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A genetic insight to the population of African savannah elephants (*Loxodonta africana*) in the Serengeti Ecosystem, Tanzania.

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PROBLEM DESCRIPTION

The African elephants (*Loxodonta africana*) have had a turbulent past, especially the last two centuries. Prior to the CITES ivory ban in 1989, the elephants saw a drastic reduction and is now confined mostly to protected areas. One of the areas which were hit the hardest is the Serengeti ecosystem, with a severe history of elephant poaching. And now, with the increasing elephant population and the exponential human population growth, human-elephant conflicts are a new major problem in the communities adjacent to the protected areas in the ecosystem. Therefore it is important to catalogue the genetic composition of the elephants to understand their evolutionary history, their overall diversity and if there are any signs of compartmentalization inside the population. An insight into the answers to these questions will act as a backbone for further studies with wider approaches, and act as a catalyst for wanting to learn more about the Serengeti elephants. It will also add a new dimension to the conservation of the elephants and add new helpful information to future management plans.

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ABSTRACT

African savannah elephants play a vital role in the Serengeti ecosystem, with the opportunity to alter the entire ecosystem by its sheer number. Management of these animals are therefore of high importance, but little genetic research has been done thus far in the Serengeti. Their recent traumatic history of poaching serves as a template for intriguing evolutionary theories and further understanding of elephant behavior. In this study it was investigated on the genetic structure and spatial differentiation of the elephants in Serengeti using a mitochondrial DNA (mtDNA) marker. A widespread sample size of 55 elephants were collected in three zones of the Serengeti National Park (West, North and Seronera) and analyzed for genetic diversity. The results gave the impression of a slightly outbreeding population with no ongoing subdivision ($F_{ST} = -0.04864$, $p = 0.92082$). A total of 7 haplotypes were obtained with one clearly being dominant (78.2 %). All collected haplotypes were compared to earlier studies that use the same mitochondrial marker and have a wider perspective, with samples ranging all across the sub-Saharan Africa. Results show that there is a possibility that the elephants now inhabiting the Serengeti are primary descendants of Northern populations coming from Kenya and Uganda, with additional individuals giving the impression that the Serengeti elephants are a mixture of individuals with historical connections from all over sub-Saharan Africa.

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1 Introduction

1.1 Implications of wildlife protected areas

With heavy population growth and human expansion on the African continent, wildlife is now more confined to restricted areas such as national parks and game reserves (Harmon & Brechin 1994). Species may be segregated into sub-populations because of this; as the human settlements surrounding these areas might prevent animals from migrating freely in and out, thus downgrading the gene flow between populations. Gene flow from outside populations assures higher genetic diversity among the populations and is an advantage in case of sudden severe environmental changes. A lack of it may lead to genetic isolation, leaving populations more vulnerable to change (O'Brien 1994; Futuyama 2005). In addition, by confining animals to smaller areas unnatural in size compared to what once was; ecological consequences are likely to arise in the long run (Holdo et al. 2009). For this very reason it is highly important to have management plans in these areas. This is especially true for the larger mammals, which have, for millennia, wandered across the African continent without the severe human interruptions of today. The African savannah elephant (*Loxodonta africana*) is such an animal, and is capable of migrating over enormous distances (Nyakaana et al. 2002). It is then reasonable to suggest that freer roaming possibilities for larger animals might serve to uphold healthier populations and steadier environments. With the rate the African population is growing in, such scenarios are highly unlikely at present time.

1.2 Key species

The study and conservation of the big impact animals (so called key species) in an ecosystem is important to keep a healthy balance between possible detrimental factors for that given system (Dublin et al. 1990). In Africa there are several such impact species beside the already mentioned elephants, with large herds of grazers, such as various species of antelopes, African buffaloes (*Syncerus caffer*) and zebras which help maintain the vegetation, and large carnivores such as lions (*Panthera leo*), spotted hyenas (*Crocuta crocuta*) and leopards (*Panthera pardus*) which help maintain the herbivorous population (Dobson 2009). The elephants are, among the animals in the African savannah ecosystem, maybe the biggest game changers of today (Dublin et al. 1990). This might have been true for the rhinos in the past as well, but as their numbers are dramatically reduced all over the world, their impact is not as obvious as their pachyderm cousins (Fyumagwa & Nyahongo 2010). Because of their

browsing behavior and large size, elephants can quickly transform the vegetative state of an area (Dublin et al. 1990). One important role they have is to maintain landscape heterogeneity by promoting the coexistence of trees and grass species. The elephants suppress the tree cover, and the tree-grass balance is regulated by the number of elephants. With a total lack of elephants, the woodlands will take over, and with too many elephants the ecosystem may be transformed into open grasslands (Goheen & Palmer 2010).

1.2.1 Examples from the Serengeti-Mara ecosystem

In addition to the living factors of a typical African savannah ecosystem there are also the abiotic factors of drought and fires. Drought, being a seasonal event of intolerable heat and lack of water, is changing entire ecosystems, making animals wander great distances to find what water is available, and fires, which are frequent and often used by man to help regulate vegetation, may also have great impacts on the ecosystem. Abnormal increase in the frequency of burning may affect and alter ecosystems noticeably, which was the case in the 1960's, when fires decreased large amounts of woodlands in the Serengeti-Mara ecosystem (Dublin et al. 1990). The woodlands of the Serengeti-Mara had prior to this already increased heavily, a result of rinderpest severely decreasing the wildebeest population in the 1890's, making them less capable of regulating woodland growth (Dublin et al 1990). To promote woodlands even further, there were also few elephants present at this time, due to heavy poaching (Sharam & Estes 2000; Fyumagwa & Nyohongo 2010). After becoming more of an open grassland ecosystem after the 1960's fires, the 1980's saw little or no woodland recruitment, as the elephants managed to effectively prevent woodland growth. Even though the wildebeest population had now risen to more than 1 million animals, studies have found that elephants seem to be the most important biological factor when it comes to preventing woodland recovery. Wildebeest, on the other hand, seems more effective in maintaining the status quo, rather than preventing growth (Dublin et al. 1990). As the aforementioned environmental factors of an African ecosystem have the ability to impact a system in such ways, it is important for us to help uphold the natural balance when nature cannot regulate itself in a healthy direction in confined areas.

