with the calf are rare on a population level. If the true father at the same time is un-sampled this is likely to lead to a high probability of paternity for the non-father using the likelihood approach in the Cervus© program. Such suspected well matching non-fathers were identified based on the level of incompatibilities using the following procedure:

Candidate fathers with one or nil mismatches to a calf and a higher than 95% confidence of paternity assigned by Cervus© were identified and considered to be “true” fathers. In the group of calves with identified “true” fathers the second most likely candidate father was identified and assumed to be a by chance well-matching “non-father”. 25 well matching “non-fathers” were identified in this way. From this group the distribution of CF/C and CF/C/M mismatches were obtained (Figure 8.4). Only 3 of the 25 had less than 3 CF/C and 1 less than 5 CF/C/M genotype mismatches whereas combination of 3 and 5 CF/C/M or higher was frequent. Based on this candidate fathers assigned by Cervus to be the most likely father with more than 80% probability but with more than 3 CF/C and 5 CF/C/M mismatches were considered to be well matching non-fathers and excluded as true fathers.

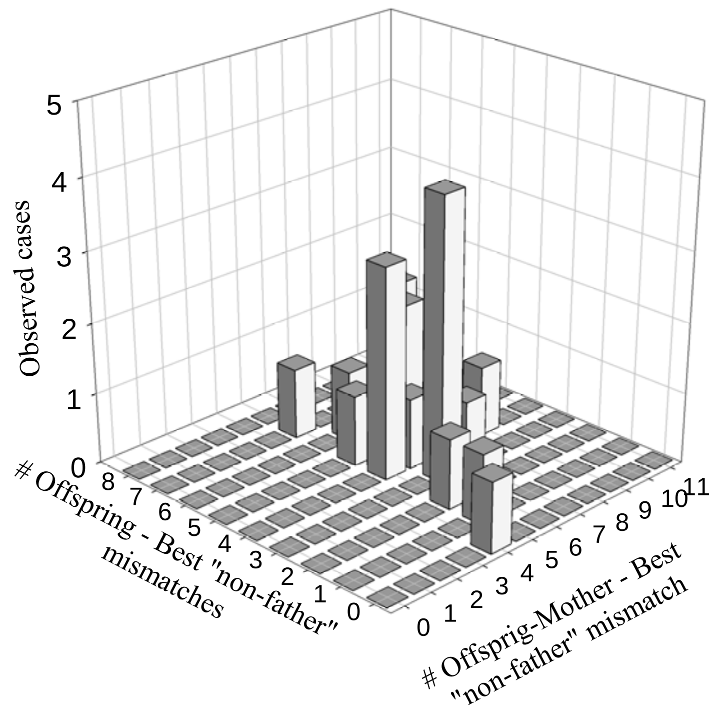


Figure 8.4: Number of Candidate father/calf and Candidate father/calf/mother mismatches within the group of second most likely fathers where the most likely father was considered the true father (one or less mismatches and <95% confidence of being father) (N=25).

8.2.6.3 Relatedness

The confidence on paternity assigned by Cervus© will overestimate the true confidence if the candidate father and the calf are related via a common maternal or paternal heritage. This could especially be the case among younger bulls that may have part siblings among the calves which paternity was in question. However any relation other than full sibs (or half sibs if the mother is un-sampled) will only marginally affect this confidence (Marshall et al., 1998). To assess if assigned paternities were influenced by shared maternal heritage the relatedness between all assigned fathers and the mothers (if available) of analysed calves were investigated using the program Relatedness 5.0 (Queller & Goodnight, 1989).

8.3 Results

8.3.2 Genotyping Errors and Data Quality

John Okello conducted the analysis of data quality. Based on analysis of 133 mother-offspring pairs and repeated genotyping the levels of genotyping errors and null alleles were shown to be comparable or lower than that reported from other studies using high quality samples from tissue or blood (Jeffery et al., 2001) and well below levels that would seriously affect any conclusions drawn:

> *A total of 98.6% consensus genotypes were successfully obtained, with allelic dropout and false alleles rates at 1.6% (n = 46) and 0.9% (n = 37) of heterozygous and total consensus genotypes respectively, and an overall genotyping error rate of 2.5% based on repeat typing. Mendelian analysis revealed consistent inheritance in all but 38 allelic pairs from known mother-offspring, giving an average mismatch error rate of 2.06%, a possible attribute of null alleles, genotyping errors or inaccuracy in maternity assignment. We detected no evidence for large allele dropout, stuttering, or scoring error in the dataset and significant Hardy-Weinberg deviations at only two loci due to heterozygosity deficiency. Across loci, estimated null allele frequencies were low (range: 0.000 - 0.042), and below the 0.20 threshold that would significantly bias individual-based studies.*(Okello et al., submitted)

8.3.3 Paternity assignment

Initially all 88 sampled males (including the very young individuals) were included in the analysis. Paternity assignment was run on the 79 calves sired between 1996 and 2001in Cervus using the four different simulations and obtaining the two most likely fathers. The simulation using a proportion of sampled fathers of 0.40 gave an approximate match between predicted and actually resolved paternities (Table 8.1) whereas proportions of 0.60 & 0.80 resulted in far less resolved paternities than expected, suggesting a too high value whereas 0.20 resulted in more resolved than expected suggesting a too low value. Hence this simulation was used for further analysis. None of the 79 calves were assigned a father younger than 20 years (in 2001,year of the last sired calf) with confidence above 80%. Including a large number of individuals that are not candidate fathers (very young individuals) will affect the confidence level of realistic candidate fathers as some of these young males may by chance have high LOD scores and hence reduce the Delta value of the most likely candidate father. Therefore the 40 sampled males belonging to this young age group were removed as potential candidate fathers and the paternity assignment was rerun on the remaining 49 candidate males.

Table 8.1 Assigned and predicted (in brackets) paternities using simulation parameters of 80 candidate fathers and 0.40 sampled including all sampled males (N=88) or males older than 20 years (n=49).

One parent known:	Candidates: All Sampled Males (n=88)		Candidates: Males 20+ years (N=49)	
Confidence level	Tests	Success Rate	Tests	Success Rate
Strict 95%	24 (27)	36% (41%)	29 (27)	44% (41%)
Relaxed 80%	31 (33)	47% (49%)	37 (33)	56% (49%)
Unresolved	39 (35)	59% (52%)	29 (33)	44% (51%)
Neither parent known:				
Strict 95%	1 (4)	8% (30%)	2 (4)	15% (30%)
Relaxed 80%	4 (6)	31% (44%)	5 (6)	38% (44%)
Unresolved	9 (7)	69% (56%)	8 (7)	62% (56%)

This resulted in an increase in the number of calves assigned a father with 80+% confidence to a level slightly above that predicted from the simulation (Table 8.1). Of the 42 calves (53%) assigned a likely father with more than 80% confidence none of the second most likely fathers appeared to be likely candidates, all having considerably higher levels of incompatibilities. Of these 42 assigned paternities, 6 (all assigned under relaxed 80% confidence) had more than 3 CF/C and/or 5 CF/C/M mismatches and were excluded as true fathers. None of the remaining likely fathers were closely related to the mother of the calf. Hence 36 of the 79 calves (46%) were considered to have resolved paternity with 31 at 95% confidence and 5 at 80% confidence. All of the 36 calves with resolved paternity were observed less than 2 weeks after their estimated birth hence providing an accurate estimate of the date of conception (by subtracting the average 656 days of gestation) and thus enabling their conception to be matched to the observed reproductive state of their fathers at that time.

8.3.4 Age and tactic specific reproductive success

Of the 36 calves considered to have resolved paternity 33 (92%) belonged to the group of 48 males regularly occurring within the study area during the time the calves were sired. The number of resolved calves per male in relation to the age of the males in 1998 is shown in fig 8.5 and the age specific reproductive success of males is shown in figure 8.6.

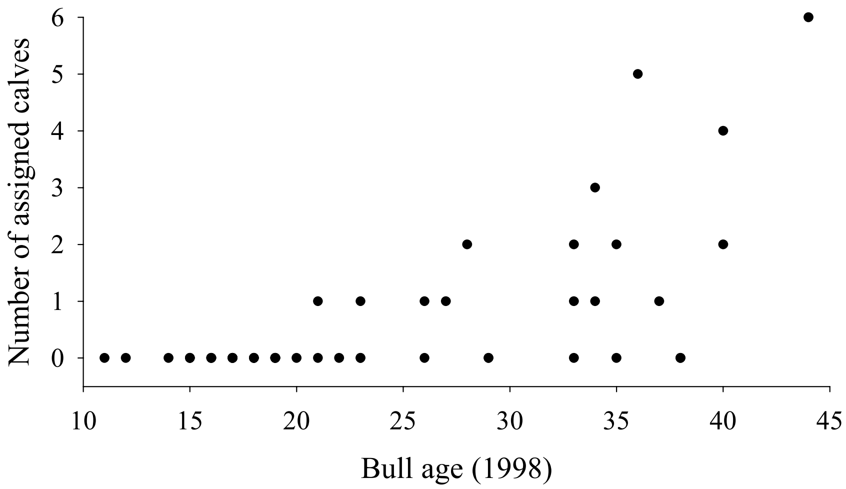


Figure 8.5: Number of assigned calves per male vs. age of sire within the group of regularly observed males (calves = 33; males = 48)

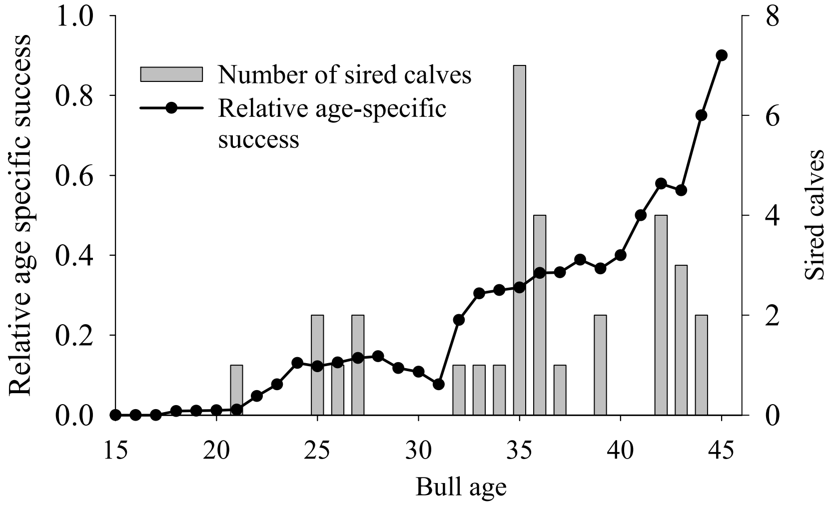


Figure 8.6: The number of calves vs. the age of the sire (Bar, secondary y-axis) Relative age specific reproductive success (line, primary y-axis) calculated as the running average based on bulls +/-3 years of indicated age (bulls=48, calves =33)

The average number of assigned offspring increased dramatic after 40 years of age. (Figure 8.6). Corrected for the number of individuals in each age group, approximately 30% of the reproduction could be assigned to males less than 30 years and 60% to males older than 35 years (Table 8.2 and Figure 8.7) with approximately 20-25% resulting from bulls not in musth. Interesting a 45-year-old regular musth bull sired two calves outside his musth period.

