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Conservation genetics of African wild dogs *Lycaon pictus* (Temminck, 1820) in South Africa

By

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Submitted in partial fulfilment of the requirements for the degree

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Declaration

I declare that this dissertation, which I hereby submit for the degree Magister Scientiae at the University of Pretoria, is my own work and has not been previously submitted by me for a degree at this or any other tertiary institution.

Date: …… Signature: ……

Dissertation summary

The African wild dog *Lycaon pictus* is Africa's second most endangered carnivore. Only 14 out of 39 countries in Africa still have wild dogs present. This makes the populations of wild dogs in South Africa very valuable with respect to the entire species. Kruger National Park (Kruger) has the only self-sustaining and viable population of wild dogs in South Africa, making Kruger the core area of conservation for South African wild dogs. It is of vital importance to know the numbers of wild dogs present in Kruger. In chapter 2 of this dissertation I monitored and gathered demographic information from as many southern Kruger wild dog packs and individuals as possible over a three month period. I used real time text messaging to collect the information. A wild dog hotline number was used for tourists to contact immediately after they sighted a pack, noting location, time and number of wild dogs sighted. This new technique resulted in more than 300 reported wild dog sightings in three months enabling a count of individuals and packs. This also created an opportunity to take identification photographs and to collect DNA samples.

In 1997 it was decided to establish and manage several small wild dog populations in various geographically isolated reserves in South Africa as one large managed metapopulation. In order to simulate the natural dispersal patterns of wild dogs, individuals are translocated between the managed metapopulation reserves, imitating natural gene flow and hopefully preventing inbreeding. To date, all decisions have been made using demographic data only. This in time is likely to result in a loss of genetic diversity and subsequent inbreeding. The aim of chapter 3 was to obtain genetic information from wild dogs in the managed metapopulation and Kruger (chapter 2) to provide a basis for sound population management including monitoring of inbreeding and maintaining levels of genetic diversity similar to those found in large self-sustaining populations (such as Kruger). This study included both mitochondrial DNA (mtDNA) and nuclear microsatellite loci to determine the genetic structure of South Africa's wild dogs specifically with regards to genetic diversity, population structure and relatedness. The results showed a difference in historical and recent diversity between the managed metapopulation and Kruger. Two genetic clusters were evident in South Africa, however one was due to wild dogs from Botswana being translocated into the managed metapopulation. After the Botswana influence was removed from the analysis, three genetic clusters were observed in the South African wild dogs. These three genetic clusters comprise too few wild dogs to manage them as separate units. Relatedness between and within

populations, reserves and packs were estimated and can in future be used to guide translocations of wild dogs to maximise their genetic variability. It is suggested that due to the low numbers, and historical and recent trends in genetic structure of South Africa's wild dogs, they should be managed as one unit, allowing movements to and from neighbouring countries. All translocations should follow an isolation-by-distance pattern.

For Mum, Dad, Megan

& Nanny

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"If we do not do something to prevent it, Africa's animals, and the places in which they live, will be lost to our world."

~ Nelson Mandela

A wild dog being sampled by me in the Kruger National Park.

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Chapter 1

General introduction

1.1 Wild dog – the species

The African wild dog *Lycaon pictus* (Temminck 1820) is South Africa's most endangered carnivore (Friedman & Daly 2004). *Lycaon pictus* means painted wolf - aptly named due to their tan, black and white patchwork coat. Each wild dog has a unique coat pattern allowing for identification of individuals (Frame *et al.* 1979; Smithers 1983). Wild dogs are medium sized canids standing 65 – 75 cm tall at the shoulder and weighing 18 – 28 kg (Smithers 1983). They are known for their highly gregarious and cooperative pack living characteristics (Estes & Goddard 1967; Fanshawe *et al.* 1991; Girman *et al.* 1997). Wild dogs prey mostly on ungulates specifically impala (*Aepyceros melampus*) and wildebeest (*Connochaetes taurinus*; Hayward *et al.* 2006). The classic wild dog pack consists of a breeding pair, their offspring and non-breeding subdominant adults (usually relatives of one or both of the breeding pair individuals; Fanshaw *et al.* 1991; Girman *et al.* 1997). The pups (and alpha female during lactation) are provided for by the other adult and yearling wild dogs in the pack. Dispersal of wild dogs from their natal packs occurs on average after 19 months of age (Estes 1991; McNutt 1996). Female wild dogs disperse more often than males, however, male wild dogs disperse further distances from their natal pack than females (Frame & Frame 1976; Frame *et al.* 1979; McNutt 1996; Girman *et al.* 1997).

1.1.1 Taxonomy

The more phylogenetically distinct an endangered species is, the higher its conservation value (Frankham *et al.* 2005). In 1820, Temminck first described the African wild dog as part of the hyaena family and named it *Hyena picta*. In 1930, Matthew altered this taxonomic placement and grouped wild dogs with the dhole (*Cuon alpinus*) and bush dog (*Speothos venaticus*) in Simocyoninae, a subfamily of the Canidae. *Lycaon*, *Cuon* and *Speothos* were grouped together only because of a similar shaped lower carnassial molar (Van Valkenburgh 1989). Bush dogs differ in appearance to both dholes and wild dogs that are alike in morphology, behaviour and ecology (Johnsingh 1982; Venkataraman 1995). However, in

1954, Thenius suggested that the dhole originated from an Early Pleistocene jackal of Asian descent. *Lycaon* fossils, which are very similar to contemporary wild dogs, have been dated to the mid-Pleistocene (Savage 1978). The origin of the African wild dog has been greatly debated. It has been suggested that skull fragments found in Europe in Late Pleistocene sites were that of *Lycaon* (Kurtén 1968). Alternatively Thenius (1972) and Malcolm (1979) believe that the fragments were *Canis*, thus indicating that the oldest *Lycaon* evidence may be of African origin. Savage & Russell (1983) showed that wild dogs may have arisen two to three million years ago in Africa.

Using molecular genetics, Girman *et al.* (1993) showed that wolves and jackals of the genus *Canis* are distinct from wild dogs, which are classified within a separate genus. Wild dogs are the only known extant species of a distant lineage of wolf-like canids (Girman & Wayne 1997). The taxonomic status of wild dog subspecies is currently unclear (Girman & Wayne 1997); although much research has been conducted, there remains uncertainty as to what constitutes a wild dog subspecies. Previous research by Girman *et al.* (1993) showed that populations of southern and East African wild dogs were genetically and morphologically distinct. However, more recent research using a greater number of samples has shown that genetic exchange between these southern and East African populations has occurred in the past (Girman *et al.* 2001). Unique nuclear and mitochondrial alleles are present in the South African and the northeast African populations with transitional populations in Botswana, Zimbabwe and south-eastern Tanzania. These populations contain a mixture of the alleles found in the southern and eastern regions (Girman 1996; Girman *et al.* 2001). Conversely, it has been shown that West African wild dogs may have a unique haplotype that is distinct from the eastern and southern African populations (Roy *et al.* 1994; Girman 1996). Future research on the West African wild dogs is pertinent as these dogs are likely to be distinct from the other African wild dogs and may thus represent a separate subspecies. Girman and Wayne (1997) suggest that even though no separate subspecies are currently acknowledged, the confirmed genetic differences between the southern, eastern and West African wild dogs illustrates that all of the populations must be conserved if the wild dogs' genetic diversity is to be preserved.

1.1.2 Distribution

The historical range of wild dogs is thought to have included most of sub-Saharan Africa, with the exception of rain forest and the driest desert biomes (Skinner & Smithers 1990). They are

thought to prefer savannah and acacia woodland habitats (Skinner & Smithers 1990; Fanshawe *et al.* 1991; Girman *et al.* 2001). In recent years, many wild dog individuals and even entire populations have been wiped out from within protected areas and countries (Fanshawe *et al.* 1991). Wild dog numbers have decreased because of an endless, yet sporadic, conflict with human activities, habitat fragmentation and infectious disease (Woodroffe *et al.* 2004). Of the 39 countries where wild dogs occurred in the past, 25 no longer have wild populations (Fanshawe *et al.* 1997; Woodroffe *et al.* 2005). This decrease in their distribution and total number of populations has affected the overall number of African wild dogs.

1.2 Conservation status

1.2.1 Conservation issues

Lycaon pictus is one of the world's most endangered large carnivore species (Fanshawe *et al.* 1997; Woodroffe *et al.* 2005). Wild dogs are the second most threatened carnivore in Africa and are classified as endangered in South Africa (Mills *et al.* 1998; IUCN 2009). It is estimated that fewer than 5000 wild dogs exist in the world today and that this number is declining annually (Fanshawe *et al.* 1991; Woodroffe & Ginsberg 1997, Woodroffe *et al.* 2004).

In South Africa there are four distributions of wild dogs: a contiguous viable population of roughly 120 individuals occurs in the Kruger National Park (2009 wild dog census); seven reserves constituting the wild dog metapopulation, have about 121 wild dogs (WAG minutes 2009); around 350 wild dogs occur in captivity (Rehse 2006); and there are roughly 104 free-ranging wild dogs living outside of protected areas (Lindsey *et al.* in prep).

1.2.2 Management paradigm in South Africa

In South Africa it was decided to improve conservation efforts and try to increase the local free-ranging wild dog numbers. As a result, in 1997 a Population and Habitat Viability Assessment (PHVA) was conducted for wild dogs. The outcomes of this meeting led to a comprehensive strategy of actions necessary to improve the conservation status of the South African wild dogs. Kruger National Park is home to the only contiguous viable wild dog population in the country and no other single reserve or conservation area is large enough to

sustain another viable population. It was thus decided to manage several small wild dog populations in various reserves as one large "metapopulation". The programme was defined by the way the metapopulation was to be run which involved managing at least nine separate wild dog subpopulations on several isolated reserves (Mills *et al.* 1998). The PHVA plan was to introduce wild dogs into several reserves and to simulate the natural dispersal patterns of wild dogs by artificially translocating wild dogs between these metapopulation reserves, thus imitating natural gene flow and preventing inbreeding (Mills *et al.* 1998; Davies-Mostert *et al.* 2009). The Wild dog Advisory Group (WAG) was created as a result of the PHVA to oversee the wild dog metapopulation expansion and management approach throughout South Africa. In less than ten years, nine reserves across the country became part of the managed metapopulation.

Two reserves introduced wild dogs before the managed metapopulation approach was decided upon. In 1980, 14 wild dogs were reintroduced into Hluhluwe-iMfolozi Park (Hluhluwe), a 900 km^2 reserve in northern KwaZulu-Natal and in 1995, 20 wild dogs were translocated into Madikwe Game Reserve (Madikwe), a 620 km^2 reserve in the North West Province (Davies-Mostert *et al.* 2009). After the decision to start a managed metapopulation several conservation areas (provincial and private) accepted wild dogs namely Pilanesberg National Park (Pilanesberg; 1999), Venetia Limpopo Nature Reserve (Venetia; 2002), Tswalu Kalahari Reserve (Tswalu; 2004), Balule (2005), Mkhuze Game Reserve (Mkhuze; 2005) and Thanda Private Reserve (Thanda; 2006). For more information regarding these conservation areas see Table 1.1. By mid-2009 only seven reserves remain as two reserves, Marakele and Balule, no longer participate in the metapopulation management action. Marakele's wild dogs were translocated to the Tuli Game Reserve in Botswana (WAG minutes 2006) and several of Balule's wild dogs were seen in Kruger having dispersed there on their own accord (Wild dog census 2009). No samples were obtained from Tswalu and thus Tswalu is not included as a metapopulation reserve in this study. Figure 1.1 shows the sampling localities of the managed metapopulation reserves and southern Kruger.

Table 1.1 The conservation areas, which make up the managed metapopulation, where wild dogs were reintroduced. Tswalu Kalahari Reserve was not included in the present study.

Year	Conservation area name	Province	Available habitat size (km^2)	No. of founders
1980	Hluhluwe-iMfolozi Park	KwaZulu-Natal	900	14
1995	Madikwe Game Reserve	North West	620	20
1999	Pilanesberg National Park	North West	500	13
2002	Venetia Limpopo Nature Reserve	Limpopo	320	20
2003	Marakele National Park*	Limpopo	740	17
2004	Tswalu Kalahari Reserve	Northern Cape	200	16
2005	Balule Game Reserve*	Limpopo	200	7
2005	Mkhuze Game Reserve	KwaZulu-Natal	400	13
2006	Thanda Private Reserve	KwaZulu-Natal	50	4

* Conservation area that no longer belongs to the managed metapopulation.

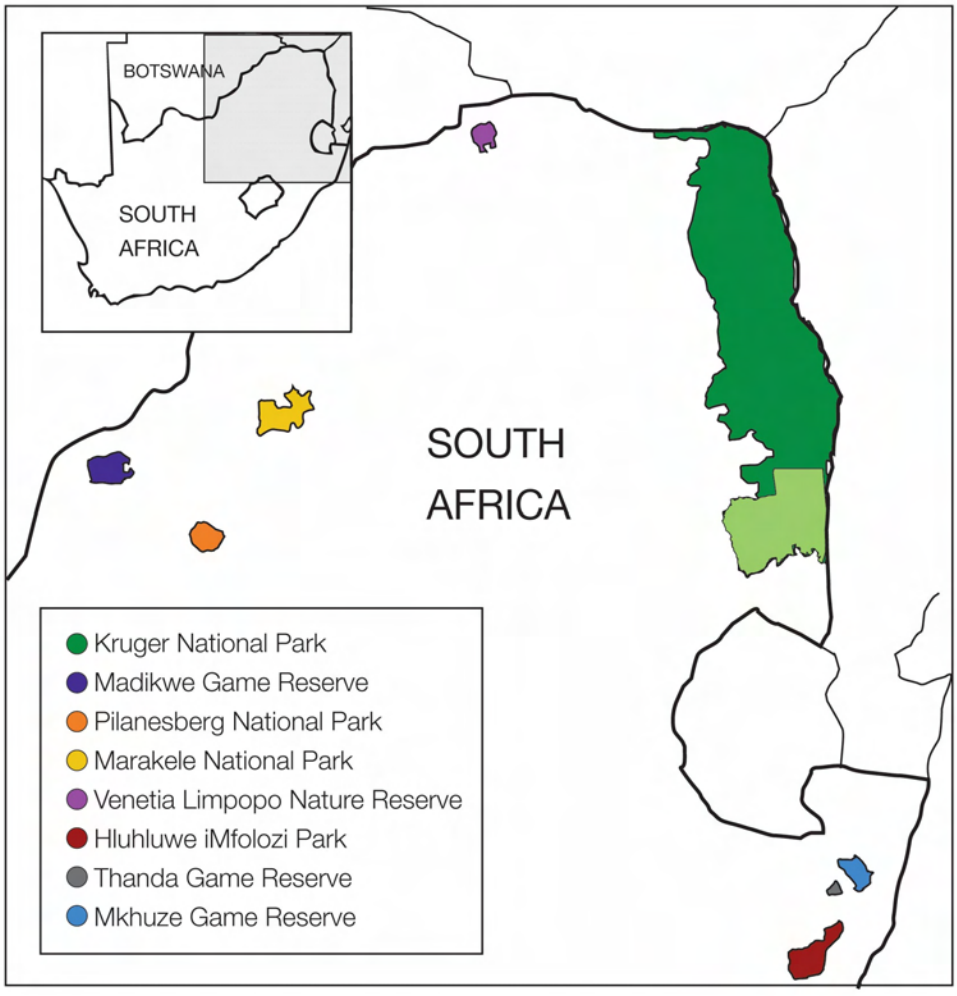

Figure 1.1 Map showing the locations of the South African wild dog metapopulation reserves and Kruger National Park. Southern Kruger National Park is shown in light green.

1.3 Conservation genetics

Molecular techniques are continuously being developed for all levels of interactions in population studies, for example, molecular barcoding at species level (Hebert *et al.* 2004), and microsatellites at population (Dematteo *et al.* 2009) and individual level (Girman *et al.* 1997). The simultaneous expansion of mathematical models to illustrate and infer changes in populations, both temporally and spatially, has also advanced the field (e.g. Beaumont 1999). These techniques have made the new subject of conservation genetics possible which comprises population genetics, systematics, molecular ecology and evolutionary biology (Avise *et al.* 1997; Frankham *et al.* 2002). Conservation genetics is a fast growing field which merges genetic data and biological concepts to improve the management of vulnerable, endangered and critically endangered species (Bertorelle *et al.* 2004). It aids the understanding of issues such as the deleterious effects of inbreeding on reproduction and survival of a species, the loss of genetic diversity and the ability of a species to adapt in response to changes in the environment, the reduction in gene flow and the fragmentation of populations, the determination of taxonomic uncertainties, and the defining of management units within a species (Frankham *et al.* 2005).

When making informed decisions about population management, it is important to understand the demographic history of the population concerned (Beaumont & Bruford 1999). In order to retain an accurate representation of a population's genetic diversity, a study of its genetic structure is vital (Frantzen *et al.* 1998). There are many approaches to quantifying the effects of demographic history on contemporary populations and many are dependant on the classification of a population. In this study I followed Futuyma's (1998) definition of a population: "A group of conspecific organisms that occupy a more or less well-defined geographical region and exhibit reproductive continuity from generation to generation."

The movement of animals between different populations may increase the genetic variability of the species and subsequently aid the avoidance of inbreeding. However, this raises questions such as which populations should be used to source individuals for translocations, and which should be maintained as genetically distinct (Beaumont & Bruford 1999).

1.3.1 Gene flow

Gene flow is the quantity of newly immigrant genes moving into a given population (Endler 1977). It is a crucial element with regards to the increase in numbers of a species, and may be attained through any individual of the species. The linkage of genes between populations of a species is maintained only through gene flow (Lowe *et al.* 2004).

Intrinsic and extrinsic factors influence gene flow. The former can be broken down into several components, including reproductive processes, mobility of individuals, behaviour of the animals (Lowe *et al.* 2004), and population density (Franceseschinelli & Bawa 2000). Extrinsic factors such as physical barriers and environmental conditions can have large effects on the ability of populations of the same species to connect and could thus impede or promote gene flow (Lowe *et al.* 2004).