1.3 Human-wildlife conflicts

When human settlements and manmade barriers prevent animals to migrate freely between areas, it leads to negative consequences for both the local people and the wildlife. This results in human-wildlife conflicts, which is a growing problem in Africa because of the steadily increasing human population (Sarker 2010). Even though the human settlements are outside the areas in mention, they are also an important factor to consider during management and conservation when they are in close vicinity to protected areas. There is a continued struggle to keep nearby settlements content with their situation and at peace with the surrounding wildlife. Because of wide-spread poverty among the African societies, exploitations of wildlife are always a quick resolution to earn money. Poaching or illegal hunting of bush meat is a problem throughout Africa, and are, despite increasing efforts, hard to prevent (Nyahongo 2010 (B)). There are also the problems of wildlife moving into human settlements outside of the protected areas, interfering with human livelihood. Large carnivores, and especially lions, have taken livestock from humans for centuries, and in the more serious events, even humans, which in most cases turn into retaliation from the local population and leads to the killing of numerous lions (Ikanda 2010). Another huge conflict is the human-elephant conflict, also present in Asia, where herds of elephants completely devours entire fields of crops during the night, and sometimes even destroys property by trampling, leaving the injured party with a devastating loss in both valued goods and personal housing (Sarker 2010).

As the human population continues to expand, conflicts like this is inevitable and is only likely to increase in frequency. Continued research is therefore important to help reduce the damage done when these two worlds collide, and in many cases resolutions are underway, such as the honeybee fences in Eastern Africa. These help in keeping the elephants at bay, as elephants seem to avoid the presence of honeybees (Vollrath & Douglas-Hamilton 2002; King et al. 2010). Additional solutions involve getting the local society more associated in the conservation and management of the protected areas (Hanks 2001). Educating them in the important role that animals play in the society and ecosystem provides important knowledge. It is further important to find measures to alleviate poverty, and to consider the cultural viewpoints and ecological state of the area when involving the local communities in conservation (Nyahongo 2010 (A)).

1.4 Conservation genetics

Conservation and management plans are often associated with ecological studies, but in fact the best way to successfully conserve an ecosystem is to bring different disciplines together (O'Brien 1994). One of these is the area of genetic studies. Population genetics has been on the rise in recent time, but is still not as widespread on the African continent because of the technological disadvantage. Genetic studies have many usable elements in conservational studies, and can be used to tell how genetically diverse a population is, its evolutionary history, the creation of in situ sub-populations and speciation, and problems such as inbreeding and isolation (O'Brien 1994). For many management areas it is very important to establish a consensus regarding these subjects. The genetic diversity of a species in a given ecosystem is important to establish if the population is undergoing inbreeding (showing abnormal amounts of homozygosity), and to declare how fit the population is at a whole, meaning how well they will react to drastic change and diseases. The evolutionary history of a population is important to obtain, as one can find out how long a species, or population, has been residing in a certain area, and from where it once came. The establishment of sub-populations in situ is also important to recognize because it might indicate evolutionary processes happening in an enclosed area, and if there are certain ecological factors encouraging the creation of sub-populations (O'Brien 1994; Costantini et al. 2009). Such factors may be certain niches exploited by certain individuals of the species, or ecological wastelands between resource rich areas. The latter creates a non-physical barrier individuals seldom cross, as the cost of crossing it is not preferential, and hence leads to the creation of sub-populations (Costantini et al. 2009). Isolation between populations from different areas undergoes the same process as the creation of sub-populations, only in a grander scale, and may lead to speciation if there is sufficient amount genetic diversity or deprivation when diversity is low (Futuyama 2005). It is important to detect this genetically to detect if the segregation process should be deemed unviable for a given population. Countermeasures may then be commenced.

1.5 The African elephant

As already mentioned, the African elephant is one of the most influential factors of the African savannah ecosystem, and by its sheer impact is one of the most important species to conserve and manage. It is the largest terrestrial animal living today and an icon of the African wilderness (Blanc 2008). Being subjected to considerable research during the years, a lot of information has been gathered on the giant pachyderm. Even the suggestion to separate the African elephant into two species, the African savannah elephant (*L. africana*) and African forest elephant (*L. cyclotis*) has been considered in various studies (Eggert et al. 2002). Because of the vast range and wide variety of habitats covered by the elephant across the African continent, distribution, density and number estimates have been hard to obtain. Adding to the fact that the history of the species has been quite variable across the continent, the cross-continental status of the African elephant is highly inconsistent. Since the mid 1990's the knowledge regarding its status has been steadily increasing, but even though large gaps still remain, it is, at present time, considered by IUCN to be vulnerable across its current range (Blanc 2008).

1.5.1 Social structure

Genetic studies of the African savannah elephant are an important factor in its conservation program, but an overview in their social behavior is essential to get a basic view in how they relate to each other, by way of genetically relatedness and social relationships. The social make-up of the African savannah elephants is a complex matter, consisting of multiple levels of social and spatial relationships between groups and individuals (Douglas-Hamilton 1972; Moss & Poole 1983; Moss 1988). The “family group” is the most basic form of social units and is made up by one or more adult females and their offspring. The individuals of these groups tend to spend most of their time together in the same activities, it being foraging, bathing or resting, under the leadership of a sole matriarch. As most females keeps to her family group the entire life, it is reasonable to say all members are closely related. It is reported, however, that unrelated groups may fuse on rare occasions (Moss 1988). The home range of a family group is mainly issued by factors such as the distribution of water, vegetation and human presence and varies between the dry and wet seasons (Harris et al. 2008).

In contrast to the females, male elephants leave their natal groups in their mid-teens when reaching sexual maturity. The males will then spend the rest of their life wandering alone, having associations with different family groups and sometimes coalesce into short-term male-groups. Rivaling confrontations among male elephants increase as they mature, and they frequently engage in contests to establish their rank and dominance in the local population. Their aggressiveness towards each other and their mating success is at its peak upon reaching musth, a hormonal condition experienced every year by males, starting in their late twenties (Poole & Moss 1981; Poole 1987). The home range of males is much larger than that of a family group, as they move between family groups and are never in any need to protect family members. The latter makes the males more prone to wander larger distances between ideal habitats (rich on water and vegetation), than female led family groups, and increases their likeliness to get closer to human settlements (Harris et al. 2008). The males also avoid population inbreeding effectively by engaging in less sexual behavior towards related females (Archie et al. 2007).

