Conservation genetics for the management of black rhinoceros (*Diceros bicornis michaeli*) in Tanzania

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Abstract

As anthropogenic impacts continue to rise, many species are confined to small, isolated populations. Conservation efforts aimed at reducing extinction risks often involve strategies like enhancing habitat connectivity, translocating individuals from captive populations, reintroductions, or closely monitoring highly protected closed populations. Despite potential variations in individual fitness resulting from different selection pressures in these scenarios, the genetic consequences of these strategies are frequently overlooked.

Eastern black rhinoceros (*Diceros bicornis michaeli*) are critically endangered mega herbivores that had suffered 96% decline in in their native range due to poaching and now persist as small and fragmented populations totaling less than 2300 individuals across the globe. Although there have been studies on genetic diversity in some geographic regions, no previous studies have assessed genetic variation of extant populations in Tanzania. The purpose of this study was to use conservation genetic techniques to assist the management of the eastern black rhinoceros in Tanzania.

I used the mitochondrial DNA (mtDNA) control region to investigate the genetic impacts of past management interventions on mitochondrial control region diversity in extant subpopulations in Tanzania. Six maternal haplotypes were identified, with an overall haplotype diversity of $h = 0.72$, but lower nucleotide diversity within populations ($\pi = 0.017$) compared to historical populations ($\pi = 0.021$). Translocated populations did not share haplotypes with native populations, although all haplotypes from translocated individuals were found among historic samples from Kenya, indicating successful restoration of previous diversity but restricted female movement between subpopulations due to current management practices. The extant haplotypes were distributed among three East African haplogroups, suggesting preservation of multiple lineages despite the loss of historical haplotypes. A recommendation is made to enhance previous translocations by facilitating natural movements between subpopulations, which could be a more cost-effective and welfare-conscious management strategy compared to targeting specific animals for translocation based on genetic data.

We used whole genome sequencing data to assess the scale of inbreeding that has been induced by the severe bottlenecks and subsequent expansion of native populations as well as what impacts previous attempts at population supplementation have had on the accumulation of potentially deleterious mutations. We found that offspring from individuals dispersing from native populations or translocated from captive ones had lower inbreeding levels compared to a closed native population. However, compared to native individuals, offspring resulting from captive parents or hybridisation between wild and native parents had a larger relative abundance of deleterious mutations, and this load was sheltered by higher heterozygosity. Our work underlines the value of maintenance of habitat corridors between populations and emphasizes the significance of natural dispersal in managing the trade-off between supplementing variation and introducing potentially harmful mutations if populations are allowed to inbreed following targeted translocations.

I assessed the demographic parameters of the eastern black rhino population in Tanzania and explored different management options that minimize the risk of extinction of rhinos using a count-based Population Viability Analysis (PVA). Given the current demographic parameters and the current management efforts, there is a low probability of extinction by 2050 for the indigenous native

populations, except for Nyamalumbwa (but this is a transborder population where observational records are less accurate), and for both the reintroduced subpopulations of Ndasiata and Grumeti, which exhibit the highest probability of extinction by 2050 (i.e. they do not exceed the 20 animal benchmark of a viable population size as set out by IUCN). Overall, my analyses suggest that translocated populations have not reduced the risk of extinction for black rhinos in Tanzania. With the current protection efforts and demographic performance, Moru and Ngorongoro could serve as a source population for reintroduction to other areas. This approach would reduce the cost and risks associated with international translocation efforts.

Overall, I demonstrated the value of adding ongoing genetic surveillance to conservation management strategic plans, to allow monitoring of both the short- and long-term impacts of different management strategies used to protect small and threatened populations.

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Author's Declaration

I declare that the work recorded in this thesis is entirely my own and is of my own composition except were noted for collaborative papers. No part of this thesis has been submitted for another degree.

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1 Introduction

1.1 General introduction on Biodiversity Conservation

Biodiversity is the variety of life on Earth, it includes all organisms (species diversity), the genetic variation among and within the species (genetic diversity), and their complex assemblages of communities and ecosystems (ecosystem diversity) (Norse et al., 1986; Caughley, 1994). It encompasses the ecological processes that hold the key to the evolutionary processes for sustaining life, therefore the need for conservation is justified for four main reasons: (1) economic value of bioresources such as our food, drugs, natural fibres, timber; (2) the ecosystem services which are essential biological functioning of living organisms such as nutrient recycling, pollination and oxygen production by plants; (3) the right for living organisms to exist independently of human valuation; (4) aesthetic value derived from living organisms such as keeping pets, visiting zoos and nature reserves (Bruyn 2014; L & Vineeta 2015; Rawat & Agarwal 2016). Because of this, developing countries have benefited economically from conservation initiatives. For example, Tanzania has devoted approximately 28% of the land for conservation, with the tourism industry contributing 10.7 % of the Gross Domestic Product (GDP) and 11.1% of the country's labor force employment income (WTTC, 2021).

Despite its importance, global biodiversity is under serious threat as a result of anthropogenic and natural factors such as habitat loss, habitat fragmentation, invasive species, overexploitation, pollution, climate change, and natural disasters such as earthquakes (Sechrest & Brooks 2002). These may result in a loss of biodiversity, a decline in population size of individual species or populations, reduction in genetic variability, variety of species, and overall diversity of biological communities (Caughley, 1994). The need to conserve species arises because the biological diversity of the planet is rapidly being depleted; according to the International Union for the Conservation of Nature assessment 25% of mammals, 41% of amphibians, and 14% of birds are listed as threatened species (IUCN, 2015). Genetic diversity is a key consideration in conservation for three main reasons: diversity is required for populations to evolve and to adapt to environmental changes; the loss of genetic diversity is associated with inbreeding and reduction in reproductive fitness; genetic diversity contributes to ecosystem diversity (Frankham, 1995a). The current genetic diversity in a population is derived from cumulative effects of past generations mutations, natural selection and migration of individuals between populations (Caughley, 1994; Frankham, 1995a). For endangered species to persist, adapting to environmental changes is important, given that most instances of species extinction result from the inability of a species to respond to changes in their environment, such as new predators or diseases or changes in weather conditions. However, higher diversity is also correlated with greater resilience, meaning that critically endangered species may be better able to recover after a bottleneck.

This is important as high genetic diversity of species can mitigate negative inbreeding effects and provide species with the potential to adopt to environmental changes (Frankham, 2005; Mable, 2018). Populations with high genetic diversity might be particularly important to maintain for conservation because they have a higher chance to adapt to environmental changes (Barrett

and Schluter, 2008; Kardos and Luikart, 2021), and those with low diversity or evidence of inbreeding might require genetic management, such as augmented gene flow and genetic rescue (IUCN, 2013; Frankham et al., 2019).
Therefore, conservation genetics is an aspect of conservation biology which uses genetic theories and data to address the genetic consequences arising from reduction of once large, outbreeding populations to smaller units. This knowledge helps us to; resolve taxonomic uncertainties, resolving fragmented population structure, understanding species biology, forensics and reducing extinction risk by minimizing inbreeding and loss of genetic diversity (Mable, 2018). Considering conservation threats, the use of genetic information is important because: 1) most species requiring conservation attention persist in small, isolated units susceptible to inbreeding, genetic drift, loss of genetic diversity, and accumulation of deleterious mutations; and 2) the implementation of some conservation programs could increase genetic threats, for example, if reintroductions introduce deleterious mutations or combine populations locally adapted to different conditions. Thus, this study will focus on the use of conservation genetic knowledge to assists to in conservation of the eastern black rhino in Tanzania.

1.1.1 Genetic consequences of small population sizes

Small populations, which have resulted from population bottlenecks from larger populations, are more likely to experience inbreeding, loss of diversity leading to reduced reproductive fitness, and reduced ability to adopt to environmental changes (Frankham et al., 2017). These effects will depend upon the effective population size rather than on the actual population size (Frankham, 1995b). Loss of genetic diversity is unavoidable in small populations and is further intensified by the strength of genetic drift and inbreeding (Armstrong et al., 2021). Therefore, this is thought to be one of the greatest threats to the persistence of species because evolutionary change is reliant on the presence of adaptive genetic variation (Lacy, 1997). So, assessment of levels of diversity in endangered species will be a useful tool for conservation for these species, with other genetic threats to small populations including inbreeding, which occurs due to production of offspring from the mating of individuals that are closely related genetically. Subsequently, it increases the likelihood that an individual will have two alleles identical by descendant at any given locus, and therefore has the effect of increasing homozygosity and chances of offspring being affected by deleterious alleles, which can decrease biological fitness (inbreeding depression) of species (Frankham, 1995b). Inbreeding is unavoidable and more rapid in small than large populations (Frankham et al., 2002). For example, a study conducted on a small isolated population of adder, with less than 40 individuals using allozyme variability and DNA fingerprinting revealed low levels of genetic diversity, and inbreeding, relative to the main population (Madsen, Stille & Shine 1996). Consequently, the small population showed evidence of inbreeding depression, evidenced by lower litter size and more abnormal offspring than in the larger population. However, the introduction of 20 adult males from another population reduced the frequency of abnormalities and increased recruitment. Thus, inbreeding and inbreeding depression increase the risk of extension of small populations, but through conservation genetics knowledge it can be minimized by informing strategies for outbreeding (Frankham 1995a).
Furthermore, population fragmentation causes separation of a population into isolated small islands, causing a total or partial restriction of gene flow between

populations (Gaines et al., 1997) (Proctor et al., 2001; Fahrig, 2003). The genetic impacts of these fragmented populations will depend upon gene flow among subunits, number of populations, their structure and their distance apart (Gaines et al., 1997). Cessation of gene flow between populations in the long term can result in greater inbreeding, more loss of genetic diversity and elevated extinction risk, when compared to a single population of the same total size (Mitton, 2013). Additionally, species existing in small populations are likely to be affected by genetic drift which causes a population's allele frequencies to change from one generation to the next simply as a result of chance (Frankham, 1995a). As a result, rare alleles can be completely lost in a population. Although genetic drift happens in populations of all sizes, these effects are most profound in small populations, and can have major effects when a population is sharply reduced in size by a natural disaster (bottleneck effect) or when a small group splits off from the main population to found a colony (founder effect) (Masel, 2011; Star and Spencer, 2013).

Moreover, small populations are more likely to accumulate deleterious mutations, expressed as genetic load. Genetic load is defined as the reduction in fitness due to the segregation and fixation of deleterious mutations. The availability of whole-genome sequencing data has facilitated the indirect estimation of genetic load in individuals(Ewens, 2013; Bertorelle et al., 2022). In diploid organisms, the genetic load can be divided into two components: the realized load, and the masked load, known as inbreeding load/potential load (Ewens, 2013). The realized load diminishes the fitness in the present generation, while the masked load assesses the potential fitness decline caused by (partially) recessive deleterious mutations (Agrawal and Whitlock, 2012). These mutations may manifest in future generations, contingent on factors like the population's demography (e.g., inbreeding, population decline, or subdivision).

1.1.2 Quantifying genetic diversity

Genetic diversity represents the total genetic variation among individuals within a population. There are many ways to assay and analyses genetic variation. The choice of analytical method mostly will depend on the type of genetic marker used, whether the marker is subjected to selection (non-neutral) or selectively neutral (Agrawal and Shrivastava, 2014). Estimates of genetic variation are based on the expected relationship between allele and genotype frequencies when a population is in Hardy-Weinberg equilibrium (HWE) (Hartl and Clark, 1997). The HWE principle proposed that allele and genotype frequencies in a population will remain at an equilibrium over time when the following conditions are met: no new mutations are occurring; no migration; no natural selection; the population is infinitive large; mating is random; all organisms are diploid and sexual reproducing. Therefore, violations of the HWE assumptions can cause deviations from expectation and the effects will depend on which of the assumptions that have been violated (Frankham et al., 2004). Genetic diversity of a population can be described by the allelic richness (***A***), which represents the average number of alleles per locus rarefied to match the number of observations in the population with the lowest sample size; this measure is sensitive to sample size. The percentage of polymorphic loci (***P***) expresses the percentage of variable loci in a population. This estimate is also sensitive to sample size, and it cannot be applied to variable markers such as microsatellites (Frankham et al., 2002; Freeland, 2012). Observed heterozygosity is the mean of the observed proportions of

heterozygotes in the sampled population. It is informative about inbreeding and relative allele frequencies but not really a measure of diversity at the population level (Frankham, 1995a; Fairbairn, 1998). However, expected heterozygosity (H_e), also referred to as gene diversity, describes the proportion of heterozygous genotypes expected under Hardy-Weinberg equilibrium and so provides a measure of diversity because it is based on allele frequencies. When the observed heterozygosity is lower than expected, we can suspect the population might be inbreeding. If observed heterozygosity is higher than expected, we might suspect the mixing of two previously isolated populations, disassortative mating, or selection (Hartl and Clark, 1997; Fairbairn, 1998). This measure is very useful and can tell us about the population structure and demographic history of a population including bottleneck and founder events, population expansion and stable populations. For example, a low level of observed heterozygosity may indicate past bottlenecks in the population history and an increase in observed heterozygosity may indicate population expansion as the genetic diversity is stored through new mutation and gene flow. Furthermore, whole genome sequencing has enabled scientist to measure runs of homozygosity (ROH), which are continuous homozygosity segments of the DNA sequence, measured in successive windows across the genome (Gibson et al., 2006). ROHs are often used as a measure of autozygosity, which is the probability that two alleles at a given locus are inherited from a common ancestor (Wright, 1922). It occurs when parents with a common ancestor pass shared DNA segments on to their offspring, which will result in homozygous segments in the offspring's genome that give rise to ROH that could be caused by inbreeding or genetic drift (Broman and Weber, 1999). ROH facilitates the estimation of the levels of autozygosity at the individual and population levels by quantifying the degree of inbreeding and the genetic relationships between individuals (Brüniche-Olsen et al., 2018; Hasselgren et al., 2021).

1.1.3 Use of conservation genetics in management of threatened species

Molecular genetic analyses provide a means to study some unknown critical aspects of species biology which are difficult to determine directly and are important for conservation and management of threatened species (Allendorf, 2017). Below I summarise some of these aspects.

1.1.4 Determining population size and demographic history

Obtaining direct estimates of population size for some rare or nocturnal species may be quite challenging and expensive, but based on a few hairs or faeces, estimates of population size can be obtained from the number of unique multilocus genotypes using molecular markers (Luikart et al., 2010; Salmona et al., 2019). The distribution of the number of sequence differences between pairs of alleles has characteristic shapes which can be used to infer demographic histories of the population such as: distinguishing populations with stable size or exponential growth; estimating the timing of bottleneck secondary contact and fusion events (Beichman et al., 2018). Molecular markers allow us to estimate the effective population size (Ne), which represents the number of breeding individuals in a population (Jiang et al., 2019). This is crucial because a larger

effective population size typically preserves greater genetic diversity, which is crucial for the health, adaptability, and long-term survival of populations. However, a smaller Ne can result in lower genetic diversity, increased inbreeding, and a higher risk of genetic drift, all of which can jeopardize population viability.

1.1.5 Gene flow and population structure

The degree of population differentiation is determined by the amount of gene flow between populations which is also influenced by dispersal abilities, physical barriers, reproductive compatibility and metapopulation structure (Frankham 1995a,(Whitlock and McCauley, 1999). Therefore, we can use genetic markers to infer dispersal patterns from among populations and detect migrants in a population, which are difficult to measure by direct observation (Whitlock and McCauley, 1999; Qu et al., 2004). For threatened species gene flow is important for transferring genetic diversity among populations and inference of population structure is thus useful for genetic management (Sbordoni et al., 2012).

1.1.6 Managing reintroduction and translocations

Limited gene flow and habitat fragmentation can lead to genetic erosion of species when reproductive individuals die off before reproducing with others in their endangered small population (Frankham et al., 2002; Qu et al., 2004). To alleviate this, re-establishment of gene flow by moving individuals (translocation), and/or by establishing migration corridors to maximize genetic diversity and minimizing inbreeding is often necessary (Mitton, 2013). Molecular marker can assist us to evaluate candidates to relocate and identify sites of reintroduction. This will help managers to design translocation manuals for management of endangered species (Bertola et al., 2022). For example, a study conducted in southern white rhinoceros populations in Botswana identified parents for 29 out of 45 offspring and suggested 8 non breeding bulls with high mean kinship as potential candidates for translocation (Purisotayo *et al*. 2019). This was inferred by combining microsatellite genotypes with an incomplete, field-based observational pedigree to improve accuracy of parentage assignment. The study demonstrated the value of combining genetic information with ongoing surveillance to inform management of threatened populations. In a study conducted by (Bertola et al., 2022) on guidelines for the translocation of the African lion, a decision-making tool for managers when planning lion translocations was introduced. This tool lists 132 lion populations/lion conservation units and provides details on genetic assignment, uncertainty, and suitability for translocation for each source/target combination. By offering four levels of suitability, ranging from 'first choice' to 'no option,' the study provides managers with a diverse range of options. This study demonstrated the importance of developing tools which consider genetics for translocation decision.

1.1.7 Forensics

Poaching and illegal harvesting threaten a wide variety of species, especially large cats, bears, elephants, rhinoceroses, and parrots (Armstrong et al., 2017; Haines et al., 2021). However, it is often difficult to obtain evidence to convict

individuals illegally taking, or trading in, protected species. Molecular markers can be used to aid in detection of illegal hunting and identify the origin of biological material including feathers, hair, horns, ivory, meat, turtle shells and plant materials (Alacs et al., 2010; Linacre and Tobe, 2011). Application of molecular technology is now a standard tool to assist law enforcement through provision of robust evidence in the court of laws and DNA testing is being used widely to provide forensic evidence on wildlife trade law enforcement cases (Caniglia et al., 2010; Linacre and Tobe, 2011; Gristwood, 2019). In countries like Tanzania, however, there are limitations on the use of DNA technology for cases involving particular wildlife species because individual identification through genetic tools are not well developed and standardized for target species. For example, for black rhinos (*Diceros bicornis*), despite their high profile as a conservation icon with few remaining natural populations, surprisingly little effort has been done to inform management through population genetics or establish data bases that can be used in forensic cases to inform poaching and other management issues.

1.1.8 Molecular techniques used in conservation genetics

Conservation genetics studies rely on molecular markers that allow us to generate data from the infinitely variable deoxyribose nucleic acid (DNA) molecules; to quantify genetic diversity, track the movements of individuals, measure inbreeding, identify the remains of individuals, characterize new species and retrace historical patterns of dispersal (Anne, 2006; Agrawal and Shrivastava, 2014). Molecular marker technology has developed rapidly over the last few decades and a range of markers are available for studying populations in the wild and can be classified on the basis of mode of gene action (co-dominant or dominant markers), method of detection (hybridization-based molecular markers or polymerase chain reaction based markers) and mode of inheritance (paternal organelle vs maternal organelle, bi-parental nuclear inheritance or maternal nuclear inheritance) (Kirk and Freeland, 2011; Agrawal and Shrivastava, 2014). Additionally, the choice of markers to use will depend on the biological question and availability of resources including time, expertise, and money (Allendorf, 2017). Table 1.1 shows the properties of different markers that have been applied in conservation genetics (Anne, 2006; Agrawal and Shrivastava, 2014; Allendorf, 2017; Supple and Shapiro, 2018; Bertola et al., 2024).

1.1.9 Predicting extinction probabilities through population viability analysis

A Population Viability Analysis (PVA) is a model that projects the likely future status of a population by evaluating the long-term demographic and genetic sustainability and extinction risk, identify key factors impacting a population's dynamics, and compare alternative management strategies (Drake, 2008; Pierson et al., 2015). Therefore, PVA act as a tool for assisting management of threatened species by allowing iterative planning to determine sensitivity and to compare recovery options, rather than providing a precise prediction of extinction risk (Tian et al., 2011; Kimanzi, 2018). For example. Chinook salmon populations in Oregon, USA have declined, primarily due to habitat degradation associated with siltation from road building and forestry. Ratner et al.,1997 conducted a PVA study

on the spring chinook population in the South Umpqua River, projecting that extinction risks over 100 and 200 years were very low, assuming no further habitat degradation (Ratner et al., 1997). However, integration of genetic data to inform levels of inbreeding or genetic diversity that could be incorporated into PVA models can increase insights for the impacts of different management interventions (Lacy, 2000; Brook et al., 2002; Zilko et al., 2021).

Table 1.1 Properties of various markers that have been used to inform conservation genetic studies, including: Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs), microsatellites, Single Nucleotide Polymorphisms (SNPs) based on genotyping arrays, and Whole Genome Sequencing (WGS).

Marker property	RFLPs	RAPDs	AFLPs	Microsatellites	SNPs	WGS
Inheritance	Co-dominant	Dominant	Dominant	Co-dominant	Co-dominant	Co-dominant
Development time	Moderate	Low	Moderate	High	High	Low
Cost	Low-moderate	Low	Moderate	Moderate-high	Moderate-high	Low
Require prior information (Primer/reference Genome)	Yes	No	No	Yes	Yes	Yes
suitability for evolutionary studies	Limited	Limited	Limited	Limited	High	High
Polymorphism level	Low-moderate	High	High	High	Moderate	Yes
Comparison of data between studies	Limited	Limited	Limited	Yes	Yes	Limited
Quantity of DNA	High	Medium	Low	Low	Low	Low
Quality of DNA	High	High	High	Low	High	High

1.2 Study species: Eastern black rhinoceros

The focus of this thesis will be on conservation management of eastern black rhinoceros (Diceros *bicornis*) in Tanzania. Rhinoceros belong to the family Rhinocerotidae of the order Perissodactyla, from which five species are known to survive in the world today. Three species - the Indian (*Rhinocerosunicornis*), Sumatran (*Diceros sumatrensis*) and the Javan (*Rhinoceros sondaicus*) rhinoceros - are indigenous to Asia and the other two species - black (*Diceros bicornis*) and

white (*Ceratotherium simum*) rhinoceros - are native to Africa (Steiner and Ryder, 2011). In Tanzania, two of the five recognized black rhino sub-species occur: *Diceros bicornis minor*, distributed in the south and Diceros *bicornis michaeli*, in the north and central regions of the country. However, currently, *Diceros bicornis minor* consists of 5 individuals imperfectly known in the vast Selous Game Reserve (Burgess et al., 2022).

1.2.1 Black rhinoceros biology

Eastern black rhinoceros inhabits a range of habitats that have shrubs, trees, and herbs, along with water sources and mineral licks nearby, including savanna, shrubland, woodland, dense forest, and wetlands (Oloo et al., 1994; Anderson et al., 2018). Black rhinos are normally active in the early morning and late evening, potentially engaging in feeding during the night. These solitary and territorial herbivores are selective browsers, primarily feeding on leaves, shoots, and fruits. Territorial behaviour is observed in both male and female black rhinos, with males potentially overlapping territories with multiple females. Black rhino bulls are extremely territorial and will fight other males found in their territory, often causing significant injuries to each other. They reach age of first reproduction (maturity) from 5 to 7 years old for females, and 7 to 8 years for males. Black rhinos are polygynous (Garnier et al., 2001). Reproduction involves a gestation period of 15-16 months, with females giving birth to a single calf every 2-3 years.

1.2.2 Black rhino population performance indicators

Population performance indicators provide wildlife managers with essential tools to assess the dynamics of a population over time, offering insights into growth and recruitment. For black rhinos, the African Rhino Specialist Group has identified key indicators to gauge population performance and understand factors contributing to performance below or above the internationally-accepted minimum annual growth rate of 5% (Du Toit, 2006). These demographic indicators include:

- **Average Age at First Reproduction:** This crucial indicator of breeding performance reveals when females begin breeding. In rapidly growing rhino populations, females may have their first calves as early as 6.5 years as compared to 6 years in captive individuals.
- **Average Inter-calving Interval (Homburger et al.):** This metric, measuring the period between birthing events, serves as a robust indicator of population dynamics. An inter calving interval exceeding 3.5 years suggests poor to very poor fecundity. This calculation, unaffected by sex ratio, involves observing the dates between two successive calves and averaging these values for the entire population.
- **Annual Growth Rates:** Calculating these rates allows rhino managers to pinpoint populations performing below the 5% target. Identifying such populations underscores the need for closer examination to understand the reasons for their poor performance. This assessment is particularly crucial for the rhino population in Tanzania, where previous interventions have been lacking.

1.2.3 Conservation status of black rhinos

All five species of rhinoceros have been facing some common threats from anthropogenic-derived activities and are the major reasons for species decline and extinction (Caughley, 1994). Poaching being the single greatest threat to the rhino population all over the world and has led to the massive population decline of these species in the past decades (Emslie et al., 2016). Rhino poaching is mainly driven by the demands for their horn, which is used for traditional medicine and has been superstitiously believed to cure a variety of ailments that range from impotence, snake poisoning, headaches and cancer. So far, this has not been proven scientifically but traditional use continues to drive the demand on which poachers thrive and hence leading to the increase in poaching (Martin & Vigne 2003). The black rhinoceros is classified as Critically Endangered on the IUCN Red List (2015).This status is primarily attributed to the persistent threats of poaching for their horns and habitat (Cumming et al., 1990; Emslie and Brooks, 1999) .

1.2.4 Black rhinoceros conservation efforts in Tanzania

In Tanzania, the population of the eastern black rhinoceros plummeted from around 10,000 in the 1960s to a mere 46 individuals by 1997, with remaining populations confined to small, isolated groups (Fyumagwa and Nyahongo, 2010). By the 1990s, only three subpopulations remained, including individuals in the Moru kopjes in the southern Serengeti National Park, the Nyamalumbwa-Maasai Mara in the northern Serengeti-Mara ecosystem (transboundary with Kenya), and the Ngorongoro Crater (Cumming et al., 1990).

In 1997, efforts were initiated to reintroduce eastern black rhinoceroses to Tanzania, with two females translocated from Addo Elephant National Park. Further reintroductions followed, establishing new populations in Mkomazi National Park and two additional areas in the Serengeti-Mara Ecosystem. Native and reintroduced individuals have been managed in Intensive Protection Zones (IPZs), employing enhanced security measures like specialized anti-poaching patrols, advanced tracking technology, and community engagement (Fyumagwa and Nyahongo, 2010).

Tanzania has set aside the black rhino conservation programme as a matter of national responsibility to conserve the species and contribute to international conservation efforts of these species (Fyumagwa and Nyahongo, 2010). The main objective of the programme is to attain a minimum rate of 5% per annum growth rate through using a sub-population management approach(TAWIRI, 2019) To achieve this goal a national rhino genetic diversity study would assist generation of genetic information which can be used to design specific translocation strategies for maximising distribution of genetic variation across subpopulations and provide critical forensic evidence in the case of poached animals (Fyumagwa and Nyahongo, 2010).

Physical protection of individual populations and remote monitoring of individuals with satellite and radio collars has been adopted in the conservation and management of the black rhino. This technique
has yielded successful impacts on rhino conservation to date (Linklater, 2003). Despite being useful, this strategy is difficult to implement and expensive because rhinos are often dispersed over wide geographical areas, making individual monitoring and protection impossible as the population size increases (Toit, 1996).

So far in Tanzania, the number of outlier individuals (rhinos which move outside their protection boundary) has increased (Fyumagwa and Nyahongo, 2010). Pushing the rhinos back to their subpopulation has been the current technique used to deal with the outliers, which will probably not be a permanent solution since the number of individuals is increasing, creating a demand for more space for browsing and avoiding frequent aggression. Frequent pushing back of individuals when they move out of their original areas causes stress, which may have a negative effect on breeding success, especially for small populations that are likely to be subjected to an inbreeding problem and it also prevents gene flow between populations. Therefore, this study will focus on quantifying genetic relationships among the Eastern black rhinoceros in Tanzania, which is currently unknown for assessing historical movements, inferring gene flow between the subpopulations, quantifying changes in population genetic metrics over time and informing PVA models of extinctions risks under different management scenarios

The conservation authorities need to be proactive in establishing more habitats and increasing security for the rhinos and restricting human activity in these areas in anticipation that individuals will disperse more within the ecosystems. As black rhino recovery continues, the focus on population growth (number of individuals) needs to be combined with that of population quality (population size and genetic variation) so as to produce a population with long-term capacity to respond to changes in environment. Thus, a conservation genetic study will be a beneficial tool for management of these population in Tanzania (Fyumagwa and Nyahongo, 2010).

Currently in Tanzania, translocation has been one of the conservation tools that has been used in the reintroduction of rhinos to areas where they have been suppressed by natural catastrophes and/or anthropogenic factors. Several translocations between 1997 and 2019 (Table 1.2) involved moving a total of 34 rhinos in the past 27 years to: Ngorongoro Crater (2), Mkomazi National Park (15), Grumeti Game Reserve (9) and Ndasiata (5). Despite the success of the re-introductions in increasing the number of individuals these techniques have to be practiced with caution as introduced animals often die and may also contain deleterious genetic material and carry infectious diseases and also genetic risks associated with hybridization and loss of locally adapted genes if genetically distant populations are introduced (Batson et al., 2015). In Tanzania, rhino reintroductions have been conducted to enhance diversity by bringing individuals from various captive populations. However, these translocations were based solely on phenotypic traits. This study seeks to evaluate the consequences of such reintroductions using molecular markers, as well as assessing the efficacy of translocations in mitigating the risk of extinction. The focal point of this study was to thus conduct a genetic assessment of the black rhino population in Tanzania to address the impacts of past translocations on the management of the black rhino in Tanzania. More generally, I used critically endangered eastern black rhinoceros (*Diceros bicornis michaeli*) populations in Tanzania as a model for predicting the relative fitness impacts of reintroductions from captive populations to the wild compared to natural dispersal but focusing on ancestry cohorts of individuals rather than whole populations.

Table 1.2 History of the translocation of Tanzanian black rhinoceroses from captive populations located outside of East Africa, covering the years 1997 through 2024. The table presents information on the individual's destination, the year it was moved, the population of origin, the number of male and female participants in the move, and the overall number of rhinos moved during each translocation event.

Destination Population	Year	Origin population	Males	Female	Total
Ngorongoro	1997	Addo National Park, South Africa	-	2	2
Mkomazi	1997	Addo National Park, South Africa	2	2	4
Mkomazi	2001	Addo National Park, South Africa	2	2	4
Grumeti	2007	Port Lympne wild animal park, UK	1	1	2
Grumeti	2008	San Diego Zoo, USA	1	-	1
Mkomazi	2009	Dvur Kralove Zoo, Czech	2	1	3
Ndasiata	2010	Thaba Tholo, South Africa	2	3	5
Mkomazi	2012	Port Lympne wild animal park, UK	1	2	3
Mkomazi	2016	Dvur Kralove Zoo, Czech	0	1	1
Grumeti	2019	Thaba Tholo, South Africa	4	5	9
Total			**15**	**19**	**34**

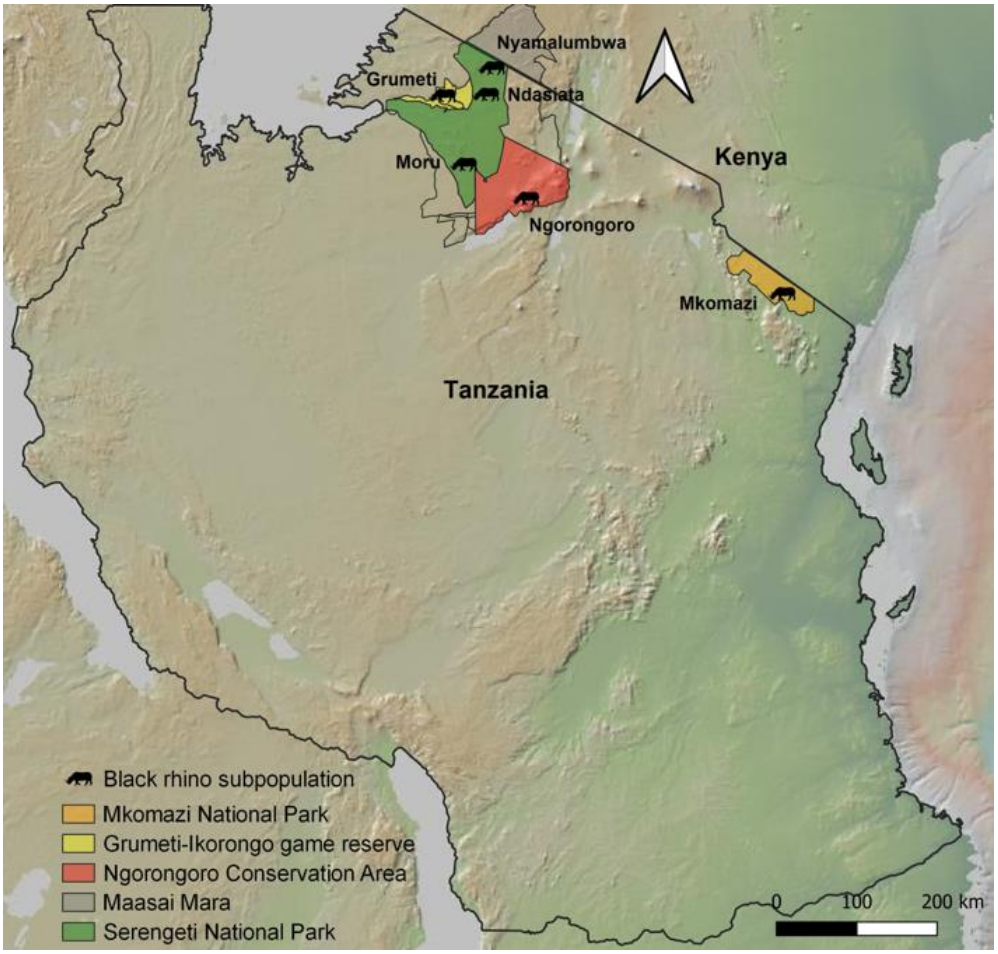


Figure 1.1 A map of Tanzania showing locations of the extant six black rhinoceros subpopulations within Tanzania. The Serengeti–Mara ecosystem allows for free movement of rhinos because there are no fences and includes the Serengeti National Park (light green), Ngorongoro Conservation Area (red), Ikorongo-Grumeti Game Reserve (yellow) and Masai-Mara National Reserve in Kenya (grey). The black rhino symbols indicate subpopulations: Moru a native subpopulation founded by three individuals, two females and one male (the male migrated from Ngorongoro); Nyamalumbwa- also a native subpopulation utilizing areas between Northern Serengeti and Maasai Mara-Kenya and so involving separate monitoring schemes on the two sides of the border; Ndasiata and Ikorongo-Grumeti Game Reserve both were initiated by reintroducing individuals from Thaba Tholo private ranch in South Africa, San Diego Zoo in the USA and Port Lympne Wild Animal Park in the UK. Despite the lack of fences, individuals are currently not allowed to move between subpopulations, due to the Intensive Protection Zone (IPZ) management scheme. The dark green polygon represents Mkomazi National Park in eastern Tanzania, which includes a rhino sanctuary that is the only fenced population in Tanzania for maximum security and consists exclusively of rhinos from different zoos: Addo Elephant National Park in South Africa, Dvur Kravole Zoo in the Czech Republic and Port Lympne Wild animal Park in the UK.