Two general approaches to the estimation of gene flow have been classified. Indirect methods use the distribution of genetic variation within current adult populations to estimate the amount of gene flow between them, i.e. gene flow over long time periods (Lowe *et al.* 2004). Microsatellites are often the preferred indirect marker for estimations of gene flow (see below and also Parker *et al.* 1998). Mitochondrial DNA is a chosen indirect marker in animal gene flow estimation because of its high and ordered variation. It is, however, maternally inherited in most mammals, thus inferences are limited to female-mediated gene flow (Zhang & Hewitt 1996). Direct methods use genetic variation in offspring groups to identify parental contributions or variability, i.e. gene flow at a specific time (Lowe *et al.* 2004). In the present study I predominantly made use of an indirect approach and the analysis of microsatellite DNA variation.

1.3.1.1 Nuclear Markers: Microsatellites

Microsatellites, otherwise known as simple sequence repeats (SSRs), are relatively uniformly distributed in all eukaryotic genomes. They are tandemly repeated motifs of one to six base pairs present in coding and non-coding regions and are usually highly polymorphic in length due to variation in the number of repeats (Hancock 1999; Zane *et al.* 2002). Microsatellites are considered very powerful genetic markers due to their high variability, co-dominant inheritance, high mutation rate, and reasonable simplicity to score (Schlötterer & Pemberton 1998). Microsatellite applications extend over different fields, ranging from genome mapping in many organisms (e.g. Schuler *et al.* 1996; Knapik *et al.* 1998), historic and forensic DNA

studies (e.g. Hedmark & Ellegren 2005), population genetics (e.g. Simonsen *et al.* 1998; Nyakaana & Arctander 1999; Jones *et al.* 2004), to conservation and management of biological resources (e.g. Eizirik *et al.* 2001; Girman *et al.* 1993, 1997, 2001; Alpers *et al.* 2004, Dalén *et al.* 2006; Koblmüller *et al.* 2009).

Microsatellites are one of the favoured markers used when studying endangered species as they provide important information to conservation. First, in many species they are relatively simple to obtain either by the isolation of species-specific markers (Hammond *et al.* 1998) or through the application of markers originally isolated from related species (Rico *et al.* 1996). Second, different loci can be used according to their amount of variation. Third, they are relatively easy to score. Finally, they can be used on non-invasively sampled material and are amplified by the polymerase chain reaction (PCR; Beaumont & Bruford 1999).

There are several shortcomings when working with microsatellites. They are challenging to isolate from certain groups of organisms, i.e. insects and birds (Saccheri & Bruford 1993; Beaumont & Bruford 1999). The identification of species-specific polymorphic microsatellites and the development of primers is an expensive and time consuming procedure (Rassmann *et al.* 1991; Zane *et al.* 2002). Microsatellites are inappropriate for phylogenetic studies because of their high mutation rates which could lead to identity by state rather than identity by descent (Beaumont & Bruford 1999). Null alleles occur with non-amplification of certain alleles due to substitutions, insertions or deletions within the priming sites. This results in an increase of homozygous individuals (Callen *et al.* 1993; Pemberton *et al.* 1995). Last, non-invasively sampled material such as hair and faeces have proven to be difficult sources for analysis as stochastic amplification problems sometimes can arise (Beaumont & Bruford 1999). Analyses programmes can overcome most of these problems.

1.3.1.2 Mitochondrial DNA

Mitochondrial DNA (mtDNA) is a circular, haploid DNA molecule that is found in the mitochondria and is usually 15 – 20 kb in size. It is non-recombining (Avise *et al.* 1987) and in mammals it is maternally inherited (Boore 1999). MtDNA has a high mutation rate and is highly variable. It can be used to trace female lines of descent or migration patterns (Frankham *et al.* 2005). Rapid evolution is experienced at the nucleotide sequence level of mtDNA (up to 10 times faster than that of typical single-copy nuclear DNA), allowing for variation within and between populations (Simonsen *et al.* 1998). Different sections of the

mtDNA genome evolve at different rates. Thus depending on the resolution necessary for a specific study and how related the taxa are, a certain part of the mtDNA most suited for that specific study may be used (Brown *et al.* 1979). In mammals the most variable section of the mtDNA genome is the control region as it is characterised by rapid changes in sequence and length (Saccone *et al.* 1991). Thus the control region is an appropriate marker for population genetic studies. Furthermore, by complementing nuclear markers such as microsatellites with a mtDNA marker a more accurate and complete picture of population structure can be achieved (Simonsen *et al.* 1998).

1.4 Research objectives

Only a handful of population genetic studies have been conducted on the African wild dog (Girman *et al.* 1993, 1997, 2001), and only one of them (Girman *et al.* 1997) was specific to South Africa. Girman *et al.* (1997), using mtDNA and microsatellite markers on 92 wild dogs from southern Kruger, showed that wild dog packs generally do consist of a breeding pair, their offspring and non-breeding subdominant adults (close relatives to the breeding pair). They also showed that on occasion subdominant individuals may reproduce but the resulting offspring usually do not live to one year of age. However, Girman *et al.*'s (1997) major findings were that timing and location of dispersals are influenced by relatedness, and that dispersers often move to areas where there are many close relatives present. In 2001, Girman *et al.* looked at the population subdivision, gene flow and genetic variability of wild dogs throughout Africa. They suggested that wild dog populations have always been smaller than other carnivore populations and that recent wild dog population declines have not decreased their genetic diversity severely. They found two highly divergent genetic clusters in African wild dogs. These clusters were not restricted to eastern and southern African populations due to a large admixture zone found in Botswana, Zimbabwe and south-eastern Tanzania. These findings of Girman *et al.* in 1997 and 2001 indicate that it is imperative to obtain genetic data on the South African wild dogs in order to actively manage these endangered carnivores as naturally as possible.

The aim of the present study was to obtain genetic information on wild dogs comprising South Africa's managed metapopulation in order to provide a basis for sound population

management, to reduce inbreeding and maintain levels of genetic diversity similar to that found in large, less-intensively managed populations such as the Kruger National Park.

The short term objectives therefore were to:

1. Sample as many wild dogs within Kruger and the managed metapopulation in a limited time frame so as to use genetic variation among Kruger's wild dogs as a guideline for the genetic management of the metapopulation's wild dogs.
2. Determine the genetic structure, including relatedness, parentage and inbreeding levels, of South Africa's wild dogs (limited to the free-ranging wild dogs inside protected areas) by testing the following predictions:
    a. Kruger has the only self-sustaining population of wild dogs in South Africa and has existed for many wild dog generations longer than the managed metapopulation. It can thus be predicted that the Kruger population would have higher levels of genetic diversity at both mtDNA and nuclear DNA loci than the managed metapopulation.
    b. Girman *et al.* (2001) reported mtDNA and microsatellite variation in African wild dogs over a large geographic area in southern and East Africa. Their analyses suggested the existence of historical gene flow connections between South Africa and other regions within southern Africa (Botswana, Zimbabwe and possibly Mozambique). Evidence of such a connection is thus also expected in the present analysis of variation among South African wild dogs.
    c. Kruger has a self-sustaining wild dog population and the tenure of each pack's alpha pair is likely to be longer than for packs from the managed metapopulation. This lead to the prediction that a higher relatedness within packs is expected in Kruger as it has greater pack stability than the managed metapopulation.
    d. Due to previously documented patterns of male and female dispersal, it was predicted that relatedness between packs in Kruger would show an isolation-by-distance pattern.

The long term objectives were to:

1. Develop a standard technique for genetic analysis that will enable future spatial and temporal comparisons of genetic structure among subpopulations within the metapopulation, and other free-ranging wild dog populations.

2. Determine the influence of past management practises on the genetic structure of the metapopulation (in instances where data exist for a number of generations) in order to provide recommendations to improve future management strategies.
3. Integrate the metapopulation genetic data within the framework of the broader scale variability among southern African wild dog populations.

1.5 Dissertation outline

Chapter 2: Using real time text messaging to monitor large carnivores: Population demographics of the African wild dog *Lycaon pictus* in southern Kruger National Park, South Africa

This chapter takes a brief look at a successful technique using modern text messaging technology to monitor the endangered African wild dog. I used real time text messaging to collect information from park visitors on the wild dog population in southern KNP. A wild dog hotline number was used for tourists to contact immediately after they sighted a pack, noting location, time and number of wild dogs sighted. This technique resulted in more than 300 reported wild dog sightings within three months enabling a count of individuals and packs. This created an opportunity to take identification photographs and to collect DNA samples and other biological and demographic information. The technique can be applied to other species and is suitable for use in any reserve, game park or farm, which meets the prerequisite conditions of having large numbers of tourists and adequate mobile phone reception.

This chapter is intended for publication as a small tools and technology note in the *Journal of Wildlife Management*. The section on management issues will be omitted for publication purposes.

Chapter 3: Conservation genetics of the endangered African wild dog (*Lycaon pictus*) in South Africa

The aim of this chapter was to obtain genetic information from wild dogs in the managed metapopulation and Kruger National Park to provide a basis for sound population management including monitoring of inbreeding and maintaining levels of genetic diversity similar to those found in large free-ranging populations (such as Kruger). This study included

both mtDNA (control region) and nuclear microsatellite loci, to determine the genetic structure, including genetic diversity, population structure and relatedness, of South Africa's wild dogs. Three mtDNA haplotypes were observed; haplotype and nucleotide diversity for South African wild dogs were 0.484 and 0.4%, respectively. For the microsatellite data expected heterozygosity ranged from 0.438 to 0.657 which, when compared to other canids, falls in the lower end of the diversity spectrum. It was found that the managed metapopulation was more diverse with regards to the nuclear DNA than the Kruger population. The structure analysis revealed two separate clusters in South Africa, one of Botswana origin and the other, South African. After removing the Botswana influence, three population clusters were evident. Relatedness between and within populations, reserves and packs were estimated and can in future be used to guide translocations to maximise genetic variability.

This chapter is intended for publication in *Animal Conservation*.

Chapter 4: Dissertation synthesis

This chapter synthesises the major findings and suggests future research recommendations based on the research reported in chapters 2 and 3.

General notes

For publishing purposes, Kruger National Park in chapter 2 is abbreviated to KNP, whereas in all other chapters it is shortened to Kruger. The authors for the publications will include Janet Edwards, Harriet Davies-Mostert, Michael Somers and Paulette Bloomer. A reference list occurs at the end of each chapter rather than at the end of the dissertation, thus duplications may occur. The style and referencing format of *Molecular Ecology* was followed for this thesis.

1.6 References

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Chapter 2

Using real time text messaging to monitor large carnivores: Demographics of the African wild dog *Lycaon pictus* in southern Kruger National Park, South Africa

Abstract

Conserving self-sustaining populations of large carnivore species is of fundamental importance as they play significant roles in their native ecosystems. However, monitoring of large carnivores often proves challenging and novel techniques are required. The African wild dog (*Lycaon pictus*) is the most endangered carnivore in South Africa. The only self-sustaining population in South Africa occurs in the Kruger National Park (KNP). In this study, I used real time text messaging to collect information on the wild dog population in southern KNP. A wild dog hotline number was used for tourists to contact immediately after they sighted a pack, noting location, time and number of wild dogs sighted. This technique resulted in more than 300 reported wild dog sightings within three months enabling a count of individuals and packs. This created an opportunity to take identification photographs and to collect DNA samples and other biological and demographic information. The technique can be applied to other species and is suitable for use in any reserve, game park or farm, which meets with the prerequisite conditions of having large numbers of tourists and adequate mobile phone reception.

Keywords African wild dog, data collection techniques, GSM, *Lycaon pictus*, monitoring large carnivores

2.1 Introduction

The large wild areas in Africa that are needed to conserve integral carnivore guilds are becoming increasingly limited (Ray *et al.* 2005; Dalerum *et al.* 2008). Human demands for natural resources are escalating and stringent tactics are necessary to set priorities for conservation of carnivores because insufficient resources are distributed over a large land area (Ray *et al.* 2005). Carnivores are particularly sensitive to human disturbance, due to their need for large areas of suitable habitat, relatively slow reproduction rates and mutual exclusiveness with people (Ray *et al.* 2005) and are among the most challenging species to conserve (Linnell *et al.* 2001; Woodroffe 2001). Subsequently, monitoring becomes essential and thus new and novel techniques of monitoring are required.

The African wild dog *Lycaon pictus* is a high-risk candidate for future extinction with 5000 - 6000 individuals occurring naturally in the wild (Fanshawe *et al.* 1991; Woodroffe *et al.* 1997, 2004). As a result, wild dogs have been classified as Endangered on the IUCN Red Data List of Threatened Species (2009). The species was once distributed throughout most of sub-Saharan Africa but has been eradicated in most west and central African countries and exists almost exclusively in conservation and protected areas in the east and the south (Fanshawe *et al.* 1997). The main reasons for their demise are human-induced persecution, habitat destruction and fragmentation, loss of natural prey, interspecific competition with other large carnivores, and exposure to transmittable diseases (Fanshawe *et al.* 1991; Woodroffe & Ginsberg 1997, 1999; Creel & Creel 1998, 2002; Woodroffe *et al.* 2004; Gusset *et al.* 2006). South Africa is one of six countries containing potentially viable populations of wild dogs (Ginsberg & MacDonald 1990; Fanshawe *et al.* 1991; Woodroffe *et al.* 1997). The only self-sustaining and viable population of wild dogs in South Africa occurs in the Kruger National Park (KNP; Maddock & Mills 1994; Davies 2000; Lindsey *et al.* 2005).

It is of vital importance to know the numbers of wild dogs in KNP as the park is the core area of conservation for wild dogs in South Africa. Contributions from human observers (ranging from specialists to laymen) have been shown to significantly aid and improve the ability to collect scientific data (Sullivan *et al.* 2009). Previous photographic surveys and published observations have varied from 45 to 157 individuals in eight to 12 packs in the southern region of KNP (Table 2.1).

Table 2.1 The estimated number of wild dogs during the relevant years of study in southern KNP.

Year	Number of wild dogs	Study
1964	100 - 120	Pienaar 1969
1978	70 +	Reich 1981
1989	84	Maddock 1989
1995	157	Wilkinson 1995
2000	77	Davies 2000
2005	45	Kemp & Mills 2005

In this chapter I determine the wild dog population structure in southern KNP with aid of text message notifications and tourist reports over the period September to November 2007. This work demonstrates the usefulness of this technology for wildlife monitoring, and emphasises the need to conduct another photographic census in KNP to enable a scientific estimation of the total number of wild dogs within the whole of KNP.

2.2 Materials and Methods

I collected wild dog sighting reports from September to November 2007 in the southern district of KNP, mainly concentrating on the 4280 km^2 area (Mills & Gorman 1997) south of the Sabie River but in one incidence extending 5 km north of the Tshokwane picnic site (Fig. 2.1). Individual wild dogs can be identified from their unique coat patterns of black, white and tan (Frame *et al.* 1979; Smithers 1983). As the time frame for this study was short and the probability of tracking down wild dogs in a large thick-bushed area was low I relied on aid from as many volunteers as possible. This was achieved thanks to the 115 000 tourists who visit the KNP every month (R. Travers, media relations, South African National Parks, personal communication), the field rangers, KNP staff and research staff. A cell phone and number were selected to create the "Wild Dog Hotline" and an information brochure was designed and printed (Fig. 2.2) asking for assistance in finding wild dogs in the study area. These information brochures were attached to all entry permits given to tourists at the five southern KNP tourist entrance gates and were available and displayed at all the camps and picnic sights in southern KNP. Tourists and staff were encouraged to text or phone the Wild Dog Hotline number if they located wild dogs south of Satara Camp. They were also asked to

take note of the location, time and number of wild dogs seen. Tourists were encouraged to email photographs of the wild dogs to a central database. Visitors are restricted to the tar and gravel roads in KNP (see tar roads in Fig 2.1). This may limit the distribution and frequency of wild dog sightings especially in areas where there are fewer roads. However, wild dogs do tend to travel on average 10 km/day (Estes 1991) and take advantage of open roads (Reich 1981). Maddock & Mills (1994) maintain that even though an individual's chances of sighting wild dogs on any given day are low, the chances that somebody somewhere within the study area will do so is statistically higher than zero.

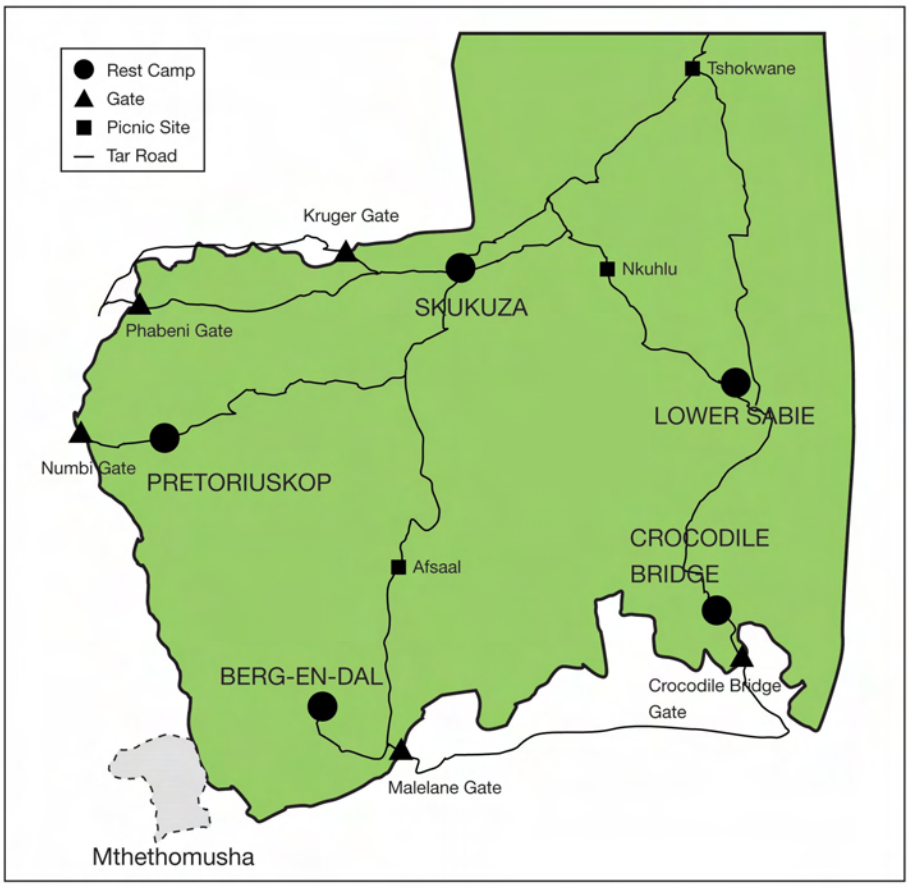

Figure 2.1 Map illustrating the camps, gates and picnic sites of southern Kruger National Park, South Africa. Mthethomusha, a private game reserve bordering Kruger, is shown in grey with a dashed border line.

