GENETIC STRUCTURING BETWEEN GEMSBOK (*ORYX GAZELLA*) POPULATIONS AND THE IMPACT OF THE FOUNDER EFFECT ON ISOLATED POPULATIONS

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DECLARATION

I declare that **GENETIC STRUCTURING BETWEEN GEMSBOK (*Oryx gazella*) POPULATIONS AND THE IMPACT OF THE FOUNDER EFFECT ON ISOLATED POPULATIONS** is my own work and that all the sources used or quoted have been indicated and acknowledged by means of complete references and that this work has not been submitted for any other degree at any other institution.

2012/03/19

Karl Benjamin Osmers ....

Date

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Signature

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Abstract

A microsatellite-based study was performed on five populations of Gemsbok (*Oryx gazella*). This study was aimed at estimating genetic diversity in introduced South African gemsbok populations (an opportunity that arose when additional animals from the same source were imported into South Africa), and determine genetic structure. Population sizes at the time of sampling were: Namibia (n = 6500), Cohen (n = 70), Tempelhof (n = 55), STS Kalahari Game Ranch (n = 1000) and Elias (n = 35). The purpose of the study was to determine the genetic structure of the aforementioned *O. Gazelle* populations, and to assess the impact of the founder effect on isolated populations. The following primers (BMS1237, MAF46, OARFC304, OARHH64, ETH225, RBP3, MAF50, HDZ8) developed for commercial purposes in the bovine group were used. Genetic diversity were calculated as Expected Heterozygosity (H_e), proportion of polymorphic loc (P) and number of alleles per locus (A). Conformation to expected Hardy-Weinberg equilibrium of genotypes was also determined, using a Chi-square test. Tests for the signature of bottlenecks in the populations studied were also performed. Genetic drift/differentiation was tested by using F_{ST} and R_{ST} coefficients. Assignment tests were performed to identify the true number of genetic populations (clusters). Genetic distance was used as an additional measure of differentiation. The results indicated that all loci showed allelic polymorphism in all the populations except one (at the OARHH64 locus). The South African Cohen population displayed the highest level of genetic diversity, with $H_e = 0.595 \pm 0.247$. This population also did not show evidence of a bottleneck. Genetic distance values indicated the greatest similarity between the Cohen and Namibian populations, in line with the Namibian origin of the Cohen group. Greatest distance was observed between the STS and Tempelhof populations. conclusion, results from this study reflects the origins of populations and suggest that inbreeding in small isolated populations may be less than previously estimated.

Introduction

Gemsbok (*Oryx gazella*) are aesthetically attractive animals favoured by many tourists and local inhabitants, whether for hunting purposes or photographic safaris. The species is thus widely kept on nature reserves as well as private game farms. The taxonomic position of this species is presented in Table 1.

Table 1 – The taxonomic designation of *Oryx gazella*

Taxonomic Designation	
Kingdom:	*Animalia*
Phylum:	*Chordata*
Class:	*Mammalia*
Order:	*Ruminantia*
Suborder:	*Pecora*
Superfamily:	*Bovoidea*
Family:	*Bovidae*
Subfamily:	*Antilopinae*
Tribe:	*Hippotragini*
Genus:	*Oryx*
Species:	*O. gazella*

a. Distribution and biology of *Oryx gazella*

Oryx gazella occurs in two discrete areas on the African continent, one in the southern African subregion, and another in East Africa (Figure 1). In the southern subregion, *O. gazella* occurs in Namibia, Botswana and various parts of South Africa (Northern Cape, Free State, Eastern and Western Cape Provinces) (Figure 1). In East Africa, the species is found from northern Tanzania to Eritrea.

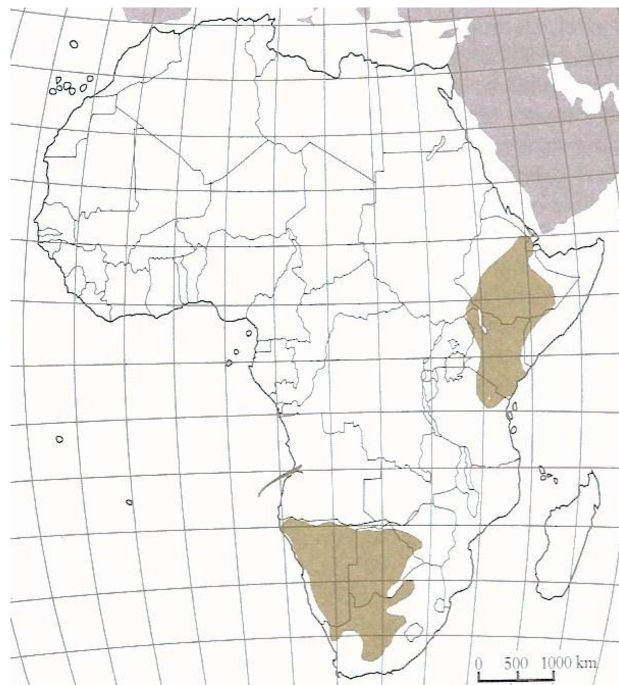

Figure 1 – The geographical distribution of *Oryx gazella* in southern and East Africa. From Skinner & Chimimba (2005).

Gemsbok males have mean territories of approximately 26km^2, while female home ranges are on average about 1,430km^2 in size (Skinner & Chimimba 2005). Gemsbok are primarily grazers, usually moving in small loose herds, feeding on the most nutritious grasses and herbs, and during the dry season will turn to browsing and digging for succulent tap roots to

supplement its water intake (Mills & Hes 1997). Sexual maturity for females is reached after two years, and calves are born throughout the year after a gestation period of nine months (Mills & Hes 1997). Males are territorial, with female herds moving between territories in search of food and non-territorial bulls moving with the female herds. During periods of drought, territories are abandoned by the males to follow female herds that have migrated in search of better feeding grounds (Mills & Hes 1997).

From a commercial (game farming) point of view, gemsbok is a highly sought after species due to its popularity with tourists and hunters. Data from the Unit for Livestock- and Wildlife-Economy at the University of the Free State show a steady growth in the live retail value of Gemsbok (*O. gazella*). Mean annual prices paid for *O. gazella* are shown in Figure 2. These amounts are based on a selection of game auctions across South Africa. A continued increase in the value of live *O.gazella* can clearly be observed from these data.

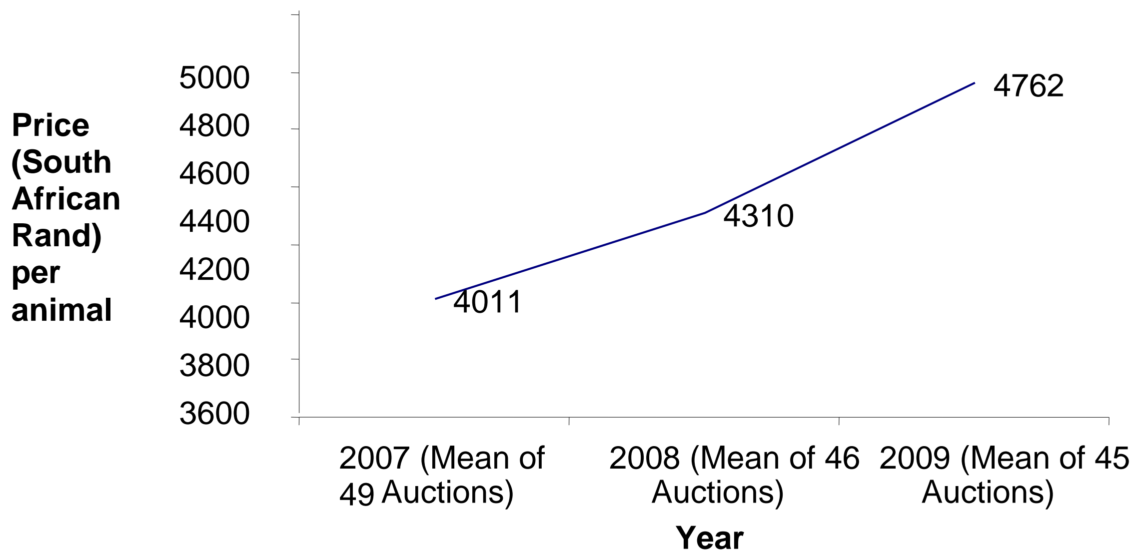

Figure 2 – Mean annual prices paid for *Oryx gazella* sold at game auctions in South Africa for the period 2007-2009. Data obtained from Vleissentraal Limited, South Africa. (1 US$ =approximately 7 ZAR in November 2011)

In the trophy hunting industry, Gemsbok are highly sought after by hunters from across the world. Two organizations - Safari Club International and Rowland Ward - publish record books recording the best trophy animals hunted internationally. The publications of both these organizations are therefore good measures of the geographic areas where trophy animals have been hunted. These organizations both have minimum requirements for an animal to be entered into these databases. Therefore these databases can be used as an indicator of areas that host animal populations with good phenotypic characteristics, which presumably translate to favourable genetic characteristics.

The system used by Safari Club International requires a Gemsbok to have a score of more than 81 points, for inclusion in record books. This score is calculated by taking a range of measurements of the particular set of horns (in inches). The total score represents the sum of all of the following measurements: the length of both horns (in the front from base to tip to the closest 1/16th of an inch), as well as the circumference of the bases of each horn. The sum of these four measurements must meet 81 points. The Rowland Ward system requires only the length of the longest horn from the base to the tip and this must exceed 41 5/8 inches. By using the data from these two organizations, an estimate of areas that yield the biggest trophies can be obtained. Data of the top ten greatest trophies ever hunted are presented in Table 2.

Table 2 – The top ten *Oryx gazella* hunted with a rifle, scored under the Safari Club International method, with the areas where these animals were hunted (www.scirecordbook.org).

Ranking	Name	Date	Location	Score
1	W.J. Ray Jr.	05/1981	Botswana Kalahari	111 5/8
1	Lloyd Douglas	09/2004	RSA Rooipoort	111 5/8
2	Ivor Karan	11/2007	RSA Bloemhof	108 1/8
3	John W. Zang III	04/1992	RSA Orange Free State	107 7/8
4	Dr. Jerry Dollar	05/2005	Botswana Kalahari	107 2/8
5	Dan L. Duncan	06/1980	Botswana Kalahari	107
6	Dick Nelson	07/1975	Botswana	106 4/8
7	Daniel Alsager	04/2009	Botswana Ghanzi	105 4/8
8	James Williams	06/1991	RSA Bhala Bhala	105 3/8
8	Phillip J. Netznik	08/1999	RSA Kalahari	105 3/8
9	Col. John H. Roush Jr.	05/1992	RSA Victoria West	104 5/8
10	Angel Antonio Fullana	04/1993	Botswana	104 4/8

Due to the increased pressure from selective hunting on this species for trophies, there may be negative genetic consequences (relating to horn length and horn thickness). Selective hunting may lead to a change in allele frequencies and eventually to the loss of rare alleles, as shown by Hartl *et al.* (1991). It may also inadvertently decrease fitness by removing animals with desirable genotypes (Harris *et al.* 2002).

b. Genetic management of game farm populations

Effective management of populations is of great importance in conservation, because of an increase in hunting and the ecotourism industry, and the artificial management that goes with this (Grobler 1994). In addition game farmers are realizing the importance of improving their wildlife stock to an extent that will make a real contribution to game conservation. These improvements include factors like trophy quality and adaptive ability. Trophy quality is an important factor because trophy animals are more sought after and attain higher prices on game auctions. Managing of populations on game farms also help to maintain strong healthy populations (Young 1984). Genetic management of wildlife in fenced areas is therefore an important task, because it ensures the sustainable utilization of the wildlife in that specific habitat.

Increased knowledge on genetic management of wildlife has resulted in more specialized game management techniques compared to previous years. The goal is to maintain genetic diversity, which represents the essential evolutionary potential for species to respond to changing environments (Frankham *et al*. 2002). With changing environmental conditions animals need to adapt, but genetic diversity is required to maintain the ability to

adapt. In the past, game farmers primarily used phenotypic characteristics (body size and horn length) as an indicator of “good genes” and a lack of inbreeding. Genetic management of wildlife is now practiced much more scientifically, with genetic laboratory studies increasingly forming the basis for management decisions. An early example of genetic management in an intensively utilized species was reported by Hartl *et al.* (1991). These authors studied the effect of selective hunting on Red deer (*Cervus elaphus*), and found that selective hunting does not lead to obvious differences in the overall values of polymorphism or heterozygosity between the populations. There were however changes in allele frequencies that could eventually lead to the loss of one or the other rare allele.

It is important to introduce and keep population sizes that are large enough to ensure that populations do not enter an extinction vortex (Gilpin & Soule 1986) under the specific circumstances. Small population size can lead to a loss of genetic variation in several ways:

- The effect of genetic drift is more pronounced in small populations, and one of the consequences of genetic drift can be the loss or fixation of alleles (Krohne 2001).
- Small populations are subjected to inbreeding effects. When relatives mate, the probability of offspring containing genes that are identical by descent increases. Inbreeding does not change allele frequencies, but it change the frequencies of the genotypes, in particular it increases the frequency of homozygotes at the expense of heterozygotes at a rate of 0.5N per generation (N = Population size). This will result in the

accumulation of deleterious homozygous recessive genotypes more frequently resulting in a decreased fitness (Krohne 2001).

Another aspect that needs to be taken into account is the social structure and group size, both of which play a role in the mating success of species. In a study done by Mattiello *et al.* (2004) on the habitat use and group size of African wild ungulates, it was shown that variations in group size and habitat use throughout time may give useful indications about the status of animal populations. These authors found that gemsbok in the bushveld area predominantly occurred in smaller groups. This observation provides researchers with a better understanding of the processes that contribute to the maintenance of genetic diversity. Smaller groups in the bushveld-type habitat may result in a divergence of breeding and thus maintain a genetic diversity for the population as a whole.

c. Forensics as an important subdivision during genetic studies

In recent years, there has been a steady increase in incidents of poaching. While this is most widely publicized in the case of rare and endangered animals, particularly rhinoceroses, the problem also affects common game species. Forensic techniques can also be used to monitor compliance with rules and regulations governing the wildlife industry. For example, Kotze *et al.* (2008) reported on the results of a study to determine the origin of Cheetah (*Acinonyx jubatus*) that were confiscated on suspicion of illegal import into South Africa. These authors used microsatellite markers and assignment tests, but could not find convincing evidence of foul play.

Genetic studies like this study are important to resolve illegal trade in endangered animals and stock identification (Grobler *et al.* 2005c).

d. Population Genetic Structure

It is vital to determine the nature of genetic structure among populations before important decisions on management, such as translocations or introduction of animals between populations, are implemented (Grobler *et al*. 2011). More than a decade after the first discussion of the ESU concept by authors such as Waples (1995), Moritz (1994; 2002) and Waples & Gaggiotti (2006), it remains a challenge to define the borders of true genetic populations and describe connectivity among them (Grobler *et al*. 2011). According to these authors, evidence of genetic structure suggests that translocations of individuals between distant populations should be discouraged in order to maintain evolutionarily significant units (ESUs).

Phylogeographic information on a wide range free-ranging species has been conducted in recent years, but such data is still available for comparatively few southern African antelope species. There is thus a great need for such studies on local game species to determine true population structure and also determine whether structure result from vicariance or have real adaptive significance. Such studies to examine the spatial distribution of genetic variation in species will be useful to inform legislation and regulate translocations (Grobler *et al*. 2005a).