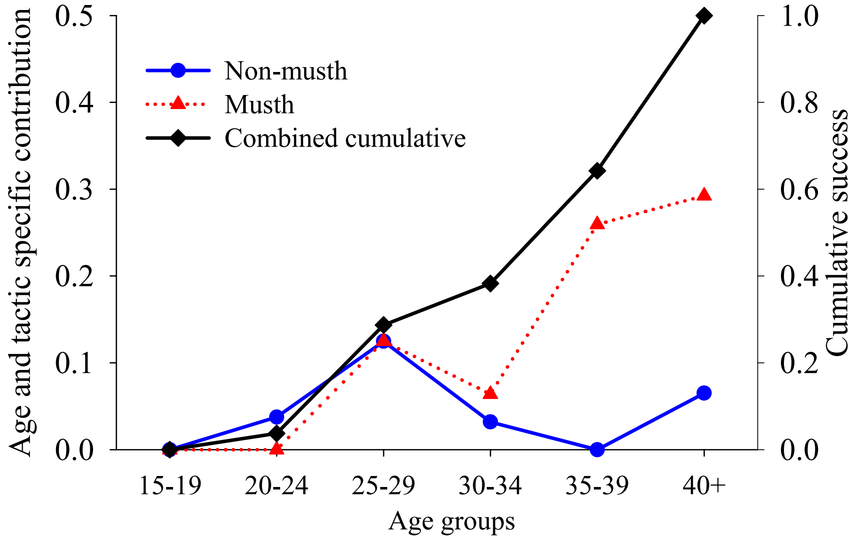


Figure 8.7: The relative contribution of musth and non-musth in each age group corrected for the total number of individuals in each group (sampled + un-sampled regular males, primary y-axis) and the combined cumulative contribution to reproduction (secondary y-axis) The musth reproduction in the 25-29 year age group belong to opportunistic musth bulls that cease signalling when confronted by older musth bulls

Table 8.2: Number of bulls and resolved paternities within age groups (age in 1998) *) = Calves sired outside musth period. **) If excluding calves sired outside musth by oldest agegroup

Age Group	15-19 years	20-24 years	25-29 years	30-34 years	35-39 years	40+ years	Total
Number of regular males in group (2002)	30	17	14	10	6	6	83
# regular sampled males (age 1998)	21	7	6	5	6	3	48
# Offspring assigned to age-group	0	2	4	7	8	12	33
Average Individual success	0	0.02	0.19	0.10	0.47	0.64	
Estimated relative success of age-group	0	0.04	0.25	0.09	0.26	0.36	1
# Offspring from Musth	0	0	1	4	8	10	23
# Offspring from Non-Musth	0	2	1	2	0	2*	5 (7)
# Offspring Unknown tactic	0	0	2	1	0	0	3
Estimated Fraction of reproduction from non-musth ***	0	1	0.5	0.33	0	0.17	0.24 0.20**

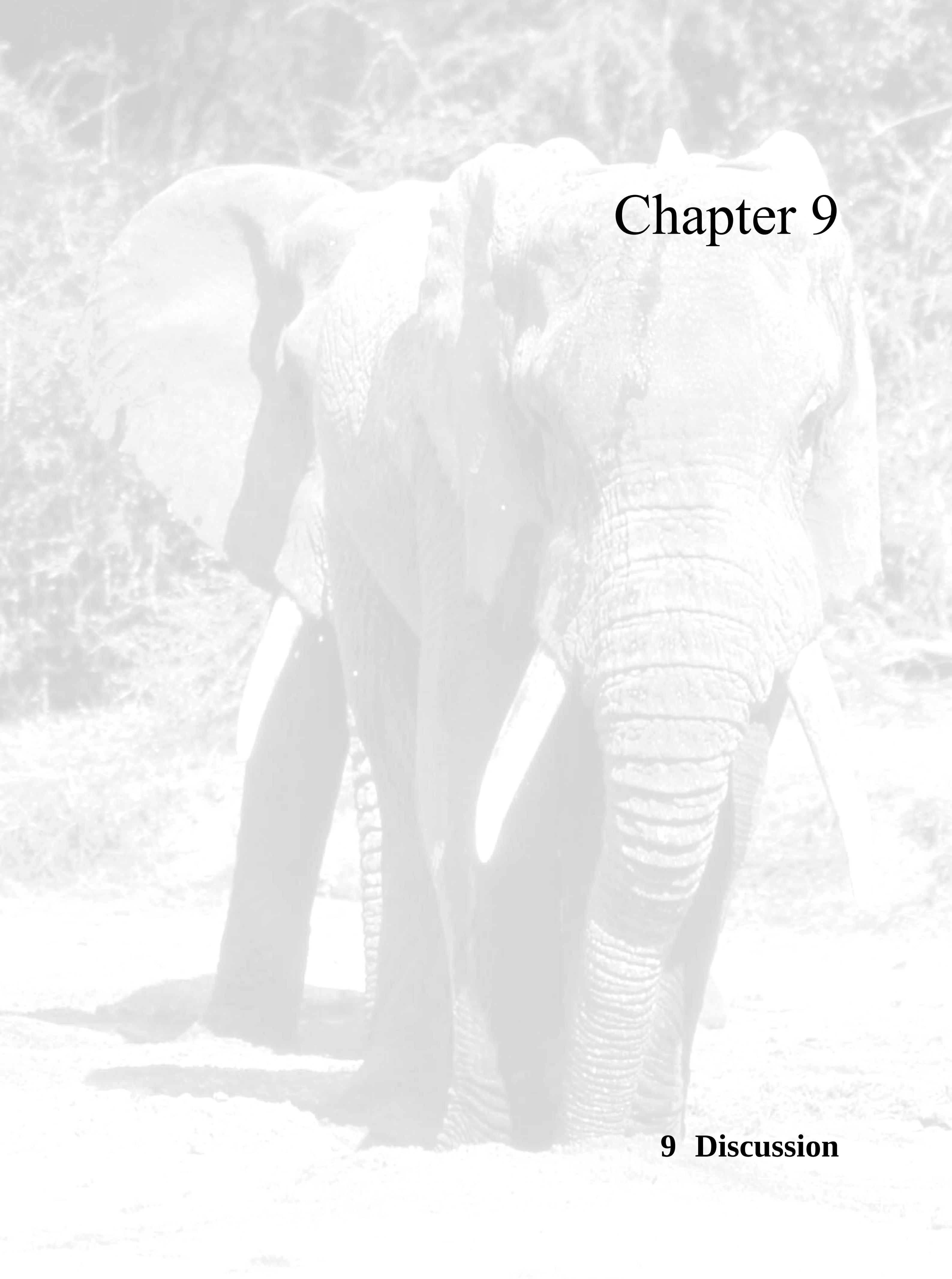
8.4 Discussion

The present study present for the first time results on age and tactic specific reproductive success in free ranging African bull elephants. The long gestation period combined with the difficulties in obtaining samples from very young calves unfortunately prevented the analysis of calves sired during the majority of the study period between 1999 and 2003 where the most detailed data on bulls exists. Of the calves sired between 1996 and 2001 samples were obtained from 79 (25%) with paternity resolved from approximately half. The majority of resolved paternities belonged to the group of sampled bulls known to have been regularly around during the time where the calves were sired whereas very few calves (3) were assigned to the group of less frequently observed bulls probably representing males mainly staying in adjacently lying areas. This suggests that the sampled calves with unresolved paternities are less likely to have been sired by bulls from surrounding areas instead these calves have likely been sired by regularly occurring un-sampled males. This is promising for future analysis of calves sired after 2000 where almost all males that occurred in the study area have been sampled.

The majority of the reproduction could be assigned to bulls in the oldest age groups and was almost exclusively obtained during their period of musth, hence partly supporting the earlier predictions by Poole (Poole, 1989c) that musth bulls are the most successful males. However the results show that bulls already in their early twenties start siring offspring with an estimated 30% contributed to bulls less than 30 years and 20-25% to bulls not in musth. Hence older musth bulls do not monopolise breeding and males below 30 cannot be disregarded within a reproductive context.

The fraction of reproduction obtained by younger males and males not in musth may be influenced by specific conditions during each season. Older dominant musth bulls may be less able to monopolise reproduction during seasons with many oestrus females as seen during May 2002 where sometime 3-4 females were in oestrus at the same time, creating general confusion and pandemonium. Contrary, they may forgo breeding during very poor seasons leaving the few receptive females to younger males as seen during April/May 2000 where almost no older males was seen in musth. Sampling and analysis of calves sired during the main study period between 1999 and 2003 where large variations in inter seasonal quality was observed is planed to be carried out in the near future and will hopefully reveal if such inter seasonal variation exists. With the current data set it was not possible to test if the reproductive payoff of the musth and non-musth tactics are approximately equal around the age of 30-35 where the shift occur between the two tactics as would be predicted from the hypothesis on status dependent tactic switch point with equal fitness (Gross, 1996).

A reduction in age specific success occurred around the age of 30 years (Figure 8.6). This age coincides in the Samburu population with the first occurrence of regular musth periods not associated with specific events like the encounter of an oestrus females, which often triggers the short and sporadic musth in younger males. This reduction is suggestive of a temporary reduction in reproductive success associated with shifting mating tactics. Bulls may need to learn the ’tricks of the new trade’ and establish the reliability of their musth signals among other bulls before becoming successful. Alternatively bulls may at that age reach a size where they are considered a treat by more dominate musth bulls. Hence, the bulls enter a period where they are too strong to be allowed close, but too weak to attain full dominance during attractive reproductive periods resulting in a loss of opportunities compared to the slightly younger 25-year-old bulls that are not perceived as a threat by the highest ranking bulls. Further analysis of the patterns seen during 1999-2003 and/or from other populations where these transition points may occur at other age points is needed to examine this.