This new method of information transfer allowed me to attempt to get to the located pack of wild dogs before they moved off the road or resumed hunting. This in turn improved my ability to identify the number of individuals in a pack, and to sex and divide the individuals into age

classes (juvenile or adult) where possible and applicable. I defined juveniles as pups born during the whelping phase of 2007. This was easily identifiable as the pups were roughly three to five months old during the study period and thus much smaller than the adults. I classified all other age classes as adults. Photographs were taken of all wild dog packs and individuals seen by the researcher. Location of the packs (using a Garmin eTrex GPS, coordinate system WGS1984 or, in the case of most tourist sightings, verbal/written descriptions stating distance to nearest intersection), number of pack individuals (adults and juveniles), and sex of pack adults were observed and recorded when possible. I named packs according to the area in which they were most commonly observed. Two adult male dogs from different packs were immobilized using a cocktail of Xylazine and Fentanyl and fitted with radio collars (African Wildlife Tracking) operating at 148-150 MHz to track the wild dogs at a later stage and sample more individuals from within the collared dogs' packs. A combination of Atipamezole and Naltrezone was administered to reverse the effects of the tranquilising drugs. While on the move wild dogs can cover vast distances. Thus I did not attempt to find the wild dogs if they were sighted >1 hour away from the base camp in Skukuza during possible hunting sessions (early morning or late afternoon). After a while I was able to recognise packs due to their reported numbers and locations and if these packs had previously been sampled and photographed I did not attempt to obtain another visual.

Figure 2.2 The flyer requesting tourists to phone or text the "wild dog hotline" if wild dogs were sighted anywhere south of Satara camp. The flyers were handed out to all tourists at the camps and gates in the southern Kruger National Park between September and November 2007.

2.3 Results

2.3.1 Observations

I received more than 230 text messages, 40 phone calls and 30 emails with photographs attached were received from the public reporting wild dog sightings in southern KNP between September and November 2007. Seventy-seven percent of reported sightings were of wild dogs occurring within 15 km of a tourist camp, picnic site or entrance gate. This gave an estimated 60 days worth of reported sightings during the project. Only five reports were sightings of wild dogs from outside the study area. Twelve of the southern sightings were made specifically by the researcher to obtain samples, identification photographs and other necessary demographic information. All reported sightings following the identification of packs were used to verify the numbers of individuals within the packs and the areas utilized by them.

I identified ten wild dog packs south of the Sabie River with an additional pack identified just north of this area (Table 2.2). A total of 87 individuals were recorded. Just under half (45%) of these wild dogs were juveniles born during the 2007 denning season, three months prior to the commencement of this study. Two of the packs were single-sex groups and thus non-breeding. The term “pack” is conventionally only used for a potential breeding group (Malcolm 1979; Reich 1981; Childes 1988). Of the eight packs potentially able to reproduce, six were reproductively successful. Group size ranged from the single Skukuza Loner to the two Skukuza disperser males. Pack size ranged from three individuals in the Mathekenyane pack to 19 individuals in the Phabeni pack. The average pack size (including the two single-sex groups) was 8.7 individuals per pack and 4.8 adults per pack in southern KNP.

Table 2.2 The age and sex structure of wild dog packs in southern KNP.

Pack	Males	Females	Unknown	Pups	Total in pack
Afsaal	2	5			7
Berg en Dal	1	2		4	7
Croc Bridge			7	2	9
Mathekenyane	3				3
Mthethamusho	3*	1	4	5	13
Phabeni	2	1	5	11	19
Skukuza			7	11	18
Skukuza Dispersers	2*				2
Skukuza Loner		1			1
Tshokwane	2			6	8
Total individuals					87

*One individual was fitted with a radio-collar

2.3.2 Comparison with earlier KNP wild dog census numbers

Previous wild dog census counts were recorded on the 1st of January, however, this study finished one month earlier. To compensate for potential attrition during December, I used Creel *et al.*'s (2004) annual mortality rate for KNP to estimate the number of wild dogs that may die in a worst case scenario per any random month in an "average" year. Using the number of wild dogs at the end of November 2007, it was estimated that in this extreme case, one adult, two sub-adults and two pups could die per month within the southern Kruger packs. Thus the population of wild dogs south of the Sabie River in 2007 contained at least 29 individuals more than in 2005 (Fig. 2.3). However under this extreme mortality the number of individuals dropped below what was found in the 2000 census. The average pack size of the eight wild dog packs residing south of the Sabie River in the KNP by the end of November 2007 was 8.8 wild dogs and in the case of extreme mortality this dropped to 8.2. Both of these figures are more than what was found in the previous two KNP wild dog censuses (Fig. 2.3).

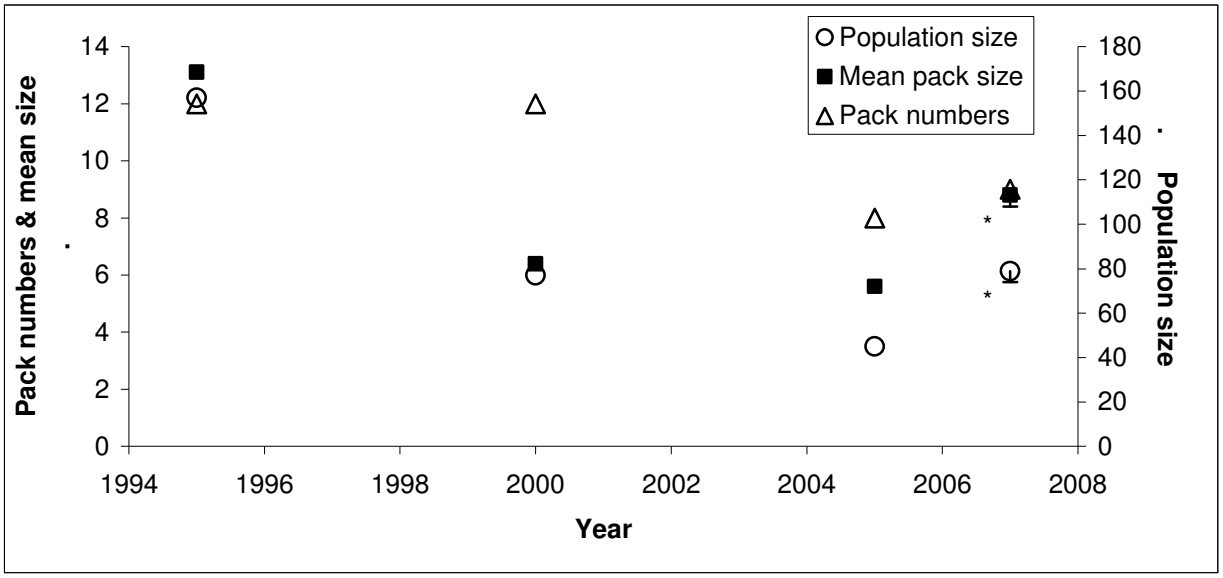

Figure 2.3 A comparison of the number of wild dog packs, their mean sizes and the wild dog population sizes observed during the previous years' census counts and this study in southern Kruger National Park. The estimated effects of mortality in an extreme case during one month (making 2007 compatible with the previous census data) are represented by y-error bars highlighted by *.

2.3.3 Other management issues: snare removal

During this study, five wild dogs from two packs had wire snares around their necks and one had a snare around its tail. Three wild dogs from the Phabeni pack were assumed to have been snared in the Sabie River Road area. The other three wild dogs with snares were from the Mthethomusha Pack and were likely snared whilst moving between KNP and Mthethomusha, a private game reserve which borders the south west KNP perimeter fence. The KNP Veterinary Wildlife Services team removed the neck snares and treated the wounds of five dogs. It is assumed the snare on the tail of the one wild dog fell off on its own as no tail snare was seen again and the number of wild dogs in the pack remained the same. No wild dogs were carrying snares at the conclusion of fieldwork.

2.4 Discussion

Although the use of cell phone technology has been successfully used on GSM wildlife tracking collars this study used a new system of instant information transfer that worked rapidly and effectively at relaying wild dog location data from tourists (from the moment of the sighting) to a central point. The new technique of using mobile phone technology and utilising the public allowed for visuals of most individuals and biopsy darting for further genetic studies (Chapter 3) of some of the individuals within the sighted wild dog packs. Individual and pack sightings were augmented through tourist and staff (received via email) photographs. This technique allowed me to determine a minimum count of all individuals and assess rudimentary demographic information such as pack size and pup survival.

The study shows that the number of wild dogs in southern KNP has increased between the years 2005 and 2007. This may possibly signify that wild dog numbers throughout KNP have increased as well. I strongly recommend a follow up park-wide wild dog census to count the number of wild dogs within the entire KNP and to observe their spatial occurrence throughout the park.

As tourist sightings are restricted to roads not all wild dog packs in southern KNP may have been observed, limiting the effective survey area. Neither tourists nor roads are evenly distributed in southern KNP (Maddock & Mills 1994), and the influence of this on the results of

this study was evident as wild dog packs in higher tourist density areas were sighted much more frequently than those in lower tourist density areas (pers. obs.).

Data collection ended one month earlier than the standardised census date (Maddock & Mills 1994). Creel *et al.* (2004) showed that only 35% of wild dog pups in KNP survive their first year. By the end of November most of the pups observed were roughly five months old. This is a time in their lives where they are trailing the adults in hunts and thus become most vulnerable to predation (Creel & Creel 2002). The chances of several of them dying (even within a one month period) are high. For this reason an average mortality was estimated (considering a worst case scenario) in order to make up for the one month discrepancy between this study and the previous censuses.

This study quickly generated basic distribution and demographic data for southern KNP's wild dogs and also played a crucial public relations role. The public was requested to help one of South Africa's most endangered mammals. This active, yet non-monetary (except for the negligible cost of sending a text message) involvement from many tourists and staff of KNP is an important "good deed" and a way for the people who saw wild dogs to "do their bit for conservation". The publicity generated by the study brought the predicament of the South African wild dogs to the attention of the general public, creating awareness and facilitating education.

This new technique of having immediate knowledge of sightings via a "hotline" benefited both the information gathering process and the level of wild dog awareness within the KNP. It is suggested that a similar technique be incorporated into the next KNP wild dog census to aid the process of data collection. This technique can also be applied to other species and is suitable for use by any other reserve, game park or farm meeting the prerequisite conditions of large numbers of tourists and adequate mobile phone reception.

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Chapter 3

Conservation genetics of the endangered African wild dog (*Lycaon pictus*) in South Africa

Abstract

The African wild dog (*Lycaon pictus*) is classified as endangered and in South Africa the Kruger National Park (Kruger) represents the only local conservation area with a self-sustaining population. In 1997 it was decided to actively increase conservation efforts for wild dogs and manage another population, consisting of several isolated reserves around the country, as a managed metapopulation. Here I report genetic diversity of African wild dogs in South Africa based on mitochondrial DNA (mtDNA) control region sequence variation in 20 individuals and variability at 18 nuclear microsatellite loci in 139 individuals from seven managed metapopulation reserves and Kruger. Only three mtDNA haplotypes were observed; haplotype and nucleotide diversity for South African wild dogs were 0.484 and 0.4%, respectively. For the microsatellite data expected heterozygosity ranged from 0.438 to 0.657 which, when compared to other canids, falls at the lower end of the diversity spectrum. It was found that the managed metapopulation was more diverse with regards to the nuclear DNA than the Kruger population, possibly due to its pack instability and high individual turnover. The Bayesian population structure analysis revealed two separate clusters, one of Botswana origin and the other, South African. After removing the Botswana influence, three population clusters were evident. These three genetic clusters comprise too few wild dogs to manage them as separate units. Relatedness between and within populations, reserves and packs were estimated and can in future be used to guide translocations to maximise genetic variability. It is suggested that due to the low numbers, and historical and recent trends in genetic structure of South Africa's wild dogs, they should be managed as one unit, allowing movements to and from neighbouring countries. All translocations should follow an isolation-by-distance pattern.

Keywords African wild dog, control region, genetic diversity, *Lycaon pictus*, managed metapopulation, microsatellites, population structure, relatedness

3.1 Introduction

The core of wildlife management has been defined by Sinclair *et al.* (2006) as "the management of wildlife populations in the context of the ecosystem". The knowledge of a species and its habitat are of vital importance in order to best manage wildlife. For threatened and endangered species the critical goal of management is to conserve the species by increasing their numbers (Sinclair *et al*. 2006). In most countries, national parks and protected areas are key locations for the conservation of free-ranging wildlife. Zoos and captive breeding facilities can also be considered as places of conservation for captive-bred individuals. One main advantage of a national park or protected area is that it offers protection for species that cannot coexist with humans. However, often these areas are too small to sustain viable populations, especially for species with large territories, that migrate or that occur in large groups. The effects that may occur due to these habitat constraints may be lessened by active management strategies.

In order for the development of effective conservation and management strategies for an endangered species, it is important to have a good understanding of the species' population structure (and history), the existence of physical or behavioural barriers to dispersal throughout its geographic range, and the distribution of its genetic diversity (Avise 1989; O'Brien 1994). It may, however, be more important to understand the process generating the diversity than only preserving the pattern (Moritz 1999, 2002). Populations within a species may differ considerably in adaptive features or genetic structure, thus requiring separate management (Frankham *et al.* 2005). Such management units can be determined using Fraser & Bernatchez's (2001) definition of evolutionary significant units: "A lineage demonstrating highly restricted gene flow from other such lineages within the higher organizational level (lineage) of the species."

Population structures have been comprehensively investigated using mitochondrial DNA (mtDNA; e.g. Avise *et al.* 1987; Smith & Wayne 1996) for the reason that sections of the genome evolve fast enough to reveal variation within and between populations. In mammals, the most variable section of the mtDNA genome is the control region as it is characterised by rapid changes in sequence and length (Saccone *et al.* 1991), and this makes it suitable for population genetic analyses. The mtDNA genome is maternally inherited in mammals thus yielding a female-biased assessment of population structure (Zhang & Hewitt 1996). A more

accurate and complete picture of population structure can be obtained by complementing mtDNA data with nuclear markers such as microsatellites (Simonsen *et al.* 1998). Microsatellites are tandem groups of short repeats which mutate through changes in the number of repeats. This and the fact that they have high rates of mutation make microsatellites convenient for the analysis of fine-scale population structure (Bruford & Wayne 1993; Avise & Hamrick 1996; Smith & Wayne 1996; Simonsen *et al.* 1998).

Wild dogs (*Lycaon pictus*) are the only extant representatives of the genus *Lycaon* (Girman *et al.* 1993). The ancestry of these wolf-like canids is unique (Girman & Wayne 1997) and wild dogs are distinct from the genus *Canis* which comprises wolves and jackals (Girman *et al.* 1993). Historically wild dogs were recorded from 34 sub-Saharan African countries in every habitat, except rain forests and some desert areas (Fanshawe *et al.* 1991; Smithers 1983). More recently, only 15 countries have resident populations and most of these comprise small fragmented packs in national parks and conservation areas (Skinner & Smithers 1990). Only six countries have viable populations (Fanshaw *et al.* 1991), including South Africa (Ginsberg & MacDonald 1990; Fanshawe *et al.* 1991; Woodroffe *et al.* 1997). The African wild dog is a high-risk candidate for future extinction with less than 5000 individuals occurring naturally in the wild (Fanshawe *et al.* 1991; Woodroffe *et al.* 1997). As a result, wild dogs have been classified as Endangered on the IUCN Red Data List of Threatened Species (2009). The main reasons for their demise are human-induced persecution, habitat destruction and fragmentation, loss of natural prey, interspecific competition with other large carnivores and exposure to transmittable diseases (Fanshawe *et al.* 1991; Woodroffe & Ginsberg 1997, 1999; Creel & Creel 1996, 2002; Woodroffe *et al.* 2004; Gusset *et al.* 2006). The African wild dog is the most endangered carnivore in South Africa (Friedman & Daly 2005), with the only self-sustaining and viable population occurring in the Kruger National Park (Kruger; Maddock & Mills 1994; Davies 2000; Lindsey *et al.* 2005). In addition 146 wild dogs occur in captivity (Rehse 2006) and free roaming wild dogs have been documented (WAG minutes 1998 - 2009). Hluhluwe-iMfolozi Park and Madikwe Game Reserve have had wild dogs prior to a Population and Habitat Viability Assessment (PHVA) in 1997 (Mills *et al.* 1998).

Wild dogs are known for their gregarious and cooperative pack living (Estes & Goddard 1967; Fanshawe *et al.* 1991; Girman *et al.* 1997). The classic wild dog pack consists of a breeding pair (alpha male and female), their offspring and non-breeding subdominant adults (usually relatives of one or both breeding individuals). At reproductive age, groups of similar sexed

individuals often disperse from their natal pack (Estes 1991). Two eastern African studies suggest that female wild dogs disperse more often than males (Frame & Frame 1976; Frame *et al.* 1979); however, male wild dogs disperse further distances from their natal pack than females (McNutt 1996). In Kruger, male wild dogs immigrated into other more distant packs more frequently than females who generally dispersed into packs near their relatives (Girman *et al.* 1997). Pack structure and sex-biased dispersal of Kruger's wild dogs were suggested to play a very important role in maintaining the genetic variability of populations by limiting the chances of inbreeding (Girman *et al.* 1997). However, because vast numbers of the southern African wild dogs have disappeared due to extermination and habitat loss, it is likely that the current Kruger population originated from a smaller, less genetically diverse population (Girman *et al.* 2001).