Many family groups are associated and often share home ranges. These family groups are often referred to as “clans”. This form of associations has no basis in possible genetic relationships and is defined purely by the geographical area the groups are confined to (Moss & Poole 1983; Moss 1988). Molecular studies have confirmed the non-relative status of these clans (Charif et al. 2005). Inside these clans, some family groups tend to associate more with each other. Such associated groups are called “bond groups”, often fusing and engaging in the same behaviors (Moss & Poole 1983; Moss 1988). The greeting behaviors expressed as these family groups meet after separation is similar to the way family members greet each other, which give the impression that these bond groups are genetically related (Douglas-Hamilton 1972; Moss 1988). This has been found to be the case in most situations. However in poached populations bond groups may evolve between unrelated groups because of lack of kin (Gobush et al. 2009).

1.5.2 Implications of poaching

Poaching has had a huge impact on elephant populations across the African continent, with the total number being more than halved in less than a decade from 1.3 million individuals to 600,000 between 1979 and 1987 (Gobush et al. 2008). Because of the longer and more developed tusks of older females and males, they are prime targets of poachers, leaving heavy poached areas with interrupted social structures. Family groups in such populations are often

deprived of matriarchs, causing internal disruptions on a group level. Without the safety and the leadership of an older and wiser elephant, the remaining individuals show an increase in stress and a decrease in reproductivity (Gobush et al. 2008). Unrelated, reduced family groups therefore create bond groups to increase their success. Such impacts on an elephant population are sustained and are clearly visible at present time, where the aforementioned consequences are still visible. Genetic studies have further confirmed the disrupted social structure in elephant populations affected by poaching (Gobush et al. 2009). This, sadly, has been a truth for many conservation areas throughout Africa.

1.6. Serengeti National Park

One of the most iconic and famous conservation areas in Africa is the Serengeti National Park, situated in Northern Tanzania, and has provided the inspiration for many documentaries over the years. It has been a symbol for the African wildlife as a whole for the outside world, and is for many a naturalist seen as the ultimate African dream. If one looks closer one sees that the Serengeti is not without its flaws, and has been a victim of illegal human activities for many years. With the expanding human population surrounding the park, it has seen its share of human-wildlife conflicts, especially because of its proximity to the heavily populated areas surrounding Lake Victoria. Work to satisfy both parties has been going on for years, but conflicts are ongoing still (Nyahongo 2010 (B)). The entire Serengeti ecosystem encompasses more than 30 000 km² of savannah and woodland, and it holds, together with the adjoining Masai Mara in Kenya, one of the largest densities of big mammals in the world (Sinclair & Arcese 1995; Holdo et al. 2009). It has a unique fauna, with the blue wildebeest (*Connochaetes taurinus*) migration as its most famous addition. The Serengeti ecosystem is balanced with the effects of the large herds of grazers, fires and browsers (mainly elephants) (Holdo et al. 2009). Of these three factors, the African savannah elephant is the least studied in the Serengeti ecosystem, and it is therefore crucial to study the local elephant populations closer. New data may then be implemented into future studies and management plans, and will give a better understanding on how this unique ecosystem works. As already mentioned, it has been shown that the elephants have a large effect on the woodlands in the Serengeti-Mara region because of their browsing habits, added by their tendency to break down trees (Dublin et al. 1990). They therefore seem to be upholding a balance in the ecosystem at their present number, furthering the importance of them being closely managed and monitored. Additionally we picked up on the presence of a serious ongoing human-elephant conflict, with

herds of elephants creating havoc when entering nearby human settlements outside of the park, making the focus on elephants a personal matter for the local residents.

1.6.1 The Serengeti elephants

The Serengeti has a healthy elephant population of 2100 individuals at present, but as in so many other areas; the elephants of the Serengeti have had a dark history of intense poaching. With ivory trade peaking in the 1880's and 1890's, most elephants were killed or chased away from the Serengeti. The elephants slowly began their return into the park in the 1930's, as the ivory trade decreased. The population then increased to a grand total of 2460 elephants up until the mid 1970's, where the Serengeti elephants went into its second mass slaughtering. The price of ivory increased and the poaching went on recklessly up until the CITES ivory ban in 1989. At that time, only 500 elephants were left in the park (Sharam & Estes 2000; Gobush et al. 2008). By connecting the history of the Serengeti elephants with the natural elephant social structure, it would be reasonable to expect some unrelated bond groups because of the potential lack of kin. Further, the population of today is solely based on the elephants which migrated back into the park in the 1930's, making the assumption that the genetic diversity is not as diverse as an untouched population, and even more so after the second depopulation in the 1970's and 1980's.

The Serengeti elephants seems to belong to a gene pool consisting of several populations (of those tested) in the nearby Northern vicinity, ranging up to central Kenya, based on earlier microsatellite studies (Comstock et al. 2002), giving the impression that the influx of elephants during the 1930's came mainly from the North. The nearby Ngorongoro and Tarangire populations show to be of a slightly different gene pool (Comstock et al. 2002). This may be an indication that the large southern plains of the Serengeti, and maybe the numerous Maasai settlements in the Ngorongoro Conservation Area near the border to Serengeti National Park, act as a barrier for elephant movement. This is further strengthened by the fact that most elephants tend to be located north of the plains, in the woodlands of the Western, Central and Northern parts of the park.

1.7 Elephant genetics

The importance of cataloguing the genetic material of elephants is clear when it comes to conservation and management plans. Still, there is a lack of detailed studies done in this area, despite its importance (Okello et al. 2008). Genetic studies can, as mentioned, tell if populations are differentiating from other populations or if they are in isolation, with no genetic influx from nearby populations. Microsatellites and mitochondrial DNA (mtDNA) are the most commonly used markers when cataloguing elephant populations by genetic methods, with microsatellites being able to check for homozygosity and possible inbreeding as they are found in the homologous nuclear DNA, and mtDNA, being matrilineally passed on to the offspring without recombination, have higher mutation rate than nuclear DNA and are well suited to track genetic relationships of groups within a species. An earlier study on East African elephant populations revealed that the degree of population subdivision measured with mitochondrial DNA was twice that measured with microsatellites (Nyakaana et al. 2002). In many areas in Eastern Africa, elephants are coming into conflict with humans, and places with high elephant density show huge impacts on the environment. In such cases, translocation of elephants to less populated areas may be a necessity. To have knowledge of the genetic composition in populations at such situations are of great importance. When the genetic distance between translocated elephants and the receiving population are measured to be high, a healthy and more genetically diverse population will often be the result of the union. Such translocation projects are also useful when you have populations showing low numbers in genetic diversity, and not only during pending ecological collapse and human-elephant conflicts. The necessity of actions such as this is increasing, and the genetic structure should always be high on the list of considerations before they are to be undertaken (Okello et al 2008).