Chapter 9

9 Discussion

9.1 Discussion

The phenomenon of musth seen in older male elephants is referred to in numerous works of both fictional and non-fictional literature and has for the Asian elephant been recognised since antiquity (Eisenberg et al., 1971) and become part of the folklore. Musth has also attracted considerable attention in the scientific community within a wide range of disciplines ranging from behaviour, endocrinology, physiology, ecology and conservation to vocal communications and chemical signalling. Musth are often used as synonymous to male reproductive behaviour an bulls not yet exhibiting periodic musth have been termed “sociological immaturity” (Poole et al., 1984; Sukumar, 2003). However, the existence of sexually active periods in younger non-musth bulls have been suggested (Poole, 1994b; Poole et al., 1984) but has neither been quantified, confirmed nor obtained much attention. The age when musth signals first occur varies greatly between individuals within the same population, ranging from early twenties to mid thirties in the Amboseli population (Poole, 1987) and similarly in the Samburu population (this thesis). In captivity musth signals and elevated androgen levels are seen in individuals as young as 13 years (Ganswindt et al., 2005 (in press)). In a wild population lacking older individuals long musth periods were seen in bulls around 20 years of age but disappeared after the introduction of older bulls (Slotow et al., 2000). This variation in the occurrence of musth suggests that this state is conditional dependent and not linked to a specific post pubescent age where the bulls become socially mature.

The objectives of this thesis has been to investigate the reproductive strategy of males within a broader lifelong perspective and to evaluate if varying reproductive tactics exist in this long-lived species. More specifically the aims have been i) to assess the existence and occurrence of alternative reproductive tactics ii) to evaluate the underlying hormonal changes linked to these tactics iii) to compare the investment levels between alternative tactics and finally iiii) to assess the reproductive success and contribution from the different tactics.

Any assessment of reproductive state (active / inactive) must be based on data unrelated to tactic specific behaviours or signals. Older bulls have been shown to associate more with females during musth than out of musth. Therefore the temporal changes in association patterns of individual bulls were evaluated to see if shifts in such association could be used as an indication of the underlying reproductive mode. Hidden Markov models were used as analytical tool and proved highly suited for investigating temporal changes in such time structured behavioural data. From the age of 20 years, bulls started to have distinct alternating periods of predominant male and predominate females associations (Chapter 5). The associational preferences within these periods were similar between younger bulls not observed in musth and older bulls with regular musth periods. Musth signals started to occur around the age of 25-30 within already established, recurrent periods of high female associations and with musth periods becoming synonymous with these periods in older males. These periods occurred throughout the year in individual bulls but were, on a population level, in synchrony with the peaks in oestrus females but not with variation in the ecological conditions. This strongly suggests that these shifts were linked to alternating sexually active and inactive periods. During periods of high female associations both musth and non-musth bulls foraged less and spent more time walking and increased the daily walking distance and displacement between areas (Chapter 7). Likewise both musth and non-musth bulls had a high and comparable level of shifts between female groups (Chapter 7) showing that the affinity to females were not directed towards specific groups. Bulls were also often observed examining female urine to evaluate their reproductive state (pers observations no data shown). These changes are all in agreement with a sexually active state with active search for receptive females. Thus periods with high females association can be viewed as sexually active periods regardless of the presence of the musth signals of urine dribbling (UD and temporal gland secretion (TGS)

The androgen levels (epiandrosterone, EA) were significantly different between bulls in sexually inactive (SI) and sexually active (SA) periods. Furthermore, a large difference in EA levels existed between SA bulls with UD and TGS signals (musth) and SA bulls without signals (SAN) (Chapter 6). SAN bulls had an approximate doubling of their EA levels compared to a four to six fold increase in musth bulls. The occurrence and duration of periods with musth signals were positively correlated with the age of the individual. This has also been reported from other populations (Poole, 1987; Poole, 1989a). However, the differences in EA levels were unlinked to the age of the individual but rather linked to the presence or absence of signals. The musth and SAN states could to a large extent be viewed as on/off states and short periods of signaling within an otherwise SAN period were associated with a distinct rise in EA levels. Thus Musth and SAN are distinct states within sexually active periods with different state specific levels of androgens. These results in combination with the associational shifts show the existence of two different age-related reproductive states.

Musth bulls have been shown to do the vast majority of mate guarding (Poole, 1989c) and have a high level of aggression directed towards competing males with the majority of fights between males having at least one of the contestants in musth whereas non-musth bulls seldom engage in physical confrontations (Hall-Martin, 1987; Poole, 1989c). The high aggression in musth is likely linked to the very high androgen levels (Brain, 1979). Thus the alternative states of musth and SAN can be viewed as alternative tactics with musth being a competitive tactic aimed at defending and monopolizing the receptive female (reproductive resource) whereas SAN is a non-competitive tactic attempting to sneak copulations with limited defense of the reproductive resource. Musth bulls are often challenged by other musth bulls leading either to one of them ceasing their signals (often seen in young musth bulls) or one of them leaving to an adjacent area. In addition the high androgen levels associated with musth signals are likely to be metabolically costly suggesting that musth signals are honest (Poole, 1989a). Females have been shown to solicit guarding by musth bulls and may prefere to mate with musth males (Poole, 1989c; Moss, 1983). Whether this is i) female choice of mating partner based on an honest signal ii) a result of male/male competitions with musth bulls trying to prevent females from mating with other bulls or iii) simply the female seeking protection from the harassment by multiple males is difficult to assess.

The shifts between inactive and active periods (determined by associational shifts) were preceded by changes in androgen levels with a rise in epiandrosterone occurring 10-20 days prior to the shift in associations (Figure 6.11 & 6.12). Likewise the onset and termination of musth signals were closely linked to EA levels within SA periods. The relative plasticity hypothesis suggests that sex steroids have an activiational role of reproductive behaviour (Moore, 1991; Moore et al., 1998; Knapp, 2004). This have been shown in other systems where alternative reproductive tactics can be partially altered by administrating or blocking sex steroids (Wikelski et al., 2004). Hence androgens are likely to play an activational role in both the onset of active periods and if highly elevated, activating the onset of signals and the shift to a competitive tactic in elephants.

The two tactics, SAN and musth differ in how to obtain access to a given female once located. However it is likely that the majority of SA periods are spend on searching for females rather than within a competitive context around a receptive female due to the very low densities of receptive females and the short duration of oestrus (Moss, 1983). Musth bulls had a higher time specific investment in search compared to SAN bulls, measured by the level of reduction in foraging and increase in walking. However SAN bulls spent a significantly larger proportion of their time sexually active, suggesting that the lower time specific investment by SAN bulls were, at least partly, counter balanced by the duration of investment. Hence the lower investment seems to result from a short-term optimization of payoff per unit investment, rather than a long-term optimization between current and future reproduction. This difference in investment tactic is likely linked to a basic cost associated with musth, which predict a higher optimal time specific investment. This emphasizes the importance of looking at duration of reproductive bouts when comparing overall investment levels.

Paternity analysis revealed that approximately 20-25% of the reproduction could be attributed to SAN bulls and around 30% to bulls less than 30 years and with almost 60% of all males above 20 years reproducing successful during a 5-year period. Hence the reproductive success in bull elephants is less skewed than previously though and musth bulls do not monopolize the breeding. Interestingly a reduction in age specific success occurred around the age of 30, the time when bulls shift from the SAN to the musth tactic. This could be due to at least two different, non-exclusive scenarios. 1) Bulls around 30 years may start to be perceived as a threat by older musth bulls, regardless of not signaling musth, and hence not allowed close to the receptive females preventing the success of a sneaker tactic and at the same time being too young to assume the competitive musth tactic, resulting in a reduction in their success. 2) Bulls may also have to learn the new tactic and establish the reliability of their signals to other bulls and hence experiencing a temporary reduction in success compared to the success they could obtain by engaging in the SAN tactic. If the later plays a role this will have implications for the understanding of conditional dependent shifts between tactics where current theory assumes that alternative tactics have equal fitness at the tactic switch point (Gross, 1996). Further analysis of our GPS data on inter distances between bulls during mate guarding situations and further analysis of the reproductive success of bulls between 2000-2004 will hopefully elucidate the dynamics occurring around the time of the tactic switch point. Varying densities of oestrus females may also affect tactic specific success. This could not be assessed with the current genetic data set but will hopefully be reveal by the planed analysis of all calves sired between 1999 and 2004 where large inter-seasonal variations in the number of conceptions where observed (Chapter 4)