1.3 Aims of this thesis

The aim of this study is to use applied conservation genetics to generate information on the genetic status of all six extant black rhinoceros subpopulations from four protected areas in Tanzania (Serengeti National Park, Ngorongoro Conservation Authority, Mkomazi National Park, and Grumeti Game Reserve) and one neighbouring population from Kenya (Maasai Mara), to address the following specific questions:

I. Has the demographic decline affected the genetic variation of the black rhino population in Tanzania?
II. To what extent are the black rhinoceros subpopulations genetically differentiated from each other?
III. What is the extent of inbreeding and genetic load in individual black rhinoceros with different histories of management?
IV. What are the impacts of the recent reintroductions on genetic diversity, inbreeding and genetic load?
V. What is the probability of extinction of the black rhinoceros subpopulations under the current management scenario and how can this be reduced?

The thesis is organised into three data chapters and a general discussion, which integrates resulting management recommendations.

Chapter 2: Mitochondrial DNA diversity of the eastern black rhinoceros (*Diceros bicornis michaeli*) in Tanzania: implications for future conservation. In order to compare current patterns of genetic variation in Tanzania with the widest geographic sampling from other studies, this study focused on the maternally inherited mtDNA control region. Our specific aims were to investigate: (1) the impacts of past translocations on diversity of maternal lineages in extant subpopulations; (2) whether there has been evidence of dispersal of females between populations based on haplotype sharing; and (3) how current haplotype diversity relates to historical patterns. This chapter has been published in *Conservation Genetics* (Mellya et al., 2023a).

Chapter 3: Natural dispersal is better than translocation for reducing risks of inbreeding depression in eastern black rhinoceros (*Diceros bicornis michaeli)*. Using whole genome sequences from critically endangered eastern black rhinoceros as a model, we compare the consequences of different types of conservation efforts. We assessed the impacts of various management practices on: (1) genetic diversity; (2) genome-wide inbreeding; (3) accumulation of deleterious mutational load (relative load); and (4) homozygosity of deleterious alleles (realized load). A preliminary version of this chapter was published on the preprint server bioRviv (Mellya et al., 2023b) and submitted for consideration in Current Biology, but it has not yet been published in a peer-reviewed journal.

Chapter 4: Assessment of demographic parameters and population viability analysis of the eastern black rhinoceros in Tanzania for future management. The main objective of our analysis is to assess the demographic parameters of the eastern black rhino population in Tanzania and to explore different management options that minimize the risk of extinction of rhinos using a count-based PVA. Specifically, we use long-term rhino monitoring data to assess: (1) population performance indicators such as age of first reproduction and inter-calving interval; and (2) the effects of inbreeding on these population performance indicators. Using the variance around the observed population growth rates, we then explore (3) the four different management options on the probability of extinction of eastern black rhinoceros in Tanzania by 2050 in which: (i) each subpopulation is managed independently (i.e. with no dispersal between subpopulations and animals pushed back into their management zones); (Hoffmann et al.) subpopulations are managed as separate zones (i.e. animals are allowed to disperse between adjacent subpopulations but not across the entire ecosystem); and (iii) subpopulations are managed at the ecosystem level (i.e. animals are allowed to disperse freely between all subpopulations with the same ecosystem). Furthermore, we assess: (4) the impact that translocations have had on changing the probability of extinction. Finally, using the count-based population viability analysis, we assess (5) if the Serengeti could be used as a source population of rhinos for reintroductions into other ecosystems in the future. This chapter is in preparation for publication but has not been submitted yet.

Chapter 5: General discussion. In this chapter, I bring the key findings of the present study together and contrast them with recent policies and findings for the conservation of the Eastern black rhinoceros populations. I discuss alternatives for the species´ conservation at both the international and local Tanzanian scales. I conclude with proposals for future research on the conservation of the species.

2 Mitochondrial DNA diversity of the eastern black rhinoceros (*Diceros bicornis michaeli*) in Tanzania: implications for future conservation

Abstract

There has been a drastic decline in the number of eastern black rhinoceros (*Diceros bicornis michaeli*) across Africa, leaving individuals restricted to small, isolated populations that are vulnerable to extinction. Focusing on highly threatened populations in Tanzania, this study investigated the genetic impacts of past management interventions on mitochondrial control region diversity in extant subpopulations, assessed whether there has been evidence of dispersal of females between populations based on haplotype sharing, and related current haplotype diversity to historical patterns. Across extant subpopulations in Tanzania, six maternal haplotypes were identified, with an overall haplotype diversity of $h = 0.72$ but lower overall nucleotide diversity within populations ($\pi = 0.017$) compared to historical populations ($\pi = 0.021$). Translocated populations did not share haplotypes with native populations, even though all haplotypes from translocated individuals had been found among historic samples from Kenya. This suggests that translocations have been successful at restoring previous diversity to the region but that the current Intensive Protection Zone (IPZ) management practices have restricted the movement of females between subpopulations. Extant haplotypes were distributed among three East African haplogroups described in previous studies, suggesting that multiple lineages have been preserved despite the loss of historical haplotypes. Our recommendation is to enhance the utilisation of previous translocations by enabling the natural movements of individuals between subpopulations. Such a change in management strategy could be less costly both economically and in terms of animal welfare than the alternative of using genetic data to target specific animals for translocation in order to supplement diversity.

2.1 Introduction

Understanding how animal populations vary within their environment is essential for developing effective conservation and managements plans; this becomes critical when dealing with endangered species. Incorporating genetic information into conservation management plans can help to reduce extinction risks by minimizing loss of genetic diversity through inbreeding, identifying populations of conservation concern, inferring population structure, resolving taxonomic uncertainties to define management units within species, detecting hybridization, defining sites for reintroductions, and choosing the best populations for reintroduction and forensics (Caughley, 1994; Frankham, 1995a). There is, therefore, no doubt that the field of conservation genetics is key in efforts to attain sustainable biodiversity conservation.

The eastern black rhinoceros (*Diceros bicornis michaeli*; also known as the eastern hook-lipped rhinoceros) is a subspecies that was once widely distributed throughout South Sudan, Uganda, Ethiopia, Kenya and north-central Tanzania (Groves, 1967; Hillman-Smith and Groves, 1994). However, the population has declined by 90% in the last three generations, from an estimate of 70,000 individuals across Africa in the late 1960s to only 3,800 in 1987, due to intensive poaching for their horns and habitat loss (Cumming et al., 1990) . In Tanzania, the eastern black rhinoceros population had dropped from approximately 10,000 in the 1960s to only 46 by 1997 (Brooks and Emslie, 1999). The few remaining individuals were restricted to a series of small and isolated populations (Makacha et al., 1982; Sinclair and Arcese, 1995). By the 1990s in Tanzania, only three subpopulations remained: 1) three individuals in the Moru kopjes in the southern part of the Serengeti National Park; 2) 10 individuals in the Nyamalumbwa-Maasai Mara in the northern Serengeti-Mara ecosystem - a transboundary population between Kenya and Tanzania; and 3) 13 individuals in the Ngorongoro Crater. In 1997, eastern black rhinoceroses were first reintroduced to Tanzania. Two females were translocated from Addo Elephant National Park to the Ngorongoro Crater but their ancestors originally had been introduced to Addo Elephant National Park from Kenya in 1961 and 1962 (Hall-Martin, 1984), so they were originally of east African origin. This was followed by further reintroductions to establish new populations in Mkomazi National Park and two additional populations in the Serengeti-Mara Ecosystem (Ndasiata and Ikorongo-Grumeti) from five captive populations, including: Port Lympne Wild Animal Park in the United Kingdom; Dvůr Králové Zoo in the Czech Republic; San Diego Zoo Safari Park in the United States of America; and Thaba Tholo private game ranch and Addo Elephant National Park in South Africa. Since then, both the native and reintroduced individuals have been managed in Intensive Protection Zones (IPZ) separate subpopulations (Fyumagwa and Nyahongo, 2010). IPZs are designed to provide an area of enhanced security by increasing protection and monitoring measures, such as specialized anti-poaching patrols, the use of advanced technology for identifying and tracking individual animals, and engaging local communities in conservation efforts. The only fences associated with IPZs are in Mkomazi and Grumeti; Mkomazi has a large, fenced enclosure to constrain eastern black rhinoceros to the IPZ, and Grumeti has a fence only on the western boundary of the protected area to minimise wildlife-livestock conflicts but is open on the eastern side that borders the Serengeti. The other four IPZs (Moru, Ngorongoro, Nyamalumbwa, and Maasai Mara) do not have physical barriers but animals are restricted within the IPZ using GPS collars and geo-fencing technology (i.e. a

virtual boundary). Rangers track movements of individuals within the IPZ and receive alerts when an animal moves out of their native zone. Eastern black rhinoceros outside the IPZ are pushed back into their designated area. However, this intensive protection strategy could come at a cost to natural dispersal.

Eastern black rhinoceros are solitary animals that establish and defend individual territories. While males are known for their territorial behaviour and active defence of their range, females are more tolerant of each other's presence (Tatman et al., 2000). Female dispersal in this species is a behavioural pattern where individuals leave their birth area to establish their own territories and breeding opportunities (Reid et al., 2007). They may either stay near their natal range (philopatry) or move away to find vacant territories (dispersal). Dispersing females typically leave their natal area before reaching sexual maturity (around 3-6 years of age) and undertake movements covering significant distances, searching for unoccupied territories to establish their own home ranges (Hillman-Smith and Groves, 1994). In the Serengeti, the average size of eastern black rhinoceros home ranges vary from approximately 40 to 133 square kilometres, whereas in the Ngorongoro area, it spans from 2.6 to 58.0 square kilometres (Frame, 1980). Therefore, restricting dispersal could compromise genetic diversity, increase inbreeding, and reduce the spatial distribution of the eastern black rhinoceros populations in Tanzania.

As a result of past re-introductions, coupled with intensive protection and monitoring, the number of eastern black rhinoceroses in Tanzania has increased from 24 individuals in 1995 to 177 by the end of 2019 (TAWIRI, 2019). Whilst this approach has yielded success in rehabilitating these closed subpopulations, the potential impacts of inbreeding depression are unknown because empirical genetic information was not considered in the selection of the founder individuals. The consequences of this demographic bottleneck on the genetic diversity for the small remote subpopulations could also result in additional impacts, including reduced viability of the population to evolve in response to extreme climates, parasitic burden or diseases epidemics (Gaines et al., 1997; Frankham et al., 2019).

Inbreeding can put populations at risk of extinction by increasing levels of homozygosity and exposing deleterious recessive alleles that could weaken reproductive fitness and ability to survive, resulting in inbreeding depression (Frankham, 1995a). Furthermore, by virtue of it being stronger than selection, genetic drift can cause unpredictable loss of adaptive alleles or retention of deleterious alleles (Hartl and Clark, 1997). Small, isolated populations are often also characterised by restricted gene flow, as there is less chance of immigration and emigration (Frankham, 1995a). Apart from re-introductions from captive populations, translocation of wild individuals between different populations is another strategic management intervention (Fyumagwa and Nyahongo, 2010). Such interventions are often used to balance the harmful effects of small population size and maintain natural evolutionary processes (Sinclair and Arcese, 1995; Seddon et al., 2014). However, both reintroductions and translocations are only effective if the individuals being moved are sufficiently different from the host population to offset the effects of inbreeding (Jackson and Hobbs, 2009). Therefore, genetic relatedness between the donor and the recipient populations is used as a key tool to inform suitability of different management interventions. It is for these reasons that establishing the current genetic health of the isolated sub-populations within Tanzania and that of the neighbouring cross-border population of Maasai Mara in Kenya becomes of paramount importance.

A pan-African assessment of the genetic status of black rhinoceros populations using microsatellite markers and mitochondrial DNA (mtDNA) sequencing revealed a 69% loss of mtDNA variation of the species. Low genetic diversity and high inbreeding were also established in the Maasai Mara sub-population in Kenya compared to other larger subpopulations in a previous study (Muya et al., 2011). Across the entire species range, seven haplogroups have been identified, based on a combination of geographic distribution and phylogenetic clustering: WW, West Africa (west of the Shari-Logone River system); CV, Chari-Victoria (east of the Shari-Logone River to East Africa); NE, North-East Africa; EA, East Africa; CE, Central Africa (separated from EA by the Zambezi River); RU, Ruvuma region between Kilombero and Shire Rivers; and Southern Africa, which was subdivided further based on spatial distribution into Northern (SN), Eastern (SE) and Western (SW) lineages (Moodley et al., 2017). Most recently, *de novo* sequence analysis of genomes from all five extant and three extinct rhinoceros species has shown strong support of the geographical hypothesis of rhinoceros evolution and confirmed low genomic diversity in all extant rhinoceroses (Liu et al. 2021). However, none of these studies included representative samples from the current populations in Tanzania, so little is known about the genetic impacts of the severe population declines and subsequent management practices to increase numbers in this region. Thus, revealing the maternal diversity will help conservation efforts with regards to current management practices focused on translocations of individuals and to inform population viability assessments.

In order to compare current patterns of genetic variation in Tanzania with the widest geographic sampling from other studies, this study focused on the maternally inherited mtDNA control region. Our specific aims were to investigate: 1) the impacts of past translocations on diversity of maternal lineages in extant subpopulations; 2) whether there has been evidence of dispersal of females between populations based on haplotype sharing; and 3) how current haplotype diversity relates to historical patterns.

2.2 Materials and Methods

2.2.1 Study area description

We sampled the East African subspecies of eastern black rhinoceros, *D. b. michaeli,* from the six extant protected subpopulations in Tanzania and one transboundary population in the Maasai Mara in Kenya (Figure 1.1). Each subpopulation has had a different history of demographic changes and re-introduction strategies, as detailed below.

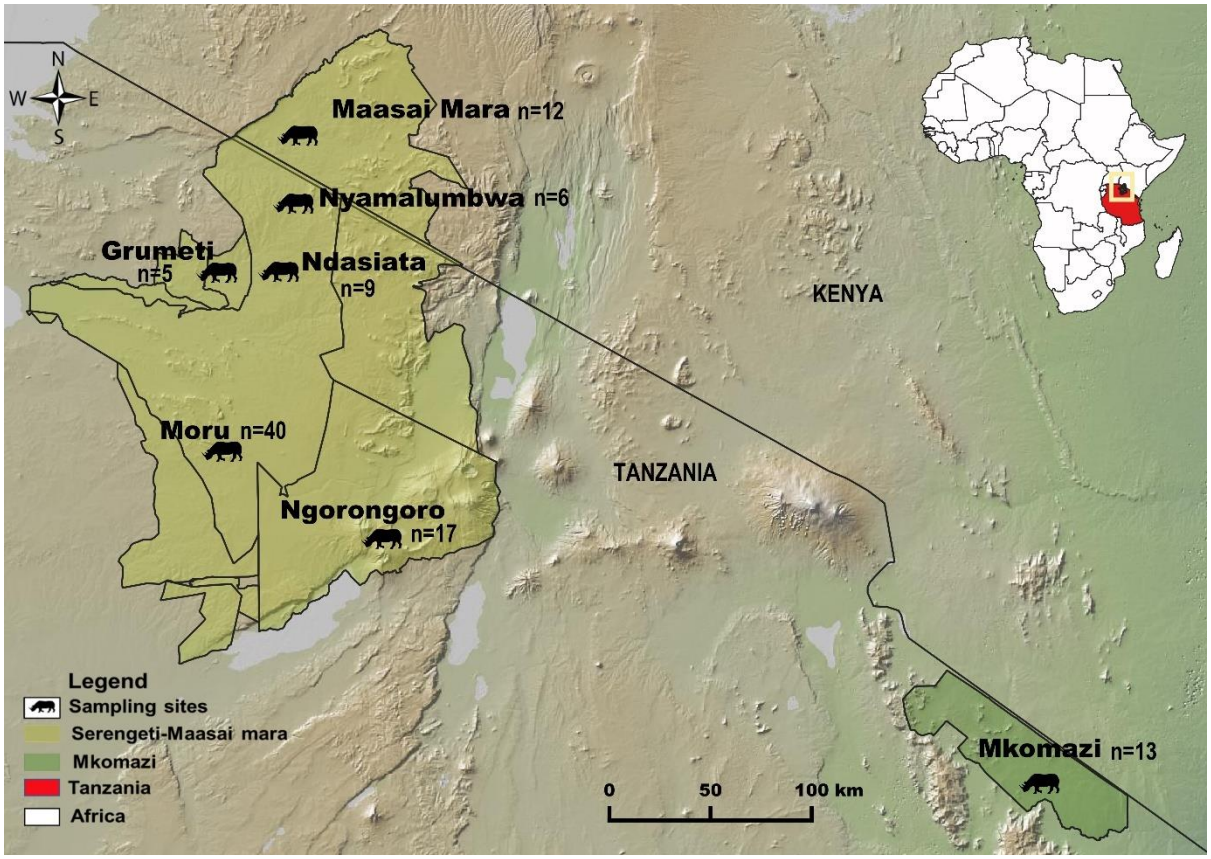


Figure 2.1. Seven populations of eastern black rhinoceros (n=102 individuals) sampled for mtDNA analysis from the Serengeti-Mara ecosystem and the Mkomazi ecosystem in Tanzania and Kenya, East Africa. The inset shows the location of Tanzania (red) in Africa and the sampling area.

2.2.1.1 Maasai Mara

The Maasai Mara Game Reserve in Kenya is located in the northern portion of the Serengeti-Mara ecosystem (Figure 1.1). There were approximately 120 eastern black rhinoceros in 1971 but this number plummeted to 18 individuals by 1984 due to poaching (Moehlman et al., 1996). It is the only population in Kenya with free-ranging indigenous inhabitants unaffected by translocations (Muya et al., 2011). At present, there are 25 eastern black rhinoceros in this population (Table 2.1) that utilize areas across the border between Tanzania and Kenya. Although there are separate monitoring programmes on the two sides of the border, animals are known to move between the Maasai Mara IPZ and the Nyamalumbwa IPZ within the Serengeti National Park.

2.2.1.2 Nyamalumbwa

The Nyamalumbwa rhinoceros project works to conserve the eastern black rhinoceros inhabiting the cross-border area between northern Serengeti in Tanzania and the Maasai Mara National Reserve in Kenya (TAWIRI, 2019). The project started in 1999, with only four pioneer native individuals; i.e. one male and three females (Fyumagwa and Nyahongo, 2010). The Nyamalumbwa population moves freely across the international border and often interacts with the Maasai Mara population to the north and is adjacent to the re-introduced

population at Ndasiata to the south. There are currently 20 individuals in the population (Table 2.1).

2.2.1.3 Ndasiata

The Ndasiata rhinoceros project (Serengeti Rhinoceros Repatriation Project) is situated in the north-eastern part of the Serengeti National Park (Figure 2.1). The population was re-introduced in 2009, with the main objective to return indigenous animals to their native habitat. Five eastern black rhinoceros (two males and three females) were reintroduced from a captive population in Thaba Tholo, Thabazimbi, South Africa. The original animals in the Thaba Tholo captive population came from Tsavo National Park in Kenya and had been caught in 1961, during a period of high poaching (Hall-Martin, 1984). The population has increased to 9 individuals (Hall-Martin, 1984). The population has increased to 9 individuals (Table 2.1).

2.2.1.4 Moru kopjes

The Moru rhinoceros project strives to conserve eastern black rhinoceros inhabiting the southern part of the Serengeti National Park (Figure 2.1). The population started with only one male and two females. While the two females were residents who survived poaching crises in the late 1980's and early 1990's, the male migrated from the Ngorongoro Crater in 1994 (TAWIRI, 2019). The three founders successfully reproduced to generate 40 individuals (Table 1.1) in the current population (TAWIRI, 2019).

2.2.1.5 Ngorongoro Crater

The Ngorongoro Conservation Area (Harrisson et al.) occupies the southern side of the Serengeti-Mara ecosystem. Between 1964-1966, there were 108 eastern black rhinoceros in the NCA but, due to poaching in the 1990's, only 10 individuals remained in the area. In 1997, two female eastern black rhinoceroses were introduced from Addo Elephant National Park in South Africa. The parental stock of these individuals were initially sourced from the Kibodo area in Kenya in 1961 and 1962 (Hall-Martin, 1984). Currently, the NCA holds the largest population of free ranging eastern black rhinoceros in Tanzania (Table 2.1).

2.2.1.6 Grumeti

Grumeti-Ikorongo is a conservation area situated in the northern part of Tanzania, adjacent to the Serengeti National Park, and is part of the larger Serengeti-Mara ecosystem. The area includes the Grumeti Game Reserve and the Ikorongo Game Reserve. The Grumeti Rhino Reintroduction project initiative combined habitat restoration, anti-poaching measures, with the reintroduction of eastern black rhinoceros to the reserve from various sources. As of September 2021, the project had successfully reintroduced 18 individuals that had been maintained in captive conditions, from San Diego Zoo, Port Lympne Park in the UK and Thaba Tholo in South Africa.

2.2.1.7 Mkomazi

The Mkomazi Rhinoceros Sanctuary is in Mkomazi National Park. This is actually the southern extension of Kenya's Tsavo West National Park ecosystem (Mbeyale and Songorwa, 2008). Historically, eastern black rhinoceros would have moved between these two areas; however, fencing now restricts their movements (Homewood and Brockington, 1999). The sanctuary was established in 1997 as a breeding ground for eastern black rhinoceros, with the aim of restoring a wild population. The starting population was composed of individuals from a collection of different zoos around the world: five from Addo Elephant National Park in South Africa; three from Dvur Kravole Zoo in the Czech Republic; and three from Port Lympne Wild animal Park, UK (Fyumagwa and Nyahongo, 2010). The population currently has 30 individuals (Table 2.1).

Table 2.1. The number and type of samples collected from each population, n = number of individuals sequenced, N = estimated population size based on 2019-2021 census (TAWIRI, 2019) TANAPA and TAWA annual rhino census reports 2021.

Population						n	N
	Serum	Ear tissue	FTA card	Blood EDTA	Skin biopsy		
Maasai Mara	0	12	0	0	0	12	25
Nyamalumbwa	0	6	0	0	0	6	20
Ndasiata	0	3	0	6	0	9	9
Grumeti	0	0	5	0	0	5	18
Moru	4	30	0	6	0	40	40
Ngorongoro	0	8	0	9	0	17	60
Mkomazi	2	7	0	1	3	13	30
Total	**6**	**66**	**5**	**22**	**3**	**102**	**202**

2.2.2 Sample collection

For each subpopulation, samples were collected opportunistically during ear-notching operations designed to provide unique individual identification or during routine veterinary interventions. Samples include ear tissue, whole blood in EDTA and serum. In addition, biopsy darts were used to collect tissue samples from three young individuals from Mkomazi that had not yet been included in the ear notching campaigns (Table 2.1). Our strategy was to sample as many of the extant individuals as possible, rather than targeting individuals that may be related or occupy adjacent home ranges.

2.2.3 DNA extraction, mtDNA amplification and sequencing

Total genomic DNA from serum, EDTA blood or tissue samples was extracted using DNeasy® DNA kits following the manufacturer's protocol (Qiagen Inc., Valencia, CA, USA, 2014). A 532 bp fragment of the mtDNA control region was amplified using the mt15996L (5'-TCCACCATCAGCACCCAAAGC-3') and mt16502H (5'-TTTGATGGCCCTGAAGTAAGAACCA-3') primers, as described by (Brown and Houlden, 2000). The primers target the *D. b. michaeli* mtDNA control region at positions 15408 and 15939 (Moodley et al., 2017).

Polymerase chain reactions were carried out in a 20 µl reaction containing 2 µl of DNA diluted to 1/100, 2 µl of 1x PCR buffer, 1.2 µl of 50 mM MgCl2, 2 µl of 25 mM dNTP, 0.2 mg/µl purified BSA, 0.4 µl of each primer (10 µM), 0.2 µl of Taq polymerase (5 U/ µl) and 11.6 µl of purified water. Reactions were denatured at 95°C for 5 min, followed by 45 cycles of 94°C for 30 sec, 60°C for 1 min, 72°C for 1 min and a final extension of 72° C for 10 min. Amplified products were sent to the University of Dundee Sequencing Service for Sanger sequencing on an ABI 3730 automated sequencer; samples were sequenced in both directions using the PCR primers. The resultant sequences were manually cleaned and the contigs assembled using Sequencher version 4.5 (Gene Codes Inc; Ann Arbor, Michigan).

2.2.4 Impacts of past translocations on genetic variation

The sequences obtained from the samples collected from extant populations were aligned with one another and grouped into unique haplotypes using Sequencher 4.5 (Gene Codes Inc; Ann Arbor, Michigan). The identity of each unique haplotype was determined using a Basic Local Alignment Search Tool (BLAST) search against the National Centre for Biotechnology Information (NCBI) database. The sequences were aligned using Clustal Omega (Sievers et al., 2011) and manually optimised using Se-Al version 2.0 (Rambaut 2002; http://tree.bio.ed.ac.uk/software/seal/). Thereafter, the sequences were collapsed into unique haplotypes using DNAsp v6 (Rozas et al., 2017) and haplotype frequencies for each population were calculated (see Online Resource 1).

Relationships among the extant and historical haplotypes were visualized using a minimum spanning haplotype network generated with PopArt version 1.7 (Leigh et al., 2015). Branch lengths were scaled according to the number of mutations separating linked haplotypes in the network.

Genetic diversity of the mtDNA control region for the entire population, as well as for each subpopulation, was independently assessed by calculating haplotype diversity (*h*) and nucleotide diversity (π) in Arlequin version 3.5 (Excoffier and Lischer, 2010). Haplotype diversity (*h*) is the probability that two randomly sampled haplotypes from a population will be different from one another (Nei, 1987). Nucleotide diversity (π) is the average number of nucleotide differences per site between two DNA sequences across all possible pairs in the sample population (Nei, 1987). To assess changes in diversity over time, we compared the values from the extant Tanzanian and Maasai Mara populations to the historical populations sampled by Moodley et al 2017, Thuo et al. 2019 and Muya et al 2011.

2.2.5 Differentiation between subpopulations in Tanzania

For the current Tanzanian mtDNA control region sequences, population structure was assessed using analysis of molecular variance (AMOVA) in Arlequin 3.5 (Excoffier and Lischer, 2010). Population differentiation was further assessed using pairwise genetic distances between each population based on F_{st}.

2.2.6 Phylogenetic context of Tanzanian haplotypes

For comparative analysis, mtDNA D-Loop data from captive and wild black rhinoceros populations were obtained from GenBank. These sequences were

deposited by (Akçakaya and Sjögren-Gulve, 2000; Brown and Houlden, 2000; Muya et al., 2011; Kotzé et al., 2014; Githui et al., 2017; Moodley et al., 2017) (see Online Resource 1). Only Moodley et al, 2017 included samples from Tanzania but the other two studies were focused on samples from Kenya, allowing a broader context for the relative frequency of East African haplotypes; Thuo et al. (2019) provided 25 samples from Lake Nakuru National Park and Muya et al. (2011) included samples from 12 Kenyan subpopulations but only deposited unique haplotypes to Genbank. We used the Clustal W multiple alignment package in the BioEdit software version 7 (Hall, 2017; https://thalljiscience.github.io/page2.html) to align sequences obtained from the current study with a total of 444 other sequences retrieved from GenBank from these studies. The sequences were then collapsed into unique haplotypes and their frequencies recorded. The geographical region for each sample was identified (where that information was available; Online Resource 1) and each haplotype classified into one of the haplogroups identified by Moodley et al, 2017. Where possible, haplotypes were further classified into either historical or modern groups (i.e., originating from museum archives, as opposed to being sampled from an extant population). No sampling dates were provided for the Muya et al. (2011) and Thuo et al. (2019) sequences but they were sampled from extant populations so they were considered as "modern". Phylogenetic relationships among the haplotypes were analysed using black rhinoceros haplotypes recovered in our data set and from previous studies with a white rhinoceros (*Ceratotherium simum simum*) sequence from GenBank as an outgroup (FJ004916.1; Online Resource 1). This analysis was done in BEAST v 2.5 (Bouckaert et al., 2019) under a Bayesian skyline model for lineage coalescence and TN93 (Tamura and Nei, 1993) nucleotide substitution model, as determined by model selection in the MEGA X software (Kumar et al., 2018). The analysis was run for 100 million MCMC steps, sampling the posterior distribution every 10,000 steps. The initial 10% of steps were discarded to ensure we sampled from the stationary part of the distribution. The final tree was visualised in Evolview software version 3 (Subramanian et al., 2019) and annotated using: the relative frequency of each haplotype, whether the haplotype was sampled from the extant (modern) or historical (museum samples) populations, the geographical regions of the haplotypes, and the haplogroups (WW, NE, CV, EA, CE, RU and South African) described by Moodley et al. (Moodley et al.). For our samples and those lacking spatial data from other studies, we assigned haplogroups based on the positions in the phylogenetic tree (Online Resource 1). To further visualise relationships between the extant and historical haplotypes, we generated a minimum spanning haplotype network using PopArt version 1.7 (Leigh et al., 2015).

2.3 Results

2.3.1 mtDNA haplotype distribution

A total of 90 samples were sequenced successfully from Tanzania, with 12 more sequenced from the Maasai Mara in Kenya. The sequences included 25 polymorphic sites with no insertions or deletions and 438 monomorphic sites. Six haplotypes were found among the samples, which differed in frequency and distribution among the populations (Figure 2.2). A comparison of the sampled mtDNA haplotypes with sequences in GenBank showed 100% similarity to published

sequences for *D. b. michaeli* from Kenya for all the haplotypes except Haplotype 2, which most closely matched a sequence from Uganda that had been classified as *D. b. ladoensis* by the submitting authors (Table 2.2). Mkomazi had the highest number of haplotypes (four) while Maasai Mara had three and Ndasiata had a single haplotype. Haplotype 1 was found at the highest frequency and was shared among the five populations from Moru, Ngorongoro, Nyamalumbwa, Maasai Mara and Mkomazi. Haplotype 6 was shared among the three populations that were formed entirely from translocated individuals (Grumeti, Mkomazi and Ndasiata), but also a native population in Ngorongoro, which contains some translocated individuals. Haplotype 2 was found only in the native Moru and Maasai Mara populations, haplotype 4 was restricted to Ngorongoro, Nyamalumbwa and Maasai Mara while haplotype 5 was found in the Mkomazi and Grumeti populations (Figure 2.2). The minimum spanning network showed that haplotype 2 had the highest number of mutations separating it from all others, whereas haplotype 6 and haplotype 3 were separated by only one mutation. Haplotype 4 was separated from haplotype 1 and haplotype 5 by the same number of mutations, forming a triangle (Figure 2.3).

Table 2.2. NCBI Blast results of the six mtDNA control region haplotypes from the extant eastern black rhinoceros populations in Tanzania, showing the most similarly matching sequence in the GenBank database (100% similarity in each case). Query cover % = the percentage of overlap between the input sequence and the sequences identified in the database (out of 478bp). The species identity and geographical origin of the closest match, along with the GenBank accession numbers are also shown.