In a study by Alpers *et al.* (2004), sequences of the mitochondrial DNA (mtDNA), and microsatellite genotypes, were used to quantify the genetic variability within and among populations of roan antelope (*Hippotragus equinus*). Results of this study showed that historical separation has resulted

in significant divergence between populations in West Africa and the rest of the distribution area. The populations in West Africa should therefore be managed as a separate ESU in comparison with the populations in the rest of Africa. Studies such as this need to be performed to identify genetic units for conservation, before translocations of animals over long distances are made. Movements of animals therefore need to be put on hold and only be continued when accurate genetic data is available for the species involved.

Artificial Translocations

Translocations are a regular occurrence in the southern African wildlife management environment. Numerous game farm owners re-establish populations of *O. gazella* on their farms from founder populations from either the Northern Cape or Namibian populations. A very important question is from where to where translocations of animals should be allowed. In a study by Grobler *et al.* (2005a), it was found that Nyala (*Tragelaphus angasii*) from different geographical locations showed unique genetic characteristics, and that translocations should be discouraged. Information on the genetic structure of most South African antelopes is not readily available. It is therefore essential to create databases on the genetic structure of different species of animals across various geographical locations. Spear & Chown (2009) commented on how significant translocations of indigenous ungulate species altered the range sizes of these species. A very big and important impact of translocations according to Spear & Chown (2009) is the alteration of range sizes of a particular species. Through climate change vegetation has changed and with the aid of numerous translocations the range sizes of the species have increased. The

long distances these translocations have taken, places the concern for genetic homogenization.

Evolutionary Movements

According to Campbell & Reece (2002) species transplants are normally restricted by dispersal barriers, which could include various biotic or abiotic factors. In recent times, man-made barriers affect natural dispersal more actively than natural barriers. This is particularly seen in the case of *O. gazella* where natural migrating routes from Namibia across Botswana and the northern parts of South Africa are restricted by international borders. Overexploitation of the *O. gazella* populations in many areas in southern Africa has destroyed the natural occurring populations.

e. Genetic Diversity

Fragmentation and small population size are the main reasons for loss of genetic diversity (Frankham *et al*. 2002). Loss of diversity may result in reduced fitness, i.e. the relative ability for reproductive success under selection pressure (Fairbanks & Andersen 1999). The occurrence of Founder events determines the probability that a population will survive; small isolated founder populations have a great probability to enter an extinction vortex and large introductory populations gives you a higher probability on a viable population. In isolated populations losses depend on the effective population size (N_e). The N_e is usually much less than the number of adults in that population (Frankham *et al*. 2002). According to Freeman & Herron (2004) the N_e is particularly sensitive to differences in the number of reproductively active females versus males. The value of N_e is defined by

Freeman & Herron (2004) as the size of an ideal random mating population (with no selection, mutation, or migration) that would lose genetic variation via drift at the same rate as is observed in an actual population. As an example of how genetic diversity can give us a better understanding of the genetic assembly of a population, consider the following example: four populations of Impala (*Aepyceros melampus*) were compared using protein/gel – electrophoresis to determine the influence of different management strategies on genetic variability and differentiation (Grobler & Van der Bank, 1994). The four study groups were one large population, two small isolated populations and one small isolated but well managed population. The results indicated as expected that a large natural population (Klaserie Nature Reserve) had a higher heterozygosity (H_e=4.6) and a proportion of polymorphic loci (PPL) of 14.29%. The only other population that retained comparable level of diversity was the small isolated but well managed population from the Pietersburg (Polokwane) Nature Reserve (H_e = 4.43% and PPL = 14.29%). A small isolated population which started with a founder population of 150 and no further management revealed the lowest values of H_e = 2.41% and PPL = 5.71%, these suggest reduced genetic variation as a result of inbreeding. The other small population were introduced in three batches over three years of 7, 7 and 21 individuals. This way of introduction of individuals, can be seen as a way of managing a population. That is why the H_e = 3.21% and PPL = 11.43% estimate of this population show an improved level of genetic variation when compared with the isolated once-off founder population of n = 150. From these results it is clear that a founder effect is evident in the small population with

founder population of n = 150. The importance of exchanging new individuals between populations is clearly demonstrated in this study.

A population of highly heterozygous individuals should be more viable than a population of less heterozygous individuals because individuals of the former population would have higher growth, fecundity, and survival rates than individuals of the latter population (Leberg 1990). The following example describes an alternative scenario where a population's numbers recover very quickly and without a seriously reduced survival rate or fecundity. According to Hartl & Pucek (1994) a single genetic bottleneck with a subsequent rapid recovery of population size often does not lead to a serious reduction of heterozygous loci, even when the minimum number of individuals passing through that bottleneck is very small. The European bison (*Bison bonasus*) is a species with a present world population of about 3,200 individuals, which originated from only 12 founder genomes, yet the current population still represents a reasonable good level of genetic diversity. The pattern of change in genetic variability largely depends on the size of the bottleneck, rate of population growth, and the mutation rate. It has however been shown that mutation has a negligible effect in the timespans relevant to conservation actions (Frankham *et al*. 2002). A decrease in founder population size does however result in an accelerated increase in genetic distance in the early generations (Chakraborty & Nei 1977).

The founder effect refers to a change in allele frequencies that occurs after a new population is established, due to genetic drift in the form of sampling error in drawing founders from the source population (Freeman & Herron 2004). Translocations in the live game industry in South Africa are an

extensive industry, and more landowners are converting their land into game farms, because of the low input high output potential of the game industry. The farms are populated with the maximum number of species, in small population sizes, to accommodate more species and thus enable farmers to enter the hunting industry. Some owners hunt newly founded populations as soon as a viable population is present on the farm. Others turn towards the relocation of these animals that are in excess to new areas with decreased population sizes. Good management of game farm populations is required to ensure genetic diversity. Inbreeding on a given farm, and relocating these animals to recipient farms could in the end result in a downward spiralling decrease in heterozygosity within the species. The founder effect can have different effects whether it is to cause a rapid decrease in genetic diversity or that it can give an initial rise in genetic diversity. The time period during which the effect of this phenomenon can be observed can vary and a real timescale cannot be correlated with it (Freeman & Herron 2004).

The founder effect is also linked with the population size and in the end a viable population size can have a dramatic effect on the survival of such a population, and the genetic effects it has on the population.

An artificial population bottleneck can occur when new populations are introduced, because a small group has been selected from a donor population and populations like these often have very limited genetic variability as a result of the founder effect. Small populations that narrowly survive demographic contraction may undergo close inbreeding, genetic drift, and loss of overall genomic variation due to allelic loss or reduction to homozygosity (O’Brien 1994). Population bottlenecks, like the artificial

bottleneck caused by the founder effect, can be the cause of an extinction event (Figure 3).

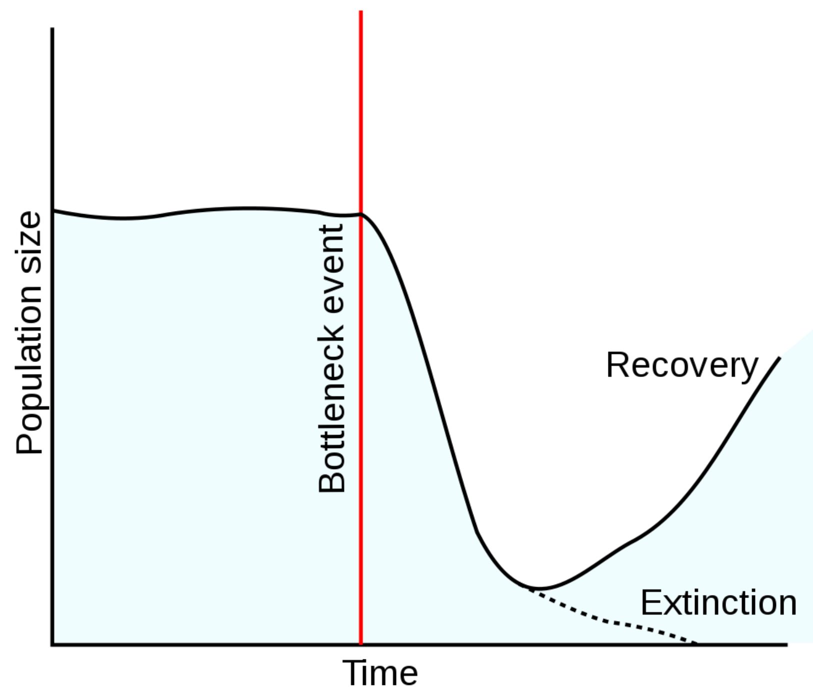

Figure 3 – The impact of a bottleneck on population size, showing two possible outcomes (from Fairbanks & Andersen 1999).

Conversely, continuous introduction of animals from different areas could lead to an outbreeding depression (Edmands 2006). This could lead to decreased adaptability of animals to the specific environment of introduction.

A good example of where highly intensive genetic management of wildlife is taking place is the numerous projects with endangered species found across South Africa. Examples of this include Sable antelope (*Hippotragus niger*), Roan antelope (*Hippotragus equinus)* or disease-free African buffalo (*Syncerus caffer*). In all cases, correct genetic management of these animals

is important to ensure that the animals that are relocated from breeding projects are animals with high genetic fitness.

Theory behind Inbreeding

Inbreeding decreases the frequency of heterozygotes and increase the frequency of homozygotes compared to expectations under Hardy-Weinberg assumptions. In the Hardy-Weinberg analysis the final assumption states that individuals mate at random, which is not the case during inbreeding (Freeman & Herron 2004). Within population gene pools recessive alleles continuously arise through mutation. These alleles are usually deleterious if they are expressed in a homozygous state in individual phenotypes. In large populations where random mating occur these deleterious alleles occur at very low frequencies and are thus not easily found in the homozygous state. However, in a smaller population where mating between relatives commonly occur, there is an increased probability of such homozygotes occurring. Extensive inbreeding reduces the average fitness of populations, because an increasing number of individuals may be suffering from genetic abnormalities. Ideally, inbreeding can be a temporary phenomenon, as the deleterious alleles will be selected against and ultimately purged from the populations as these alleles are expressed. If the population survives the severity of the initial inbreeding depression, the effects of this depression should decline over time. In the long run this can cause a different situation where loss of genetic variability is accompanied by an increase in average fitness (Pullin 2002). It must be taken into consideration that all these effects are only seen in an evolutionary timescale, not the short-term timescale of game farms. In the context of long-term ability to adapt to

changing environmental conditions, this increase in fitness is however probably only temporary (Pullin 2002). Inbreeding results in a decreased reproductive success in mammals, and is one of the problems associated with trying to breed from small populations (Krebs & Davies 2004). Since the founder effect on a new population is prone to show a high level of inbreeding, the current study is aimed to determine the level of inbreeding in a population.

Large free-ranging populations of Gemsbok are still abundant. Free-ranging refers to populations unrestricted by fences, or populations that roam freely in areas larger than their required home range size determined by their social structure of larger than 26 km^2 (Skinner & Chimimba 2005). Data obtained from the current study is important in providing more data on genetic diversity within Gemsbok, because sufficient data on this particular species is not readily available (Grobler 1994). Furthermore it would be important to establish whether the loss of alleles in isolated populations could be observed, and whether certain alleles are more prone to be the first to be lost from the genome in the isolated populations. This refers to the direct result of the founder effect on small isolated populations.

f. What is the role played by conservation genetics?

According to Holsinger (1996) genetics have five important roles it plays in conservation biology:

- To investigate the extent and importance of genetic variability within populations;
- To document patterns of parentage and kinship within populations;

- Identify patterns of divergence among populations;
- Delimiting species boundaries and;
- Resolving phylogenetic relationships among species and higher taxa.

For the purpose of this discussion a further subdivision of these five roles have been made to give the following ten roles:

i. Minimize inbreeding and loss of genetic variation

All natural environments are challenging to survive in. The genotype and the gene pool of populations are evolutionary responses to this challenge. The ability of a population to adapt to a constantly changing environment greatly rests on mutation within the gene pool which creates genetic diversity which in the end is responsible for the adaptation to the changing environment. If the variability in the gene pool is lost the flexibility to adapt is lost. In a large population the loss of alleles through genetic drift is balanced by the gain in new alleles through mutation. In small isolated populations the rate of loss of alleles increase through drift, and the gain of new alleles through mutation decreases, resulting in a rapid loss of alleles and essentially a loss in genetic diversity. Inbreeding in small populations causes the deleterious recessive alleles that are always present in population gene pools, to become expressed in a homozygous state in the individual phenotypes. This can result in a decrease of the average fitness of a population in that more and more individuals suffer from genetic abnormalities. By detecting populations suffering from an inbreeding depression through conservation genetics techniques, rapid management decisions can be made. For example in the study done by Flagstad *et al.*

2000) on Swayne's Hartebeest (*Alcelaphus buselaphus swaynei*) these authors could detect a population that needed management before losing too much genetic diversity and entering an extinction vortex. By introducing more individuals from larger populations it could be possible to increase the genetic diversity of this population and in the end save the population. From this observation, a conclusion can be made that genetic studies are important in the management of small populations to counter inbreeding.

ii. Identify populations of concern and resolving population structure

Techniques of genetics can contribute valuable data to aid in identifying evolutionary significant units (ESUs) worthy of conservation in delimiting reproductively and demographically independent units. This can be done by drawing species or subspecies boundaries and in confirming threats to rare species posed by reproduction with widespread compatible relatives (Holsinger 1996). Alpers *et al.* (2004) performed a study on the Roan antelope (*Hippotragus equinus*) to resolve the population structure and give some suggestions for conservation of the species. The data suggested that the West African populations differed significantly from the rest of the populations across Africa. By resolving the structure of each species significant management decisions can be made that is in the best interest of the species. The West African populations could be recognized together as an ESU. Samples from the rest of the continent constituted a geographically more diverse assemblage with genetic associations not strictly corresponding to the other recognized subspecies. Another significant study done by Lorenzen *et al.* (2006) on Impala (*Aepyceros melampus*) gave important results. Two subspecies of Impala are presently recognized

namely: Black-faced Impala (*Aepyceros melampus petersi*) and Common Impala (*Aepyceros melampus*). After completing this study a third important group could be genetically considered distinct, namely the Samburu population (East African Impala *Aepyceros melampus rendilis*) in Kenya. Looking at these examples important decisions could be made by resolving population structure by using conservation genetics.

iii. Detecting hybridization

A study done by Masembe *et al.* (2006) on three genetically divergent lineages of the Oryx in eastern Africa suggested evidence for an ancient introgressive hybridization. Another study done by Grobler *et al.* (2005b) on Black Wildebeest (*Connochaetes gnou*) also indicated hybridization between the closely related sister group the Blue Wildebeest (*C. taurinus*). By knowing information like this important translocation decisions can be made by keeping particular populations or species away from each other to avoid genetic "pollution". A recent study by Grobler *et al.* (2011a) on the management of hybridization in a population of black wildebeest (*Connochaetes gnou*) showed hybridization between Black Wildebeest (*C. gnou*) and Blue Wildebeest (*Connochaetes taurinus*).

Hybridization between the species was observed at various localities in South Africa. Various approaches were suggested on the management of these herds to ensure the well-being of the population, for example:

- The purist approach which is to cull all the herds with hybrids followed by a rigorous legislative regime to prevent new hybridization events. Unfortunately this approach will cause a

serious bottleneck event within the species. This species has survived two bottleneck events already (Grobler *et al.* 2011a).

- The second approach would be to keep certified pure herds in government-controlled protected areas, private protected areas and accredited private game ranches. All the other herds with moderate introgression of blue wildebeest genetic material would still be allowed on game ranches for sport hunting which will allow time to make the correct decision for the species and prevent a bottleneck.
- The third approach will be to introduce pure animals to hybrid herds thus leading to the genetic swamping of introgressed alleles. Although this sounds good, the number of pure Black Wildebeest in South Africa is not high enough to practice this approach.