In 1997, a PHVA for wild dogs was conducted in South Africa. The outcomes of this meeting led to a comprehensive strategy of actions necessary to improve the conservation status of the South African wild dogs. It was decided to establish and manage several small wild dog populations in various reserves in South Africa as one large metapopulation. A conventional metapopulation is defined as a collection of spatially discrete subpopulations that exhibit asynchronous population dynamics and where migration between one or more patches is feasible (Levins 1969; Hanski & Simberloff 1997). Harrison (1994) suggested other scenarios such as patchy populations (panmictic populations that result from high rates of recolonization), source-sink metapopulations (one subpopulation is a continuous source for the colonization of other sites acting as sinks), and non-equilibrium/declining metapopulations (extinction exceeds recolonization). However, probable reintroduction sites in South Africa are isolated and confined (predator-fenced) resulting in drastically inhibited rates of natural dispersal and colonization of wild dogs (Davies-Mostert *et al.* 2009). Thus the concept of a managed metapopulation was formed where extinction would be balanced with artificial recolonization (Mills *et al.* 1998). Translocations (as a surrogate for natural dispersal) would counteract mortality due to environmental and demographic stochasticity, and significantly improve dispersal success (Davies-Mostert *et al.* 2009). Mills *et al.* (1998) defined the South African managed metapopulation approach as a programme which involved the coordinated management of at least nine separate wild dog subpopulations on several isolated reserves. Packs of wild dogs have been introduced into several reserves constituting the managed metapopulation (Fig. 3.1). In order to simulate the natural dispersal patterns of wild dogs, animals were translocated between these managed metapopulation reserves, imitating

natural gene flow and presumably preventing inbreeding (Mills *et al.* 1998; Gusset *et al.* 2008; Davies-Mostert *et al.* 2009).

To date, management decisions involving translocations and reintroductions have been made solely on demographic data collected by the various managed metapopulation reserves (WAG minutes 1998-2006), the collection of which is facilitated by active monitoring of wild dogs in these reserves. Unfortunately, the quality of the data collected is inconsistent as some reserves are more intensely monitored than others. These discrepancies, combined with the rapid rate of expansion of the metapopulation since monitoring programs were initiated, make the maintenance of accurate lineages within the metapopulation reserves increasingly difficult. Thus, relying only on demographic information to conduct metapopulation management is likely to result in loss of genetic diversity over time and subsequent inbreeding.

The aim of this study was therefore to obtain genetic information from wild dogs in the managed metapopulation and Kruger to provide a basis for sound population management, including monitoring of inbreeding and maintaining levels of genetic diversity similar to those found in large self-sustaining populations (such as Kruger). This study included both mtDNA (control region) and nuclear microsatellite loci, to determine the genetic structure, including genetic diversity, population structure and relatedness, of South Africa's wild dogs. Four predictions were considered:

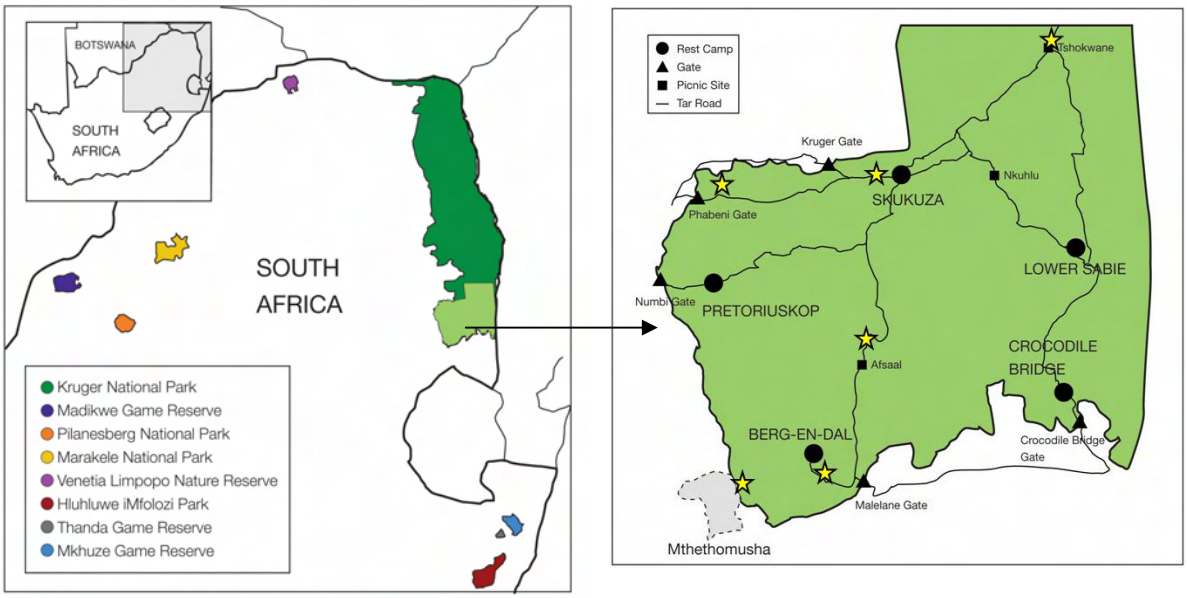
i) Kruger has the only self-sustaining population of wild dogs in South Africa and has existed for many wild dog generations longer than the managed metapopulation. It can thus be predicted that the Kruger population would have higher levels of genetic diversity at both mtDNA and nuclear DNA level than the managed metapopulation.

ii) Girman *et al*. (2001) reported mtDNA and microsatellite variation in African wild dogs over a large geographic area in southern and East Africa. Their analyses suggested the existence of historical gene flow connections between South Africa and other regions within southern Africa (Botswana, Zimbabwe and possibly Mozambique). Evidence of such a connection is thus also expected in the present analysis of variation among South African wild dogs.

iii) Kruger has a self-sustaining wild dog population and the tenure of each pack's alpha pair is likely to be longer than for packs from the managed metapopulation.

This lead to the prediction that a higher relatedness within packs is expected in Kruger as it has greater pack stability than the managed metapopulation.

iv) Due to previously documented patterns of male and female dispersal, it is predicted that relatedness between packs in Kruger would show an isolation-by-distance pattern.



(a) **(b)**

Figure 3.1 Maps showing the sampled managed metapopulation reserves and Kruger National Park in South Africa, and (b) the sampling localities (depicted as yellow stars) within southern Kruger National Park.

3.2 Materials and Methods

Whole blood, epithelial tissue and/or hair samples were collected from 139 wild dogs from eight different reserves: Kruger National Park (Kruger), Pilanesberg National Park (Pilanesberg), Madikwe Game Reserve (Madikwe), De Beers Venetia Limpopo Game Reserve (Venetia), Hluhluwe-iMfolozi Park (HiP), Mkhuze Game Reserve (Mkhuze), Thanda Game Reserve (Thanda) and Marakele National Park (Marakele; Fig. 3.1). Most sampling took place during translocations or veterinary procedures while the wild dogs were sedated. However 17 tissue samples were collected via biopsy darting (Karesh *et al.* 1987).

Three different methods for total genomic DNA extraction were performed on the three types of tissue collected. Whole blood DNA was isolated as follows: Whole blood (500 µl) was centrifuged with 2 volumes of red blood cell lysis solution (10mM NaCl, 10mM EDTA, pH 7.0) and the supernatant discarded. This step was repeated. The remaining pellet was mixed with 0.4 volumes of cell lysis solution (10 mM Tris – HCl pH 8.0, 50 mM NaCl, 10 mM EDTA), 0.02 volumes of 20% SDS and 0.5 mg Proteinase K and incubated at 65°C for 2 hours. Total genomic DNA from biopsied tissue (ear or biopsy dart sample) was extracted as follows: 2 mm^2 of tissue was cut into small pieces using a sterile scalpel blade and mixed with 0.4 volumes of cell lysis solution (10 mM Tris – HCl pH 8.0, 50 mM NaCl, 10 mM EDTA), 0.02 volumes of 20% SDS and 0.5 mg of Proteinase K. The tissue was digested at 56°C overnight.

Both the whole blood and biopsy DNA extraction solutions were incubated at 94°C for 10 minutes to inactivate the Proteinase K. Impurities were removed by phenol (repeated twice) and subsequently any remaining phenol was removed with a chloroform:isoamyl alcohol (24:1) solution (Sambrook *et al.* 1989). The aqueous layer was removed and 2.5 volumes of cold 99.9 % sequencing grade EtOH and 0.1 volumes of 3 M NaAc were added (Sambrook *et al.* 1989). The solution was inverted several times and placed in the freezer at –20°C to precipitate the DNA. The whole blood solutions were kept at this temperature for 30 minutes whereas the tissue solutions remained in the freezer overnight. The precipitated DNA was centrifuged at 10 000 rpm for 15 minutes and then washed with 70% EtOH. The last step was repeated but for only 5 minutes. The DNA pellets were air-dried, resuspended in 100 µl Tris-EDTA buffer (pH8.0) and rehydrated for 2 hours at 56°C, and subsequently at room temperature overnight.

Genomic DNA was extracted from six hair roots per sampled individual with 200mM NaOH and 200mM HCl, 100mM Tris–HCl, pH 8.5.

3.2.1 Primer design, mtDNA amplification and sequencing

Following Girman *et al.* (2001), a light strand primer, ThrL (5' CGA AGC TTG ATA TGA AAA AAC CAT C 3'; Kocher *et al.* 1989) in combination with a heavy strand primer, DLH (5' CCT GAA GTA GGA ACC AGA TG 3'; Kocher *et al.* 1989) were used in a Polymerase Chain Reaction (PCR, Saiki *et al.* 1988) in order to amplify the 5' end of the control region of the mtDNA. Two specific primers were designed for a better and more consistent amplification of the control region by using the domestic dog (*Canis lupus familiaris*; Kim *et al.* 1998,

GenBank accession # NC_002008), grey wolf (*Canis lupus*; Arnason *et al.* 2007, GenBank accession # AM711902), coyote (*Canis latrans*; Bjornerfeldt *et al.* 2006, GenBank accession # NC_008093) and wild dog (Girman *et al.* 2001, GenBank accession # AF335724) mtDNA sequences. The light strand primer L15424 (5' AGC TCT TGC TCC ACC ATC AG 3') anneals within the tRNA-Pro sequence (flanking the 5' end of the control region). The heavy strand primer was an internal primer, H15844 (5' CCA TCG AGA TGT CCC ATT TG 3'), which when combined with the light primer amplified 458 bp of the 5' end of control region.

DNA amplifications using PCR were performed in a total reaction volume of 25 µl. Each reaction contained approximately 120 ng of genomic DNA as template and in the case of the negative control reactions, Sabax® water was used instead of DNA. Each reaction also included 1 x PCR reaction buffer (Southern Cross Biotechnology), 1mM $MgCl_2$, 0.2mM of each of the four deoxyribonucleotides (Promega), 2.5 pmol of each primer and 0.75 U of SuperTherm® DNA Polymerase (Southern Cross Biotechnology). PCR conditions were as follows: Initial denaturation at 94°C for 2 minutes, 35 cycles of denaturation at 94°C for 30 seconds, primer annealing at 53°C for 30 seconds, elongation at 72°C for 1 minute, and an extended final elongation at 72°C for 5 minutes in a GeneAmp® PCR System 9700 (Applied Biosystems). The PCR fragment amplification was verified by running 4 µl of the PCR product through 1% agarose gels (Roche Diagnostics) using electrophoresis. No contamination of the reagents used in the PCR process was apparent from the absence of bands in the negative controls. The PCR products were precipitated using 0.08 volumes 3mM NaAc, 2.6 volumes 99.9 % sequencing grade EtOH and 0.4 volumes Sabax® water. Precipitated DNA was eluted in 20 µl Sabax® water. Success of the precipitation was evaluated by electrophoresis through 1% agarose gels.

The precipitated DNA fragments were sequenced in both directions with the primers used in the amplification step. Cycle sequencing was performed in a GeneAmp® PCR System 9700 using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit V3.1 (Applied Biosystems). Each cycle sequencing reaction contained 100 – 120 ng of purified DNA as template, 3.2 pmol primer and 2 µl of the Big Dye reaction mix. Cycle sequencing reactions were precipitated using the same protocol as described above for PCR products. Sequences were separated through an ABI 3130 capillary automated DNA sequencer (Applied Biosystems).

The raw sequence data were inspected for quality and background peaks using Sequence Analysis version 3 (Applied Biosystems). The successful sequences were imported into Sequence Navigator version 1.0.1 (Applied Biosystems). The light and heavy strand sequences of each individual were aligned in order to obtain a consensus sequence for each individual. The consensus sequences of all the individuals were aligned in Clustal X (Thompson *et al.* 1997). All sequences were trimmed to 435 bp to minimize missing data.

3.2.2 Microsatellite genotyping

A panel of 20 microsatellite markers regularly used for domestic dog identification and parentage testing, and recommended by the International Society of Animal Genetics (ISAG) was used to genotype the wild dog samples. A number of samples were run more than once to ascertain genotyping error. Microsatellite primers were obtained from Applied Biosystems. The 5'-end of each forward primer was labelled with one of the following fluorescent dyes: FAM, NED, VIC or PET. Multiplex PCRs were performed in four panels of three, three, four and ten loci.

Multiplex PCR was carried out in a 10 µl reaction volume using a GeneAmp PCR System 9700 (Applied Biosystems). AmpliTaq Gold DNA polymerase (Applied Biosystems) and approximately 100 ng of extracted DNA were added to the PCR mix. PCR cycles included an initial denaturing step at 95°C for 10 minutes; 30 cycles of denaturing at 95°C for 30 seconds, primer annealing at 56°C for 30 seconds, elongation at 72°C for 1 minute and an extended final elongation step at 72°C for an hour in a GeneAmp PCR System 9700 (Aplied Biosystems). Fragment amplification was verified by running 4 µl of the PCR product through 2% agarose gels (Roche Diagnostics) using electrophoresis. No contamination of the reagents used in the PCR process was apparent from the absence of bands in the negative controls.

An amount of 1 µl PCR product, 0.25 µl Genescan Liz500 size standard (Applied Biosystems) and 10 µl HiDi formamide (Applied Biosystems) were analysed by a 3130 XL Genetic Analyser (Applied Biosystems). STRand Software (version 2.3.94, Board of Regents, University of California, Davis) was used to determine allele sizes.

3.2.3 Statistical analyses

3.2.3.1 Genetic diversity

The genetic diversity among wild dog mtDNA sequences was quantified as nucleotide diversity (π) and haplotype diversity (H_D). Nucleotide diversity is the average pairwise nucleotide difference between individuals within samples (Nei 1987) and haplotype diversity is the probability that two randomly selected individuals have different alleles (Nei & Tajima 1981). It is very important to test for selective neutrality of mtDNA before making deductions from results because even though the control region is a non-coding section of mtDNA it can sometimes be influenced indirectly by other sections of the genome (Ballard & Whitlock 2004). It is recommended to use more than one test of neutrality as neutrality tests are often based on different models of evolution (Wall 1999). However Ramos-Onsins and Rozas (2002) found that when dealing with small sample sizes the R_2 test was the most appropriate test when compared to other statistical tests. Thus neutrality was tested using Ramos-Onsins and Rozas R_2 test. Nucleotide and haplotype diversity as well as neutrality were calculated using DnaSP version 4.9 (Rozas *et al.* 2003).

Microsatellite genotypes were tested for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium by means of a Markov chain algorithm (10 000 dememorisation steps, 1 000 batches and 10 000 iterations per batch) using GENEPOP version 4 (Rousset 2000). Statistical significance of these tests was modified for multiple comparisons by applying the Bonferroni correction (Rice 1989). Observed (H_O) and expected heterozygosity (H_E), number of alleles, and allele frequencies were calculated using Fstat Version 2.9.3.2 (Goudet 2001) and Arlequin version 3.0 (Excoffier *et al.* 2005).

3.2.3.2 Population structure

In this study I followed Futuyma's (1998) definition of a population: "A group of conspecific organisms that occupy a more or less well-defined geographical region and exhibit reproductive continuity from generation to generation." Thus a population is demographically linked and not necessarily an ecological population – "A group of organisms of the same species occupying a particular space at a particular time" (Krebs 1994; Roughgarden *et al.* 1989). For most population analyses, sampling location was regarded as the population. However not all analyses followed these assumptions and the latter analyses were conducted by considering individuals as data points.

Pairwise population differentiation estimates of the mitochondrial and microsatellite data were calculated with the F_{ST} estimators (Φ and θ, respectively; Weir & Cockerham 1984) and using Arlequin version 3.0 (Excoffier *et al.* 2005). Significance levels for multiple comparisons were modified using Bonferroni corrections (Rice 1989). Analysis of MOlecular VAriance (AMOVA; Excoffier *et al.* 1992) was used to produce estimates of genetic variance components, taking into account the number of mutations that are found between different alleles, using Arlequin version 3.0 (Excoffier *et al.* 2005). An allele network using only the mitochondrial data was constructed using statistical parsimony as implemented in TCS version 1.21 (Clement *et al.* 2000).