1.8 Objectives

This study had the goal of obtaining a genetic insight into the elephants of the Serengeti, using a mitochondrial marker. By sampling different individuals from different parts of the Serengeti, it would be possible to show if there are any distinction between the populations in the central area of Seronera, the Western Corridor and the North (Figure 1), and if there might be indications of sub-populations in the making. As mentioned, there are few elephants on the Great Plains in the South, resulting in no data collection from areas south of the central area of Seronera. The goal was to establish a backbone for further genetic studies of elephants in

the Serengeti. By providing the mitochondrial DNA of a population-wide sample size of 55, one can begin drawing conclusions and set up new hypotheses for future studies. Additionally, several similar large scale cross-continental studies have been conducted on the elephant in Africa using mitochondrial markers, to which our results were to be measured up against. Other studies give an impression that the Serengeti elephants of today have come from the North. This study aimed to validate this hypothesis by examining how the Serengeti elephants aligned with other elephant populations across Africa. Thus, some conclusions are drawn on the evolutionary history of these elephants, and on where they are situated on the evolutionary tree of the African elephant as a whole.

2 Methods

2.1 Fieldwork

A Land Rover Discovery was used to drive through the areas of Seronera, the Western Corridor and the North in Serengeti National Park during dung sample collection (Figure 1), in the period early April until mid July, 2010. Using the Serengeti Wildlife Research Centre (SWRC) in Seronera as base area, all driving through the park were commenced from there. Two day trips were used when travelling through the Western Corridor and the Northern areas, with sleepovers in Lamadi or Bunda outside the park in the West, and in the Maasai village of Ololosokwan outside the park in the North. Tenting was also done occasionally in the North. Travelling around the Seronera areas were done in one day trips. An equal amount of trips in each area were accomplished. Trips along roads deviating from the main road were done when there was time. As elephants were spotted, they were slowly approached off road, when possible, and observed from a safe distance. Elephants defecating were spotted and noted, and the feces were retrieved when the elephants had moved a safe distance away from the area. The stool samples were picked up in rubber gloves and kept in a cooler during fieldwork, and stored later on in a freezer at the SWRC Veterinary laboratory. All samples were further transferred to containers filled with pure ethanol.

With the elephants being observed from the moving vehicle, most samples were collected nearby the main roads. Not all spotted elephants could be sampled, most often because of rough off-road terrain and conditions, and sometimes because of lacking dung samples and time restraints. The source elephants were identified during fieldwork and catalogued with

photographs, and the exact location of the droppings was obtained using Global Positioning System (GPS) (Figure 2). Additional information such as size, gender, group size and group structure were noted when possible. Especially the smaller individuals were tough to sex, and the exact group size was hard to obtain when the elephants were spread in dense woodlands and when the number of individuals was too big for counting (often during the fusing of several bond groups). Pseudoreplication was believed to be successfully evaded as matriarchs were catalogued with recognizable traits written down and photographed. Probable causes of error may have come in cases when several groups are fused together into 50 animals or more and several matriarchs may have been unaccounted for.

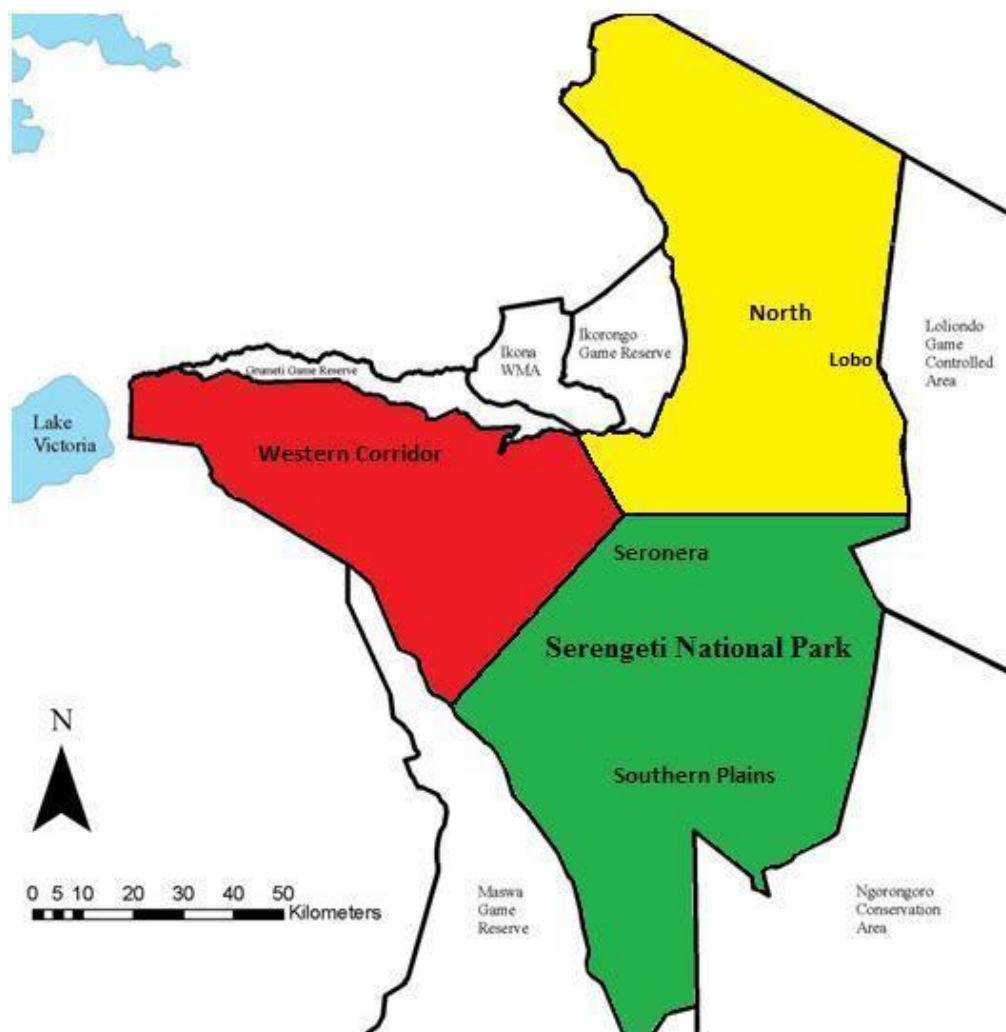


Figure 1: A map depicting Serengeti National Park with adjoining management areas. Each area in which we looked for elephants are marked with a color, red being the Western Corridor, yellow marking the North and green marking Seronera and the southern areas of the park. With it being considerably less elephants south of Seronera, centered in the middle, we rarely traveled further south than where the park name is situated on the map. The borders are unofficial and made solely by us to easily categorize the elephants into the different geographical areas.