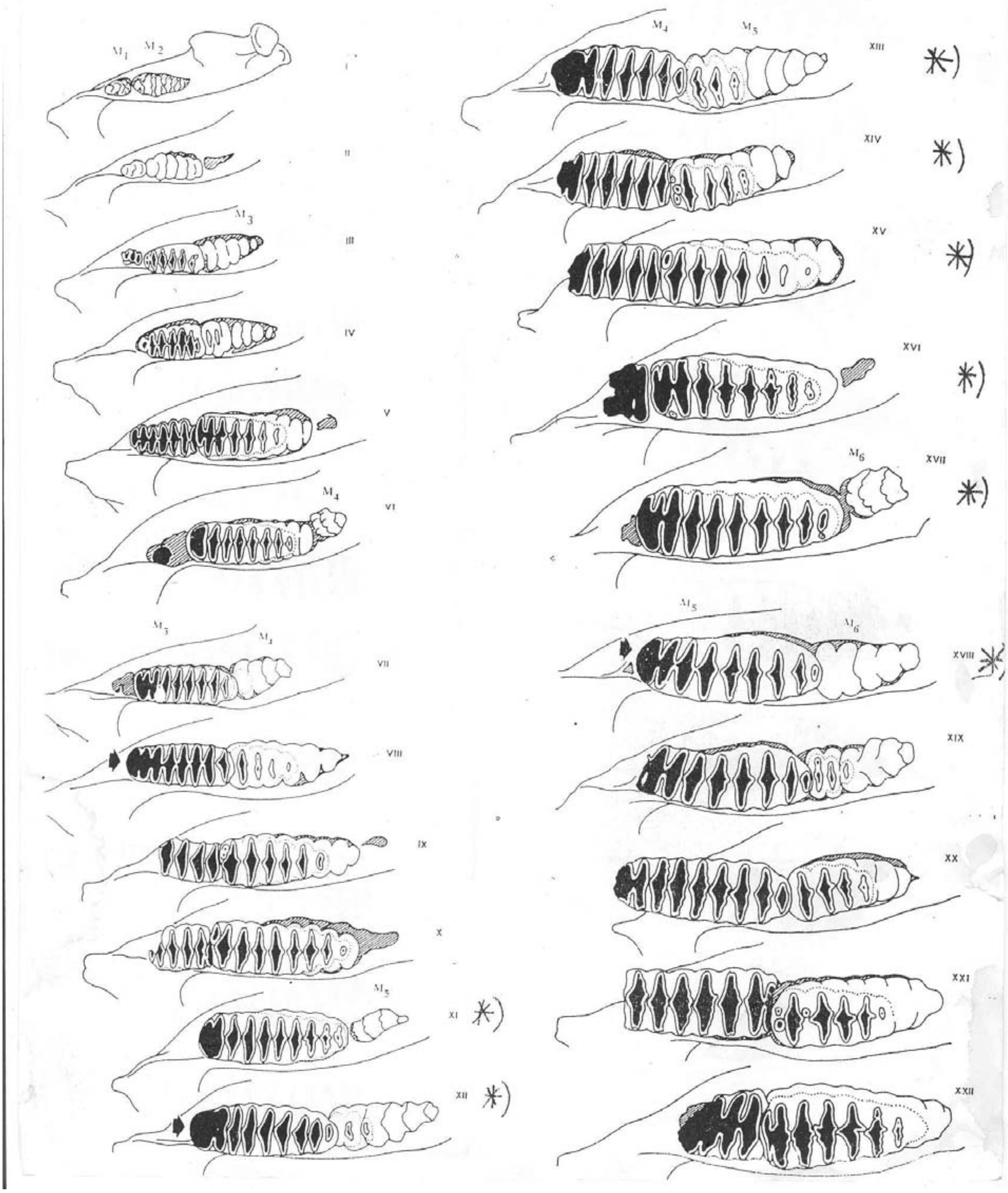
In conclusion, the African male elephant alternate between sexually active and inactive periods from the age of 20 and onwards. Sexually active bulls have two different alternative reproductive tactics: i) The sexually active non-musth tactic (SAN), a non-competitive tactic seen in less dominant, often younger males which is associated with low prolonged investment and ii) The musth tactic, a competitive tactic seen on dominant often older individuals associated with short periods of high investments. The shifts between both sexual states and tactics may be hormone driven. In the Samburu population approximately 20-25% of the reproduction could be assigned to SAN bulls and the remaining to musth.

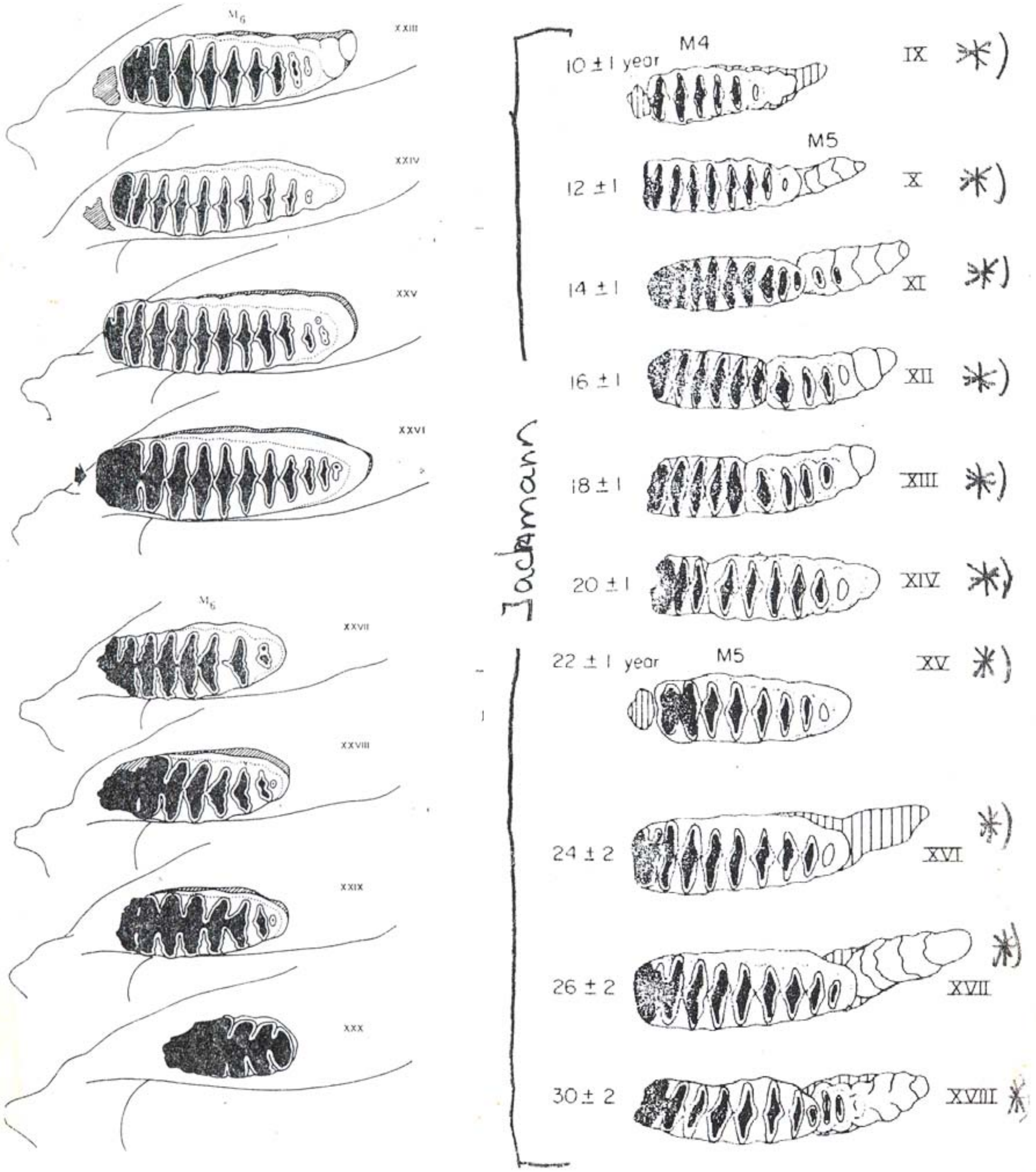
Bulls generally increase in dominance rank as they become older and larger. With an increase in rank it would be expected that bulls shift their SA periods towards more attractive periods (times with more oestrus females). This does not seem to be the case. Within the Samburu population bulls with regular musth periods generally have one SA period per year with a high degree of synchrony between years. This stability in the annual timing of individual musth periods is also seen in the Amboseli population (Poole, 1987). Although a few individuals in that population have been reported to shift in apparent response to the death of other higher-ranking individuals the majority do not change their timing with some having had the same “time slot” for more than 25 years. Preliminary analysis of bull/bull associations (not presented) suggests that bulls with overlapping SA periods have the closest associations during inactive (SI) periods, spending up to 20% of their SI time together. Further these associations tend to be tighter between age-mates and last longer just prior to their SA periods (GPS data not presented). The relatively few serious fights observed during the 4-year study period were all between bulls using different areas and known not to associate during SI periods. This suggests that one of the underlying reasons for bulls/bull associations during SI periods may be (in a non-competitive environment) to establish rank and obtain social knowledge of the bulls they compete with during SA periods. Hence shifting SA period may result in the encounter of “unknown” competitor forcing the bull to establish his rank via costly fights. This cost may outweigh the benefits from shifting to a slightly better period. Further analysis of bull/bull associations, genetic relatedness and duration and timing of associations between individual males are planed to evaluate this hypothesis.

The existing GPS data set collected since 2000 in combination with the results presented in this thesis will form the background for more in-depth analysis of movement patterns. One of the interesting avenues to explore is the actual spatial search for resources. Bulls shift from searching for forage during inactive periods to searching for females during sexually active periods. These two resources are distributed differently thus SA and SI periods are likely associated with different optimal search strategies.

10 Appendix A

Molar age groups used for assigning age to tooth moulds (Chapter 3) (Reproduced from (Laws, 1966; Jachmann, 1988). *) Jackmann's corrections applied correction





Age Group	Mean Age	Probable age Range	Age Group	Mean Age	Probable Age Range
I	0	-	XVI	26	2
II	½	-	XVII	28	2
III	1	-	XVIII	30	2
IV	2	<½	XIX	32	2
V	3	<½	XX	34	2
VI	4	<1	XXI	36	2
VII	5	1	XXII	39	2
VIII	6	1	XXIII	43	2
IX	8	1	XXIV	45	2
X	10	1	XXV	47	2
XI	13	1	XXVI	49	2
XII	15	1	XXVII	53	2
XIII	18	2	XXVIII	55	4
XIV	20	2	XXIX	57	4
XV	22	2	XXX	60	4

11 Appendix B

Indiv. ID	Est Born	Collar Type	Functional Period	Functional Days	Functional Hours	Hourly positions	Total positions	Hourly acquired	Total acquired	Reason for termination
B1001	1954	GPS1000	26/01/2001 to 29/04/2001	93	2233	2212		99.06		Collar destroyed
		GPS2200XL	26/03/2002 to 09/04/2003	379	9097	9056		99.55		Memory full, replaced
		GPS2200XL	09/04/2003 to 08/05/2003	30	703	700		99.57		Belting ripped
B1005	1961	Telonics	25/03/2002 to current		n/a	n/a		n/a		Removed
B1009	1958	Telonics	07/06/2002 to 29/10/2002		n/a	n/a		n/a		Replaced
		TVP-Simplex	29/10/2002 to 21/02/2004	480	11519	11293	21037	98.04	97.4	Belting ripped
B1011	1964	GPS2000	27/04/2001 to 07/07/2001	71	1712	1690		98.71		Electronic failure, wire breakage
B1013	1960	GPS2200XL	03/02/2002 to 17/07/2002	163	3899	3764		96.54		Belt connection broken
B1016	1965	AWT	12/11/2002 to 26/04/2003	165	3937	3510		89.15		Power depleted prematurely
B1018	1963	GPS2200XL	02/11/2002 to 03/01/2003	61	1468	1466		99.86		Belting ripped
B1019	1970	GPS1000	13/01/2000 to 08/05/2000	116	2798	302		10.79		Collar flipped, removed
B1023	1960	TVP-Simplex	28/10/2002 to 30/11/2003	397	9550	8575	15935	89.79	90.1	Collar still running, incomplete downloads
B1026	1971	GPS1000	13/01/2000 to 11/10/2000	272	6531	6301		96.48		Power depleted, expected
		GPS2000	08/02/2001 to 09/05/2001	90	2170	1897		87.42		Belting ripped
		TVP-Simplex	28/10/2002 to 19/12/2003	417	9999	9631	17924	96.32	96.4	Belting ripped
B1027	1964	GPS1000	16/01/2000 to 27/12/2000	346	8303	7367		88.73		Power depleted, expected
		GPS1000	02/02/2002 to 02/02/2002	1	0	0		0.00		Electronic failure
		TVP-Simplex	28/10/2002 to 14/01/2004	443	10633	10444	19489	98.22	98.4	Belting ripped
B1033	1965	GPS1000	05/02/2002 to 08/12/2002	305	7343	7199		98.04		Power depleted, expected
		GPS2200XL	20/01/2003 to 02/01/2004	347	8329	8297		99.62		Power depleted, expected
B1039	1977	GPS2000	10/02/2000 to 14/12/2000	308	7393	7210		97.52		Power depleted, expected
		GPS1000	23/01/2001 to 28/10/2001	278	6671	6548		98.16		Power depleted, expected
		Telonics	26/03/2002 to current		n/a	n/a		n/a		Running
B1041	1972	Telonics	08/06/2002 to		n/a	n/a		n/a		Replaced
B1044	1976	TVP-Simplex	30/10/2002 to 16/03/2003	137	3280	3213	5886	97.96	97.8	Belting ripped
B1045	1977	GPS2000	09/07/2001 to 29/01/2002	204	4888	4364		89.28		Removed
B1122	1981	TVP-Simplex	24/01/2003 to 13/06/03	140	3354	2852	5371	85.03	86.2	Lost, incomplete download