A very similar scenario can be seen in the North American Bison (*Bison bison*), where through overexploitation, the species went through a population bottleneck (N = 100) and although the population is now 500,000 individuals strong only 4% are in conservation areas and the rest contain strains of cattle due to the promotion of hybridization with cattle by the ranchers in the late 1800's and early 1900's (Hedrick 2009).

iv. Defining sites and genotypes for reintroduction

The importance to manage a species as a whole is becoming a more and more practical solution to ensure that the species as a whole do not enter an extinction vortex. Managing populations in correlation to others can save a species as a whole. A study done by Flagstad *et al.* (2000) on Swayne's Hartebeest (*Alcelaphus buselaphus swaynei*) suggested with the aid of mitochondrial (D-loop) and nuclear (microsatellite) variability, that

translocation of animals for enhancement of population size as well as genetic variation in one of the populations should be considered. The species survives in only five relict populations. With the studies they performed important managing decisions could be made.

v. Estimating population size and sex ratios

Population sizes and sex ratios can be determined by using genetic techniques. This kind of information gives the owner or the wildlife manager the reference to make important management decisions. These include controlling population size and setup. Knowing this helps the manager of a game farm or ranch the benefit of knowing the ratio of males to females. This in turn helps them with the decision making for introduction of more animals to this population (Young 1984).

vi. Establishing parentage: pedigree analysis

Parentage/pedigree genetic studies are important in everyday life in the livestock environment as well as the equine environment where horse racing have become a very genetically orientated sport. Pedigree/parentage analysis in the current study can be used to confirm source populations of the founder populations.

vii. Understanding population connectivity

Understanding the phylogeographic processes affecting endangered species is crucial both to interpreting their evolutionary history and to the establishment of conservation strategies. As in a study done by Barnett *et al.* (2006) on Lions (*Panthera leo*) they found that the 'modern' lion

currently consist of three geographic populations on the basis of their recent evolutionary history. This information should be taken into consideration for future conservation strategies. By understanding how these populations are interlinked important management decisions could be made. A very good example of successful wildlife restoration is that of the White-tailed deer (*Odocoileus virginianus*) in Kentucky. Through intensive restocking efforts since the 1940's the population in Kentucky has been successfully restored (Doemer *et al*. 2005).

viii. Use in the management of captive populations and increasing the reproductive capacity of animals

The Sable antelope (*Hippotragus niger*) and disease free Buffalo (*Syncerus caffer*) are good examples of where conservation genetics are important. Because of the monetary value of these species more and more game farms are starting breeding programmes with these species. These species are then specifically bred with by selecting for specific traits and also increased reproductivity. The high selection pressure on these species makes it very important that decisions are made carefully on which traits to select for. Selection of which individual animal breeds with which other individual are being done more and more by game farmers. A specific selection for an increased reproductive fitness is done by introducing genetics into conservation biology.

g. Molecular techniques

Methods based on microsatellite DNA regions' allozyme diversity and mitochondrial DNA control region sequencing are useful in determining the

genetic diversity within and between populations (Grobler *et al*. 2005a). In a study done by Grobler (2006) on 14 southern African mammal species, the accuracy of allozymes as indicators of genomic DNA variability were confirmed although it is not the method of choice anymore. Due to the sampling nature and type samples used, microsatellites were used as the preferred method. Microsatellites have both positive and negative attributes. Microsatellites are short segments of DNA in which a specific motif of 1-6 bases is repeated up to a usual maximum of approximately 60. Due to their exceptional variability and relative ease of scoring microsatellites are generally considered the most powerful genetic marker (Dib *et al*. 1996). It is typical to observe loci with more than 10 alleles and heterozygosities above 0.60, even in relatively small samples (Bowcock *et al*. 1994, Deka *et al*. 1995), while certain loci can be considerably more variable (Primmer *et al*. 1996). In addition to being highly variable, microsatellites are also densely distributed throughout eukaryotic genomes, making them the preferred marker for very-high resolution genetic mapping (Dib *et* al. 1996, Dietrich *et al*. 1996). Microsatellites have rapidly replaced RFLPs in most applications in population biology, from identifying relatives to inferring demographic parameters (Bowcock *et al*. 1994, Goldstein *et al*. 1996, Jame & Lagoda 1996). Part of the appeal of microsatellites over RFLPs and RAPDs is that the genetic basis of microsatellite variability is readily apparent: unique primers amplify a genomic region including a well-defined repeat structure that is responsible for the observed variation. This allows the development of inferential methods based on explicit models of microsatellite evolution (Slatkin 1995a,b; Goldstein *et al*. 1995a,b; Goldstein *et al.* 1996; Feldman *et al.* 1996; Pollock *et al.* 1996). These

advantages suggest that microsatellites will enjoy a lengthy reign in population studies.

Microsatellite loci are standard genetic markers for population genetic analysis, whereas single nucleotide polymorphisms (SNPs) are more recent tools that require assessment of neutrality and appropriate use in population genetics. Single nucleotide polymorphisms are single base substitutions found at a single genomic locus. In a study done by Coates *et al*. (2009) on the western corn rootworm (*Diabrotica virgifera virgifera*) to compare between SNPs and microsatellite markers for population genetic analysis they came to the conclusion that SNP marker loci are viable tools for characterization of natural populations. Analyses of SNP and microsatellite marker data resulted in similar conclusions with respect to population structure. Single nucleotide polymorphism marker loci provided a higher estimate of F_{st} that may reflect the lack of systematic downward bias due to numerous alleles and possibly the lower reversion rate of substitution mutations compared with microsatellite repeats. Because SNP marker loci are less susceptible to these effects, they may provide better estimates of F_{st}.

One perceived difficulty with microsatellites is the long lead time in identifying and characterising microsatellites in new taxonomic groups. This problem is partially alleviated, however, by the continuing popularity of microsatellites in genetic mapping. Dense microsatellite maps are now available in nearly all organisms of genetic and/or economic interest including humans, mice, fruit flies, cows, sheep, chickens, pigs, tomatoes, soybeans, rice, etc. (Xiao *et al*. 1994, Akkaya *et al*. 1995, Crawford *et al*. 1995, Goldstein & Clark 1995, Broun & Tanksley 1996, Crooijmans *et al*. 1996, Dib *et al*. 1996, Dietrich *et al*. 1996, Postlethwait *et al*. 1994, Rohrer

et al. 1996, Su & Willems 1996, Taramino & Tingey 1996). This allows us to use microsatellites across species boundaries. Various studies have been successfully executed by using cross-species amplification of bovid microsatellites. For example Ntie *et al*. (2010) successfully amplified 16 bovid microsatellites in six species of Central African duikers. Beja-Pereira *et al*. (2004) found 20 polymorphic markers that amplified well in two threatened species of ungulates (*Gazella dorcas* & *Ammotragus lervia*) and will facilitate conservation and genetic studies in these two species, and promise to be widely useful across divergent ungulate taxa.

h. Aims of the current study

The following outcomes are envisaged for the current study:

a) To determine the level of genetic diversity (heterozygosity, allele richness, and number of unique alleles) in each of the five Gemsbok (*O. gazella*) populations, and thus investigate the possible effect of the founder effect on small Gemsbok populations.
b) Determine the structural genetic grouping of the populations included in the study, by studying at the current patterns of differentiation.
c) To identify suitable microsatellite loci to quantify genetic diversity and differentiation in Gemsbok.

The work presented in this dissertation contributed significantly (±60%) to a manuscript accepted for publication in the internationally accredited scientific journal Mammalian Biology (Elsevier). The details of the article follow:

Osmers B., Petersen B.S., Hartl G.B., Grobler J.P., Kotze A., Van Aswegen E. and Zachos F.E. (2012) Genetic analysis of southern African gemsbok (*Oryx gazella*) reveals high variability, distinct lineages and strong divergence from the East African *Oryx beisa*. *Mammalian Biology* **77 (1),** 60-66.

Materials and Methods

a. Populations and Sampling

The populations sampled during this study consisted of small isolated populations and populations derived from large free-ranging populations. Specifically, the populations sampled were as follows (See also Figure 4 and Table 3):

(i) Otjiwarongo area (Namibia). This population numbers approximately 6,500 animals that are descendent from gemsbok occurring naturally in the area.

(ii) Cohen Game Farm, Limpopo Province, South Africa. An introduced population, founded from (i) above. In 2010, this population numbered 70 animals, descendent from eight founders.

(iii) Tempelhof Game Farm (n = 11), Limpopo Province, South Africa. A second introduced population, founded from (i) above. Records on the exact size and year of introduction of the founder populations are not available, but it is known that the population was founded with less than 10 animals and numbered 55 by 2010.

(iv) STS Kalahari Game Ranch, Northern Cape Province, South Africa. This population numbers approximately 1,000 animals, descendent from naturally occurring gemsbok in the area.

(v) Elias Game Farm, Northern Cape Province, South Africa. An isolated population with an approximate population size of 35 animals. Specific details on the year of introduction and founder size are unknown.

Due to the logistic, financial and time constrains of sampling animals specifically for this project, alternative methods (compared to darting or other forms of physical handling of animals) were sought to sample statistically significant numbers of individuals from these populations. For this study coordination with hunting activities was therefore used as a sampling method, because hunting activities were already happening on these areas. Sampling kits were thus delivered to farm owners in the Northern Cape and Namibia. The individual farm owners them completed the sampling during the hunting season. A pre-prepared sampling kit was sent to each farm owner and the samples were collected when animals were randomly hunted (See Appendix A).

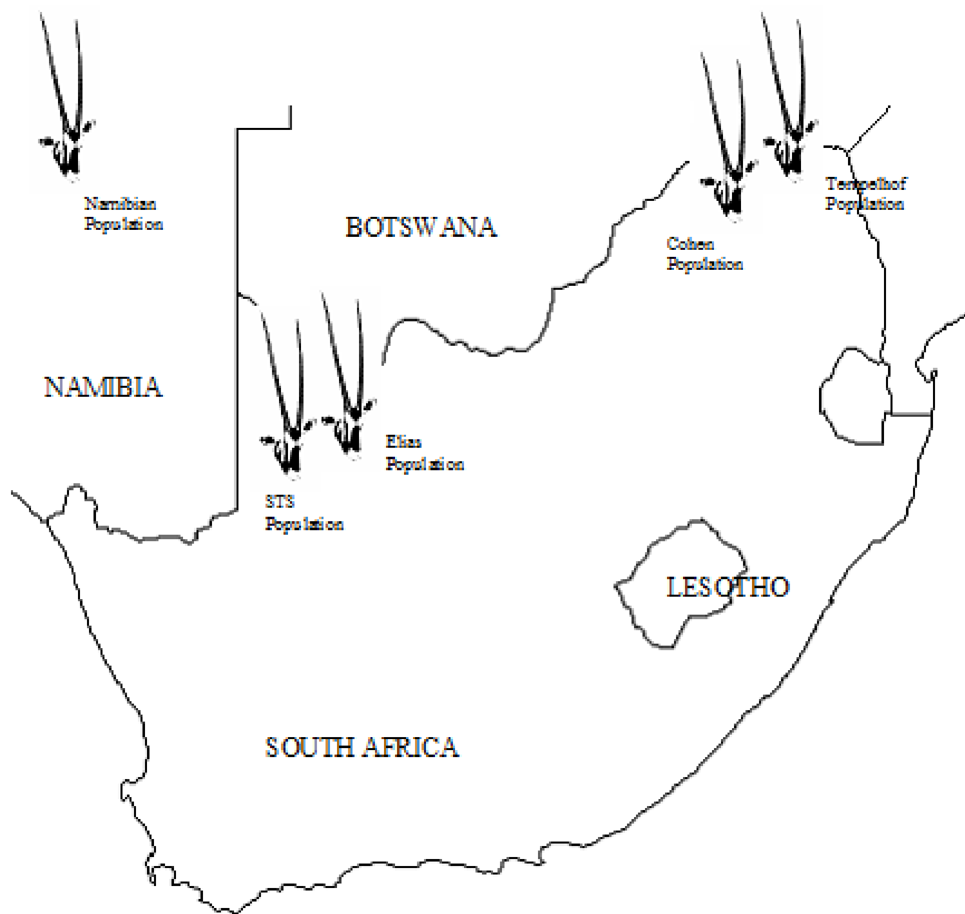

Figure 4 – Geographical location of the five *O. gazella* populations sampled during this study.

Sampling kits (see appendix) contained sample tubes (n = 20) that were filled with 90% ethanol. Other components of the kit were pencil, labels and a measuring tape to measure horn length.

Table 3 – Current population size, origin and period of isolation of each of the populations sampled.

Population	# Samples	Population Size (n) (with period since founding in brackets)	District
Cohen	15	70 (9 years)	Musina
Tempelhof	11	55 (10 years)	Musina
STS Kalahari Game Ranch	20	1,000 (Natural)	Askham
Askham	9	35 (8 years)	Askham
Namibian (Source)	20	6,500 (Natural)	Otjiwarongo

b. Genetic Analyses

i. DNA Isolation

Nucleic acids were isolated from tissue samples with the aid of the High Pure PCR Template Preparation Kit from Roche Applied Science ©. The procedure followed was as follows:

- A sample of the gemsbok tissue of about 40 mg was cut into smaller segments and then mixed with tissue lysis buffer and the enzyme proteinase K. It was then immediately mixed and incubated overnight at 55°C. The long incubation period allowed for total digestion of the tissue samples.
- A binding buffer was added, the solution mixed, and the sample incubated at 70°C for 10 minutes.

- Isopropanol was added and the entire content was emptied into a filter tube, which fits into a collection tube, and was then centrifuged for 1 minute at 8,000 rpm.
- The collection tube was discarded and the filter tube was placed in a new collection tube. An Inhibitor Removal Buffer was added and the filter and collection tube combination was centrifuged again for 1 minute at 8,000 rpm.
- The flow-through and collection tube was discarded and a new collection tube was used. A wash buffer was added and the mixture was centrifuged for 1 minute at 8,000 rpm. This process was repeated twice.
- The flow-through was then discarded and within the same collection tube centrifuged at maximum speed (14,000 rpm) for 10 seconds to remove any wash buffer still present.
- The collection tube was then discarded. The filter tube was placed in a new 1.5ml reaction tube. Prewarmed ($70^{o}C$) Elution Buffer was added to the filter tube, and centrifuged for 1 minute at 8,000 rpm.
- The filter tube was discarded. The remaining microfuge (Eppendorf) tube then contained the eluted DNA.

After completion of the extractions, 1 µl droplets of the extracted DNA were quantified on a NanoDrop® ND 1000 Spectrophotometer to determine the DNA concentrations at a wavelength of 230 nm. Dilution factors were then

calculated to uniformly standardize all the DNA samples to a concentration of 25 ng/µl.

ii. Fragment (Microsatellite) Analysis

According to Grobler *et al.* (2005b) no specific microsatellite markers have been published for most South African antelope species, but cross-species application of primers between taxonomically close groups is well accepted. Gemsbok belongs to the Bovidae, and primers developed for commercial purposes in the bovine group were tested for expression in gemsbok during this study. The following five primers were used: BMS1237; MAF46; OARFC304; OARHH64; ETH225. These primers gave promising results during an unpublished study by Osmers (2005) on *O. gazella* samples. A further three loci were screened at the Zoologisches Institut, Christian–Albrechts–Universität, Kiel, Germany, namely: RBP3; MAF50 and HDZ8 (Table 4). All the forward primers were fluorescently labeled with 6-FAM. ABI 377 and ABI 310 sequencers were used to resolve microsatellite fragments. Genotyper (ABI©) and Genemarker (SoftGenetics 2007) software were used to visualize the fragments.

