Behavioural ecology, genetics and success of African wild dogs *Lycaon pictus* (Temminck, 1820) in KwaZulu-Natal, South Africa

by
Penny Ann Spiering

Submitted in partial fulfilment of the requirements of the degree
Doctor of Philosophy (Wildlife Management)
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Supervisors: Prof. M.J. Somers
Dr. M. Szykman Gunther
Dr. J.E. Maldonado
Dr. D.E. Wildt

Declaration

I, Penny A. Spiering, declare that the thesis hereby submitted in partial fulfilment of the requirements of the degree Doctor of Philosophy (Wildlife Management) at the University of Pretoria has not been submitted by me for any other degree at any other institution.

Signature:

Date: 9 June 2010

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Abstract

The African wild dog (*Lycaon pictus*) is one of Africa's most endangered species, with fewer than 5,000 individuals surviving in the wild. Most of the remaining populations are increasingly fragmented and small, making loss of genetic diversity, reduced gene flow and inbreeding depression major concerns for the species. Recent efforts to conserve and increase severely declined numbers of wild dogs in South Africa have been focused on reintroductions to small protected areas to form a large metapopulation. The subpopulation in KwaZulu-Natal (KZN) was the first created and is currently the second largest population in the country with approximately 114 individuals. The overall goal

for wild dog conservation in KZN is to establish a large, self-sustaining population within the province. This study evaluated the genetics and behaviour of the wild dogs in the province and explored how these factors influence long-term population health. An assessment of the progress made through reintroduction efforts to date was conducted by examining the current genetic diversity of the population and the pack and population dynamics shaping it. These findings were also used to investigate strategies for advancing the population to viable levels in the future.

The scope of the project was divided into five topics to address the most crucial and intriguing aspects of African wild dog biology, conservation and management. First, non-invasive faecal genetic techniques were refined for field sampling of wild dogs in order to accurately genotype individuals and assess population heterozygosity. Second, the influences of inbreeding and genetic heterozygosity on fitness traits were investigated to determine if effects of inbreeding depression were apparent in the current population. Third, reproductive sharing among dominant and subordinate wild dogs was evaluated using genetic parentage analyses and long-term behavioural and demographic data. Fourth, the extent of inbreeding avoidance in wild dog reproduction was examined and the impact of this behaviour on future population persistence explored. Lastly, a population viability assessment using genetic inputs and goals was conducted for the current KZN wild dog population and strategies for the establishment of a viable future population were suggested.

Genotyping and sampling errors were identified as significant sources of error in non-invasive analyses using faecal samples from wild dogs. Software assessments and replications were used to detect and correct genotyping errors. Sampling errors occurring in the field were corrected by evaluating multiple faecal samples from individual wild dogs, comparing matching individual blood and faecal genotypes, and using a large number of microsatellite loci to the identification of unique individuals in the dataset. Results showed that although genotyping and sampling errors were significant in our faecal DNA investigation of wild dogs, it is possible to correct these errors to generate consistent and accurate genotypes. Whenever possible, future genetic studies on African wild dogs and other endangered carnivores should apply these non-invasive techniques as they avoid handling of these stress-sensitive animals and have proven to be a reliable method of attaining good quality DNA.

Though some cases of incest have been observed in the KZN wild dog population, the deleterious effects of inbreeding depression have only moderately begun to show on the lifespans of inbred individuals. Compared to non-inbred individuals, inbred wild dogs ($f \geq 0.25$) had significantly shorter lifespans. However, this trend was limited to a small number of packs, indicating that although this seems to be evidence of inbreeding depression, a larger sample size is needed to confirm this. These results provide baseline data on the genetic diversity and fitness of the population and highlight the importance of tracking these characteristics in the future to ensure a healthy population.

Within the parentage analyses, a substantial portion of reproductive sharing between dominants and subordinates was discovered in packs that contained siblings of the alpha pair. Alpha females mated annually, whereas subordinate betas bred in over half of all breeding years. Regardless of the number of adult males in the pack, litters of mixed paternity occurred in over 50% of years. Alpha males sired just over half of pups while one or two subordinate males sired the remaining pups in each pack. A skewed

adult sex-ratio and frequent alpha mortalities for females and behavioural aggression in males allowed most individuals to attain dominant status in their lifetime. These findings suggest that more individuals than expected were able to contribute to reproduction in the population. In addition, reproductive partitioning in wild dogs is mediated through a combination of demographic, behavioural and hormonal factors.

Extensive behavioural and genetic data confirm that wild dogs largely avoid mating with kin and suggest that avoidance is based on familiarity since only one inbreeding pair had prior association with each other. Computer-simulated populations revealed that preventing relatives from breeding could cause demographic declines and extinction due to an absence of unrelated mates. Although stronger inbreeding avoidance maintained significantly more genetic variation, small, isolated wild dog populations could encounter potentially severe demographic impacts of these behavioural mechanisms.

Population viability analysis simulations including genetic data and goals revealed that wild dog populations were influenced most by the proportion of adult females that had the capacity to begin breeding, the mortality of females 3 yr and older, and the severity of disease outbreaks. Within the habitat now available, the current population failed to meet demographic or genetic viability criteria. The model suggested that demographic viability could be maintained for 50 years by translocating a dispersal group into the area every 4 yr, while retaining at least 90% of genetic diversity involved more intensive management (supplementation of two dispersal groups every 2 yr). My findings illustrate that securing more habitat and constant supplementations are critical to achieving wild dog conservation in KZN. This study also highlights the value of including genetic information for wild populations and incorporating such data into PVA models to produce more realistic expectations for guiding reintroduction strategies.

This project offers new insights into African wild dog population genetic structure and behavioural ecology contributing to the overall knowledge of the complex biology of cooperative breeders. More importantly, the findings on several of these topics have huge management implications. With new information on the wild dog social system and the current genetic status of an endangered carnivore population, some significant management recommendations are made in effort to greatly contribute to the conservation of African wild dogs and other reintroduced carnivores.

ACKNOWLEDGEMENTS

I would like to thank Ezemvelo KZN Wildlife, especially the management teams at Hluhluwe-iMfolozi Park and the uMkhuze section of iSimangaliso Wetland Park for permission and support while working in their protected areas. I also appreciate the assistance of Thanda Private Game Reserve and the wildlife management team. Thank you to my supervisors and advisors, Michael Somers, Micaela Szykman Gunther, David Wildt and Jesus Maldonado for their encouragement, time, patience and dedication. Thanks also to my other co-authors, Phil Miller, Michele Walters, and Amy Wilson who helped to contribute to at least one manuscript from my dissertation. Many thanks to JoGayle Howard and Joan and David Challinor for generously allowing me to stay with them while doing laboratory work in Washington, D.C.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Genetics of Small Populations

Small populations are prone to a loss in genetic diversity that may negatively impact individuals through inbreeding depression. These populations are susceptible to inbreeding events due to a lack of availability of unrelated individuals for breeding in the area, which is restricted due to habitat loss and fragmentation (McNutt 1996). Gene diversity is potentially lost quickly in such situations due to occasional incestuous matings, genetic drift (random fluctuations in allelic frequencies) and few immigrants entering into the area (Girman *et al.* 1997). Intensive management of an endangered species often can require one or more infusions of new genes from unrelated individuals and populations (Pimm *et al.* 2006). If possible, reintroduction programs should utilise animals that are genetically unrelated to avoid inbreeding, while promoting genetic diversity to enhance the population's ability to respond to selection pressures.

Genetic diversity (heterozygosity) among populations and individuals can be measured by assessing microsatellites from blood or tissue, as well as from sloughed cells in faecal samples (Coltman & Slate 2003). There is evidence for strong correlations between microsatellite heterozygosity and fitness in the Florida panther (*Puma concolor coryi*: O'Brien *et al.* 1990), grey wolf (*Canis lupus:* Bensch *et al.* 2006) and red deer (*Cervus elaphus*: Slate & Pemberton 2002, Coltman & Slate 2003). When gene diversity is lost due to mating between related individuals, inbreeding depression can result in the expression of deleterious alleles that can: 1) reduce survival of young (Shoemaker 1982); 2) cause sterility, altered gamete structure/function (Fitzpatrick & Evans 2009), reduced fecundity and neonatal and juvenile survival (Wildt *et al.* 1982, Ralls *et al.* 1988); 3) contribute to an array of developmental defects (Hedrick 1995); 4) lower ability to withstand stress (Miller 1994); 5) reduce intra- and interspecific competitive ability (Lacy 1997); 6) decrease longevity (Laikre 1999); 7) slow growth; and 8) increase disease susceptibility (Allendorf & Leary 1986). Many of these deleterious effects on fitness have been linked to allozyme and microsatellite homozygosity (Reed & Frankham 2003). Although mostly found in non-coding regions of the genome, microsatellites may reflect genome-wide heterozygosity levels, may be associated with specific fitness-related loci (identity disequilibrium) or may be in linkage disequilibrium (associative overdominance) with fitness-related loci (Hedrick *et al.* 2001, Slate & Pemberton 2002).

Even if low genetic diversity has not already adversely affected the population's persistence in the short-term, loss of heterozygosity will compromise a population's long-term ability to adapt to changing environments (Lacy 1997). Small populations are especially prone to extinction due to loss in adaptive capacity (Burger & Lynch 1995). The small number of remaining African wild dog individuals in nature combined with rapidly changing environmental conditions makes genetic diversity and adaptability critical factors in regulating the survival of wild dog populations.

1.2 Influence of Behaviour on Genetics and Demographics

In small, isolated populations, it is easier for individuals to mate with relatives, since inbreeding with nearby kin means that individuals avoid the risks associated with dispersal to find unrelated mates (e.g. predation, competition and starvation; Frankham *et al.* 2002). In addition, in the absence of unrelated mates, high levels of inbreeding are known to occur (e.g., Keller 1998; Roldan *et al.* 1998; Jamieson *et al.* 2007), including in canids (e.g. Raikkonen *et al.* 2006; Liberg *et al.* 2005). However, whenever possible, most mammals are thought to avoid inbreeding (Ralls *et al.* 1986). This highlights the importance of maintaining larger populations or translocating an adequate number of unrelated founders to begin reintroduced populations. Furthermore, making decisions about importing unrelated individuals to supplement small populations can only be made when there is basic information on contemporary levels of genetic variation (Miller *et al.* 1999).

Continual monitoring of how behaviours shape the availability of mates and the demographic makeup of population is essential to conserving undersized populations. Since the maintenance of genetic diversity is vital to the survival of small and reintroduced populations (Frankham 2009) such as the KZN wild dog population, assessing the level of inbreeding that is occurring, as well as the behavioural mechanisms functioning in the species that reduce or conserve gene diversity is critical. Animals have evolved a variety of behaviours to avoid inbreeding including: 1) sex-biased dispersal (Pusey *et al*. 1987); 2) dispersal patterns that vary for males and females (Koenig *et al*. 1996); and 3) recognition of individuals with similar phenotypes or MHC alleles (Penn & Potts 1999). Some species have developed additional behaviours to maintain or increase genetic diversity, such as extra-pair (e.g., alpine marmot *Marmota marmota*, Cohas *et al*. 2007) or extra-group copulations (e.g., meerkat *Suricata suricatta*, Young *et al*. 2007) and selection for more heterozygous mates (e.g., grey wolf *C. lupus*, Bensch *et al*. 2006). While some behaviours maintain gene diversity and have positive effects on future population viability, others may dramatically affect the demographic makeup of the population and eventually lead to population decline. For example, greater sexual aggression by common lizard (*Lacerta vivipara*) males decreases the fecundity and increases the mortality of females, leading to the amplification of male bias and the rapid decline of small populations (LeGalliard *et al*. 2005). Therefore, investigations focused on understanding the interactions between demographics, behaviour and genetics, especially of small, endangered populations of species such as the African wild dog, are necessary to properly conserve these animals.

1.3 Objectives and Scope of the Study

The overall aim of this study was to collect comprehensive demographic, behavioural and genetic information on African wild dog packs in the KwaZulu-Natal (KZN) province of South Africa. Determining the level of genetic variability within and among the packs, combined with insight into the social behaviour and demographic structure of the population, produced an effective longitudinal assessment to further understand wild dog biology and promote conservation efforts. In addition, results from this dissertation will be used to advise managers in wild dog (and other endangered carnivore) reintroduction programs.

Specific objectives and conservation outcomes expected from this project include:

1) The validation and refinement of non-invasive faecal genetic techniques for African wild dogs in order to accurately genotype individuals and assess population heterozygosity. This allowed for a more complete and conservative collection of genetic material from this vulnerable species. (Chapter 2) Published manuscript:

   Spiering, P.A., Szykman Gunther, M., Wildt, D.E., Somers, M.J. & Maldonado, J.E. (2009) Sampling error in non-invasive genetic analyses of an endangered social carnivore. *Conservation Genetics*, **10**, 2005-2007.)

2) An investigation of the effects of inbreeding on genetic heterozygosity and fitness in the current population, including the creation of a genetic database for the wild dogs of KZN useful for population management and conservation in the province. Using an extensive demographic database combined with genetic information, this study explored the

sensitivity of the wild dog to decreased heterozygosity that is likely to occur as the population continues to grow in an area with restricted and fragmented dispersal space. (Chapter 3) Submitted manuscript:

Spiering, P.A., Szykman Gunther, M., Wildt, D.E., Somers, M.J., Walters, M. & Maldonado, J.E. Inbreeding, heterozygosity and fitness in a free-ranging population of endangered African wild dogs (*Lycaon pictus*). In review at *Conservation Genetics*

3) An analysis of the reproductive strategies and trends within packs of wild dogs. Including genetic parentage analyses and long-term behavioural and demographic data, this research evaluated reproductive sharing and skew within the dominance hierarchies of breeding packs. These results further the understanding of wild dog social biology and in turn, our knowledge of how genetic diversity may be maintained within populations of these cooperative breeders. (Chapter 4) Published manuscript:

   Spiering, P.A., Somers, M.J., Maldonado, J.E., Wildt, D.E. & Szykman Gunther, M. (2010) Reproductive sharing and proximate factors mediating cooperative breeding in the African wild dog (*Lycaon pictus*). *Behavioral Ecology and Sociobiology*, 64, 583–592.

4) Exploration of the extent of behavioural inbreeding avoidance in African wild dogs and its potential effects on reproductive success and population growth. Due to the fact that mate choice plays a large role in determining which individuals breed within the population, these findings highlight how active inbreeding avoidance can affect genetic diversity and population persistence of the KZN wild dog population. (Chapter 5) Submitted manuscript:

Spiering, P.A., Maldonado, J.E., Wildt, D.E., Somers, M.J. Szykman Gunther, M. & Miller, P. Inbreeding avoidance influences the viability of reintroduced populations of African wild dogs. In review at *Animal Behaviour*

5) A population viability assessment for the current KZN wild dog population and recommendations of strategies for establishing a demographically and genetically viable future population. Resulting data were provided to provincial wildlife authorities in order to (a) predict the current population's trajectory for the next 50 years, (b) explain the level of management intervention and resources required to ensure viability of the population in the region and (c) provide supplementation strategies, such as the frequency of translocations needed using genetically variant packs or dispersers. (Chapter 6) Submitted manuscript:

   Spiering, P.A., Szykman Gunther, M., Wildt, D.E., Somers, M.J., Maldonado, J.E. & Miller, P. Integrating genetics into population models improves viability assessments for endangered species management. In review at *Animal Conservation*

1.4 Natural History of African Wild Dogs

1.4.1 Description, taxonomy and life history

The African wild dog (*Lycaon pictus)* is a highly social cooperative breeding canid. The Latin name literally translates as "painted wolf" (Temminck 1820), which refers to their unique patchwork pelage of brown, black and white. While domestic dogs (*Canis familiaris*), wolves and jackals belong to the genus *Canis*, African wild dogs diverged from this group several million years ago into the genus *Lycaon* (Girman *et al.* 1993; Chen *et al.* 2000). A phylogenetic tree based on DNA sequence analyses suggests that African wild dogs diverged before *Canis* species, raccoon dogs (*Nyctereutes procyonoides*), red foxes (*Vulpes vulpes*), and blue foxes (*Alopex lagopus*) (Chen *et al.* 2000).

This species has distinctively large, rounded ears, four toes on the forelegs and does not have the vestigial dewclaw found in other canid species (Estes 1991; Creel & Creel 2002). The average wild dog measures 60-75 cm tall at the shoulder, and weighs 20-25 kg (Smithers 1983; Estes 1991). Wild dogs have evolved physiological adaptations that make them highly specialized and successful hunters that catch as much as 85% of the prey they chase (Estes 1991). Compared to domestic dogs, African wild dogs display an increased tolerance for high body temperature and a lower evaporation rate (Taylor *et al.* 1971) which may allow an increase in the pursuit distance of wild dogs and make it an effective chasing predator. In addition, dentition selected for increased bite capacity, increased mechanical advantage of the jaw and enhanced meat slicing capabilities ensures quick prey consumption (Van Valkenburgh & Koepfli 1993) and reduction of kleptoparasitism from other predators (Creel & Creel 1996).

Pack sizes range from two to 30 individuals in most populations, but were recorded as large as 40 individuals in Serengeti (Estes 1991). Within a pack, wild dogs have tight social bonds and are rarely apart from other pack members for more than a few minutes while hunting. Established breeding packs have home ranges as large as 1,300 km² in some areas (Fuller & Kat 1990), making large protected areas and corridors essential to accommodate the far-ranging lifestyle of the African wild dog.

Wild dogs are naturally found in lower densities and utilize larger areas than other canid species and other African carnivores (Creel & Creel 2002). Males and females in breeding packs have separate social hierarchies and the socially dominant alpha male and female are thought to be responsible for most of the annual breeding (Malcolm & Marten 1982; Girman *et al.* 1997).

Upon reaching one year of age, offspring begin to leave their natal pack in single-sex sibling groups, dispersing huge distances from their familiar home territory (McNutt 1996). Successful dogs find opposite-sex dispersal groups or infrequently take over an existing pack to form a new breeding pack (Frame *et al.* 1979). This emigration away from the birthplace to novel areas provides new opportunities for breeding, prevents inbreeding and promotes gene diversity to ensure viable, reproductively sound and healthy populations (McNutt 1996).

1.4.2 Worldwide status, distribution and decline

Within Africa, the African wild dog is the second most endangered carnivore (IUCN Redlist; McNutt *et al.* 2006). Historically, this species ranged throughout much of sub-Saharan Africa (Creel & Creel 2002), but most of the remaining populations are confined to isolated reserves in parts of southern and eastern Africa (Smithers 1986, Woodroffe *et al.* 1997: Fig. 1.1). There are approximately 3,000 to 5,500 free-ranging wild dogs remaining in all of nature (Woodroffe *et al.* 2004), largely due to habitat fragmentation, disease and human-induced mortality (Mills & Gorman 1997, Woodroffe *et al.* 2007).