12 Appendix C

12.1 Parameter estimation

The Baum-Welch algorithm is a particular case of the Expectation-Maximization algorithm for finding parameter values that (locally) optimises the likelihood. It iterates between an Expectation step, calculating the expected number of times that each transition and emission is used, and a Maximization step, optimising the model parameters given the set of expected counts. In the first step, the expectation is taken over all hidden state paths, using the probability distribution over all such paths given by the current parameters and the observed data. This distribution is calculated using the Forward and Backward algorithms for HMMs. We write *x*(*i*,*j*) for the *i*th emitted symbol in the *j*th training sequence; here *i*=1,...,*L*. The algorithm is as follows:

$$f_s^j(0) = \pi_s$$

$$f_s^j(i) = \left(\sum_t f_t^j(i-1) A_{ts} \right) B_{s,x(i,j)} \quad (i = 1 \ldots L) \qquad \text{(Forward algorithm)}$$

$$P(j) = \sum_s f_s^j(L)$$

$$b_s^j(L) = 1$$

$$b_s^j(i) = \left(\sum_t A_{st} B_{t,x(i+1,j)} b_t^j(i+1) \right) \quad (i = L-1 \ldots 1) \qquad \text{(Backward algorithm)}$$

$$\overline{A}_{st} = 1 + \sum_j \frac{1}{P(j)} \sum_{i=1}^{L} f_s^j(i-1) A_{st} B_{t,x(i,j)} b_t^j(i)$$

$$\overline{B}_{se} = 1 + \sum_j \frac{1}{P(j)} \sum_{\substack{i \le i \le L \\ x(i,j)=e}} f_s^j(i) b_s^j(i) \qquad \text{(Expectation step)}$$

$$\hat{A}_{st} = \frac{\overline{A}_{st}}{\sum_t \overline{A}_{st}}; \quad \hat{B}_{se} = \frac{\overline{B}_{se}}{\sum_e \overline{B}_{se}} \qquad \text{(Maximization step)}$$

The Forward algorithm computes the probability of emitting symbols up to position *i* and ending up in state *s*, while the Backward algorithm computes the probability of, starting from state *s*, emitting all symbols beyond position *i*. The quantity *P(j)* is the total likelihood of emitting the *j*th training sequence. $\overline{A}_{st}$ and $\overline{B}_{se}$ are the expected usage counts for the transitions and emissions respectively; the additional pseudocount term corresponds to a uniform prior distribution for all parameters. Finally, $\hat{A}_{st}$ and $\hat{B}_{se}$ are the new parameter estimates, which can be shown to always improve the model likelihood. These steps are repeated until the likelihood improvement falls below a preset threshold. (For more details see (Durbin et al., 1998). The algorithms given here are for fixed-length sequences and thus require no additional End state.)

12.2 Recovering the hidden state path

The Viterbi algorithm calculates the state path that is most likely to give rise to the observed sequence of symbols. The algorithm is as follows (we write *x(i)* for the *i*th observed symbol):

$$
\begin{aligned}
&v_s(0) = \pi_s \\
&v_s(i) = B_{s,x(i)} \max v_t(i-1)\, A_{ts} \quad (i = 1 \ldots L) \\
&p(L) = \arg\max_s v_s(L) \\
&p(i) = \arg\max_s v_s(i) A_{s,p(i+1)} \quad (i = L-1 \ldots 0)
\end{aligned}
$$

The first two lines constitute the Viterbi recursion, computing the probability of the most likely state path that emits the first *i* symbols and ends up in state *s*. The final two lines form the traceback part of the algorithm, and compute the most likely state path for the full sequence in *p(i)*. (For more details see (Durbin et al., 1998) We note that the Viterbi algorithm as given here does not use traceback pointers, making it slightly more memory efficient than standard versions.

12.3 Time-inhomogeneous hidden Markov models

To deal with variable time intervals between successive states, the transition probabilities A_{ts} are made dependent on the position within the sequence. The Viterbi, Forward and Backward algorithms given above remain valid, with obvious changes. We model the behaviour of the discrete sequence of hidden states by supposing that it is generated by an underlying continuous-time Markov chain. Changes of state occur according to a Poisson process. Analogous to discrete-time Markov chains, the rates per unit of time, R_{ij}, at which these transitions occur depend on the current state i and target state j. By convention, $R_{ii} = -\sum_j R_{ij}$, and it can be shown that, if the system is in state i at time 0, the probability that the system is in state j after time T is equal to $\exp(RT)_{ij}$ (see Ross, 1997). We used these as our time-dependent transition probabilities, treating the transition rates R_{ij} as system parameters.

13 Appendix D

DNA extraction, Micro-satellite Screening and Optimisation were carried out by John Okello at University of Copenhagen and University of Kampala using the following procedure described in: "Non-invasive Genotyping and Mendelian Analysis of Microsatellites in African Savannah Elephants" J. B. A. Okello, G. Wittemyer, H. B. Rasmussen, I. Douglas-Hamilton, S. Nyakaana, P. Arctander, and H. R. Siegismund. Submitted to Journal of Heredity 2005.

13.1 DNA extraction

Total genomic DNA was extracted in the laboratory from the samples using standard procedures for animal tissues in DNeasy® tissue kit, with a slight modification (OR with the following modification to adapt to faecal samples). From each dung sample, approximately 250 µL of the mucosal part of dung was placed in a 1.5 µl microcentrifuge tube, 180 µl of AE buffer and 20 µl of proteinase K (20mg/ µl) solutions added, and incubated overnight at 70 °C in a 100 rpm rotor. Digests were spun at 8000 rpm for 1 min in a microcentrifuge and the supernatants pipetted leaving out the undigested plant materials in the tubes. The supernatants were then extracted following the standard procedure for animal tissues (step 3) of the protocol for extracting DNA from animal tissues (QIAGEN® 1999). To minimize contaminations, DNA extractions, amplifications and handling of PCR products where conducted at separate locations with dedicated instruments, reagents, and negative controls incorporated in all steps.

13.2 Micro-satellite Screening and Optimisation

As a pilot study, forty micro-satellite primer sets (LaT05, LaT06, LaT07, LaT08, LaT13, LaT16, LaT17, LaT18, LaT24, LaT25, LaT26 (Archie *et al.* 2003); FH1, FH19,

FH39, FH40, FH48, FH60, FH67, FH71, FH94, FH102, FH103 (Comstock *et al.* 2000); FH126, FH127, FH153 (Comstock *et al.* 2002); LA1, LA2, LA3, LA4, LA5, LA6 (Eggert *et al.* 2000); LafMS01, LafMS02, LafMS03, LafMS04, LafMS05 (Nyakaana & Arctander 1998); and LafMS06, LafMS07, LafMS08, LafMS09 (Nyakaana *et al.* in press) isolated from the elephant genome and known to amplify DNA derived from elephant tissues were end-labelled with fluorescent dyes and tested for their amplifications on DNA extracted from fresh elephant dung samples. Out of the 40 micosatellile loci screened, 20 very highly polymorphic and easy to score loci were selected for use in the rest of analysis (See supplementary data in Table 1 for details of annealing temperature and the number of cycles used for each of the 20 loci whose results are presented hereafter). All amplifications were done on a HYBAID PCR express thermocycler, each in a 10 μl reaction volume containing 1-2 μl of undiluted DNA extract solution, 1.5 mM $MgCl_2$, 10 mM Tris buffer, 50 mM KCl, 5 ng BSA, 200 μM of each dNTP, 2 pmol of each primer and 0.5U AmpliTaq Gold DNA polymerase (Boehringer Mannheim GmbH). All PCR products were first visualised after electrophoresis on agarose gels to check for visible signs of contamination and spurious amplification profiles. The intensity of amplified PCR products on agarose gels were ignored because our experience show that even if there were no visible bands, clear results could still be obtained on ABI analysis.

An automated system of multiplexing was developed depending on the fragment sizes and then used throughout the analysis to reduce the cost of genotyping. Primers LaT08(FAM) + LaT05(FAM) + LaT17(FAM) + LaT26(JOE); FH67(FAM) + LaT06(FAM) + LafMS06(JOE) + LaT24(JOE); FH40(FAM) + FH103(JOE) + LaT16(JOE) + La4(TAMRA); FH39(FAM) + LaT07(FAM) + LafMSO2(JOE) + LaT18(JOE); La6(FAM) + LaT25(FAM) + FH1(JOE) + LaT13(JOE) were always multiplexed by pooling 1μl of each amplification product, 3 µl of formamide loading buffer and 0.5 µl of Rox-500 internal size standard (Applied Biosystems, USA). The multiplex was heated at 96 ^{0}C for 5 min and super-cooled on ice before loading 1.75 µl into each lane of a 4% polyacrylamide gel, polymerized for a minimum of 1h and pre-ran for 1h to overcome electrophoresis artefacts (Fernando *et al.* 2001). After optimization, these loci were screened on fifteen individual samples randomly selected from each of the different elephant family units (as defined in Wittemyer *et al.* in press). Samples in this pilot study were run in three replicates, alleles separated on an ABI 377 automated DNA sequencer, and analyzed with the programs Genescan 3.0 and Genotyper 2.1 (Applied Biosystems, USA). Both negative and positive controls were included in each run to check for contaminations and ABI gel electrophoresis consistency, respectively.