Table 4 – Primer sequences for the eight microsatellite loci used in Gemsbok, with the relevant annealing temperatures (T_a) (All primers are listed with the forward primers first followed by the reverse primer; 5' – 3')

Primer	Sequences (5' - 3')	T_a (°C)	Source
BMS1237	GTTTTCACTAGCACCCTGTGG CCCAGTTAACCCTAGAGTCGG	60	Stone *et al*, 1995
ETH225	GATCACCTTGCCACTATTTCCT TCATCGTCGACCGACAGTACA	60	Steffen *et al*, 1993
MAF46	AAATACCCTATAAGGCACAGTACCAC CACCATGGCCACCTGGAATCAGG	60	Swarbrick *et al*, 1992
OarHH64	CGTTCCCTCACTATGGAAAGTTATATATGC CACTCTATTGTAAGAATTTGAATGAGAGC	60	Henry *et al*, 1993
OarFCB304	CCCTAGGAGCTTTCAATAAAGAATCGG CGCTGCTGTCAACTGGGTCAGGG	60	Buchanan & Crawford, 1993
RBP3	TGTATGATCACCTTCTATGCTTC GCTTTAGGTAATCATCAGATAGC	45	MacHugh *et al,* 1997
MAF50	GTAGACTACTCATGAAAATCAGGTCTTAGG GGGACATGCAGCTATACACTTGAG	49	Swarbrick *et al,* 1992
HDZ8	GACAAACACTCAGAAGGCAAAG GGTGGCAGGACTGAGCAAG	52	Huebinger *et al,* 2006

iii. Microsatellite Primer Preparation

Newly synthesized primers were hydrated overnight by the amounts of water (ddH_2O) specified by the manufacturer to achieve a concentration of 100 µM. Because of the light sensitivity of labels, the primers were kept covered in aluminium foil and in a dark place. To create a working solution 20 µl of primer were added to 180 µl of ddH_20 to provide a working solution of 10 µM.

Reaction mixtures for microsatellites contained the following:

- DNA (2ul), which was standardized at 25 ng/μl
- Reaction Buffer (1x) with 15 mM $MgCl_2$ (no further $MgCl_2$ were added)
- The dNTP mix containing 5mM of each dNTP (dATP; dCTP; dGTP; dTTP) at pH 7.0. The stock dNTP concentration was 20 mM, and a working solution with a concentration of 2mM was created.
- Taq polymerase (Super-therm Gold DNA polymerase) with a stock concentration of 5 U/μl.
- Forward and reverse primers, with a base working solution of 10 μM.
- ddH2O

iv. Reaction Conditions

The PCR (Polymerase Chain Reaction) were performed on the GeneAmp PCR System 9600 by Applied Biosystems. PCR reactions were conducted as follows:

The samples went through the following cycles:

1. 94°C for 10 minutes - denaturation
2. 95°C for 30 seconds - denaturation

   60°C for 30 seconds - annealing

   72°C for 60 seconds - extention
3. 72°C for 60 minutes – final Extention
4. Hold at 4°C

a. Statistical Analyses

i. Genetic Diversity

Heterozygosity and proportion of polymorphic loci were applied as measures of diversity during the current study. Heterozygosity is used to inform researchers about the genetic status of populations; specifically, the heterozygosity values increase proportional to the level of genetic diversity in a population. It is therefore a widely used measure to compare genetic variation between various populations of the same species. Expected heterozygosity is the (expected) probability that an individual will be heterozygous at a given locus. Expected heterozygosity (H_e) (Nei 1987) is also known as gene diversity and is calculated as 1.0 minus the sum of the squared gene frequencies.

Proportion of polymorphic loci or polymorphism (P) and the number of alleles per locus (A) are additional indicators of the genetic diversity in populations. A higher proportion of polymorphic loci percentage is seen as a biologically significant indication of retained diversity, since it suggests retained diversity at a range of loci, rather than high values at one or two loci.

Expected heterozygosity, and P and A were calculated using POPGENE (Version 1.32) (Yeh *et al* 1997).

ii. Hardy-Weinberg Equilibrium

Hardy-Weinberg equilibrium refers to a situation where allele frequencies and genotypic frequencies remain constant from one generation to the next, and it suggests that certain assumptions (Freeman & Herron 2004) are met in the specific population:

- There must be no mutation, because mutation can alter allele frequencies.
- There must be no migration, because individuals migrating in and out of the population can cause the infusion of alleles or the loss of alleles in the migrants, and can thus alter allele frequencies in the population.
- Individuals must mate at random with respect to genotype. Non-random mating tends to favour either homozygotes or heterozygotes and in the end disrupt the equilibrium.
- There must be no selection, because selection for or against an allele may increase or decrease the frequency of that allele.
- The population must be infinitely large to counter the effects of sampling error.

In practise, very few populations are in Hardy-Weinberg equilibrium. In most real populations, each gene naturally mutates at a very low rate, migrants often move in and out of natural populations, no population is infinitely large, random mating does not always take place and selection (natural or artificial) commonly takes place.

Nevertheless, the relative degree to which populations conform to Hardy-Weinberg expectations can provide valuable insight into the relative magnitude of population genetic processes in each population. Conformation to expected Hardy-Weinberg equilibrium in Gemsbok was determined by using a Chi-square test, as implemented in the PopGene Software package.

iii. Testing for population bottlenecks

Bottleneck Software (Plenert 1993) was used to test for the signature of bottlenecks in the populations studied. Two mutation models, namely the Infinite Alleles Model (IAM) and the Stepwise Mutation Model (SMM), were used. The two methods used by the Bottleneck software are based on the following assumptions:

- The Infinite Alleles Model (IAM)

  The IAM follows the assumption that each mutation is unique and that mutations are not reversible, it means that two alleles identical in state must also be identical by decent.

- Stepwise Mutation Model (SMM)

  This model describes that when microsatellites mutate, they only gain or lose one repeat (i.e. they mutate in a step-wise fashion). This suggests that two alleles that differ by one repeat are more closely related than alleles that differ by many repeats. This model is satisfactory in calculating relatedness but there is the problem of homoplasy. Homoplasy refers to

the phenomenon when to alleles are identical in state but not identical by descent, this could be due to convergent evolution, parallelism or reversal (Freeman & Herron 2004).

iv. Genetic drift/differentiation

The F_{st} and R_{st} coefficients are commonly used as indicators of relatedness. F_{st} (Fixation index) was developed by Sewal Wright in the 1920's and is still widely used (Freeman & Herron 2004). It is a measure of population differentiation and genetic distance, based on genetic polymorphism data such as single-nucleotide polymorphisms (SNPs) or microsatellites. The R_{st} statistic is very similar to F_{st} but is based on the stepwise mutation model. Alleles are therefore closer related if they differ by a smaller number of repeats. In this study, F_{st} values were calculated by using Arlequin Software (Excoffier & Schneider 2005), whereas R_{st} values were calculated using RSTCalc by Goodman (1996).

Analysis of Molecular Variance (AMOVA) is a method of estimating population differentiation that gives a hierarchical distribution of overall diversity (Excoffier *et al*. 1992). Specifically, the relative contributions of different levels of diversity within and between taxa are quantified.

Bonferroni (Abdi 2007) corrections were made throughout the study. Since numerous pairwise tests were performed on the data set, the likelihood of a Type I error increased. This occurs when the null hypothesis is rejected when it is in fact true. To prevent this kind of error, the P-value is lowered. This makes the test more stringent, with

fewer expected errors, but it may also make it harder to detect real effects.

v. Assignment tests

The rationale behind assignment tests is to use individual genotypes to assign individuals to populations or clusters without prior knowledge on population of origin or population boundaries. Paetkau *et al*. (1995) developed the first assignment test approach. The principle of an assignment test is as follows: given a set of populations, and the allele frequencies of those populations, what is the likelihood of a given individual's genotype in the population in which it was sampled versus its likelihood in the other populations in the set? An individual is assigned to the population for which it has the highest likelihood. In the current study, a fully Bayesian method (Pritchard *et al*. 2000) was used to identify populations (clusters) and assign individuals using a probability approach to each cluster (using STRUCTURE software – Pritchard *et al.* (2000) and Falush *et al.* (2003)). The parameter ln Pr(X\K) was calculated for K values (number of populations) of 1-5, with five independent runs for each K, to estimate the true number of populations. All runs consisted of a burn-in period of 10,000 steps, followed by a MCMC simulation of 100,000 iterations.

Genetic distances (Nei, 1972) between the Gemsbok populations were calculated with the aid of PopGene Software. A dendrogram indicating the clustering of groups was then constructed from the genetic distance values with the aid of the PopGene Software.

Results

Sampling resulted in the accumulated number of 75 samples, 15 samples from the population on Cohen Game Farm, 11 samples from Tempelhof Game Farm, 20 samples from the ancestral population in Namibia, 9 samples from the farm Elias near Askham, and 20 samples from STS Kalahari Game Ranch, also near Askham. The allele frequencies of these populations at eight microsatellite loci are presented in Table 5. All the loci showed allelic polymorphism in all the different populations, except for the Elias population at the OARHH64 locus. This can however be purely the result of sampling error, due to the small sample size for this population.

Table 5 – Allelic Richness values for all five populations (Cohen, Namibia, Tempelhof, STS and Elias)

Population	Allelic Richness	Standard Deviation
Cohen	3.890	1.696
Namibia	3.878	2.071
Tempelhof	3.292	1.277
STS	2.745	1.397
Elias	3.205	1.406

The allelic richness of the populations were determined and the Cohen (3.890 ± 1.696) and Namibian (3.878 ± 2.071) populations had the highest values (Table 5). The STS population showed a low allelic richness of 2.745 ± 1.397 alleles per locus.

Expected heterozygosity values indicated that the Cohen population had the highest expected heterozygosity, H_e = 0.595±0.247 (Table 6). The expected heterozygosity values (Table 6) for the remaining populations were: Namibian population = 0.566±0.260, Tempelhof = 0.571±0.158, Elias = 0.452±0.238, STS = 0.547±0.263.

The average number of alleles per locus in each population was: Namibian Population = 5.125±3.182, Tempelhof = 3.75±1.669, Cohen = 4.75±2.252, Elias = 3.5± 1.604 and STS = 5.625±2.615 (Table 6).

Genetic distance indicated the greatest similarity between the Cohen and Namibian population as seen in Tables 12 and 13. The greatest genetic distance was observed between the STS and Tempelhof Populations.

Table **6** – Allele frequencies of five populations and 75 samples of *Oryx gazella* at eight loci. Highlighted allele frequencies are unique alleles for that specific population.

		Population				
Locus	**Allele**	**Namibia (n=20)**	**Tempelhof (n=11)**	**Cohen (n=15)**	**Elias (n=9)**	**STS (n=20)**
BMS1237	**251**	0.059		0.091		
	253	**0.059**				
	255	0.147	0.273	0.227	0.056	0.028
	257	0.088		0.046	0.111	0.139
	261	0.059	0.318	0.227		
	262	**0.029**				
	263	0.118	0.136	0.273	0.556	0.444
	265				0.111	0.167
	267	0.059			0.056	
	269					**0.028**
	271	0.206	0.227	0.046		
	273	0.088				0.167
	275	0.029	0.046	0.091		
	277	0.059				0.028
	279				**0.056**	
MAF46	**89**	0.083	0.182		0.167	0.111

		Population				
Locus	**Allele**	**Namibia (n=20)**	**Tempelhof (n=11)**	**Cohen (n=15)**	**Elias (n=9)**	**STS (n=20)**
	103	0.306	0.182	0.227		
	105	0.361	0.182	0.227		0.028
	107	0.167	0.273	0.227	0.500	0.667
	109		0.046	0.046	0.222	0.111
	111	0.083	0.136	0.227	0.111	0.028
	113					**0.056**
	115			**0.046**		
OARFC304	**113**	0.750	0.727	0.875	0.722	0.650
	115	0.250	0.273	0.125	0.167	0.225
	117				0.111	0.125
OARHH64	**106**	0.118	0.455	0.417		0.031
	108	0.029		0.167		
	110	0.824	0.455	0.417	1.000	0.969
	112	**0.029**				
ETH225	**136**	0.611	0.727	0.583	0.611	0.237
	138	0.361	0.273	0.417		
	140	0.028			0.111	0.237
	142				0.222	0.237
	144				0.056	0.263
	148					**0.026**
RBP3	**123**		0.056		0.278	0.100
	125					**0.025**
	127	0.025			0.056	0.100
	129	0.200	0.444	0.071		0.025
	131			**0.036**		
	141	0.125	0.111	0.143		0.025
	143	0.125		0.250		0.400
	145	0.325	0.333	0.357	0.667	0.275

Locus	Allele	Population Namibia (n=20)	Tempelhof (n=11)	Cohen (n=15)	Elias (n=9)	STS (n=20)
	149	0.125	0.056	0.036		0.025
	151	0.075		0.107		
	157					**0.025**
MAF50	**128**		0.125	0.036		
	148				**0.111**	
	152					**0.025**
	154	0.050	0.188			0.050
	156	0.900	0.688	0.857	0.889	0.850
	158	0.050		0.036		0.075
	168			**0.071**		
HDZ8	**145**			0.071		0.050
	147				0.056	0.175
	149		0.556	0.107		
	151	0.100		0.036		0.050
	153	0.275	0.056	0.286	0.556	0.050
	155				0.167	0.300
	157					**0.025**
	159	0.375	0.611	0.143	0.167	0.300
	161	0.100		0.143		0.025
	163	0.150	0.056	0.214	0.056	0.025
	169		**0.222**			
Nei's Expected Heterozygosity (1973)		0.566 ± 0.256	0.571 ± 0.158	0.595 ± 0.247	0.452 ± 0.238	0.547 ± 0.263
% Polymorphic loci		100	100	100	87.5	100
Average number of alleles per locus		5.125 ± 3.182	3.75 ± 1.669	4.75 ± 2.252	3.5 ± 1.604	5.625 ± 2.615

Table 7 – Chi square test probability values for each locus at each population to determine whether Hardy-Weinberg equilibrium was maintained. Shadowed loci showed P-values below 0.05 and these were consequently not in Hardy-Weinberg equilibrium

	Locus								
Population	BMS1237	MAF46	OARFC304	OARHH64	ETH225	RBP3	MAF50	HDZ8	# Alleles**
Namibia	0.000	0.104	0.212	0.000	0.418	0.436	0.000	0.141	5
Tempelhof	0.202	0.042	0.264	0.009	0.655	0.301	0.000	0.916	5
Cohen	0.203	0.139	0.692	0.000	0.959	0.001	1.000	0.852	6
Elias	0.474	0.001	0.795	Monomorphic	0.071	0.611	0.796	0.702	6
STS	0.000	0.016	0.298	1.000	0.001	0.000	0.000	0.570	3

** Number of loci diverging significantly from Hardy-Weinberg Equilibrium

Conformation to expected Hardy-Weinberg equilibrium is indicated in Table 7. In this table, the highlighted loci are considered to deviate significantly from equilibrium and these are thus considered not to meet H-W equilibrium based on the P-values indicated.