For the microsatellite data only, population structure, individual assignments and admixture proportions were estimated using STRUCTURE 2.2 (Falush *et al.* 2007) which is implemented by means of a Bayesian approach. No locality information was used and individuals were placed into *K* populations, with membership coefficients adding up to 1 across clusters. The statistic ΔK (Evanno *et al.* 2005) was used to provide the likely value of *K.* Multiple runs with values of K from one to six were repeated 20 times as suggested by Pritchard *et al.* (2000) with a burn-in period of 10^4 steps followed by a 10^5 Markov chain Monte Carlo chain. Then, for each *K* value, the average and standard deviation of the 'log estimated likelihood' (Ln*K*) across the 20 runs was calculated. The values of ΔK statistics were obtained as $\Delta K = m(|\text{Ln}(K+1) - 2\text{Ln}(K) + \text{Ln}(K-1)|) / s(\text{Ln}K)$, where *m* and *s* represent the average and standard deviation of the corresponding values across 20 runs, respectively. The LnK and ΔK statistics were then used to determine the uppermost level of population structure. An admixture model with correlated allele frequencies was used. Chains of 10^6 were run three times to ensure convergence when individuals were assigned to their inferred clusters. Individuals assigning with a probability of membership of $q \geq 0.80$ to a specific cluster were regarded as belonging to that single cluster, whereas individuals with $q < 0.80$ were considered to be admixed (Lecis *et al.* 2006).

3.2.3.3 Relatedness

Pairwise relationships between individuals, and between and within packs, the managed metapopulation, and Kruger, and within South Africa were determined using the program GenAlEx (Peakall & Smouse 2006). The relatedness value (*R*) according to Queller & Goodnight (1989) shows that first order relatives (parent-offspring and full sib relationships) should produce a *R*-value of on average ≈ 0.5, second order relatives should produce *R*-

values ≈ 0.25, whilst any *R*-value less than 0.125 indicates unrelated individuals. Known relationships of sampled individuals were used to evaluate the *R*-value estimates. The computational programme, Cervus (Marshall *et al.* 1998), was used to check the parentage of known parent-offspring relationships.

3.3 Results

3.3.1 Genetic diversity

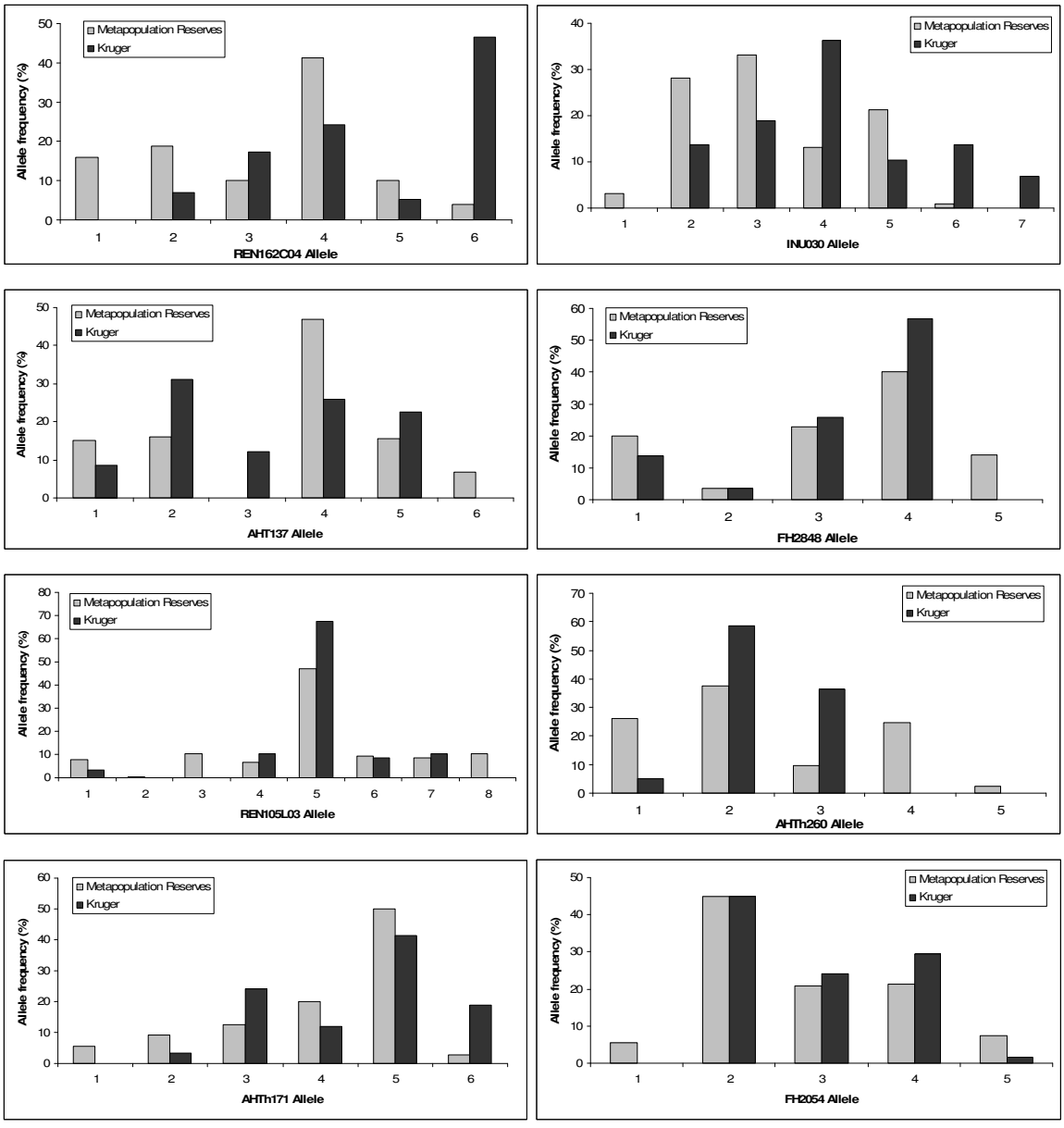
A 435 bp segment of the 5' end of the control region was compared for 12 wild dogs from the managed metapopulation and eight from Kruger. Six sites were variable, defining three unique haplotypes. At these sites, three changes were specific to the Kruger haplotype S1 and three to the Botswana haplotype Z1 (see section 3.3.2). Overall nucleotide diversity was 0.4% and haplotype diversity was 0.484. The Ramos-Onsins and Rozas R_2 test showed no significant departure from neutrality (R_2 = 0.132, P = 0.377). The summary statistics (Table 3.1) based on the mtDNA sequences of the 20 individuals show that the Kruger population may be more variable than the managed metapopulation. The variation in both populations followed neutral molecular evolution expectations (Table 3.1).

Table 3.1 Estimates of nucleotide and haplotype diversity, and results of Ramos-Onsins and Rozas's R_2 neutrality tests (and *P*-value) for the wild dogs within South Africa, the Kruger National Park (Kruger) and the managed metapopulation.

	Total SA	Kruger	Managed metapopulation
Nucleotide diversity	0.004 ± 0.001	0.005 ± 0.001	0.002 ± 0.001
Haplotype diversity	0.484 ± 0.113	0.571 ± 0.094	0.303 ± 0.147
R2 neutrality test (*P*)	0.132 (0.377)	0.286 (0.945)	0.151 (0.132)

For the nuclear data, two loci were removed post-genotyping: AMEL and INU005. AMEL, the sex-linked locus, allowed for the assignment of gender to all sampled individuals but was not used when performing most of the genetic analyses. INU005 proved to be monomorphic for wild dogs and was not informative in this study. A total number of 89 alleles were detected across the 18 microsatellite loci from 139 individual wild dogs. The number of alleles per locus varied between two at loci AHTk211 and REN247M23, and eight at locus REN105L03

(Fig. 3.2). Three and 23 alleles (out of 86) were unique to Kruger and the managed metapopulation, respectively, even though 14 of the 18 loci had the same common allele for both populations (Fig. 3.2). Expected and observed heterozygosity values were mid-range numbers and the estimates for the reserves were similar (Appendix 3.1). Mean expected values (H_E) ranged from 0.438 to 0.657 and mean observed (H_O) values ranged from 0.454 to 0.636. A total number of 18 samples were rerun twice to determine genotyping error. No genotypic errors were observed between the first and second run.



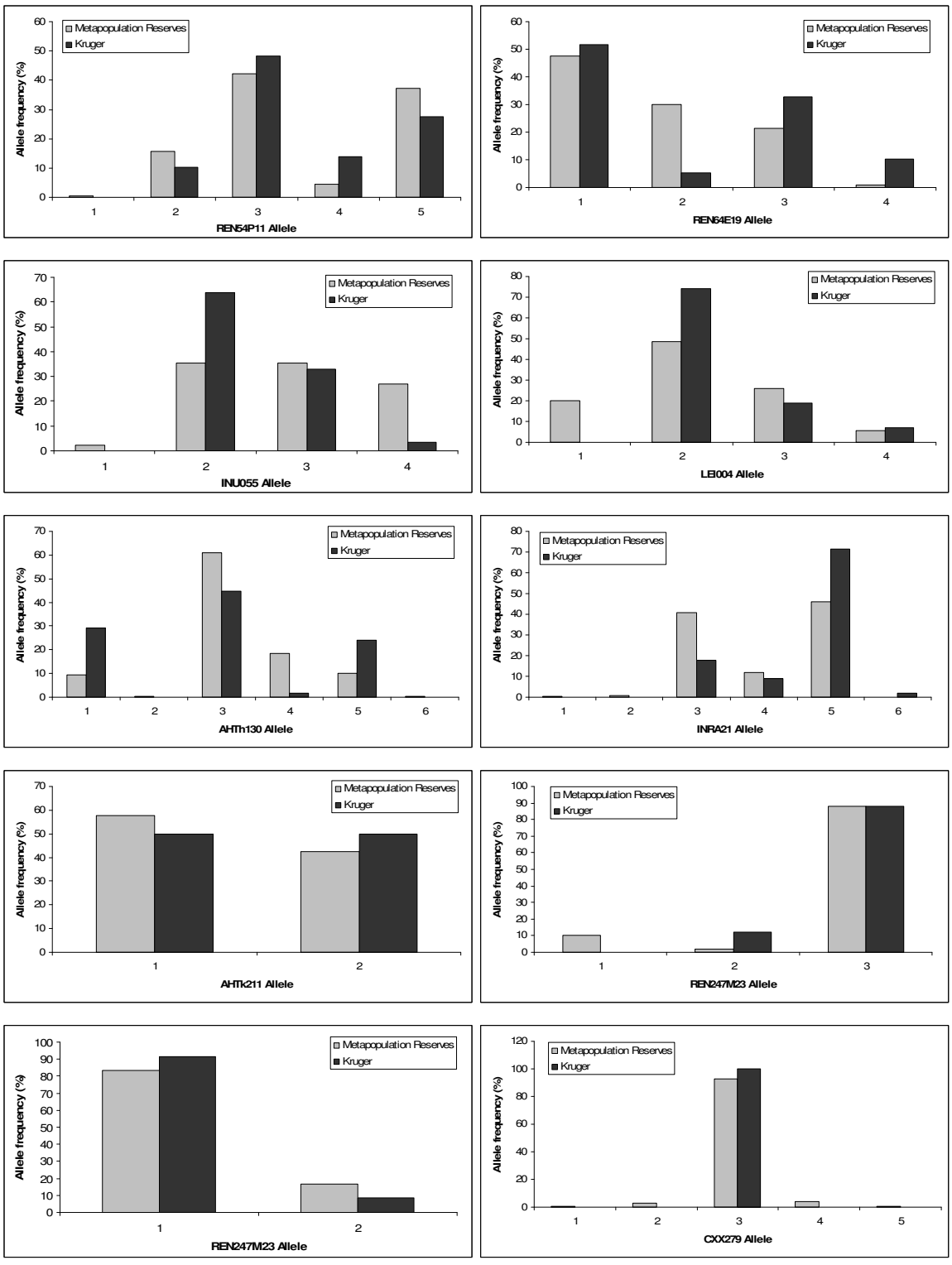


Figure 3.2 A comparison of allele frequency distributions at 18 loci for wild dogs in the managed metapopulation and Kruger.

Overall, eight of the 18 loci deviated significantly from Hardy-Weinberg equilibrium (REN162C04, INU030, AHT137, REN105L03, AHTh260, AHTh171, REN64E19 and LEI00; Appendix 3.1). However, when looking at the Kruger population and managed metapopulation separately, the Kruger population only had one locus that significantly deviated from Hardy-Weinberg equilibrium (REN54P11: H_E = 0.623, p = 0.001) whilst the managed metapopulation had six loci (REN162C04: H_E = 0.750, p = 0.000, INU030: H_E = 0.750, p = 0.000, AHT137: H_E = 0.708, p = 0.000, REN105L03: H_E = 0.736, p = 0.001, AHTh171: H_E = 0.685, p = 0.003 and LEI00: H_E = 0.657, p = 0.001). The deficit of heterozygotes in the overall and managed metapopulation analyses could be due to slight inbreeding or null alleles. However, when delving even deeper and looking at the separate reserves within the managed metapopulation and packs within Kruger, only one reserve, Marakele, had one locus that deviated significantly from Hardy-Weinberg equilibrium (AHTh260: H_E = 0.662, p = 0.000). Even though there were slight deviations from Hardy-Weinberg equilibrium, the same loci were not consistently showing the deviations, therefore all loci were used in the subsequent analyses.

Permutation tests indicated that there were large numbers of pairwise comparisons of loci that showed significant linkage disequilibrium, even after Bonferroni correction ($p < 0.0028$). The South African population had 57.5% of the pairwise comparisons of loci that showed significant linkage, while after splitting the population into Kruger and the managed metapopulation, Kruger had 7.8% and the managed metapopulation had 56.2%. Across all the populations and reserves, no loci were consistently in linkage disequilibrium. The classic wild dog pack consists of a breeding pair, their offspring and non-breeding subdominant adults (usually relatives of the breeding pair; Frame *et al.* 1979; Estes 1991; Girman *et al.* 2001). This closely related pack structure could lead to over sampling of related individuals which in turn could result in the deceptively high percentages of linkage disequilibrium. In order to test for this, only the alpha pairs of wild dogs (n = 14), or in situations where the hierarchy was not known an adult male and female, were selected and linkage disequilibrium was again tested. This resulted in a change from 57.5% to only 1.31% of the possible 153 loci comparisons being significant. All loci were thus retained for subsequent analyses.

3.3.2 Population structure

The three closely related haplotypes represented in the mtDNA control region sequences matched those identified by Girman *et al.* (2001), namely S1, S2 and Z1 (Fig. 3.3). Haplotype

S2 had the highest frequency (70%) and was present in both the Kruger and the managed metapopulation. S1 was unique to the Kruger population and occurred in 50% of the Kruger individuals. Z1 was unique to two of the managed metapopulation reserves: Marakele and Mkhuze (both reserves with dogs originating from Botswana). The mitochondrial and microsatellite analyses of population structure both showed that Kruger and the managed metapopulation were significantly different (Table 3.2). However, most of the variation occurred within the populations for all of the analyses (Table 3.2).

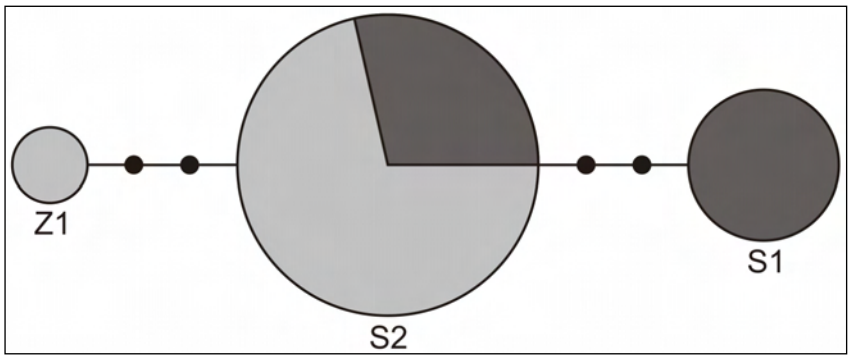


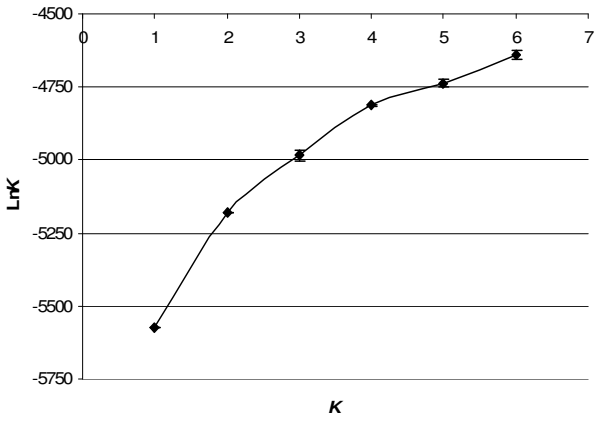
Figure 3.3 Allele network of the three haplotypes found in the wild dogs of South Africa. S1 was unique to Kruger, Z1 was unique to two reserves of the managed metapopulation and S2 was shared by both populations.

Table 3.2 An analysis of molecular variance (AMOVA) based on a 435 bp segment of the 5' end of the mitochondrial control region and microsatellite data between populations (i.e. Kruger versus the managed metapopulation) of wild dogs in South Africa. The mtDNA and first microsatellite analyses used the same 20 individuals whereas the latter microsatellite analysis used the total number of individuals (n = 139).