13.3 Genotyping Criteria

We used rigid criteria in scoring and accepting consensus genotypes to minimize potential genotyping errors (Schlötterer and Tautz 1992), including scoring of alleles by two experienced DNA analyzers and validation by a third person. False peaks due to leakages from strongly amplified products loaded in the neighboring lanes were not scored e.g. peaks in lanes A and C are leaked from lane B (Fig 1A-C); and weaker peaks (less than 100 fluorescent units of electrophoresis peak heights) were considered failed since they could not be reliably scored (Fig. 1D).

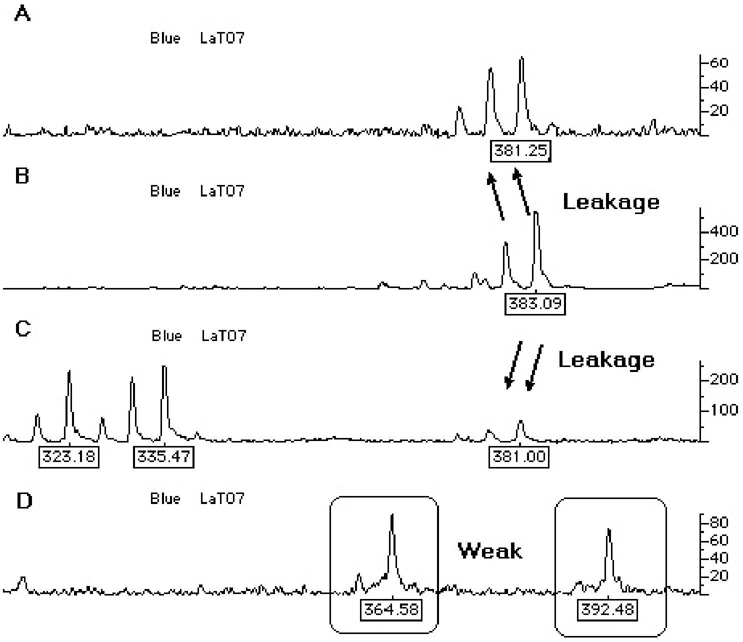


Figure 1

Following a preliminary amplification success rate of 92.2% (70 failures out of 900 PCRs) and false alleles frequency of 0.36% (3 false genotypes), we adopted recommendations in Bayes et al. (2000), Ernest et al. (2000) and Parsons (2001), and genotyped each individual locus at least twice to obtain a reliable genotype. Where an inconsistency or amplification failure occurred, that individual would be re-genotyped at least twice at the locus in question to verify alleles, and a majority consensus taken (Taberlet et al. 1996). However, a genotype at a particular locus that consistently yielded ambiguous or indistinguishable stutter peaks would not be scored, and those that successfully amplified in only a few of the repeat typing were considered failed. Consensus genotypes were assessed and designated as having had allelic dropout if it was typed homozygous once and subsequently reproduced as heterozygous of the same allele in replicate typing or, false alleles if one or more false alleles were detected in any of the amplifications at that locus (either homozygous or heterozygous)

Table 1: Annealing temperature (AT), number of PCR cycles, mother-offspring pairs (Mo-Cf), genotypic mismatches (N, due to null alleles), estimated error rate, null allele frequency (Null F) based on Van Oosterhout et al. (2004), probability for Hardy-Weinberg proportions (H-W) and, Weir and Cockerham's (1984) analog of Wright's fixation index for each locus analyzed on 202 individual elephants from Samburu, Kenya. ***** Significant at 5% level after Bonferroni correction.

Locus	AT (°C)	Cycles	Mo-Cf	M-M (N)	Null F	Errors	H-W (*P*)	F_{IS}
LaT05	58	28	133	4 (1)	0.011	0.028	0.037	0.027
LaT06	52	27	130	0 (0)	0.000	0.000	0.243	0.000
LaT07	58	28	130	2 (0)	0.033	0.012	0.002*	0.069
LaT08	58	28	131	2 (0)	0.00	0.016	0.400	0.003
LaT13	56	33	133	1 (0)	0.000	0.009	0.244	0.000
LaT16	54	33	122	2 (1)	0.019	0.019	0.193	0.043
LaT17	56	28	132	3 (0)	0.006	0.025	0.704	0.017
LaT18	56	32	132	2 (0)	0.025	0.017	0.374	0.05
LaT24	52	28	132	3 (0)	0.020	0.021	0.002*	0.043
LaT25	52	38	113	3 (0)	0.017	0.026	0.061	0.037
LaT26	54	40	125	2 (0)	0.042	0.015	0.231	0.084
FH1	52	28	132	1 (1)	0.000	0.024	0.125	-0.006
FH39	58	34	133	3 (1)	0.018	0.028	0.334	0.037
FH40	57	30	132	0 (0)	0.000	0.000	0.224	-0.089
FH67	60	25	131	2 (0)	0.000	0.03	0.884	-0.001
FH103	57	38	131	1 (0)	0.000	0.024	0.263	-0.038
La4	55	40	132	3 (0)	0.000	0.049	0.044	-0.089
La6	59	25	133	1 (1)	0.037	0.027	0.022	0.08
LafMS02	56	28	133	0 (0)	0.000	0.000	0.661	-0.047
LafMS06	58	28	133	3 (0)	0.000	0.043	0.426	-0.022

14 References

Abbott, D. H., Saltzman, W., Schulz-Darken, N. J. & Smith, T. E. 1997. Specific neuroendocrine mechanisms not involving generalized stress mediate social regulation of female reproduction in cooperatively breeding marmoset monkeys. *Ann. N.Y. Acad. Sci.*, **807,** 219– 238.

Amos, W. & Hoezel, A. R. 1991. Long term preservation of whale skin for DNA analysis. In: *Genetic Ecology of Whales and Dolphins (Special issue 13)* (Ed. by Hoelzel AR), pp. 99-104: International Whaling Commission.

Amos, W., Twiss, S., Pomeroy, P. P. & Anderson, S. S. 1993. Male mating success and paternity in the grey seal, *Halichoerus grypus*: a study using DNA fingerprinting. *Proc. R. Soc. Lond. B*, **252,** 199-207.

Barkham, J. P. & Rainy, M. E. 1976. The vegetation of the Samburu-Isiolo Game Reserve. *Afr. J. Ecol.*, **14,** 297-329.

Barnes, R. F. W. 1982. Mate searching behaviour of the elephant bulls in a semi-arid environment. *Anim. Behav.*, **30,** 1217-1223.

Baum, L. E. & Petrie, T. 1966. Statistical inference for probabilistic functions of finite state Markov chains. *Ann. Math. Stat.*, **37,** 1554-1563.

Bayes, M. K., Smith, K. L., Alberts, S. C., Altmann, J. & Bruford, M. W. 2000. Testing the reliability of microsatellite typing from faecal DNA in the savannah baboon. Conserv Genet 1:173-176. *Conserv. Genet.*, **1,** 173-176.

Blanc, J. J., Thouless, C. R., Hart, J. A., Dublin, H. T., Douglas-Hamilton, I., Craig, G. C. & Barnes, R. F. W. 2003. African Elephant Status Report 2002: An update from the African elephant database., pp. 229. Gland, Switzerland and Cambridge, UK: UCN/SSC/-AfESG.

Brain, P. F. 1979. Effects of the pituitary-gonadal axis on behaviour. In: *Chemical Influences on Behaviour* (Ed. by Brown, K. & Cooper, S. J.), pp. 255– 329. London: Academic Press.

Brannian, J. D., Griffin, F. & Terranova, P. F. 1989. Urinary androstenedione and luteinizing hormone concentration during musth in a mature African elephant. *Zoo Biology*, **8,** 165-170.

Broquet, T. & Petit, E. 2004. Quantifying genotyping errors in non-invasive population genetics. *Mol. Ecol.*, **13,** 3601-3608.

Clubb, R. & Mason, G. 2002. A review of the welfare of zoo elephants in Europe. Report pp. 303. Oxford: RSPCA.

Clutton-Brock, T. H. 1989. Mammalian mating systems. *Proc. R. Soc. Lond. B*, **236,** 339-372.

Clutton-Brock, T. H., Guiness, F. E. & Albon, S. D. 1982. *Red deer- behaviour and ecology of two sexes*. Chicago: University of Chicago Press.

Clutton-Brock, T. H. & Harvey, P. H. 1978. Mammals, resources and reproductive strategies. *Nature*, **273,** 191-195.

Coltman, D. W., Bancroft, D. R., Robertson, A., Smith, J. A., Clutton-Brock, T. H. & Pemberton, J. M. 1999. Male reproductive success in a promiscuous mammal: behavioural estimates compared with genetic paternity. *Mol. Ecol.*, **8,** 1199-1209.

Coltman, D. W., Festa-Bianchet, M., Jorgenson, J. T. & Strobeck, C. 2002. Age-dependent sexual selection in bighorn rams. *Proc. R. Soc. Lond. B*, **269,** 165-172.

Comstock, K. E., Georgiadis, N., Pecon-Slattery, J., Roca, A. L., Ostrander, E. A., O'Brien, S. J. & Wasser, S. K. 2002. Patterns of molecular genetic variation among African elephant populations. *Mol. Ecol.*, **11,** 2489-2498.

Cooper, K. A., Harder, J. D., Clawson, D. H., Fredrick, D. L., Lodge, G. A., Peachey, H. C., Spellmire, T. J. & Winstel, D. P. 1990. Serum testosterone and musth in captive male African and Asian elephants. *Zoo Biology*, **9,** 297-306.

Dakin, E. E. & Avise, J. C. 2004. Microsatellite null alleles in parentage analysis. *Heredity*, **93,** 504-509.

Darwin, C. 1871. *The decent of man, and selection in relation to sex*. London: John Murray.

Douglas-Hamilton, I. 1972. On the ecology and behaviour of the African elephant.: D.Phil thesis: University of Oxford.

Douglas-Hamilton, I. 1998. Tracking African elephants with a global positioning system (GPS) radio collar. *Pachyderm*, **5,** 81-92.

du Plessis, W. P. 2001. Effective rainfall defined using measurements of grass growth in the Etosha National Park, Namibia. *J. Arid Environ.*, **48,** 397-417.