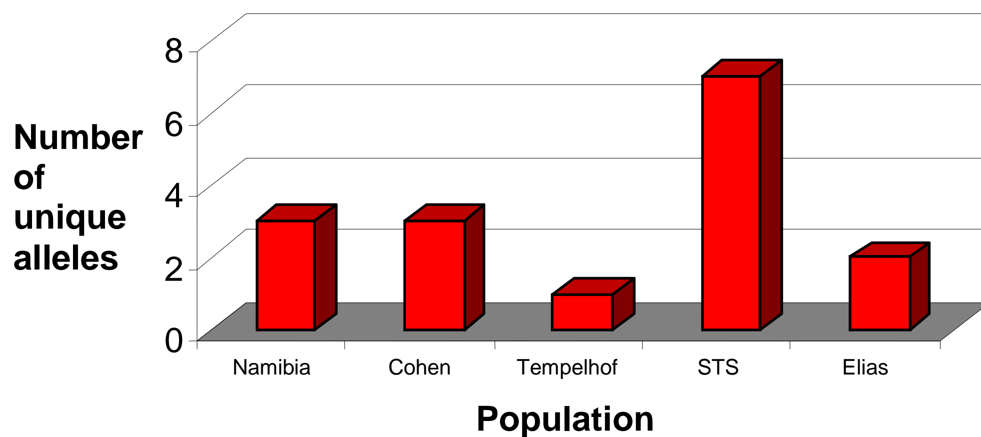

Figure 5 – The number of unique alleles for each population, from allele frequencies in Table 2.

Table 8 – Heterozygosity values obtained from the analysis by using the Bottleneck software

	Population				
Locus	**Namibia (n=20)**	**Tempelhof (n=11)**	**Cohen (n=15)**	**Elias (n=9)**	**STS (n=20)**
BMS1237	0.914	0.788	0.840	0.693	0.746
MAF46	0.756	0.844	0.827	0.699	0.541
OARFC304	0.386	0.416	0.228	0.464	0.524
OARHH64	0.316	0.519	0.652	0.000	0.063
ETH225	0.510	0.416	0.507	0.595	0.782
RBP3	0.822	0.660	0.799	0.503	0.760
MAF50	0.190	0.508	0.267	0.209	0.276
HDZ8	0.760	0.601	0.844	0.667	0.800
$\mathbf{H_e}$	0.582 ± 0.267	0.594 ± 0.161	0.621 ± 0.258	0.479 ± 0.252	0.562 ± 0.270

Table 9 – Wilcoxon test results to indicate probability (Two tails for heterozygote excess and deficiency) using the Infinite Alleles Model (I.A.M.) and the Stepwise Mutation Model (S.M.M.)

	Mutation Model	
Population	**I.A.M.**	**S.M.M.**
Namibia	0.641	0.742
Cohen	0.250	0.547
Tempelhof	0.020	0.383
STS	0.742	0.020
Elias	0.469	0.023

The results from the Bottleneck © software indicated population bottlenecks for the Tempelhof ($P = 0.020 < P = 0.05$) under the I.A.M. and for the STS ($P = 0.020 < P = 0.05$) and the Elias ($P = 0.023 < P = 0.05$) populations under the S.M.M. (Table 9). All three of these populations where the null hypothesis is not rejected thus show the signature of a population bottleneck. Conversely, the Cohen population did not undergo a population bottleneck based on Bottleneck output.

Table 10 – Differentiation among population pairs, based on Fst

	Namibia	**Cohen**	**Tempelhof**	**STS**	**Elias**
Namibia	*				
Cohen	0.023	*			
Tempelhof	0.057	0.068	*		
STS	0.097	0.108	0.154	*	
Elias	0.095	0.102	0.157	0.064	*

F_{ST} values indicated that there were high similarity between the Cohen population and the Namibian population, with $F_{st} = 0.02297$. The expected results were that there was much more similarity between the Cohen and the Namibian population because of the relatedness (Table 10). The highest genetic distance value was observed between the Tempelhof and Elias populations (Table 14).

Table 11 – Probability values calculated for the high number of pairwise comparisons to calculate F_{st} values (P-value > 0.03, considered significant).

	Namibia	Cohen	Tempelhof	STS	Elias
Namibia	*				
Cohen	0.171	*			
Tempelhof	0.009	0	*		
STS	0	0	0	*	
Elias	0	0	0	0.009	*

The high number of pairwise comparisons to calculate the F_{st} values required a Bonferroni correction in that the critical P-value of 0.05 was lowered to 0.03. From the results that were then obtained (Table 11) the only pairwise comparison that were higher than the critical P = 0.03 were between the Cohen and Namibian populations, thus this comparison were the only one that was rejected, or where differentiation was considered significantly different from zero.

Table 12 – Gene flow (Nm) values and P-values (Above diagonal) obtained from the RSTCalc software. Unbiased estimates of Slatkin's Rst genetic distance values (Below diagonal) calculated with RSTCalc software (Goodman 1996)

Population	Namibia	Cohen	Tempelhof	STS	Elias
Namibia	*	0.076	0.087	0.162	0.028
	3.042				
Cohen	P = 0.009	*	0.078	0.228	0.140
	2.625	2.976			
Tempelhof	P = 0.016	P = 0.057	*	0.227	0.123
	1.296	0.845	0.853		
STS	P = 0.000	P = 0.000	P = 0.000	*	0.028
	8.569	1.536	1.780	8.641	
Elias	P = 0.171	P = 0.002	P = 0.015	P = 0.157	*

R_{st} values (Table 12) that were considered to be statistically significant after performing a Bonferroni correction (Abdi 2007), by lowering the critical P-value of P = 0.05 to P = 0.003, were the following: between the STS and Namibia population R_{st} = 0.087; STS and Cohen R_{st} = 0.228; STS and Tempelhof R_{st} = 0.227; Elias and Cohen R_{st} = 0.140. There is more similarity between the STS and Namibia populations than between the STS and Cohen Populations. The data observed here result in the same grouping as seen in previous sections. Only four values can be considered statistically significant and can be used to proof a hypothesis: Between the STS and Namibia populations Nm = 1.296; STS and Cohen Nm = 0.845; STS and Tempelhof Nm = 0.853; Elias and Cohen Nm = 1.780 (Table 12).

Table 13 – Nei's Original Measures of Genetic Identity (above diagonal) and Genetic distance (below diagonal). (Nei 1972)

Population	Namibia	Tempelhof	Cohen	Elias	STS
Namibia	***	0.890	0.909	0.826	0.782
Tempelhof	0.117	***	0.861	0.729	0.688
Cohen	0.096	0.149	***	0.780	0.719
Elias	0.19	0.316	0.248	***	0.881
STS	0.246	0.374	0.330	0.127	***

Table 14 – Nei's unbiased measures of genetic identity (above diagonal) & genetic distance (below diagonal). (Nei 1978)

Population	Namibia	Tempelhof	Cohen	Elias	STS
Namibia	***	0.914	0.931	0.844	0.796
Tempelhof	0.090	***	0.890	0.750	0.705
Cohen	0.071	0.117	***	0.802	0.736
Elias	0.170	0.287	0.221	***	0.899
STS	0.228	0.349	0.307	0.107	***

Genetic distance and genetic identity measures (Table 13 & 14) indicated corresponding results, indicating that the Cohen and Namibian populations were the most closely related populations, with genetic distance values of 0.071 (Table 14) and 0.096 (Table 13).

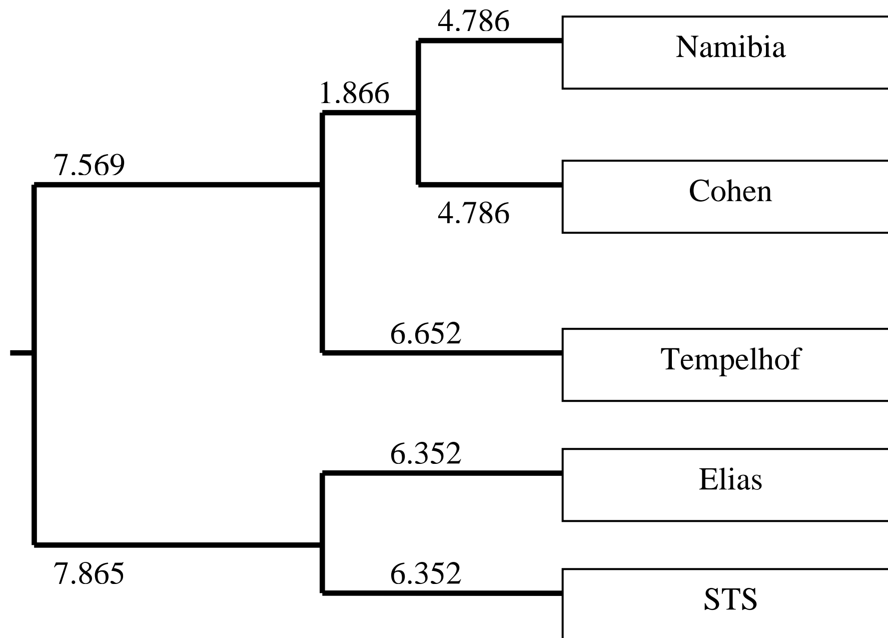

Figure 6 – Dendrogram indicating the clustering of the populations according to genetic distance, including "Bootstrap values".

The dendrogram presented in Figure 6 resulted in groupings that reflect known population histories, with the Namibian, Cohen and Tempelhof showing the closest similarity and relatedness, and the Elias and STS populations more related. The patterns of genetic distance thus reflect the actual geographic distribution of populations. Overall, the Cohen and Namibian populations are the most closely related populations.

Table 15 – True population structure: the probability for 1-5 real genetic clusters: (as -Ln probability)

	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD
K=1	-1491.1	-1494.9	-1496.2	-1495.2	-1493.3	-1494.1	1.99
K=2	-1342.3	-1342.7	-1342.4	-1342.2	-1343.2	-1342.6	0.40
K=3	-1353.2	-1363.4	-1349.5	-1346	-1348.6	-1352.1	6.80
K=4	-1392.1	-1413	-1405.1	-1400.4	-1392.5	-1400.6	8.83
K=5	-1415.8	-1586.1	-1417.3	-1444	-1423.7	-1457.4	72.83

Table 16 – Proportion of membership of each pre-defined population in each of the two clusters from a fully Bayesian clustering approach, following Pritchard *et al.* (2000)

	Inferrred Clusters	
Population	**Cluster 1**	**Cluster 2**
Namibia	0.935	0.065
Cohen	0.979	0.021
Tempelhof	0.963	0.037
STS	0.048	0.952
Elias	0.095	0.905

Calculation of the posterior probabilities of K showed the highest probability for a real structure consisting of two *O.gazella* populations (Table 16). After finding that there was two true clusters another simulation was run with a burn-in of 10,000 steps and followed by 200,000 true steps. The output can be seen in Table 16.

From the results in Table 16 two clusters can be clearly seen namely the Namibian (93.5% assigned to the first cluster), Cohen (97.9%) and Tempelhof (96.3%) cluster and then the STS (95.2% assigned to the second cluster) and Elias (90.5%) cluster.

The estimated membership coefficients for all individuals were also clustered into a bar plot (Figure 7).

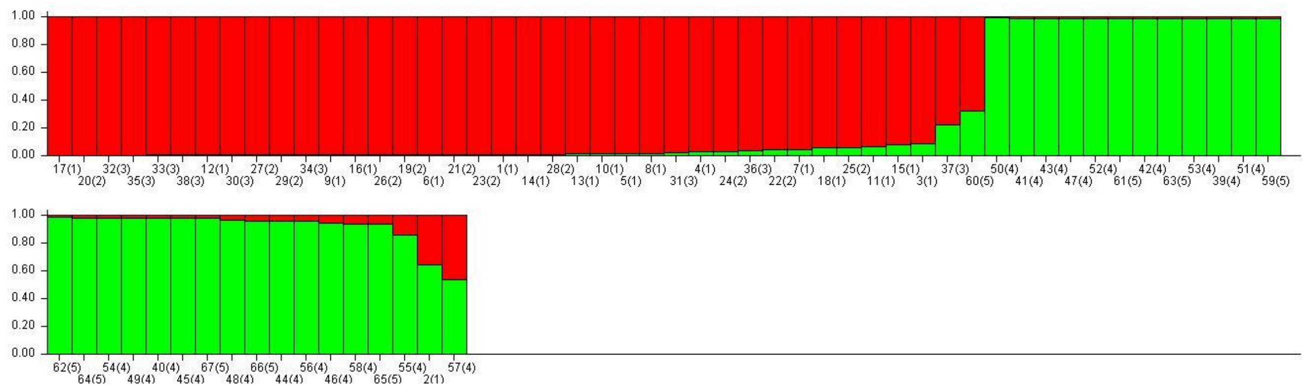

Figure 7 – Bar plot (from STRUCTURE) with two clusters of *O.gazella*. Each individual is represented by a single vertical line broken into 1-2 coloured segments, with lengths proportional to each of the two inferred clusters. Abbreviations used: Sample number (Population number). Populations: 1 = Namibia; 2 = Cohen; 3 = Tempelhof; 4 = STS and 5 = Elias. Only 67 samples are seen in the bar plot, because the other individuals had missing data at more than two loci.

Discussion

The most favourable level of genetic diversity was observed in the Cohen population with a value for H_e as 0.595±0.247. The Namibian population possessed a value for H_e as 0.566±0.260. These values are sufficiently close to each other to suggest that there is in fact no real difference.

The STS and Namibian populations showed a much higher average number of alleles per locus than any of the other populations. This is most likely due to the fact that these two populations are large natural populations, or descendent from such populations without historical bottlenecks. Allelic richness values for the populations were not too low regarding the small populations (Table 5). The STS population showed a low value of 2.745±1.397 alleles per locus which is a low value. The current study did not show significant variation in the allelic richness between the Namibian population and the other populations except the STS population. Expected results were that the Cohen, STS, Tempelhof and Elias populations would have significantly lower levels of allelic richness through sampling error and the effect of the founder event.

A study done by Webley *et al*. (2004) focussed on a population of Javan Rusa Deer (*Cervus timorensis russa*), where the population introduced in Australia originated from a founder population of only seven individuals. This population resulted in average allelic richness values of 2.29±0.095 alleles per locus, whereas the source population of the introduced individuals had average allelic richness values of 7.60±0.933 alleles per locus. Results from this study therefore provided prove that there are a loss of alleles through the founder effect and inbreeding. Small populations of a particular species are commonly found on smaller game ranches, where it is almost impossible to introduce large founder populations to populate the

area (due to cost, availability and other factors). These small founder populations only contain a sample size of the entire genomic diversity of the species. Such introduced populations are thus not comparable to large natural populations that contain sufficient genetic material to withstand inbreeding and provide adaptibility. Decisions on inbreeding have thus always been an important factor in the game ranching industry. Genetic studies like the current study is thus critical in order to maintain the genetic well-being of individuals, populations and species in the wildlife industry.

Various studies have indicated that genetics affect the physical well-being of animals under both normal and harsh environmental conditions. Increase in homozygosity, i.e. inbreeding, cause animals to be less able to cope with their environment than non-inbred animals, with an increased susceptibility for diseases and a decreased ability for adaptation (Ralls *et al*. 1979). Completing studies like the current one contribute to the goal of understanding the complicated genetic structure of various species, and help to guide management decisions. Similar studies have previously assisted to let endangered species like Sable antelope (*Hippotragus niger*) recover from a near extinction occurrence.

An important concept in small and fragmented populations is random genetic drift, which indicates a change in allele frequency due to sampling error. Inbreeding results in the loss of genetic diversity (Fairbanks & Anderson 1999). The results of the current study indicated an influence that could demonstrate the effects of the founder effect. In the Tempelhof and Elias populations a great decrease in the amount of alleles at each locus was observed, whereas the Namibian, Cohen and STS populations displayed more alleles on average at each locus. In the Namibian population there are 16 alleles that are lost from the Cohen population. The loss of these alleles could be due to inbreeding or genetic drift or through ‘miss-sampling’

during the founding population (Freeman & Herron 2004). In other words, individuals introduced during the founding event just did not possess that particular allele present in their genetic make-up. In the Cohen population there are five alleles that are not present in the Namibian population; this could be the result of sampling error.