A total of 98 fresh dung samples were collected from different individuals, representing 27 family groups and 43 lone males. The sampling was aimed to be balanced between the geographical areas. The end result gave 36 samples in Seronera, 28 in the Western Corridor and 34 in the North. Additionally, the samples were wide spread throughout each area, but with certain clusters where elephants were most likely to be found, such as by rivers and preferable vegetation (Figure 2). A balance between the number of family groups and male groups were tried to be upheld as well, which were successful in all areas except for the West. The samples from Seronera came from 11 groups and 12 males and those in the North from 12 groups and 11 males. The samples from the West were obtained from 4 groups and 20 males. With the West giving an unbalanced number between groups and males, and the fact that most individuals in a family group are closely related, the number of samples was reduced to 60 before work was continued. Each area was reduced to 20 samples, with one sample from each of the 27 family groups, and the remaining samples from the most geographically widespread males.

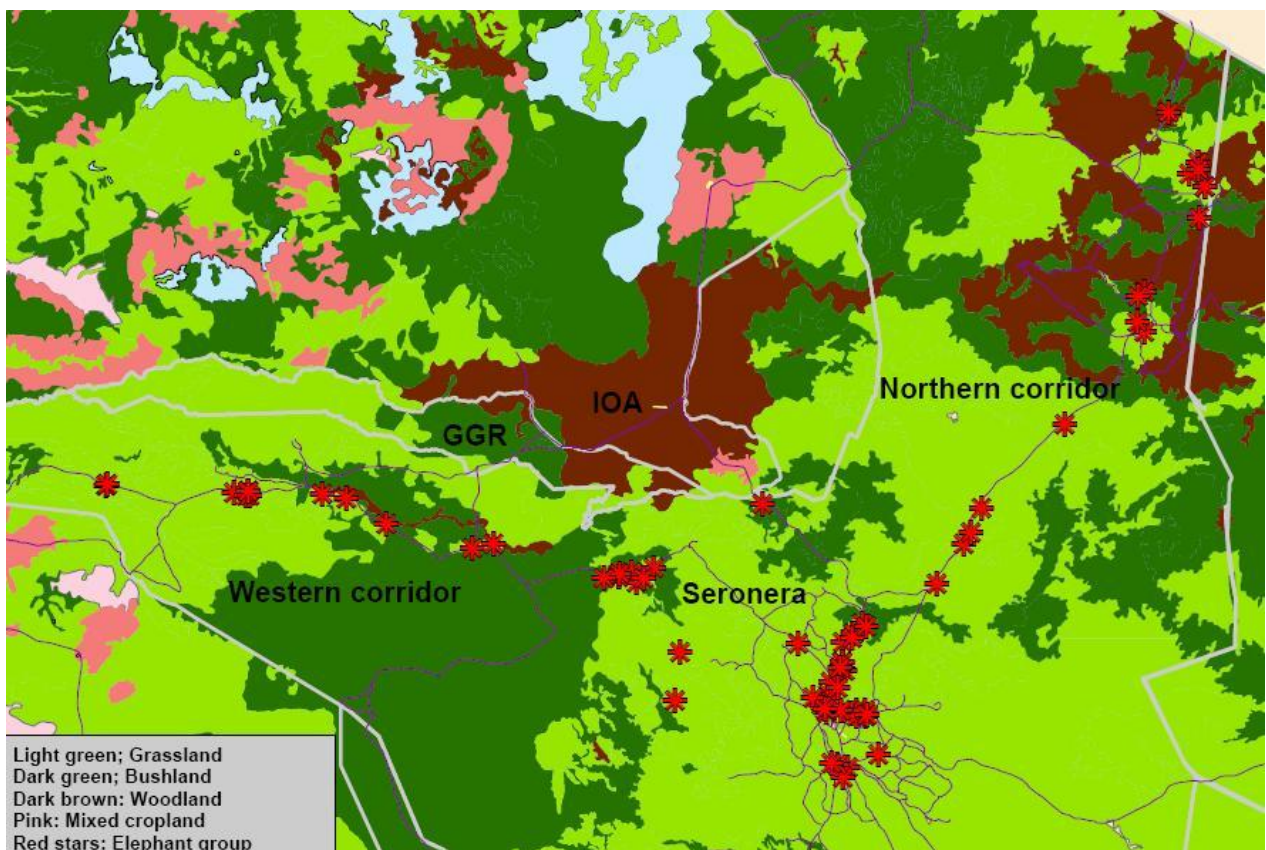


Figure 2: This is a map showing all the samples collected in this study, before reduction, as red stars. All samples after reduction upheld the original spreading depicted here. Only geographically overlapping samples were removed. The colors indicate different vegetation types and is explained in the gray box down at the left.

2.2 Laboratory methods

Dung sample preparations were done in the laboratory at the field station. Frozen dung samples were laid to defrost hours before further preparations. Only the parts rich in mucus, hence containing most DNA, of the thawed dung samples were transferred to hard plastic containers and marked with their respective numbers. The containers were filled with concentrated ethanol to cover all dung for preservation. Back-up containers were also made and marked. The leftover dung samples were dried and stored in marked rubber gloves for back-up purposes. For DNA extraction and further analyses the samples were sent to the commercial DNA laboratory Ecogenics GmbH (Schlieren, Switzerland).

Ecogenics GmbH used the QIAGEN stool kit (QIAGEN, Venlo, The Netherlands) with an adapted protocol. 200 mg stool sample was vacuum dried and incubated with 1 ml buffer ASL and 20 µl ProteinaseK o.n. at 55°C with shaking. After lysis the centrifuged supernatant was supplemented with one half of an InhibitEX tablet (QIAGEN). After centrifugation, 400 µl supernatant was supplemented with 400 µl buffer AL and 400 µl ethanol before application to the column. From here on, the standard protocol (QIAGEN booklet) was used. For application with polymerase chain reaction (PCR), the cycle program was first set to 95°C for 10 seconds, before a repetition of 35 cycles with a set up with denaturation at 94°C for 30 seconds, annealing at 58°C for 60 seconds and extension at 72°C for 90 seconds. After the completed cycles, the PCR tubes were held at 72°C for an additional 10 seconds. The PCR product was sequenced from both sides using an ABI3730 sequencer (Applied Biosystems, Foster City, USA).