Durbin, R., Eddy, S., Krogh, A. & Mitchinson, G. 1998. *Biological sequence analysis: Probabilistic models of protein and nucleic acids*. Cambridge: Cambridge University Press.

Eggert, L. S., Rasner, C. A. & Woodruff, D. S. 2002. The evolution and phylogeography of the African elephant inferred from mitochondrial DNA sequence and nuclear microsatellite markers. *Proc. R. Soc. Lond. B*, **269,** 1993-2006.

Eisenberg, J. F., McKay, G. M. & Jainudeen, M. R. 1971. Reproductive behaviour of the Asiatic elephant (*Elphas maximus maximus* L.). *Behaviour*, **38,** 192-225.

Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection and the evolution of mating systems. *Science*, **197,** 215-223.

Fayrer-Hosken, R. A., Grobler, D., Van Altena, J. J., Bertschinger, H. J. & Kirkpatrick, J. F. 2000. Immunocontraception of African elephants. *Nature*, **407,** 149.

Foley, C. A. H., Papageorge, S. & Wasser, S. K. 2001. Noninvasive stress and reproductive measures of social and ecological presurres in free-ranging African elephants. *Conserv.. Biol.*, **15,** 1134-1142.

Forchhammer, M. C. & Boomsma, J. J. 1998. Optimal mating strategies in nonterritorial ungulates: a general model tested on muskoxen. *Behav. Ecol.*, **9,** 136-143.

Forney, G. D. 1973. The Viterbi algorithm. *Proc.IEEE*, **61,** 268-278.

Gadgil, M. 1972. Male dimorphism as a consequence of sexual selection. *Am. Nat.*, **106,** 574-580.

Gagneux, P., Boesch, C. & Woodruff, D. S. 1997. Microsatellite scoring errors associated with non-invasive genotyping based on nuclear DNA amplified from shed hair. *Mol. Ecol.*, **6,** 861-868.

Ganswindt, A. 2004. Endocrine,Physical and Behavioural Correlates of Musth in African Elephants (*Loxodonta africana*). Ph.D. thesis, pp. 91. Munster: University Westfalen, Munster.

Ganswindt, A., Heistermann, M., Borragan, S. & Hodges, J. K. 2002. Assesment of tesicular endocrine function in captive African elephants by measurements of urinary and fecal androgens. *Zoo Biology*, **21,** 27-36.

Ganswindt, A., Heistermann, M. & Hodges, J. K. 2005 (in press). Physical, physiological and behavioural correlates of musth in captive African elephants (*Loxodonta africana*). *Physiol. Biochem. Zoo.*.

Ganswindt, A., Heistermann, M., Palm, R., Borragan, S. & Hodges, J. K. 2003. Non-invasive assesment of adrenal function in the male African elephant (*Loxodonato africana*) and its relation to musth. *Gen. Comp. Endocr.*, **134,** 156-166.

Gasaway, W. C., Gasaway, T. K. & Berry, H. H. 1996. Persistent low densities of plains ungulates in Etosha National Park, Namibia: Testing the food-regulating hypothesis. *Can. J. Zool*, pp. 1556-1572.

Georgiadis, N., Bischof, L., Templeton, A., Patton, J., Karesh, W. & Western, D. 1994. Structure and history of the African elephant population: 1. Eastern and Southern Africa. *J. Hered.*, **85,** 100-104.

Georgiadis, N., Hack, M. & Turpin, K. 2003. The influence of rainfall on zebra population dynamics: implications for management. *J. Appl. Ecol.*, **40,** 125–136.

Goward, S. M. & Prince, S. D. J. 1995. Transient effects of climate on vegetation dynamics: satellite observations. *J. Biogeogr.*, **22,** 549-563.

Gross, M. R. 1996. Alternative reproductive strategies and tactics: diversity within sexes. *TREE*, **11,** 92-98.

Hall-Martin, A. J. 1984. Plasma testosterone levels in relation to musth in the male African elephant. *Koedoe*, **27,** 147-149.

Hall-Martin, A. J. 1987. Role of musth in the reproductive strategy of the African elephants (*Loxodonta africana*). *S. Afr. J Sci.*, **83,** 616-620.

Hanks, J. 1972a. Aspects of dentation of the African elephant, *Loxodonta africana*. *Arnoldia*, **5,** 1-8.

Hanks, J. 1972b. Growth of the African elephant (*Loxodonta africana*). *E. Afr. Wildlife J.*, **10,** 251-272.

Hodges, J. K. 1998. Endocrinology of the ovarian cycle and pregnancy in the Asian (Elephas maximus) and African (Loxodonta africana) elephant. In: *Anim. Repro. Sci.*, pp. 3-18.

Hoffman, J. I. & Amos, W. 2005. Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. *Mol. Ecol.*, **14,** 599-612.

Huber, S., Palm, R. & Arnold, W. 2003. Effects of season,sex and sample collection on concentrations of fecal cortisol metabolites in red deer (*Cervus elaphus*). *Gen. Comp. Endocr.*, **130,** 48-54.

Jachmann, H. 1985. Estimating age in African elephants. *Afr. J. Ecol*, **23,** 199-202.

Jachmann, H. 1988. Estimating age in African elephants: a revision of Law's molar evaluation technique. *Afr J. Ecol.*, **26,** 51-56.

Jainudeen, M. R., Katongole, C. B. & Short, R. V. 1972. Plasma testosterone levels in relation to musth and sexual activity in the male Asiatic elephant, *Elphas maximus*. *J. Reprod. Fert.*, **29,** 99-103.

Jarman, P. J. & Southwell, C. J. 1986. Grouping, associations and reproductive strategies in the eastern grey kanaroos. In: *Ecological aspects of social evolution* (Ed. by Rubenstein, D. I. & Wrangham, R. W.), pp. 399-428: Princeton University Press.

Jeffery, K. J., Keller, L. F., Arcese, P. & Bruford, M. W. 2001. The development of microsatellite loci for song sparrow, Melospiza melodia (Aves) and genotyping errors associated with good quality DNA. *Mol. Ecol. Notes*, **1,** 11-13.

JoGayle, H., Bush, M., de Vos, V. & Wildt, D. E. 1984. Electroejaculation, semen characteristics and serum testosterone concentrations of free-ranging African elephants (*Loxodonta africana*). *J. Reprod. Fert.*, **72,** 187-195.

Kahl, M. P. & Armstrong, B. D. 2002. Visual displays of wild African elephants during musth. *Mammalia*, **66,** 159-171.

Kenagy, G. J., Place, N. J. & Veloso, C. 1999. Relation of glucocorticosteroids and testosterone to the annual cycle of free-living Degus in semiarid central Chile. *Gen. Comp. Endocr.*, **115,** 236-243.

Knapp, R. 2004. Endocrine mediation of vertebrate male alternative reproductive tactics: The next generation of studies. *Integr. Comp. Biol.*, **43,** 658-668.

Kwok, S., Kellog, D. E., McKinney, N., Spasic, D., Goda, L., Levenson, C. & Sninsky, J. J. 1990. Effects of primer-template mismatches on the polymerase chain reaction: human immunodeficiency virus 1 model studies. *Nucleic Acids Research*, **18,** 999-1005.

Laws, R. M. 1966. Age criteria for the African elephant, *Loxodonta africana*. *E. Afr. Wildlife J.*, **4,** 1-37.

Laws, R. M., Parker, I. S. C. & Johnstone, R. C. B. 1975. *Elephants and their habitat:the ecology of elephants in North Bunyoro, Uganda*. Oxford: Clarendon Press.

Le Boeuf, B. J. 1974. Male-male competition and reproductive success in elephant seals. *Am. Zool.,*, **14,** 163-176.

Lee, P. C. & Moss, C. J. 1995. Statural growth in known-age African elephants (*Loxodonta africana*). *J. Zool. ,Lond.*, **236,** 29-41.

Leshner, A. I. & Moyer, J. A. 1975. Androgens and agonistic behavior in mice: relevance to aggression and irrelevance to avoidance-of-attack. *Physiol Behav.*, **15,** 695– 699.

Lincoln, G. A. & Ratnasooriya, W. D. 1996. Testosterone secretion, musth behaviour and social dominance in captive male Asian elephants living near the equator. *J. Reprod. Fert.*, **108,** 107-113.

Lindeque, M. 1993. Post-natal growth of elephants *Loxodonta africana* in Etosha National Park, Namibia. *J.Zool.,Lond.*, **229,** 319-330.

Macdonald, D. E. 2001. *The New Encyclopedia of Mammals*. Oxford: Oxford University Press.

Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.*, **7,** 639-655.

Maynard Smith, J. 1976. Evolution and the theory of games. *Am. Sci.*, **64,** 41-45.

Moore, M. 1991. Application of organization-activation theory to alternative reproductive strategies: a review. *Horm. Behav.*, **25,** 154-179.

Moore, M., Hews, D. K. & Knapp, R. 1998. Hormonal control and evolution of alternative male phenotypes: Generalizations of models for sexual differentiation. *Am. Zool*, **38,** 133-151.

Moss, C. J. 1983. Oestrus behaviour and female choice in the African elephant. *Behaviour*, **86,** 167-196.

Moss, C. J. 1996. Getting to know a population. In: *Studying elephants* (Ed. by Kangwana, K. F.), pp. Chapter 7. Nairobi: African Wildlife Foundation.

Moss, C. J. 2001. The demography of an African elephant (*Loxodonat africana*) population in Amboseli, Kenya. *J. Zool, Lond.*, **255,** 145-156.

Moss, C. J. & Poole, J. H. 1983. Relationships and social structure of the African elephants. In: *Primate social relationships: An integrated approach* (Ed. by Hinde, R. A.), pp. 315-325. Oxford: Blachwell Scientific.

Möstl, E., Maggs, J. L., Schrötter, G., Besenfelder, U. & Palm, R. 2002. Measurement of Cortisol Metabolites in Faeces of Ruminants. *Vet. Res. Com.*, **30,** 74-84.

Ogawa, S., Robbins, A., Kumar, N., Pfaff, D. W., Sundaram, K., Bardin & C.W. 1996. Effects of testosterone and 7a-methyl-19-nortestosterone (MENT) on sexual behaviors in two inbred strains of male mice. *Horm. Behav.*, **30,** 74-84.

Ogutu, J. O. & Owen-Smith, N. 2003. ENSO, rainfall and temperature influences on extreme population declines among African savanna ungulates. In: *Ecol. Lett.*, pp. 412-419.

Okello, J., Wittemyer, G., Rasmussen, H. B., Douglas-Hamilton, I., Nyakaana, P., Arctander, P. & Siegismund, H. R. submitted. Non-invasive Genotyping and Mendelian Analysis of Microsatellites in African Savannah Elephants. *Heredity*, **XX,** XX-XX.