Haplotype	Accession number	Query cover %	Origin	GenBank accession
Haplotype 1	OQ095383	100	Kenya	KU569501.1
Haplotype 2	OQ095384	98	Uganda	KY472411.1
Haplotype 3	OQ095385	98	Kenya	KY472430.1
Haplotype 4	OQ095386	98	Kenya	KY472540.1
Haplotype 5	OQ095387	98	Kenya	KY472506.1
Haplotype 6	OQ095388	98	Kenya	KY472425.1

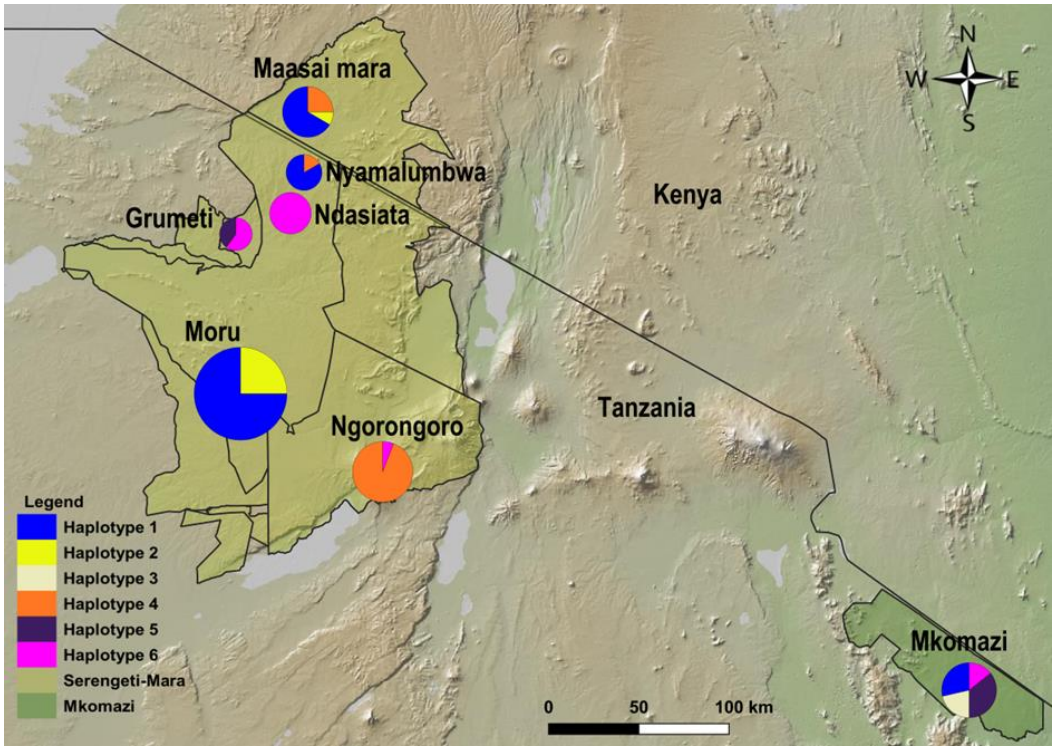


Figure 2.2. A map of relative frequency and geographical distribution of the six mtDNA haplotypes in populations of eastern black rhinoceros in Tanzania and Kenya. Size of the circles correlates with the number of individuals sampled from each population.

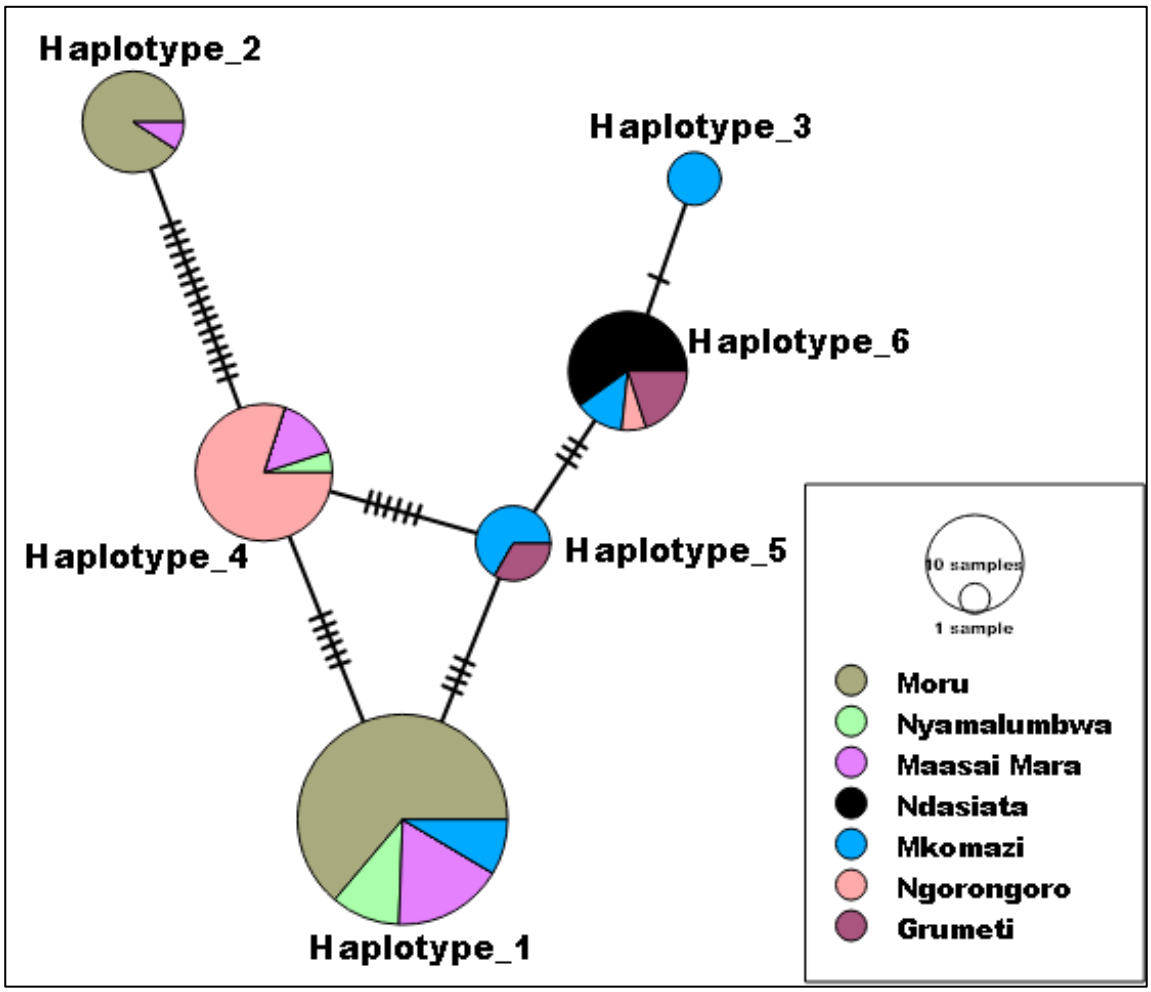


Figure 2.3. A minimum spanning network joining the six mtDNA control region haplotypes found in Tanzania. Circles represent haplotypes and the size is proportional to the haplotype

frequency; ticks on branches show the number of mutations separating linked haplotypes; colours indicate the relative frequency of the haplotypes in each population.

The overall mtDNA haplotype diversity across all extant eastern black rhinoceros sampled (n = 102) was 0.72, but the values varied considerably when each population was considered alone (Table 2.3). Mkomazi (n=13) had the highest haplotype diversity (0.78), while Ndasiata had no haplotype diversity because it had only a single haplotype. Despite having only two haplotypes, Moru had the highest nucleotide diversity ($\pi = 0.016$), followed by Maasai Mara ($\pi = 0.012$); they shared the highly divergent haplotype 2 (Table 2.3).

Table 2.3. Mitochondrial DNA control region diversity of the current eastern black rhinoceros populations in Tanzania and the Maasai Mara compared to historical samples described by Moodley et al, 2017(Moodley et al.). n=number of individuals sampled; nhap=number of haplotypes; S = number of segregating sites; h = haplotype diversity; π = nucleotide diversity. Historical diversity estimates were obtained from (Chipman et al., 2008; Moodley et al., 2017).

Population	n	nhap	S	h	π
Maasai Mara	12	3	21	0.53	0.012
Nyamalumbwa	6	2	6	0.33	0.004
Ndasiata	9	1	0	0	0
Grumeti	5	2	3	0.6	0.004
Moru	40	2	19	0.38	0.016
Ngorongoro	17	2	7	0.12	0.002
Mkomazi	13	4	7	0.78	0.007
Total Extant	**102**	**6**	**25**	**0.72**	**0.017**
Tanzania historical	**29**	**19**	**36**	**0.95**	**0.021**

The 19 haplotypes identified among 29 individuals sampled from historical populations in Tanzania by Moodley et al, 2017included five of the haplotypes found in the current populations (Table 2.3). Haplotype 5 was not found among the historic samples from Tanzania, but it had been identified among recent Kenyan samples (n=4) and a Ugandan historic sample in Moodley et al 2017. Of the three Tanzanian samples that Moodley et al, 2017 classified as "modern", two had haplotype 4 and one had an additional haplotype not found in our extant samples (haplotype 44; Online Resource 1). Moodley et al. (2017) identified three haplotypes from eight "modern" individuals from the Maasai Mara (all collected in 1989); however, they did not find haplotype 2 in this population. Instead, they found an additional haplotype that was not found in the current samples analyzed by this study (haplotype 63; Online Resource 1). Haplotype diversity for Moodley's historical samples from Tanzania was higher (h = 0.95) than for current populations in this study (h = 0.72;Table 2.3). The average nucleotide diversity across populations in the current study ($\pi = 0.017$) was less than the average of the previously described historical samples from Tanzania ($\pi = 0.021$).

2.3.2 Differentiation between subpopulations in Tanzania

For the comparison using AMOVA analyses, substantially more variation was explained within (60.2%) than among populations (39.8%), which may indicate lack of female migration between populations. However, comparison of pairwise Fst indicated substantial differentiation among individual subpopulations (Figure 2.4),

including between geographically proximate subpopulations such as Ndasiata and Nyamalumbwa.

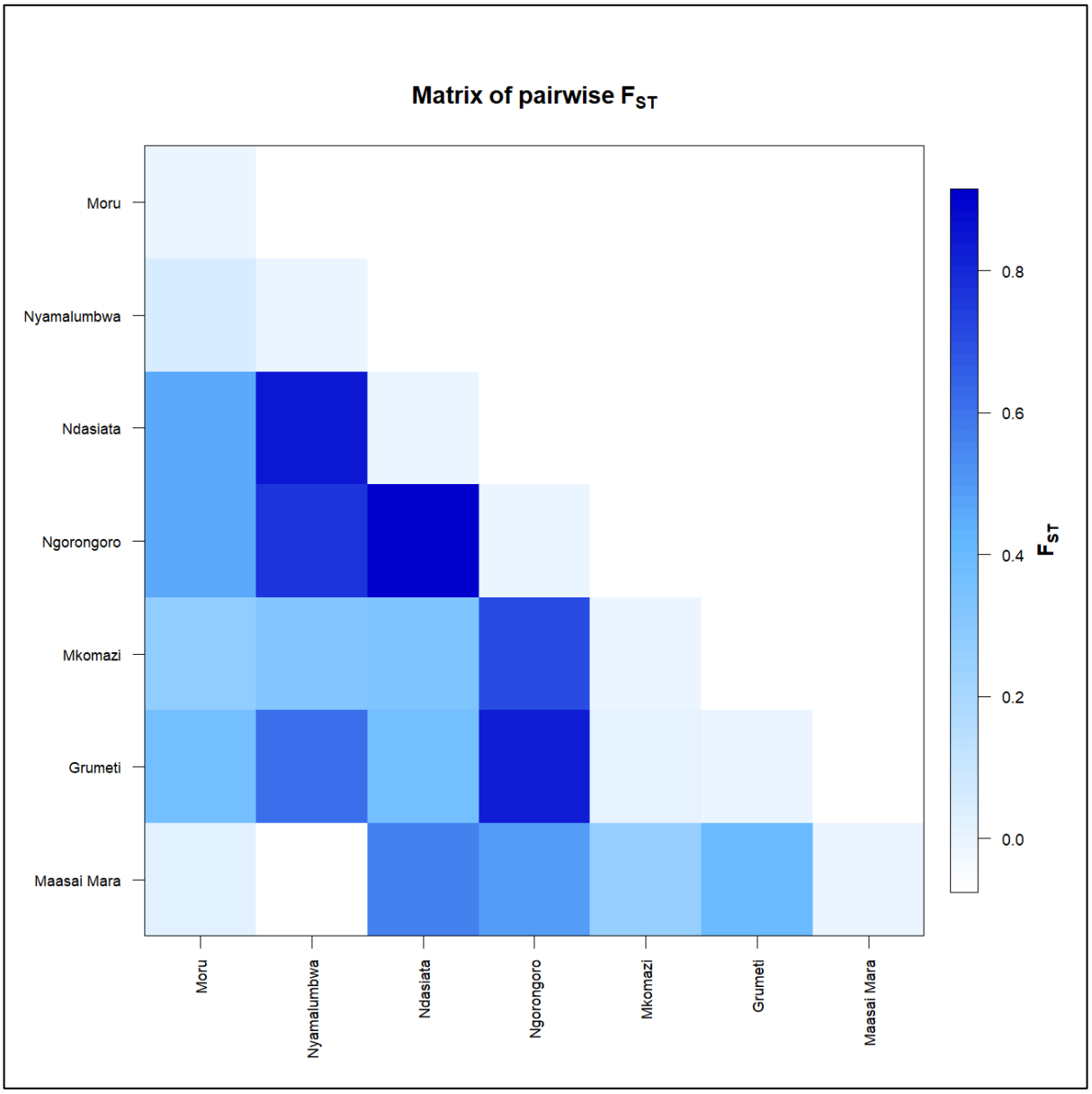


Figure 2.4. Matrix of the pairwise Fst between subpopulations of eastern black rhinoceros in Tanzania. Fst values range from 0 for no differentiation to 1.0 for complete differentiation among subpopulations fixed for different alleles, with the intensity of colours in the heatmap showing higher differentiation.

2.3.3 Phylogenetic context of Tanzanian haplotypes

Alignment to all available published sequences confirmed that all six of the Tanzanian haplotypes identified in this study had been found in other East African populations. Among the 146 sequences available from Kenya (including those newly sequenced here), 34 haplotypes were found; for samples classified by Moodley et al. (2017) nine haplotypes were identified only in historic samples (although they tended to be found in only a single individual), one (haplotype 42) only in samples classified as modern and nine were shared between time periods. The sequences from Muya et al. (2011) and Thuo et al. (2019) included 14 haplotypes that were not identical to any of those described in the Moodley et al.

(2017) study. Haplotype 1 (haplotype 2 in Thuo et al, (2019) was found at a substantially higher frequency in Kenya than all other haplotypes (n = 38; 26% of samples). The next most frequent were haplotype 33 (n = 15; not found in Tanzania) and haplotype 3 (n = 14; found only in the translocated populations Ndasiata and Mkomazi in Tanzania). All three of these haplotypes were found in historic and recent samples from Kenya. As in our study, Moodley et al. (2017) found haplotypes 1, 2 and 4 in modern samples from the Maasai Mara population; Muya et al. (Muya et al.) also identified an additional 7 haplotypes (including haplotypes 3 and 6). Haplotype 2 was also found in historical populations from Uganda. Haplotype 6, which was found in the populations that had been reintroduced (Ndasiata, Grumeti, Ngorongoro and Mkomazi) from South Africa and Europe, was also detected in Tanzanian historical populations and modern and historic Kenyan populations. Haplotype 5 was not detected in historical samples from Tanzania but was found in Kenyan modern populations and in a historic sample from Uganda that had been described as *D. b. ladoensis*.

Phylogenetic reconstruction of the mtDNA haplotypes using all published sequences (Figure 2.5) showed three divergent lineages (using the classifications described in Moodley et al. (2017)), the most distinct of which (L1) comprised haplotypes sampled from West Africa (haplogroup WW from Nigeria and Cameroon). The second lineage (L2) was separable into two haplogroups: North-eastern (NE) and east of the Shari-Logone River system (Chari Victoria; CV). The last lineage (L3) is broadly distributed in eastern and southern sub-Saharan Africa and includes four haplogroups: EA, Eastern Africa; CE, Eastern Africa (Central); RU, Ruvuma (Eastern Africa South); and Southern Africa (including SN, SE and SW geographically defined lineages). The Tanzanian extant population haplotypes were mostly distributed into L3: haplotypes 1, 3, 5 and 6 in EA; haplotype 4 in CE. However, the distinctive haplotype 2 was in the CV haplogroup from L2.

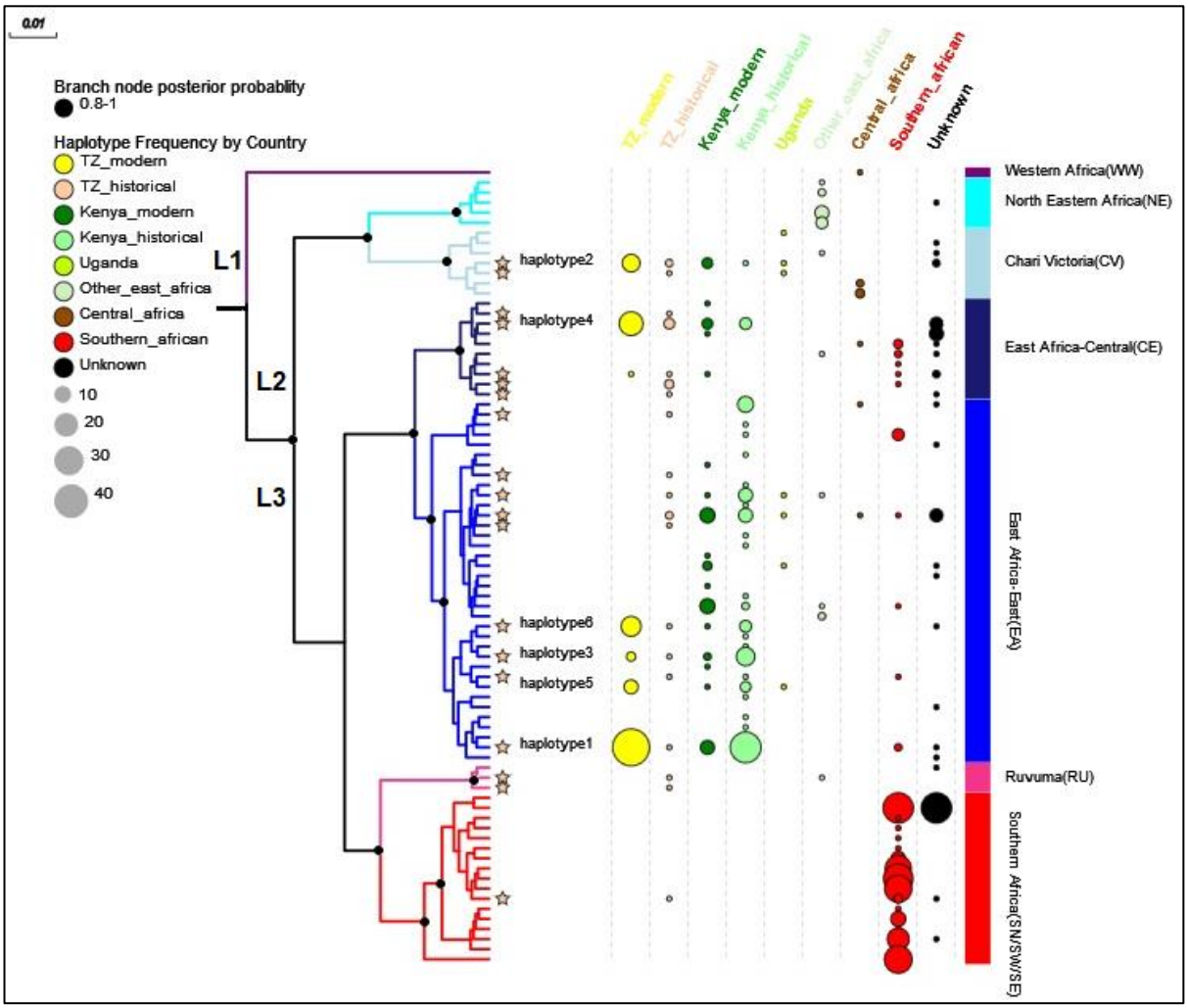


Figure 2.5. Bayesian phylogenetic tree of 79 mitochondrial DNA (mtDNA) control region haplotypes, obtained from a sample of 545 individual black rhinoceros sequences, with white rhinoceros (*Ceratotherium simum simum*) used as an outgroup. Branches with a posterior probability greater than 80% are indicated with a dot on the node. The relative frequency of each haplotype (proportional to the size of the circles) in various geographic regions (indicated with colours) is indicated to the right of the tree. Stars signify haplotypes from Tanzania, with the yellow circles indicating relative frequency in modern samples and peach circles historic. Relative frequency of haplotypes from other East African populations are indicated in various shades of green. Other East Africa includes Malawi, Ethiopia, Sudan, Eritrea and Somalia; Central Africa includes Cameroon, Chad, Nigeria and the Democratic Republic of Congo. Haplotypes from individuals sampled from Southern Africa are all indicated in red combining lineages from Southern Africa (Northern SN); Southern Africa (Eastern SE); SW, Southern Africa (Western SW). Note that haplotypes that had been translocated to Tanzania from South African captive populations (haplotypes 3, 5 and 6) all were found among East African historic samples. Haplotypes for which their locations were not specified by the original authors are indicated by black dots.

Examining the relative frequency of extant East African haplotypes in a phylogenetic context (Figure 2.5 and Figure 2.6) clearly indicates a substantial loss of genetic diversity compared to historic samples, but the remaining haplotypes span multiple lineages within in the CE and EA haplogroups. The phylogenetic tree also confirms observational records that the animals that had been translocated from South Africa were originally of East African origin; however, the introduced haplotypes (3, 5, and 6) were all closely related and from a single EA cluster. None of the haplotypes at high frequency in the southern region of Africa (red circles, Figure 2.5) were detected in the east Africa region.

The tree and network also indicate that some of the diversity that has been lost in Tanzania has been found in modern samples from Kenya.

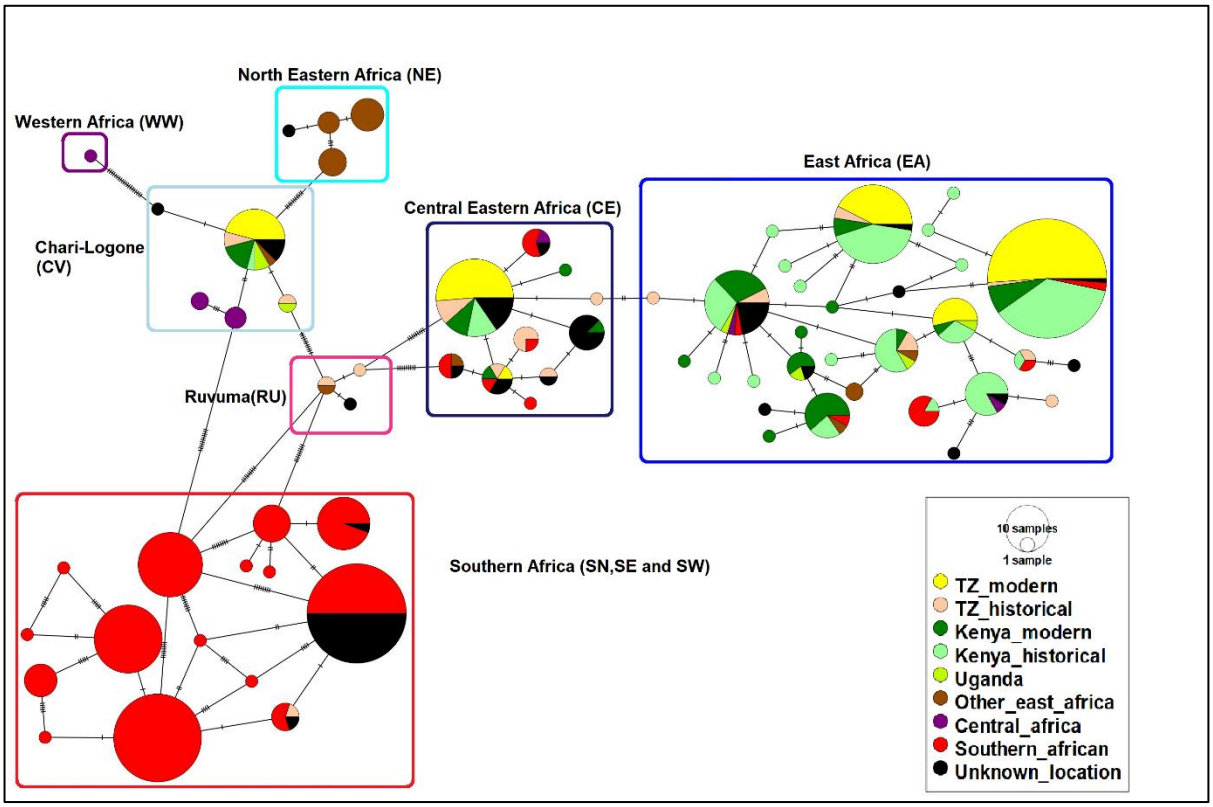


Figure 2.6. Minimum spanning network of 79 mitochondrial DNA (mtDNA) control region haplotypes, indicating the population groupings shown on Figure 2.5. Each coloured circle denotes a haplotype, the size of each circle is proportional to the frequency at which that haplotype was observed in the data set, and the colours represent the country of origin of the samples.

2.4 Discussion

This study presents the first assessment of diversity of mitochondria control region DNA of extant eastern black rhinoceros populations in Tanzania and a neighboring population in Maasai Mara, Kenya. The study adds to the assessment of the global distribution of mtDNA diversity described by Moodley et al. (2017) and provides critical information about maternal diversity that can inform conservation management of the current rhinoceros and other wildlife populations within the region. Although the comprehensive study by Moodley et al. (2017) had included three "recent" samples from Tanzania, only one was obtained from an animal which could still be alive. Therefore, our study fills a major gap in the knowledge about current maternal diversity of eastern black rhinoceros in two East African countries. As predicted from the drastic bottleneck that the extant populations experienced, with founding by only a few individuals, we found that current nucleotide diversity within the extant mtDNA control region was substantially lower than extant samples from Kenya and historical samples from Tanzania. This suggests that the current populations in Tanzania have lost genetic variation; therefore, more research utilising bioparental markers will be helpful to clearly assess the issue. The absence of haplotype sharing between the native and reintroduced populations may be influenced by the management strategies for

Intensive Protection Zones, which limit the ability of rhinos to disperse naturally. This has likely impeded the potential beneficial effects of previous translocations. Although we found that recent translocations from captive populations have restored some of the former maternal lineages that were present historically, the IPZ strategy means that this only benefits the recipient population. Allowing animals to dispersal naturally across the greater Serengeti-Mara could spread the supplemented variation across populations at little cost, rather than relying only on more expensive and riskier managed translocations. In addition, some of the haplotypes that had been lost from Tanzania are still present in extant Kenyan samples; therefore, integrated cross-border management could provide a genetic "rescue" in both countries without introducing genetic variants from outside East Africa. We recognise that free movement could increase risks of poaching, but animals already move outside of their IPZ regions and are forced to return; if dispersing animals were allowed to remain where they choose, this could allow better mixing without targeted interventions.

2.4.1 Current levels of genetic variation in Tanzania

Despite the recent decline in eastern black rhinoceros populations, moderate haplotype diversity (0.72) has been maintained, which is consistent with findings in other regional populations such as those in Zimbabwe (0.76) and Kenya (0.88) (Muya et al., 2011; Moodley et al., 2017). Haplotype diversity, nucleotide diversity and the number of haplotypes are all influenced by the proportion of the population sampled (Goodall-Copestake et al., 2012); in our study we sampled varying proportions of each subpopulation but we had the advantage of knowing how many maternal lineages were expected, due to detailed information on the founders. For example, we sampled all the individuals (40/40) of the current population from Moru but only two haplotypes (Haplotypes 1 and 2) were identified, which is consistent with founding from two females. Nevertheless, this population had the highest nucleotide diversity among the populations sampled (π= 0.017), which was comparable to pre-bottleneck historic patterns (π=0.021), but was due to the large number of mutations separating haplotypes 1 and 2. In the Maasai Mara population, we found the same three haplotypes (haplotypes 1, 2 and 4; n=12) as found by Moodley (2017) with n=15. However, the study conducted by Muya et al (2011) found eight additional haplotypes in that population (including haplotypes 3 and 6); although they didn't provide a detailed sample list, these appear to have been collected in the past decade, suggesting that diversity could be higher than our sampling suggested. We sampled all five individuals found in the other native subpopulation in this region (Nyamalumbwa) but identified only two haplotypes (haplotypes 1 and 4). Because haplotype 1 was the most frequent and is shared with several other native populations from Tanzania and Kenya (Moodley et al., 2017; Thuo et al., 2019), this suggests it could be an ancestral allele that reflects historical, rather than recent connectivity among these populations. Moodley et al. (2017) also sampled historic individuals from Zambia that had haplotype 1, despite being classified as a different subspecies (*D. b. nyasae*). Whether this reflects admixture between subspecies or misclassification would require further investigation.

The uneven distribution of haplotypes across our seven sampled populations means that allowing natural movements and dispersal between contiguous subpopulations (such as those in the greater Serengeti-Mara ecosystem) could enhance the genetic diversity. A similar approach has been suggested for bison

herds in the USA and Canada in order to restore gene flow and enhance genetic diversity (Davies et al., 2022). An alternative approach could be to allow specific animals to move. For example, the relatively high presence of haplotype 1 could suggest over-representation of particular maternal lineages in the native populations (leading to increased inbreeding). Allowing animals that do not have haplotype 1 to move or to be translocated to populations where haplotype 1 is already present could be worthwhile and more effective for restoring rarer haplotypes and lost genetic diversity. Despite retaining highly differentiated haplotypes, the concern for sustainability of the current populations is the low number of maternal lineages confirmed by the mtDNA variation. This means that allowing movements of native individuals might not be enough to maintain sufficient genetic diversity. For example, the Moru population was formed by three native eastern black rhinoceros, two females who survived the poaching catastrophe and one male which migrated from Ngorongoro. Moru has retained both maternal haplotypes from 40 sampled individuals, which is encouraging; however, it also illustrates the risk of inbreeding by maintaining isolated populations. The advantage of translocating animals is illustrated by Mkomazi, which had the highest number of haplotypes (four). Mkomazi had haplotypes that are shared with Grumeti, Ndasiata and Ngorongoro despite being isolated from the Serengeti-Mara ecosystem by a large geographical distance. Therefore, reintroducing animals from captive populations has clear advantages for enhancing genetic variation, and may be best utilized when animals from captive populations are reintroduced to populations that also include native individuals, such as Ngorongoro (Fyumagwa and Nyahongo, 2010).

2.4.2 Differentiation between subpopulations in Tanzania

The AMOVA analysis revealed that a high percentage of variation exists among individuals within populations, but the overall differentiation was moderate, which could reflect historical sharing of alleles and movement between populations. In Kenya, (2011) found the highest Fst (0.729) between Chyulu and the Masai Mara, neither of which included introduced individuals. However, the Chyulu population had been bottlenecked to only two individuals, consistent with the presence of only two mtDNA haplotypes. Since the two populations are within the same ecosystem, this suggests recent restriction of movement, similar to in our study. The lack of sharing of the introduced haplotypes in geographically close populations suggests that there is more restriction of maternal gene flow than home ranges would predict. Black rhinoceros are solitary and highly mobile; their estimated home ranges in the Serengeti are between 40 and 133 km^2, regardless of sex (Frame, 1980). However, the Intensive Protection Zones (IPZs) strategy, with no movement of individuals allowed between populations (Fyumagwa and Nyahongo, 2010), means that these home range sizes are not realized. A vivid example is a comparison between the Ndasiata and Nyamalumbwa subpopulations, which didn't share any haplotypes and so had the highest Fst value (0.97), despite being located very close in the Serengeti-Mara ecosystem. This means that the advantages of the previous translocations to Ndasiata have not been extended to the native Nyamalumbwa population, likely due to the IPZ management strategy. Observational data suggests that the animals would move further if left more unconstrained. For example, on several occasions, individuals (especially bulls) from the Ngorongoro crater (Harrisson et al.) have left their IPZ in search of new habitat or to escape from territorial fights; likewise, Moru individuals have

escaped to Mwiba-Makoa areas. However, the management requires pushing them back into their respective IPZ. For example, in 2004 a young bull from NCA was sighted near lake Eyasi, 100 km away, but was immobilized and returned back(Fyumagwa and Nyahongo, 2010). If individuals were allowed to naturally disperse, this could allow mixing of genetically distinct mating partners, without physical translocations.