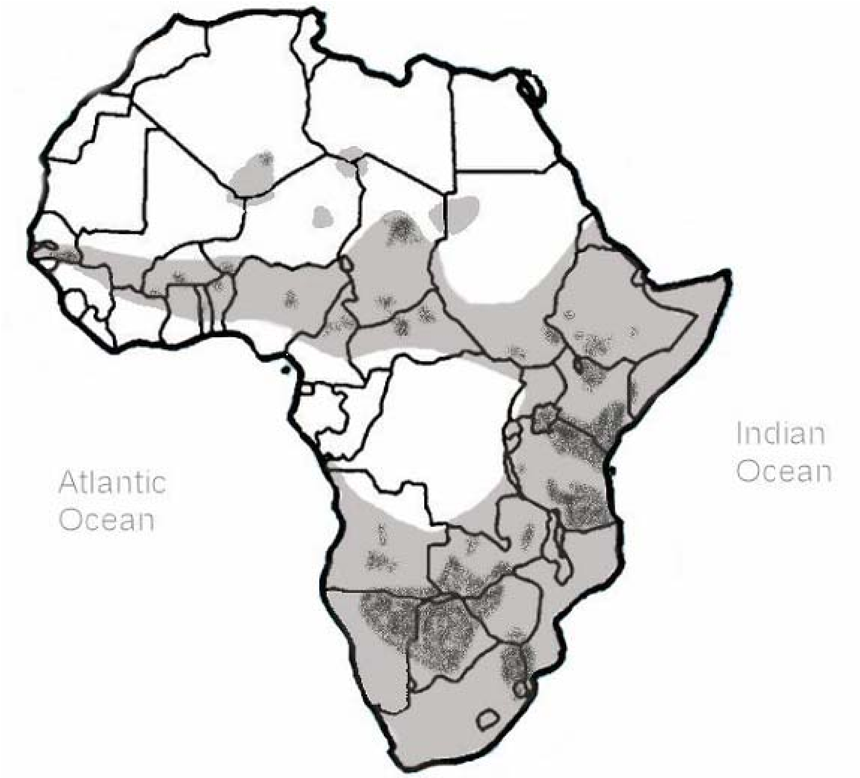


Figure 1.1 Historical (gray) and current (dark shading) distribution of *Lycaon pictus* in Africa. Current distribution indicates general regions inclusive of both vagrant and resident populations. Map reprinted with permission from Leigh (2005) and data drawn from Woodroffe *et al*. (1997), Ginsberg (1993), and Fanshawe *et al*. (1991).

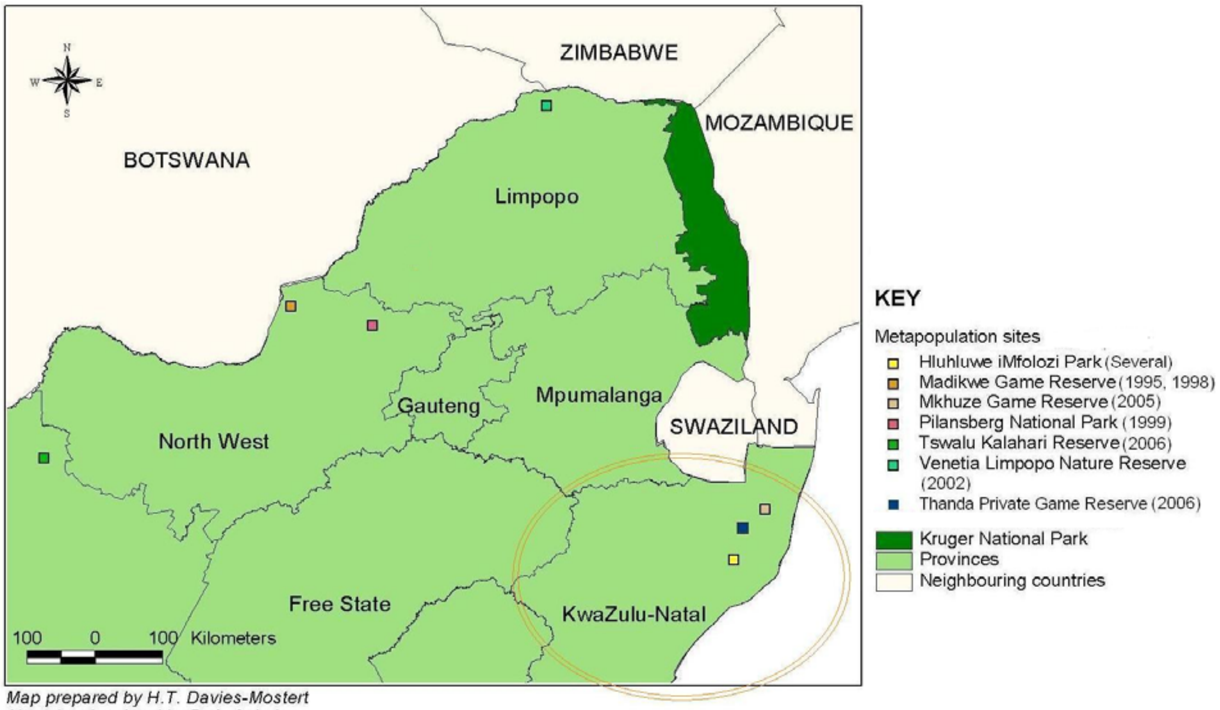
The decline of wild dogs throughout the continent reflects patterns of human population growth, as viable populations are only found in areas with low human population densities (Woodroffe & Ginsberg 1998). As land is settled and turned into agricultural area, prey populations are depleted and wild dogs are not able to sustain themselves. Prey and habitat loss is further exacerbated by humans through vehicle accidents or direct persecution such as snaring, poisoning and shooting (Woodroffe *et al*. 1997). Wild dogs often use open game paths and roads while hunting and therefore are especially prone to vehicle collisions. Most damaging to the species is the fact that several African governments considered wild dogs to be vermin that suppressed antelope populations. In some countries, persecution of wild dogs was official National Parks policy until as late as 1979 and extermination attempts killing hundreds of wild dogs were carried out in some areas until the mid 1980's (Woodroffe *et al.* 1997; Creel & Creel 2002).

Disease has also been a major reason for the decline of several African wild dog populations. Rabies and canine distemper have been implicated as a cause of the disappearance of wild dogs from the Serengeti National Park (Tanzania; Gascoyne *et al.* 1993), Masai Mara Game Reserve (Kenya; Kat *et al.* 1995), Etosha National Park (Namibia; Scheepers & Venzke 1995), Madikwe Game Reserve (South Africa; Hofmeyr *et al.* 2000) and Tswalu Kalahari Reserve (South Africa; G. Van Dyk, pers. comm.). In most cases, the source of these disease outbreaks has been determined as 'spill over' from reservoir hosts such as domestic dogs (Alexander & Appel 1994), jackals (*Canis aureus and C. mesomelas*; Alexander *et al.* 1993), and bat-eared foxes (*Otocyon megalotis*; Maas 1993). These diseases are easily transmitted from one wild dog to pack mates through social interactions that include licking each other's mouths and a large amount of physical contact (Mills 1993).

Another cause of African wild dog mortality is naturally occurring predation by lions (*Panthera leo*), and less often by spotted hyaenas (*Crocuta crocuta*). Lions have caused up to 12% of adult and 31% of pup mortalities, while hyaenas have been implicated in up to 4% of adult and 6% of pup annual deaths (Woodroffe & Ginsberg 1998; Woodroffe *et al.* 2007). Competition with and predation by these carnivores has been shown to limit wild dog populations in several range areas (Mills & Gorman 1997).

1.4.3 African wild dogs in South Africa

In South Africa, half of the estimated 3-400 free-ranging African wild dogs are found in small, fenced protected areas managed as a metapopulation (Fig. 1.2). These subpopulations are scattered throughout the country, with the majority comprised of only one to two wild dog packs each (Mills *et al.* 1998). This largely is the result of inadequate carrying capacity within reserves, with the result being a country-wide population that is (1) not yet self-sustaining and (2) highly vulnerable to perturbations that can cause rapid extinctions.



Map prepared by H.T. Davies-Mostert
Altered and updated by P. A. Spiering

Figure 1.2 African wild dog metapopulation reserves in South Africa as of 2009 with corresponding years of reintroductions. Circle is drawn over general study area

Although single-sex groups disperse from their natal packs and travel as much as several hundred kilometers, the large distances and human-dominated landscape between protected areas prevent most dispersers from reaching other reserves (Woodroffe *et al.* 1997). Kruger National Park, which holds approximately half of South Africa's African wild dogs, currently has the only 'viable' population in the country (Mills *et al.* 1998). This population is said to be sufficiently large and diverse to persist into the future "despite the foreseeable effects of demographic, environmental and genetic stochasticity and natural catastrophes" (Shaffer 1981), yet this population has recently declined to just 140 as of 2009 (Lindsey 2009). Meanwhile, for the remainder of the country, specialists have agreed that wild dogs must be regularly translocated among subpopulation reserves to mimic natural emigration and immigration while maintaining critical genetic diversity to sustain the species (Mills *et al.* 1998).

1.5 Study Area and Population

1.5.1 Study area

KwaZulu-Natal (KZN) has the potential to host a viable population of African wild dogs due to the large number of both private and provincially owned protected areas in the province. The provincial parks are under the control of Ezemvelo KZN Wildlife, while many privately-owned protected areas are former cattle grazing areas converted to game ranches for ecotourism purposes. With many protected areas in northern KZN adjacent to each other and some tracts of land between others that are not yet heavily populated with humans, when all the protected areas are combined, there is a considerable amount of habitat for wild dogs available. In addition, unlike the situation in many other provinces in South Africa, there is little hostility toward and persecution of wild dogs from farmers and villagers living adjacent to protected areas in KZN.

The current project study area includes all protected areas with breeding packs of African wild dogs between 2001-2010 (Hluhluwe-iMfolozi Park, uMkhuze section of iSimangaliso Wetland Park, Thanda Private Game Reserve, Hlambanyathi Private Game Reserve and Tembe Elephant Park). Within the context of this thesis the study population is referred to as the KZN population. The existing and potential wild dog habitat in KZN has a moderate coastal climate (Whateley & Porter 1983) and has a summer rainfall ranging between 700 - 1250 mm per annum (Walters *et al.* 2005). Average monthly temperatures range between 13 - 33°C (Grobler 1984). Two vegetation types dominate throughout KZN's protected areas, namely the Zululand thornveld subcategory of coastal tropical forest types and the lowveld subcategory of tropical bush and savanna types (Acocks 1988). The larger vegetation species include predominately *Acacia karroo*, *A. nilotica*, *A. nigrescens* and *A. tortilis* (Walters *et al.* 2005).

KZN protected areas support a large variety of potential prey species for African wild dogs, including nyala (*Tragelaphus angasi*), impala (*Aepyceros melampus*), red duiker (*Cephalophus natalensis*), grey duiker (*Sylvicapra grimmia*), bushbuck (*Tragelaphus scriptus*), kudu (*Tragelaphus strepsiceros*), blue wildebeest (*Connochaetes taurinus*), common reedbuck (*Redunca arundinum*) and waterbuck (*Kobus ellipsiprymnus*; Kruger *et al.* 1999). Due to this abundance of prey, several other large and small predators occur in KZN also. The carnivores belonging to the same feeding guild as the wild dog include spotted hyaena, lion, leopard (*Panthera pardus*) and cheetah (*Acinonyx jubatus*; Kruger *et al.* 1999). Numbers of hyaenas and lions are especially important to wild dogs, as these species have been documented to limit wild dog populations through competition and predation (Mills & Gorman 1997).

1.5.2 Study population

The formation of the earliest and largest African wild dog subpopulation outside of Kruger National Park began in 1980 and 1981 with the reintroduction of 22 captive-bred wild dogs into KZN's 900 km² Hluhluwe-iMfolozi Park (HiP). Wild dogs in this province had been extirpated by the 1940s largely as a result of human persecution. Because the reintroduced population in HiP had declined to only eight individuals by 1997, the decision was made to initiate a more intensive reintroduction strategy that primarily involved translocating wild born individuals from other reserves and some captive born individuals from breeding centres (Maddock 1999). About the same time, reintroductions of wild dogs were being made into other reserves in South Africa and translocations began to occur between reserves. In HiP, subsequent wild dog reintroductions were made in 2000 and 2003 from other remnant wild South African populations for the purpose of boosting the local population and enhancing genetic diversity (Somers & Maddock 1999, Gusset *et al.* 2006; Somers *et al.* 2008), though no genetic analyses were completed prior to that decision.

These more-recently reintroduced wild dogs, along with natural pack formations among their offspring, boosted the HiP population to nine packs comprised of approximately 110 African wild dogs. This is an extraordinary accomplishment for this historically important range of this endangered species. However, wild dog numbers had only increased within HiP, a single isolated area with limited carrying capacity. Conservation authorities decided to work with University of Pretoria, Smithsonian's National Zoological Park and other stakeholders to create a viable wild dog population in KZN that would have adequate protection, habitat and genetic diversity to ensure viability. Sustaining a large wild dog population long-term would require establishment of additional wild dog habitats and breeding areas and linking reintroduced populations through corridors (Maddock 1999; Miller *et al.* 1999).

Since 2004, actions have been taken to increase wild dog numbers outside HiP, specifically by introducing breeding packs to the uMkhuze section of the iSimangaliso Wetland Park in 2005 and to the Thanda Private Game Reserve in 2006 (Fig. 1.3). It is also encouraging that several dispersal groups have been able to leave their natal protected areas, safely traverse the province and find other wild dogs in protected areas up to 200 km away. Because of this successful dispersal and due to human-induced and natural mortalities, uMkhuze was again without a wild dog population by 2009. In the same year, Hlambanyathi Private Game Reserve agreed to reintroduce a breeding pack to the area. As a result, the total provincial population now stands at 114 wild dogs comprising nine breeding packs. Efforts continue to reintroduce breeding adults to other provincial and private protected areas in order to increase numbers and generate presumably adequate genetic diversity (Fig. 1.3).

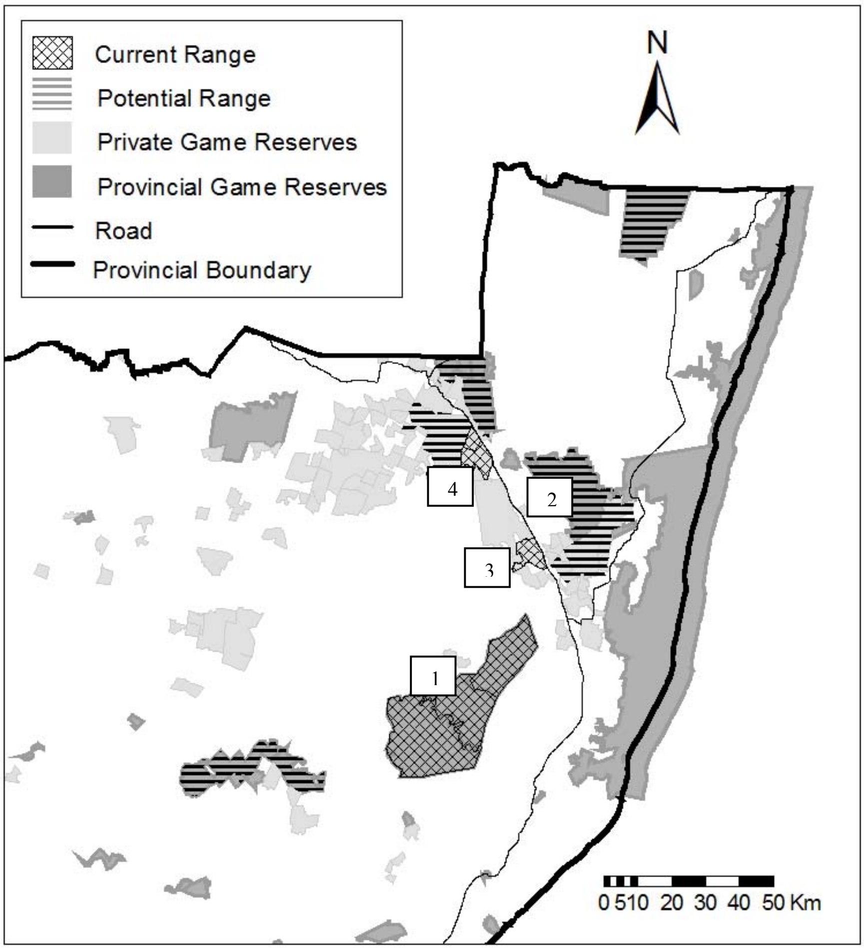


Figure 1.3 Current African wild dog subpopulations (crosshatch) and potential habitat (horizontal lines) for future reintroductions of breeding packs into private (light grey) and provincial (dark grey) protected areas in northern KwaZulu-Natal, South Africa. Numbers represent protected areas as follows: 1) HiP, 2) Mkhuze, 3) Thanda, and 4) Hlambanyathi.

It should be emphasized here that, despite efforts to source wild dogs from a variety of wild packs, all relocations and reintroductions to date have been done with no direct genetic evaluation or monitoring. Therefore, this dissertation will provide baseline genetic data for this important wild dog population, as well as contribute to the already extensive demographic and behavioural databases available for the KZN population. These new data should provide new insight into management and conservation of the population as well as contribute to the overall understanding of African wild dogs as a species.

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CHAPTER 2: SAMPLING ERROR IN NON-INVASIVE GENETIC ANALYSES OF AN ENDANGERED SOCIAL CARNIVORE

2.1 Abstract

Modern non-invasive genetic technologies are useful in studies of rare and difficult-to-observe species. An examination of endangered African wild dog (*Lycaon pictus*) faecal DNA revealed that 11.4% of samples were assigned incorrectly to an individual. Sampling mistakes in the field are not normally considered in non-invasive genetic assessments, but can be a significant source of error. To ensure meticulous data interpretation, non-invasive genetic studies should track and report sampling inaccuracies.

2.2 Introduction

Faecal genetic analysis is a powerful tool that eliminates handling of stress-sensitive animals (Kohn & Wayne 1997) while allowing studies of social organization and mating systems (Archie *et al.* 2008), but only when errors mis-assigning kinship are eliminated. Under rigorous field conditions, investigators may incorrectly identify an individual or scat or erroneously label the sample storage container, resulting in sampling error. As error likelihood increases when studying large, dynamic groups defecating in the same area, non-invasive genetic analyses have been under-exploited for studying kinship within social groups. Furthermore, minimal attention has been dedicated to sampling error (Bonin *et al.* 2004), potentially resulting in data misinterpretation.

The African wild dogs of KwaZulu-Natal (KZN), South Africa originated from ongoing reintroduction efforts initiated in the 1980s (see Gusset *et al.* 2008) and currently comprise eight packs distributed across three protected areas. This is one of the most endangered (Woodroffe *et* *al.* 1997) and socially complex species in Africa (Estes & Goddard 1967). Wild dogs live as cooperative breeders in large-sized packs consisting of as many as 27 individuals (Creel & Creel 2002). Minimizing pack disruption is essential for these sensitive animals, and faecal sampling provides an excellent opportunity for understanding genetic relatedness.

To investigate errors in faecal genetic sampling techniques, we compared 42 individual tissue/blood DNA genotypes to faecal DNA genotypes to determine the level of sampling error in our field studies. To further reduce inaccuracies, we evaluated multiple samples collected from 22 individuals and screened a large number of microsatellite loci to ensure sufficient power to identify unique individuals in our dataset (Waits *et al.* 2001).

2.3 Methods

2.3.1 Sample collection

From January 2003 through January 2008, defecating wild dogs were observed from ≥ 15m away and samples collected after the animals moved away to minimize disturbance. Since reintroduction or birth, each pack of wild dogs has been monitored intensively, and individuals are recognized by unique natural markings (e.g., coat patterns, ear tears) documented in photographs. Samples of questionable identification or samples possibly mixed with another individual's faeces due to over-marking were not collected. Faeces was collected in plastic bags and transported to a -20°C freezer within 4 hr of collection. Invasive samples were collected from a subset of the population during translocation and collaring. During anaesthesia, an ear biopsy was taken and placed in DMSO solution, while blood was aspirated from the femoral vein into a vacutainer tube containing EDTA anticoagulant and stored at -20°C. Based on field observations, we predicted that 136 faecal samples were derived from 114 individuals, with 22 sampled twice; 42 animals also contributed an invasive sample (Table 2.1).

Table 2.1 Summary of sampling error results for 136 faecal samples.