2.3 mtDNA analysis

The sequences used in this study are all found in the Hypervariable region 1 (HVR1) region of the mitochondrial DNA. From the 60 mtDNA sequences obtained from Ecogenics GmbH after DNA extraction, only 55 were workable. They were all trimmed and edited for better compatibility, before being aligned in the software GENEIOUS v. 5.3.4 (Drummond et al. 2009) and each sample was given their respective individual number, provided during fieldwork. The aligned sequences were further transferred to GenAlEx (Peakall & Smouse 2006), where they were grouped into the 3 different geographical areas of Western Corridor, North and Seronera. The sequences were then saved in an ARLEQUIN v. 3.5 (Excoffier et al. 2005) compatible format and an AMOVA (Analysis of Molecular Variance) was run in

ARLEQUIN. Results were provided in the web browser as a HyperText Markup Language (HTML) file. Haplotype network were constructed using the software Network (www.fluxus-engineering.com) (Figure 3).

To compare our sequences to other previous studies, we used the integrated GenBank search engine in GENEIOUS to search for studies matching our mitochondrial sequences. Using the search results, we found a couple of papers including publications by Eggert et al. (2002), and Johnson et al. (2007). Eggert et al. (2002) had collected samples from several areas across Africa, such as different protected areas in the Ivory Coast, Ghana, Mali, Cameroon, South Africa, Namibia, Botswana, Zimbabwe, Mozambique, Uganda and Kenya (including the Serengeti-adjoining Masai Mara). Johnston et al. had a wider coverage; in addition to their own results they used sequences from Debruyne (2005); Nyakaana et al. (2002) and Eggert et al. (2002), adding sequences from Sudan, Chad, the Democratic Republic of Congo, Zambia and central Tanzania. The sequences used in these studies were downloaded and added to GENEIOUS for alignment with our own Serengeti sequences. This provided us with several HVR1 sequences which we compared to our own sequences, and gave us the opportunity to construct a neighbor-joining phylogenetic map (Figure 4), by using included tree-making software in GENEIOUS. Genetic distance was based on the Tamura-Nei model.

3 Results

3.1 Haplotype variability

The mitochondrial marker DNA, with a sequence region of 576 base pairs, gave a total of 7 haplotypes defined by 36 polymorphic sites (Figure 3) in the Serengeti elephants. One haplotype (SERENGETI 1) represented 78.2 % of all sequences recovered, and was evenly distributed among all areas, with 14 individuals in both Seronera and the North, and 15 in the Western Corridor. A second haplotype (SERENGETI 3) stood for 12.7 % of the sequences, and had, in addition to a series of base substitutions, a deletion distinguishing it from the larger group. Three individuals from both the West and the North had this haplotype, as did one from Seronera. The last five haplotypes were recovered from single individuals evenly spread throughout the park, with each representing 1.8 %. These less frequent haplotypes had all, except one Western individual, the same deletion as represented in SERENGETI 3.

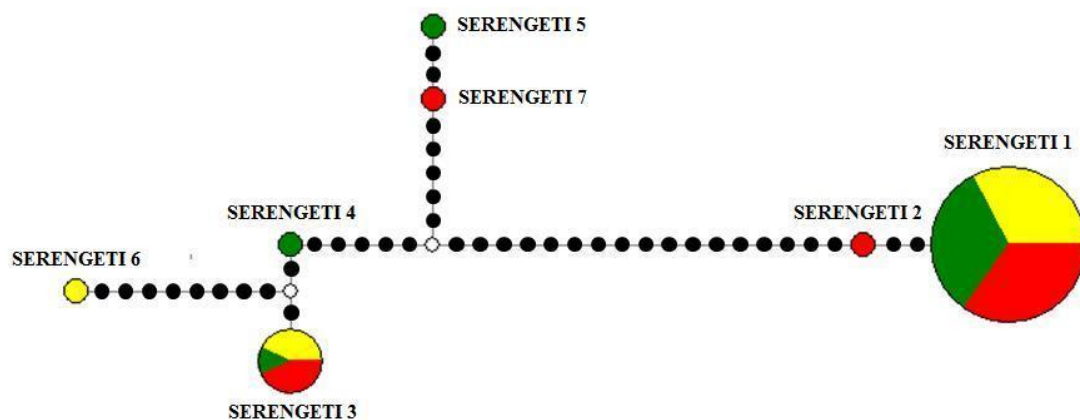


Figure 3: The different haplotypes are represented here, with designated names, as colored circles in a haplotype network, with each color representing an area of the park, as depicted in Figure 1. Green, yellow and red represents Seronera, North and the Western Corridor, respectively. The largest multi-colored circle represents the most frequent haplotype; with 78.2 % of all sampled individuals, and the other smaller multi-colored circle represent 12.7 %. The smaller single-colored circles are single individuals with unique haplotypes as far as this study goes. All the black dots connecting the different haplotypes represent mutations, and thereby evolutionary divergence. The two white dots represent intermediate haplotypes, and act as branching points.

3.2 Statistical analyses

There was no significant genetic differentiation detected in the HVR1 sequence among the three different areas ($F_{ST} = -0.04864$, $p = 0.92082$). The fixation index being closer to 0 than 1 show that there is free interbreeding across the entire elephant population and a negative value indicates a small tendency towards outbreeding. With the addition of such a high p-value, it is safe to say the null hypothesis of zero differentiation stands firmly. Further, a pairwise F_{ST} comparison between the three different areas proved no area was significantly different than any other, as shown in Table 1.

Table 1: Pairwise F_{ST} values between the different areas of Serengeti included in this study.

	North	Seronera	West
North	0.00000	-	-
Seronera	-0.04389	0.00000	-
West	-0.04937	-0.05254	0.00000

3.3 Location comparison

Referring the HVR1 sequence comparison to earlier studies by taking in haplotypes from all across the sub-Saharan Africa, resulted in a phylogenetic map depicted in Figure 4. Because each of the earlier studies had differing sequence lengths and ranges from the HVR1 region, attempting to compare these statistically, with significant results, proved difficult, and hence are not included in this study. The phylogenetic map, thus, might not be entirely accurate, but works as an indicator of possible evolutionary history of the Serengeti elephants.