2.4.3 Phylogenetic context of Tanzanian haplotypes

In Tanzania, translocation or assisted dispersal has been used as a tool for increasing the size of the eastern black rhinoceros population across the country. In previous years (1997-2018), a total of 23 individuals were translocated to Tanzania from areas outside East Africa but this was done without consideration of genetic variation (Fyumagwa and Nyahongo, 2010). Only four haplotypes (haplotypes 1, 3, 5 and 6) were found from reintroduced eastern black rhinoceros sampled in this study. The parental stock of these individuals were captured from the Kibodo area, Tsavo National Park, Isiolo and Tana River in Kenya between 1960 and 1980 and taken outside East Africa to highly protected areas such as zoos and closed sanctuaries as a measure to rescue them from poaching in the wild (Hall-Martin, 1984). This was confirmed by phylogenetic reconstruction of the mtDNA, which demonstrated that the maternal lineages introduced to Ndasiata, Mkomazi and Ngorongoro were of East African origin, despite individuals being translocated from European zoos or captive population in South Africa. However, three of the introduced haplotypes were closely related and clustered together on the tree, suggesting that the previous translocations achieved limited augmentation of genetic diversity in the extant populations. Furthermore, the presence of a wide range of the “lost” Tanzanian haplotypes in modern Kenyan samples (Muya et al., 2011; Moodley et al., 2017) suggests that translocations within East Africa could be more beneficial, less costly, and less risky than long-distance translocations from Europe and South Africa.

The phylogenetic tree illustrates that mitochondrial control region sequence variation is highly structured, suggesting that careful consideration of which lineages to reintroduce could be beneficial (Figure 2.5). Tanzanian extant populations haplotypes were distributed into all three haplogroups (CV, CE and EA) found in East Africa but there were notable gaps in the presence of particular clades that were present historically. The next step for specifically identifying individuals for translocation will be to assess the nuclear genome, not only to confirm the status of individuals with rare maternal haplotypes, but also to identify paternal contributions to the genetic diversity. We are currently taking a whole genome sequencing approach to address this question, based on a subset of the individuals used in this study.

2.4.4 Conservation implications

Our study has shown that the Tanzanian eastern black rhinoceros populations have lost substantial variation in the mtDNA from the recent population decline but still maintain moderate genetic diversity across the subpopulations. Recently translocated populations (such as Mkomazi and Ndasiata) have restored some of haplotypes that were previously present; however, the genetic benefit of translocation has been under-realized because animals are not permitted to move

between Intensive Protection Zones (IPZ). Based on our results we recommend a combined management approach: (i) subpopulations that occur in the same ecosystem (such as the greater Serengeti-Mara ecosystem) should be managed as a single metapopulation rather than isolated IPZs. This would allow movement of individuals between regions (such as Ngorongoro to Maasai Mara) and could enhance supplementation of the native populations with additional genetic variation from past translocations; (Hoffmann et al.)for populations that do not occur in the same ecosystem, such as Mkomazi, targeted reintroductions based on genetic variation may be the most effective way to reduce the effects of inbreeding and maintain genetic diversity; (iii) we recommend whole genome sequencing of nuclear DNA to further inform which individuals to translocate because this would provide evidence of both maternal and paternal contribution to genetic diversity; and finally (iv) we recommend that translocated animals be selected from extant populations within East Africa because there appears to be plenty of genetic variation in the mitochondrial DNA in these populations that were once historically connected. This also could be important to avoid translocation catastrophes, which can occur when animals that have been kept under benign captive conditions are released to wild environments (Chipman et al., 2008).

Data Availability

The six unique haplotypes generated during the current study are available in the GenBank repository, accession numbers: OQ095383-OQ095388.

Supplementary Information

Online Resource 1. Haplotype assignment for all available black rhino sequences available on Genbank as of May 2021, along with new samples collected in Tanzania in Kenya for this study. The accession number, name of the sample from the original paper, and country of origin are listed. For samples included in the Moodley et al. (2017) study, locality information, sample sources and date of sampling are also indicated, as described in the supplementary data provided with that paper. Classification of samples as historic or modern, regional classifications (E = East Africa; SC = South-Central Africa; SW = Southwest Africa; W = West Africa), and mtDNA haplogroups (CE = Central Africa; CV = Chari-Victoria; EA = East Africa; NE = North-east Africa; RU = Ruvuma; SE = Southern-east; SN = Southern-north; SW = Southern-west; WW = West Africa) were also based on Moodley et al. (2017). For nine of the Moodley et al. (2017) samples, it was not possible to match accessions to sample codes, so they have been included as unknown in the calculation of haplotype frequencies. Sampling dates were not provided for the samples from the Githui et al. (2014) and Muya et al. (Muya et al.) studies but these have been classified as modern, because they were sampled from extant populations in Kenya for those studies; they have thus also been classified as E in the regional classifications and mtDNA haplogroups were assigned based on the phylogenetic tree in this study.

3 Natural dispersal is better than translocation for reducing risks of inbreeding depression in eastern black rhinoceros (*Diceros bicornis michaeli)*

Abstract

Due to ever increasing anthropogenic impacts, many species survive only in small and isolated populations. Active conservation management to reduce extinction risk includes increasing habitat connectivity, translocations from captive populations, or intense surveillance of highly protected closed populations. The fitness of individuals born under these scenarios may vary due to differences in selection pressures. However, the genetic impacts of such strategies are rarely assessed. Using whole genome sequences from critically endangered eastern black rhinoceros as a model, we compare the consequences of different types of conservation efforts. We found lower inbreeding in offspring of individuals that had either dispersed from native populations ($F_{ROH>100Kb}$ = 0.13) or been translocated from captive populations ($F_{ROH>100Kb}$ = 0.08) compared to a closed native population ($F_{ROH>100Kb}$ = 0.17). However, the relative abundance of highly deleterious mutations was higher for offspring resulting from translocation compared to the other groups and this load was sheltered by higher heterozygosity. This could increase risks of inbreeding depression if captive founders subsequently inbreed after translocation. In contrast, native dispersers reduced the negative effects of inbreeding without compromising the benefits of past purging of deleterious mutations. Our study highlights the importance of natural dispersal and reiterates the importance of maintaining habitat corridors between populations.

3.1 Introduction

Many highly threatened animal species persist in small, isolated patches that are susceptible to inbreeding and loss of genetic variation due to drastic reductions in population size (i.e. bottlenecks or founder effects), which are warning signals of populations at threat of extinction (Hoban et al., 2022). When too few individuals remain in the wild or when there is insufficient genetic variation for natural dispersal to have a substantial impact, active human interventions have been used for genetic rescue (Weeks et al., 2011; Frankham, 2015; Berger-Tal et al., 2020). This includes translocation of individuals between native populations (*in situ*) or (re)-introduction from captive populations (*ex situ)* (Berger-Tal et al., 2020). However, such assisted movement has often been conducted without explicitly assessing existing patterns of genetic variation or basing decisions on only a handful of genetic markers (Mable, 2018; Purisotayo et al., 2019), which has not allowed assessment of the long-term fitness impacts of past or future management decisions (Ralls et al., 2018; Ralls et al., 2020).
Whole genome sequencing data has the potential to revolutionise genetic rescue because of the expanded inferences possible compared to single-gene approaches. Genomic-scale data can not only inform selection of individuals that could be most beneficial to move for genetic rescue but can also be used to assess the genome-wide consequences of particular management practices (Hedrick et al., 2019; Saremi et al., 2019; Grossen et al., 2020b; Hoffmann et al., 2021; Khan et al., 2021; Alvarez-Estape et al., 2022). Additionally, inferences about demographic history can be modelled more accurately based on millions of single nucleotide polymorphism (SNP) loci compared to markers like microsatellites. This is important to enable assessment of the potential impacts of previous bottlenecks to purge deleterious recessive mutations (Frankham et al., 2001; Hedrick and Garcia-Dorado, 2016; Khan et al., 2021; Mathur et al., 2023). However, artificial management strategies designed to boost wild populations such as establishing founder sub-populations or reintroducing captive animals into the wild could inadvertently negate the long-term benefits of purging deleterious alleles in surviving populations. There has been an increasing focus on assessing the genome-wide impacts of inbreeding on the deleterious mutation load (as a proxy for fitness) but that has most often been considered at the population level, where different individuals within populations might have experienced different types of ancestry due to differences in past management practices (Khan et al., 2021; Humble et al., 2023). However, it is important to be able to disentangle the impacts of historical processes on current fitness and evolutionary potential of wild populations (Mathur et al., 2023; Robinson et al., 2023b).

Here we use critically endangered eastern black rhinoceros (*Diceros bicornis michaeli*) populations in Tanzania as a model for predicting the relative fitness impacts of *ex situ* conservation (i.e. translocations from captive populations to the wild) compared to natural dispersal but focusing on ancestry cohorts of individuals rather than whole populations. The species is critically endangered due to extensive poaching across their natural range, with most surviving individuals restricted to highly protected areas such as zoos, closed sanctuaries and intensive protection zones (IPZ) (Emslie et al., 2009; Fyumagwa and Nyahongo, 2010; Moodley et al., 2017; Liu et al., 2021). Active management has so far succeeded in preventing their extinction in the short term, but previous translocations have

not been informed by genetics (Mellya et al., 2023a) and long-term consequences remain unknown.

Specifically, we use whole genome sequencing data to assess: 1) the scale of inbreeding that has been induced by the severe bottlenecks and subsequent expansion of native populations; and 2) what impacts previous attempts at population supplementation have had on the accumulation of potentially deleterious mutations. Our main aim is to question the potential trade-offs between 1) increasing adaptive potential by introducing new variation into threatened populations; and 2) increasing risks of inbreeding depression (reduction in fitness due to inbreeding) caused by introducing deleterious mutations (genetic load). We hypothesise that the genetic load might have been purged from inbred wild populations (due to severe past bottlenecks and ongoing inbreeding) but "hidden" in captive populations (due to reduced selection under the benign conditions under which they are kept or increased heterozygosity due to mixing individuals from different sources). We consider the impacts of various management practices on: (i) genetic diversity; (Hoffmann et al.) genome-wide inbreeding; (iii) accumulation of deleterious mutational load (relative load); and (iv) homozygosity of deleterious alleles (realised load). We also assess the potential for purging by using the whole genome sequence data from native individuals to estimate the timing and extent of previous bottlenecks. We provide recommendations for managing eastern black rhinoceros populations, as well as more general strategies for genetic rescue of other highly endangered species.

3.2 Methods

3.2.1 Study Area

We focus on the Serengeti-Mara ecosystem on the border of Tanzania and Kenya because the extant populations represent a range of different management scenarios. Black rhinoceros are restricted to five intensive protection zone (IPZ) regions (Figure 3.1), where individuals are free-ranging and unfenced but actively monitored by dedicated wardens (Fyumagwa and Nyahongo, 2010). This intensive management strategy, along with past translocations, has allowed for population size to increase from a low of 24 in 1995 to 171 in 2021. It also means that there are reliable details on population founders and observational pedigrees, as well as detailed information about reproductive rates and survival of all individuals. We collated these data and constructed pedigrees for each geographic location in our study using the R package visPedigree (https://github.com/luansheng/visPedigree). Three subpopulations were established by a small number of native founders after a severe bottleneck: 1) **Moru kopjes** (Moru) in the central part of the Serengeti National Park (Serengeti), was founded by two females native to the area and one male that dispersed from the Ngorongoro Crater in 1994; 2) in 1990 the **Ngorongoro Crater** subpopulation consisted of 13 native individuals, with two females (mother and calf) being reintroduced from Addo Elephant National Park in 1997; 3) in 1999 **Nyamalumbwa-Maasai Mara** (Nyamalumbwa) in northern Serengeti (a transboundary population between Kenya and Tanzania) consisted of 10 native individuals (on the Serengeti side of the border). These two populations originated from native black rhinoceros that endured the poaching era. No rhinos have been artificially introduced into

this region in either Tanzania or Kenya and there is continuous movement between the two sides of the border, with each individual being closely monitored by both countries. Two populations were formed by reintroduced semi wild and captive individuals: 4) Ndasiata, was founded by individuals translocated from Thaba Tholo Game Farm in South Africa in 2010 but there has been subsequent mixing with native individuals who had migrated from Moru; and 5) Ikorongo-Grumeti, on the western border of the Serengeti, was founded by a young bull (Limpopo) and cow (Laikipia), born at Port Lympne Wild Animal Park in England. The individuals were translocated to Grumeti Reserve in 2007. Unfortunately, Limpopo was killed in a fight with a bull elephant in 2009. Therefore, in 2018, a young bull from San Diego Zoo Safari Park in the USA joined the original female (Laikipia) in the sanctuary but they have not yet produced a calf. Furthermore, in 2019, nine eastern black rhinoceros from South Africa's Thaba Tholo Game Farm were translocated and released to the wild, comprising of five cows and four bulls (two of whom were calves). The Grumeti population does not yet have a pedigree as all the rhinos were part of the first generation resulting from translocations from South Africa.

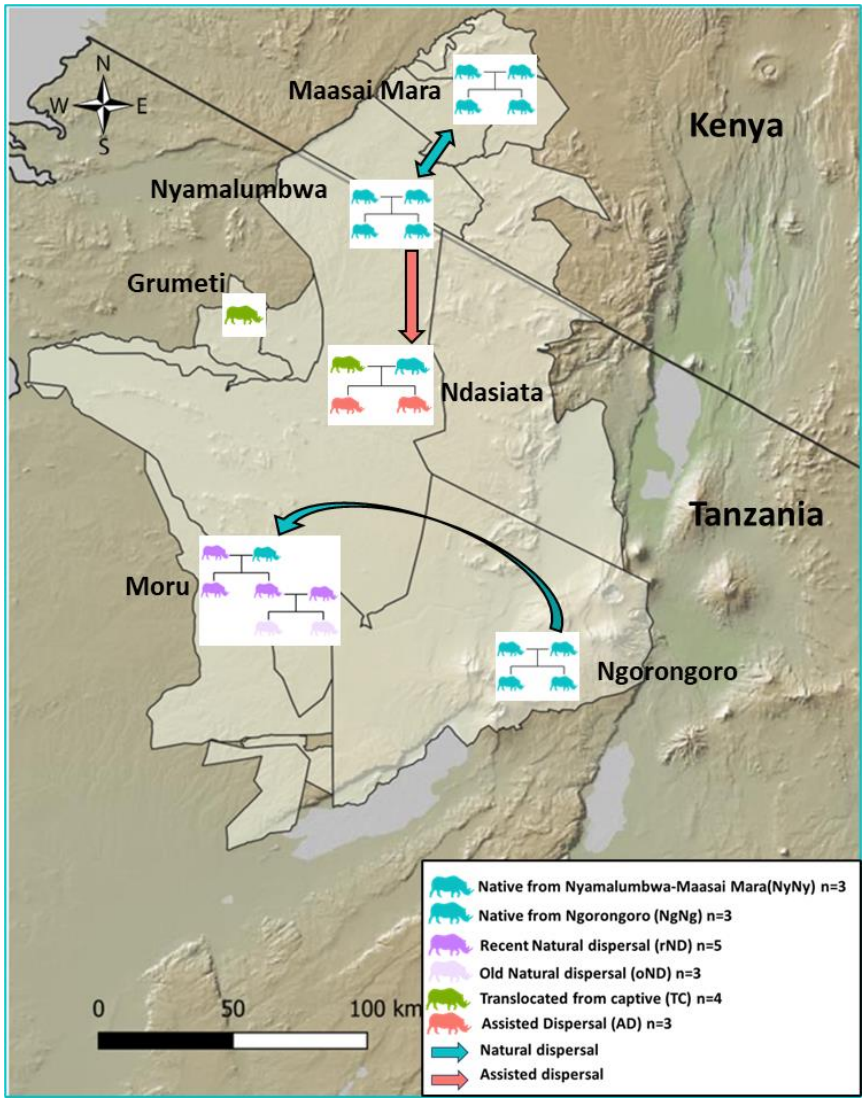


Figure 3.1 An illustrative map of the Serengeti-Mara ecosystem, situated on the Tanzania-Kenya border in East Africa, displaying sampled locations of black rhinoceros populations. The map also features a schematic overview of Ancestry cohorts within each population, and in the map legend we have indicate the number (n) of individuals sampled for each cohort.

3.2.2 Sample collection and sequencing

Tissue samples from ears of rhinos were collected opportunistically when the individuals were chemically captured/immobilized for marking, translocation, ear notching operations, fitting of telemetric gadgets, health treatment, or rescue (Supplementary Table 1). The samples were stored in absolute ethanol in 30 ml vials and transported to a laboratory, where they were stored in -20°C until further use. Blood samples were collected for three individuals from Grumeti and stored on FTA cards (Flinders Technology Associates) at 20°C until further use. DNA from tissue samples was extracted using DNeasy® Blood & Tissue Kits, following the manufacturer's protocol (Qiagen Inc., Paisley UK).

Libraries for Illumina short-read sequencing were prepared by Novogene, using their in-house DNA Library Prep Set kit. Briefly, genomic DNA was randomly sheared into short fragments (350bp) which were end repaired, A-tailed and further ligated with Illumina adapters. The fragments with adapters were amplified using PCR, size selected, and purified. The library was quality checked with a Qubit® 2.0 Fluorometer 2010 (Thermo Fisher Scientific, Cambridge), real-time PCR for quantification and a bioanalyzer (Agilent Technologies Inc., Cambridge) for size distribution detection. Libraries were sequenced on Illumina short-read platforms (NovaSeq PE 150) with an aim to obtain at least 30Gbp data per sample.

3.2.3 Data processing, variant calling and filtering

The raw FASTQ reads obtained from the Illumina platform were end trimmed using default settings of Trim Galore for Illumina (https://github.com/FelixKrueger/TrimGalore). The trimmed reads were mapped to the black rhinoceros reference genome (2.6Gb size), with 99x assembly coverage (https://www.dnazoo.org/assemblies/Diceros_bicornis) using bwa mem (Armstrong et al., 2021). The mapped reads were sorted, and duplicates were marked with samtools (Danecek et al., 2011). This created the final binary alignment map (BAM) files. The BAM files were then indexed and Strelka2 germline variant caller (Grossen et al., 2020a) was used for identifying variants. We limited variant calling only to the long chromosomal scaffolds of the rhino genome assembly to avoid biases later when estimating runs of homozygosity (Shukla et al., 2022). This, however, covers more than 90% of the rhino genome. Overall, we obtained 98,736,095 single nucleotide polymorphism (SNP) loci.

The raw variants identified were filtered using vcftools (Danecek et al., 2011). We removed all indel variants. Any base with a PHRED quality score less than 30 was removed and genotypes with quality score less than 30 were set as missing. Any site with a minor allele counts less than 3 and deviating from Hardy Weinberg equilibrium with a chi-squared p-value of less than 0.05 were removed. We also removed any individual that had more than 80% missing data. We then removed any loci that were missing in at least 25% of the individuals. We identified sex chromosomes, as described in Armstrong et al. (2021), which were also removed from analyses to maintain consistency in the estimates for males and females. We then estimated the mean sequencing depth at each locus and retained only those loci that had the mid 95 percentile depth of sequencing. Although there was

variation among individuals in terms of sequencing depth and retained loci (Supplementary Table 1), overall, we retained 1,649,646 SNP loci after all the filtering.

3.2.4 Estimating genetic diversity, inbreeding and heterozygosity

Genetic diversity, as represented by pairwise nucleotide diversity (pi), was estimated using the filtered SNP loci using the *--site-pi* option in vcftools (Danecek et al., 2011) for each cohort; to standardise sample sizes, three random samples from each cohort were used. We used a rarefaction-based approach as implemented in ADZE (Szpiech et al. 2008) to estimate allelic richness. The filtered set of SNPs were input to ADZE for a maximum haploid sample size G of seven and a TOLERANCE of 0.7. The populations were grouped by ancestry cohorts for the calculations. We used the set of filtered SNPs to estimate pairwise relatedness (PI_HAT). The filtered set of SNPs were input to PLINK v1.9 (Chang et al., 2015) and the option —genome was selected to estimate pairwise relatedness (PI_HAT). To visualise genetic differentiation between cohorts and populations, a Principal Components analysis was conducted using the filtered set of SNPs, as implemented in PLINK v1.9 (Chang et al., 2015) using the option —pca.

Levels of inbreeding can be predicted across different historical time periods, based on the length of homozygous tracts spread throughout the genome (runs of homozygosity, ROH), under the assumption that recombination will break up linked variants over time (Broman and Weber, 1999). We used BCFtools ROH (Narasimhan et al., 2016) to estimate ROH and then calculate F_{ROH} to measure inbreeding, as described in Armstrong et al. (2021). For each sample we used the ROH option of bcftools and set –G 30 and the allele frequencies were estimated on the "fly with by setting" *-e* -. We then estimated F_{ROH} for each size class using the formula F_{ROH} = length of genome in ROH/total autosomal length.

Overall estimates of heterozygosity of individuals in different cohort categories were defined as the number of heterozygous loci divided by the total loci with data for that individual, as calculated using RTGtools (https://github.com/RealTimeGenomics/rtg-tools).

We tested whether ancestry cohort significantly explained variation in the summary statistics using one-way ANOVA, followed by Tukey's tests to determine which levels of the variables explained the variation. All statistical analyses were performed in R 4.3.3 (R core Team, 2024).

3.2.5 Estimating recent demographic history

We estimated the recent demographic history of eastern black rhinoceros using only the native individuals, by combining the NN, oND and rND cohorts. We used the default parameters in GONE (https://github.com/esrud/GONE) (Santiago et al., 2020) for estimating demographic history. We plotted the harmonic mean of effective population size (Ne) obtained for the first 350 generations, using a generation time of 24 years (Liu et al., 2021).

3.2.6 Identifying ancestral alleles, mutation load and homozygosity of deleterious mutations

We defined ancestral alleles as the most common variant present in taxa related to the black rhino. For this, the genome assembly of the northern white rhinoceros (*Ceratotherium simum* genbank assembly: GCA_004027795.1), greater Indian rhinoceros (*Rhinocerous unicornis* genbank assembly: GCA_018403435.1) and Sumatran rhinoceros (*Dicerorhinus sumatrensis* genbank assembly: GCA_014189135.1) were downloaded and used. The data from these three related taxa were mapped to the black rhino genome (https://www.dnazoo.org/assemblies/Diceros_bicornis). From the mapped sequences, the alleles that are most common in the three species were identified as ancestral, as described in Khan et al, (2021).

Under the assumption that derived alleles are expected to more often be deleterious than ancestral variants (Khan, 2023), we used the approach described in Khan et al, (2021) to quantify the genetic load. Briefly, the initial set of variants identified from Strelka2 (Grossen et al., 2020a) were filtered to remove indels, bases below PHRED quality 30 and genotypes below a score of 30. Individuals with more than 80% missing data were removed. Then, we removed any loci missing in at least 25% individuals. Loci with minor allele count of at least 1 and falling within the mean depth across all loci in the mid95th percentile were retained. Loci with F_{IS} values of $-0.5>F_{IS}>0.95$ were retained and sex chromosome scaffolds were removed.

The relative genetic load (i.e. relative fitness reduction due to accumulation of deleterious mutations) of pairs of donor and recipient populations was predicted for each cohort type based on both missense (i.e. amino acid changes that retain the function of a protein) and more serious loss of function (LOF) mutations (Hedrick et al., 2019; Saremi et al., 2019; Armstrong et al., 2021). The SNPs obtained were annotated using the black rhinoceros assembly annotation files using ensembl VEP (McLaren et al., 2016). Loci with missense mutations or LOF, as defined in (Liu et al., 2021), were identified and the derived allele at these loci was classified as deleterious. All intergenic regions were classified as neutral sites. We randomly selected three individuals from each cohort for estimating mutation loads for all pairs of cohorts to control for the differences in sample size. The R_{XY} method, described by Do et al. (2015) and as implemented by Xue et al. (2015), was used to estimate load. Standard deviations were obtained by 100 rounds of bootstrap. Although this is a simplification (Bertorelle et al., 2022; Robinson et al., 2023a), we used homozygosity of derived LOF and missense mutations as a proxy for the realised genetic load, by estimating the number of loci with homozygous derived LOF and derived missense mutations and then dividing by the total number of loci hosting derived LOF and missense mutations.

3.2.7 Enrichment analysis

In order to determine what types of processes might be associated with deleterious mutations, WebGestalt (Liao et al., 2019) (http://www.webgestalt.org) was used for gene enrichment analysis of both the LOF and missense alleles. *Homo sapiens* was set as the organism of interest, the

Over-Representation Analysis was chosen as the method of interest and gene ontology Biological Process, Cellular Component and Molecular Function was the functional database that was chosen. The black rhinoceros genome annotation was uploaded as the reference set. We then uploaded the list of genes containing LOF and missense mutations to run the analysis, with default parameters. The enrichment ratio compares the observed number of genes in the set of interest (e.g. genes with LOF mutations) for a particular category (e.g. myosin complex) to the expected number, which is calculated by dividing the overall number of genes in the set of interest by that in the reference set, multiplied by the number in the reference set for the particular category (Zhang et al., 2005). A hypergeometric test is then used to estimate the significance of the enrichment.

3.3 Results

3.3.1 Observational pedigrees

For each of the five populations we sampled in the Serengeti-Mara ecosystem (Figure 3.1), we utilised the observational pedigree data from the intensive IPZ monitoring to trace the ancestral origins of the rhinos and categorize individuals into five cohorts (Figure 3.2 and; Table 3.4): 1) native, no dispersal (native) - individuals with two wild parents from the same native subpopulation; 2) recent natural dispersal (rND), - first generation offspring of individuals who dispersed from a native subpopulation and mated with an individual in their new resident subpopulation; 3) old natural dispersal (oND) – 2nd or 3rd generation offspring of a parent that had dispersed into a different native subpopulation and mated with residents but where there has been no subsequent gene flow into that lineage; 4) assisted dispersal (AD) - individuals where one parent was native to the population and the other was born in a captive breeding facility; and 5) translocated captive (TC) - individuals that were translocated to Tanzania but whose parents were both born in captivity.

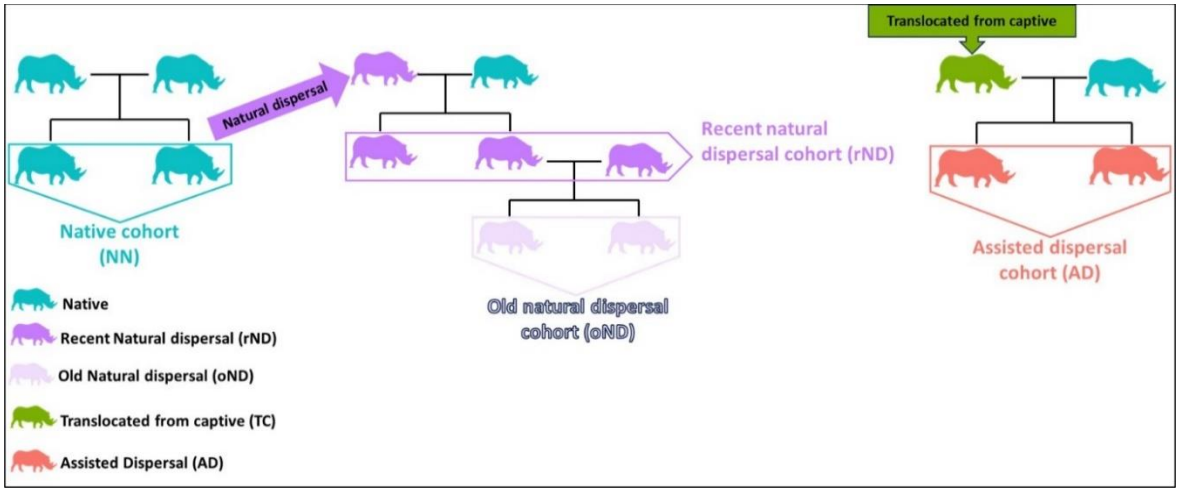


Figure 3.2 Schematic diagram defining the ancestry cohorts in the observational pedigree of the sampled subpopulations in the Serengeti-Mara ecosystem in Tanzania. The native cohort comprises indigenous black rhinoceros that remained in the area following poaching incidents in the 1970s and 1980s. The two natural dispersal categories are intended to investigate the impacts of individuals moving naturally between native populations, assessed across multiple generations after the dispersal event. Due to the male dominance hierarchy, in this case the impacts are of a male that dispersed and remained dominant across multiple

generations; he mated with his daughters, grand-daughters and great-granddaughters. Recent Natural Dispersal (rND) denotes the first-generation offspring resulting from mating between this dominant male, who had natural dispersed from Ngorongoro to Moru in 1994 and mated with the two remaining females in that subpopulation; included in this category is a mating between the male and a daughter produced by his mating with the resident females. Old Natural Dispersal (oND) encompasses the 2nd generation offspring resulting from mating between the first-generation offspring of the founders, along with an offspring produced by the founding male mating with his granddaughter. The Translocation Cohort (TC) are individuals reintroduced to Ndasiata and Grumeti from captive populations in South Africa between 2007-2022, while the Assisted Dispersal Cohort (AD) represents offspring resulting from mating between an individual reintroduced from captivity after translocation and native individuals (effectively, hybrids between captive and native).

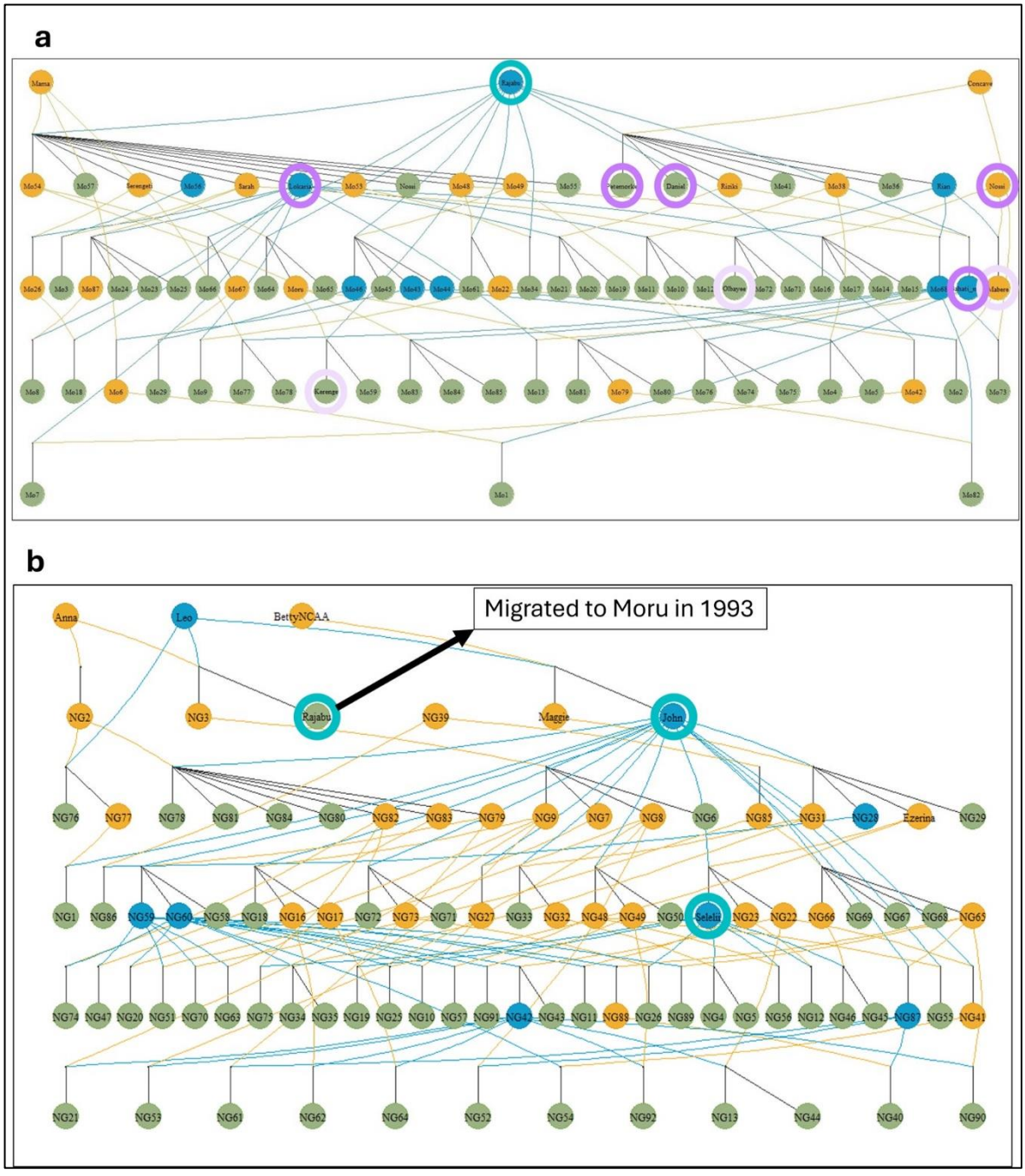
Table 3.4 Ancestry of sampled cohorts, indicating the source population of the parents, the type of ancestry (NN = native, no dispersal; rND = recent natural dispersal; oND = old natural dispersal; AD = assisted dispersal; TC = translocated individuals whose parents were from captive populations), the subpopulation from which the individual was sampled, and the number of individuals sequenced.