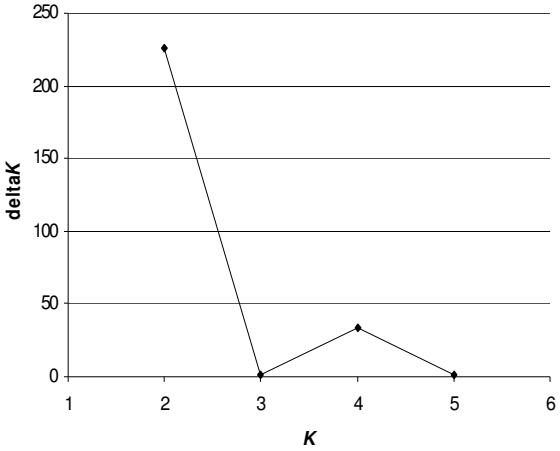
	mtDNA	Microsatellite	Microsatellite
Sample size	20	20	139
Number of populations	2	2	2
Among populations	27.17%*	5.46%***	6.29%***
Within populations	72.83%*	94.54%***	93.71%***
Φ_{ST} and F_{ST}	0.272*	0.055***	0.063***

* P < 0.05, ** P < 0.01, *** P < 0.001

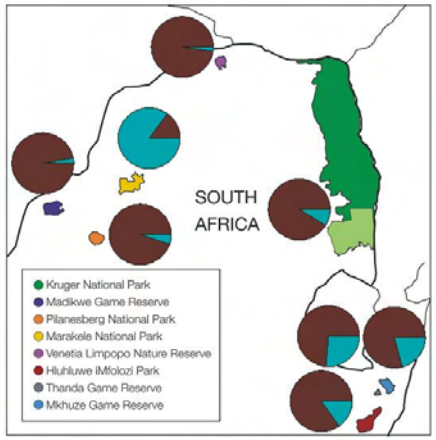
Based on only the microsatellite data, $K = 2$ produced the highest ΔK and split the individuals into a 'Marakele' group and a 'rest of South Africa' group (Fig. 3.4). From the history of previous translocations it was known that the Marakele founders originated from Botswana. Thus to fully understand the South African wild dog population structure, a very conservative approach was taken and all wild dogs in the first STRUCTURE results with a proportion of 'Marakele' membership $q > 0.05$ were removed and the STRUCTURE runs were repeated exactly as previously executed. This resulted in the identification of three groups ($K = 3$ produced the highest ΔK, Fig. 3.5).



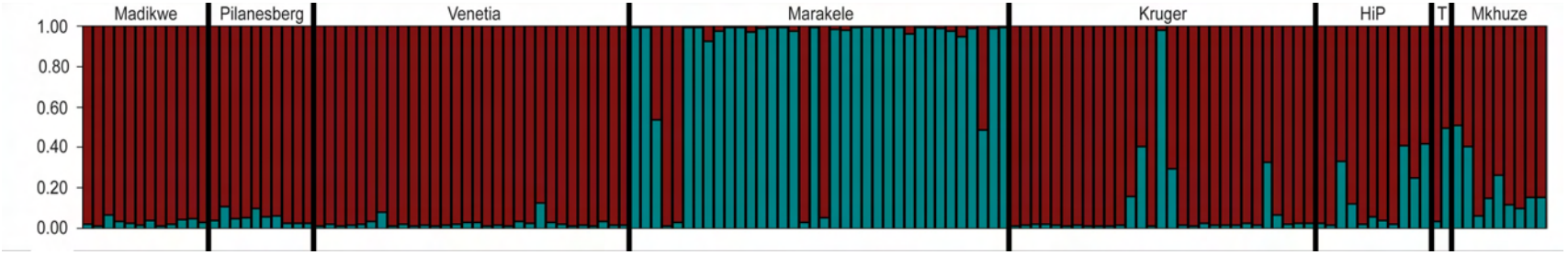
(a)



(b)



(c)



d)

Figure 3.4 Bayesian analysis of the genetic structure of wild dogs in South Africa based on 18 microsatellite loci. (a) Mean likelihood over 20 runs assuming *K* clusters (*K* = 1 - 6). (b) ΔK, where the modal value of the distribution is considered as the maximum level of structuring. (c) Map of South Africa showing the average proportion of each cluster (*K* = 2) within the managed metapopulation reserves and Kruger. (d) Individual assignment to each of the clusters (K = 2). Each column represents an individual, with coloured divisions indicating the likelihood of assignment to the corresponding cluster (T represents Thanda).

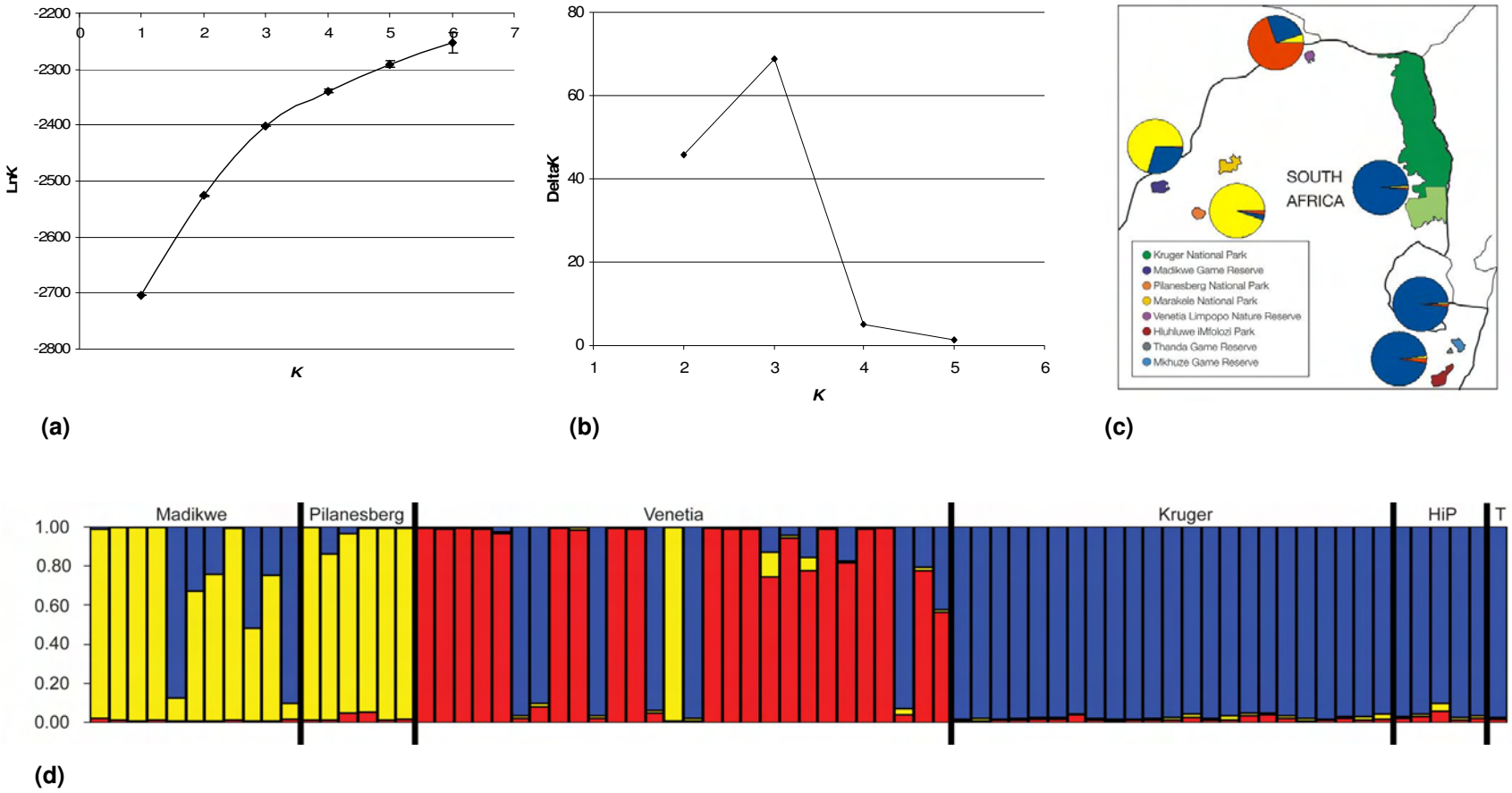


Figure 3.5 Bayesian analysis of the genetic structure of wild dogs (n = 78) in South Africa after individuals with more than 5% contribution of the 'Marakele' cluster from Fig. 3.4 was removed, based on 18 microsatellite loci. (a) Mean likelihood over 20 runs assuming *K* clusters (*K* = 1 - 6). (b) Δ*K*, where the modal value of the distribution is considered as the maximum level of structuring. (c) Map of South Africa showing the average proportion of each cluster (*K* = 3) within the associated managed metapopulation reserves and Kruger. The large circles' colours correlate with the colours in the following structure diagram. (d) Individual assignment to each of the clusters (K = 3). Each column represents an individual, with coloured divisions indicating the likelihood of assignment to the corresponding cluster (T represents Thanda).

3.3.3 Relatedness

The mean relatedness values based on the Queller & Goodnight (1989) estimator in GenAlEx (Peakall & Smouse 2006) were used to determine the relationship between the sampled individuals. Known relationships in this study between individuals based on field observations and their mean relatedness values are shown in Table 3.3. The known estimates were evaluated to better understand the relatedness of the unknown individuals. Relatedness values within the populations, reserves and packs ranged considerably (Table 3.4). The managed metapopulation had the lowest relatedness value, whilst Phabeni, Tshokwane and Pilanesberg had the highest values. Relatedness between the managed metapopulation reserves and Kruger showed a low value of R = -0.038. Between the managed metapopulation reserves, only the comparison between Pilanesberg and Madikwe had a relatedness value greater than Queller and Goodnight's unrelated value and fell between first and second order relatedness (Table 3.5). The relationship comparisons between Kruger's packs ranged from R = -0.198 to R = 0.283 (Table 3.6). Three packs fell within second order relatedness.

Table 3.3 Known individuals, their relationships in three managed metapopulation reserves (Marakele, Venetia and Mkhuze) and one Kruger pack (Berg en Dal), sample size (n) and their mean relatedness values (R) according to Queller and Goodnight (1989).

Reserve / Pack	Known relationship	n	*R*
Marakele	Mother - Offspring	10	0.515 ± 0.069
	Father - Offspring	10	0.523 ± 0.052
	Full sibs	9	0.449 ± 0.137
	Alpha male - Alpha female	2	0.175
Venetia	Mother - Offspring	6	0.544 ± 0.099
Mkhuze	Mother - Offspring	8	0.384 ± 0.102
Berg en Dal	Mother - Offspring	5	0.525 ± 0.079
	Full sibs	4	0.603 ± 0.099
	Alpha male - Alpha female	2	0.084

Table 3.4 Number of individuals (n) and mean relatedness values (*R*) within all populations and packs. The managed metapopulation reserves and Kruger packs are shown in descending order of relatedness.

Population/Reserve/Pack	n	*R*
South Africa	139	-0.006
Metapopulation	110	-0.001
Kruger	29	0.172
Phabeni	6	0.491
Tshokwane	2	0.479
Pilanesberg	10	0.443
Skukuza	7	0.437
Mthethamusho	4	0.432
Berg en Dal	7	0.390
Afsaal	3	0.373
Madikwe	12	0.295
Venetia	30	0.261
Mkhuze	9	0.246
Marakele	36	0.176
Hluhluwe iMfolozi *	11	0.125
Thanda	2	-0.134

* An unknown number of packs contribute to this sample.

Table 3.5 Mean relatedness values for pairwise comparisons between the eight managed metapopulation reserves. Sample sizes (n) are shown in parentheses.

	Madikwe (n = 12)	Pilanesberg (n = 10)	Venetia (n = 30)	Marakele (n = 36)	Kruger (n = 29)	HiP (n = 11)	Thanda (n = 2)	Mkhuze (n = 9)
Madikwe								
Pilanesberg	0.329							
Venetia	-0.063	0.023						
Marakele	-0.112	-0.076	-0.146					
Kruger	-0.001	-0.011	-0.005	-0.103				
HiP	-0.106	-0.021	0.095	-0.108	-0.016			
Thanda	-0.159	-0.102	-0.010	-0.091	-0.066	0.010		
Mkhuze	-0.062	-0.022	-0.005	-0.045	0.019	-0.070	-0.016	

Table 3.6 Mean relatedness values of pairwise comparisons between the six Kruger packs. Sample sizes (n) are shown in parentheses.

	Mthethamusho (n = 4)	Skukuza (n = 7)	Afsaal (n = 3)	Tshokwane (n = 2)	Phabeni (n = 6)	Berg en Dal (n = 7)
Mthethamusho						
Skukuza	0.100					
Afsaal	0.112	0.055				
Tshokwane	0.003	0.111	0.140			
Phabeni	0.126	0.100	0.283	-0.198		
Berg en Dal	0.195	0.051	0.275	0.068	0.228	

3.4 Discussion

The results of the present analysis of mtDNA and microsatellite variability among South African wild dogs suggest that the managed metapopulation may be more diverse than the Kruger population, potentially due to the high turn-over of individuals in these reserves. Both populations, however, retain reasonable levels of neutral variation with no indication of significant levels of inbreeding. Small sample sizes may affect the interpretation of the mtDNA data, but the Kruger appears to hold higher levels of historical diversity. Currently genotyped samples comprise two genetic clusters, one from Botswana and the other from South Africa. The 'South Africa' cluster can be further divided into three sub-clusters. A higher relatedness was seen within packs of wild dogs in Kruger than that of the managed metapopulation, again a reflection of the higher turn-over in metapopulation packs. Relatedness between packs within Kruger shows an isolation-by-distance pattern. These findings can be integrated in optimising the management of South Africa's most endangered carnivore.

3.4.1 Genetic diversity

Through investigation of both nuclear and mtDNA it is possible to look at diversity on two temporal scales. MtDNA diversity shows the diversity that has occurred historically in female (maternal) lineages (Boore 1999; Frankham *et al.* 2005), whereas the nuclear diversity is indicative of the current diversity across both male and female lineages (Schlötterer & Pemberton 1998).

Population sizes of the endangered African wild dog are low due to direct and/or indirect human persecution and also intra- and inter-specific competition (Creel & Creel 2002). This

has led to their low population densities (Creel & Creel 1996; Mills & Gorman 1997; Gorman *et al.* 1998). Such a low density of small populations would lead to reduced genetic diversity in a species and this can be a major component in increasing the risks of extinction (Frankham *et al.* 2005). In this study, the nucleotide diversity in Kruger was slightly higher than that found previously in Kruger (Girman *et al.* 2001), even though the number of haplotypes remained the same. Estimates of haplotype diversity (H_D) and nucleotide diversity (π) for the South African wild dogs were lower than that found by Koblmüller *et al.* (2009) in North America for coyotes (Eastern: $H_D = 0.780$, $\pi = 0.016$; Western: $H_D = 0.98$, $\pi = 0.018$), gray wolves ($H_D = 0.575$, $\pi = 0.006$) and Great Lakes wolves ($H_D = 0.741$, $\pi = 0.045$).

The genetic diversity of the South African wild dog population is directly comparable to other carnivores on a range of geographic scales. The endangered New World jaguar, *Panthera onca*, for example displays similar patterns of nucleotide diversity on both a continental ($H_D = 0.940$, $\pi = 0.008$, number of haplotypes (n_H) = 22) and regional scale (e.g. Central America: $H_D = 0.846$, $\pi = 0.004$, $n_H = 7$; Eizirik *et al.* 2001) when compared to the wild dog across the whole of Africa ($H_D = 0.788$, $\pi = 0.015$, $n_H = 8$; Girman *et al.* 2001) and just the South African region.

The heterozygosity levels based on microsatellite loci falls within the range of diversity reported for canids e.g. Roy *et al.* (1994) reported on the gray wolf (H_E: 0.565 – 0.741), coyote (H_E: 0.627 – 0.709), red wolf (*Canis rufus*; $H_E = 0.548$) and Kenyan golden jackal (*Canis aureus*; $H_E = 0.520$). The wild dog, however, falls at the lower end of the spectrum of diversity observed among these canids. It is important to consider the reproductive biology (family structure and breeding hierarchy) of an animal such as the wild dog. For instance, a highly successful breeding pair will influence the genetic diversity found within a pack and across a small population. However, this influence may not be evident in a regional population (encompassing multiple reserves) as packs and reserves within the population may differ with regards to breeding pairs in consecutive years. Whilst the reproductive success of the pack can be linked to pack size (Buettner *et al.* 2006), the reproductive output is restricted to a single pair. This reduces the effective population size of the species to being approximately equal to the number of breeding pairs and has a direct impact on the level of genetic diversity present. The disproportionate success of individual packs can contribute to this paucity of genetic diversity within individual reserves, due to the increased potential of a

single lineage to colonise empty habitat areas, and join neighbouring packs. A similar principle is likely to apply over broader geographic scales.

Wolves and wild dogs are pack animals with similar family structure and breeding hierarchies, whereas both coyotes and jackals are not. Mean allele numbers and sample sizes are of importance when comparing genetic diversity of species with different reproductive strategies (Hedrick 2005, and references therein). Wild dogs have a lower diversity than the other canids as, even though the mean allele number increases as the sample size increases (r = 0.877), the slope of this correlation in wild dogs is low and at high sample sizes the wild dogs' mean allele number is still lower than the other canids (Fig 3.6).

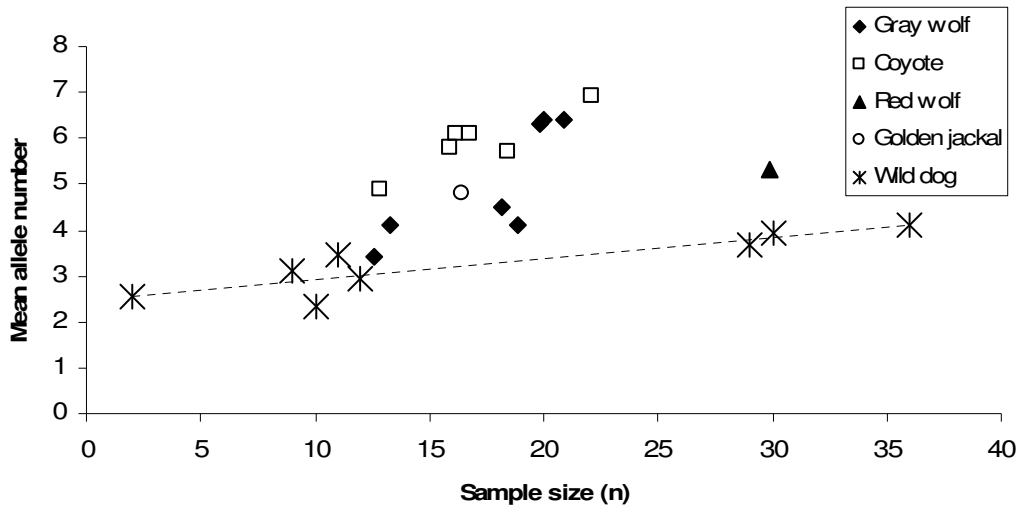


Figure 3.6 Relationship between sample size (n) and mean number of alleles (Â) for South African wild dogs (present study) and other canids (Roy *et al.* 1994). The line of best fit for the wild dogs is shown as a dashed line (Pearson's correlation, r = 0.877, P < 0.05, Â = 0.045n + 2.475) and shows a positive linear relationship between the variables.