	Number of individuals (Incorrect samples)	Error (%)
Individuals observed in field	114	
Replicate samples	22 (3)	13.6
Blood/tissue and faecal comparison	42 (4)	9.5
Probability of identity (CERVUS)	110 (6)	5.5
Overall sampling error	114 (13)	11.4

2.3.2 DNA extraction and amplification

Faecal DNA was extracted with the QIAamp DNA Stool Mini Kit and tissue and blood DNA extracted with the QIAamp Tissue and Blood Kit (QIAGEN) in a separate room from PCR products (Eggert *et al.* 2005). We screened 19 microsatellite loci from the 2006 International Society for Animal Genetics domestic dog panel (Table 2.2). For all loci, faecal samples were genotyped at least three times for heterozygotes and five times for homozygotes. Invasive samples were genotyped once for each locus and replicated when allelic scores were questionable or failed to match faecal genotypes. Each 25 µL PCR reaction contained 3.0 µL of template DNA, 1.0 µL of forward and reverse primers, 2.0 µL $MgCl_2$, 0.2 µL *Taq Gold* polymerase, 2.5 µL *Taq* buffer (Applied Biosystems), 2.0 µL BSA and 2.5 µL dNTPs. The PCR profile included 10 min at 95°C, followed by 38 cycles for faecal DNA or 30 cycles for tissue/blood DNA of 1 min at 95°C, 1 min at 60°C and 1 min at 72°C, and an extension of 10 min at 72°C. Samples were run with GS-500 ROX size standard on ABI PRISM 3100 or 3130 automatic sequencers (Applied Biosystems) and alleles determined with Genotyper (Perkin Elmer) or GeneMapper (Applied Biosystems) software.

2.4 Results

The mean amplification success rate for non-invasive samples was 63% (Table 2.2). Errors associated with allelic dropout (37%; Table 2.2) and false alleles (3%) accounted for most of the genotyping inaccuracies in this study. Consensus faecal genotypes were assessed and genotypes deemed <95% reliable by RelioType software (Miller *et al.* 2002) were replicated at specific loci. Although genotyping errors were significant in our faecal DNA investigation, as well as those of others (Bonin *et al.* 2004), it was possible to correct allelic dropout errors to generate consistent genotypes.

Table 2.2 Locus names, size ranges (base pairs), amplification success and allelic dropout rates for African wild dog faecal samples.

Locus	Size range	Amp. success	Allelic dropout
AHT137	131-147	67%	24%
AHTh130	117-125	88%	9%
AHTh171	217-225	42%	49%
AHTh260	246-254	37%	44%
AHTk211	89-91	73%	27%
AHTk253	298-306	65%	35%
CXX279	116-118	85%	22%
FH2054	128-140	59%	32%
FH2328	194-220	68%	24%
FH2848	232-240	54%	41%
INRA21	97-101	59%	29%
INU030	144-150	64%	40%
INU055	208-216	62%	51%
LEI004	95-99	57%	49%
REN54P11	234-246	43%	34%
REN105L03	235-245	83%	41%
REN162C04	194-200	72%	55%
REN169DO1	208-212	68%	48%
REN247M23	254-256	51%	44%
Mean	234-246	63%	37%

Once reliable genotypes were achieved, we checked for further errors that would have occurred in the field at the time of sample collection. Of the 22 individuals with two faecal samples for comparison, we found inconsistent genotypes for three samples (13.6%), but were able to elucidate identities of these individuals with invasive samples. Comparing genotypes from tissue/blood and faeces showed that four of 42 (9.5%) individual donors were sampled in error. All erroneously identified samples matched another individual in the pack. Lastly, CERVUS 2.0 (Marshall *et al.* 1998) determined that our loci yielded highly significant probability of identity (4.78×10^{-14}) and probability of identity among siblings (2.1×10^{-6}) values. Assessing genotypes of 110 faecal samples labelled as different individuals (excluding the four samples not matching invasive samples) revealed that only 104 were unique individuals, resulting in 5.5% sampling error. In summary, three evaluative methods detected sampling errors due to incorrectly identifying faeces donors or mislabelling containers 13 times in 114 faecal samples (11.4%; Table 2.1).

2.5 Discussion

Although genotyping errors from faecal DNA analyses were anticipated, sampling inaccuracies were unexpected. Previously, faecal DNA studies have not considered the confounding influence of observer error and mis-assignment of sample source, although this is well addressed in endocrine (Stavisky *et al.* 2001; Keay 2006) and demographic studies (Gabriele *et al.* 2001; Kelly 2001; Milligan *et al.* 2003). Difficult field conditions common to wildlife research can introduce mistakes to faecal sample recovery. For example, in our study, the investigator must wait for the animal to move away and then leave the vantage point to collect the sample. Also this species lives in a social, tight-knit group, and it is common to encounter a significant number of wild dogs defecating within the same area and in dense bush. A semi-natural captive environment apparently increased the likelihood of misidentification, as four sampling inaccuracies occurred while animals were in large enclosures for reintroduction and defecated in the same area daily.

We demonstrate that sampling error in faecal genetic studies can be reduced and allow the resolution of genotypes of misidentified animals to ensure accurate data. We recommend photographing defecating animals, collecting multiple samples from individuals, comparing invasive and faecal samples, and using sufficient loci for adequate power to detect duplicated individuals.

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CHAPTER 3: INBREEDING, HETEROZYGOSITY AND FITNESS IN A REINTRODUCED POPULATION OF ENDANGERED AFRICAN WILD DOGS (*LYCAON PICTUS*)

3.1 Abstract

It is crucial to understand the genetic health and implications of inbreeding in populations of vulnerable wildlife species. Using extensive demographic and genetic data, we investigated the relationships among pedigree inbreeding coefficients, metrics of molecular heterozygosity and fitness for a population of endangered African wild dogs (*Lycaon pictus*) in South Africa. Molecular metrics based on 19 microsatellite loci were significantly, but weakly correlated to inbreeding coefficients in this population. Inbred wild dogs with inbreeding coefficients of ≥ 0.25 and subordinate individuals had shorter lifespans than outbred and dominant contemporaries, suggesting some deleterious effects of inbreeding. However, this trend was confounded by pack-specific effects as many inbred individuals originated from one large pack. Although heterozygosity-fitness correlations are more detectable in endangered species and small populations, molecular metrics were not significant predictors in models of fitness based on breeding pack formation, dominance, reproductive success or lifespan in this study. Given that inbreeding can result in severe morphological and genetic abnormalities in a short time in endangered canids, a further understanding of heterozygosity and additional fitness traits is urgently needed to guide future management decisions and to sustain wild dog populations.

3.2 Introduction

As habitats shrink and fragment, inbreeding increasingly becomes a serious threat to local wildlife populations, often resulting in the loss of genetic diversity and inbreeding depression

(Frankham *et al*. 2002). The consequences of increased homozygosity for individual fitness have been shown repeatedly within captive populations, leading to increased neonatal and juvenile mortality (Ralls *et al*. 1979) as well as compromised reproduction (Fredrickson *et al*. 2007) and longevity (Charpentier *et al*. 2008). Due to inherent logistical challenges, much less is known about the influence of inbreeding within natural populations, an issue which now has become a major priority in conservation genetics (Frankham *et al*. 2002; Keller & Waller 2002).

There has been substantial interest in determining the relationships among inbreeding coefficients (Keller & Waller 2002) and levels of molecular genetic diversity (heterozygosity-fitness correlations, or HFCs; Coltman & Slate 2003) to fitness. Molecular metrics are commonly used as a surrogate for pedigree inbreeding coefficients (*f*), because the latter can be logistically difficult to obtain within natural populations. The most frequently used molecular metrics of heterozygosity in HFCs analyses include standardized multilocus heterozygosity (stMLH; Coltman *et al*. 1999), internal relatedness (IR; Amos *et al*. 2001) and standardized mean d^2 (Coulson *et al*. 1998). However, it has been suggested that the correlation between molecular heterozygosity and pedigree inbreeding coefficients is too weak to be of biological significance (Coltman & Slate 2003; Balloux *et al*. 2004; Slate *et al*. 2004; Hansson & Westerberg 2008). For wild canids, results vary even between studies of the same species. For example, Hedrick *et al*. (2001) reported a significant negative correlation between heterozygosity and inbreeding coefficients for the Scandinavian gray wolf (*Canis lupus*). However, Bensch *et al*. (2006) found no significant relationship between these metrics in the same species, which the authors attributed to natural selection for heterozygosity in their study population.

The presence of significant, albeit modest HFCs has been reported in numerous studies, while a few investigations have found no relationships (Coltman & Slate 2003). Molecular metric assessments have led to HFC discoveries involving fitness traits such as fecundity (Amos *et al*. 2001), immunocompetence (Coltman *et al*. 1999), recruitment (Jensen *et al*. 2007) and survival (Da Silva *et al*. 2006). Although small populations of endangered species are most vulnerable to inbreeding and fitness reduction (Fitzpatrick & Evans 2009), most HFC studies have focused on large populations of common species (see Grueber *et al*. 2008 for review). Common species offer benefits in terms of large sample sizes and well understood natural histories, but studies of these species will not likely translate to bottlenecked and endangered populations that have the most potential of being detrimentally affected by genetic diversity loss. HFCs are more likely to be detected in populations of such species because these populations are prone to: 1) experience an increased mean and variance of inbreeding coefficients due to a prevalence of incestuous matings (Grueber *et al*. 2008); 2) more environmental stress that can intensify inbreeding depression, thereby increasing variance in fitness responses (DaSilva *et al*. 2006); and 3) a greater degree of linkage disequilibrium due to bottlenecks, selection or population admixture. These characteristics can cause HFCs as a result of linkage with fitness loci (the ‘local effects hypothesis’; Hansson *et al*. 2004) or might provide heterozygosity information for a larger proportion of the genome (the ‘general effects hypothesis’; Pemberton 2004). While significant HFCs resulting from general effects can be attributed to inbreeding that has reduced genome-wide heterozygosity, significant HFCs due to local effects can be detected in the absence of inbreeding (Hansson & Westerberg 2008). Distinguishing between these two hypotheses is challenging as researchers must screen many polymorphic loci, have complete pedigrees with high variance in inbreeding coefficients, and secure detailed fitness metrics (Slate *et al*. 2004).

In this study, we tested the hypothesis that inbreeding coefficients and molecular heterozygosity predict fitness in a free-ranging, endangered species, the African wild dog (*Lycaon pictus*) in KwaZulu-Natal (KZN) province, South Africa. Approximately 3,000-5,500 wild dogs remain in Africa, living in a small fraction of their former range, with most populations isolated due to human encroachment, habitat loss and fragmentation (Woodroffe *et al*. 1997). In South Africa, nearly half of the estimated 400 wild dogs are found in small, fenced, protected areas managed as a metapopulation (Davies-Mostert *et al*. 2009). These reserves are scattered throughout the country and have limited carrying capacities, usually accommodating only one or two packs each (Mills *et al*. 1998). Fencing generally is sufficiently 'porous' to allow single-sex groups to disperse from the natal pack and travel as far as hundreds of kilometers. However, the sparsity of protected areas and the human-dominated landscape prevent most dispersers from reaching a suitable, alternative reserve in other parts of the country (Woodroffe *et al*. 1997). Therefore, for the metapopulation conservation plan to be effective, wild dogs often are physically translocated between areas to mimic natural dispersal with the objective to enhance overall species demography while sustaining genetic diversity (Mills *et al*. 1998).

Interestingly, this coordinated and intensive management plan is conducted without information on the genetic status or viability of the African wild dog metapopulation. There is a general assumption that some inbreeding has occurred in selected packs, although at unknown levels, and that wild dogs with reduced genetic diversity are 'less fit' than more heterozygous counterparts. The expression of deleterious effects has been demonstrated in inbreeding studies of wild canids (Liberg *et al*. 2005; Bensch *et al*. 2006; Hedrick & Fredrickson 2008), but these results are based on small sample sizes and low statistical power (Frankham 2009). The evidence is more definitive from studies of captive canids where inbreeding has been associated with increased proportions of malformed spermatozoa (Mexican wolf, *C. l. baileyi*; Asa *et al*. 2007), reduced fecundity (Fredrickson *et al*. 2007), blindness and reductions in juvenile weight and longevity (Scandinavian wolf; Laikre & Ryman 1991). Inbreeding has been linked to reduced immunocompetence in some non-canid species (O'Brien & Evermann 1988; Acevedo-Whitehouse *et al*. 2003). If inbreeding affects disease susceptibility in African wild dogs as well, this could dramatically impact species persistence since rabies, canine distemper and parvo viruses have already caused significant mortalities in many populations. Decreases in immunocompetence may leave wild dogs particularly vulnerable to disease-related mortalities considering they have reduced diversity in the genes of the major histocompatibility complex (MHC) that regulates pathogen and parasite resistance compared to other canids (Marsden *et al*. 2009).

In this study, we used detailed information generated from our long-term involvement in establishing and monitoring a reintroduced population of wild dogs in South Africa. We worked with conservation authorities to translocate animals and then monitored 257 individuals representing 10 distinctive packs over the study period. Although translocations and dispersal events resulting in natural pack formations have increased numbers of the KZN population since 2001, occasional observations of interbreeding between close relatives have been made in the field. To explore if these events impacted either genetic diversity or fitness traits, we analyzed the relationships between inbreeding coefficients and molecular metrics and then determined if either metric was predictive of fitness. For the purpose of this study, we considered fitness of wild dogs to be largely reliant on the ability of an individual to find a breeding pack (McNutt 1996), be dominant (Creel & Creel 2002), produce offspring and survive (Buettner *et al*. 2007).

We included inclusive reproductive success in our analyses because wild dogs are obligate cooperative breeders (Courchamp *et al*. 1999) and we sought a precise measure of lifetime reproduction through each individual's own breeding success as well as their contribution in helping to raise related kin. This study begins a substantial database useful for long-term genetic and fitness monitoring of a reintroduced wild carnivore, while also providing objective data informative to conservation managers on the minimal best practices to sustain genetic diversity and future population health.

3.3 Methods

3.3.1 Study population

Intensive demographic and behavioural monitoring was conducted for the reintroduced wild dog population in KZN province, South Africa from January 2001 through October 2008. The population was established in the 1980s with initial reintroductions to Hluhluwe-iMfolozi Park (HiP). Additional translocations were made to HiP in 1997 (Somers & Maddock 1999), 2001 and 2003 (Gusset *et al*. 2006), as well as to two other semi-connected protected areas in KZN province in 2005 and 2006 (Davies-Mostert *et al*. 2009). Most of these approximately 20 founders were wild-caught dogs captured on private farmlands after conflict with livestock and game farmers, while two individuals were sourced from Kruger National Park, and still others were captive-bred dogs that were bonded with wild-caught dogs to improve the hunting skills of reintroduced packs (Gusset *et al*. 2006). Each founder pack was comprised of males derived from a different locality than the females in the pack, and founders of newer packs were not chosen from areas where previous founders were sourced. When pedigree information was known, only sources of wild dogs without a probable history of inbreeding were selected as founders (Mills *et al*. 1998). In addition, it is unlikely that source populations included high numbers of inbred dogs, as several studied populations have displayed minimal inbreeding due to avoidance mechanisms such as long-distance dispersal (McNutt 1996; Girman *et al*. 1997).

Over the 7 year period of this study, the KZN wild dog population grew steadily through reintroductions and natural pack formations and from one protected area with two breeding packs comprising seven individuals to three protected areas with nine breeding packs and 88 dogs (Somers *et al*. 2008). In all, a total of 257 individuals were identified and present in the KZN study population over our study interval. One to four individuals per pack were fitted with VHF radio-collars so that groups (including dispersers) could be located by radio-telemetry. Data on pack composition (number of animals, age and gender structure), life histories (births, dispersals, pack formations, deaths), dominance (the hierarchy of each sex in a pack) and breeding status (mating, denning) were collected at least once and as often as 10 times monthly. Individual wild dogs were recognized by unique coat patterns and photographs to facilitate identifications. Pups were first counted, identified and sexed upon emergence from the den at 3 mo of age. Within each pack, the alpha male and female were determined based on: 1) reciprocal male and female scent marking behaviours; 2) obvious coincidental male and female movements; and 3) dominance and mutual offense and defence in agonistic encounters with other adult pack members (Girman *et al*. 1997). Dominance tenure was measured as the proportion of the time spent as an alpha-ranked individual in a breeding pack and was categorized as ‘always dominant’, ‘dominant at sometime’, or ‘never dominant’. The lifespan of each individual in the population was usually known with precision to within 1 mo of birth and death. Additionally, it was often possible to differentiate between dispersal and mortality events, in part, because the lack of prey and potential mates available made it extremely unlikely for dispersing individuals to survive for long periods of time outside of protected areas. It was also assumed that any dominant female not seen with her pack for a period of time had died because alpha females do not disperse (Creel & Creel 2002).

3.3.2 Genetic sampling and genotyping

Sampling for genetic analyses was conducted from January 2003 through January 2008 using a combination of hands-on and non-invasive approaches. Specific sample collection, immobilization techniques and DNA extraction methods are described in Spiering *et al*. (2009; 2010). Briefly, tissue and blood samples were obtained opportunistically during immobilization procedures for translocation and collaring, or when the occasional wild dog carcass was discovered. A larger and more representative sample was obtained by using non-invasive faecal DNA assessments (n = 113 individuals from 10 packs). Faecal DNA was extracted from scat with the QIAamp DNA Stool Mini Kit and from tissue and blood with the QIAamp Tissue and Blood Kit (QIAGEN). Genotyping was conducted using 19 microsatellite loci selected from the 2006 ISAG (International Society for Animal Genetics) domestic dog (*Canis familiaris*) panel that were consistent with those used in other wild dog genetic studies in southern Africa. All individuals were typed at 17 dinucleotide microsatellite loci and two tetranucleotide loci (Table 3.1). These loci are commonly used for determining parentage in domestic dogs and, therefore, were known to be highly polymorphic and distributed throughout the genome. Polymerase chain reaction (PCR) protocols and the elimination of genotyping and sampling errors in our faecal DNA analysis are addressed in detail in Spiering *et al*. (2009). In all, 87.6% of individuals (n = 99) were genotyped at all 19 loci, 8.9% at 17 or 18 loci and the remaining four individuals at 13 or more loci.

Table 3.1 Microsatellite loci used for estimating molecular heterozygosity metrics and determining parentage in free-ranging African wild dogs.

Locus	Chromosome	Allele sizes	Number of alleles	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)
AHT137	CFA11	131-147	5	0.81	0.71
AHTH130	CFA36	117-125	6	0.46	0.47
AHTH171	CFA06	217-225	6	0.80	0.72
AHTH260	CFA16	246-254	5	0.52	0.61
AHTK211	CFA26	89-91	2	0.61	0.49
AHTK253	CFA23	298-306	6	0.77	0.73
CXX279	CFA22	116-118	3	0.26	0.23
FH2054	CFA12	128-140	4	0.80	0.66
FH2328	CFA29	194-220	10	0.76	0.81
FH2848	CFA02	232-240	5	0.72	0.71
INRA21	CFA21	97-101	3	0.49	0.41
INU030	**CFA12**	**144-150**	**6**	**0.55**	**0.60**
INU055	CFA10	208-216	4	0.76	0.67
LEI004	CFA37	95-99	3	0.58	0.61
REN54P11	CFA18	234-246	5	0.71	0.67
REN105L03	CFA11	235-245	7	0.81	0.80
REN162C04	CFA07	194-200	6	0.74	0.73
REN169D01	CFA14	208-212	3	0.29	0.28
REN247M23	CFA15	254-256	2	0.27	0.27

Bold font indicates a locus not in Hardy-Weinberg equilibrium and, therefore, excluded from parentage analyses.

3.3.3 Parentage analysis

Parentage of offspring was assumed from pack composition only in cases where a single pair of adults was present and behavioural observations confirmed breeding status. All other parentage assignments were made based on a combination of longitudinal behavioural observations plus molecular genetic data. Tests for deviation from Hardy-Weinberg equilibrium and parentage analyses were completed using the likelihood-based approach in the program CERVUS (version 3.0) and are described in detail in Spiering *et al.* (2010). Locus INU030 was excluded from the parentage analyses because a significantly lower than expected frequency of heterozygotes was detected, indicating a high incidence of null alleles. No other locus deviated from Hardy-Weinberg equilibrium. The simulation program in CERVUS was used to establish the critical difference in LOD scores (natural logarithm of the likelihood ratio) between the first and second most likely candidate parents at > 95% confidence. For the 18 loci used in the analysis, the overall probability of exclusion was 0.991 for the first parent and 0.999 for the second.