Sire	Dame	Ancestry type	Subpopulation	N
Ngorongoro	Ngorongoro	NgNg	Ngorongoro	3
Ngorongoro	Moru	rND	Moru	5
Ngorongoro	Moru	oND	Moru	3
Nyamalumbwa	Nyamalumbwa	NyNy	Nyamalumbwa	3
Nyamalumbwa	Ndasiata	AD	Ndasiata	3
Captive	Captive	TC	Grumeti/Ndasiata*	4

* Since only a single translocated individual was available from Ndasiata, this was combined with the adjacent Grumeti population, which was established from the same captive population in South Africa (Thaba Tholo Game Farm).

Due to the complexity of the pedigrees with more than two generations of data available, the full observational pedigrees are provided in Figure 3.3 and simplified versions to include only the individuals sequenced and their direct ancestors are provided for Moru and Ngorongoro. Individuals presently in Moru are the most inbred because the population had only three founders and the pedigree clearly demonstrates transgenerational mating (Figure 3.3a). However, the single founding male (Rajabu) had dispersed naturally from the native Ngorongoro population and remained the dominant male across multiple generations in Moru, meaning that all rND and oND individuals descended from that sire (Figure 3.3a). The native Ngorongoro population experienced a milder bottleneck than Moru but is still characterised by multiple generations of inbreeding (Figure 3.3b). The records for the other native population in Tanzania (Nyamalumbwa) are not as complete because it is a transborder population connected to Masai Mara National Reserve in Kenya (Figure 3.3c), and there has not been a common system of monitoring developed between the two countries. However, all individuals are classified as native (NyNy). For the Ndasiata subpopulation, founded by South African captive individuals, the samples sequenced were offspring of a native individual who had dispersed from Nyamalumbwa (Msafiri) and mated with captive founders from Thaba Tholo Game Farm in the Republic of South Africa; these were classified as assisted dispersal (AD, Figure 3.3d). We also had a sample available from one of the captive founders (Lunar) from Ndasiata in Tanzania, but this was grouped with the translocated captive (TC) individuals from the adjacent Grumeti

population (Grumeti Game Reserve) for subsequent analyses because they were sourced from the same captive population.



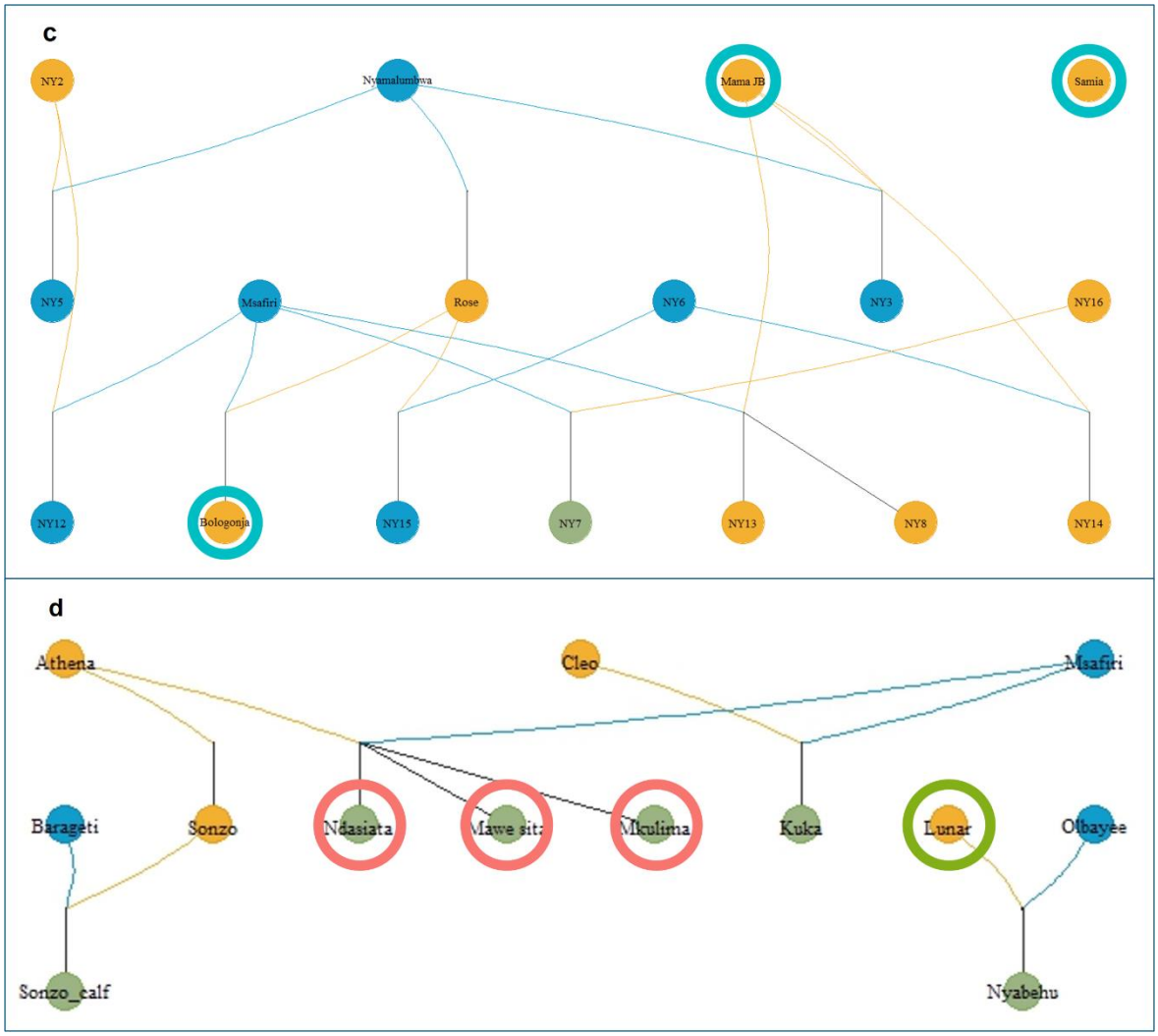


Figure 3.3 Observational pedigree of the sampled subpopulations in Tanzania. For each pedigree, the dark sky-blue circles denote males, dark golden yellow circles females, and dark olive-green circles nonbreeders, which are either sub-adults or adults without offspring. The lines extending from each parent are color-coded according to the gender of the parent; when two parents breed and produce an offspring, the lines converge to create a unified dark grey line connected to the offspring. The individuals sampled for this study are circled with the colour of their cohort type (teal = native individuals whose parents have not dispersed from another population; purple = individuals whose parent dispersed between native populations, rND; red = individuals whose grandparent or great-grandparent dispersed between native populations, oND; orange = offspring resulting from mating between translocated and native individuals and so classified as assisted dispersal, AD; green = translocated individuals whose parents had both been raised in captivity outside of Tanzania, TC). Names are provided for the individuals that were sequenced, along with their parents; other individuals are indicated by their population code and a number. (a) the Moru subpopulation was founded by two females native to Moru (Mama and Concave) and one male (Rajabu), who had migrated by natural dispersal from Ngorongoro; he is classified as native (NgNg) because his parents had been born and reproduced in Ngorongoro, but his offspring produced in Moru are classified as either oND or rND, depending on the generation. (b) the Ngorongoro subpopulation consists of offspring from both native (n = 13 remaining in 1990) and translocated individuals (a mother NG39 and daughter NG85 translocated from Addo National Park in South Africa). but for this study we only sequenced individuals that were classified as native (NgNg). (c) the Nyamalumbwa subpopulation, which is a transborder population between Tanzania (Serengeti National Park) and Kenya (Maasai Mara), was founded exclusively by native eastern black rhinoceros; the individuals we sampled (Bologonja, Mama JB and Samia) were thus all classified as native (NyNy). (d) The Ndasiata subpopulation was founded by the reintroduction of three captive females (Lunar, Athena, Cleo) from Thaba Tholo Game Farm in South Africa in 2010. Subsequently, native males from

Moru (Olbayee, who was himself the offspring of an individual who had migrated from Ngorongoro and so classified as rND in our sequencing; and Msafiri), migrated to the area and bred with the captive females. We sequenced three offspring (Ndasiata, Mkulima and Mawesita) resulting from matings between translocated (Athena) and native (Msafiri) individuals and so classified as AD. The founder we sequenced (Lunar; classified as TC) was grouped with the Grumeti population for the cohort analyses, since she was the only one in this cohort type from Ndasiata but was also sourced from the Thaba Tholo Game Farm. The pedigree from Grumeti is not shown, since all individuals are currently first-generation TC.

3.3.2 Genetic diversity

Using whole genome sequencing (aiming for a range of at least 10x coverage, with an average sequencing depth range after filtering of 7-21x) of 3-5 individuals per cohort, we found similar genetic diversity across cohort types, except for the TC individuals (Grumeti/Ndasiata), which showed higher pairwise nucleotide diversity (Figure 3.4) and allelic richness (Figure 3.5) than the others. One of the native cohorts (NgNg) consistently showed the lowest genetic diversity but differences were small.

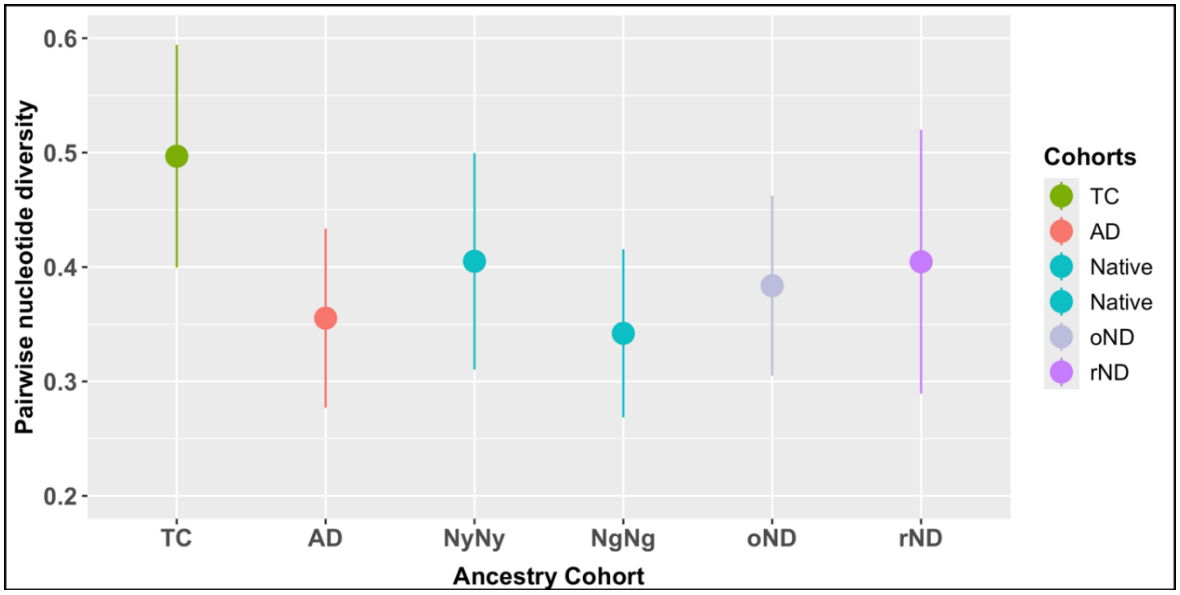


Figure 3.4 The impact of different translocations on genetic diversity, measured as the average number of pairwise differences ± variance between individuals in a cohort (pi).

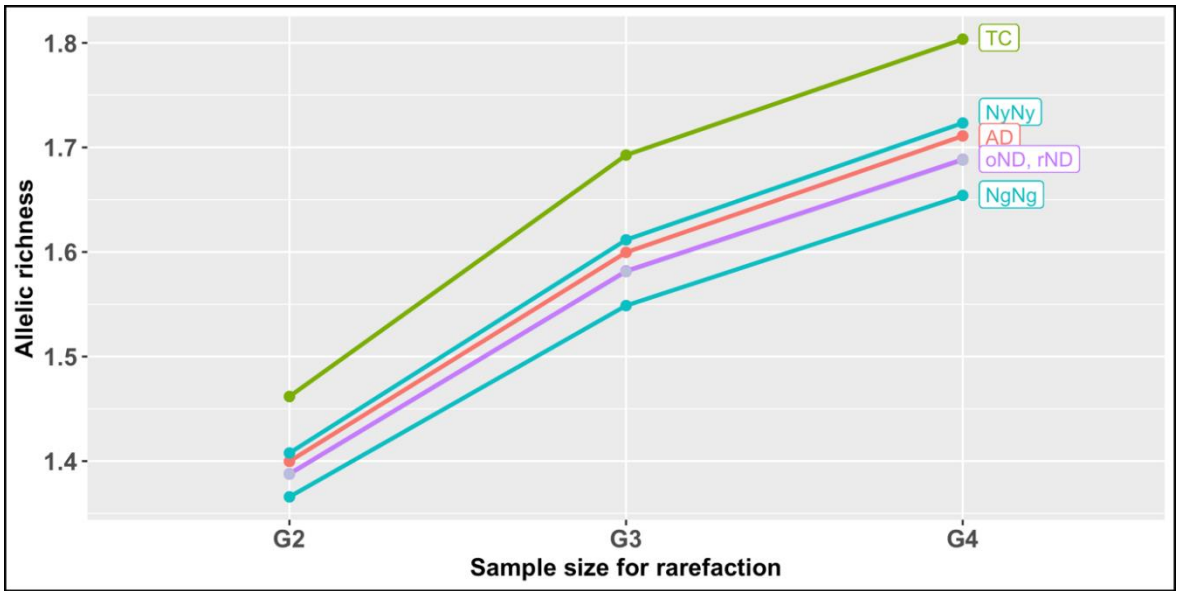


Figure 3.5 Allelic richness in each cohort type. The Y axis represents the average number of distinct alleles per locus and the x axis represents the sample size taken from each cohort during a round of rarefaction. Each line is coloured based on ancestry cohort, but the lines representing oND and rND overlap.

There was a significant effect of ancestry cohort on pairwise relatedness within cohorts ($F_{(5, 22)} = 5.31$, $p = 0.002$; Figure 3.6), which confirms expectations from the pedigree: relatedness was significantly higher (based on Tukey's tests) for the assisted dispersal cohort (AD), which are all siblings, compared to individuals native to the transborder Nyamalumbwa population (NyNy; difference = -0.295) and the two natural dispersal cohorts (oND = -0.217; rND = -0.193). None of the other pairwise differences between ancestry cohorts were significant.

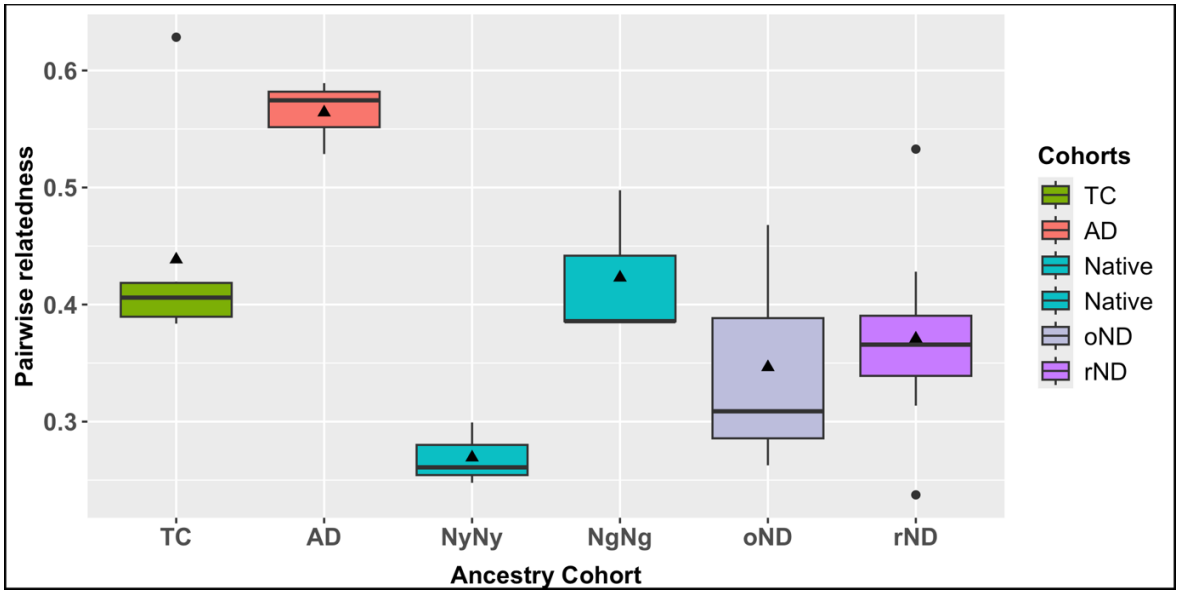


Figure 3.6 Pairwise relatedness among individuals in each ancestry cohort. The length of the box signifies the interquartile range, with the horizontal line representing the median value within each cohort group. Means are indicated by triangles. Whiskers extending from the box depict the range of the majority of the data in each cohort, and black circles beyond the whiskers represent outlier data points.

The Principal Components Analysis (Figure 3.7) indicated separation of the two native populations along both PC1, which explained 19% of the variation, and PC2, which explained 9% of the variation. As might be expected given the geographic

proximity of the Ngorongoro and Moru populations (Figure 3.1), there was little separation of the NgNg, oND and rND cohort from one another, but they were separated from the AD cohort along both axes. The TC cohort were separated from all other cohorts along PC2 but overlapped with both the AD and NyNy cohorts along PC1.

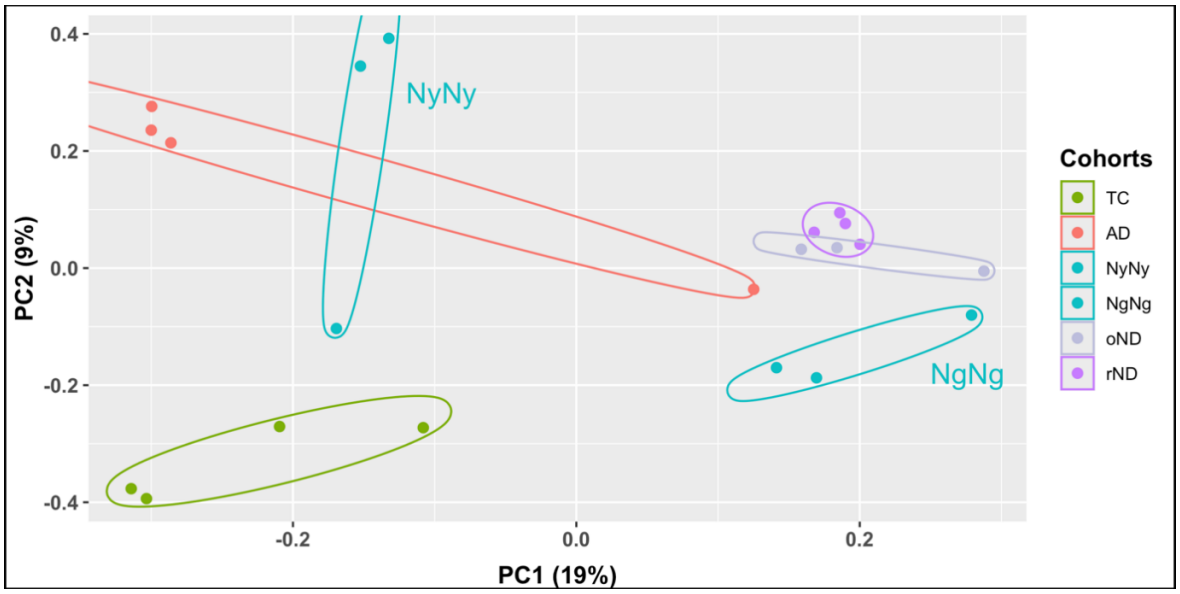


Figure 3.7 Principal components analysis (PCA) based on single nucleotide polymorphisms (SNPs) of all individuals in the dataset. Ellipses indicate variation among individuals within cohorts. The two native cohorts (NyNy and NgNg) are labelled.

3.3.3 Genome-wide inbreeding

Based on runs of homozygosity (ROH) longer than 100kb, reflective of historical timescales, the native cohorts showed higher evidence of inbreeding than cohorts involving captive parents (AD and TC; Figure 3.8a). Although there was a significant effect of ancestry cohort on ROH>100kb ($F_{5,15}$ = 3.25, p =0.0344), no individual pairwise comparisons were significant based on Tukey's tests, reflecting the small sample sizes and large individual variation observed (Appendix Figure 4A.2). However, for ROH>1Mb, evidence for historical inbreeding ($F_{5,15}$ = 3.18, p =0.039) was significantly higher for the oND cohort compared to the AD cohort; interestingly, the means for the TC and rND cohorts were similar, suggesting that natural dispersal can also reduce impacts of past inbreeding (Figure 3.8b). Levels of historical inbreeding in the native individuals were ranked as predicted by the observational pedigrees, with Nyamalumbwa individuals (NyNy), which have ongoing gene flow from the Maasai Mara, showing less historical inbreeding than Ngorongoro individuals (NgNg; Figure 3.8a and Figure 3.8b). However, the offspring of older dispersal from Ngorongoro to Moru (oND) were as inbred as the offspring of native Ngorongoro individuals (NgNg; Figure 3.8a and b; Appendix Table 4A.1).

Considering lengths of ROH reflecting more recent inbreeding (ROH>10 Mb and 20 Mb; Figure 3.8c and d), while there was no significant effect of ancestry cohort on their frequencies ($F_{5,15}$ = 1.88, p =0.157; $F_{5,15}$ = 2.14, p =0.116), the patterns suggest that the highest levels of inbreeding remain in the oND cohort, whereas the rND cohort showed a substantially lower level of inbreeding that is similar to the translocated cohorts (TC and AD). This is consistent with the biology: oND are

the descendants of a single dominant male who mated with his daughters and grand-daughters across generations, which could have reduced the benefits of natural dispersal apparent in the first generation (rND). The rND cohort also showed less inbreeding than the native population that the sire moved from (NgNg) at ROH>10 Mb. While both native cohorts showed higher inbreeding than the translocated cohorts at ROH>10Mb (Figure 3.8c), this effect was reduced for the most recent inbreeding (ROH>10Mb; Figure 3.8d). Maximum ROH, reflecting the most recent inbreeding,

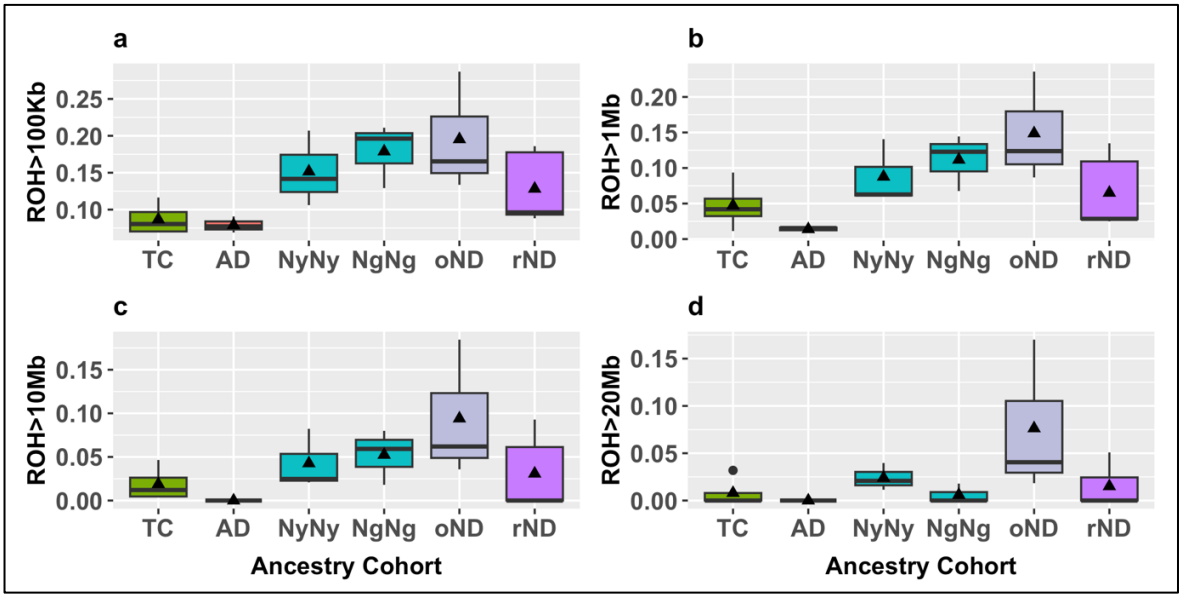


Figure 3.8 Boxplots of frequency of runs of homozygosity (ROH) of varying lengths in relation to ancestry cohorts. (a) ROH>100kb and (b) ROH > 1 Mb are reflective of historical inbreeding; (c) ROH >10 Mb and (d) ROH >20 Mb are reflective of recent inbreeding.

Although there was no overall significant effect of cohort ancestry on the proportion of single nucleotide variants (SNVs) that are heterozygous ($F_{5,15} = 2.63$, $p = 0.067$), there were some interesting patterns (Figure 3.9). Both recent (rND) and old natural dispersal (oND) cohorts showed higher heterozygosity than individuals that had not dispersed from the population that the dispersing sire had come from (NgNg), which included individuals with the lowest values. However, while assisted dispersal (AD) did not substantially alter heterozygosity compared to the native NyNy cohort from which one of the parents had dispersed, the cohort involving only translocated individuals (TC) had higher average heterozygosity than the others.

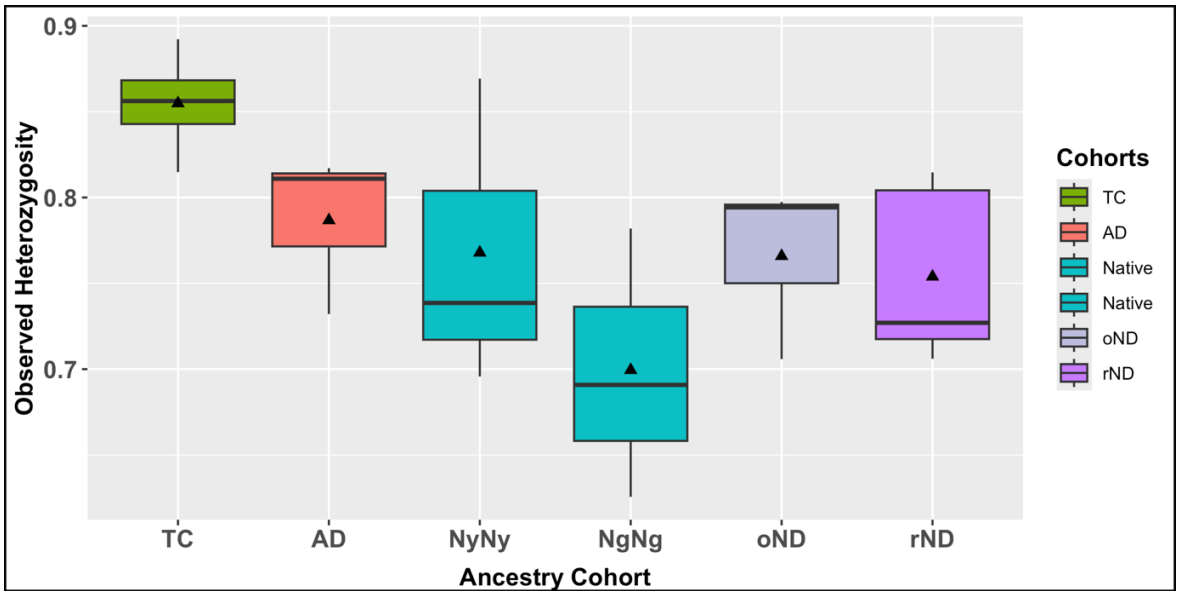


Figure 3.9 Boxplots of the proportion of heterozygous single nucleotide variants (SNV) in polymorphism-containing loci in relation to ancestry cohorts. Based on the data there was no no overall significant effect of ancestry cohort, the translocated cohort showed higher genome-wide heterozygosity than the others, whereas one of the native cohorts included individuals with the lowest values (NgNg).

3.3.4 Recent demographic history

Estimates of recent demographic history using only the individuals with two native parents (NN, rND, oND cohorts) suggest that eastern black rhino populations had an effective population size less than 3500 in the last 350 generations (8400 years) (Figure 3.10). Additionally, the population faced a severe bottleneck 80 generations (~1900 years) ago and then again 7 generations (~168 years) ago.

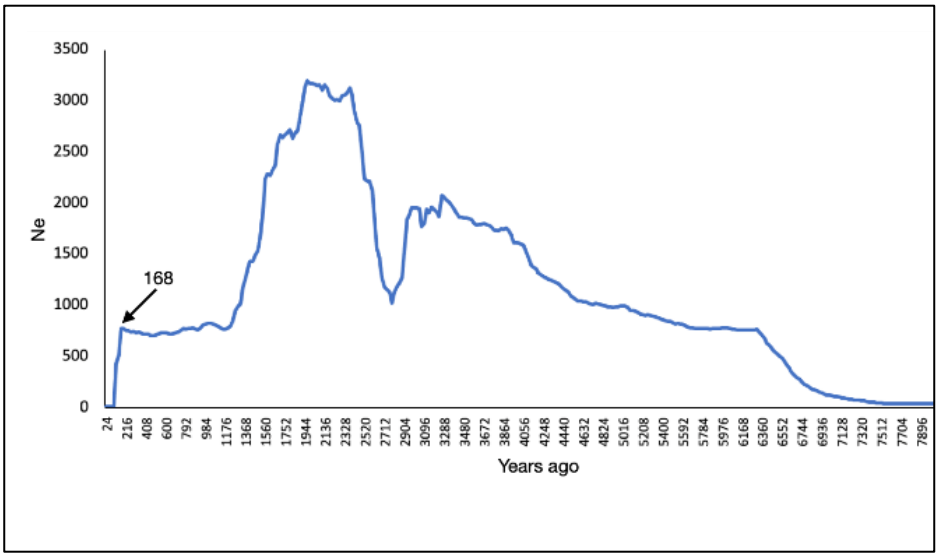


Figure 3.10 Plot of the recent demographic history of eastern black rhinoceros populations, showing changes in effective population size (*Ne*) in relation to time, with the arrow indicating the time of the most recent bottleneck, approximately 7 generations ago (168 years ago, assuming a generation time of 24 years).

3.3.5 Accumulation of genetic load

Based on loss of function mutations (LOF), relative genetic load (as measured by Rxy) differed between ancestry cohorts (Figure 3.11). Notably, although the oND cohort had the highest levels of inbreeding (Figure 3.8), it showed a significantly reduced load compared to all of the other cohorts, including native individuals from the sire's source population (NgNg; Figure 3.11a). Although the earlier generation relatives of the oND individuals (rND) showed a lower load than the cohorts involving individuals translocated from captive populations (TC and AD) and showed a slightly higher load than one of the native cohorts (NyNy), they did not differ from the native cohort of their father (NgNg; Figure 3.11b). Both the AD and TC cohorts showed increased LOF loads compared to the native cohorts, which did not differ substantially from one another, but the "hybrid" AD cohort had lower load than the first-generation translocated individuals (TC; Figure 3.11c). For derived missense mutations, whose impacts are less clear but are often assumed to be more mildly deleterious (Robinson et al., 2023a), there were fewer significant differences between cohorts (Figure 3.11). The exception was the cohort from the intensively managed ("closed") native population (NgNg), which showed significantly lower loads than the transborder ("open") native population (NyNy), both natural dispersal cohorts (oND and rND) and both cohorts involving captive individuals (AD and TC).