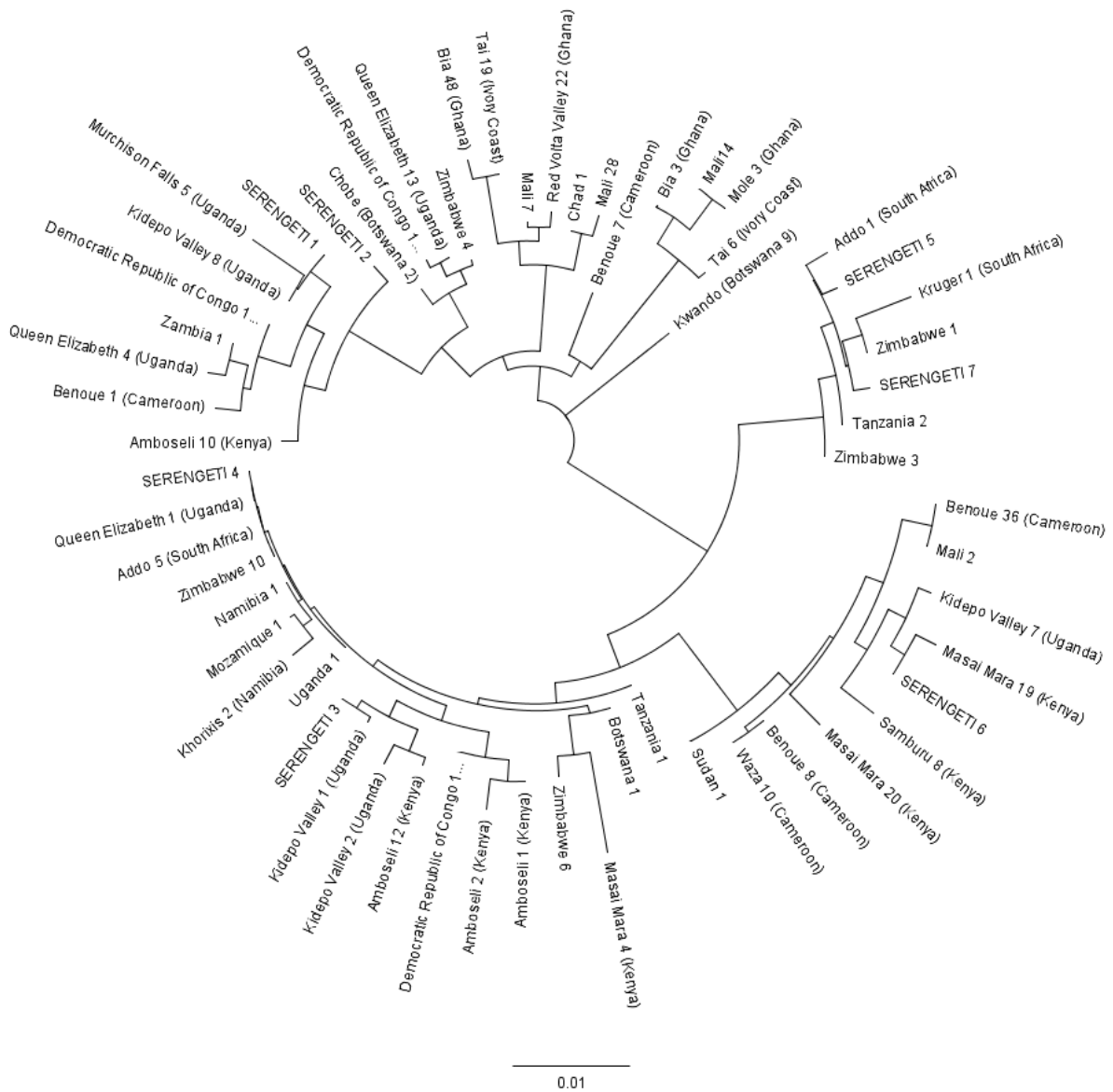


Figure 4: This is a phylogenetic map with genetic distance based on the Tamura-Nei model, where we have compared our 7 different haplotypes of the HVR1 sequence on the elephant mitochondrial DNA with 54 haplotypes from earlier studies, from elephants sampled across the sub-Saharan Africa. The Serengeti elephants are all written with capital letters with their designated names (see Figure 3) to distinguish them from the rest of the haplotypes. There are two main branches springing out on each side of a haplotype from Kwando, Botswana. SERENGETI 1 and SERENGETI 2 are found on the left branch. SERENGETI 1 is the most numerous of our haplotypes and falls into proximity with a group with other East African populations from Uganda and Kenya. SERENGETI 2, the haplotype closest in similarity to SERENGETI 1, is found relatively close by, earlier down on the tree. A large group of Western and Central African haplotypes span the map before the next Serengeti haplotypes occur on the right branch. SERENGETI 5 and 7 are situated on a branch accompanied by haplotypes from central Tanzania, South Africa and Zimbabwe. These haplotypes includes the deletion spanning 5 of the 7 Serengeti haplotypes (SERENGETI 1 & 2 are deletion free). Further, SERENGETI 6 is on a later branch accompanied by haplotypes from Kenya (most notably the ecologically attached Masai Mara) and Uganda. More

Western/Central African haplotypes are found earlier in the same branch. The final two haplotypes (SERENGETI 3 & 4) find themselves on the rightmost branches furthest away from the mid-section of the map, with SERENGETI 3 being the next most numerous haplotype in Serengeti (see Figure 2). SERENGETI 3 has neighboring haplotypes mostly of East African origin, and SERENGETI 4, though closely related, is widely surrounded by Southern haplotypes from Mozambique, Zimbabwe and Namibia, with a Ugandan exception.

4 Discussion

This study presents an overview of matrilineal descent in the Serengeti elephants by way of mtDNA extraction and comparison, with the goal of detecting population subdivision inside the national park and theorizing the evolutionary history of the local elephants. Such studies have never been done on elephants in this park, and it is essential for future studies of the Serengeti elephants. Understanding the genetic history and composition of animals with the ability to drastically change an entire ecosystem will help future conservation and management plans in the Serengeti ecosystem.

4.1 Elephant expansion and variation

The different haplotypes of the HVR1 region seemed to be evenly distributed across the Serengeti, but for those individuals with exclusive haplotypes it is hard to tell if they are more prone to occur at the area they were collected. The statistical results present a more precise picture, clearly giving the impression that there is no population subdivision going on among the elephants in the Serengeti, and with high probability ($p > 0.9$). With the ability to move across long distances and with no clear ecological barriers to keep elephants separated, the results were no surprise. There was a certain gap between the southern part of the North and halfway up towards Lobo (see Figure 1), along the eastbound main road, where no elephants were spotted, which made an impression that there was an ecological barrier of sorts that kept the elephants separated. This is more likely explained by the lack of water streams running through that area (the Grumeti River flows across the North to the West of that area and is a likely candidate for attracting elephants away from the East), and less favorable vegetation (some areas have high amounts of ant-infested whistling-thorn trees (*Acacia drepanolobium*), which repel elephants (Goheen & Palmer 2010)). Signs of elephants traversing this area were present, but sparse compared to other areas of the park. All other visited areas saw evidence of frequent elephant presence, with broken trees and dried up dung, which indicates a wide range across the majority of the park.