The high P-values seen in Table 6 indicate that very few natural populations are actually in Hardy-Weinberg equilibrium. Restricted population size, migration and selection play an important role in natural populations thus making it impossible to maintain Hardy-Weinberg equilibrium.

Dramatic reductions in genetic variability as an apparent consequence of isolation and the "founder effect" are known in natural populations of several organisms. It is also possible that a small isolated population reproduces and genetically differentiate from the rest of the group, by means of genetic drift (Bonneli & Selander 1974). A study done by Grobler *et al.* (1996) on African Buffalo (*Syncerus caffer*) highlighted the trend as the results found in the current study, in that small isolated populations tend to be less polymorphic.

In the Elias and the Tempelhof populations (Table 8) the effect can clearly be seen from the rapid loss of alleles. The Cohen population was introduced 27 April 1999. Effectively only 10 generations are involved, and even in this short time a rapid decrease in the amount of alleles is visible in the Cohen population.

Human interference has resulted in the fragmentation of large open habitat into small isolated pieces of land. This has resulted in a situation where populations of species are maintained in fragments, often with very small numbers. This is a consequence of the desire by farm owners to keep wide variety of animal species on farms. However, because of limited availability, habitat or cost only low numbers of

the particular species can often be accommodated. Because of this phenomenon processes like inbreeding should be kept in mind.

To counter the effect of inbreeding, a system should be in place to know the exact origin of animals (founder populations), and to know when and from where a population of animals should receive new genetic material. This is necessary to maintain the genetic diversity and decrease the risk of inbreeding but also outbreeding depression. To quantify such values is complex. It is therefore essential to determine genetic characteristics of species using appropriate molecular and statistical approaches, and also to make the founder population as large as possible. In this study the founder population only started with eight individuals in the case of Cohen population. Eight individuals is not a number that will constitute an ideal founder population, because the effective population size from this group is probably even lower.

Minimum viable population size has become a very important factor with the introduction of a new population, because with a decrease in population size an increased number of problems of demographic and genetic stochasticity occur together with the possibility of Allee effects (Extinction vortices) (Pullin 2002). As populations drop below a critical threshold the possibility of a population becoming extinct increases exponentially. Thus if a population decreases below a certain size it would be impossible for this population to recover and it would go extinct. However it is not always possible to introduce a large enough population as a founder group to exclude all of the above factors, and other methods of preventing extinction of the particular population should be explored.

The original aims and objectives of this study were achieved as follows:

a. Determine the genetic diversity of each of the five Gemsbok (*O. gazella*) populations through making use of microsatellite techniques, and determine if any population bottlenecks could be observed from the data

Each population was sampled and various statistical approaches implemented to quantify genetic diversity in all five populations. From these results, a good indication of the genetic status of each population was obtained, as described above.

Sampling error and sampling size are important additional factors that should be considered to reach conclusions of the genetic status of a population.

- Sampling error or bias

  It is important to consider the method of sampling, specifically whether it takes place in a completely random fashion or whether selection plays an important role. Selection during sampling is possible, for example, during trophy hunting where specific trophy animals are hunted, thus selection for specific phenotypic traits is occurring. In this particular study sampling were mostly done at random, but on large ranches like STS Kalahari Game Ranch the sampling were more selective. Trophy hunting was the way samples were collected on this property, which is a highly selective way of collecting samples. This is because with trophy hunting only certain animals which fulfil specific criteria are hunted.

- Sample Size

  The use of small sample sizes introduces bigger rates of error during genetic studies, notably the calculation of genetic diversity, rates of drift and the determination of true population structure. Sampling error will cause the observed results to vary from the real or predicted results.

The Tempelhof and the Elias populations were expected to test positive for a population bottleneck, because they both did underwent a founder effect during the formation of these populations. The reason for the positive test in the STS population remains a mystery; a possible explanation could be sampling error. It should be remembered that sampling was performed from hunting excursions and on the STS Kalahari game ranch hunting can mostly be categorized as trophy hunting, thus making the sampling less random. Each individual is particularly looked at to meet certain requirements to be regarded as a trophy. The Tempelhof population is a population that grew after going through an artificial founder event, experienced during the introduction of *O. Gazella* to the property in low numbers. The Elias population is also a population that was introduced as a small initial population, thus experiencing a founder event as a population. The STS population gave results that indicated a population bottleneck. As discussed in the previous section the STS population's sampling were however not carried out randomly and thus fluctuations from the expected outcome can be expected.

A founder effect normally results in a loss of genetic variation because of the growth of population numbers from a low initial population size. A

serious reduction in population size causes a population to become prone to the long term effects of drift and also certain genotypes may be lost by chance. When the population then increases in size some of the previous genetic variation may no longer be present (Krohne 2001). It is also important to keep in mind that low genetic variation does not necessary result from a recent bottleneck, and it is not necessarily an indication of the level of endangerment (Zhang *et al*. 2002). In a study done by Spencer *et al.* (2000) it was found that when microsatellites are used to detect a demographic bottleneck, heterozygosity, temporal variance in allele frequency and allelic diversity tend to be more sensitive than proportion of polymorphic loci. It was also found that heterozygosity excess and expected heterozygosity was useful in varying degrees in the detection of bottlenecks. In the current study a founder effect occurred which will give the same signature as seen during a population bottleneck.

Isolation of populations can have a drastic effect on genetic status. By looking at the allele frequencies recorded during this study, it can be seen that small populations that are isolated with no migration taking place can experience changes in allele frequencies. Thus through inbreeding and population bottlenecks changes are observed in the genetic status of the small isolated populations. Furthermore, small populations can effectively be even smaller than is immediately apparent. In this regard, the effective population (N_e) size is important in the sense that very small population sizes or distorted demographics result in even smaller effective population sizes, ending in populations with a very low probability of surviving. It is important to get an idea of the minimum viable population size of a population under such circumstances. Founder populations are normally

very small and to keep these populations from entering an extinction vortex they need to be managed on a genetic, ecological and behavioural level. To get an idea of the probability of survival over different time scales has led, for example, to the concept of population viability analysis (PVA). This technique attempts to obtain an estimate/prediction of what the population size would be like in the future or what will happen to the population.

b. Determine the structural genetic grouping of all the populations

Predictions on how the populations are related were based on the assumption that the Cohen, Tempelhof and Namibian populations can be considered one group and the STS and Elias populations another group. These predictions were based on the known histories of the populations and furthermore the geographic location of these populations. The results of the study supported the prior assumptions. The separation can be clearly seen from the STRUCTURE results. The populations were grouped into two very distinct genetic clusters. The Cohen, Namibian and Tempelhof were grouped together and the STS and Elias populations were grouped together. The clustering of the populations grouped together in accordance with the geographical localities of the populations.

Similarly, the grouping of the populations based on genetic distance values showed a strong correlation between the molecular / statistical data and the known history of each population. The Cohen population originated from a small founder population of eight individuals from the Namibian population nine years prior to the sampling for the current study took place. The Tempelhof population also had its founder population originating from the

Namibian population ten years prior to sampling. These three populations (Namibian, Cohen and Tempelhof) are thus more closely related and grouped together, separate from the other two populations (STS and Elias). Geographically the STS and the Elias populations are spatially close together and from the data, these two populations are also grouped together and separate from the other three populations (Cohen, Namibia, Tempelhof).

c. Management implications and recommendations:

The Cohen, Elias and Tempelhof populations were isolated after founding, thus resulting in a lack of gene flow between these populations. Over time two things may potentially happen to these populations:

- The allele frequencies will change in these populations purely by chance, due to genetic drift. Some will increase in frequency at the expense of others and some alleles may be lost completely. The allele frequencies in fragmented populations will become increasingly distinct over time (Pullin 2002).
- Natural selection (and potentially hunting selection) will differ between these populations, thus each population will be exposed to different environments. Predation pressure, food availability, climate and competition will differ between the populations and will cause further divergent changes in the genetic makeup of the populations.

In fragmented populations like the ones in this study, the pattern of genetic diversity will therefore be the consequence of four factors:

- The historical distribution of diversity before fragmentation;

- The contemporary distribution and size of the fragmented populations;
- The level of isolation of the populations (rate of exchange of individuals relative to generation time);
- The reproductive ecology and demography (like the mating system, dispersal behaviour etc.)

The effects of inbreeding may only appear after a significant period of time. Therefore, it is important to keep the following recommendations in mind while managing wildlife populations on a game farm and establishing populations of new species:

- Population size is potentially a critical factor in the game farming milieu, with introductions, it is suggested that game-owners start-off with as large of a founder population as possible, to ensure that the effective population size (N_e) is as sufficient. This should contribute significantly to keeping future effects of inbreeding to a minimum.
- It should also be attempted to add genetic material from a source population with a very large population size periodically, to ensure that bottlenecking or drift through hunting and game capture does not influence the genetic pool on a very large scale.
- Lastly, attempts should be made to add individuals from a very large or well managed source population every couple of years to enrich the genetic pool to compensate for alleles lost through selection (hunting) or mutation. In this regards, attempt to monitor the possible phenotypic effects of selective hunting should also be made, through careful keeping of records on horn length and carcass weight.

Management plans guided by the above recommendations stand a good chance of contributing to the maintenance of genetically sound populations, at least in theory. Further studies like the current study should be encouraged to give the above recommendations more credibility and to ensure sustainability of wildlife on game farms, because the hunting and tourism industry has grown to such a big industry in South Africa.

Cross-species application of microsatellites and the use of microsatellites in general have been very successful. DNA microsatellites have proven useful as markers in studies of gene mapping due to their high level of polymorphism and broad genomic distribution. These properties suggest that they will also be useful for studies of population structure. This success in using heterologous PCR primers to amplify microsatellite loci in several different species eliminates the need to develop sets of primers for each species and therefore facilitates the use of DNA microsatellites as markers in studies of population genetics (Engel *et al* 1996) as seen in the current study.

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Appendix

a. Sampling kits

b. Raw Data

Population	BMS1237		MAF46		OARFC304		OARHH64		ETH225		RBP3		MAF50		HDZ8	
Namibia																
N1	*	*	105	105	113	113	110	110	136	138	143	149	156	156	151	163
N2	*	*	*	*	*	*	*	*	*	*	145	145	156	156	159	163
N3	257	257	107	107	113	113	110	110	136	136	141	145	156	156	153	163
N4	251	251	103	107	113	115	110	110	136	136	145	145	156	156	153	153
N5	255	263	105	105	115	115	110	110	136	138	127	129	156	156	153	161
N6	263	271	105	105	115	115	108	112	136	138	141	145	156	158	153	159
N7	263	271	103	103	113	115	106	110	136	136	145	151	156	156	153	159
N8	255	257	107	111	113	115	110	110	138	138	145	149	156	156	151	151
N9	255	261	89	107	113	113	106	110	136	136	149	149	156	156	153	159
N10	255	263	103	105	113	113	110	110	138	138	129	129	156	156	159	159
N11	267	273	103	105	113	113	110	110	136	138	129	129	156	156	153	163
N12	273	267	89	103	113	113	110	110	136	136	129	145	156	156	159	159
N13	271	271	103	105	113	113	110	110	136	136	129	145	156	156	159	163
N14	255	273	105	105	113	115	110	110	136	136	149	151	156	156	159	161
N15	261	277	103	103	113	113	110	110	136	138	143	143	156	156	161	161

N16	253	262	89	107	113	115	110	110	136	138	141	141	156	156	153	153
N17	271	277	103	111	113	113	106	110	136	136	141	145	156	156	153	163
N18	271	271	105	105	113	113	*	*	138	138	145	151	156	156	151	159
N19	253	275	103	111	113	113	106	110	138	140	143	145	156	158	159	159
N20	*	*	*	*	*	*	*	*	*	*	129	143	154	154	159	159
Cohen																
C1	251	275	111	111	113	113	106	110	136	138	143	143	156	156	149	153
C2	*	*	105	111	113	113	108	108	138	138	143	151	128	156	149	161
C3	251	263	107	107	113	113	108	108	136	136	*	*	*	*	*	*
C4	261	261	*	*	113	113	106	110	136	138	145	145	156	156	159	159
C5	271	275	107	107	113	115	106	110	136	138	143	145	156	156	153	163
C6	255	263	111	111	113	113	106	110	136	136	145	151	156	156	153	161
C7	263	263	103	107	113	115	106	110	138	138	131	151	156	156	153	161
C8	255	257	105	109	113	113	106	110	136	136	145	145	156	156	161	163
C9	255	263	103	105	113	113	106	110	136	138	129	143	156	168	151	163
C10	255	255	103	115	113	115	106	110	136	138	145	145	156	168	153	163
C11	261	263	103	103	113	113	106	110	136	136	145	145	156	156	159	163
C12	261	261	105	105	113	113	106	110	136	138	143	143	156	156	149	153
C13	*	*	*	*	*	*	*	*	*	*	141	141	156	158	145	153
C14	*	*	*	*	*	*	*	*	*	*	141	141	156	156	145	163
C15	*	*	*	*	*	*	*	*	*	*	129	149	156	156	153	159
Tempelhof																
T1	255	263	103	111	113	115	106	110	138	138	129	129	156	156	159	159
T2	255	271	107	107	113	115	106	110	136	136	129	129	*	*	159	169
T3	261	271	103	105	113	115	106	110	136	138	129	145	156	156	159	169
T4	261	271	103	105	113	113	110	110	136	136	129	129	154	156	153	169
T5	255	271	89	111	113	115	106	110	136	138	129	141	154	154	159	159
T6	255	261	105	105	113	115	106	110	136	136	*	*	*	*	*	*
T7	271	275	89	111	113	113	106	110	136	138	145	149	128	128	159	169
T8	261	261	89	109	113	113	106	110	136	136	145	145	156	156	159	163
T9	255	263	107	107	113	113	106	110	136	136	141	145	156	156	159	159
T10	261	261	89	103	113	115	106	110	136	138	129	145	156	156	149	159
T11	255	263	107	107	113	113	106	110	136	136	*	*	*	*	*	*
STS																
S1	263	273	107	107	113	113	110	110	142	142	127	145	156	156	159	159

S2	257	263	107	107	113	115	110	110	136	148	143	145	156	158	147	151
S3	263	273	107	107	113	113	110	110	140	140	143	143	156	156	155	157
S4	263	263	109	109	113	115	*	*	136	142	123	145	156	156	147	147
S5	265	273	89	89	113	115	*	*	142	142	123	145	156	158	147	155
S6	257	263	107	109	113	115	110	110	136	136	143	143	156	156	159	159
S7	257	277	107	107	113	117	*	*	144	144	143	143	156	156	151	155
S8	*	*	*	*	113	117	*	*	144	144	143	145	152	156	153	163
S9	*	*	*	*	113	117	110	110	144	144	127	143	156	156	155	155
S10	263	263	105	107	113	117	110	110	144	144	145	145	156	156	147	159
S11	255	269	89	109	115	115	110	110	140	142	127	143	156	156	155	155
S12	265	265	107	113	113	117	110	110	144	144	123	145	156	156	147	155
S13	263	263	107	107	113	113	110	110	140	142	123	145	154	154	145	155
S14	263	263	107	107	115	115	110	110	140	140	125	157	156	156	147	155
S15	257	265	107	107	113	115	110	110	136	140	143	143	156	156	155	159
S16	263	273	107	107	113	113	110	110	136	140	143	145	156	156	153	159
S17	257	263	89	107	113	113	110	110	136	140	129	143	156	158	159	159
S18	263	273	107	107	113	113	110	110	136	136	127	145	156	156	159	159
S19	265	265	111	113	113	113	110	110	*	*	141	149	156	156	145	159
S20	263	273	107	107	113	113	106	110	142	142	143	143	156	156	155	161