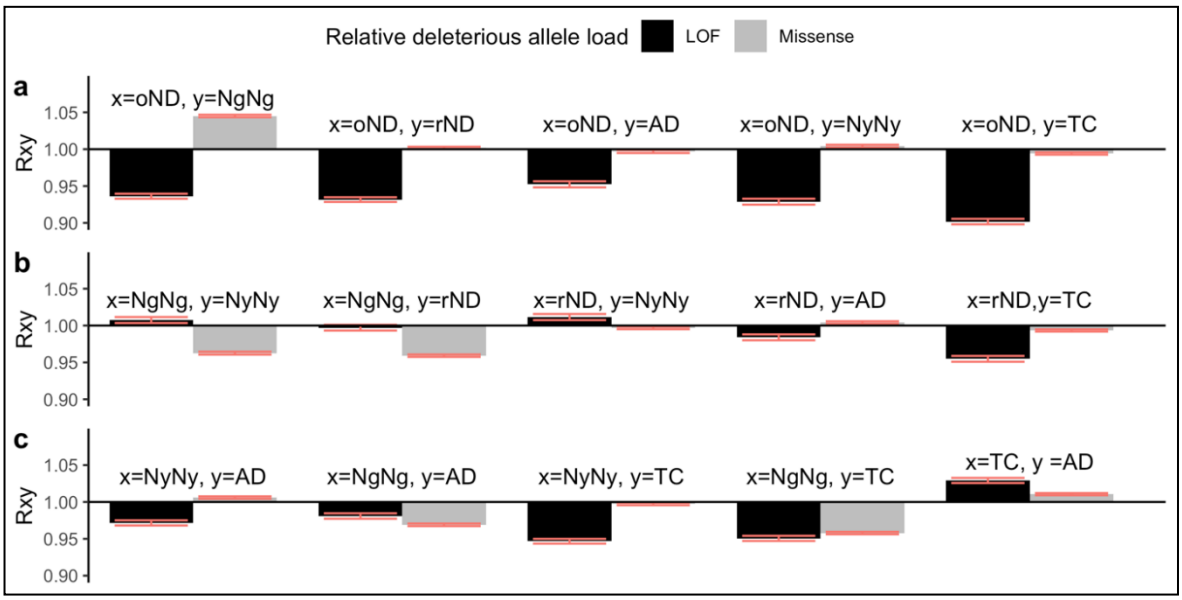


Figure 3.11 Relative deleterious allele load (Rxy) for both mildly (missense; grey bars) and loss of function (LOF; black bars) derived mutations, comparing cohorts of individuals with different ancestries. For each pair of cohorts, relative load is assessed by comparing cohort "x" compared to "y", with values less than one indicating a lower load in the former compared to the latter and values above one *vice versa*. (a) The oND cohort showed significantly lower genetic load for LOF than any of the other cohorts, consistent with purging of the most deleterious mutations. However, missense mutations were similar to the other cohorts, except for NyNy, which showed lower load than the oND cohort. (b) The rND cohort (earlier generation relatives of the oND cohort) did not show evidence of purging of LOF compared to the native cohorts (and in fact had a slightly higher load than NyNy) but it did show reduced load compared to the cohorts including captive individuals (AD and TC). The NgNg cohort showed a slightly higher LOF load than the NyNy cohort but again showed a significantly lower missense load compared to both NyNy and rND. (c) Native cohorts showed a consistently lower LOF load than both cohorts including captive individuals, but the load was

higher for both LOF and missense mutations in the first-generation translocated individuals (TC) compared to offspring that were hybrids between translocated and native individuals (AD). The NyNy cohort showed a slightly higher missense load than the AD cohort but not the TC cohort. As for the other comparisons, the NgNg cohort showed significantly lower missense load than both AD and TC.

Enrichment analysis of the LOF mutations showed the highest enrichment ratios associated with myosin complex and energy related functions, as well as cytoskeletal functions and anion binding (Table 3.5). Missense mutations showed higher associations with olfactory and chemical stimulus related functions, as well as cytoskeletal functions.

Table 3.5 Gene enrichment analysis for loss of function (Bertola et al., 2024) and missense mutations, showing the enrichment ratio for particular biological Process, Cellular Component and Molecular Function categories, the significance of the enrichment, and the mutation type.

Function	Enrichment Ratio	P Value	Mutation type
myosin complex	4.68	4.9E-06	LOF
ATPase activity	2.40	6.4E-09	LOF
ATPase activity, coupled	2.27	1.8E-06	LOF
actin binding	2.207	2.0E-06	LOF
nucleoside-triphosphatase activity	1.787	4.7E-06	LOF
protein-containing complex binding	1.747	6.1E-07	LOF
cytoskeletal protein binding	1.73	4.9E-06	LOF
cytoskeletal part	1.560	6.5E-07	LOF
cytoskeleton	1.52	2.9E-07	LOF
anion binding	1.38	6.6E-06	LOF
detection of chemical stimulus involved in sensory perception of smell	2.19	<2.2e-16	missense
olfactory receptor activity	2.19	<2.2e-16	missense
sensory perception of smell	2.19	<2.2e-16	missense
detection of chemical stimulus involved in sensory perception	2.11	<2.2e-16	missense
detection of chemical stimulus	2.05	<2.2e-16	missense
sensory perception of chemical stimulus	2.03	<2.2e-16	missense
detection of stimulus involved in sensory perception	1.20	<2.2e-16	missense
detection of stimulus	1.81	<2.2e-16	missense
sensory perception	1.55	1.8E-13	missense
cytoskeleton	1.32	2.0E-11	missense

3.3.6 Homozygosity of derived mutations

Although there was no overall significant effect of ancestry cohort on homozygosity of derived LOF mutations (Figure 3.12a), the patterns were generally similar to missense (Figure 3.12b) mutations, where there was a

significant effect ($F_{5,15}$ = 4.023, p =0.0163). Homozygosity of derived mutations was highest for the native NgNg cohort and descendants of the male who had moved from that population to Moru (rND and oND) and lowest for the first generation translocated from captive population (TC). However, the differences were only significant for NgNg vs TC and oND vs TC (Figure 3.12b). There was no difference for either type of mutation between the AD cohort and the population that the native father came from (NyNy; Figure 3.12a and b). Overall, the data has shown no significant differences in homozygosity between cohorts, with smaller differences observed for LOF mutations compared to missense mutations.

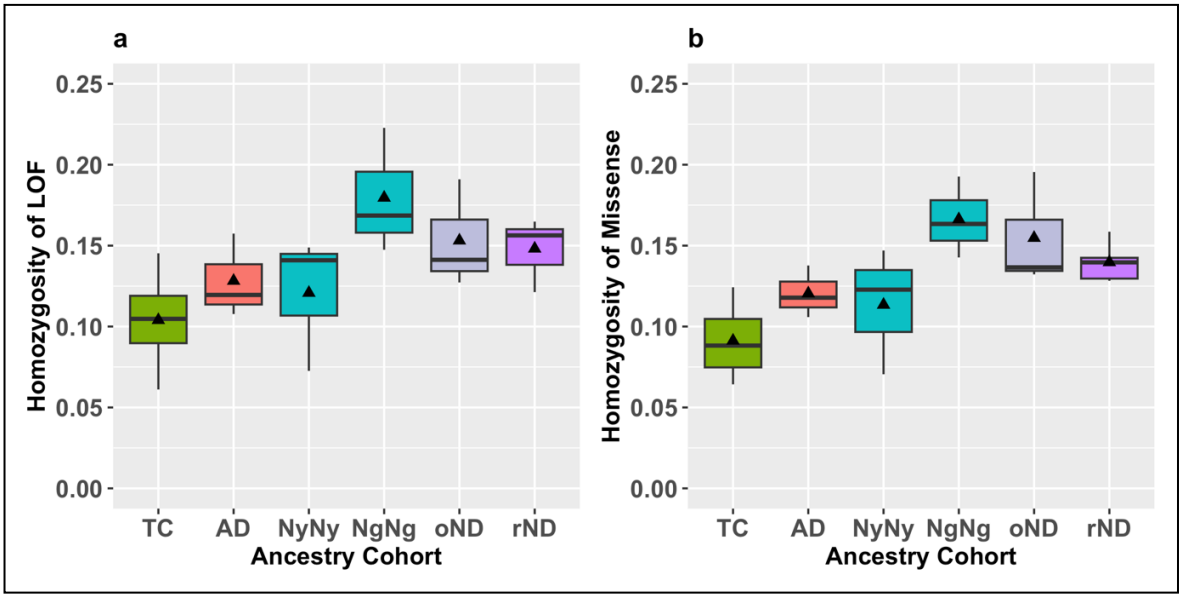


Figure 3.12 Homozygosity of derived mutations in various ancestries for: (a) loss of function (LOF) mutations expected to be under the strongest selection; and (b) missense mutations, with unknown effects on fitness. There was only a significant effect of ancestry cohort for missense mutations, and this was driven by the difference between the NgNg/oND and TC cohorts.

3.4 Discussion

Overall, our results question some of the assumptions of *ex situ* conservation strategies; past translocations from captive populations have achieved the goals of increasing the overall population size, as well as the genetic variation and heterozygosity, but at what cost? Introducing new alleles and increased heterozygosity of beneficial alleles could increase adaptive potential but our results also emphasise that introduction of deleterious alleles that have been sheltered in heterozygotes could result in increased inbreeding depression if exposed as homozygotes in the wild, i.e. if active management does not prevent subsequent inbreeding after translocations. This is consistent with the observation from meta-analyses that translocation of captive-born individuals is often less successful than translocation of wild-born individuals (Rummel et al., 2016; Berger-Tal et al., 2020). In reality, many translocation programmes do not assess the genetic impacts beyond the translocated generation but our results, and others (Miller et al., 2012) reviewed by (Bubac et al., 2019) emphasise the critical importance of monitoring fitness consequences for multiple generations after translocations. An important contribution of our study is comparison of the two cohorts from the Moru population that shared a male ancestor who had

translocated from Ngorongoro: the noted differences in levels of inbreeding and reduction of genetic load across only a few generations emphasise the initial benefits of allowing natural movement of individuals between native populations but also the consequences of allowing mating between close relatives after any type of genetic mixing. However, the very severe bottleneck experienced by the Moru population (down to only two females and the one migrant male in 1994) could explain the strong evidence we found for purging of mutations expected to have deleterious consequences (Armstrong et al., 2017; Ralls et al., 2020; Perez-Pereira et al., 2022)) several generations of inbreeding after the natural dispersal event. Since fitness is challenging to assess in natural populations, such assessment of genome-wide genetic loads in relation to management could provide important management perspectives (Hohenlohe et al., 2021). For example, the pedigrees for most black rhinoceros populations are too shallow to directly estimate fitness after translocations because of their long generation times. The short-term benefits of introducing genetic variation from different sources could thus be compromised in the long term unless there are sustained efforts to reduce subsequent inbreeding and monitor impacts on fitness (Pérez-Pereira et al., 2022). Critically, our results emphasise the value of monitoring the potential impacts of individuals rather than just focusing on population-level parameters when considering costs and benefits of management interventions.

Long-distance translocations are expensive not only in terms of financial costs and logistics but also can come at a cost to animal welfare (Teixeira et al., 2007). Encouragingly, at least for highly threatened eastern black rhinoceros, we found that both assisted and natural dispersal reduce inbreeding in the target population. However, natural movement of highly inbred individuals between subpopulations results in a lower high-impact mutational load of deleterious alleles than mating between wild and translocated individuals. Native cohorts also consistently showed a lower LOF load than cohorts involving captive individuals, which is consistent with past reductions in population size. Our demographic analysis suggests that there have been both historic (~1900 years ago) and recent (~170 years ago) bottlenecks in the native eastern black rhino populations. This is consistent with global analyses suggesting that black rhino populations have been persisting in small populations for at least last 2,500 years and have been declining for the last 200,000 years (Liu et al., 2021). Such bottlenecks are expected to reduce deleterious allele loads but also drive deleterious mutations to fixation and increase homozygosity (Kyriazis et al., 2023). Interestingly, there was also evidence of purging for mutations expected to be under weaker selection (missense) in the native Ngorongoro cohort compared to all others, even though this was not observed for LOF.

Enrichment analysis of LOF mutations provides a warning that potential fitness consequences should be monitored in wild populations that hybridised with captive-bred individuals. This is because deleterious mutations can be “hidden” both by heterozygosity in captive populations and because the selection pressures on wild individuals is very different from captive populations, emphasising the role of the environment in expression of inbreeding depression (Keller and Waller, 2002). For example, deleterious alleles associated with the myosin and energy-related functions that we identified could potentially affect muscle-related activities, including heart disease, developmental defects or anatomical anomalies (Finno et al., 2009; Finno, 2020). This could help to explain the observation that a translocated individual in Grumeti was suspected to have died due to a muscle-related problem (Eblate Mjingo, personal observation).

Unfortunately, we were not able to obtain a sample for this individual. Such health problems might not be apparent in captive populations due to the high nutrition and benign environmental conditions under which they are kept, as well as increased heterozygosity resulting from mixing of individuals from different source populations. However, there also could be impacts of the physical stress of translocating individuals to a novel environment. For example, hemosiderosis, which results from accumulation of iron deposits (hemosiderin) in tissues, was found to be prevalent in captive black rhinos from a UK zoo but also in individuals that had been translocated within Zimbabwe from the wild to managed ranches (Kock et al., 1992). In contrast, high levels of hemosiderin were not observed in free-ranging individuals. Such diseases could also be exacerbated by the diet in captive populations. For example, a serious skin disease (similar to necrolytic dermatitis) was identified in nearly 50% of captive black rhinoceros individuals across the 21 zoo populations that were held in the U.S.A. in the late 1990s (Munson et al., 1998). Since the disease had not been reported in wild individuals, the authors suggested that it could have resulted from metabolic changes due to the rich captive diet. The small size of the native populations could also lead to introduced deleterious alleles reaching fixation rapidly, which may push populations to extinction (Whitlock, 2000). Moreover, a further caution comes from the history of the South African captive populations that have been used for translocations to Ndasiata and Grumeti: there are reports of hybridisation between eastern black rhinoceros (*D. b. michaeli*) individuals from Kenya and southern black rhinoceros (*D. bicornis minor*) from Zululand (Hall-Martin, 1984). Such admixture between subspecies could have introduced deleterious alleles or contributed to sheltering of the load due to increased genome-wide heterozygosity.

Even though the Serengeti-Mara consists of continuous, unfenced protected areas where movement of individuals with the large home ranges typical of rhinoceros should be possible (Sinclair and Arcese, 1995), the intensive protection zone (IPZ) strategy, in which animals are artificially pushed back into specific areas of the landscape where they can be easily monitored, reduces any prospect of natural dispersal between different subpopulations (Fyumagwa and Nyahongo, 2010). Thus, the current management strategy would require modifying so that animals are allowed to mix across management boundaries. Previous translocations of eastern black rhinoceros have not considered genetics, but our results suggest the potential benefits of capitalizing on the existing variation in the local native populations, rather than relying only on long-distance translocations. This is further emphasized by the observation that the transborder Nyamalumbwa subpopulation, for which mixing is allowed with the Kenyan Maasai Mara subpopulation, shows lower levels of inbreeding, within cohort relatedness and homozygosity of deleterious mutations than the other Serengeti populations. The observational pedigrees could be used to identify unrelated individuals for translocation (Moodley et al., 2017), but the approach would be more powerful if combined with an assessment of the genetic load. For example, removing dominant males that have contributed multiple generations of offspring (e.g. in Moru and Ngorongoro) could allow a wider range of individuals to breed, as has been suggested for southern white rhinoceros managed in a metapopulation structure in Botswana (Purisotayo et al., 2019). Genome-wide sequencing data then could be used to model what impacts such a strategy could have on purged deleterious mutations. Nevertheless, our results suggest that sustainable strategies for inbreeding reduction through natural dispersal may be more

important than supplementing variation (e.g. increasing heterozygosity) through translocation and reintroduction of captive animals.

Our results showed little effect of management strategies on genetic diversity within cohorts, with a substantial increase in pairwise nucleotide diversity only for individuals whose parents were from captive populations. This is consistent with a recent study using the D-loop of mitochondrial DNA, which suggested that some of the historical maternal diversity in Tanzania had been restored in the populations that included translocated individuals from South Africa or European zoos, rather than introducing completely new variants (Mellya et al., 2023a). The study also confirmed that the translocated individuals were from maternal ancestors originally captured from wild populations in Kenya, where many of the lineages persist. Since Kenyan populations have been found to harbour higher genetic diversity than the Tanzanian populations (Moodley et al. 2017; Mellya et al. 2023) and there is no fence between the Serengeti (TAWIRI, 2019) and Maasai Mara (Kenya) management areas, cross-border gene flow could enhance genetic variation. There has already been increased collaboration between rhinoceros management teams in the recent translocation of five eastern black rhinoceros from Ngulia Rhino Sanctuary in Kenya to Ngorongoro in Tanzania for the purpose of increasing diversity, but this was conducted without first obtaining genetic profiles of the translocated rhinos, which would have allowed for prediction of the genetic impacts.

In conclusion, facilitating natural dispersal seems to be the best strategy for managing threatened wild populations like black rhinos, which have been sufficiently bottlenecked to purge out some of the most serious genetic load. Corridors that facilitate animal dispersal have the combined benefits of reducing inbreeding without increasing the genetic load while maximizing breeding opportunities with unrelated individuals. While translocations from managed game reserves (like captive populations) do reduce inbreeding and increase genetic diversity, they risk increasing deleterious mutation loads. As the genetic load is sheltered due to potentially benign environments and high heterozygosity in captive populations, there is a risk of future exposure of fitness-reducing deleterious mutations after translocation, unless sustained efforts are made to ensure inbreeding avoidance (Pérez-Pereira et al., 2022). This could be accomplished by changing management practices to allow for natural mixing, which would incur a lower financial cost and improved animal welfare. Alternatively, targeted translocations in each generation could reduce the risk of inbreeding within the reintroduced population but this would be costly both financially and in terms of animal stress. This study focused on analyzing individual cohorts within each rhino population in the Serengeti ecosystem. As a result, only a small number of individuals were sequenced, which was insufficient for a thorough population-level analysis. We recommend that future research take a population-level approach by sequencing a larger and more representative number of individuals from each population to ensure adequate coverage. The power provided by whole genome sequence data offers the opportunity to move away from an original assumption of *ex situ* conservation that supplementation of any genetic variation will reduce extinction risks; instead, we should consider the functional consequences of population mixing on the re-emergence of deleterious alleles, particularly for highly threatened populations (Mable, 2018).

4 Assessment of demographic parameters and population viability analysis of the eastern black rhinoceros in Tanzania for future management

4.1 Introduction

4.1.1 General – management of endangered species

Understanding population dynamics, particularly for endangered species, requires an understanding of the factors that influence demographic parameters and how those factors function (Sæther, 1997). For example, we can use demographic data to quantify multiple threats by the relative impact of each threat on population growth, so that management actions may be prioritized (Rhodes et al., 2011). The demography of populations of large herbivores has received considerable attention, especially as regards dynamics and the effects of density and environmental influences (Bonenfant et al., 2009; Owen-Smith, 2009). Key demographic indicators of performance are crucial parameters used to assess and evaluate aspects of a population to provide insights into trends and behaviour, which are essential for decision making in management of wildlife. Some key indicators include age of first reproduction, Inter-calving interval, reproductive sex ratio, mortality rates, population growth rates, inbreeding coefficient, and population structure (Caughley, 1977). For individual endangered species, such as rhinos, these parameters can be used to identify factors contributing to population performance below or above internationally accepted minimum annual growth rate (e.g. 5% for rhinos) and reproductive rate standards, which can then be used to conduct population viability assessment (Subedi et al., 2017).

One key performance indicator is age at first reproduction, particularly for females. For rhinos, females in rapidly growing populations can have their first calves as young as 6.5 years, while populations with poor performance may have an age of first reproduction over 7.5 years (Du Toit., 2001). This variation could be dependent on density and resource availability but also could be an important indicator of fitness (Okita-Ouma et al., 2021). The average birth period is also a reliable indicator of population performance, largely independent of sex ratio, determined by observing known female calving frequency and averaging these values. For rhinos, less than 2.5 years between calves indicates good to excellent fecundity whereas greater than 3.5 years indicates poor performance (Du Toit., 2001). Average annual growth rates are another reliable indicator of population performance, allowing for meaningful numerical predictions for a wide range of users in management. For rhinos, intrinsic growth rates range from 9-11% (Okita-Ouma et al., 2021).

Although hard to relate directly to fitness for the reasons described in chapter 3, inbreeding is also often viewed as an important factor potentially contributing to variation in population or individual performance. The inbreeding coefficient measures genetic similarity between individuals, with higher values indicating higher likelihood of inherited alleles being identical, potentially leading to

deleterious traits resulting from loss of genetic diversity and inbreeding depression, defined as reduced fitness of inbred compared to outbred individuals (Wright, 1922).

4.1.2 Historic rhino populations in East Africa

Large herbivore population demographics have attracted a lot of study, particularly in relation to dynamics, the effects of density, and environmental factors. Black rhinoceros were historically distributed across various regions of Africa, including Tanzania, and they inhabited a range of habitats, from grasslands to savannas and dense forests. However, black rhino populations suffered a drastic decline at the end of the 20th century due to poaching and habitat loss. Between 1970 and 1993, the population of black rhinos decreased by 96% from approximately 65,000 to only 2,300 surviving in the wild. Since 1996, intense anti-poaching efforts and strategic translocations to safer areas have allowed the species to slowly recovering at a growth rate of 3% and declining by 1.6% from the period between 1997-2021. By the end of 2021, an estimate of 6,195 individuals remained in the wild across Africa, and 218 in ex site populations (Burgess et al., 2022). In Tanzania, the black rhino population had declined to 46; 24 eastern black rhino and 22 southern black rhinos by 1997. Since then the population had increased to 177 by the end of 2019 (Brooks and Emslie, 1999; Mellya et al., 2023a). As a result of the demographic decline, black rhinoceros are now categorized as Critically Endangered on the IUCN Red List (Emslie, 2020). The remaining extant subpopulations are now managed in fenced sanctuaries, private lands, zoos and Intensive protection zones (IPZ) for maximum protection and growth (Du Toit, 2006). Understanding the status and drivers of metapopulation dynamics is crucial for management to achieve optimal growth targets. The standard guidelines set by the African rhino specialist group can be used to compare metrics that can assess the efficiency of management options (Du Toit, 2006). For example, an examination of reproductive parameters in the Hluhluwe–iMfolozi Park, KwaZulu-Natal, South Africa black rhinoceroses between 1998 and 2013 found a mean age of first reproduction of 12, which surpassed the targets set in the Guidelines for Implementing SADC Rhino Conservation Strategies of 7 years and 5 months and an inter-calving interval of 3 years and 8 months, which was longer than the recommended 3 years (Nhleko et al., 2017). This could have been due to poor habitat quality, animal condition, loss of females, predation, or negative social effects.

Black rhinos were previously abundant in the Serengeti ecosystem, with approximately 450 individuals estimated in the Serengeti National Park alone in 1974 (Frame, 1980; Metzger et al., 2007) . However, poaching intensified in the area, resulting in a drastic decline in numbers to just 24 rhinos by 1997 (Brooks and Emslie, 1999).

4.1.3 Current management strategy in Tanzania

The main goal for the rhino conservation policy in Tanzania is “To increase black rhino population at a minimum rate of 5% per annum to reach at least 205 black rhinos by the end of 2023 using a meta-population management approach, in line with internationally best practiced standards” (TAWIRI, 2019). Currently, the management strategy for black rhinos in Tanzania involves independently

managing subpopulations within intensive protection zones (IPZs) across five subpopulations in the Serengeti ecosystem and one in the Tsavo ecosystem (Mellya et al., 2023a). Historically, prior to the poaching crisis in the 1950s, the black rhino population in Serengeti was about 700 individuals by 1974, dispersed throughout the region. However, due to poaching, only a few native rhinos remained isolated in Nyamalumbwa, Moru, and Ngorongoro (Frame, 1980; Metzger et al., 2007). In 1993, these areas were officially designated as IPZs to enhance the security of the remaining rhinos. In 1997, in an effort to boost rhino populations in Tanzania, individuals were reintroduced through translocations to establish new subpopulations in Grumeti and Ndasiata within the Serengeti ecosystem, and the Mkomazi Sanctuary in the Tsavo ecosystem (Fyumagwa and Nyahongo, 2010).

This study employs annual growth rates and variance to evaluate the viability of rhino subpopulations under the current management strategy and explore potential enhancements for the black rhino population in Tanzania. Therefore, in this study I will use demographic data to assess the performance of the rhino subpopulations in Tanzania towards attaining the minimum growth of 5% and the viability of the metapopulation approach in minimizing risks of extinction of rhinos in Tanzania.

4.1.4 Population Viability Analyses

Population viability analysis (PVA) is a technique used to estimate extinction probabilities and population declines by incorporating threats to survival into stochastic models. It predicts future population size, estimates extinction probability, and evaluates conservation strategies for population persistence (Akçakaya and Sjögren-Gulve, 2000). It is well established that when populations become small and isolated genetic, demographic, and environmental stochasticity increase the probability of extinction, making populations more vulnerable. Identifying factors that affect demographic parameters and how those factors act is vital for understanding population dynamics, especially of endangered species. Moreover, specific ideas in the population dynamics of large herbivores underpin the management of the critically endangered black rhinoceros (*Diceros bicornis*). While the Tanzania rhino program produces annual summaries of population numbers and translocations, along with some basic calculations of growth rates and initial documentation of certain reproductive indicators, only one study has been conducted to provide a comprehensive quantification and comparison of the relative performance of black rhino populations in Tanzania but that was a retrospective study from the 1970s (Metzger et al. 2007). An understanding of key demographic parameters assists in guiding management interventions to ensure their recovery and persistence over the longer term. Estimating the population parameters, performance and factors that influence reproduction from long-term, individual-based monitoring data is the gold standard for effective wildlife management and conservation. The aim of this study is to guide conservation investment priorities by identifying critical threats and their thresholds. This would help in adaptive management and ensure the long-term survival of the Tanzania rhino population.

4.1.5 Eastern black rhinoceros monitoring and management in Tanzania

Black rhino monitoring and management in Tanzania are integral components of the country's conservation strategy, aiming to safeguard the endangered species from poaching and habitat threats. The Tanzanian government, in collaboration with international organizations and local stakeholders, has implemented comprehensive programs to ensure the well-being and survival of black rhinos (TAWIRI, 2019). Monitoring efforts involve the use of advanced technologies such as GPS tracking devices and aerial surveys to keep a close eye on rhino populations in various protected areas across the country (TAWIRI, 2019). This enables conservationists to gather critical data on rhino movements, habitat utilization, and population dynamics. Regular monitoring not only helps in assessing the effectiveness of conservation measures but also identifies potential threats and challenges.

Management strategies include the establishment and maintenance of well-protected reserves and national parks where black rhinos can thrive. Anti-poaching units equipped with modern technology and trained personnel are deployed to deter and combat illegal activities. Furthermore, community engagement initiatives promote local awareness and support for rhino conservation, fostering a sense of responsibility among nearby communities. Tanzania's commitment to black rhino monitoring and management reflects a broader global effort to preserve biodiversity and protect endangered species. By employing a multifaceted approach that combines cutting-edge technology, legislative measures, and community involvement, Tanzania strives to secure a sustainable future for the black rhino population within its borders.

The process of using annual status reporting to monitor and contrast the population performance of black rhino reserves in a meta-population framework, rather than individually protecting subpopulations has proved to be very beneficial in South Africa and Namibia (where this has been underway since 1989) (Britz and Loutit, 1989). Apart from the production of annual summaries of population numbers and translocations by the Tanzania rhino programme, and some limited calculations of growth rates, a more in-depth quantification and comparison of the relative performance of Kenyan black rhino populations has not yet been undertaken to determine whether it could be beneficial to consider changing management strategies from intensively protected subpopulations to a larger management units involving multiple populations.

4.2 Objectives

The main objective of our analysis is to assess the demographic parameters of the eastern black rhino population in Tanzania and to explore different management options that minimize the risk of extinction of rhinos using a count-based PVA. Specifically, we use long-term rhino monitoring data to assess: (1) population performance indicators such as age of first reproduction and inter-calving interval; and (2) the effects of inbreeding on these population performance indicators. Using the variance around the observed population growth rates, we then explore (3) the four different management options on the probability of extinction of eastern black rhinoceros in Tanzania by 2050 in which: (i) each subpopulation is

managed independently (i.e. with no dispersal between subpopulations and animals pushed back into their management zones); subpopulations are managed as separate zones (i.e. animals are allowed to disperse between adjacent subpopulations but not across the entire ecosystem); and (iii) subpopulations are managed at the ecosystem level (i.e. animals are allowed to disperse freely between all subpopulations with the same ecosystem). Furthermore, we assess: (4) the impact that translocations have had on changing the probability of extinction. Finally, using the count-based population viability analysis, we assess (5) if the Serengeti could be used as a source population of rhinos for reintroductions into in other ecosystems in the future.

4.3 Materials and Methods

4.3.1 Study area

The study was carried out in two protected areas in Tanzania, the Serengeti ecosystem and Mkomazi National Park. The ecosystems are approximately 420km apart and are separated by large areas of human-dominated agricultural and pastoralist land as well as the Rift Valley. Although rhinos may once have moved freely throughout the entire area, human density and land use changes mean this is no longer possible. The Serengeti ecosystem lies approximately 2º south and 35º east along the border of Tanzania and Kenya (Figure 4.1). The protected area encompasses around 30,000 km^2 of territory, which includes the Serengeti National Park (SENAPA), Ngorongoro Conservation Area (Harrisson et al.), Maswa Game Reserve, Grumeti-Ikorongo Game Reserve and the Pololeti Game Reserve. Kenya's Masai Mara Game Reserve lies directly to the north. The Serengeti ecosystem features the world's second greatest terrestrial mammal migration (over 1,300,000 wildebeest), which significantly influences all other ecological dynamics in the ecosystem (Sinclair et al., 2015). The habitat is comprised of grassland plains in the south and east, open *Acacia* and *Commiphora* woodlands to the west and north, interspersed with closed canopy riverine forests. The Serengeti ecosystem contains five rhino populations (Nyamalumbwa, Ndasiata, Moru, Ngorongoro and Grumeti) which are currently managed as independent subpopulations (as per chapter two). Although there is also a subpopulation in the Masai Mara that is managed separately by the Kenyan Wildlife Authority, animals move freely across the border it shares with Nyamalumbwa because there are no fences; although I did not include it in my analyses because the observational data are not complete, demographic parameters in this subpopulation could affect viability on the SENAPA side.

Mkomazi National Park is about 3,500 km^2 and lies 4º south and 38º east along the Tanzania-Kenya border. The habitat is arid open *Acacia* woodland, with the closed canopy forests of the Usambara Mountains occurring directly to the south. The Usambara's are a centre of biodiversity and high endemism and were likely an important rhino refuge historically. Mkomazi was originally created as the Umba Game Reserve in 1951 but was upgraded to a national park in 2006. Together with the neighbouring Tsavo National Park in Kenya, Mkomazi National Park forms the Tsavo-Mkomazi ecosystem.

The six subpopulations of rhinos included in this study have different management regimes: five from the Serengeti ecosystem and the one from Mkomazi National Park. The Ndasiata, Grumeti, and Mkomazi subpopulations were established

through reintroduction via translocation of rhinos from zoos and sanctuaries in South Africa and the United Kingdom. Grumeti is partially fenced in areas bordering human settlements, while Mkomazi is completely fenced. Nyamalumbwa, Ndasiata, Moru and Ngorongoro do not have physical boundaries but are geofenced, allowing rhinos to move freely within designated areas but carefully monitored using the Intensive Protection Zone (IPZ) strategy described in chapters 2 and 3, which is also applied to Grumeti. If a rhino ventures beyond these boundaries, it is returned to the designated population.

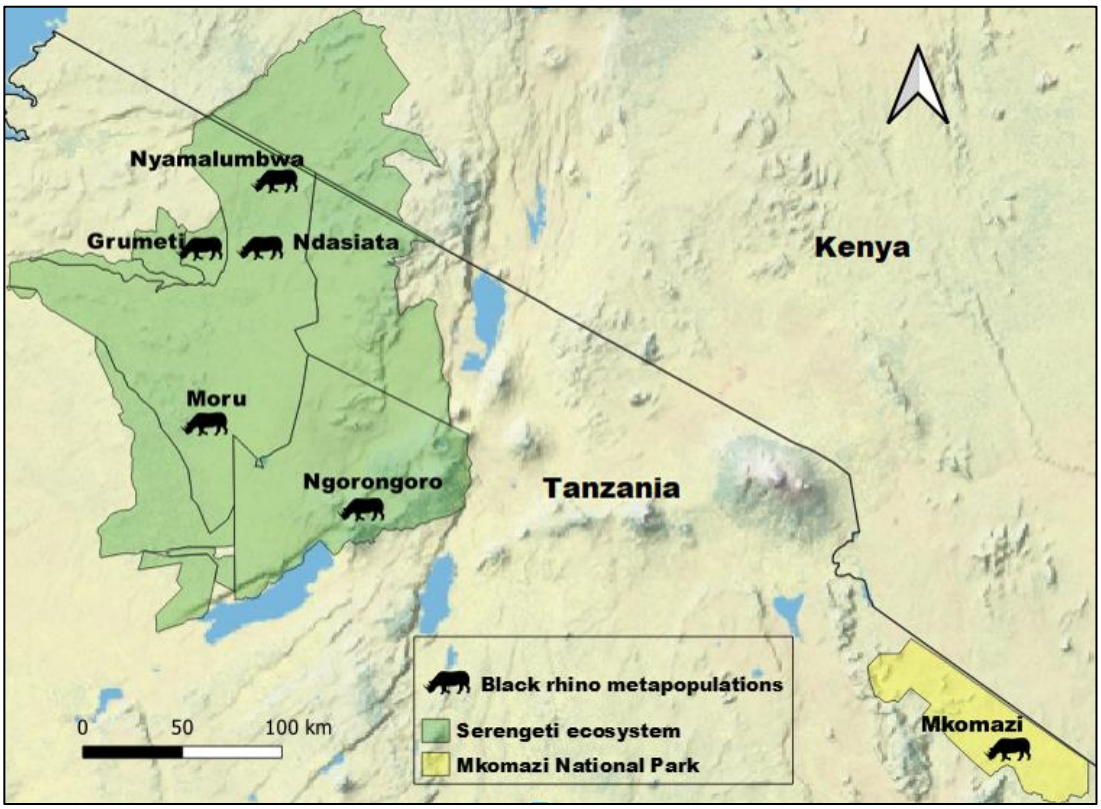


Figure 4.1. A map of the Serengeti Ecosystems and Mkomazi National Park indicating the locations of the black rhino subpopulations used in this study. In the Serengeti ecosystem, data were collected from five black rhino populations managed as intensive protection zones: Nyamalumbwa, Ndasiata, Grumeti, Moru, and Ngorongoro. In Mkomazi National Park, data were collected from one population managed in a sanctuary. The Grumeti and Ndasiata populations in the Serengeti ecosystem were formed by reintroduction of black rhinos from zoos and sanctuaries in South Africa and the United Kingdom.