The Kruger population appears to have been more genetically diverse historically than the managed metapopulation, whereas in recent times the managed metapopulation is more diverse than Kruger. Only three mtDNA haplotypes were found in this study and their relationship suggests a recent shared history of the maternal wild dog lineages in South Africa (see Fig. 3.3). These three haplotypes are in accordance with earlier genetic work in South Africa (Girman *et al.* 2001). This is interesting as even though Girman *et al.*'s samples were up to 70 years older (roughly 15.5 wild dog generations), there seems to be little

difference when compared with contemporary samples. Girman *et al.* (2001) only had one individual with the Z1 haplotype occurring in South Africa which they sampled from a museum specimen from the former Transvaal province. They presumed the sample was a historical Kruger wild dog. This could suggest that since that time (1930s) Kruger has lost genetic diversity as neither Girman *et al.* (2001) nor this study found that haplotype present in Kruger. However, the Z1 haplotype was found in two reserves in the managed metapopulation (Marakele and Mkhuze). Both of these reserves have individuals which originated from Botswana. This could indicate historical gene flow between Botswana and Kruger which may not be continuing between these two areas and is hence suggestive of a change in migration patterns or even implying that an important habitat corridor is no longer in use or currently unavailable.

The managed metapopulation has grown considerably in the last 10 years (WAG minutes 2009). Management of this population included founder wild dogs being actively placed where necessary to augment or reintroduce wild dog packs by specifically taking into account unrelated individuals. The fact that this population is managed along with its growth could be the reason for its higher genetic diversity compared to the Kruger population. The Kruger population is self-sustaining with very little human involvement. Another possible explanation for the difference of nuclear DNA diversity between these two populations is that the number of wild dogs in Kruger has decreased, leading to a loss of diversity.

3.4.2 Population structure

It is difficult to draw robust conclusions about population structure at a fine scale from the 435 bp segment of the mtDNA control region due to the presence of only three haplotypes and our analysis of only 20 individuals. However, one haplotype (S1) was unique to Kruger and similarly another haplotype (Z1) was unique to two of the managed metapopulation reserves. This supports the historical separation of these populations. The former presence of the Z1 haplotype in South Africa likely reflects low levels of historical female gene flow across southern Africa.

Koblmüller *et al.* (2009) tested nuclear data at Hardy-Weinberg equilibrium to identify potential subpopulations, demonstrating an effective method of revealing cryptic population structure. This type of approach is implemented in STRUCTURE (Falush *et al.* 2007). Limitations are inevitable in all analyses, and any clustering method can be vulnerable to

over-representation. For example, a single individual from a divergent breeding population in an analysis will be grouped within the most similar genetic cluster represented in the data. I found that some reserve sub-populations grouped within the same genetic cluster. A strong genetic group originated from one particular reserve, Marakele (Fig. 3.4), of which the founder dogs were of Botswana origin. This explains the Botswana cluster that was found in the Mkhuze wild dogs, whose alpha female originated from Marakele. Wild dogs with membership to multiple genetic clusters can largely be explained through managed metapopulation translocations. Surprisingly Kruger has three admixed individuals ($q < 0.80$) and one that grouped with $q = 0.98$ to the Marakele cluster. From photographic evidence I know that none of the sampled wild dogs in this study were founder wild dogs from Marakele. However, the founder wild dogs (male) in Balule, a private game reserve which borders a section of the eastern perimeter of central Kruger (see Fig. 3.7), originated in Marakele. In the 2009 wild dog census it was apparent that some of these Balule wild dogs had dispersed into Kruger, potentially providing the source of the admixed Kruger individuals. Due to the Botswana founder wild dogs in Marakele (and subsequently elsewhere in South Africa), the STRUCTURE analysis was rerun without the influence of this genetic cluster.

The re-analysis of wild dogs excluding those individuals containing a 5% or greater membership to the Marakele cluster restricted the analysis to South African individuals without possible introgression from wild dogs originating in Botswana. Three genetic clusters were evident. The cluster with the highest membership contained individuals from Kruger, Hluhluwe and Thanda (mean $q = 0.97$). Despite a high membership to the Kruger/Hluhluwe cluster being evident in the other reserves (more than 20% in 14 individuals in the other reserves), there was a strong membership of Venetia to its own genetic cluster, and a shared membership of Madikwe and Pilanesberg to a third genetic cluster. The genetic cluster found in Venetia was not significantly represented in any other reserve (Fig 3.5). Venetia's founder wild dogs were free-ranging individuals from farmland roughly 20 km south of Venetia (Davies-Mostert unpublished data). One of these wild dogs became the alpha female for the next few years, breeding with another free-ranging male caught in Hoedspruit (see Fig. 3.7). Both of these wild dogs belong to the Venetia genetic cluster. However, the male has a high membership to both the Kruger/Hluhluwe and Pilanesberg genetic clusters. All of these alphas' offspring group into the Venetia cluster. The one individual in Venetia which groups to the Pilanesberg cluster is a wild dog which was translocated from Pilanesberg into Venetia but never bred (Fig. 3.7). The remaining wild dogs which group into the Kruger/Hluhluwe

cluster are individuals that have been translocated from Hluhluwe into Venetia. The founder wild dogs of Pilanesberg were two free-ranging adult females (plus four pups) from a farm near Venetia and three males from Cango Wildlife Ranch, a captive facility in the Eastern Cape Province, South Africa. Both of these origins or the subsequent mixture of these individuals could be the explanation for the Pilanesberg genetic cluster. This cluster is not surprisingly shared between Pilanesberg and Madikwe as five males from Pilanesberg were translocated to Madikwe leaving their sisters in Pilanesberg. The Kruger/Hluhluwe cluster present in the Madikwe wild dogs can be explained by translocations from both Kruger and a captive breeding facility, De Wildt Cheetah and Wildlife Centre.

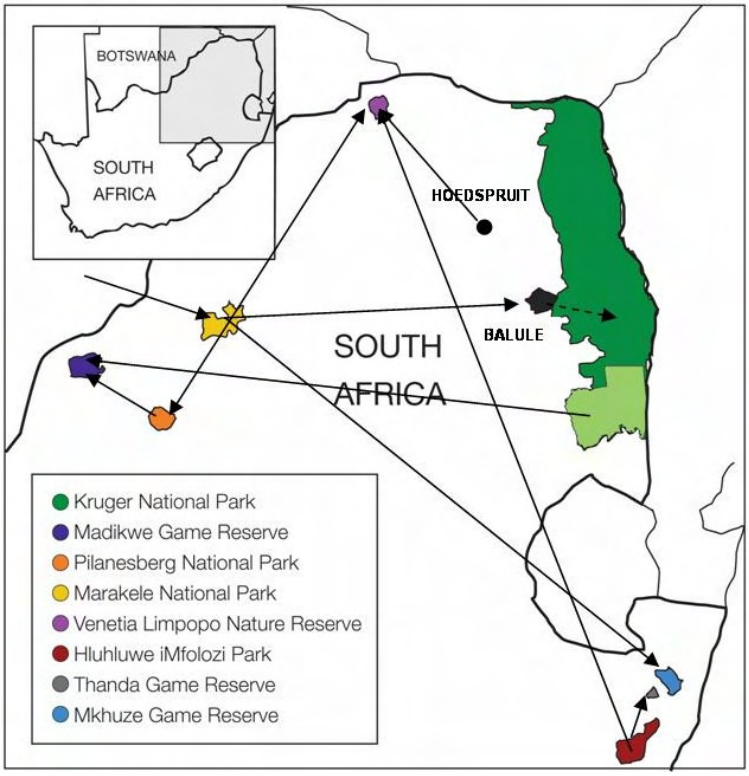


Figure 3.7 Reintroductions and translocations of wild dogs in South Africa that were evident in the findings of this study. Known movements are represented by solid arrows. The dashed arrow represents a possible dispersal of wild dogs from Balule into Kruger.

3.4.3 Relatedness

The pairwise relatedness values between the managed metapopulation packs were lower than those observed between the Kruger packs (Table 3.6). This indicates that inbreeding

avoidance has been effectively achieved through active management. It also shows that, under conditions as seen in Kruger, the relatedness between packs can be as high as that of a second order relative. This could be expected in packs occurring reasonably close to each other, for example, any pack within southern Kruger where the possibility of dispersers finding other dispersers or packs is relatively high, such as dispersers from Berg en Dal, Afsaal and Phabeni. The opposite would be expected for packs further away from each other (e.g. Tshokwane and Mthethomusha, R = 0.003). Interestingly, when relatedness values were considered at individual level (results not shown), pairwise relatedness between several individuals from Berg en Dal, Afsaal and Phabeni, was at the level of first order relatives. This suggests that siblings (not necessarily litter mates) have dispersed between and settled in these packs. This is unlikely to happen in the managed metapopulation due to the management and control over which wild dogs are translocated and the reserves involved. Although, unlikely natural dispersal can on occasion happen between managed metapopulation reserves such as when Marakele females dispersed (on their own accord) to Pilanesberg where sibling wild dogs were already residing (Davies-Mostert unpublished data).

Individual relatedness values can confirm relatedness between unknown wild dogs, for instance the wild dog in Kruger that groups into the Marakele cluster (Fig. 3.4). Due to photographic evidence (via unique coat pattern identification) it is known that this individual is not a translocated dog. However, if it is not related to any of the translocated individuals within a first order relatedness, it could possibly be that this individual dispersed into Kruger from either Botswana or Marakele. This particular individual was related to the Marakele males in Balule with either first or second order relatedness. This is indicative that the individual in Kruger could be an offspring of the first order related Balule male.

The Kruger packs fell into a range between first order and second order relatedness. This may be closer to the expected relatedness value for wild dog packs in natural conditions given the species' family structure. This can thus serve as a relatedness guideline for managing the managed metapopulation reserves. However, in order to avoid future inbreeding events, very strict management regimes will have to be set. The managed metapopulation reserves (except Pilanesberg) ranged from second order relatedness to unrelated, as was the case for Thanda. As the metapopulation reserves are managed and non-family individuals are brought in and other family individuals are removed for translocation purposes, this will break down the family structures and reduce the relatedness

within the pack. Sample size and sampling bias will also have effects on relatedness, e.g. only two individuals were sampled in Thanda and it just so happened that they were unrelated wild dogs, giving Thanda the appearance of having the least related wild dogs. This stresses the importance of knowing relatedness at an individual level before translocating dogs between reserves. In addition, the recent photographic survey count of only approximately 120 wild dogs in Kruger raises the question as to whether Kruger is the best self-sustaining population of wild dogs to base management strategies on for the managed metapopulation. Another self-sustaining viable population elsewhere in southern Africa with a larger population of individuals may prove to be of more long term guidance for the managed metapopulation.

3.4.4 Management

Even though the management interventions are intended to mimic natural dispersals, this does not guarantee breeding opportunities for translocated individuals and their genetic legacy is not always passed on. The current genetic diversity in a reserve will thus not necessarily be represented in future generations. Effective management requires an awareness of the parentage/ origins of individuals, particularly of dispersers. For example, if a single reproductively successful pack is producing most of the dispersers in a region, this will have implications for possible over-representation of this pack and subsequent inbreeding in that area. Additionally, while a pack as a whole may appear reproductively successful, it is in fact generally the single mating pair that is reproducing, i.e. an effective population size of two per pack. Careful translocation management combined with genetic monitoring would provide a solution to this diminished diversity.

Current management strategies in South Africa are considering two conservation units: the Kruger and the managed metapopulation. One aim of this study was to evaluate the justification of keeping those units separate. Despite the lack of informativeness at the finer scale, the mtDNA tentatively supports the maintenance of two management units (see population structure section). The nuclear data indicate that the separation between the Kruger population and the managed metapopulation represents two reservoirs of genetic diversity. When considering private alleles, three alleles are unique to Kruger and 23 unique to the managed metapopulation (out of 86 alleles). It is likely that this reflects a recent trend in the population dynamics since the managed translocations began.

The three apparent genetic clusters found in the fine scale population structure (excluding individuals in the Botswana cluster) could be used as the management units themselves. This raises the question of the importance of the free-ranging wild dogs in South Africa. They may represent one of the only sources of gene flow among Kruger and the other populations (apart from the actively managed translocations). Also, if they constitute the Venetia cluster then they too are an important population and should not be overlooked when conserving the wild dogs of South Africa. However, when does one draw the lines with management units and conserve the South African wild dogs as one population? With so few wild dogs left in South Africa, the best solution may be to manage one population concentrating on increasing the future wild dogs' genetic variability. However, a pack of wild dogs in a reserve is not a population as it is effectively two breeding alpha individuals. Too little genetic exchange between small subpopulations will result in genetic drift. Too much will result in overall homogeneity which in turn could lead to fewer genetic combinations thus decreasing local adaptation to diseases. I suggest an intermediate management method whereby historical inter-regional gene flow is mimicked in an isolation-by-distance pattern.

The STRUCTURE analysis of the entire dataset suggests that there are two genetic clusters within South African wild dogs: Marakele and the rest of South Africa. As this split represents the strongest genetic differentiation present, these two genetic clusters could form potential management units. Knowing that the Marakele cluster is evident in Botswana (due to translocation of the Marakele wild dogs to Tuli Game Reserve, Botswana; WAG minutes 2007), South African management strategies may be to prioritise the genetic cluster originating in South African wild dogs. This would preclude the inclusion of Marakele individuals in a breeding program. The alternative consideration is that if it is feasible that gene flow can occur naturally between Botswana and the eastern side (KwaZulu-Natal) of South Africa, then it may be rationalised that the Botswana animals represent part of a greater breeding population that extends into South Africa. One could expect gene flow to occur between Botswana and the Limpopo Province (central northern side of South Africa). However, due to translocations the Marakele cluster is now present in Mkhuze (in KwaZulu-Natal). This raises two questions: first whether wild dogs would ever have moved naturally from the west into KwaZulu-Natal and second whether South African wild dogs should be managed on a fine scale or regionally ?

From a management perspective I am interested in identifying individuals for translocations and genetic compatibility. This means I need to identify related individuals for inbreeding avoidance in the absence of a complete and accurate pedigree. For instance, Pilanesberg and Madikwe are the two reserves with the highest pairwise relatedness value (Table 3.5). This can be explained through the knowledge of previous translocations. However, without that knowledge, just the fact that their relatedness value is high suggests that they would be poor candidates for future translocations. Many of the reserves are on average unrelated suggesting that future translocations between them would be optimal. However, the pairwise comparisons of the reserves should only be used as an indicator for potential translocations due to averaging the individuals' relatedness values. A pairwise comparison of specific individuals should rule the decision for translocations of optimum genetic variability.

3.4.5 Conclusions

Breeding programs and translocation issues need a genetic component for adequate management planning and long term conservation of species, even if this does not simplify the situation. The genetic investigation of variability and cryptic population structure reported in this dissertation again raises concern over the defining of management units and what is best for the survival of the wild dog.

I set out to test four predictions. First, I predicted that the Kruger population would have higher levels of genetic diversity at both mtDNA and nuclear DNA level than the managed metapopulation. Based on microsatellites the managed metapopulation currently is more diverse than the Kruger population. This contrasts with the mtDNA evidence that suggests that Kruger was historically more diverse than the managed metapopulation. One possible reason for this is that the number of wild dogs in Kruger has decreased and this decline may have led to the loss of some of Kruger's diversity. Alternatively the high turnover of individuals and instability of packs in the managed metapopulation has led to an increased nuclear DNA diversity.

Second, I expected evidence of gene flow from other southern African countries in the analysis of variation among South African wild dogs. The S2 haplotype that is most common in the mtDNA analysis was also found by Girman *et al.* (2001) in Botswana, Zimbabwe and Namibia. In this current study the Z1 haplotype, previously recorded in Botswana and Zimbabwe (Girman *et al.* 2001), was recorded in South Africa. However, this haplotype was

traced to translocated founder wild dogs from Botswana. On the nuclear DNA level, when looking at population structure, introgression from Botswana (brought in through translocations) was observed in the South African wild dogs. This was evident through clustering among those individuals and the separation from other South African wild dogs. When the individuals forming the Botswana cluster were removed from the analysis, three clusters of wild dogs were observed, suggesting three subpopulations of South African wild dogs. Currently South Africa is managing two conservation units, namely Kruger and the managed metapopulation. These units are supported by both mtDNA and nuclear DNA when considering unique haplotypes and alleles. However, over a greater geographical scale South African wild dogs have historically been linked to wild dogs from other southern African countries (see Girman *et al.* 2001). When we looked at the nuclear DNA of the South African wild dogs without the influence of the Botswana three genetic clusters were revealed.

The number of wild dogs in South Africa is very low; this should prompt a shift from individual population management towards national management of the South African dogs as a single unit, focussing on increasing the genetic variability of future individuals. The stepwise method of gene exchange between neighbouring packs and reserves should be taken into account. The management plan should possibly be more scientific and less opportunistic than that currently being practised.

Third, I predicted that a higher relatedness within packs is expected in Kruger as it has greater pack stability than the managed metapopulation. The relatedness value within packs in Kruger was higher than that found in the managed metapopulation. The family structures in the managed metapopulation have been broken down due to managed translocations between the reserves whereby non-family individuals are brought in and other family individuals are moved out.