3.3.4 Inclusive fitness

Total lifetime reproductive success or inclusive fitness was based on the number of offspring parented by each animal (direct fitness) plus the number of offspring belonging to closely-related pack members that the individual assisted in raising (indirect fitness). For this calculation, we used relatedness values of 0.50 for offspring and full siblings, 0.25 for half-siblings, nieces and nephews and 0.12 for grand-offspring, then multiplied these values by 2 to convert to units of offspring equivalents (Grafen 1982). All pairwise genetic relatedness values were estimated using the program KINSHIP (version 1.3.1).

3.3.5 Inbreeding coefficients

The inbreeding coefficient (f) of each wild dog was calculated from the pedigree using FSPEED (version 2.04; Tenset Technologies Limited). Because limited pedigree information may lead to underestimations of f (Keller 1998), individuals were assigned inbreeding coefficient values only in cases where grandparents were confirmed using parentage analyses or when behavioural and demographic records allowed confidence in pedigree data (n = 181 wild dogs, 10 packs). Given the origin and history of reintroduction of this population, inbreeding levels were likely roughly f

= 0 before the last two generations. As 257 wild dogs were present during our monitoring interval, we derived inbreeding coefficients for 70.4% of the collective population.

3.3.6 Molecular metric of heterozygosity

Heterozygosity was calculated for all genotyped individuals (n = 113) for three different molecular measures of genome-wide diversity. Of the 181 wild dogs assigned *f* values, 85 of them (from eight packs) were also genotyped to include corresponding molecular metrics. Standardized multilocus heterozygosity (stMLH) was calculated based on the proportion of loci genotyped for a given individual that was heterozygous, divided by mean heterozygosity in the population at the same loci (Coltman *et al*. 1999; Slate *et al*. 2004). IR reflects the relatedness of the parents of an individual by determining the degree of allele-sharing relative to random expectations across all loci (Amos *et al*. 2001). Standardized mean d^2 (hereafter referred to as d^2) is a measure focused on events deeper in the pedigree than individual heterozygosity (Hedrick *et al*. 2001) and is based on the genetic distance between parental gamete genomes (Coulson *et al*. 1998, 1999). d^2 was calculated for each individual as the squared distance in repeat units between two alleles at a given locus, averaged over all loci typed for that individual (Coulson *et al*. 1999) and then standardized by dividing each value by the maximum observed at that locus (Hedrick *et al*. 2001). This metric is used with the assumption that microsatellites evolve under the stepwise mutation model (SMM; Valdes *et al*. 1993) and therefore departure from SMM was tested for all loci with the program BOTTLENECK (Cornuet & Luikart 1996). The stMLH, IR and d^2 values were calculated using an EXCEL macro written by Amos *et al*. (2001).

3.3.7 Statistical analyses

The relationship between pedigree inbreeding coefficients and microsatellite-based metrics were compared by linear regression. To test the strength of the correlation between *f* and stMLH, we compared our observed linear correlation to the predicted relationship using the model of Slate *et al*. (2004) based on the mean population *f* as well as the variance in *f* and heterozygosity. We used generalized linear mixed models (GLMMs) to evaluate the relationship between molecular metrics (stMLH, IR and d^2) or inbreeding coefficients (*f*) on: *i*) breeding pack membership, *ii)* tenure of pack dominance, *iii)* individual inclusive fitness, and *iv*) lifespan. High correlations between the molecular metrics (Table 3.2) precluded joint inclusion into a single model. Molecular metrics and *f* were not considered within a single joint model due to too few individuals with available data for both measures and fitness traits.

All models used maximum likelihood and not restricted maximum likelihood, such that models with different fixed effect structures could be compared. GLMMs were used to evaluate breeding pack membership (binomial, logit), inclusive fitness (poisson, log-link) and lifespan (poisson, log-link) in relation to the fixed effect predictors of sex, dominance and one molecular metric (stMLH, IR or d^2) or *f*. All models included pack identity as a random effect. Pack dominance was treated as a multinomial response variable in generalized linear models incorporating sex, one molecular metric (stMLH, IR or d^2), or *f*, and pack as fixed effect predictors. For lifespan analyses, inbreeding coefficients were coded as two categorical predictors of inbred ($f \geq 0.25$) or outbred ($f = 0$), because our dataset primarily consisted of individuals with $f = 0.25$ or $f = 0$.

Breeding pack membership and dominance models included dead animals only, as both of these life history events may occur at any time in the life of a wild dog. Animals that died before joining a breeding pack were excluded from models for dominance, as wild dogs rarely achieve dominance in their natal packs (Spiering *et al.* unpublished data). For models of inclusive fitness, only individuals that were dead and had survived to at least 12 mo (yearlings) were included in analyses, as this allowed calculating lifetime reproductive success of mature individuals. In the lifespan analyses, only individuals that had survived beyond 12 months and for which sex was known were included. Means are presented ± standard error of the mean.

3.3.8 Model selection

We used Akaike's Information Criterion (AIC) to evaluate among candidate models based on all combinations of predictor variables in addition to an 'intercept-only' model. All combinations represented reasonable *a priori* hypotheses and were required to calculate factor weights. Model selection was based on AIC for small samples (AIC_c), where we considered models with $\Delta AIC_c \leq 2$ to be well supported by the data (Burnham & Anderson 2002). All statistical analyses were conducted using the Program R (version 2.8.1).

3.4 Results

Pedigrees and parentage assignments confirmed via genetic analyses allowed us to estimate inbreeding coefficients for 181 African wild dogs using a minimum of two generations of data. The mean and variance in f for this population was 0.074 and 0.008, respectively, with 37.5% of wild dogs with known pedigrees having a non-zero inbreeding coefficient. Values of f ranged from 0 (n = 115 animals) to 0.281 (n = 4; Fig. 3.1a).

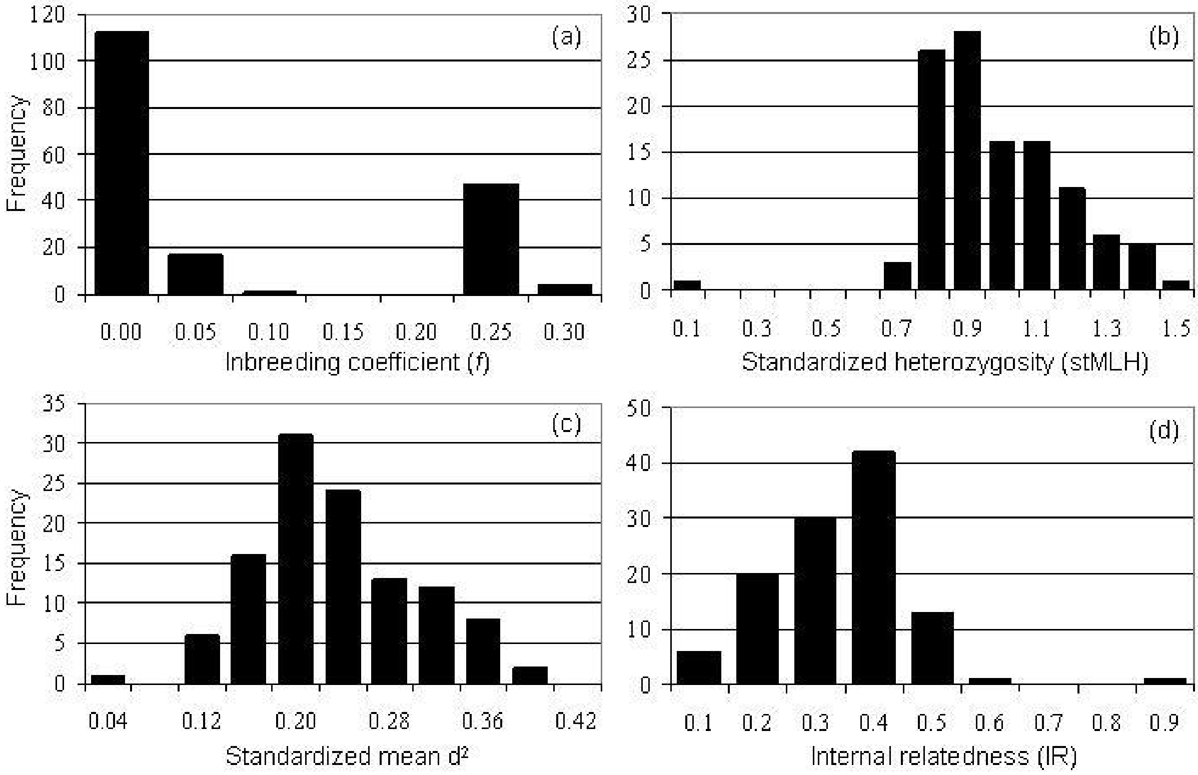

Figure 3.1 Frequency distributions of (a) inbreeding coefficients (*f*; n = 181 African wild dogs); (b) standardized multilocus heterozygosity (stMLH); (c) standardized mean d^2 (d^2); and (d) internal relatedness (IR; n = 113 African wild dogs).

A total of 113 wild dogs were genotyped using the panel of polymorphic microsatellite loci to quantify individual heterozygosity using stMLH, IR and d^2. Tests of mutation models using BOTTLENECK revealed that the distribution of the microsatellites did not depart from the distribution expected under the stepwise mutation model (P = 0.73, n = 113). Within the study population, the mean stMLH was 1.01 ± 0.02, the mean IR was 0.39 ± 0.01 and d^2 was 0.21 ± 0.01 with distributions of the molecular metrics approximately normal (Figs. 1b, c, d).

In terms of life history metrics, we observed that 25.3% (n = 65) of wild dogs in this population successfully joined or formed a breeding pack, and 13.3% (n = 34) of all individuals in the population secured the alpha position in the dominance hierarchy during their lifetime.

Mean offspring number per wild dog was 1.8 ± 0.5 pups with inclusive reproductive success of individuals averaging 9.6 ± 1.0 total young. Average lifespan for all dogs was 2.0 ± 0.2 years, with individuals that attained dominance status in breeding packs living longer (4.7 ± 0.5 years; Wilcoxon test, $T_{176} = -6.0$, $P < 0.0001$).

3.4.1 Relationship between inbreeding coefficient and heterozygosity

The correlation between f and d^2 was not statistically significant, though the remaining correlations were ($P < 0.05$), with stMLH and IR most strongly correlated ($r = -0.99$; Table 3.2). Linear correlations also revealed that stMLH was significantly and negatively correlated to f ($r = -0.37$, $P < 0.001$; Fig. 3.2), with the relationship similar to but slightly weaker than predicted under the model by Slate *et al*. (2004) ($r_{predicted} = -0.44$).

Table 3.2 Linear correlation coefficients (r) for inbreeding coefficients and molecular metrics of heterozygosity in African wild dogs (n = 85).

	stMLH	d^2	IR
F	-0.37**	-0.18	0.35*
stMLH	---	0.75**	-0.99**
d^2	---	---	-0.76**

An asterisk indicates a correlation that is significant from zero with * = $P < 0.05$ and ** = $P < 0.001$.

As multilocus heterozygosity can only reflect genome-wide diversity (i.e. general effects of inbreeding) if individual loci are correlated (Balloux *et al*. 2004), we further tested if stMLH was correlated between loci in our panel. Upon estimating heterozygosity of ten random subsets of loci from our panel, results revealed only a modest, non-significant correlation between genetic markers ($r = 0.16$, $r^2 = 2.4\%$). Thus, it appears that heterozygosity is an inadequate predictor of inbreeding coefficients in our population of wild dogs.

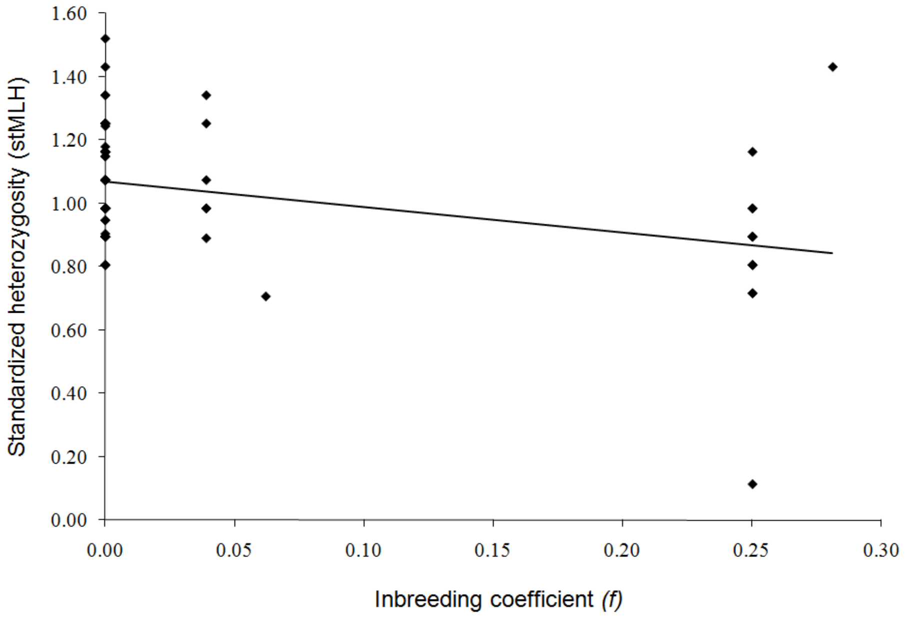

Figure 3.2 The relationship between standardized multilocus heterozygosity (stMLH) measured using 19 microsatellites and inbreeding coefficient (*f*) of 85 KZN African wild dogs.

3.4.2 Influence of molecular metrics and f on fitness

The GLMM analyses to evaluate the relationship between all three molecular metrics and fitness measures yielded the same models both in rank and magnitude of support. Therefore, to avoid redundancy, we present here only the stMLH results.

Breeding pack membership. The most informative model of the GLMM analyses of molecular metrics and breeding pack membership was the intercept only model (Table 3.3a). In analyses that only included *f*-coefficients, the most supported model included sex and pack (Table 3.3e), with males having a lower probability of joining a breeding pack than females ($\hat{\beta}$ = -0.08 ± 0.54).

Tenure of pack dominance. For both the molecular metric and *f* GLMM analyses, the top model predicting duration of pack dominance was the single predictor model that included sex (Table 3.3b, f), where females tended to have longer durations of dominance than males ($\hat{\beta}$ = 0.4

± 0.45), as previously reported (Spiering *et al*. 2010). When only females were considered, neither *f* nor pack identity was predictive of the duration of dominance.

Inclusive fitness. The most informative model resulting from our GLMM analyses of molecular metrics describing inclusive fitness included dominance and pack (Table 3.3c). Dominance increased inclusive fitness ($\hat{\beta}$ = 0.8 ± 0.25), and pack effects accounted for 14% of the total variance. Similarly, in *f*-coefficient only analyses, the top model included dominance and pack (Table 3.3g), with dominance increasing inclusive fitness ($\hat{\beta}$ = 0.51 ± 0.29) and pack effects accounting for 29% of the variance. However, models that included sex had similar support, where males had lower inclusive fitness than females ($\hat{\beta}$ = -0.35 ± 0.23).

Lifespan. When lifespan was examined in relation to molecular metrics, the most supported model included dominance (Table 3.3d), where dominant individuals had longer lifespans than subordinates ($\hat{\beta}$ = 0.41 ± 0.12). Pack effects accounted for 5.8% of the total variance in lifespan. For the lifespan analyses in relation to *f*, the most supported model incorporated dominance and *f* (Table 3.3h). Individuals that became dominant for at least part of their time in a breeding pack had longer lifespans than subordinates ($\hat{\beta}$ = 0.34 ± 0.14). Outbred wild dogs with $f = 0$ also lived longer than inbred counterparts ($\hat{\beta}$ = 0.61 ± 0.14) with the latter compromised in longevity by 0.83 years on average. Pack effects accounted for 6.8% of the total variance in lifespan.

Table 3.3 Summary of most informative models from molecular metric analyses (a-d) and *f*-coefficient analyses (e-h).

Models	AIC$_c$	ΔAIC$_c$	K
Molecular metric analyses			
a) Breeding pack membership			
intercept-only	167.9	0.0	6
Sex	78.4	1.49	2
b) Tenure of dominance			
Sex	126.6	0.0	4
intercept-only	127.3	0.8	2
stMLH	128.1	1.5	6
c) Inclusive fitness			
dominance + pack	172.0	0.0	4
Dominance	173.8	1.8	3
sex + dominance + pack	173.9	1.9	5
d) Lifespan			
dominance + pack	83.3	0.0	4
sex + dominance + pack	85.0	1.1	5
stMLH + dominance + pack	85.8	1.9	5
***f* - coefficient analyses**			
e) Breeding pack membership			
sex + pack	99.5	0.0	4
f) Tenure of dominance			
Sex	96.2	0.0	4
g) Inclusive fitness			
dominance + pack	204.9	0.0	4
sex + dominance + pack	205.0	0.1	5
sex + pack	205.2	0.3	4
Pack	205.5	0.6	3
f + dominance + pack	206.7	1.8	6
h) Lifespan			
f + dominance + pack	92.9	0.0	5

3.5 Discussion

Here we present one of the few studies of an endangered species that integrates data on inbreeding coefficients from pedigrees, molecular metrics of heterozygosity and multiple fitness traits. The remaining small, segregated populations of African wild dogs, like the reintroduced population we are studying in South Africa, are vulnerable to matings between close relatives that can lead to loss of genetic diversity and inbreeding depression. Even within the few short generations since reintroductions (i.e., most founders released after 2000), observations and genetic analyses confirm that inbreeding has indeed occurred, but is not yet widespread in the KZN population. Perhaps because of this, inbreeding depression was not detected in the fitness traits of breeding pack membership, tenure of dominance or inclusive fitness. Results suggest that inbreeding may already reduce longevity of inbred wild dogs by as much as 10 months. However, as many individuals with an $f \geq 0.25$ originated from a single pack (called iMfolozi), pack-specific effects and inbreeding are confounded. Much of the inbreeding in the KZN population has been associated with the iMfolozi pack as a mother-son pair produced large litters of offspring in four consecutive years. Although our interpretation of the fitness effects due to inbreeding are mostly confined to this subset of the population, it is nonetheless an important example of how rapidly inbreeding depression can affect fitness.

Our analyses indicate that non-genetic or non-inbreeding related traits, including dominance, sex and pack, are also important predictors of fitness metrics in this population. Dominance was included in the top model for inclusive fitness, which is logical considering that dominant individuals parent a majority of offspring (Girman *et al*. 1997; Spiering *et al*. 2010), and tend to live longer (Spiering *et al*. 2010). Dominance also predicted lifespan in our analyses, an expected trend, as the chance of becoming an alpha individual increases with age in this and other wild dog populations (Creel & Creel 2002; Spiering *et al*. 2010). Two possible reasons as to why male wild dogs were less likely to establish breeding packs than females are: 1) males dispersed further distances than females, as reported in other regions (McNutt 1996), and may have died before finding mates since survival rates decrease with distance from the source population (Lindsey *et al*. 2004); and 2) male bias in adult age classes (Spiering *et al*. 2010) created a situation where male disperser groups were less successful in finding similar female groups due to their scarcity. Pack association was an important influence on inclusive fitness, with the highest values belonging to individuals within two packs. This is not likely due to habitat quality differences as inbred and outbred packs overlapped in portions of their home ranges and were not temporally separated. Instead, these differences are likely attributed to the tenure of groups, with the bigger, established breeding packs containing individuals with the highest inclusive fitness from raising multiple large litters of related pups. Newer packs were still small and may not have been able to produce large litters of pups (McNutt & Silk 2008), thereby limiting the indirect fitness of pack members.