4.3.2 Rhinoceros monitoring data

We collected demographic data from daily rhino monitoring activities in each of the subpopulations since 1990. Monitoring of the rhino populations in Tanzania has been conducted by special rangers who receive regular training in ID-based rhino monitoring using the IUCN African Rhino Specialist Group accredited course (Mukinya, 1976; Du Toit, 2006; Balfour, 2019). Individual rhinos were identified using a combination of features such as ear notches, distinctive body marks, horn shapes, age, and sex and as part of the daily black rhino management activities in each subpopulation (Klingel, 1966; Du Toit, 2006). The ear notches were cut during marking operations, which take place when the calves are still with their mothers, which is part of routine rhino management activities. Standardized field recording forms are used for each sighting, recording individual(s), general area, and mortality information. After collecting the data in the field, the data were then

verified by accredited observers following the standard protocols, before they were entered into the Tanzania Black Rhino Information Management System, maintained at the metapopulation level, which is currently the entire Tanzanian part of the Serengeti ecosystem. Only sightings where identity could be confirmed were used for analysis. For each subpopulation, I gathered data from 1990 to 2023 on individual rhinos, including their ID, gender, parentage (father and mother), date of birth, date of death (if applicable), original population, current population, ancestry origin (native or non-native), dates and destinations of translocations, as well as dates and destinations of dispersals.

4.3.3 Demographic performance indicators

I defined age at first reproduction as the age of the female when she gave birth to her first calf, and I only included individuals that had their first birth within the study period and for which we had accurate records of reproduction. I calculated the inter-calving intervals in years for each successive calf, beginning from the date of initial reproduction, using the calf's birthdate. For each breeding female with more than one calf I divided the difference between the dates of birth of two successive calves by 365 to get the inter-calving interval in years. Subsequently, I computed the average inter-calving interval for each subpopulation. I used ANOVA to test whether subpopulation significantly explained variation of the key parameters, followed by Tukey's tests to determine which of the subpopulations were significantly different from one another. The sex ratio was calculated as the proportion (males / (males + females)) alive in a given year, where a value of 0.5 indicates an equal sex ratio, higher values indicate a male bias, and lower values indicate a female bias (Wilson and Hardy, 2002).

4.3.4 Inbreeding

The inbreeding coefficient of an individual is the probability that two alleles chosen at random are identical by descent. For this analysis, I used observational pedigree data generated from the three subpopulations that had multiple generations of complete reproductive records available: Moru, Ngorongoro, and Mkomazi. Two of the other populations (Grumeti and Ndasiata) were founded too recently (see details in Chapters 2 and 3) to have a sufficient pedigree depth and one lacked sufficiently complete records (for Nyamalumbwa, parents could be from the Masai Mara) to infer levels of inbreeding. I utilized the parentage data for each individual (Dam and Sire) to estimate the inbreeding coefficient using the formula ($F = \sum[(1/2)^{n+n'+1}\,(1 + F\alpha)]$), employing the R package purgeR (López-Cortegano, 2021). where *F* is the inbreeding coefficient of the individual in question; n and n' represent the number of generations between the sire and dam, respectively, and their common ancestor; and *Fa* is the inbreeding coefficient of the ancestor, common to both the sire and the dam (Wright, 1922). I used generalised linear models (GLMs); using the lmer4 package in R) to test whether the age of first reproduction or the inter-calving interval for the breeding females are associated with levels of inbreeding and whether this varied by subpopulation or their interaction. For the inter calving period accounted for variation in sibling intervals by including the ID of the mother as a random effect, using generalised linear mixed models (GLMMs). I used likelihood ratio tests to test the significance of each variable.

4.3.5 Count based PVA

To generate the total count of live rhinos and gender for each year from 1990-2023 for the indigenous and translocated individuals and the total number of live rhinos for each subpopulation, I used R to generate a loop to count the number of living male and female individuals each year. I utilized the count of living rhinos in each year to derive the population growth parameters by linear regression of numbers of individuals against time to estimate: μ (population growth rate based on the slope of the regression) and σ^2 (variance of the growth rate), including the upper and lower confidential intervals, as proposed by Dennis et al, (1991). Then I used the mean, variance and the quasi- extinction threshold to construct the extinction time cumulative distribution function (CDF). The quasi-extinction threshold is the critical population size where a population faces high risk of extinction due to factors like genetic drift, inbreeding, and environmental stochasticity, crucial for conservation efforts; for my I analysis I used a threshold of 20 individuals, which is set by the IUCN African rhino specialist group (Emslie et al., 2009). The CDF is a statistical tool that estimates the probability of a population's extinction by a specific time point; it provides cumulative probabilities for extinction occurring at or before specific time points, allowing for the assessment of extinction risk over time. I used μ and δ^2 to estimate the probability of extinction for the next 26 years for the indigenous rhino population and for both indigenous and translocated rhinos in each metapopulation. Specifically, I compared the probability of extinction by 2050 of each rhino population under the current management as well as various scenarios for alternative schemes. I thus categorized the metapopulation into different management strategies. The first strategy involved managing a southern and northern zone, where the former would combine Moru and Ngorongoro as a single population, allowing dispersal between them, while Ndasiata, Nyamalumbwa, and Grumeti subpopulations would be grouped into the latter. Additionally, I considered a scenario in which all the Serengeti subpopulations would be combined into one management unit, allowing animals to disperse within the entire Serengeti ecosystem. Finally, I considered a scenario in which all the Tanzania rhino subpopulations were considered as a single management unit. For each management approach, we used an assumption that, if we manage the subpopulations under the current scenario as independent subpopulations, then what is the joint probability that no subpopulation goes extinct versus the joint probability that at least one goes extinct. Assessing the feasibility of Serengeti as a source population I assessed the possibility of using Moru and Ngorongoro (the two largest extant native subpopulations) as source populations for harvesting and reintroducing rhinos to other areas in Tanzania. This was done by assessing the difference in probability of extinction and the confidence intervals for the event to happen the size falling below a viable threshold, if animals can move freely between subpopulations. Specifically, I assessed the probability of extinction when the population starts at different abundances (i.e. removing 5, 10, 15, 20, 25, and 30 animals from each subpopulation). Therefore, I harvested varying numbers of rhinos in Moru and Ngorongoro and assessed the impact on the probability of extinction by 2050 for these populations as a result after harvesting.

4.4 Results

4.4.1 Population performance indicators

4.4.1.1 Age of first reproduction

I examined the age of first reproduction for breeding females in four reproductively active subpopulations (Moru n=20; Ngorongoro n=29; Mkomazi n=13; and Ndasiata n=3). The remaining subpopulations (Grumeti and Nyamalumbwa) had too few breeding females to calculate the age of first reproduction. The mean age of first reproduction was highest in Mkomazi at 9.2 years (SD = 2.6), followed by Ngorongoro at 8.8 years (SD = 2.4). And Ndasiata at 8.2 years (SD =0.4). The Moru subpopulation had the lowest age of first reproduction at 7.1 years (SD = 1.2) (Figure 4.2). These findings were compared with the standard benchmarks for rhino population performance set by the IUCN African Rhino Specialist Group presented in Table 4.1. None of the subpopulations exceeds the "moderately poor" threshold for the age of first reproduction. Mkomazi, Ndasiata and Ngorongoro were all classified as "poor" while Moru would be considered "moderately poor". Subpopulation significantly explained variation in the age of first reproduction ($F_{3, 62} = 3.19$, $p = 0.030$). The post-hoc Tukey's HSD test suggested that Moru had a significantly lower value than Mkomazi ($p=0.03$) but none of the other comparisons was significantly different (Figure 4.2).

4.4.1.2 Inter-calving interval

I examined the inter calving interval for the breeding females in the four reproductively active subpopulations (Ngorongoro $n_{females} = 17$, $n_{calving} = 60$, and Moru $n_{females} = 14$, $n_{calving} = 60$, Ndasiata $n_{females} = 1$, $n_{calving} = 3$, and Mkomazi, $n_{females} = 9$, $n_{calving} = 30$) and compared them with the benchmarks set out by the IUCN African Rhino Specialist Group (Table 4.1). The Grumeti and Nyamalumbwa subpopulations were not included because the breeding females did not have enough data to estimate the calving interval. The mean inter calving interval was lowest for Ndasiata (2.2 years; SD=0.5), followed by Moru (2.4 years; SD=0.6) and then Mkomazi (3.1 years; SD= 1.2) and Ngorongoro (3.4 years; SD= 1.4) (Figure 4.2a). A comparison with the IUCN benchmarks suggests that Moru and Ndasiata have "excellent" inter-calving intervals whereas Mkomazi and Ngorongoro 3 are "moderately poor" (Table 4.1).

The ANOVA results suggested that subpopulation significantly explained variation in the mean inter calving interval ($F_{3, 149} = 9.76$, $p < 0.001$) (The post-hoc Tukey's HSD test suggested that although Moru did not differ significantly from Ndasiata, it showed a significantly lower mean inter calving interval than the other subpopulations. Ndasiata showed a lower value than Moru but did not differ significantly from Mkomazi and Ngorongoro (Figure 4.2b).

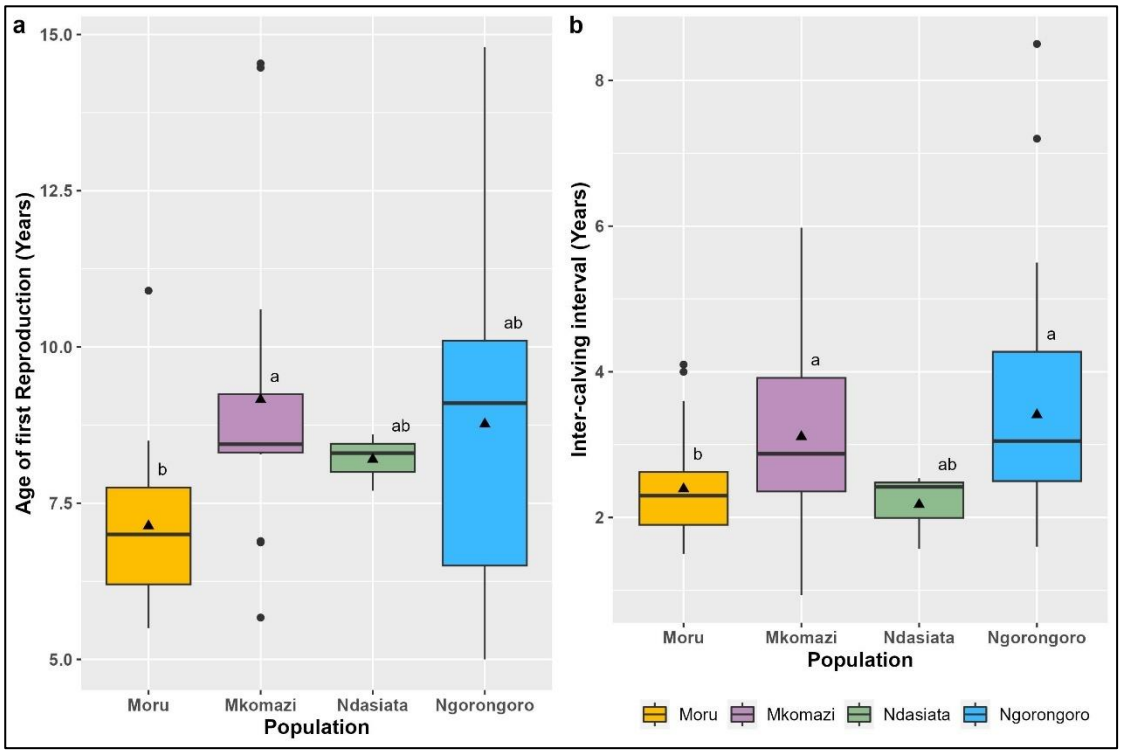


Figure 4.2 The effects of subpopulation on key performance indicator. (a) The age of first reproduction and (b) intercalving interval for female rhinos in four reproductively active subpopulations (Mkomazi, Moru, Ndasiata and Ngorongoro) measured in decimal years. The median (horizontal line), mean (black triangles), range of the data (black whiskers) and outliers (black points) are shown for each subpopulation relative to the interquartile range (coloured box). Letters above the boxplots represent the results of the Tukey's HSD pairwise comparisons between subpopulations (subpopulations with distinct letters are significantly different from each other, while subpopulations sharing the same letter are not significantly different).

Table 4.1 Population performance benchmark indicators for Ndasiata, Moru, and Ngorongoro compared to the IUCN African Rhino Specialist Group classifications. Age of first reproduction and Inter-calving interval are measures in years. Values in brackets represent the standard deviation of the mean.

Population performance	IUCN benchmark indicators				Ndasiata	Moru	Mkomazi	Ngorongoro
	Poor	Moderately Poor	Moderately Good	Excellent				
Mean age at first calving (years)	>7.5	7.5 – 7.0	7.0 – 6.5	<6.5	8.2 (0.5)	7.1 (1.2)	9.2 (2.6)	8.8 (2.5)
Mean inter-calving interval (years)	>3.5	3.5 – 3.0	3.0 – 2.5	<2.5	2.2 (0.5)	2.4 (0.6)	3.1 (1.2)	3.4 (1.4)

4.4.2 Effects of inbreeding coefficient on Age of first reproduction

The GLM exploring the effect of inbreeding on the age of first reproduction used data from a total of 28 reproductively active females from Moru (n=9), Mkomazi (n=1), and Ngorongoro (n=18). Likelihood ratio tests indicated that there was no significant interaction between inbreeding and subpopulation (chi-squared test statistic = 0.4392, df = 1, p = 0.508) and no significant effect of subpopulation on age at first reproduction (test statistic = 4.314, df = 2, p = 0.116). However, there was a weakly significant effect of inbreeding (test statistic = 3.912, df = 1, p = 0.048), with an increase in age of first reproduction with higher inbreeding coefficients (intercept = 5.828, slope = 9.421; Figure 4.3a). Effects of inbreeding coefficient on inter-calving interval

A GLMM exploring the effect of inbreeding on the inter-calving interval used 95 birthing events from 32 breeding females across three subpopulations (Mkomazi, $n_{females} = 2$, $n_{calving} = 4$ Moru $n_{females} = 13$, $n_{calving} = 43$, and Ngorongoro $n_{females} = 17$, $n_{calving} = 48$). Likelihood ratio tests indicated that there was no significant interaction between inbreeding and subpopulation (test statistic = 1.278, df = 1, p = 0.258) or subpopulation on its own (tests statistic = 5.343, df =2, p = 0.069). There was a highly significant relationship between inbreeding and inter-calving interval (test statistic = 7.208, df = 1, p =0.0073; Figure 4.3b), with inter-calving interval increasing with higher inbreeding coefficients (intercept = (1.871, slope = 4.504).

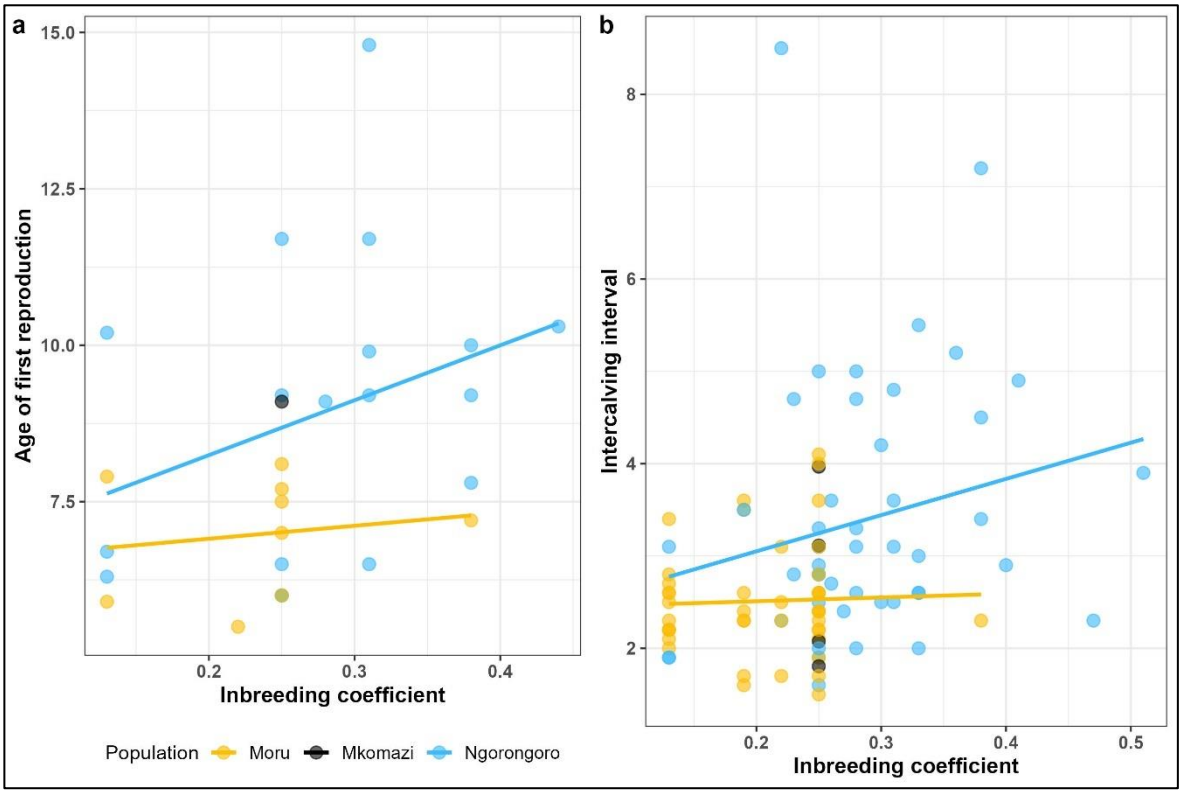


Figure 4.3 Impact of inbreeding on key performance parameters. (a) Impact of Inbreeding on age of first reproduction and (b) Intercalving interval in three reproductively active subpopulations (Moru, Mkomazi and Ngorongoro). Each dot coloured by subpopulation represents the inbreeding coefficient and corresponding age of first reproduction measured

in decimal years. The coloured lines represent the best fit line from the model. There was no significant effect of the interaction between subpopulation and inbreeding or of subpopulation on its own but there was a significantly positive association between inbreeding and both performance indicators.

4.4.3 Annual population growth

The number of individuals within each subpopulation has shown a consistent trend of increasing over time between 1990 and 2024, both when considering indigenous rhinos alone as well as both indigenous and translocated rhinos combined (Figure 4.4). Most of the populations have been biased towards females, except for Grumeti in which the annual sex ratio has been biased towards males despite the reintroductions following the IUCN guidelines (Figure 4.5).

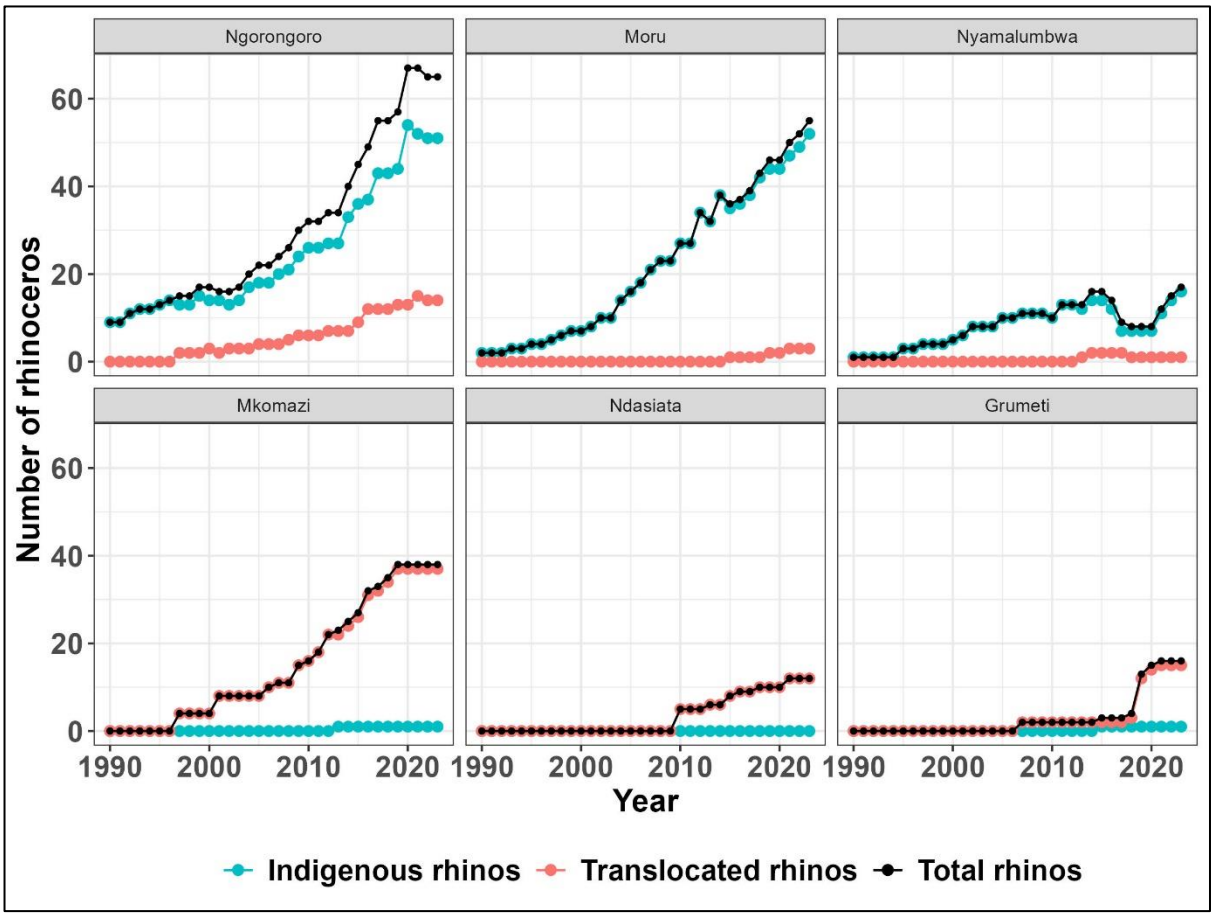


Figure 4.4 Trends of living indigenous (turquoise), translocated (coral pink), and total individuals alive (Hoffmann et al.) in each of the Tanzanian black rhinos subpopulations from 1990 to 2024.

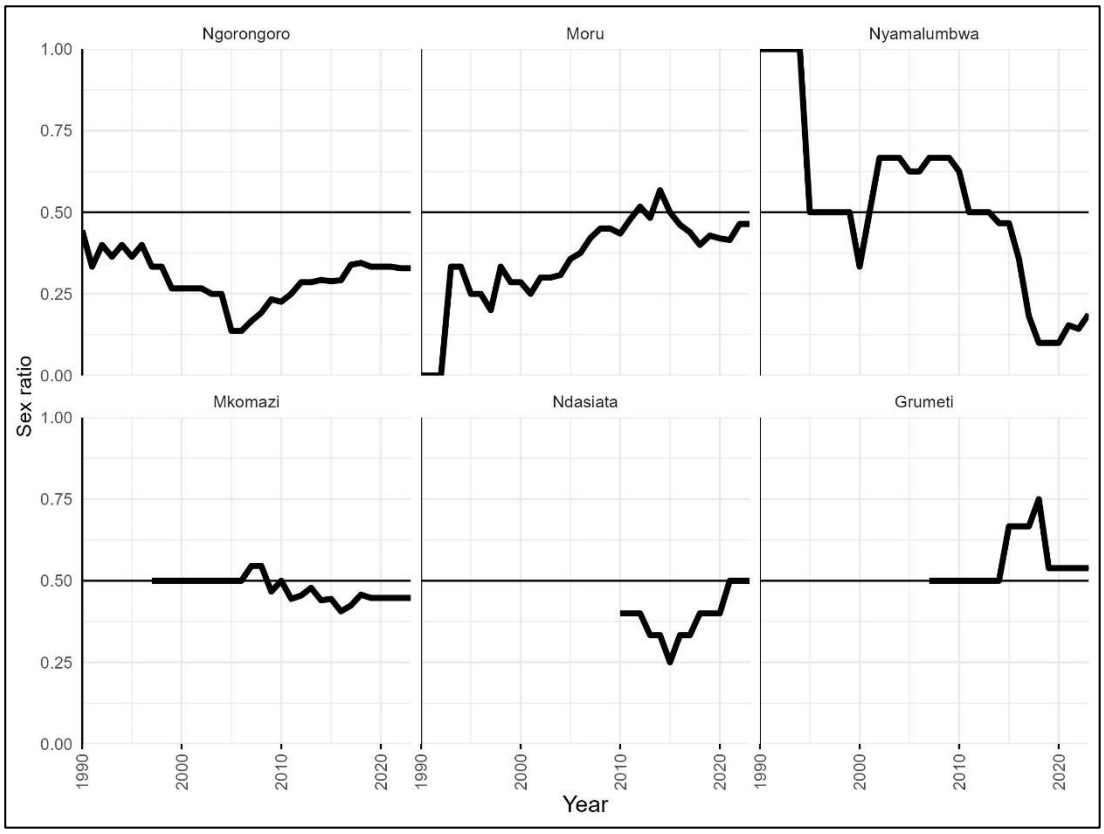


Figure 4.5 Annual sex ratio trend from six black rhino subpopulations in Tanzania from 1990 to 2024, with the black horizontal line representing equal ratios (0.5); represented as sex ratio (> 0.5 male biased; <0.5 female biased), 0.5 indicate equal ratio and higher values are male biased, and lower values are female biased.

Increasing trends were also apparent for all of the different potential management strategies considered: 1) separation of the Serengeti ecosystem into northern (Figure 4.6a) and southern (Figure 4.6b) zones, with Mkomazi managed separately; 2) the Serengeti ecosystem (Figure 4.6c) managed as a single management unit, with Mkomazi managed separately; and 4) the total population in Tanzania managed as a single management unit (Figure 4.6d).

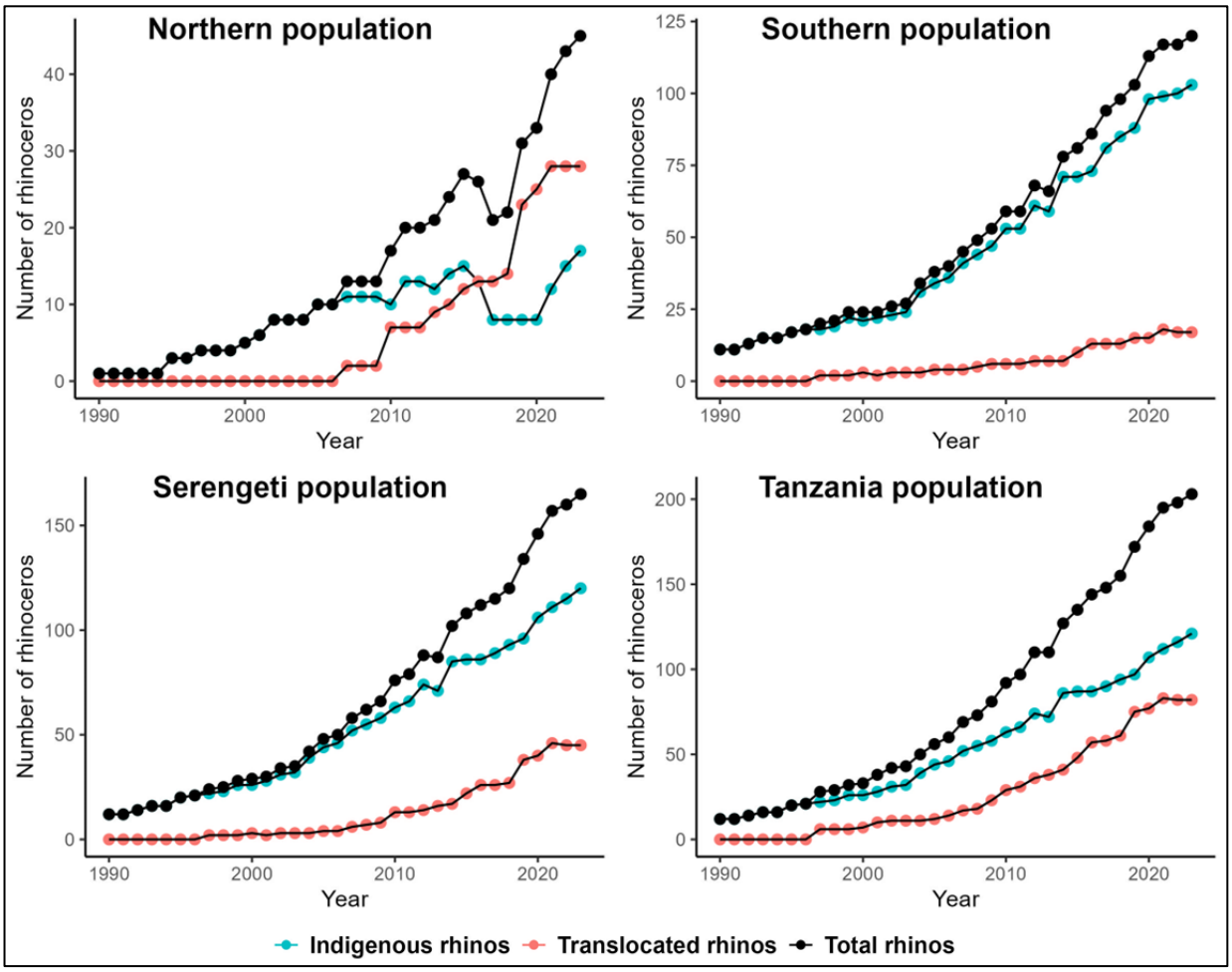


Figure 4.6 Trends of both indigenous native (turqoise), translocated (coral pink) and the total (Hoffmann et al.) living individuals under different potential management strategy scenarios, from 1990-2024. Considering separation into: (a) a northern zone, encompassing Ndasiata, Nyamalumbwa and Ngorongoro; and (b) a southern zone, encompassing Moru and Ngorongoro; alternatively, scenarios considering (c) all the rhinos in the Serengeti as a single management unit or (d) all of Tanzania as a single management unit.

All the subpopulations had a mean annual growth rate (**μ**) ranging between 5-13% (Table 4.2). This signifies good to excellent performance when compared to the IUCN benchmark guidelines.

When we ignore the effects of translocated animals and their offspring and look at the growth rate for indigenous subpopulations only, Moru has had the highest annual recruitment (μ = 9% per annum) and Ngorongoro indigenous rhinos had the lowest (μ = 5.3% per annum). Among the native subpopulations, Nyamalumbwa had the highest variation (σ^2 = 0.062) in the mean annual growth. Compared against the IUCN guidelines, Moru and Nyamalumbwa showed excellent annual growth while Ngorongoro was classified as good.

When translocated animals and their offspring were included in the calculation of annual growth rate, Grumeti showed the highest growth rate (μ = 13%), but also with the highest variance (σ^2 = 0.093) of all the subpopulations and Mkomazi showed a growth rate similar to Nyamalumbwa. Ndasiata showed a lower growth rate than the other translocated subpopulations (μ = 6.7%) and was like Ngorongoro; these two populations would be classified as "good" compared to the IUCN benchmarks while all of the others would be "excellent".

When we combine populations together by management zone scenarios, the northern zone had a higher growth rate and variance for both indigenous and total rhinos compared to the southern zone. For both indigenous and total including

translocated subpopulations, the northern zone would be classified as "excellent" compared to the IUCN benchmark whereas the southern would be considered as "good" but the lower variance in the south could indicate higher stability.
When we combine all the subpopulations in the Serengeti ecosystem (i.e. Ngorongoro, Moru, Ndasiata, Grumeti and Nyamalumbwa) the growth rate for indigenous animals (μ = 7%) was lower than that for the total rhinos including the reintroduced individuals (μ = 7.9%). The national level management strategy scenario which combines all the subpopulation in Tanzania suggested a similar growth rate for Tanzania indigenous animals (μ = 7%) as for the Serengeti scenario, but the former showed a higher growth rate (μ = 8.6%) than the latter for the total rhinos including the translocated individuals and their offspring. The mean growth rates for both strategies would be classified as "good" for the native rhinos but "excellent" when considering both translocated and native individuals.