Oliveira, R. F., Canario, A. V. M. & Grober, M. S. 2001. Male sexual polymorphism, alternative reproductive tactics, and androgens in combtooth blennies (Pisces:Blenniidae). *Horm. Behav.* , **40,** 266-275.

Palm, R. & Möstl, E. 1993. Biotin-streptavidin enzyme immunoassey for the determination of oestrogens and androgens in boar faeces. In: *Advances of Steroid Analysis* (Ed. by Görög, S.), pp. 111-117. Budapest: Akade' miai Kiado'.

Pemberton, J. M., Albon, S. D., Guinness, F. E., Clutton-Brock, T. H. & Dover, G. A. 1992. Behavioural estimates of male success tested by DNA fingerprinting in a polygynous mammal. *Behav. Ecol.*, **3,** 66-75.

Perry, J. S. 1953. The reproduction of the African elephants, *Loxodonta africana*. *Phil. Trans. B*, **237,** 93-149.

Poole, J. 1994a. Logistical and ethical considerations in the management of elephant populations through fertility control. 278-283. In: *Proceedings, 2nd International Conference on Advances in Reproductive Research in Man and Animals.* Charanjit Singh Bambra (Ed.). Institute of Primate Research, National Museums of Kenya: pp. 278-283.

Poole, J. H. 1987. Rutting behaviour in the African elephants: The phenomenon of musth. *Behaviour*, **102,** 283-316.

Poole, J. H. 1989a. Announcing intent:the aggressive state of musth in African elephants. *Anim. Behav.*, **37,** 140-152.

Poole, J. H. 1989b. Mate guarding, reproductive success and female choice in African elephants. *Anim. Behav.*, **37,** 842-849.

Poole, J. H. 1994b. Sex differences in the behaviour of African elephants. In: *The Differences Between the Sexes* (Ed. by Short, R. & Balaban, E.), pp. 331-346. Cambridge: Cambridge University Press.

Poole, J. H., Kasman, L. H., Ramsay, E. C. & Lasley, B. L. 1984. Musth and urinary testosterone concentrations in the African elephant (*Loxodonta africana*). *J. Reprod Fert.*, **70,** 255-260.

Poole, J. H. & Moss, C. J. 1981. Musth in the African elephant, *Loxodonta africana*. *Nature*, **292,** 830-831.

Poole, J. H. & Moss, C. J. 1989b. Elephant mate searching: group dynamics and vocal and olfactory communication. *Symp. Zool Soc , Lond.*, **61,** 111-125.

Queller, D. C. & Goodnight, K. F. 1989. Estimating relatedness using genetic markers. *Evolution*, **43,** 258-275.

Rabiner, L. R. 1989. A tutorial on Hidden Markov Models and selected applications in speech recognition. *Proc. IEEE*, **77,** 257-286.

Raeside, J. I., Renaud, R. L. & Marshall, D. E. 1992. Identification of 5-alpha-androstane-3-beta, 17-beta-diol and 3-beta-hydroxy-5-alpha-androstan-17-one sulfates as quantitatively significant secretory products of porcine leydig-cells and their presence in testicular venous-blood. *J. Steroid Biochem.*, **42,** 113-120.

Ramsay, M. A. & Sterling, I. 1986. On the mating system of polar bears. *Can. J. Zool*, **64,** 2142-2151.

Rasmussen, H. B., Wittemyer, G. & Douglas-Hamilton, I. Submitted. Predicting Time Specific Reproductive Rates using Remote Sensing Data. *J. Appl. Ecol.*.

Rasmussen, L. E. L., Hall-Martin, A. J. & Hess, D. L. 1996. Chemical profiles of male African elephants,*Loxodonta africana*: Physiological and ecological implications. *J. Mam.*, **77,** 422-439.

Rasmussen, L. E. L. & Perrin, T. E. 1999. Physiological correlates of musth: Lipid metabolites and chemical composition of exudates. *Phys. Behav.*, **67,** 539-549.

Roberts, M. L., Buchanan, K. L. & Evans, M. R. 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. *Anim Behav*, **68 part 2,** 227-239.

Roca, A. L., Georgiadis, N., Pecon-Slattery, J. & O'Brien, S. J. 2001. Genetic Evidence for Two Species of Elephant in Africa. *Science*, **293,** 1473-1477.

Roon, D. A., Thomas, M. E., Kendall, K. C. & Waits, L. P. 2005. Evaluating mixed samples as a source of error in non-invasive genetic studies using microsatellites. *Mol Ecol.*, **14,** 195-201.

Rose, R. M., Bernstein, I. S., Gordon, T. P. & Catlin, S. F. 1974. Androgens and aggression: a review and recent findings in primates. In: *Primate Aggression, Territoriality, and Xenophobia.* (Ed. by Holloway, R. L.), pp. 275–304. New York: Academic Press.

Rubenstein, D. I. 1980. On the evolution of alternative mating strategies. In: *Limits to action* (Ed. by Staddon, J. E. R.), pp. 65-100. New York: Academic Press.

Scanlon, T. M., Albertson, J. D., Caylor, K. K. & Williams, C. A. 2002. Determining land surface fractional cover from NDVI and rainfall time series for a savanna ecosystem. *Remote. Sens. Environ.*, **82,** 376-388.

Shchaller, G. 1967. *The deer and the Tiger. A study of wildlife in India*. Chicago: University of Chicago Press.

Short, R. V. 1969. Notes on the teeth and ovaries of an African elephant (*Loxodonta africana*) of known age. *J.Zool.,Lond.*, **158,** 421-425.

Siegismund, H. & Arctander, P. 1995. Structure of African elephant populations. *J. Hered.*, **86,** 467-468.

Sikes, S. K. 1967. The african elephant, *Loxodonta africana:* a field method for the estimation of age. *J.Zool.,Lond.*, **154,** 235-248.

Sikes, S. K. 1971. *The natural history of the African elephant*. London: Weidenfeld and Nicolson.

Sinclair, A. R. E., Dublin, H. & Borner, M. 1985. Population Regulation of Serengeti Wildebeest - a Test of the Food Hypothesis. In: *Oecologia*, pp. 266-268.

Sinclair, A. R. E., Mduma, S. A. R. & Arcese, P. 2000. What determines phenology and synchrony of ungulate breeding in Serengeti? *Ecology*, **81,** 2100-2111.

Slotow, R., van Dyk, G., Poole, J., Page, B. & Klocke, A. 2000. Older bull elephants control younger males. *Nature*, **408,** 425-426.

Stearns, C. J. 1992. *The Evolution of Life Histories*. Oxford: Oxford University Press.

Sukumar, R. 2003. *The living elephants*. Oxford: Oxford University Press.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual selection and the descent of man 1871-1971* (Ed. by Cambell, B.), pp. 136-179. Chicago: Aldine Publishing Company.

Uglem, I., Mayer, I. & Rosenquist, G. 2002. Variation in plasma steroids and reproductive traits in dimorphic males of corkwing wrasse (*Symphodus melops* L.). *Horm Behav.*, **41,** 396-404.

Viau, V. & Meaney, M. J. 2004. Testosterone-dependent variations in plasma and intrapituitary corticosteroid binding globulin and stress hypothalamic–pituitary–adrenal activity in the male rat. *J. Endocrinol.*, **181,** 223-231.

Wasser, S. K., Risler, L. & Steiner, R. A. 1988. Excretory steroids in primate feces over the menstrual cycle and pregnancy. *Biol. Reprod.*, **39,** 862-872.

Wattier, R., Engel, C. R., Saumitou-Laprade, P. & Valero, M. 1998. Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus Gv1CT in *Gracilaria gracilis* (Rhodophyta). *Mol. Ecol.*, **7,** 1569-1573.

Wessels, K. J., Prince, S. D., Frost, P. E. & van Zylc, D. 2004. Assessing the effects of human-induced land degradation in the former homelands of northern South Africa with a 1 km AVHRR NDVI time-series. *Remote. sens. environ.*, **91,** 47-67.

Whitehead, H. 1990. Rules for roving males. *J.theor.Biol.*, **145,** 355-368.

Whitehead, H. 1994. Delayed competitiv breeding in roving males. *J.theor.Biol.*, **166,** 127-133.

Whitehouse, A. M. & Hall-Martin, A. J. 2000. Elephants in Addo Elephant National Park, South Africa: reconstruction of the population's history. *Oryx*, **34,** 46-55.

Whitehouse, A. M. & Harley, E. H. 2002. Paternity in the Addo elephant population, South Africa. Is a single male monopolizing matings? *Afr. Zool.*, **37,** 247-253.

Whitehouse, A. M. & Harley, H. E. 2001. Post-bottleneck genetic diversity of elephant populations in south Africa, revealed using microsatellite analysis. *Mol. Ecol.*, **10,** 2139-2149.

Whitehouse, A. M. & Schoeman, D. S. 2003. Ranging behaviour of elephants within a small, fenced area in Addo Elephant National Park, South Africa. *Afr. Zool.*, **38,** 95-108.

Wikelski, M., Steiger, S. S., Gall, B. & Nelson, K. N. 2004. Sex, drug, and mating role: testosterone-induced phenotype-switching in Galapagos marine iguanas. *Behav. Ecol.*, **16,** 260-268.

Wingfield, J. C. 1984. Androgens and mating systems: Testosterone-induced polygony in normally monogamous birds. *Auk*, **101,** 665-671.

Wittemyer, G. 2001. The elephant population of Samburu and Buffalo Springs National Reserves, Kenya. *Afr, J. Ecol.*, **39,** 357-365.

Wittemyer, G., Daballen, D., Rasmussen, H. B., Kahindi, O. & Douglas-Hamilton, I. 2005. Demographic status of elephants in the Samburu and Buffalo Springs National Reserves, Kenya. *Afr, J. Ecol.*,**43,** 44-47.

Wittemyer, G., Douglas-Hamilton, I. & Getz, W. M. In Press-b. The socioecology of elephants: analysis of the processes creating multitiered social structures. *Anim. Behav.*.

Wittemyer, G., Rasmussen, H. B. & Douglas-Hamilton, I. submitted. The influence of variability in primary productivity on female elephant reproductive strategies *Oikos*.

Ziegler, T., Hodges, J. K., Winkler, P. & Heistermann, M. 2000. Hormonal correlates of reproductive seasonality in wild female Hanuman Langurs (*Presbytis entellus*). *Am. J. Prim.*, **51,** 119-134.