As molecular metrics of heterozygosity were correlated with inbreeding coefficients, we expected these metrics to be predictive of variation in fitness traits, especially lifespan. Standardized multilocus heterozygosity in particular was negatively correlated to *f* values and the relationship was similar to that predicted by Slate *et al*. (2004). It also is well established that the strength of the correlation between genome-wide heterozygosity and marker-based heterozygosity is influenced by the amount of historic inbreeding in a population (Aparicio *et al*. 2007; Grueber *et al*. 2008). Since small populations of endangered species such as the African wild dog have reduced effective population sizes, inbreeding is often more common. Therefore, marker-based heterozygosity should more accurately reflect genome-wide heterozygosity, and thus more precisely indicate levels of inbreeding in these populations (Fitzpatrick & Evans 2009). This, combined with a greater degree of linkage disequilibrium (due to bottlenecks, selection or population admixture) and the likelihood of increased environmental stress for threatened species that can intensify inbreeding depression (Grueber *et al*. 2008), gives substantial support for the possible detection of significant HFCs in this species. Although we observed breeding between close kin and inbreeding coefficients were negatively correlated with fitness, none of the three molecular metrics of genetic diversity were predictive of fitness traits.

The absence of HFCs suggests that the small effective population size and reported inbreeding events in this population have not occurred at high enough frequencies to create a signature of reduced genome-wide heterozygosity that is detectable by marker heterozygosity. The weak correlation among subsets of our marker loci also suggests that general effects are unlikely to be present since heterozygosity averaged across markers (stMLH) is only likely to be informative about loss of diversity due to inbreeding when heterozygosity is correlated among individual markers (Balloux *et al*. 2004). In addition, the lack of significant HFCs suggests that our microsatellite loci may not be linked to any heritable traits that influence the fitness metrics examined here (i.e., local effects; Hansson & Westerberg 2008). Lastly, while the number of individuals included in our study is a large proportion of the entire South African wild dog population, the sample size possible with these endangered animals may not provide sufficient power to detect these relationships (Coltman & Slate 2003).

Our study demonstrates that even in a small, endangered population, the conditions under which HFCs are detectable make it difficult to assume that molecular metrics are an accurate reflection of real inbreeding levels. This issue appears especially relevant as molecular metrics are commonly used as a surrogate for inbreeding coefficients because pedigree information is often unavailable and difficult to reconstruct via molecular parentage analyses (Coltman & Slate 2003). The results of our molecular metrics analyses were derived from a panel of 19 microsatellite loci, which is larger than those used in many other studies of endangered or wild species (e.g., Coulson *et al*. 1999). Nevertheless, our findings were consistent with recent studies suggesting that the correlation between marker-based heterozygosity and inbreeding levels may often be too weak to be of biological significance (Coltman & Slate 2003; Hansson & Westerberg 2008). However, we are not prepared to take this latter position because, to-date, most of our pedigrees were only two generations deep. Balloux *et al*. (2004) suggested that this level can be sufficient for making broad-scale inferences because recent events have a disproportionately large influence on inbreeding coefficients. However, in such cases, *f* values may have been underestimated if founders were derived from populations that had already accumulated substantial inbreeding or had been subject to strong genetic drift. In this case, it is unlikely, that source populations included high numbers of inbred wild dogs since previous studies have shown that inbreeding has been minimal (McNutt 1996; Girman *et al*. 1997) and, in general, the genetic diversity of wild dogs has been retained despite recent habitat loss and population declines (Girman *et al*. 2001). Furthermore, founders were carefully selected to avoid known inbred individuals and were sourced from different areas to avoid including related individuals. Including five generations or more in future studies, as recommended by Balloux *et al*. (2004), will provide invaluable data to more accurately estimate inbreeding coefficients, which should better correlate with molecular metrics. While neutral molecular markers may provide a means of rapid assessment of diversity, inbreeding coefficients remain most predictive of fitness. Therefore, pedigree information should be continually recorded for endangered populations, as inbreeding may have severe impacts on the fitness of these already struggling species (Frankham *et al*. 2002).

Concerns regarding the vulnerability of endangered canids to reduced genetic diversity are warranted. A particularly poignant example is that of an extirpated Scandinavian population of wild wolves that was repatriated with the natural immigration of four founders (Liberg *et al*.

2005). Although now having increased to approximately 150 individuals (Bensch *et al.* 2006), reduced pup survival (Liberg *et al.* 2005) and a higher incidence of physical deformities (Raikkonen *et al.* 2006) suggest that inbreeding depression is influencing the viability of this population. However, Bensch *et al.* (2006) found that loss of genetic diversity was slowed because breeding establishment was positively correlated with heterozygosity. Nonetheless, inbreeding is known to exert a significant influence in wild canids, especially those maintained *ex situ*, where genetic management protocols are lacking or fail to be appropriately implemented. For example, several generations of inbreeding within a captive Mexican wolf population led to certain males producing abnormally high proportions of severely deformed spermatozoa with poor motility (Asa *et al.* 2007). Deformed spermatozoa impede reproductive function and are commonly associated with reduced genetic variation and inbreeding (Fitzpatrick & Evans 2009). However, there is no evidence that these types of physiological anomalies are occurring yet within the KZN population. Even males with an inbred lineage have produced multiple, normal-sized litters (up to 14 pups each).

3.5.1 Conservation implications

In the past, there has been little attention directed toward recording accurate pedigrees or monitoring genetic status in the South African wild dog metapopulation, despite the heavy influence of genetic characteristics on management decisions. Wild dogs that were presumed to be inbred have been isolated and not translocated to new areas to reproduce because they were seen as undesirable, despite the lack of evidence showing that these individuals suffer from reduced fitness. Our results suggest that removing these individuals may be premature, as these ‘inbred’ animals may have demographic value in the metapopulation. Although we have provided evidence that their longevity may be slightly reduced, we did not find that these individuals were any less able to find breeding packs, become dominant, or produce healthy offspring. With so few wild dogs remaining in South Africa (and throughout Africa), avoiding inclusion of any individual may be only defensible if the data clearly indicated that there was a potential viability risk to the population (or species). However, we recommend caution when making final decisions on the conservation utility of 'inbred' wild dogs, as this will require more longitudinal data, in part, because increasing genetic diversity in populations is challenging and requires extensive management. Within each population, we strongly recommend that emphasis be placed on recording detailed pedigrees and, when possible, genetic analyses should be used to provide measures of heterozygosity and to clarify parentage of offspring. Knowledge of inbreeding levels will be an important predictor of the success of the management program and, more importantly, will provide guidance for future translocations, releases or other intercessions. In addition, we recommend that comprehensive studies of wild populations that combine deeper pedigrees, assessments of genetic status, detailed life histories and physiological metrics continue to be undertaken to determine more clearly the influence of inbreeding on all aspects of fitness, including lifespan. Armed with this fundamental information and rapid advances in autozygosity measurement, genome mapping and fitness gene identification, it may soon be possible for wildlife managers and researchers to determine required levels of genetic diversity (or even the specific genes to be conserved) to sustain long-term population viability of the African wild dog.

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CHAPTER 4: REPRODUCTIVE SHARING AND PROXIMATE FACTORS MEDIATING COOPERATIVE BREEDING IN THE AFRICAN WILD DOG (LYCAON PICTUS)

4.1 Abstract

Although dominant African wild dogs (*Lycaon pictus*) generally are believed to be the sole breeders within a pack, earlier behavioural and endocrine data suggest that reproduction could be shared with subordinates. We performed an extensive behavioural, demographic and genetic evaluation of a wild dog population in South Africa to examine the level of such sharing and the proximate mechanisms influencing reproductive contributions of each sex. While a majority of pups were born to dominants because of a lack of subordinate potential breeders, we discovered a substantial portion of reproductive sharing between dominants and subordinates. Compared to alpha females that mated annually, subordinate beta females bred in 54.5% of years whereas thetas never bred. The three top-ranking males all sired pups (56.0%, 32.0% and 12.0%, respectively) when three or more adult males were present. With only two pack males, alpha and beta individuals shared reproduction nearly equally (55.2% and 44.8%, respectively), and litters of mixed paternity were discovered on eight of 15 (53.3%) occasions. A skewed adult sex-ratio and frequent alpha mortalities for females and behavioural aggression in males allowed most individuals to attain dominant status in their lifetime, creating a constantly shifting social hierarchy. Genetic parentage results corresponded to reported hormone profiles, suggesting physiological suppression in some lower-ranked individuals of both sexes. Thus, a combination of demographic, behavioural and hormonal proximate factors mediates reproductive partitioning in wild dogs. We conclude that reproductive sharing can be significant in this species, especially for males that have less robust suppressive mechanisms than females.

4.2 Introduction

Cooperative breeders live in social groups where reproduction is partitioned among members to varying degrees, and individuals other than parents help care for offspring (Faulkes & Bennett 2001). In some such species, such as the naked mole rat (*Heterocephalus glaber*, Clarke & Faulkes 1997), dwarf mongoose (*Helogale parvula*, Rood 1990) and jackal (*Canis mesomelas* and *C. aureus*, Moehlman 1979), social dominants nearly completely monopolize copulations, and reproduction is rare or nonexistent for subordinate adults, even of prime age. Other species express intermediate levels of reproductive partitioning, with some subordinates being reproductively successful, as in the Seychelles warbler (*Acrocephalus sechellensis*, Richardson *et al*. 2001), redfronted lemur (*Eulemur fulvus rufus*, Kappeler & Port 2008) and Jamaican fruit-eating bat (*Artibeus jamaicensis,* Ortega *et al*. 2008). Still others express almost equal division in reproductive capacity among group members, as in the coati (*Nasua narica*, Russell 1983) and banded mongoose (*Mungos mungo*, Creel & Waser 1991). This substantive diversity among species suggests that there are complex mechanisms regulating reproductive sharing. Such information is crucial for understanding the processes of sexual selection, reproductive skew and inclusive fitness. Yet, studies to precisely identify these driving factors are challenging due to the lack of large-scale genetic and behavioural analyses of wild populations that include lineage relationships.

Comprehensive studies reveal that proximate mechanisms of subordinate reproductive suppression may encompass behavioural, physiological and/or demographic factors (French 1997). Behavioural mechanisms, such as aggression (Reyer *et al*. 1986), can profoundly influence distribution of reproduction with dominants physically blocking a subordinate's access to mates, as observed in the gray wolf (*Canis lupus*, Packard *et al*. 1985). Mate guarding is a similar tactic (Birkhead & Moller 1992) used by the moustached tamarin (*Saguinus mystax*, Huck *et al*. 2004*)*. Physiological suppression by means of stifling a prominent reproductive hormone (i.e., testosterone) has been effective, as seen in the naked mole-rat (Faulkes & Abbott 1991). An indirect strategy also may occur, as when subordinates produce elevated glucocorticoids (stress hormones) that, in turn, compromise normal reproduction, as reported for the song sparrow (*Melospiza melodia*, Wingfield & Silverin 1986). A combination of behavioural and hormonal cues also is well known to limit subordinate reproduction in the common marmoset (*Callithrix jacchus*, Barret *et al*. 1993) and cotton-top tamarin (*Saguinus oedipus*, Savage *et al*. 1988). In the context of demographics, group size and composition are significant. For example, chances for subordinate male savannah baboons (*Papio cynocephalus*, Alberts *et al*. 2003) to breed are vastly improved in larger troops due to increased energetic costs of mate guarding for dominants. Thus, the mechanisms associated with reproductive contributions appear as diverse as the phenotypes of the cooperatively-breeding species studied to-date.

To improve our understanding of the phenomenon of reproductive sharing, we chose the endangered African wild dog (*Lycaon pictus*) as a study species, largely because of its well-recognized, highly cooperative and complex social system (Estes & Goddard 1967). Although naturally found at low densities, packs as large as 27 individuals are known to range an average of 12 km daily to hunt prey (Creel & Creel 2002). The standard model of a wild dog pack consists of a dominant breeding pair, several subordinate non-breeding adults (usually siblings of the same-sex dominant individual) and subordinate offspring of the alpha pair (Girman *et al*. 1997). After a birth (usually once per year in a given pack), all group members cooperate in provisioning the lactating female in the den and feeding/protecting pups after emerging from underground (Malcolm & Marten 1982; Creel & Creel 2002). After one year of age, offspring disperse as far as 250 km from the natal pack territory in single-sex, sibling groups (Fuller *et al.* 1992) in search of opportunities to join other dispersers or an already established group (Frame *et al.* 1979). Once a new breeding pack forms, a clear social dominance hierarchy develops within each gender, with reproductively 'prime', middle-aged males and the oldest female usually holding dominant positions (Creel & Creel 2002).

The prevailing view has been that dominant female and male wild dogs are the sole breeders within a pack, with most pups resulting from this pairing (Malcolm & Marten 1982; Girman *et al.* 1997). One study estimated that alpha females and males achieve ~96% of total annual reproductive success through mating and offspring production compared to only ~10% for occasionally-breeding subordinates (Girman *et al.* 1997). However, exceptions to alpha reproductive dominance have been reported, with behavioural observations suggesting that subordinate females have produced 19% of litters in Kruger National Park (South Africa; Reich 1981), 24% in the Selous (Tanzania; Creel & Creel 2002) and 25% in the Serengeti ecosystem (Tanzania; Malcolm & Marten 1982). Only three small-scale genetic analyses have compared reproductive contributions between wild dog social classes. In a study of nine packs in Kruger National Park, Girman *et al.* (1997) found that subordinate females and males produced only 8% and 10% of pups, respectively. Creel and Creel (2002) examined two litters in the Selous and found one pup in each litter not sired by the alpha male. Moueix (2006) reported that at least one pup in each of five sampled litters from Madikwe Game Reserve and Pilanesburg National Park (South Africa) was not offspring of the alpha male.

We conducted the first large-scale molecular genetics, demographic and behavioural assessment of a wild dog population in KwaZulu-Natal (KZN) Province, South Africa in order to answer three important questions about the relationship between social rank and reproductive opportunities in this species. First, how much reproduction is shared between dominants and subordinates in a breeding pack? Second, what behavioural, demographic or physiological factors are influencing distribution of reproductive opportunities? And third, does the degree of reproductive sharing, or the mechanisms that control reproductive contributions, vary between sexes?

We hypothesized that the incidence of shared reproduction among dominant and subordinate African wild dogs was higher than previously reported for this species. We also expected that a combination of demographic, behavioural and hormonal factors determined the distribution of breeding opportunities, and that mechanisms mediating reproductive sharing were different for males and females. To test these assumptions, we evaluated extensive behavioural and genetic data collected through radio-telemetry monitoring, frequent behavioural observations, individual faecal sampling and occasional field immobilizations. Specifically, we compared social rank and genetic parentage results, examined behavioural processes maintaining dominance hierarchies, compared our extensive genetic results to hormonal data for dominants and subordinates collected earlier by others (Creel *et al.* 1997) and evaluated demographic processes at work in the population.

4.3 Materials and Methods

4.3.1 Study population

Demographic and behavioural information was collected on African wild dogs in KZN from January 2001 through August 2008. This population began successfully breeding and expanding in 2001 after the release of artificially-assembled packs into a single protected area. By August 2008, further reintroductions, natural dispersals and pack formations boosted the population to 88 dogs in eight different groups living in three protected areas (Spiering *et al*. 2009). During the study period, the population included 257 individuals that comprised 10 packs and 36 total pack years, with successful breeding occurring in 32 of these years.

Data on pack composition (number of dogs, sex, age classes and litter size at first emergence from the den), location and reproductive status (i.e., breeding, non-breeding, pregnant, lactating) were collected once monthly minimally and as often as 10 times per month for more accessible packs. Packs and dispersing groups were located by radio-telemetry, observations made from a vehicle or on foot and individual wild dogs identified by unique coat patterns and photographic records.

4.3.2 Determining dominance

The alpha male and female in a given pack were recognized on the basis of: 1) reciprocal male and female scent-marking behaviour (Frame *et al*. 1979); 2) obvious co-incidental male and female movement; and 3) mutual offensive and defensive maneuvers in agonistic encounters with other adult pack members (Girman *et al*. 1997). The dominance hierarchy also was inferred from gestures of subordination, including laying the ears flat against the head and/or rotating the head away from a higher ranking individual (van Lawick 1970) as well as passive submission that included a subordinate rolling onto its back in the presence of a more dominant dog (Schenkel 1967).

In this study, we considered three ways for an individual to gain the dominant position within a pack. First, a wild dog could become dominant by default as the only adult of their sex in the pack. Alternatively, there could be inter-animal competition without physical aggression with a same-sex adult at the initial pack-bonding phase or when the hierarchy was disrupted due to death of the alpha individual. Lastly, the current dominant could be overthrown by a competitor through fighting. As dominant females never disperse (Creel *et al.* 2004), we assumed that any such individuals missing from a pack had died. Subordinate siblings of the alpha pair were considered 'potential breeders', whereas subordinate offspring of the dominant pair were not because alpha individuals apparently share breeding with siblings, but rarely with offspring (Girman *et al*. 1997; McNutt & Silk 2008; Spiering *et al*., unpublished observations).

4.3.3 Genetic sampling and genotyping

Biomaterials for molecular genetic evaluations were collected from January 2003 through January 2008. Wild dog tissue and blood samples were obtained opportunistically during immobilization operations for translocation and collaring and when a wild dog carcass was located (Spiering *et al*. 2009). Non-invasive collection of faeces allowed securing representative samples from a significant-sized population (n = 113 wild dog individuals and 10 packs). Faecal samples were collected fresh from known individuals within 5 to 30 min of deposition and then kept in a cool bag for up to 4 hr before storing in labelled, plastic freezer bags at -20^{o}C until genetic analysis.

Sample collection and detailed DNA extraction protocols are described in Spiering *et al*. (2009). In brief, DNA was extracted from scat using a QIAamp DNA Stool Mini Kit and from tissue and blood using a QIAamp Tissue and Blood Kit (QIAGEN). Genetic analyses were completed using 19 microsatellites selected from the 2006 International Society for Animal Genetics domestic dog (*Canis familiaris*) panel that were consistent with other wild dog genetic studies in southern Africa (Moueix 2006). All individuals were genotyped at 17 dinucleotide microsatellite loci (AHT130, AHT137, AHTh171, AHTh260, AHTk211, AHTk253, CXX279, FH2848, INRA21, INU030, INU055, LEI004, REN54P11, REN105L03, REN162C04, REN169D01, REN247M23) and two tetranucleotide loci (FH2054 and FH2328). These loci are commonly used for determining parentage in domestic dogs and, therefore, were selected because they are widely distributed throughout the genome and highly polymorphic. The polymerase chain reaction (PCR) protocols are discussed in Spiering *et al.* (2009). A combination of the multiple tubes approach (Taberlet *et al.* 1996) and the maximum likelihood method (Miller *et al.* 2002) were used to overcome the potential for faecal DNA genotyping errors (Spiering *et al.* 2009). To detect and eliminate sampling error, we compared matched tissue or blood with faeces, analyzed duplicate samples for individuals and used a significant number of microsatellite markers to verify unique individuals in the dataset (Waits *et al.* 2001).

4.3.4 Parentage analysis

During the study period, 220 pups emerged from 30 litters, and 86 of these offspring were sampled for parentage analyses using the likelihood-based approach in the Cervus 3.0.3 software package (Marshall *et al.* 1998). The simulation program in Cervus was used to establish the critical difference in natural logarithm of the likelihood ratio (LOD score) between the first and second most likely candidate parents (at ≥ 95% confidence). Only adults from within the pack with a given set of offspring were considered candidate parents because no extra-group copulations have been reported for this species (and analyses later confirmed that all parentage was assigned to pack members). As most wild dog packs are comprised of a group of brothers and an unrelated group of sisters (Girman *et al*. 1997), we completed all simulations with and without the advanced simulation option that includes relatives among candidate parents. Assignments using the advanced option with relatives did not differ from assignments not using the function.