Elias

E1	263	279	107	107	113	115	*	*	136	136	123	145	156	156	155	155
E2	257	265	111	111	113	113	*	*	136	136	127	145	156	156	153	163
E3	263	265	107	109	113	115	110	110	140	142	123	145	148	156	153	153
E4	263	263	107	107	113	117	110	110	136	136	145	145	156	156	147	153
E5	257	263	107	107	113	115	110	110	142	142	123	145	156	156	153	159
E6	263	273	89	107	113	113	110	110	136	144	123	145	156	156	153	159
E7	263	263	107	109	113	113	*	*	136	136	145	145	156	156	153	153
E8	255	267	109	109	113	113	110	110	140	142	145	145	148	156	153	155
E9	263	263	89	89	113	117	110	110	136	136	123	145	156	156	153	159

c. POPGENE Input File

/*Gemsbuck: 1Namibia 2Tempelhof 3Cohen 4Elias 5STS*/

Number of populations = 5

Number of loci = 8

Locus names:

BMS1237 MAF46 OARFC304 OARHH64 ETH225 RBP3 MAF50 HDZ8

.. cc aa cc ab gi ee dj

.. .. hh ee hj

dd dd aa cc aa fh ee ej

aa bd ab cc aa hh ee ee

cg cc bb cc ab cd ee ei

gk cc bb bd ab fh ef eh

gk bb ab ac aa hj ee eh

cd df ab cc bb hi ee dd

ce ad aa ac aa ii ee eh

cg bc aa cc bb dd ee hh

il bc aa cc ab dd ee ej

li ab aa cc aa dh ee hh

kk bc aa cc aa dh ee hj

cl cc ab cc aa ij ee hi

en bb aa cc ab gg ee ii

bf ad ab cc ab ff ee ee

kn bf aa ac aa fh ee ej

kk cc aa .. bb hj ee dh

bm bf aa ac bc gh ef hh

.. .. dg dd hh

cg bf ab ac bb dd ee hh

ck dd ab ac aa dd .. hk

ek bc ab ac ab dh ee hk

ek bc aa cc aa dd de ek

ck af ab ac ab af dd hh

ce cc ab ac aa .. .. ..

km af aa ac ab hi aa hk

ee ae aa ac aa hh ee hj

cg dd aa ac aa fh ee hh

ee ab ab ac ab dh ee ch

cg dd aa ac aa .. .. ..

am ff aa ac ab gg ee ce

.. cf aa bb bb gj ae ci

ag dd aa bb aa .. .. ..

ee .. aa ac ab hh ee hh

km dd ab ac ab gh ee ej

cg ff aa ac aa hj ee ei

gg bd ab ac bb ej ee ei

cd ce aa ac aa hh ee ij

cg bc aa ac ab dg eg dj

cc bh ab ac ab hh eg ej

eg bb aa ac aa hh ee hj

ee cc aa ac ab gg ee ce

.. .. ff ef ae

.. .. ff ee aj

.. .. di ee eh

go dd ab .. aa ah ee ff

dh ff aa .. aa ch ee ej

gh de ab cc cd ah be ee

gg dd ac cc aa hh ee be

dg dd ab cc dd ah ee eh

gl ad aa cc ae ah ee eh

gg de aa .. aa hh ee ee

ci ee aa cc cd hh be ef

gg aa ac cc aa ah ee eh

gl dd aa cc dd ch ee hh

dg dd ab cc af gh ef bd

gl dd aa cc cc gg ee fg

gg ee ab .. ad ah ee bb

hl aa ab .. dd ah ef bf

dg de ab cc aa gg ee hh

dn dd ac .. ee gg ee df

.. .. ac .. ee gh ce ej

.. .. ac cc ee cg ee ff

gg cd ac cc ee hh ee bh

cj ae bb cc cd cg ee ff

hh dg ac cc ee ah ee bf

gg dd aa cc cd ah dd af

gg dd bb cc cc bk ee bf

dh dd ab cc ac gg ee fh

gl dd aa cc ac gh ee eh

dg ad aa cc ac dg ef hh

gl dd aa cc aa ch ee hh

hh fg aa cc .. fi ee ah

gl dd aa ac dd gg ee fi

d. Bottleneck Input File

Title line: "Gemsbuck"

Loc1

Loc2

Loc3

Loc4

Loc5

Loc6

Loc7

Loc8

POP

N , 0 105105 113113 110110 136138 143149 156156 151163

N , 0 0 0 0 0 145145 156156 159163

N , 257257 107107 113113 110110 136136 141145 156156 153163

N , 251251 103107 113115 110110 136136 145145 156156 153153

N , 255263 105105 115115 110110 136138 127129 156156 153161

N , 263271 105105 115115 108112 136138 141145 156158 153159

N , 263271 103103 113115 106110 136136 145151 156156 153159

N , 255257 107111 113115 110110 138138 145149 156156 151151

N , 255261 89107 113113 106110 136136 149149 156156 153159

N , 255263 103105 113113 110110 138138 129129 156156 159159

N , 267273 103105 113113 110110 136138 129129 156156 153163

N , 273267 89103 113113 110110 136136 129145 156156 159159

N , 271271 103105 113113 110110 136136 129145 156156 159163

N , 255273 105105 113115 110110 136136 149151 156156 159161

N , 261277 103103 113113 110110 136138 143143 156156 161161

N , 253262 89107 113115 110110 136138 141141 156156 153153
N , 271277 103111 113113 106110 136136 141145 156156 153163
N , 271271 105105 113113 0 138138 145151 156156 151159
N , 253275 103111 113113 106110 138140 143145 156158 159159
N , 0 0 0 0 0 129143 154154 159159
POP
C , 251275 111111 113113 106110 136138 143143 156156 149153
C , 0 105111 113113 108108 138138 143151 128156 149161
C , 251263 107107 113113 108108 136136 0 0 0
C , 261261 0 113113 106110 136138 145145 156156 159159
C , 271275 107107 113115 106110 136138 143145 156156 153163
C , 255263 111111 113113 106110 136136 145151 156156 153161
C , 263263 103107 113115 106110 138138 131151 156156 153161
C , 255257 105109 113113 106110 136136 145145 156156 161163
C , 255263 103105 113113 106110 136138 129143 156168 151163
C , 255255 103115 113115 106110 136138 145145 156168 153163
C , 261263 103103 113113 106110 136136 145145 156156 159163
C , 261261 105105 113113 106110 136138 143143 156156 149153
C , 0 0 0 0 0 141141 156158 145153
C , 0 0 0 0 0 141141 156156 145163
C , 0 0 0 0 0 129149 156156 153159
POP
T , 255263 103111 113115 106110 138138 129129 156156 159159
T , 255271 107107 113115 106110 136136 129129 0 159169
T , 261271 103105 113115 106110 136138 129145 156156 159169
T , 261271 103105 113113 110110 136136 129129 154156 153169
T , 255271 89111 113115 106110 136138 129141 154154 159159
T , 255261 105105 113115 106110 136136 0 0 0
T , 271275 89111 113113 106110 136138 145149 128128 159169
T , 261261 89109 113113 106110 136136 145145 156156 159163
T , 255263 107107 113113 106110 136136 141145 156156 159159
T , 261261 89103 113115 106110 136138 129145 156156 149159
T , 255263 107107 113113 106110 136136 0 0 0
POP
S , 263273 107107 113113 110110 142142 127145 156156 159159
S , 257263 107107 113115 110110 136148 143145 156158 147151
S , 263273 107107 113113 110110 140140 143143 156156 155157
S , 263263 109109 113115 0 136142 123145 156156 147147

S	,	265273	89089	113115	0	142142	123145	156158	147155
S	,	257263	107109	113115	110110	136136	143143	156156	159159
S	,	257277	107107	113117	0	144144	143143	156156	151155
S	,	0	0	113117	0	144144	143145	152156	153163
S	,	0	0	113117	110110	144144	127143	156156	155155
S	,	263263	105107	113117	110110	144144	145145	156156	147159
S	,	255269	89109	115115	110110	140142	127143	156156	155155
S	,	265265	107113	113117	110110	144144	123145	156156	147155
S	,	263263	107107	113113	110110	140142	123145	154154	145155
S	,	263263	107107	115115	110110	140140	125157	156156	147155
S	,	257265	107107	113115	110110	136140	143143	156156	155159
S	,	263273	107107	113113	110110	136140	143145	156156	153159
S	,	257263	89107	113113	110110	136140	129143	156158	159159
S	,	263273	107107	113113	110110	136136	127145	156156	159159
S	,	265265	111113	113113	110110	0	141149	156156	145159
S	,	263273	107107	113113	106110	142142	143143	156156	155161
POP									
E	,	263279	107107	113115	0	136136	123145	156156	155155
E	,	257265	111111	113113	0	136136	127145	156156	153163
E	,	263265	107109	113115	110110	140142	123145	148156	153153
E	,	263263	107107	113117	110110	136136	145145	156156	147153
E	,	257263	107107	113115	110110	142142	123145	156156	153159
E	,	263273	89107	113113	110110	136144	123145	156156	153159
E	,	263263	107109	113113	0	136136	145145	156156	153153
E	,	255267	109109	113113	110110	140142	145145	148156	153155
E	,	263263	89089	113117	110110	136136	123145	156156	153159

e. Arlequin Input File

[Profile]

Title="Gemsbok"

NbSamples= 5

DataType= MICROSAT

GenotypicData= 1

LocusSeparator= WHITESPACE

GameticPhase= 0

RecessiveData= 0

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    RecessiveAllele= null
    MissingData= '?'

[Data]

[[Samples]]

SampleName="Namibia"
SampleSize= 20
SampleData= {
N1      1   -9   105  113  110  136  143  156  151
            -9   105  113  110  138  149  156  163
N2      1   -9   -9   -9   -9   -9   145  156  159
            -9   -9   -9   -9   -9   145  156  163
N3      1   257  107  113  110  136  141  156  153
            257  107  113  110  136  145  156  163
N4      1   251  103  113  110  136  145  156  153
            251  107  115  110  136  145  156  153
N5      1   255  105  115  110  136  127  156  153
            263  105  115  110  138  129  156  161
N6      1   263  105  115  108  136  141  156  153
            271  105  115  112  138  145  158  159
N7      1   263  103  113  106  136  145  156  153
            271  103  115  110  136  151  156  159
N8      1   255  107  113  110  138  145  156  151
            257  111  115  110  138  149  156  151
N9      1   255  89   113  106  136  149  156  153
            261  107  113  110  136  149  156  159
N10     1   255  103  113  110  138  129  156  159
            263  105  113  110  138  129  156  159
N11     1   267  103  113  110  136  129  156  153
            273  105  113  110  138  129  156  163
N12     1   273  89   113  110  136  129  156  159
            267  103  113  110  136  145  156  159
N13     1   271  103  113  110  136  129  156  159
            271  105  113  110  136  145  156  163
N14     1   255  105  113  110  136  149  156  159
            273  105  115  110  136  151  156  161
```

```
N15     1   261  103  113  110  136  143  156  161
            277  103  113  110  138  143  156  161
N16     1   253  89   113  110  136  141  156  153
            262  107  115  110  138  141  156  153
N17     1   271  103  113  106  136  141  156  153
            277  111  113  110  136  145  156  163
N18     1   271  105  113  -9   138  145  156  151
            271  105  113  -9   138  151  156  159
N19     1   253  103  113  106  138  143  156  159
            275  111  113  110  140  145  158  159
N20     1   -9   -9   -9   -9   -9   129  154  159
            -9   -9   -9   -9   -9   143  154  159
}
SampleName="Cohen"
SampleSize= 15
SampleData={
C1      1   251  111  113  106  136  143  156  149
            275  111  113  110  138  143  156  153
C2      1   -9   105  113  108  138  143  128  149
            -9   111  113  108  138  151  156  161
C3      1   251  107  113  108  136  -9   -9   -9
            263  107  113  108  136  -9   -9   -9
C4      1   261  -9   113  106  136  145  156  159
            261  -9   113  110  138  145  156  159
C5      1   271  107  113  106  136  143  156  153
            275  107  115  110  138  145  156  163
C6      1   255  111  113  106  136  145  156  153
            263  111  113  110  136  151  156  161
C7      1   263  103  113  106  138  131  156  153
            263  107  115  110  138  151  156  161
C8      1   255  105  113  106  136  145  156  161
            257  109  113  110  136  145  156  163
C9      1   255  103  113  106  136  129  156  151
            263  105  113  110  138  143  168  163
C10     1   255  103  113  106  136  145  156  153
            255  115  115  110  138  145  168  163
C11     1   261  103  113  106  136  145  156  159
            263  103  113  110  136  145  156  163
```

```
C12    1    261  105  113  106  136  143  156  149
            261  105  113  110  138  143  156  153
C13    1    -9   -9   -9   -9   -9   141  156  145
            -9   -9   -9   -9   -9   141  158  153
C14    1    -9   -9   -9   -9   -9   141  156  145
            -9   -9   -9   -9   -9   141  156  163
C15    1    -9   -9   -9   -9   -9   129  156  153
            -9   -9   -9   -9   -9   149  156  159
}
SampleName="Tempelhof"
SampleSize= 11
SampleData={
T1     1    255  103  113  106  138  129  156  159
            263  111  115  110  138  129  156  159
T2     1    255  107  113  106  136  129  -9   159
            271  107  115  110  136  129  -9   169
T3     1    261  103  113  106  136  129  156  159
            271  105  115  110  138  145  156  169
T4     1    261  103  113  110  136  129  154  153
            271  105  113  110  136  129  156  169
T5     1    255  89   113  106  136  129  154  159
            271  111  115  110  138  141  154  159
T6     1    255  105  113  106  136  -9   -9   -9
            261  105  115  110  136  -9   -9   -9
T7     1    271  89   113  106  136  145  128  159
            275  111  113  110  138  149  128  169
T8     1    261  89   113  106  136  145  156  159
            261  109  113  110  136  145  156  163
T9     1    255  107  113  106  136  141  156  159
            263  107  113  110  136  145  156  159
T10    1    261  89   113  106  136  129  156  149
            261  103  115  110  138  145  156  159
T11    1    255  107  113  106  136  -9   -9   -9
            263  107  113  110  136  -9   -9   -9
}
SampleName="STS"
SampleSize= 20
SAmpleData={
```

S1	1	263	107	113	110	142	127	156	159
		273	107	113	110	142	145	156	159
S2	1	257	107	113	110	136	143	156	147
		263	107	115	110	148	145	158	151
S3	1	263	107	113	110	140	143	156	155
		273	107	113	110	140	143	156	157
S4	1	263	109	113	-9	136	123	156	147
		263	109	115	-9	142	145	156	147
S5	1	265	89	113	-9	142	123	156	147
		273	89	115	-9	142	145	158	155
S6	1	257	107	113	110	136	143	156	159
		263	109	115	110	136	143	156	159
S7	1	257	107	113	-9	144	143	156	151
		277	107	117	-9	144	143	156	155
S8	1	-9	-9	113	-9	144	143	152	153
		-9	-9	117	-9	144	145	156	163
S9	1	-9	-9	113	110	144	127	156	155
		-9	-9	117	110	144	143	156	155
S10	1	263	105	113	110	144	145	156	147
		263	107	117	110	144	145	156	159
S11	1	255	89	115	110	140	127	156	155
		269	109	115	110	142	143	156	155
S12	1	265	107	113	110	144	123	156	147
		265	113	117	110	144	145	156	155
S13	1	263	107	113	110	140	123	154	145
		263	107	113	110	142	145	154	155
S14	1	263	107	115	110	140	125	156	147
		263	107	115	110	140	157	156	155
S15	1	257	107	113	110	136	143	156	155
		265	107	115	110	140	143	156	159
S16	1	263	107	113	110	136	143	156	153
		273	107	113	110	140	145	156	159
S17	1	257	89	113	110	136	129	156	159
		263	107	113	110	140	143	158	159
S18	1	263	107	113	110	136	127	156	159
		273	107	113	110	136	145	156	159
S19	1	265	111	113	110	-9	141	156	145
		265	113	113	110	-9	149	156	159