Fourth, I predicted that relatedness between packs in Kruger would show an isolation-by-distance pattern. The Berg en Dal, Afsaal and Phabeni packs are closely residing packs in Kruger. The pairwise relatedness comparisons for these packs range from 0.228 to 0.280. These relatedness values fall within second order relatedness. If the Phabeni and Tshokwane packs, which are not closely residing packs, are compared the relatedness value is very low (R = -0.198). These differences in distances between the packs and the relatedness values confirm an isolation-by-distance pattern in Kruger.

Information gathered about relatedness within and between reserves and populations can serve as an indicator of previous translocations. More importantly such information can suggest reserves that are potentially good for future translocations. Current data allow pairwise relatedness calculations to be made between individuals. Optimal translocations can be made based on these relatedness values. In this way, inbreeding can be limited or avoided altogether despite low numbers of wild dogs in South Africa. It is suggested that for future translocations, individuals to be united should have a pairwise relatedness value of less than 0.125 as shown for most between-pack comparisons in Kruger. The preference for translocation should be a bias towards the lowest values and the likely trend of isolation-by-distance needs to be kept in mind.

After careful consideration of the low numbers and the historical and recent trends in the genetic structure of the South African wild dogs, I suggest that the South African wild dogs should be managed as a whole (the managed metapopulation and the Kruger population), keeping the translocations as localised as possible, imitating what natural dispersing wild dogs may do in the wild. Possible translocations of wild dogs from other southern African countries into South Africa (also in an isolation-by-distance pattern) should be considered when more is known about the genetic structure of the other countries' wild dogs.

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3.7 Appendix

Appendix 3.1 Microsatellite results for the 18 loci in each managed metapopulation reserve, Kruger National Park pack and population. For each sampling site the following are shown: Number of alleles (A), observed heterozygosity (Ho), expected heterozygosity (He) and probability value for Hardy-Weinberg equilibrium test (p).

	Madikwe (n = 12)				Pilanesberg (n = 10)				Venetia (n = 30)				Marakele (n = 36)			
Locus	A	Ho	He	p	A	Ho	He	p	A	Ho	He	p	A	Ho	He	p
REN162C04	5	0.636	0.706	0.140	3	0.444	0.386	1.000	5	0.556	0.551	0.087	6	0.829	0.798	0.136
INU030	3	0.750	0.518	0.292	3	0.700	0.647	1.000	5	0.700	0.702	0.012	6	0.722	0.650	0.399
AHT137	5	0.833	0.725	0.911	4	0.600	0.563	0.796	5	0.733	0.615	0.167	5	0.861	0.732	0.015
FH2848	2	0.417	0.344	1.000	2	0.400	0.356	1.000	4	0.808	0.658	0.145	5	0.722	0.720	0.032
REN105L03	2	0.455	0.368	1.000	2	0.800	0.533	0.428	6	0.708	0.757	0.413	5	0.722	0.649	0.397
AHTh260	3	0.750	0.627	0.673	3	0.143	0.538	0.021	4	0.542	0.506	1.000	5	0.771	0.662	**0.000**
AHTh171	3	0.500	0.467	0.705	*				5	0.536	0.505	0.023	6	0.917	0.772	0.082
FH2054	3	0.500	0.649	0.367	2	0.600	0.526	1.000	5	0.724	0.699	0.112	5	0.914	0.720	0.303
REN54P11	5	0.917	0.793	0.907	3	0.500	0.621	0.394	4	0.731	0.655	0.045	4	0.600	0.574	0.333
REN64E19	2	0.455	0.455	1.000	2	0.556	0.529	1.000	3	0.714	0.595	0.591	3	0.400	0.434	0.350
INU055	3	0.583	0.565	1.000	2	0.667	0.523	0.539	3	0.667	0.632	0.014	4	0.667	0.583	0.065
LEI004	4	0.833	0.736	1.000	2	0.300	0.521	0.246	4	0.621	0.564	0.336	4	0.639	0.550	0.099
AHTh130	3	0.909	0.645	0.355	3	0.556	0.582	0.711	5	0.464	0.411	0.398	3	0.629	0.557	0.296
INRA21	2	0.250	0.228	1.000	3	0.400	0.353	1.000	3	0.233	0.368	0.006	4	0.639	0.647	0.414
AHTk211	2	0.545	0.485	1.000	2	0.556	0.425	1.000	2	0.571	0.444	0.197	2	0.314	0.269	0.565
REN169D01	2	0.417	0.344	1.000	2	0.556	0.425	1.000	2	0.067	0.066	1.000	2	0.194	0.178	1.000
REN247M23	2	0.545	0.485	1.000	2	0.400	0.356	1.000	2	0.440	0.429	1.000	2	0.056	0.055	1.000
CXX279	2	0.333	0.290	1.000	*				4	0.138	0.134	1.000	3	0.086	0.137	0.017
Mean	2.944	0.590	0.524	0.797	2.333	0.454	0.438	0.758	3.944	0.553	0.516	0.364	4.111	0.593	0.538	0.306
s.d.	1.079	0.194	0.163	0.300	0.745	0.218	0.178	0.329	1.177	0.208	0.182	0.385	1.329	0.263	0.222	0.305

Appendix 3.1 (continued)

	Hluhluwe iMfolozi Park (n = 11)				Thanda (n = 2)				Mkhuze (n = 9)				Metapopulation Reserves (n = 110)			
Locus	A	Ho	He	p	A	Ho	He	p	A	Ho	He	p	A	Ho	He	P
REN162C04	2	0.273	0.506	0.214	2	0.500	0.500	1.000	6	0.778	0.745	0.448	6	0.635	0.750	**0.000**
INU030	4	0.455	0.619	0.098	3	0.500	0.833	0.329	4	0.889	0.712	1.000	6	0.700	0.750	**0.000**
AHT137	4	0.909	0.714	0.812	3	1.000	0.833	1.000	4	1.000	0.784	0.568	5	0.818	0.708	**0.000**
FH2848	4	0.727	0.688	0.255	4	1.000	1.000	1.000	4	0.778	0.706	0.683	5	0.703	0.731	0.026
REN105L03	6	1.000	0.831	0.966	4	1.000	1.000	1.000	2	0.667	0.471	0.457	8	0.724	0.736	**0.001**
AHTh260	4	0.455	0.593	0.182	2	0.500	0.500	1.000	4	1.000	0.758	0.587	5	0.650	0.726	0.005
AHTh171	5	0.818	0.749	0.680	3	1.000	0.833	1.000	4	0.778	0.575	0.756	6	0.667	0.685	**0.003**
FH2054	4	0.909	0.727	0.247	3	1.000	0.833	1.000	3	0.556	0.503	0.347	5	0.759	0.704	0.162
REN54P11	4	0.727	0.593	0.716	2	0.000	0.667	0.334	3	0.556	0.451	1.000	5	0.663	0.661	0.536
REN64E19	3	0.818	0.658	0.722	3	0.500	0.833	0.334	3	1.000	0.686	0.212	4	0.600	0.640	0.049
INU055	4	0.909	0.762	0.888	3	1.000	0.833	1.000	2	0.444	0.471	1.000	4	0.670	0.680	0.274
LEI004	3	0.636	0.567	1.000	2	0.500	0.500	1.000	4	0.750	0.592	1.000	4	0.630	0.657	**0.001**
AHTh130	4	0.455	0.519	0.232	3	0.500	0.833	0.334	3	0.556	0.660	0.183	6	0.581	0.578	0.134
INRA21	2	0.455	0.368	1.000	3	1.000	0.833	1.000	4	0.556	0.529	0.494	5	0.445	0.611	0.003
AHTk211	2	0.636	0.506	0.554	2	0.500	0.500	1.000	2	0.778	0.503	0.176	2	0.505	0.491	0.842
REN169D01	2	0.364	0.312	1.000	*				2	0.333	0.294	1.000	3	0.239	0.215	0.750
REN247M23	2	0.273	0.368	0.439	2	0.500	0.500	1.000	*				2	0.253	0.279	0.462
CXX279	3	0.273	0.255	1.000	*				*				5	0.131	0.142	0.220
Mean	3.444	0.616	0.574	0.612	2.556	0.611	0.657	0.833	3.111	0.634	0.525	0.620	4.778	0.576	0.597	0.193
s.d.	1.117	0.240	0.161	0.336	0.831	0.356	0.286	0.298	1.242	0.290	0.224	0.311	1.436	0.186	0.185	0.274

Appendix 3.1 (continued)

	Skukuza (n = 7)				Mthethomusha (n = 4)				Afsaal (n = 3)				Tshokwane (n = 2)			
Locus	A	Ho	He	p	A	Ho	He	p	A	Ho	He	p	A	Ho	He	P
REN162C04	2	0.571	0.440	1.000	3	0.750	0.607	1.000	3	1.000	0.733	1.000	3	1.000	0.833	1.000
INU030	3	0.857	0.670	1.000	3	0.750	0.714	1.000	3	0.667	0.600	1.000	3	1.000	0.833	1.000
AHT137	3	0.714	0.670	0.777	2	0.750	0.536	1.000	4	1.000	0.867	1.000	2	0.500	0.500	1.000
FH2848	3	0.857	0.703	1.000	3	0.500	0.607	0.426	2	0.667	0.533	1.000	2	1.000	0.667	1.000
REN105L03	3	0.857	0.703	1.000	*				*				3	1.000	0.833	1.000
AHTh260	3	0.714	0.615	1.000	2	0.250	0.250	1.000	2	0.667	0.533	1.000	*			
AHTh171	2	0.429	0.363	1.000	*				3	1.000	0.733	1.000	2	0.500	0.500	1.000
FH2054	3	0.571	0.582	1.000	3	0.750	0.679	1.000	3	1.000	0.733	1.000	*			
REN54P11	3	0.857	0.692	0.262	3	0.750	0.679	1.000	2	0.333	0.333	1.000	3	1.000	0.833	1.000
REN64E19	3	0.429	0.560	0.160	4	0.750	0.821	0.313	2	0.333	0.333	1.000	2	0.500	0.500	1.000
INU055	2	0.286	0.264	1.000	2	0.750	0.536	1.000	2	0.333	0.600	1.000	2	0.500	0.500	1.000
LEI004	2	0.429	0.363	1.000	2	0.500	0.429	1.000	2	0.333	0.333	1.000	2	0.500	0.500	1.000
AHTh130	3	0.857	0.670	1.000	3	0.500	0.714	0.087	3	0.667	0.733	1.000	3	1.000	0.833	1.000
INRA21	2	0.286	0.264	1.000	2	0.750	0.536	1.000	2	0.667	0.533	1.000	2	0.500	0.500	1.000
AHTk211	2	0.571	0.527	1.000	2	0.250	0.536	0.429	2	0.333	0.333	1.000	2	0.500	0.500	1.000
REN169D01	2	0.429	0.363	1.000	2	0.500	0.429	1.000	2	0.333	0.333	1.000	2	0.500	0.500	1.000
REN247M23	2	0.429	0.363	1.000	*				2	0.333	0.333	1.000	*			
CXX279	*				*				*				*			
Mean	2.444	0.563	0.490	0.894	2.222	0.472	0.448	0.804	2.278	0.537	0.478	1.000	2.056	0.556	0.491	1.000
s.d.	0.598	0.240	0.193	0.263	0.853	0.299	0.270	0.331	0.731	0.317	0.240	0.000	0.705	0.369	0.296	0.000

Appendix 3.1 (continued)

	Phabeni (n = 6)				Berg en Dal (n = 7)				Kruger (n = 29)				South Africa (n = 139)			
Locus	A	Ho	He	p	A	Ho	He	p	A	Ho	He	p	A	Ho	He	P
REN162C04	3	0.833	0.667	1.000	4	0.286	0.396	0.229	5	0.655	0.700	0.081	6	0.639	0.781	**0.000**
INU030	3	0.667	0.667	0.721	3	0.714	0.692	1.000	6	0.759	0.793	0.626	7	0.712	0.777	**0.000**
AHT137	2	0.500	0.409	1.000	4	0.857	0.659	0.535	5	0.724	0.778	0.223	6	0.799	0.735	**0.002**
FH2848	2	0.667	0.485	1.000	2	0.286	0.264	1.000	4	0.621	0.600	0.096	5	0.685	0.709	0.161
REN105L03	2	0.333	0.303	1.000	2	0.714	0.495	0.442	5	0.517	0.527	0.115	8	0.677	0.698	**0.001**
AHTh260	2	0.500	0.409	1.000	3	0.857	0.582	0.252	3	0.586	0.532	0.866	5	0.636	0.718	**0.000**
AHTh171	2	0.500	0.409	1.000	3	0.714	0.703	0.808	5	0.517	0.731	0.007	6	0.635	0.703	**0.001**
FH2054	3	0.833	0.667	1.000	3	0.857	0.692	0.761	4	0.724	0.666	1.000	5	0.752	0.696	0.247
REN54P11	3	0.667	0.682	0.686	2	0.429	0.538	1.000	4	0.655	0.673	0.353	5	0.662	0.666	0.698
REN64E19	3	0.667	0.667	0.083	3	0.571	0.604	1.000	4	0.552	0.623	**0.001**	4	0.590	0.649	**0.000**
INU055	2	0.167	0.167	1.000	2	0.429	0.495	1.000	3	0.379	0.493	0.297	4	0.609	0.662	0.102
LEI004	2	0.500	0.409	1.000	2	0.714	0.495	0.441	3	0.517	0.417	0.452	4	0.606	0.623	**0.002**
AHTh130	3	0.833	0.682	1.000	2	0.429	0.495	1.000	4	0.690	0.666	0.906	6	0.604	0.614	0.395
INRA21	2	0.400	0.356	1.000	4	0.571	0.495	1.000	4	0.500	0.458	0.791	6	0.457	0.597	0.007
AHTk211	2	0.500	0.530	1.000	2	0.714	0.495	0.441	2	0.517	0.509	1.000	2	0.507	0.495	0.861
REN169D01	*				*				2	0.241	0.216	1.000	3	0.239	0.218	0.619
REN247M23	*				2	0.143	0.143	1.000	2	0.172	0.160	1.000	2	0.234	0.254	0.477
CXX279	*				*				*				5	0.103	0.113	0.176
Mean	2.167	0.476	0.417	0.899	2.500	0.516	0.458	0.744	3.667	0.518	0.530	0.519	4.944	0.564	0.595	0.208
s.d.	0.687	0.272	0.237	0.249	0.898	0.274	0.214	0.300	1.291	0.199	0.212	0.397	1.545	0.184	0.192	0.281

* Monomorphic locus

Numbers in bold represent significant p-values after Bonferroni correction ($p < 0.0027$)

Chapter 4

Concluding synthesis

4.1 Synthesis

The African wild dog (*Lycaon pictus*) is classified as endangered (IUCN 2009) and in South Africa the Kruger National Park (Kruger) represents the only local conservation area with a self-sustaining population. In 1997 it was decided to actively increase conservation efforts for wild dogs in South Africa and manage another population, consisting of several isolated reserves around the country, as a managed metapopulation (Mills *et al.* 1998). Management programmes, especially when it comes to an endangered species and translocation issues, need a genetic component for adequate planning and long term conservation - even if this does not necessarily simplify the situation (Avise 1989; O'Brien 1994).

This dissertation had two main objectives. The first objective was to determine the wild dog population structure in southern Kruger with aid of text message notifications and tourist reports over a three month period. The second objective was to obtain genetic information from wild dogs in the managed metapopulation and Kruger to provide a basis for sound population management for the managed metapopulation similar to that found in large self-sustaining populations (such as Kruger).

More than 300 reported wild dog sightings were received in three months which enabled me to identify and make a count of individuals and packs, and sample a subset of each located pack in southern Kruger. The new technique of using mobile phone technology and utilising the public was a great success not only for the information it allowed me to collect but also for the publicity it generated, creating awareness of this endangered species and facilitating education to the public. It is imperative to involve the general public in conservation actions if we are to succeed in saving our threatened and endangered species. This new technique definitely has its advantages; it can be used to supplement other monitoring approaches, especially for elusive predators, and in any reserve, game park or farm. However, these

areas have to meet the prerequisite conditions of large numbers of tourists and adequate mobile phone reception.

Genetic methods are a valuable component of multi-disciplinary approaches within conservation and should be applied more broadly, especially for elusive species that cannot easily be completely understood through other methods alone. Conservation genetics only represents one approach and thus should not be considered by itself but rather as one of many data sets.

The genetic investigation of variability and cryptic population structure reported in this dissertation again raises concern over the defining of management units and what is best for the survival of the wild dog. After careful consideration of the low numbers and the historical and recent trends in the genetic structure of the South African wild dogs, I suggest that the South African wild dogs should be managed as a whole (the managed metapopulation and the Kruger population), keeping the translocations as localised as possible, imitating what naturally dispersing wild dogs may do in the wild. Possible translocations of wild dogs from other southern African countries into South Africa (also in an isolation-by-distance pattern) should be considered only when more is known about the genetic structure of the other countries' wild dogs.

From a genetic point of view, the management of the metapopulation has been successful thus far, especially in terms of inbreeding avoidance. However, it is too presumptuous to know if natural gene flow is being imitated. Kruger is perhaps not the only benchmark by which the management of the metapopulation should be guided, especially after the 2009 Kruger wild dog census (unpublished data) reported only ~ 120 individuals. Comparing the Kruger population, and subsequently the managed metapopulation, to another large population of wild dogs ultimately will also facilitate the definition of biologically meaningful conservation units for the species.

4.2 Future research

It is suggested that biological data be gathered and broad scale neutral and adaptive genetic variation analysed from more South African (and other countries') wild dogs, including the

free-ranging individuals outside of protected areas, as well as the captive populations. In order to improve the long term status of wild dogs the results of the analyses should be incorporated into management considerations. The wild dogs residing in northern Kruger may be of considerable conservation value and important links between neighbouring countries' wild dog populations. Wild dog microsatellite markers should be developed to optimise the resolution for population level analyses. Variability at adaptive loci should also be integrated before making a final decision as to the most appropriate scale for effective African wild dog conservation. I suggest, as a high priority, a similar project to this current study be done comparing Kruger to another large, self-sustaining population of wild dogs in another southern African country – possibly Botswana.

4.3 References

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