With slightly negative values on the fixation index, outbreeding seems to be ongoing inside the park. Although more research is needed to confirm this, it is reasonable to say the elephant population today is healthy and well preserved despite its recent and brutal history. Although they seem to be doing well genetically, the elephants themselves might be somewhat scarred by these earlier events, as we experienced increasingly circumspect behavior towards the areas closest to human settlements in the West and the North. As the haplotypes do not seem to be restricted to certain areas, this behavior is more than likely not genetic, but acquired through learning and observation. As likely as this seem, it is in no way certain and is in need of further studies for confirmation. Some elephant haplotypes may have undergone a heavier poaching history than others, and might have evolved a more wary behavior towards human presence and an “on edge” attitude in certain high risk zones. If the genetic healthiness of the elephants observed here is confirmed in future studies, relocating problem animals to other areas might not compromise the population if such is needed in the future.

Of the seven haplotypes collected there seems to be evidence of one haplotype clearly dominating in the Serengeti, with other haplotypes being sparsely spread across the park. With as many as 78.2 % of the elephants having the same haplotype it is reasonable to believe that this was the first haplotype presented in the park as the elephants reentered the Serengeti in the early 20th century, or that it was the dominant survivor of the heavy poaching in the 1970-80's. A combination of both theories may also be the case. As for the other haplotypes, all, except one, had a deletion clearly distinguishing them from the vastly more numerous deletion free haplotype. These might be haplotypes coming in with later migratory animals, or perhaps many of their relatives were removed during heavy poaching. What seems clear is that these deletion haplotypes are more diverse than the dominant (Five haplotypes compared to two). This might indicate that they spring from an older haplotype and may have been dominant in the park before the elephant depletion at the end of the 19th century. If this is correct, the dominant haplotype of today may have a more recent insertion, rather than the observed deletion in the other haplotypes, and has evolved in separation elsewhere before migrating into the park. There is no clear evidence of either to be correct, but a look at the evolutionary history of the elephants may give an impression of what might have happened.

4.2 Evolutionary history

By observing the phylogenetic map (Figure 4), there is no clear evidence of where the Serengeti elephants came from when reentering the park. All the haplotypes seem to have a close connection to other East-African countries, especially Kenya and Uganda, but haplotypes from other parts of sub-Saharan Africa are also present nearby on some branches. There is a clear separation of two groups on the phylogenetic map, with a clear branching originating at the left side of the Kwando 9 haplotype from Botswana, and a branching originating at the right. This is most likely a clear separation of the haplotypes with a deletion (to the right), and those without (to the left).

The most numerous haplotype, SERENGETI 1, is found on the left, and is in close proximity to a couple of Ugandan haplotypes, which, if one assumes this is the main haplotype that reentered the park in early 20th century, may indicate that most of the migratory elephants came from the North. This is further strengthened by the fact that this haplotype seem to originate from the Amboseli 10 haplotype of southern Kenya, but this, again, seems to originate from SERENGETI 2. The most reasonable answer then is that these haplotypes are quite common all over East Africa, and that somehow SERENGETI 1 came to be the dominant haplotype of the Serengeti. Close relatives are even found far away in Cameroon, Zambia and in the forests of the Democratic Republic of Congo, which clearly indicates that distance was not an issue before the onset of the exponential human population growth.

The Serengeti haplotypes harboring the deletion seems more difficult to place evolutionary, as they are more diverse and widespread, with no indication of originating from one place or the other. SERENGETI 3 and SERENGETI 6 are the haplotypes which most clearly show a connection to nearby populations in Kenya and Uganda, and further strengthens the theory indicating that the Serengeti elephants reentered the park from the North. SERENGETI 4 is closely related to a Ugandan haplotype, and to SERENEGTI 3, but the close connection to Southern haplotypes from Mozambique, Zimbabwe and South Africa gives no indication of origin. SERENGETI 5 and SERENGETI 7 do also show relatedness towards Southern haplotypes, but with origins in central Tanzania and Zimbabwe. It is therefore reasonable to think that the mixture of haplotypes on display in modern day Serengeti is the result of elephants migrating to the park from all directions of Africa, but with a clear dominance of migratory animals coming from the North. This, then, makes the impression that most elephant actually do not travel through the southern plains, but some might occasionally do.

The Serengeti haplotypes collected in this study is present in almost all the main branches on the phylogenetic map, and gives the impression that the Serengeti has a composition of haplotypes of cross continental representation. Another interesting observation from the map is that there is no clear distinction between the savannah elephant (*L. africana*) and the forest elephant (*L. cyclotis*) (most of the haplotypes from central Africa represent forest elephants). In the article by Eggert et al. (2002), there is an explanation for these observations: The complex phylogeographic patterns detected result from continental-scale changes repeated over the 5-6 million year old evolutionary history of the African elephant. This, then, means that the elephants have been on the move all across the continent because of environmental changes, and has caused the different haplotypes to be mixed, with no clear sense of belonging and origin, when viewed from a geographical standpoint.

4.3 Future work

This research is meant as an originator and a backbone for further genetic studies, not just on the Serengeti elephants, but on all elephant populations in Tanzania. Because of its relatively small sample size, and sole focus on mitochondrial DNA, no conclusive answers are given in this study. Therefore future studies need to take a wider approach, with focus on larger sample size and the addition of microsatellite markers. To further expand the research to other protected areas, in addition to the Serengeti, will increase the probability of getting more conclusive answers on the proposed theories discussed in this paper. Additionally it will be beneficial for the conservation of Tanzanian wildlife as a whole to catalogue and compare different elephant populations, and helpful in the human-elephant conflict because of the already mentioned solution of translocating rogue elephants to other wildlife areas. The genetic studies will further provide insight in the evolutionary history of populations, and it is important to include this information when making decisions for future management plans for elephants (Okello et al. 2008). Even though the results obtained in this study, on their own, are not sufficient enough to revolutionize the elephant management plans of the Serengeti, they provide a backbone to understand the origin and viability of these elephants.

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