A lower than expected frequency of heterozygotes indicating a high frequency of null alleles was detected at locus INU030, which, consequently, was excluded from further analysis. No other locus deviated from Hardy-Weinberg equilibrium. For the 18 loci used, the overall probability of exclusion was 0.991 for the first parent and 0.999 for the second. Critical LOD values were calculated to assign: 1) maternity, with paternity unknown (in cases where multiple females appeared to be pregnant); then 2) paternity, with known maternity (in cases where multiple adult males were present); and 3) the parent pair, with sexes known (to verify assignments). Each breeding pack was simulated and assigned parentage separately, which allowed entering pack-specific data, including the proportions of potential parents sampled and the relatedness between candidate parents. All statistical analyses were performed using JMP software version 3.2.2 (SAS Institute Incorporated) with results presented as means ± standard error of the mean.

4.4 Results

4.4.1 Reproductive sharing

4.4.1.1 Maternity

The age of first litter production for a female varied from 1.3 to 5.0 years (mean, 3.2 ± 0.3 years). The number of litters produced per breeding female ranged from one to seven (mean, 2.1

± 0.4 litters). Alpha females produced 32 litters in 36 breeding years, resulting in an 88.9 ± 5.3% annual probability of breeding for dominants. In the remaining 4 years, packs formed late in the year or a dominant died, thereby causing reproductive failure. Alpha females gave birth to 93.0% of the 86 pups that were genetically sampled (22 litters), whereas the remaining 7.0% were produced by beta counterparts. However, 68 of the 86 pups (79.1%) whelped by alpha females were born to packs comprised of only one adult female at that time.

When multiple adult females were present in a pack, physical signs of pregnancy (i.e., greatly increased weight, enlarged teats) were never observed in more than two females at one time. Although a few matings were observed in 2 years involving theta females, none subsequently appeared pregnant on the basis of physical appearance or offspring production. Alpha individuals mated every year compared to beta counterparts that bred in 6 of 11 breeding years (54.5 ± 15.7%; Table 4.1; Wilcoxon test, $T_{41} = 5.04$, $P < 0.001$). Female subordinates of all ranks gave birth to six litters in 16 individual years, resulting in a 37.5 ± 12.5% probability of a subordinate breeding. In breeding years with multiple adult females present, six alpha females whelped 12 sampled pups (66.7%), and three subordinates produced six sampled pups (33.3%; Table 4.1). There was no significant difference in average number of pups per litter for years with single (7.4 ± 0.6 pups, 26 litters) versus multiple (8.8 ± 1.4, 3 litters; Wilcoxon test, $T_{27} = 0.80$, $P = 0.43$) births. Although sample size was small, on the three occasions when two litters were living within the same den (i.e., creched), the proportion of emerging pups whelped by the alpha female (54.1 ± 21.0%) was similar to that for subordinates (45.9% ± 20.5%; t-test, $t_4 = 0.37$, $P = 0.80$). There also was no significant difference in percentage survival to 1 year for pups born to alpha versus beta mothers (83.4% ± 16.7% and 100.0% ± 0.0%, respectively; t-test, $t_2 = -1.00$, $P = 0.42$).

Table 4.1 Number of years with multiple African wild dog females pregnant and number of sampled pups (18 pups over 11 pack years) parented by dominant versus subordinate females.

Pack	Year	No. of adult ♀s	Multiple pregnant ♀s?	No. alpha ♀ offspring	No. beta ♀ offspring	Observations
iMfolozi	2001	2	No	1	0	
Crocodile	2003	3	Yes	NGD*	NGD	Multiple males mated with all ♀s. No pups emerged from den.
	2004	2	No	3	0	Males mated with both ♀s, but no pregnancy in the beta.
Mkhuze	2005	3	No	1	0	Offspring only from the alpha ♀.
	2006	3	Yes	0	1	Alpha ♀ died before whelping. Beta ♀ whelped ~2 wk later.
Thanda	2006	2	Yes	3	1	
	2007	2	No	NGD	NGD	Pups and alpha ♀ killed at den.
	2008	3	Yes	NGD	NGD	Alpha and beta ♀ litters killed by lions at den.
Ume	2007	3	Yes	2	4	Beta ♀ whelped ~2 wk after alpha ♀.
	2008	2	Yes	NGD	NGD	Large litter of 14 pups emerged. No genetic samples.
Veggie	2007	2	No	2	0	
Mean		2.5	54.5%	66.7%	33.3%	

*NGD indicates no genetic data available.

4.4.1.2 Paternity

Males first bred from 1.1 to 5.0 years of age (mean, 2.9 ± 0.2 years), which was not significantly different from females (3.2 ± 0.3 years; Wilcoxon test, $T_{31} = 0.78$, $P = 0.44$). During the study interval, individual breeding males produced from one to five litters each (mean, 1.7 ± 0.3 litters). Based on genetic analyses, alpha males were confirmed to have sired 72.1% of pups compared to 22.1% for beta and 5.8% for theta counterparts (Table 4.2). Alpha males sired offspring in 20 of 24 sampled litters, resulting in an 83.3 ± 7.7% chance of breeding annually.

However, for 32 pups in six litters (37.2% of sampled pups), only one adult male was present and was confirmed to have sired all pups.

Table 4.2 Numbers and mean percentages of sampled pups emerging from dens sired by alpha, beta and theta African wild dog males.

	No. pups	No. litters	Alpha ♂ pups (%)	Beta ♂ pups (%)	Theta ♂ pups (%)	Subordinate ♂s total (%)
All sampled pups	86	24	72.1	22.1	5.8	27.9
Alpha ♂ only packs	32	6	100.0	NP*	NP	NP
Multiple adult ♂ packs	54	17	55.5	38.9	5.6	44.5
2 adult ♂s	29	9	55.2	44.8	NP	44.8
$\geq$ 3 adult ♂s	25	8	56.0	32.0	12.0	44.0

*NP indicates individual of that rank not present.

Without exception, if present in a pack, at least one subordinate male was observed mating with the alpha female, a subordinate female or both. In the presence of multiple potentially breeding males, the dominant individual sired 55.5% compared to the beta at 38.9% and theta at 5.6% of all offspring (based on 54 pups in 17 litters; Table 4.2). When only two adult males were present, the incidence of reproductive sharing was high, and the percentage of pups sired did not differ significantly between the alpha (55.2%) and beta (44.8%; t-test, $t_{14} = 0.75$, $P = 0.47$) males, with similar annual probabilities of siring offspring (alpha, 80.0 ± 16.4% versus beta, 60.0 ± 15.7%; $t_{28} = 1.18$, $P = 0.25$). In the presence of three or more males in a pack, the dominant individual produced 24% more pups than the beta that, in turn, sired 20% more offspring than the theta (Table 4.2). The overall annual probability of breeding for the alpha males (86.4 ± 7.5%) was not significantly different than for the beta counterparts (73.3% ± 11.8%; Wilcoxon test, $T_{35} = 0.98$, $P = 0.33$), but both were higher than for theta individuals (28.6 ± 18.4%; $T_{27} = 3.45$, $P = 0.002$ and $T_{20} = 2.09$, $P = 0.048$, respectively).

In 15 breeding years when subordinate males were present, multiple sires fathered pups in eight single litters, with the alpha male siring the majority of three mixed-sire litters. Two males were present in eight breeding years where the dominant individual sired 58.8 ± 16.5% of pups, which was not significantly different than the 41.3% ± 16.5% for beta individuals (t-test, $t_{14} = 0.75$, $P = 0.47$). In the seven litters with three potential male breeders, the alpha male sired 55.4% ± 12.3% of young compared to 30.3% ± 5.5% and 14.3% ± 10.2% for the beta and theta individuals, respectively (Table 4.3). For seven litters and 35 pups, there was no statistically significant difference between the proportion of pups surviving to 1 year that had been sired by dominant (78.8% ± 12.9%) versus subordinate males (93.8% ± 5.0%; t-test, $t_{13} = -1.14$, $P = 0.30$).

Table 4.3 Percentages of litters sired by alpha, beta and theta African wild dog males in 15 breeding years with subordinate male potential breeders present. Numbers represent percentages of pups sired within single litters.

Pack	Year (no. of litters)	No. subordinate ♂s	No. sampled pups	Alpha ♂ offspring (%)	Beta ♂ offspring (%)	Theta ♂ offspring (%)
iMfolozi	2001 (1)	1	1	100.0	0	NP*
	2002 (1)	1	5	100.0	0	NP
	2003 (1)	1	10	20.0	80.0	NP
	2004 (1)	1	3	100.0	0	NP
Crocodile	2004 (1)	2	3	33.3	33.3	33.3
	2005 (1)	2	3	0	33.3	66.7
	2006 (1)	2	7	71.4	28.6	0
	2007 (1)	1	3	100.0	0	NP
Juma	2005 (1)	1	2	0	100.0	NP
	2006 (1)	1	1	0	100.0	NP
	2007 (1)	4	3	66.7	33.3	0
Mkhuze	2005 (1)	3	1	100.0	0	0
Thanda	2006 (2)	1	4	50.0	50.0	NP
Ume	2007 (2)	2	6	66.7	33.3	0
Veggie	2007 (1)	2	2	50.0	50.0	0
Total pups			54	30	21	3
Mean % of litters with 2 adult ♂s in pack				58.8	41.3	NP
Mean % of litters with ≥ 3 adult ♂s in pack				55.4	30.3	14.3

*NP indicates individual of that rank not present.

4.4.2 Factors mediating reproductive opportunities

4.4.2.1 Population demographics

Although the average lifespan of African wild dogs in our population was 2.0 ± 0.2 years, dispersing individuals that formed breeding packs lived 4.1 ± 0.3 years, which was similar to the mean of 4.7 ± 0.5 years for animals gaining dominance status (Wilcoxon test, $T_{46} = -0.91$, $P = 0.37$). During the breeding season in May, pack size ranged from two to 23 adults and yearlings (mean, 8.1 ± 0.8 individuals/pack). At emergence from the den, litter size varied from two to 14 pups (mean, 7.6 ± 0.6 pups/litter) with a sex ratio near parity (0.51 ± 0.04). The number of potentially breeding adults (intra-pack adults that were siblings, half-siblings, fathers or uncles, but not offspring of the dominant pair) varied from two to seven (mean, 3.6 ± 0.5 adults/pack). The remainder of each pack (i.e., 54.7% ± 6.5% of individuals) was comprised of offspring from the alpha pair, generally considered to be non-breeders and confirmed as such in this study. As observed in other wild dog populations (McNutt & Silk 2008), adult and yearling males outnumbered females slightly, but not significantly within packs (males, 54.9% ± 3.9% versus females, 45.1% ± 2.6%; t-test, $t_{62} = -1.13$, $P = 0.21$). However, this pattern was significant when considering only potentially breeding adults (males, 59.7% ± 3.2% versus females, 40.3% ± 5.8%; $t_{62} = -3.19$, $P = 0.003$). Of the 32 successful breeding years evaluated, only 11 (34.4%) were years when more than one adult female potential breeder was in the pack (i.e., generally there was a lack of subordinate females available for breeding). In contrast, multiple adult males were present in 22 (68.9%) of these same breeding years.

4.4.2.2 Influence of demographics and behaviour on rank

Only 25.3% of the wild dogs in our study population survived to disperse from the natal group and form a breeding pack. An even smaller percentage (13.3%) became dominant in the pack during their lifetime, which was similar to the proportion of dogs that eventually reproduced (15.2%). Of all dogs surviving dispersal and living in a breeding pack for at least 1 year, 76.5% eventually became dominant.

On average, dominance tenures lasted 2.4 ± 0.3 years, with alpha females tending to have non-significantly longer such periods (2.7 ± 0.5 years) than males (2.1 ± 0.4 years; t-test, $t_{38} = 0.17$, $P = 0.86$). Of the packs having multiple, potentially breeding adult males ($n = 8$ packs, 19 pack years), there was major variation in the frequency of alpha male replacement. One pack remained stable for more than 2 years, one changed dominants randomly three times in 4 years, and others experienced dominance switches annually or biannually. Within packs with multiple females (n = 7 packs, 14 pack years), alpha females were sustained in multiple years for three packs, but in all other cases dominants changed annually or biannually, usually as a result of alpha female mortality. In years with multiple, potentially breeding adults, male dominance changes occurred in 65.6 ± 5.3% of pack years compared to 41.7 ± 4.1% for females (Wilcoxon test, $T_{31} = 1.15$, $P = 0.31$). Although rates of hierarchical change were similar between sexes, the causes leading to reestablishment of social dominance roles were different. Changes in dominance status were more likely to occur as a result of aggression in males than in females (males, 45% versus females, 5%; Fig. 4.1; t-test, $t_{38} = -3.21$, $P = 0.003$). In contrast, females most often earned dominance via non-aggressive competition at pack formation or after the death of an alpha individual, or by default as being the sole surviving adult female in the pack (Fig. 4.1). A higher probability of achieving social dominance was observed with increasing age, as all males and females in our population surviving to 6 years of age or older were dominant. In each case, these dogs were the sole individual of their sex left in the pack and, therefore, attained dominance (and became the only reproducers) by simply outlasting competitors.

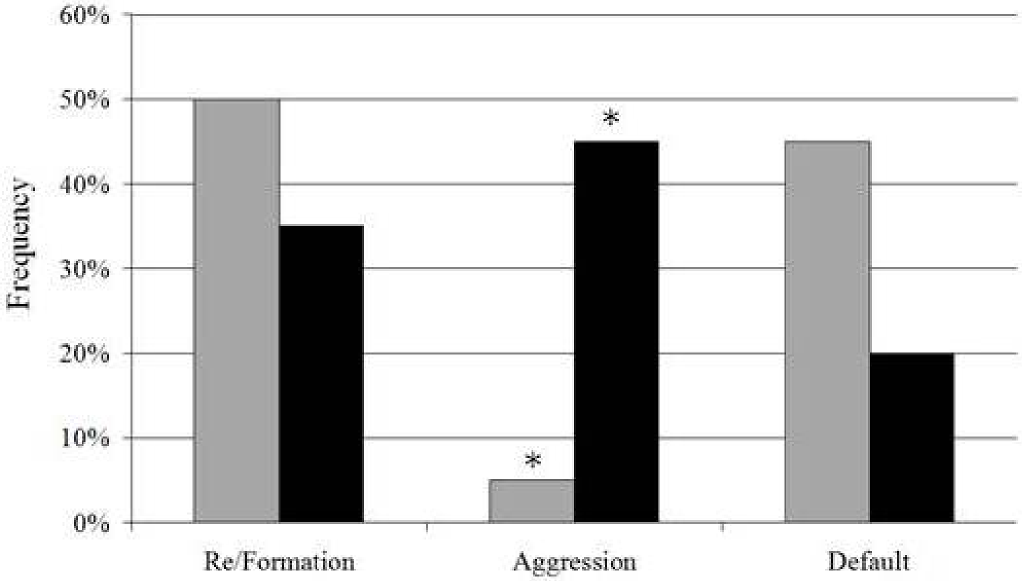

Figure 4.1 Frequency distribution of the mechanisms used to achieve dominance for 20 female (grey bars) and 20 male (black bars) African wild dogs. An asterisk indicates a significant difference (P = 0.003).

4.5 Discussion

Results from our integrated demographic, behavioural and genetic analysis of social dominance and parentage in a multi-pack population of African wild dogs supported the hypothesis that reproduction was shared among adults substantially more than previously reported. While an earlier behaviour-genetics study reported that lower ranking individuals rarely bred (subordinate females and males producing only 8% and 10% of offspring, respectively; Girman *et al*. 1997), our detailed genetic testing revealed significant reproductive sharing with, and pup production by, subordinate adults, especially males. In the presence of multiple adult potential breeders, we found that subordinate females whelped 33.3% and subordinate males sired 44.5% of pups. Subordinate females became pregnant in 37.5% of individual years with beta individuals reproductively successful in 54.5% of years. It is unclear if the litters sampled by Girman *et al*. (1997) were born into packs with several potential breeders or if packs consisted only of a single alpha pair and their offspring. This would greatly alter results of subordinate breeding, as sharing reproduction has been reported with siblings of the alpha pair, but never with adult offspring. When both single alpha pair packs and multiple potential breeder packs were considered, our results revealed female percentages (7.0%) similar to Girman *et al*. (1997), but subordinate male reproduction still was substantially higher (27.9%).

Contrary to a previous investigation that suggested that male and female subordinates reproduce at similarly low levels (Girman *et al*. 1997), our study confirmed that the proportion of subordinates able to breed and produce offspring was higher for males than females. This observation corroborated earlier speculation by Creel *et al*. (1997) who, in interpreting gonadal hormone profiles, predicted that shared paternity should be more common than shared maternity in the African wild dog. We determined that female reproduction was only shared with the beta female regardless of number of available female potential breeders. In contrast, when there were three or more adult males in a pack, each almost always sired some pups in a given litter. Reproductive partitioning extended as far as the third ranking male, a mechanism absent in females where the third position apparently offers no direct reproductive benefit.

Our discovery of shared reproduction among pack members, even including multiple sires in a given litter, suggests that this strategy contributes to ensuring the fitness of the pack and gene diversity in progeny (Gottelli *et al*. 2007). This is advantageous in an unpredictable environment or when the risk of disease-related mortality is high (Sherman *et al*. 1998). Others have maintained that increased genetic variation could simply be the result of multiple matings rather than its selective force (Wolff & Macdonald 2004). The wild dog also may have evolved a multiple mating strategy to promote post-copulatory sperm competition (Madsen *et al*. 1992) or to encourage all males to contribute equally to caring for young (Nakamura 1998).

Our results revealed that a combination of factors appeared to mediate reproductive partitioning within the African wild dog breeding pack. Within the ecological conditions of KwaZulu-Natal, both demographic and behavioural mechanisms created a constantly shifting dominance hierarchy and social system. Although genetic analyses confirmed that dominants indeed parented the majority of offspring, we found that alpha male and female dominance tenures were relatively short, with frequent within-pack hierarchical changes that permitted multiple adults to gain top-tier status and reproduce. Although only ~25% of African wild dogs born into this population survived long enough to join a breeding pack, 75% of those succeeding at this endeavor eventually became alpha. The slightly longer (non-significant) dominance tenures for females compared to males was similar to observations of Creel *et al.* (1997), but there also was substantial inter-pack variation in dominance stability for both sexes. Regardless, we were most impressed with the overall short lifespan of the average wild dog in breeding packs (ca. 4 years) that, in turn, stimulated a constantly changing society where frequent alpha deaths provoked dominance changes, ranging from inheritance of dominance to dispersals to search for new mates. The incidence of mortalities measured in our study was similar to that reported by Woodroffe *et al.* (2007) for other wild dog populations in Africa. Therefore, it is reasonable to suspect that a continually changing hierarchical social system is a common trait of this species regardless of regional location.

The basis for abbreviated dominance tenures was markedly different between males and females, with the former changing via aggression and the latter mostly by non-violent competition at pack-bonding. Whereas there was little physical antagonism among females (regardless of social status), dominant males often reaffirmed dominance by aggression with subordinates frequently challenging alpha male status. Both genders displayed the capacity to advance their status opportunistically upon the death of an alpha individual. Exactly how a wild dog individual secures the advantage to win dominance in these situations is unknown and is fascinating given that most competitive interactions often were between closely related and physically-similar individuals (i.e., siblings). More study is warranted, especially exploring the potential of mate choice by either sex in influencing who attains the alpha (and breeding) positions.