```
S20	1	263	107	113	106	142	143	156	155
		273	107	113	110	142	143	156	161
}
SampleName="Elias"
SampleSize= 9
SampleData={
E1	1	263	107	113	-9	136	123	156	155
		279	107	115	-9	136	145	156	155
E2	1	257	111	113	-9	136	127	156	153
		265	111	113	-9	136	145	156	163
E3	1	263	107	113	110	140	123	148	153
		265	109	115	110	142	145	156	153
E4	1	263	107	113	110	136	145	156	147
		263	107	117	110	136	145	156	153
E5	1	257	107	113	110	142	123	156	153
		263	107	115	110	142	145	156	159
E6	1	263	89	113	110	136	123	156	153
		273	107	113	110	144	145	156	159
E7	1	263	107	113	-9	136	145	156	153
		263	109	113	-9	136	145	156	153
E8	1	255	109	113	110	140	145	148	153
		267	109	113	110	142	145	156	155
E9	1	263	89	113	110	136	123	156	153
		263	89	117	110	136	145	156	159
}
[[Structure]]
StructureName="Per population"
NbGroups=1
Group={
		Namibia
		Cohen
		Tempelhof
		STS
		Elias
}
```

f. Structure Input File

N1	1	-9	105	113	110	136	143	156	151
N1	1	-9	105	113	110	138	149	156	163
N2	1	-9	-9	-9	-9	-9	145	156	159
N2	1	-9	-9	-9	-9	-9	145	156	163
N3	1	257	107	113	110	136	141	156	153
N3	1	257	107	113	110	136	145	156	163
N4	1	251	103	113	110	136	145	156	153
N4	1	251	107	115	110	136	145	156	153
N5	1	255	105	115	110	136	127	156	153
N5	1	263	105	115	110	138	129	156	161
N6	1	263	105	115	108	136	141	156	153
N6	1	271	105	115	112	138	145	158	159
N7	1	263	103	113	106	136	145	156	153
N7	1	271	103	115	110	136	151	156	159
N8	1	255	107	113	110	138	145	156	151
N8	1	257	111	115	110	138	149	156	151
N9	1	255	89	113	106	136	149	156	153
N9	1	261	107	113	110	136	149	156	159
N10	1	255	103	113	110	138	129	156	159
N10	1	263	105	113	110	138	129	156	159
N11	1	267	103	113	110	136	129	156	153
N11	1	273	105	113	110	138	129	156	163
N12	1	273	89	113	110	136	129	156	159
N12	1	267	103	113	110	136	145	156	159
N13	1	271	103	113	110	136	129	156	159
N13	1	271	105	113	110	136	145	156	163
N14	1	255	105	113	110	136	149	156	159
N14	1	273	105	115	110	136	151	156	161
N15	1	261	103	113	110	136	143	156	161
N15	1	277	103	113	110	138	143	156	161
N16	1	253	89	113	110	136	141	156	153
N16	1	262	107	115	110	138	141	156	153
N17	1	271	103	113	106	136	141	156	153
N17	1	277	111	113	110	136	145	156	163
N18	1	271	105	113	-9	138	145	156	151
N18	1	271	105	113	-9	138	151	156	159
N19	1	253	103	113	106	138	143	156	159
N19	1	275	111	113	110	140	145	158	159

N20	1	-9	-9	-9	-9	-9	129	154	159
N20	1	-9	-9	-9	-9	-9	143	154	159
C1	2	251	111	113	106	136	143	156	149
C1	2	275	111	113	110	138	143	156	153
C2	2	-9	105	113	108	138	143	128	149
C2	2	-9	111	113	108	138	151	156	161
C3	2	251	107	113	108	136	-9	-9	-9
C3	2	263	107	113	108	136	-9	-9	-9
C4	2	261	-9	113	106	136	145	156	159
C4	2	261	-9	113	110	138	145	156	159
C5	2	271	107	113	106	136	143	156	153
C5	2	275	107	115	110	138	145	156	163
C6	2	255	111	113	106	136	145	156	153
C6	2	263	111	113	110	136	151	156	161
C7	2	263	103	113	106	138	131	156	153
C7	2	263	107	115	110	138	151	156	161
C8	2	255	105	113	106	136	145	156	161
C8	2	257	109	113	110	136	145	156	163
C9	2	255	103	113	106	136	129	156	151
C9	2	263	105	113	110	138	143	168	163
C10	2	255	103	113	106	136	145	156	153
C10	2	255	115	115	110	138	145	168	163
C11	2	261	103	113	106	136	145	156	159
C11	2	263	103	113	110	136	145	156	163
C12	2	261	105	113	106	136	143	156	149
C12	2	261	105	113	110	138	143	156	153
C13	2	-9	-9	-9	-9	-9	141	156	145
C13	2	-9	-9	-9	-9	-9	141	158	153
C14	2	-9	-9	-9	-9	-9	141	156	145
C14	2	-9	-9	-9	-9	-9	141	156	163
C15	2	-9	-9	-9	-9	-9	129	156	153
C15	2	-9	-9	-9	-9	-9	149	156	159
T1	3	255	103	113	106	138	129	156	159
T1	3	263	111	115	110	138	129	156	159
T2	3	255	107	113	106	136	129	-9	159
T2	3	271	107	115	110	136	129	-9	169
T3	3	261	103	113	106	136	129	156	159
T3	3	271	105	115	110	138	145	156	169

T4	3	261	103	113	110	136	129	154	153
T4	3	271	105	113	110	136	129	156	169
T5	3	255	89	113	106	136	129	154	159
T5	3	271	111	115	110	138	141	154	159
T6	3	255	105	113	106	136	-9	-9	-9
T6	3	261	105	115	110	136	-9	-9	-9
T7	3	271	89	113	106	136	145	128	159
T7	3	275	111	113	110	138	149	128	169
T8	3	261	89	113	106	136	145	156	159
T8	3	261	109	113	110	136	145	156	163
T9	3	255	107	113	106	136	141	156	159
T9	3	263	107	113	110	136	145	156	159
T10	3	261	89	113	106	136	129	156	149
T10	3	261	103	115	110	138	145	156	159
T11	3	255	107	113	106	136	-9	-9	-9
T11	3	263	107	113	110	136	-9	-9	-9
S1	4	263	107	113	110	142	127	156	159
S1	4	273	107	113	110	142	145	156	159
S2	4	257	107	113	110	136	143	156	147
S2	4	263	107	115	110	148	145	158	151
S3	4	263	107	113	110	140	143	156	155
S3	4	273	107	113	110	140	143	156	157
S4	4	263	109	113	-9	136	123	156	147
S4	4	263	109	115	-9	142	145	156	147
S5	4	265	89	113	-9	142	123	156	147
S5	4	273	89	115	-9	142	145	158	155
S6	4	257	107	113	110	136	143	156	159
S6	4	263	109	115	110	136	143	156	159
S7	4	257	107	113	-9	144	143	156	151
S7	4	277	107	117	-9	144	143	156	155
S8	4	-9	-9	113	-9	144	143	152	153
S8	4	-9	-9	117	-9	144	145	156	163
S9	4	-9	-9	113	110	144	127	156	155
S9	4	-9	-9	117	110	144	143	156	155
S10	4	263	105	113	110	144	145	156	147
S10	4	263	107	117	110	144	145	156	159
S11	4	255	89	115	110	140	127	156	155
S11	4	269	109	115	110	142	143	156	155

S12	4	265	107	113	110	144	123	156	147
S12	4	265	113	117	110	144	145	156	155
S13	4	263	107	113	110	140	123	154	145
S13	4	263	107	113	110	142	145	154	155
S14	4	263	107	115	110	140	125	156	147
S14	4	263	107	115	110	140	157	156	155
S15	4	257	107	113	110	136	143	156	155
S15	4	265	107	115	110	140	143	156	159
S16	4	263	107	113	110	136	143	156	153
S16	4	273	107	113	110	140	145	156	159
S17	4	257	89	113	110	136	129	156	159
S17	4	263	107	113	110	140	143	158	159
S18	4	263	107	113	110	136	127	156	159
S18	4	273	107	113	110	136	145	156	159
S19	4	265	111	113	110	-9	141	156	145
S19	4	265	113	113	110	-9	149	156	159
S20	4	263	107	113	106	142	143	156	155
S20	4	273	107	113	110	142	143	156	161
E1	5	263	107	113	-9	136	123	156	155
E1	5	279	107	115	-9	136	145	156	155
E2	5	257	111	113	-9	136	127	156	153
E2	5	265	111	113	-9	136	145	156	163
E3	5	263	107	113	110	140	123	148	153
E3	5	265	109	115	110	142	145	156	153
E4	5	263	107	113	110	136	145	156	147
E4	5	263	107	117	110	136	145	156	153
E5	5	257	107	113	110	142	123	156	153
E5	5	263	107	115	110	142	145	156	159
E6	5	263	89	113	110	136	123	156	153
E6	5	273	107	113	110	144	145	156	159
E7	5	263	107	113	-9	136	145	156	153
E7	5	263	109	113	-9	136	145	156	153
E8	5	255	109	113	110	140	145	148	153
E8	5	267	109	113	110	142	145	156	155
E9	5	263	89	113	110	136	123	156	153
E9	5	263	89	117	110	136	145	156	159

All the samples with more than two loci with missing data were left out during the calculations. In the end 8 samples were left out.

g. RSTCalc Input file

```
Gemsbokke
8
20
5
20
15
11
20
9
BMS12
2
132
MAF46
2
134
OARFC
2
140
OARHH
2
124
ETH22
2
138
RBP3
2
144
MAF50
2
142
HDZ8
2
144
```

Pop1

000 000 105 105 113 113 110 110 136 138 143 149 156 156 151 163

000 000 000 000 000 000 000 000 000 000 145 145 156 156 159 163

257 257 107 107 113 113 110 110 136 136 141 145 156 156 153 163

251 251 103 107 113 115 110 110 136 136 145 145 156 156 153 153

255 263 105 105 115 115 110 110 136 138 127 129 156 156 153 161

263 271 105 105 115 115 108 112 136 138 141 145 156 158 153 159

263 271 103 103 113 115 106 110 136 136 145 151 156 156 153 159

255 257 107 111 113 115 110 110 138 138 145 149 156 156 151 151

255 261 089 107 113 113 106 110 136 136 149 149 156 156 153 159

255 263 103 105 113 113 110 110 138 138 129 129 156 156 159 159

267 273 103 105 113 113 110 110 136 138 129 129 156 156 153 163

273 267 089 103 113 113 110 110 136 136 129 145 156 156 159 159

271 271 103 105 113 113 110 110 136 136 129 145 156 156 159 163

255 273 105 105 113 115 110 110 136 136 149 151 156 156 159 161

261 277 103 103 113 113 110 110 136 138 143 143 156 156 161 161

253 262 089 107 113 115 110 110 136 138 141 141 156 156 153 153

271 277 103 111 113 113 106 110 136 136 141 145 156 156 153 163

271 271 105 105 113 113 000 000 138 138 145 151 156 156 151 159

253 275 103 111 113 113 106 110 138 140 143 145 156 158 159 159

000 000 000 000 000 000 000 000 000 000 129 143 154 154 159 159

Pop2

251 275 111 111 113 113 106 110 136 138 143 143 156 156 149 153

000 000 105 111 113 113 108 108 138 138 143 151 128 156 149 161

251 263 107 107 113 113 108 108 136 136 000 000 000 000 000 000

261 261 000 000 113 113 106 110 136 138 145 145 156 156 159 159

271 275 107 107 113 115 106 110 136 138 143 145 156 156 153 163

255 263 111 111 113 113 106 110 136 136 145 151 156 156 153 161

263 263 103 107 113 115 106 110 138 138 131 151 156 156 153 161

255 257 105 109 113 113 106 110 136 136 145 145 156 156 161 163

255 263 103 105 113 113 106 110 136 138 129 143 156 168 151 163

255 255 103 115 113 115 106 110 136 138 145 145 156 168 153 163

261 263 103 103 113 113 106 110 136 136 145 145 156 156 159 163

261 261 105 105 113 113 106 110 136 138 143 143 156 156 149 153

000 000 000 000 000 000 000 000 000 000 141 141 156 158 145 153

000 000 000 000 000 000 000 000 000 000 141 141 156 156 145 163

000 000 000 000 000 000 000 000 000 000 129 149 156 156 153 159

Pop3

255 263 103 111 113 115 106 110 138 138 129 129 156 156 159 159

255 271 107 107 113 115 106 110 136 136 129 129 000 000 159 169

261 271 103 105 113 115 106 110 136 138 129 145 156 156 159 169

261 271 103 105 113 113 110 110 136 136 129 129 154 156 153 169

255 271 089 111 113 115 106 110 136 138 129 141 154 154 159 159

255 261 105 105 113 115 106 110 136 136 000 000 000 000 000 000

271 275 089 111 113 113 106 110 136 138 145 149 128 128 159 169

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261 261 089 103 113 115 106 110 136 138 129 145 156 156 149 159

255 263 107 107 113 113 106 110 136 136 000 000 000 000 000 000

Pop4

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263 273 107 107 113 113 110 110 140 140 143 143 156 156 155 157

263 263 109 109 113 115 000 000 136 142 123 145 156 156 147 147

265 273 089 089 113 115 000 000 142 142 123 145 156 158 147 155

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000 000 000 000 113 117 000 000 144 144 143 145 152 156 153 163

000 000 000 000 113 117 110 110 144 144 127 143 156 156 155 155

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265 265 107 113 113 117 110 110 144 144 123 145 156 156 147 155

263 263 107 107 113 113 110 110 140 142 123 145 154 154 145 155

263 263 107 107 115 115 110 110 140 140 125 157 156 156 147 155

257 265 107 107 113 115 110 110 136 140 143 143 156 156 155 159

263 273 107 107 113 113 110 110 136 140 143 145 156 156 153 159

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265 265 111 113 113 113 110 110 000 000 141 149 156 156 145 159

263 273 107 107 113 113 106 110 142 142 143 143 156 156 155 161

Pop5

263 279 107 107 113 115 000 000 136 136 123 145 156 156 155 155

257 265 111 111 113 113 000 000 136 136 127 145 156 156 153 163

263 265 107 109 113 115 110 110 140 142 123 145 148 156 153 153

263 263 107 107 113 117 110 110 136 136 145 145 156 156 147 153

257 263 107 107 113 115 110 110 142 142 123 145 156 156 153 159

263 273 089 107 113 113 110 110 136 144 123 145 156 156 153 159

263 263 107 109 113 113 000 000 136 136 145 145 156 156 153 153

255 267 109 109 113 113 110 110 140 142 145 145 148 156 153 155

263 263 089 089 113 117 110 110 136 136 123 145 156 156 153 159