Table 4.2 The means (μ) and variances (σ^2) of annual population growth of the indigenous and total number of living rhinos within each subpopulation category, showing the lower and upper confidence intervals in parentheses. Moru showed the highest growth rate and Ngorongoro the lowest. The symbols after the mean annual population growth represent the rank of annual growth according to the IUCN standard benchmark, with ‡‡<2.5% denoting poor growth, ‡2.5-4.9% denoting moderate growth, *5-7.5% denoting good growth, and **>7.5% denoting excellent growth.

Metapopulation	Indigenous population		Indigenous and translocated	
	Mean population growth (μ)	Variance population growth (σ^2)	Mean population growth (μ)	Variance population growth (σ^2)
Moru	0.099** (0.058-0.139)	0.013 (0.009-0.023)	0.100** (0.06-0.141)	0.013 (0.008-0.022)
Ngorongoro	0.053* (0.024-0.081)	0.006 (0.004-0.011)	0.060* (0.037-0.083)	0.004 (0.003-0.007)
Nyamalumbwa	0.084** (0.0006-0.17)	0.062 (0.040-0.108)	0.086** (0.001-0.171)	0.058 (0.101-0.001)
Grumeti	-	-	0.130** (-0.032-0.29)	0.093 (0.05-0.22)
Ndasiata	-	-	0.067* (0.008-0.127)	0.010 (0.005-0.026)
Mkomazi	-	-	0.087** (0.026-0.147)	0.022 (0.014-0.042)
North zone	0.086** (0.0001-0.172)	0.058 (0.038-0.102)	0.115** (0.039-0.192)	0.046 (0.03-0.081)
South zone	0.068* (0.044-0.092)	0.005 (0.003-0.008)	0.072* (0.051-0.094)	0.004 (0.002-0.006)
Serengeti	0.07* (0.048-0.092)	0.004 (0.002-0.007)	0.079** (0.058-0.1)	0.003 (0.002-0.006)
Tanzania	0.07* (0.048-0.092)	0.004 (0.002-0.006)	0.086** (0.06-0.11)	0.004 (0.003- 0.001)

4.4.4 Count based population viability analysis.

The cumulative probability of extinction by the year 2050, both for indigenous rhinos alone and for combined populations of indigenous and translocated rhinos calculated for each subpopulation (Ngorongoro, Moru, Nyamalumbwa, Mkomazi, Ndasiata, and Grumeti) and management strategy (Northern Zone, Southern Zone, Serengeti ecosystem, Tanzania) is summarized in Table 4.3. Furthermore, the joint probability of extinction by 2050 (i.e. the probability that all subpopulations within each management zone go extinct versus at least one subpopulation within the management zone goes extinct) for subpopulations with and without translocated animals is summarised in Table 4.4.

4.4.5 Cumulative probability of extinction

The cumulative probability of extinction (in this case defined as falling below the minimum viable threshold of 20 individuals) of rhinos across Tanzania is 3.3E-32 (CI: 4.1E-57, 2.9E-15; Table 4.3). If we consider only indigenous animals and ignore the effect of translocated animals then the probability of extinction is 2.0E-30 (CI: 6.4E-58, 6.8E-16), suggesting that translocation has not made a significant difference in reducing the probability of extinction of rhinos in Tanzania, whereas natural recruitment has been very important. Under the current management strategy in which all subpopulations are managed independently, then the joint probability that all subpopulations go extinct in Tanzania is 6.6E-45, (CI:1.3E-76, 5.1E-26); however, the probability that at least one of the subpopulations goes extinct is 0.008 (CI: 1.2E-05, 0.3). The results suggest that at a national level, the chance of losing all the subpopulations by 2050 is very low; however, the chance that at least one is lost is moderately high.

4.4.6 Assessing the viability of subpopulations with and without translocations

The indigenous subpopulations of Ngorongoro and Moru have very low probability of extinction (2.0E-07, CI:5.3E-15, 0.004; 5.9E-07,CI: 8.5E-14, 0.002) and there are very few discernible effects of reintroduction to these subpopulations (total population = 3.4E-15, CI:2.6E-29, 2.4E-06; 1.3E-07, CI: 5E-14, 0.0011) (Table 4.4). The indigenous and translocated rhinos in the Ngorongoro population had the lowest probability of extinction (3.4E-15, CI: 2.6E-29, 2.4E-06) followed by the indigenous rhino population of Moru (5.9E-07, CI: 8.5E-14, 0.002) compared to all the other subpopulations.

Three subpopulations had very high probability of extinction by 2050 under the current management efforts despite reintroductions: Nyamalumbwa (1.6, CI: 1, 3.4), Ndasiata (149, CI 1, 3.8E+6) and Grumeti (2.2, CI:1, 21.0). If the current management maintains the present variation in mean annual growth and the annual variation caused by environmental stochasticity, these populations will likely fall below the extinction threshold. Mkomazi when managed on its own had a lower probability of extinction by 2050 but still with a large confidence interval (0.008, CI: 1.2E-05, 0.3). Among the translocated populations, Mkomazi was the only one deemed viable, with a lower probability of extinction by 2050 under the current annual variation rate in population growth (Table 4.4).

Table 4.3. The probability of extinction for black rhino subpopulations under current and alternative management approaches by 2050 (i.e. the probability that abundance drops below the minimum viable size of 20 individuals as suggested by the IUCN rhino specialist group). The "Total" is the probability of extinction when the progeny of both indigenous and translocated animals are included, whereas "native" does not include translocated lineages. This allows the effects of reintroductions on reducing the risk of extinction to be evaluated. 95% confidence intervals are indicated in parentheses.

Management			Subpopulation	Native	Total	Native	Total	Native	Total	Native	Total
Tanzania	Serengeti Ecosystem	Southern zone	Moru	5.9E-07 (8.5E-14, 0.002)	1.3E-07 (5E-14, 0.0011)	1.3E-21 (3.9E-38, 1.0E-12)	8.6E-32 (1.1E-56, 3.6E-17)	1.2E-29 (2.7E-53, 1.9E-15)	8.2E-43, (1.1E-71, 1.7E-25)	2.0E-30 (6.4E-58, 6.8E-16)	3.3E-32 (4.1E-57, 2.9E-15)
			Ngorongoro	2.0E-07 (5.3E-15, 0.004)	3.4E-15 (2.6E-29, 2.4E-06)						
		Northern zone	Nyamalumbwa	1.84 (1, 15.1)	1.6 (1, 3.4)	1.6 (1, 3.5)	0.02 (9.8E-5, 0.24)				
			Grumeti	n/a	n/a						
			Ndasiata	n/a	149 (1, 3.8E+6)						
			Mkomazi	5.9E-07 (8.5E-14, 0.002)	1.3E-07 (5E-14, 0.0011)						

Table 4.4. The joint probability of extinction for black rhinos subpopulations when combined under alternative management approaches by 2050 (i.e. Northern and Southern Zones, Serengeti ecosystem, total population in Tanzania). "All", is the probability of extinction by 2050 of all the subpopulations when managed under the alternatives approaches. "At least one" is the probability that at least one subpopulation will go extinct under alternative approaches. 95% confidence intervals are indicated in parentheses.

Management			Subpopulation	PE(2050) (LCL, UCL) for each Subpopulation	Joint probability for zones		Joint probability for Serengeti Ecosystem		Joint probability for Tanzania	
					All	At least one	All	At least one	All	At least one
Tanzania	Serengeti Ecosystem	South zone	Moru	1.3E-07 (5E-14, 0.0011)	4.42E-22 (1.2E-42, 2.6E-09)	1.3E-07 (4.5E-14, 0.001)	2.3E-19 (1.2E-42, 0.7)	108 (1, 1.8E+8)	1.9E-21, (1.3E-47, 0.21)	107 (1, 1.2E+8)
			Ngorongoro	3.4E-15 (2.6E-29, 2.4E-06)						
		North zone	Nyamalumbwa	1.6 (1, 3.4)	524.48 (1, 2.7E+8)	107.56 (1, 1.8E+08)				
			Grumeti	2.2 (1, 21.0)						
			Ndasiata	149 (1, 3757224)						
			Mkomazi	0.008 (1.2E-05,0.3)						

4.4.7 Assessing the viability of combining and managing the rhino subpopulations as zones

Under the current management in which all subpopulations are managed independently with limited dispersal opportunities between subpopulations (Table 4.4) then the joint probability that all of the subpopulations will fall below the extinction threshold in the northern zone is very high (524.48, CI: 1, 2.7E+8) and that at least one subpopulation will be less than the minimum viable population size by 2050 is 107.56 (CI: 1, 1.8E+08). However, for the southern zone, the joint probability that both Ngorongoro and Moru subpopulation sizes are projected to be below the minimum viable threshold by 2050 is very low (4.42E-22, CI: 1.2E-42, 2.6E-09) and the joint probability that at least one subpopulation will be under the minimum viable population size is 1.3E-07 (CI: 4.5E-14, 0.001). Conversely, when the Serengeti ecosystem is partitioned into two management zones (Northern Zone with Nyamalumbwa, Ndasiata and Grumeti and Southern Zone with Moru and Ngorongoro) and the subpopulations within each zone are permitted to disperse and are managed as a single population, then the Northern zone becomes marginally viable but only when translocated animals are included (0.02, CI: 9.8E-5, 0.24). If no translocation occurs the Northern zone has a very high probability of extinction (1.6, CI: 1, 3.5) (Table 4.3) If the subpopulations in the southern zone (Moru and Ngorongoro) are combined, then the probability of extinction is very low (1.3E-21, CI: 3.9E-38, 1.0E-12) (Table 4.4).

4.4.8 Assessing the viability of rhino with and without translocation at an ecosystem level

Under current management scenarios where each subpopulation is managed independently and with limited dispersal between them, then the probability that all subpopulations go extinct is small (2.3E-19, CI: 1.2E-42, 0.7), but the probability that at least one subpopulation goes extinct is much higher (0.02, CI: <0.001, 0.24) (Table 4.4). Translocations did not alter this effect; the joint probability of extinction of indigenous animals alone was <0.001, except for Nyamalumbwa (Table 4.3). However, if we manage Serengeti as a single population in which animals are free to move between all 5 subpopulations then the probability of extinction was very low with (8.2E-43, CI: 1.1E-71, 1.7E-25). The probability remained low despite the effect of translocations; the probability of extinction for indigenous populations alone is 1.2E-29, (CI: 2.7E-53, 1.9E-15) (Table 4.3).

4.4.9 Assessing feasibility of Serengeti as a source population

I assessed the possibility of using Serengeti as a source population to support reintroductions of rhinos into other areas in Tanzania by looking at the probability of extinction of the population and the confidence interval for the event to happen after harvesting the rhinos from either Moru or Ngorongoro (Table 4.5). Neither Moru nor Ngorongoro showed any significant change in the probability of extinction after harvesting between 5-25 rhinos. Moru showed a moderate risk of extinction

by 2050 when 30 or more individuals were removed. In contrast, we can harvest 30 rhinos in Ngorongoro and keep the population viable (1.4E-07, CI: 3.4E-13, 0.0009).

Considering both subpopulations together in the Serengeti (Table 4.5), my results indicate that up to 60 rhinos can be harvested without compromising the probability of extinction by 2050 (1.5E-33, CI: 2.9E-58, 6.6E-18). This conclusion is based on the probability of extinction and the confidence intervals, which show that the chances of the population falling below a viable size are very low under current management efforts. This suggests that the Serengeti ecosystem could be used as a source for other populations if we maintain the current management strategies.

Table 4.5. The probability of extinction for black rhinos subpopulations under current and alternative management approaches by 2050 if animals were removed from Moro or Ngorongoro to supplement smaller populations. The "Total population" is the number of rhinos in the donor subpopulation. Nc is the number of rhinos remaining after harvest in the donor subpopulation. Lower and upper confidence intervals are also displayed.

Population	Total population	Number harvested	Nc	Probability of extinction by 2050	Lower confidence interval	Upper confidence interval
Moru	55	5	50	5.6E-07	5.5E-13	0.002
Moru	55	10	45	3.0E-06	4.6E-12	0.003
Moru	55	15	40	1.9E-05	5.3E-10	0.011
Moru	55	20	35	0.0002	4.8E-08	0.02
Moru	55	25	30	0.0017	2.9E-6	0.04
Moru	55	30	25	0.03	0.001	0.23
Ngorongoro	65	5	60	3.3E-14	5.4E-27	5.3E-07
Ngorongoro	65	10	55	4.0 E-13	3.0E-23	1.3E-06
Ngorongoro	65	15	50	6.0E-12	2.9E-24	8.1E-06
Ngorongoro	65	20	45	1.2E-10	3.5E-19	7.0E-05
Ngorongoro	65	25	40	3.3E-9	3.1E-27	0.0002
Ngorongoro	65	30	35	1.4E-07	3.4E-13	0.0009
Serengeti Ecosystem	165	10	155	1.7E-41	1.0E-71	2.1E-21
Serengeti Ecosystem	165	20	145	4.1E-40	1.8E-68	2.9E-21
Serengeti Ecosystem	165	30	135	1.2E-38	9.6E-63	6.3E-20
Serengeti Ecosystem	165	40	125	4.5E-37	7.3E-65	2.0E-20
Serengeti Ecosystem	165	50	115	2.2E-35	6.7E-71	2.8E-19
Serengeti Ecosystem	165	60	105	1.5E-33	2.9E-58	6.6E-18

4.5 Discussion

This study analysed demographic data collected from daily rhino monitoring activities from six black rhino subpopulations in Tanzania over 34 years (1990-2024). The most important finding from our work suggests that rhino populations are recovering in Tanzania most likely because of natural recruitment and reduced mortality achieved by the intensive management zone (IPZ) protection strategies. Although inbreeding may have some effects on the recovery of the population, we found that the effect of translocations has been minimal. Although all populations are increasing, many of them have a very low probability of meeting IUCN threshold of 20 animals by 2050, such as Nyamalumbwa, Ndasiata and Grumeti. Translocations appear to be most beneficial when several small adjacent subpopulations are managed as a single unit in which animals are allowed to disperse. Our results thus suggest that the most effective way to ensure the recovery of rhinos in Tanzania is to facilitate natural recruitment by protecting broader areas that allow them to movement freely rather than restricting them to small IPZ management zones.

4.5.1 Key performance indicators and population growth rates

There are three potential mechanisms by which Tanzania's populations of rhino might be increasing: by increased recruitment, as a result of active reintroductions programmes, or as a result of reduced mortality. The recruitment in a population can be influenced by the age at first reproduction which can influence the potential for the population to increase because the earlier age of first reproduction the longer the lifetime productivity, as females will mature and start to reproduce at an early age, and this could increase the growth of the population. Also, longer inter-calving intervals can decrease the annual number of calves born, which lowers the rate of population recruitment and hence growth. The sex ratio holds significant importance in shaping the growth rates and population dynamics of numerous large mammal populations. In populations skewed towards females, higher growth rates are anticipated under favourable conditions. This is because favourable conditions facilitate females in reaching the necessary body mass for reproduction earlier, leading to earlier age of first reproduction. In this study, Grumeti had the highest growth rate, and the sex ratio has been skewed towards males, so sex ratio alone cannot contribute to high growth. The annual sex ratio trend indicated that most populations have more females than males; therefore, we expect to see better recruitment. Nevertheless, most of the subpopulations are performing relatively well against the IUCN rhino specialist group performance indicators.

However, our results also suggest that recruitment by translocated individuals is lower than for native individuals. Mkomazi, which was entirely reintroduced in 1997, showed a significantly later age of first reproduction and longer inter-calving interval than the indigenous Moru subpopulation and a much slower population growth rate. Although this was the only translocated population with sufficient data to compare with the native populations, the indigenous Ngorongoro subpopulation included some translocated individuals. Interestingly, it also showed significantly longer inter-calving intervals than Moru and the translocated individuals showed a lower growth rate than the native individuals in that population.

While recruitment is important, it is unlikely to be the only reason for the strong recovery we observe in Tanzania populations at this point. the high growth rates may be influenced by low mortality due to poaching, which has been effectively mitigated through strong anti-poaching efforts.

4.5.2 Impacts of inbreeding

One concern about the severe bottlenecks experienced by Tanzanian rhino populations is that they will suffer from negative effects of inbreeding. Chapter 3 suggests that some of these concerns might be mitigated by purging of the genetic load. The results presented in this chapter also suggest that inbreeding might not have strong impacts on reproductive performance. While there was a significant increase in age of first reproduction and inter-calving interval with inbreeding coefficient, the slope was relatively shallow and there was no effect of subpopulation, despite the different histories of inbreeding in the populations. Moru was reduced to only three breeding individuals but is now one of the largest populations, with the highest fitness parameters (shortest age of first reproduction and shortest inter-calving interval). As described in chapter 3, this could be due to purging but it also could be because the three founding individuals had particularly high fitness.

4.5.3 Impacts of different management strategies on extinction probabilities

If the strong population growth observed in rhino populations were the result of translocations and reintroductions of rhinos into new subpopulations, then there should be clear differences in the growth rates and population viability when translocated animals and their progeny are included in the analysis. However, our results do not support this premise. Ngorongoro indigenous animals exhibited a moderate growth rate, even when considering the inclusion of translocated rhinos, indicating that translocation had no discernible effect on growth. Also, there is no change in probability that Grumeti, Ndasiata, or Nyamalumbwa meet the minimum viable population threshold by 2050 when projections include the translocated animals and their progeny as opposed to just the indigenous. Furthermore, Moru and Ngorongoro have a very low probability that their populations drop below the viability threshold, despite including translocated animals and their offspring in the model projections. In addition, if the rhino population is managed in the current way as independent subpopulations in isolation, then the joint probability that at least one of the subpopulations in Serengeti does not meet the minimum viable population size of 20 animals is substantial. While introductions are an important management tool for establishing new populations, there is little evidence from my study that introductions alone can enable rhino populations to meet the minimum viable size. Reintroduction alone is thus unlikely to account for the population increase we observe in Tanzanian subpopulations.

However, our analysis suggests that when reintroductions are combined with the ability for animals to disperse naturally between adjacent subpopulations, the probability that the populations can exceed the minimum viable size of 20 animals by 2050 as recommended by IUCN is enhanced. For example, if we allow animals

to disperse and move freely between populations then: the northern zone becomes marginally viable but only in scenarios with translocation; they would not be viable if no translocations would have occurred. However, if managed at the ecosystem level and allowing animals to disperse between all subpopulations then there is an extremely low probability the population dips below the minimum viable size.

4.5.4 Potential for using Serengeti as a source population

The added advantage of allowing animals to move freely in the Serengeti is that the population could become a source for rhinos that could be introduced to other ecosystems in the country which have been identified by the rhino policy as future areas for reintroduction (such as Manyara, Mikumi, Burigi-Chato, Arusha and Tarangire National Parks). Our assessment suggests that as many as 25 rhinos could be harvested, particularly from Moru and Ngorongoro, without compromising the viability of the Serengeti population. Furthermore, the differences in sex ratios Figure 4.5 suggest that Moru may be a good source of males whereas Ngorongoro could be a good source of females.

4.5.5 Effects of resource protection / antipoaching

Poaching was the main reason for the 96% population decline of rhinos in the Serengeti ecosystem. Securing good rhino habitat with dedicated teams of anti-poaching rangers that track individuals every day likely has a stronger effect than recruitment and translocations on the population growth of rhinos in Tanzania. Consistent daily observations have likely reduced the risk of poaching and has reduced the overall rate of mortality. Historic evidence suggests that Serengeti and Mkomazi both were good habitats before poaching became a major source of mortality. In the early 1970s, Serengeti National Park was estimated to host approximately 450 rhinos at an overall density of 0.03 rhinoceroses per km^2, with the largest population recorded in the north (140 ± 22; density of 0.08) and the smallest in the southwest (53 ± 15; density of 0.02), indicating variation in abundance of rhinoceros within the park even before poaching (Frame, 1980; Metzger et al., 2007). Mkomazi had a population of at least 150 rhinoceros in the reserve in the mid-1960s but the species in 1970 underwent a total local extinction in Mkomazi in 1985 due to poaching (Eltringham et al., 1999). Therefore, if we manage poaching there is higher possibilities of restoring the rhino population in these ecosystems even if only managing the indigenous population without additional translocations.

4.5.6 Cost/benefit analysis of reintroductions compared to rangers

Given the benefits we observe in terms of protecting habitats and investing in intensive ranger patrols and rhino monitoring it is worth evaluating the costs and benefits of each approach. It costs approximately $108,661 US dollars for translocation of one rhino from South Africa to Tanzania, including all aspects of the operation such as capture, quarantine, transport, release and monitoring. Tanzania has translocated a total of 34 rhinos, originating from South Africa (24), the United Kingdom (5), the USA (1), and the Czech Republic (4), with 14 rhinos

relocated to Mkomazi and 19 to the Serengeti ecosystem. Unfortunately, 8 rhinos have died during translocation, representing a mortality rate of 24%, which means for every translocation there is 24% percent chance of losing the animals. In Tanzania, the average monthly salary for a ranger is $470. To effectively manage the whole Serengeti ecosystem, which spans approximately 30,000 square kilometres, there are currently about 150 rangers to protect the area. Therefore, for one year, the budget allocated for ranger salaries to safeguard rhinos amounts to $846,000 for the Serengeti ecosystem, which is approximately the cost for translocation of 8 rhinos. Thus, if our recommendations to extend management across a wider area were adopted, while it would require more investment in rangers, it could still be cheaper than relying on physically moving animals between subpopulations. As described in previous chapters, there are also costs in terms of animal welfare, as many animals have died after translocation, in other countries as well as Tanzania.

4.6 Conclusion, assumptions, and recommendations

Based on the assumption that poaching rates stay at the current level and we have no catastrophes before 2050, we recommend that the most efficient strategy could be that animals were allowed to move freely and equally between all subpopulations and provide an opportunity for dispersal and random mating. In our data collection there have been reports of rhinos attempting to move between regions in the Serengeti that are forced to return to their original subpopulation. We also know that animals move between Nyamalumbwa and the Maasai mara but the separate monitoring schemes on the two sides of the border mean that animals can't be tracked to follow their reproductive success. Including integrated cross-border management could thus be important to improve our understanding of variation in recruitment across the entire Serengeti ecosystem. For example, developing a common database could help to improve records for animals currently monitored separately.
Our recommendation is to invest in rangers in preference to translocations, in order to allow rhinos to move between subpopulations. Our PVA suggests that allowing rhinos to move freely across the Serengeti ecosystem has a great benefit in terms of lowering extinction risks compared to keeping them in the intensive protection zones. We also recommend that If translocations consider using indigenous sources before foreign; for example, using the larger and fitter populations in the Serengeti to supplement smaller populations with lower recruitment rates. Our results suggest that if we managed the rhinos in Serengeti as a single management unit the probability of losing any population becomes lower and we can harvest up to 60 rhinos without compromising the viability of the metapopulations. This would also avoid the cost of international translocation, and the risks associated with moving individuals into habitats that they might not be preadapted to.

We also recommend that the intensive monitoring of population dynamics and reproductive performance should be continued in order to get better estimates of growth in the populations with shallow pedigrees or incomplete data. This is especially true to assess the impacts of previous translocations, since Mkomazi is the only translocated population that was established long ago enough that we could estimate recruitment rates.

5 General discussion

In this final chapter, I review the main findings of this thesis, beginning with the application of mtDNA control region markers and whole genome sequencing to assess genetic diversity and ending with population viability analysis of the black rhinoceros in Tanzania. Subsequently, I examine the conservation management policies for the eastern black rhinoceros in Tanzania, highlighting key insights contributed by the study's findings. Following this discussion, I address the challenges faced by eastern black rhinoceros in Tanzania and offer conservation recommendations for management of subpopulations based on the study's results. Lastly, I propose future research directions to be pursued at both the range-wide and local scales for the future management of rhinos in Tanzania.

5.1 Application of conservation genetics in conservation of the eastern black rhino in Tanzania

Genetic information is crucial in developing management plans for threatened populations and species. Molecular genetic tools have been used to estimate population-level parameters, understand gene flow patterns, and estimate deleterious mutation accumulation. These parameters help determine necessary management actions to increase demographic numbers and genetic variation, alleviate genetic load, and track long-term viability of populations (Willi et al., 2022). Therefore, monitoring genetic variation in threatened and restored populations is also essential. In this study I have demonstrated the use of conservation genetics to assist the conservation of the black rhino in Tanzania. For example, in chapter 2, I used the mtDNA control region marker to assess genetic impacts of past management interventions on genetic diversity in Tanzania's extant eastern black rhinoceros population, assessing female dispersal and relating current haplotype diversity to historical patterns. This marker has also been used by several other black rhino studies across the globe (Brown and Houlden, 2000; Muya et al., 2011; Kotzé et al., 2014; Moodley et al., 2017). I also compared my results with 443 mtDNA sequences from these studies that had been deposited in GenBank, including 29 sequences from historical populations in Tanzania. The study enhances the global mtDNA diversity assessment conducted by Moodley et al. (2017), providing crucial information for conservation management of rhinoceros and other wildlife populations in the region. Also, the patterns of mtDNA variation confirmed that the maternal lineages introduced to Ndasiata, Mkomazi, and Ngorongoro were of East African origin, despite being translocated from European zoos or captive populations in South Africa. Also, the study revealed that, out of the 34 reintroduced rhinos in Tanzania, we have successfully restored only three historical haplotypes (haplotype 3, haplotype 5, and haplotype 6). Therefore, in the future considering genetic profiles will be crucial in order to introduce rhinos with more diverse haplotypes. Furthermore, this study has revealed that the current Intensive Protection Zone (IPZ) management practices have restricted the movement of females between subpopulations. I recommended a combined management approach, including managing subpopulations in the same ecosystem, targeting reintroductions based on genetic variation sequencing nuclear DNA to inform translocation decisions, and selecting translocated animals from extant populations within East Africa to

avoid translocation catastrophes, while allowing more natural dispersal between populations rather than restricting movements. Therefore, this chapter demonstrated the potential of mtDNA to address different management questions for adaptive management of wildlife.

In chapter 3, I used whole genome sequencing to inform the management impacts of current conservation efforts on individuals of the eastern black rhinos with different types of ancestry. Recent genomic approaches emphasise evaluating the overall effects of inbreeding on the accumulation of harmful mutations throughout the genome, which serves as an indirect proxy of fitness (Khan et al., 2021). However, in other studies, such assessments have primarily been conducted on a population-wide scale, where various individuals within populations may have encountered diverse ancestral backgrounds because of variation in past management approaches. My results focus on the impacts that individuals can make and show that offspring from dispersed or translocated individuals exhibit lower inbreeding compared to those from closed native populations. However, translocated offspring display a higher abundance of deleterious mutations, potentially leading to inbreeding depression if subsequent inbreeding occurs. Conversely, native dispersers mitigate the negative effects of inbreeding while maintaining the benefits of purging deleterious mutations. The study emphasizes the significance of natural dispersal and underscores the importance of habitat corridors for maintaining genetic diversity among populations. Hence, this chapter utilizes conservation genomics techniques to understand the effects of various conservation interventions on the trade-off between inbreeding and accumulation of genetic load in natural populations.

5.2 Tanzania rhino conservation strategic plan in relation to this study

The rhino conservation and management plan, mandated by the 2014 National Strategy to Combat Poaching and Illegal Wildlife Trade, serves as a pivotal tool for all agencies engaged in wildlife protection in Tanzania. Over a five-year span, this plan delineates strategic measures aimed at safeguarding, conserving, and fortifying the black rhino population. The goal of the plan is “To increase black rhino population at a minimum rate of 5% per annum to reach at least 205 black rhinos by the end of 2023 using a meta-population management approach, in line with internationally best practiced standards”. The primary objective of my study was to utilize conservation genetics tools for the management of the eastern black rhino in Tanzania (TAWIRI, 2019). Thus, in this section, I will identify and discuss key areas highlighted by Tanzania's rhino conservation strategy that required further research to inform the management and that I have been able to address with my study.

5.2.1 Key areas in the rhino conservation strategy of Tanzania that have been addressed by this study.

Overall, my study addresses several strategic objectives related to the overall priority of "Conducing priority research to provide information for adaptive management and protection of rhinos and their critical habitats".

Strategic objective 4.6.1.4: Carry out research on rhino genetic diversity
I have been able to address areas of research needs identified by the rhino management team in Tanzania, as outlined in their rhino policy and strategic plans (page 32). I conducted a comprehensive investigation into the genetic diversity of the eastern black rhino in Tanzania using mtDNA control region markers and whole genome sequencing. My study revealed a loss of maternal diversity, likely attributable to poaching pressure. However, recent translocations have successfully restored some previous maternal diversity haplotypes. Additionally, my study found that managing the rhino metapopulation within intensive protection zones has restricted maternal dispersal, hindering the potential benefits of translocations in enhancing diversity.
Furthermore, through whole-genome sequencing, we assessed levels of genetic diversity, inbreeding, and genetic load among native, translocated, and captive-born offspring. We found that the native rhinos in Ngorongoro, Moru and Nyamalumbwa have high inbreeding and low genetic diversity compared to the reintroduced rhinos from captive populations. Also, lower inbreeding in offspring of individuals that had either dispersed from native populations or been translocated from captive populations compared to a closed native population was observed. However, the relative abundance of highly deleterious mutations was higher for offspring resulting from translocation compared to the other groups and this load was sheltered by higher heterozygosity. This could increase risks of inbreeding depression if captive founders subsequently inbreed after translocation. In contrast, native dispersers reduced the negative effects of inbreeding without compromising the benefits of past purging of deleterious mutations. My findings underscore the significance of natural dispersal mechanisms and underscore the critical necessity of preserving habitat corridors to facilitate gene flow between populations.

Section, 4.6.2 Demography studies on rhino for appropriate management decisions enhanced
In my study I have conducted a demographic assessment of the key population performance parameters of the rhino populations in Tanzania. I have assessed age of first reproduction of the females, inter calving interval, and annual population growth.

Section, 4.1.8 Rhino population status and growth rate in each rhino area established
In chapter four I used demographic data from all the rhino subpopulations in Tanzania to estimate the annual growth rate and compare it with the standard benchmark growth rate set by IUCN. My results indicate that the current growth rate in all the metapopulations is above the minimum standard benchmark of 5% set by the rhino conservation strategy to attain a total number of 205 rhinos by 2023. My study has found that the total population of black rhinos in Tanzania is

growing at an annual rate of 8% but the total number of rhinos by 2024 is predicted to reach 203, which is just marginal below the minimum desired goal.

Section, 4.6.1.5 Conduct research on translocated and reintroduced animals
In this section the rhino management strategy identified the need to conduct research on the translocated and reintroduced rhinos. My study has partially fulfilled his gap by analysing diversity, inbreeding and genetic load of the reintroduced rhinos as well as the offspring the y have produced with the native rhinos. Although I found lower levels of inbreeding compared to the native rhinos this came at the cost of high levels of genetic load in the rhinos and the offspring they have produced with native rhinos. Furthermore, I estimated the annual growth rate of the translocated rhinos in Grumeti, Ndasiata and Mkomazi and found that the combined subpopulations had annual growth rate above the minimum standard of 5%. In conclusion, this study provided important information on genetic diversity of the black rhino in Tanzania and the demographic performance of the metapopulation in relation to the goals set by the policy.

5.3 Overall conservation management implications

The ability of threatened species to survive depends on both comprehensive adaptive management measures that can lessen or eliminate the threat of extinction and a detailed understanding of the mechanisms driving that threat. The main threat to the survival of black rhinoceros in Tanzania and elsewhere is poaching. Thus, facilitating "safe" natural dispersal appears to be the optimal strategy for managing endangered wild populations such as black rhinos, which have experienced significant genetic bottlenecks. Corridors that enable animal dispersal offer dual benefits by reducing inbreeding and homozygosity of deleterious alleles, thus minimizing the genetic load while maximizing breeding opportunities with unrelated individuals. Although translocations from managed game reserves can enhance genetic diversity, they also carry the risk of increasing deleterious allele mutation loads. To mitigate this risk, management practices should prioritize natural mixing to minimize inbreeding and reduce the likelihood of fitness-reducing mutations. While targeted translocations could also address inbreeding, they pose financial and logistical challenges. Leveraging whole genome sequence data can help move conservation efforts away from the assumption that supplementation of genetic variation alone reduces extinction risks, instead emphasizing the functional consequences of population mixing on the emergence of deleterious alleles, particularly for highly threatened populations. Future studies will be necessary perhaps using more whole genome sequencing to assess the genetic load for each subpopulation in Tanzania. Developing a genetic tool which can be used to inform the management on which individual to translocate would be highly valuable for future initiatives and to further study the impacts of past conservation efforts.

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