It was clear from our investigation that a female often achieved dominance by default when she alone joined a male dispersal group or when the death of a dominant sibling resulted in her being the sole surviving adult female in the pack. Our results supported the findings of Creel and Creel (2002) and Somers *et al.* (2008) that the probability of being socially dominant increases with female age, as all females in our population surviving to 6 years or older became dominant. In fact, there was an overall skewing of sex ratio for mature wild dogs to favor males (60%) despite there being near gender parity in the pup age class. Frequent adult mortalities led to a prevalence of packs with only a single female, with multiple adult females occurring during only about one-third of all pack breeding years. This was not surprising given the findings of others (Frame *et al.* 1979; Reich 1981; Creel & Creel 2002) who have noted progressively increasing male bias in older age classes. In the KZN population, generally males had more contemporaries than females in a breeding pack and, thus, more competitors that decreased the chance of securing dominance after the death of an alpha male. This explained why only 20% of males achieved dominance by default and rather relied on constantly striving to win dominance by aggressively and/or competitively overthrowing siblings. In contrast, females experienced less competition within the pack and perhaps developed a patient ‘waiting’ strategy, seeking out active reproductive opportunity only after the alpha female’s death. Interestingly, subordinate males reproduced more than subordinate females, indicating that more competitors were not necessarily a significant obstacle to males achieving reproductive success.

When breeding packs did contain multiple adult females, reproductive success of subordinates was noticeably higher than reported by Creel and Creel (2002), probably because our population often was comprised of newly formed packs with less stability in dominance hierarchies. In 3 of 11 breeding years, packs with multiple female potential breeders had formed shortly before the breeding season, and, on many occasions, copulations were observed between several males and females before pack dominance hierarchy was established. This is not unusual as others have observed multiple females and males copulating in earlier African wild dog studies (Malcolm & Marten 1982; Girman *et al*. 1997). Occasionally, this has resulted in births to subordinate females with the litters then sometimes killed by the alpha female (Reich 1981; Malcolm & Marten 1982; Fuller *et al*. 1992). Although our sample size for these genetic comparisons was modest, we observed no infanticide on the three occasions where two litters were produced in a pack simultaneously, and similar pup numbers were whelped by the alpha and beta females.

Our study is the first to show a parallel between the extent of reproductive sharing and behavioural and endocrine correlates of rank reported for the African wild dog in the Selous (Creel *et al*. 1997). For instance, Creel *et al*. (1997) found that female dominant African wild dogs excreted higher oestrogen and progestin concentrations in faeces and were more aggressive during mating periods than all subordinates. Although it was predicted that subordinate females in the Selous did not ovulate due to high baseline estrogens and oestrogen-to-progestin ratios (Creel *et al*. 1997), we suspect that most beta females in our population completed a normal ovarian cycle, in part because at least five of these individuals actually produced pups. However, this ovarian activity likely was temporally delayed based on betas always appearing visibly pregnant later and whelping pups 2 to 4 weeks after the alpha. Since no females that ranked third or lower in the dominance hierarchy became pregnant in our population, it was possible that these individuals were physiologically suppressed and failed to ovulate. This seemed especially likely as little aggression was observed between females to suggest behavioural mechanisms of limiting lower ranking individuals' access to males. Creel *et al*. (1997) also reported differences in androgen excretion among males in the hierarchy, the highest values being in the top-tier dog, which was believed to present advantages in reproductive capability and aggression. Our results, however, demonstrated clearly that beta and theta males were physiologically capable of siring young, but differences in gonadal steroid production may have affected fertility in males ranking third and lower. Nonetheless, most reproductive benefits probably are conferred behaviourally by dominants being more likely to block access to females in oestrus through enhanced antagonistic, female guarding activities.

In conclusion, our study has demonstrated that reproductive success of the African wild dog is influenced by (1) a continuously dynamic social system where marked hierarchical shifts ensure that a diversity of individuals eventually become alpha reproducers and (2) a greater than previously reported incidence of reproductive sharing, especially involving subordinate males. Both beta males and females played a significant role in producing viable young that appeared as robust as those from the dominant pair. Although the alpha position was considered most attractive to all pack members, this top-tier status was not necessary to contribute offspring. Nevertheless, short lifespans and an aspiration to be dominant appeared to drive a constantly shifting social order, with males mainly relying on behavioural aggression and females remaining vigilant for nonviolent opportunities to escape possible hormonal suppression. We suspect that this strategy assists in sustaining genetic diversity, and more effectively maintains heterogeneity than in other cooperative breeders (i.e., naked mole rat, Clarke & Faulkes 1997; dwarf mongoose, Rood 1990), where reproduction is almost exclusively monopolized by alpha individuals. This is not the case in the African wild dog where more individuals are contributing genes to offspring, even when only a single litter is produced annually.

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CHAPTER 5: INBREEDING AVOIDANCE INFLUENCES THE VIABILITY OF REINTRODUCED POPULATIONS OF AFRICAN WILD DOGS (*LYCAON PICTUS*)

5.1 Abstract

Because most African wild dog populations are fragmented and small, conserving this endangered species relies on understanding the natural processes affecting genetic diversity, demographics, and future viability. We used extensive behavioural, life-history, and genetic data from reintroduced African wild dogs in South Africa to (1) test for inbreeding avoidance via kin recognition and (2) model the potential consequences of avoidance on population persistence. Results suggest that wild dogs avoid mating with kin as inbreeding was rare in natal packs, after reproductive vacancies, and between sibling cohorts (observed in 0.8%, 12.5%, and 3.8% of occasions, respectively). Only one of the six (16.7%) breeding pairs confirmed as third-order (or closer) kin consisted of animals that were familiar with each other, while no other paired individuals had any prior association. Computer-simulated populations allowed to inbreed had only a 1.6% probability of extinction, whereas all populations avoiding incestuous breeding became extinct within 100 years due to the absence of unrelated mates. Populations that avoided mating with first-order relatives became extinct after 63 years compared with persistence of 37 and 19 years for those also prevented from second-order and third-order matings, respectively. Although stronger inbreeding avoidance maintains significantly more genetic variation, our results demonstrate the potentially severe demographic impacts of reduced numbers of suitable mates on the future viability of small, isolated wild dog populations. The rapid rate of population decline suggests that extinction may occur before inbreeding depression is observed.

5.2 Introduction

In a number of species, mating with kin has been shown to lead to decreased heterozygosity, expression of deleterious alleles, and reduced fitness due to inbreeding depression (Keller & Waller 2002; Reed & Frankham 2003). Although the short-term effects can be morphological abnormalities (Roelke *et al*. 1993), decreased reproductive success (Keller 1998), and greater susceptibility to disease (O'Brien & Evermann 1988), the long-term consequences can be reduced ability to adapt to environmental change (Frankham *et al*. 2002) and increased risk of extinction (Brook *et al*. 2002). As a result, natural selection should favour behavioural mechanisms for many animals to avoid mating with kin, particularly in species that could potentially suffer the most severe costs of inbreeding depression (Pusey & Wolf 1996). There are three recognized behavioural strategies associated with inbreeding avoidance. The first is that natal dispersal reduces contact among relatives, an approach commonly found in species like the black-tailed prairie dog (*Cynomys ludovicianus*) that displays male biased dispersal and female philopatry (Hoogland 1982). In the second, females seek extra-pair matings to enhance the genetic diversity of progeny, as observed in the blue tit (*Parus caeruleus*, Foerster *et al*. 2003). And in the third, individuals avoid mating with relatives via three types of kin recognition: 1) familiarity (Seychelles warbler, *Acrocephalus sechellensis*; Komdeur *et al*. 2004); 2) major histocompatibility complex comparisons (house mouse, *Mus musculus*; Penn & Potts 1999); or 3) phenotype matching, where individuals compare templates of close kin or itself to determine relatedness to unknown individuals (golden hamster, *Mesocricetus auratus*; Mateo & Johnston 2000).

Loss of genetic diversity is frequent and the threat of inbreeding depression high for endangered species (Spielman *et al*. 2004), cooperative breeders with high reproductive skew towards selected individuals (Miller *et al*. 2009), and small, reintroduced populations (Williams *et al*. 2000; Miller & Lambert 2004). Although the consequences of homozygosity are well known, little attention has been directed at the specific behaviours used to avoid inbreeding. No doubt this is due, in part, to the need for a comprehensive, longitudinal database on the life history, genetics, and breeding behaviour of targeted species. It also is possible that these behaviours are not prevalent in some species because the cost of avoiding mating between relatives outweighs the genetic consequences arising from inbreeding depression (Kokko & Ots 2006). As inbreeding avoidance further restricts numbers of suitable mates available to reproduce, avoidance costs could be quite high for species living in low density, fragmented populations and for those with mating systems involving few breeders (Legendre *et al*. 1999; Stephens & Sutherland 2000; Moller & Legendre 2001). However, it is not yet known whether endangered species (which generally are undergoing both significant habitat loss and population declines) rely on mechanisms of inbreeding avoidance, or if these behaviours affect future viability.

To improve our understanding of inbreeding avoidance and its consequences, we examined both real and simulated data based on a reintroduced population of African wild dogs (*Lycaon pictus*) in KwaZulu-Natal Province (KZN), South Africa. Previous studies by others have indicated that inbreeding with first-order relatives (parents, offspring, and siblings) may be rare in this species due to long-distance and, in some regions, sex-biased dispersal (McNutt 1996; Girman *et al*. 1997). However, wild dogs are cooperative breeders living in highly social groups with mature offspring often remaining in the natal pack to help raise pups for 1 to 3 years before dispersal (Girman *et al*. 1997). The primary factor generally believed to regulate reproductive success (and inbreeding avoidance) is behavioural dominance displayed by the alpha male and female who, in turn, behaviourally and/or physiologically suppress reproduction in remaining pack members (Cooney & Bennett 2000).

However, if only dominance prevents offspring from breeding in the natal pack, then at least three other outcomes would be common. First, when the dam or sire dies or disappears, an offspring would breed with the opposite sex parent. Second, siblings would breed together after dispersal from the natal pack. Third, offspring, siblings, and adults unrelated to the alpha pair should be equally suppressed from reproducing in the natal pack. However, this third supposition has been rejected scientifically as we recently presented evidence of significant reproductive sharing with siblings of the dominant pair in this species (Spiering *et al*. 2010). Another point of relevance is derived from the earliest efforts at reintroducing wild dogs to KZN. After release of the founders in 1980 and 1981 that led to the formation of a single pack, reproduction stopped in 1987 through 1989 and again from 1994 through 1996, leaving just five individuals remaining in the population in 1996 (Maddock 1996; Somers *et al*. 2008). Maddock (1996) speculated that reproduction may have ceased because only close relatives remained, though this was not confirmed with genetic or pedigree data.

The present study had two major aims. The first was to examine the possibility that African wild dogs avoid inbreeding through kin recognition. The second purpose was to explore the persistence of this species, given its dire status, naturally low densities, and small population sizes. These factors plus the existence of strong inbreeding aversion inevitably will cause even more challenges for wild dogs to find unrelated mates. Thus, we explored through simulation modelling the extinction risk associated with different inbreeding thresholds.

Our first hypothesis was that African wild dogs avoid inbreeding beyond the restrictions of established dominance, and that some type of inherent kin recognition prevents matings between familiar relatives. We tested for behavioural inbreeding avoidance in wild dogs between: 1) parents and reproductively mature offspring in the natal pack; 2) parents and mature offspring after a reproductive vacancy; and 3) adult siblings at or after the time of dispersal. Using an extensive genetic database, we also evaluated the influence of relatedness on mate choice by comparing the relatedness of confirmed breeding pairs to the mean pairwise values of individuals with known relationships, as well as to pairs within the population that might have mated but did not. Our second hypothesis was that inbreeding avoidance had a large negative impact on the reproductive potential of wild dogs, which would increase the likelihood of population extinction in small, fragmented populations without genetic management. We expected that simulation modelling would show these population-limiting effects given that earlier studies have demonstrated the demographic vulnerability of this species to extinction when deterministic or stochastic fluctuations in pack size or numbers fall below a critical threshold (Somers *et al*. 2008). This examination took advantage of a substantial database on wild dog population specific demographic and behavioural data. Recently developed population viability analysis tools were used to examine the sensitivity of African wild dogs to different levels of inbreeding and future population trends.

5.3 Methods

5.3.1 Study population

Intensive demographic and behavioural monitoring was conducted for the reintroduced African wild dog population in KZN Province from August 1997 through December 2008. After initial multiple releases into Hluhluwe-iMfolozi Park (HiP) in the 1980s, total numbers of wild dogs dwindled to five adult individuals in a single, non-reproducing pack by 1996 (Maddock 1995, 1999). To stimulate population growth, additional packs were translocated to HiP in 1997 (Somers & Maddock 1999), 2001, and 2003 (Graf *et al*. 2006; Gusset *et al*. 2006) as well as to two other protected areas in the province in 2005 and 2006 (Davies-Mostert *et al*. 2009). Over this 11 year period, the collective population grew steadily through translocations, reproduction, dispersal, and new, natural group formations to nine breeding packs comprised of 88 total dogs in three protected areas. Our examination here focused on adult males and females that were alive and sexually mature from 1997 through 2008 (n = 207, including 111 males and 96 females). Our estimate of sexual maturity (> 18 months old) was conservative given that we had occasionally observed that some males copulated at as young as 13 months old and females conceived at 15 months. Of our total study population, 113 wild dogs (54.6%) were physically sampled for blood, tissue, and/or voided faeces to extract DNA to produce direct evidence of levels of genetic relatedness among individuals (see below). This work was done with the permission and relevant permits from the local government authority, Ezemvelo KZN Wildlife, and was approved by the HSU IACUC, protocol no. 06/07.W.209.A.

5.3.2 Demographic and behavioural data collection

Data on pack composition (number of animals, age, and gender), life history information (births, dispersals, pack formations, deaths), dominance (hierarchy of individuals of each sex per pack), and reproductive status (mating, denning) were collected at least once and as often as 10 times per month. Details for these methods recently have been published in Spiering *et al*. (2009, 2010). In short, individual wild dogs were identified by unique coat patterns and were individually known from birth or translocation to KZN. At least one and as many as four individuals per pack were fitted with VHF radio collars to facilitate the monitoring of packs from a vehicle or on foot.

Although reproductive sharing does occur in wild dogs, a majority of pups in the KZN population were produced by alpha males and females (Spiering *et al*. 2010). Therefore, behavioural determination of the dominant pair of each pack was used as an indicator that these individuals were mating together, but we also observed mating behaviour involving subordinates and resolved genetic parentage of pups when possible. The alpha male and female in a given pack were recognized on the basis of: 1) reciprocal male and female scent-marking behaviour (Frame *et al*. 1979); 2) obvious co-incidental male and female movement; and 3) mutual offensive and defensive manoeuvres in agonistic encounters with other adult pack members (Girman *et al*. 1997).

5.3.3 Genetic analyses

Rather than assuming familial relationships within these cooperative breeding groups strictly on the basis of observing behaviours, we combined our longitudinal behavioural observations with molecular genetic data to determine specific pack member interrelationships. Biomaterials sampling for genetic evaluations was conducted from January 2003 through January 2008 using a combination of invasive and non-invasive approaches. Wild dog tissue and blood samples were obtained opportunistically during immobilization operations for translocation and collaring and when a wild dog carcass was located (Spiering *et al*. 2009). Faecal samples were collected fresh from known individuals within 5 to 30 min of deposition and then stored in labelled, plastic freezer bags at -20^{o}C until genetic analysis. In total, wild dog blood, tissue, and faeces were collected from 113 individuals representing 10 packs.

All individuals were genotyped at 17 dinucleotide microsatellite loci and two tetranucleotide loci that yielded 4.8 alleles per locus on average. These markers were consistent with other wild dog genetic studies and are commonly used for determining parentage in domestic dogs (Spiering *et al*. 2010). Specifics on DNA extraction, polymerase chain reaction (PCR) protocols, and methods used to eliminate genotyping and sampling errors are discussed in Spiering *et al*. (2009, 2010). Tests for deviation from Hardy-Weinberg equilibrium and tests for parentage in the present evaluation relied on the likelihood based approach in CERVUS software (Marshall *et al*. 1998). Locus INU030 was excluded from the parentage analyses because a significantly lower than expected frequency of heterozygotes was detected, indicating a high incidence of null alleles. No other locus deviated from Hardy-Weinberg equilibrium. The simulation program in CERVUS was used to establish the critical difference in natural logarithm of the likelihood ratio (LOD score) between the first and second most likely candidate parents (at ≥ 95% confidence). Only adults from within the pack with a given set of offspring were considered candidate parents because no extra-group copulations have been reported for this species (and analyses later confirmed that all parentage was assigned to pack members). We included genotypes for all genetically sampled individuals from the population to calculate pairwise relatedness estimates (r) with the program KINSHIP (version 1.3.1; Goodnight & Queller 1999) and used the observed r values to determine Wright's inbreeding coefficients (F; Wright 1922).

5.3.4 Tests of inbreeding avoidance

For our evaluation, and based on the observed r values derived from our population allelic frequencies, we considered first-order relationships to be parent-offspring or full sibling pairs.

Half sibling and aunt/uncle-nieces/nephew pairs were second-order kin, and first cousin pairs were third-order relatives. Since all breeding takes place within established wild dog packs, we tested for inbreeding avoidance by determining the frequency of situations in which packs included breeding pairs that were related. Specifically, the number of situations in which inbreeding might have occurred (both individuals were alive, sexually mature, and in the same group) were compared to behavioural observations of mating between (1) parents and offspring in natal packs, (2) parents and offspring after reproductive vacancies, and (3) mature siblings at dispersal. Secondly, to determine if mating occurred between close kin, we compared the pairwise genetic relatedness of breeding pairs to the mean pairwise values of individuals with known relationships in our population. Lastly, as there is a relatively stable group structure within African wild dog packs (i.e., a separate social hierarchy for males and females with a dominant breeding pair; Creel & Creel 2002), we also assessed the influence of pairwise genetic relatedness on mate choice by comparing the relatedness in breeding pairs with pairs within the population that did not breed with each other.

5.3.5 Statistical analyses

We assessed the relationships between opportunities for inbreeding, observed incestuous matings and population size by means of linear regression. We compared pairwise relatedness values of dyads of known relationships, breeding pairs and the entire population with Wilcoxon signed-rank tests. We used Student's t-tests to assess the maintenance of genetic diversity across varying inbreeding thresholds in our models. All statistical analyses were performed with JMP software version 3.2.2 (SAS Institute Incorporated) and means given ± standard error of the mean, except where indicated.

5.3.6 Population modelling parameters

We used VORTEX (Version 9.95; Lacy *et al*. 2005; Miller & Lacy 2005) population viability modelling software to evaluate the influence of inbreeding avoidance behaviours on population trends and extinction risk for the African wild dogs. Each simulation was repeated 1,000 times and results predicted over 100 years. Demographic rates reported below include measures of annual environmental variation, expressed as standard deviations around the mean values of variables (Miller & Lacy 2005).

The existing extensive database on the KZN African wild dog population was used for model development, with input from the published literature (Creel & Creel 2002; McNutt *et al*. 2008), as appropriate. To mimic a realistic population demographic structure and pedigree, a studbook file that included all individuals alive in the KZN population in December 2008 was used as input to the model. To simulate the social and reproductive characteristics of African wild dogs within VORTEX, we used a combination of settings in the model to reflect accurate reproductive rates, including proportions of animals within packs and across the population that actually bred. First, to reflect that wild dog packs generally are relatively stable with the same groups of individuals mating over several years and the dominant individuals often breeding repeatedly (Girman *et al*. 1997; Creel & Creel 2002), 'long-term monogamy' was selected as the reproductive system. Although more reproductive sharing than previously expected was discovered in breeding packs that contained adult siblings of the alpha pair, most pups in the KZN population were still produced by dominants (93% whelped by alpha females and 72% sired by alpha males) because many packs were comprised of only the alpha pair and their offspring (Spiering *et al*. 2010). Therefore, long-term monogamy captures the most important aspects of the wild dog reproduction scenario and is the closest approximation of the breeding system. We then calculated the percentages of males and females in the breeding pool, thereby dealing with the normative that only adult members of the pack have the opportunity to reproduce (i.e., excluding dispersing individuals or offspring remaining in natal packs). Thus, it was determined that an average of 48% of all adult males in the KZN population comprise the breeding pool, a value used throughout the analysis. For females, we added a function that would allow us to incorporate the percentage of individuals breeding based on specific age classes and whether or not a female had produced offspring in previous years. Only 7.7 ± 3.0% (SD) of 2 year old females normally have reproduced because younger wild dogs are less likely to have already dispersed and joined a breeding pack. While only 29.7 ± 10.0% of females >2 years old that had not whelped pups in previous years produce young, 95.6 ± 10.0% of females that had already reproduced previously as the alpha or beta individual continue to breed, most often until death (Spiering *et al*. 2010). In KZN, African wild dog females whelp pups at 1.3 to 10 years old, and males sire offspring from 1.1 to 10 years old (Spiering *et al*. 2010). As most individuals did not breed before 2 years old, this was set as the age at first offspring production with maximum breeding age fixed at 10 years. Mean litter size in this population is 7.6 ± 0.6 pups, with the largest litter recorded as 14 pups and near gender parity at this age class (0.51 ± 0.04; Spiering *et al*. 2010).

Using our demographic database, we calculated that age-specific mortality rates in the KZN population were similar to those reported by Creel and Creel (2002) for wild dogs in the Selous Game Reserve, Tanzania. Pup mortality (emergence through 1 year) in our region was 24.4 ± 8.0% for females and 22.5 ± 7.3% for males. Yearling mortality was 23.0 ± 7.0% for females and 8.2 ± 7.5% for males, with this rate remaining similar for 2 year old females and increasing for males (females, 22.6 ± 8.0%; males, 23.8 ± 4.8%). However, the incidence of mortality in females aged 3 years (42.9 ± 8.0%) and older (50.0 ± 8.0%) was higher than for counterpart males (27.3 ± 4.8% and 32.0 ± 4.8%, respectively). The latter finding is known to lead to increasing male bias in this species in older age classes (Frame *et al*. 1979; Reich 1981; Creel & Creel 2002; Spiering *et al*. 2010).

Inbreeding depression was simulated as a reduction in pup survival of inbred individuals according to the general model of Morton *et al*. (1956):

$$S_f = S_0 e^{-Bf}$$

where S_f and S_0 are survival rates for individuals with inbreeding coefficients equal to f and 0, respectively, and B is a constant describing the rate of decline in survival with increasing coefficient of inbreeding. The severity of inbreeding depression is typically expressed in terms of the number of lethal equivalents per diploid genome in the population of interest, which is conveniently expressed as $2B$. We used the nonlinear maximum likelihood approach of Kalinowski & Hedrick (1998) to estimate the number of lethal equivalents, using data on inbreeding coefficients derived from studbook records and observed survival rates. The maximum likelihood method produces results that are more statistically robust than more traditional linear regression methods. From this analysis, we estimate a total of $2B = 1.7$ lethal equivalents per diploid genome for the KZN wild dog population. We assume that 50% of this genetic load is attributable to lethal alleles, with the remainder composed of detrimental alleles, or those alleles that confer only a probability of dying for an individual that is homozygous for the allele in question. The distribution of the genetic load of a mammal population (i.e., lethal vs. detrimental) is impossible to determine through field observation. The choice of 50% of the genetic load due to lethal alleles is derived from extensive research in *Drosophila* that is routinely applied to analyses across mammals, birds, and other animals of conservation concern (Simmons & Crow 1977). Therefore, given this information, VORTEX reduces the survival probability of an inbred individual during their first year of life by the factor:

$$e^{-b(1-\Pr[Lethals])F}$$

where Pr[Lethals] is the proportion of the total inbreeding effect (number of lethal equivalents) attributable to lethal alleles. The VORTEX model explicitly allows for purging of deleterious alleles over time, with the rate of purging related to the type of genetic load (i.e., a higher proportion of lethal alleles leads to a faster rate of purging).

We initially set the carrying capacity in the model to twice the size of the KZN population as of December 2008 (initial population size = 81; carrying capacity = 162). The carrying capacity was altered in later scenarios to allow exploring its relationship to the probability of extinction in the presence of inbreeding avoidance. We purposely did not include supplementation from human management into the model because of our focus on understanding the future viability of the extant population. Natural immigration from other areas also was not included, because there have been no reports of immigrant wild dogs entering KZN or emigrants successfully reaching populations in other South African provinces.

Although pathogens are known to adversely affect the long-term persistence of African wild dog populations (Vucetich & Creel 1999), disease frequency and severity is difficult to ascertain due to limited access to dead individuals to determine cause of death and few historical records (Flacke 2007). Therefore, this variable was excluded from the models because the inclusion of these uncertain data could obscure our focus on the demographic effects of inbreeding avoidance behaviours.

We conducted a sensitivity analysis of our model to specifically identify the demographic and genetic variables that most greatly influenced the growth rates and viability of simulated African wild dog populations. This was accomplished by varying the percentages of females breeding, mortality rates, inbreeding thresholds, carrying capacities, or the percentage of males in the breeding pool by ± 25% individually, while keeping all other variables constant. The standard sensitivity index for each variable was calculated as $S = [(\lambda_{Base - 25\%} - \lambda_{Base + 25\%}) / (0.5* \lambda_{Base})]$, where λ was the annual rate of population growth calculated from the simulation, and subscripts *Base, Base - 25%, and Base + 25%,* referred to growth rates from models using the baseline parameter value and those increasing or decreasing that parameter value by 25%, respectively (Heppell *et al*. 2000). For incidence of mortality and percentage of females breeding, age classes were altered separately and together to determine whether age-specific characteristics most affected the population. These analyses included restrictions on inbreeding by preventing matings that would result in offspring of $F > 0.20$ ($r > 0.40$), which would prevent matings with first-order relatives only in the simulation.

To explore the effect on the wild dog population, inbreeding avoidance levels were varied to prevent matings with inbreeding coefficient values (F) greater than 0.20, 0.123 ($r = 0.246$), and 0.063 ($r = 0.126$) which prevent breeding between first-order relatives only, second-order (and more related) kin, and third-order (and more related) kin, respectively. VORTEX determines the suitability of mates by calculating the kinship between individuals based on the pedigree information in the studbook file that is continually updated by the program (Miller & Lacy 2005). Therefore, mating is restricted by familial relatedness and not by allelic similarities that may accumulate over time through genetic drift. These models preventing mating among kin were compared to the baseline scenario that did not include an inbreeding threshold (i.e., one that allowed all relatives to breed). Finally, we examined the influence of carrying capacity on population growth and persistence over time in the presence of inbreeding avoidance behaviours by conducting additional analyses varying this parameter in relation to initial population size.

5.4 Results

5.4.1 Behavioural evidence for inbreeding avoidance

From 1997 through 2008, we were able to observe 156 situations in which a female had the opportunity to mate with first-order adult kin of the opposite sex. Of these, 122 existed between a parent and offspring in the natal pack, eight between a parent and offspring after the death of a dominant adult (a reproductive vacancy), and 26 between siblings after dispersal (Table 5.1). As the population expanded over time, there was a corresponding increase in the number of opportunities for inbreeding between parents and offspring in natal packs ($R^2 = 0.68$, $P < 0.001$) and among siblings ($R^2 = 0.88$, $P < 0.001$), but not in number of reproductive vacancies occurring ($R^2 = 0.30$, $P = 0.06$). Most importantly, the frequency of observed incestuous pairings did not rise even while opportunities for inbreeding with close relatives increased (parent-offspring in natal pack: $R^2 = 0.003$, $P = 0.86$; siblings: $R^2 = 0.20$, $P = 0.15$). Over the course of the 11 year interval, inbreeding was rarely detected via behavioural observations. In one instance, a full sibling cohort comprised of two males and one female was unknown to each other; these individuals were born into the natal pack at different times and subsequently joined together after dispersal and produced pups. In the second case, a son mated with his mother while in the natal pack, which led to a litter comprised of offspring sired by the son and others sired by the alpha male (his father; Spiering *et al*. 2010). The son in this situation went on to displace his father and fill the reproductive vacancy to continue breeding with his mother (Table

5.1). The only other inbreeding circumstances were associated with two pairs of third order relatives, one aunt-nephew and one cousin-cousin (or half cousin) coupling, each occurring in different packs. In both of these latter cases, neither of these dogs was familiar with the other, having been raised in different natal packs. Otherwise, opposite sex siblings dispersed and generally formed temporary groups for up to 2 years, but these cohorts never interbred and later joined other groups. Collectively, these observations suggest that African wild dogs are actively recognizing and avoiding breeding with familiar kin.

Table 5.1 Occasions where inbreeding was possible versus observed in the field within natal packs, after reproductive vacancies, and among siblings in the KZN African wild dog population (adults and yearlings) from 1997 through 2008.

			Parent-offspring in natal pack		Parent-offspring after reproductive vacancy		Siblings after dispersing	
Year	Pop. Size	No. packs	Inbreeding possibilities	Inbreeding observed	Inbreeding possibilities	Inbreeding observed	Inbreeding possibilities	Inbreeding observed
1997	9	2	0	0	0	0	1	0
1998	10	2	2	0	0	0	1	0
1999	15	2	10	0	2	0	1	0
2000	5	1	6	0	0	0	1	0
2001	6	2	0	0	0	0	1	0
2002	14	2	7	0	0	0	2	0
2003	24	3	13	1[§]	0	0	2	0
2004	31	3	12	0	1	1[§]	2	0
2005	44	5	17	0	0	0	3	0
2006	59	5	13	0	3	0	3	1[†]
2007	54	6	12	0	0	0	4	0
2008	64	7	30	0	2	0	5	0
Proportion of cases with inbreeding			1/122 = 0.8%		1/8 = 12.5%		1/26 = 3.8%	

[§] Mother-son pair that bred together multiple years
[†] Full siblings unfamiliar to each other due to being born 2 years apart and present in the natal pack at different times

5.4.2 Genetic evidence for inbreeding avoidance

Mean pairwise relatedness values calculated for dyads with known relationships were slightly lower than the expected theoretical value of 0.50 for parent-offspring (r = 0.40 ± 0.03) and full siblings (*r* = 0.42 ± 0.01), while relatedness values for half siblings were consistent to the theoretical value of 0.25 (*r* = 0.25 ± 0.04; Fig. 5.1). The mean pairwise relatedness of all dyads in the population was -0.003 ± 0.004 (n = 81 individuals), which was higher than the mean *r* value comparing only unrelated individuals (n = 58 individuals, *r* = -0.12 ± 0.01; Wilcoxon test: T_{4879} = 17.10, P < 0.0001).

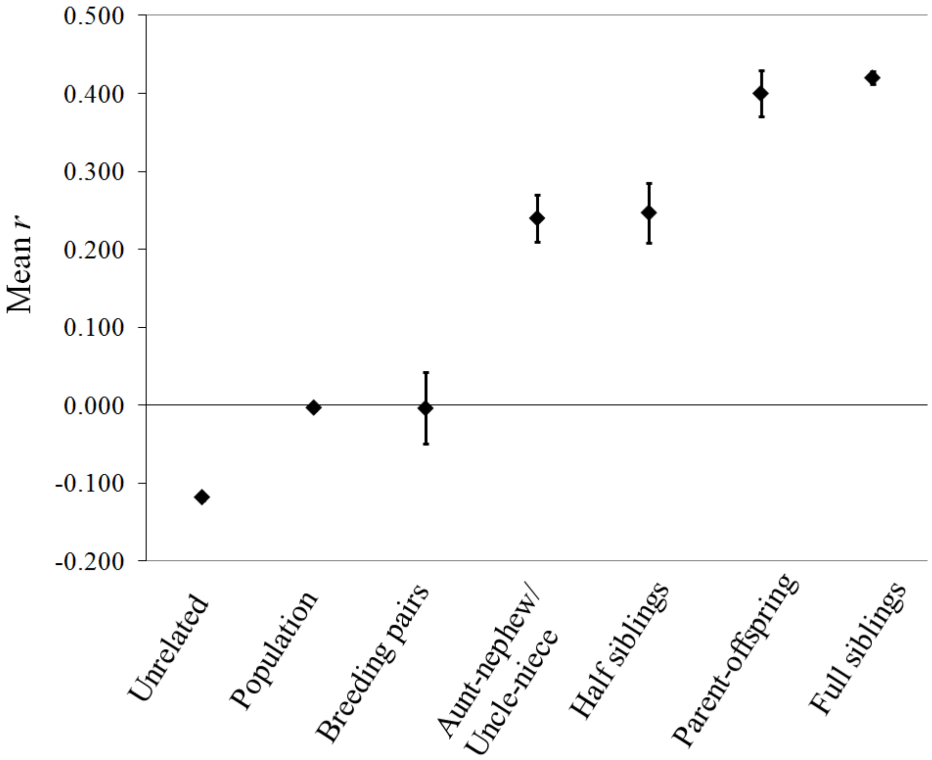

Figure 5.1 Mean pairwise relatedness (*r*) for different relationships in the KZN wild dog population with numbers of dyads examined for each category indicated.

To determine whether confirmed breeding pairs of individuals were closely related, we estimated *r* for 23 dyads confirmed via genetic analyses to have produced offspring. Pairwise relatedness of breeding males and females ranged from -0.36 to 0.45 with a mean of -0.04 ± 0.05 (Figs. 5.1 and 5.2). The latter did not differ from an *r* = 0 or from the mean of all other population pairwise *r* values (*r* = -0.006 ± 0.004; Wilcoxon test: T_{4751} = -0.630, P = 0.52).

Because the distribution of all possible *r* values excluding breeding pairs was centred near zero and was approximately normal (Fig. 5.2), it was possible to affirm that mating pairs were not closely related. Of the 23 confirmed breeding pairs, 73.9% (n = 17) were more distantly related than third-order kin in the population. In contrast, there were only two pairs (8.7%) that had *r* values similar to first-order relatives, two pairs (8.7%) with *r* values comparable to second-order kin and two pairs (8.7%) with relatedness values consistent to third-order relationships. Of the six breeding pairs that were confirmed to be related at the third-order kin level or higher (via behavioural or genetic analysis), only one (16.7%) was comprised of individuals who were familiar with one another, involving a mother and her son. This mother-son pair bred for 4 consecutive years, both in the intact natal pack and after a reproductive vacancy. The other five related pairings were genetic relatives but consisted of individuals that were never simultaneously in a common natal pack before joining together to breed.

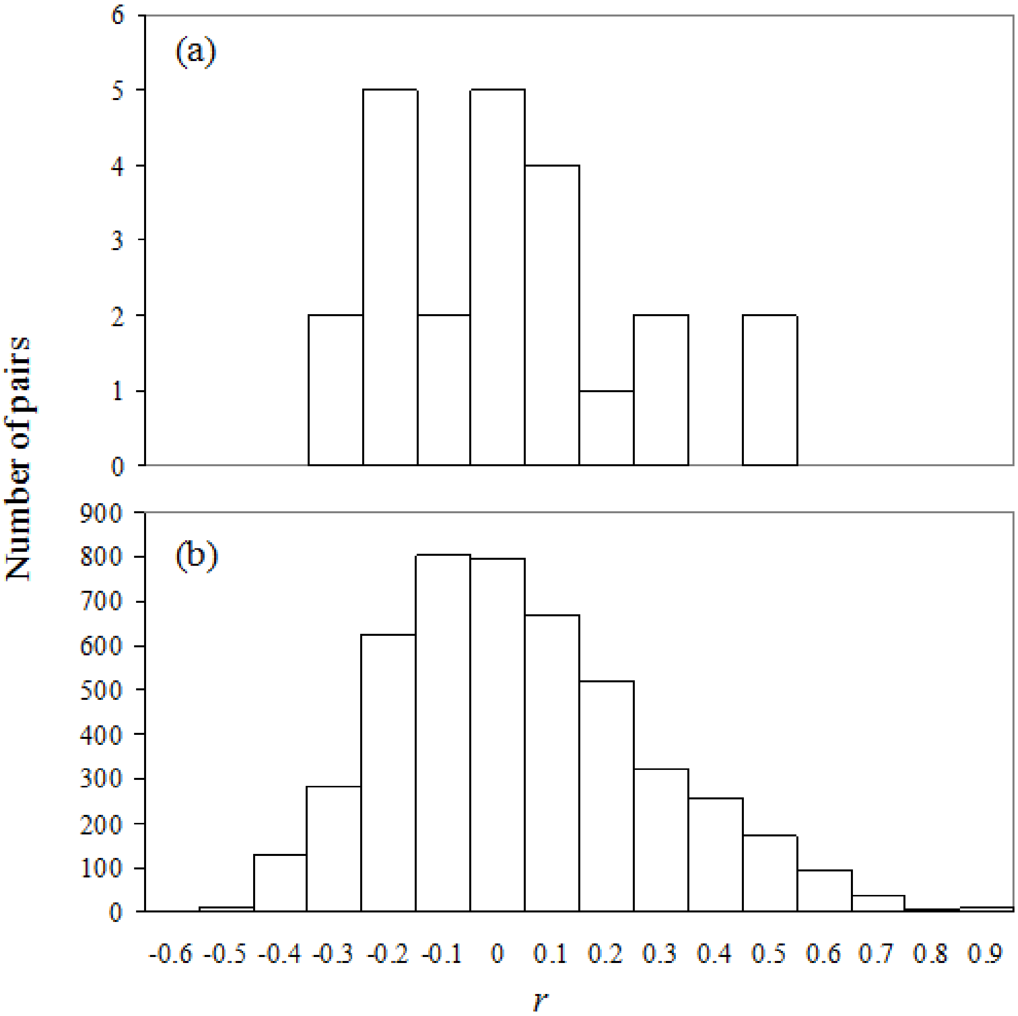

Figure 5.2 Relatedness (*r*) of mated pairs (a) compared with relatedness values for all possible pairs in the KZN population (b).

5.4.3 Population modelling analyses

In general, simulated African wild dog populations were influenced most by the (1) proportion of adult females (>2 years) that were able to begin breeding, (2) mortality of females 3 years and older, and (3) inbreeding thresholds that limited the number of suitable mates (Table 5.2). Other tested factors (e.g., 2 year old female mortality, adult male mortality, carrying capacity, pup mortality and percentage of males in the breeding pool) were less sensitive to variation in the model (Table 5.2).

Table 5.2 Sensitivity analyses for selected model input variables with a ± 25% variation range in values for a simulated African wild dog population.

Model Parameter	-25%	Baseline	+25%	S
*Females >2 yr first breeding (%)	22.3	29.7	37.1	-0.1523
*3 yr old female mortality (%)	32.2	42.9	53.6	0.0935
*>3 yr old female mortality (%)	37.7	50	62.5	0.0929
*Inbreeding avoidance (F threshold)	0.15	0.2	0.25	-0.0795
2 yr old female mortality (%)	17	22.6	28.3	0.0560
Adult male mortality (%)	2 yr = 17.9 3 yr = 20.5 >3 yr = 24.0	2 yr = 23.0 3 yr = 27.3 >3 yr = 32.0	2 yr = 29.8 3 yr = 34.1 >3 yr = 40.0	0.0358
Carrying capacity (individuals)	122	162	203	-0.0312
Pup mortality (%)	F = 18.3 M = 16.9	F = 24.4 M = 22.5	F = 30.5 M = 28.1	0.0303
Males in breeding pool (%)	36	48	60	-0.0093

*Indicates the variables with the highest model sensitivity (S).

In support of the hypothesis that inbreeding avoidance is a significant predictor of population persistence over time, all models that included an inbreeding threshold demonstrated a probability of extinction of 100% within 100 years. The model with mild inbreeding avoidance (to exclude only first-order relative matings) revealed that simulated populations went extinct within 63.1 ± 0.2 years (Fig. 5.3a). Populations avoiding mating with second-order and more related kin survived 37.0 ± 0.1 years, whereas those that also did not pair with third-order kin became extinct after only 18.7 ± 0.08 years (Fig. 5.3a).

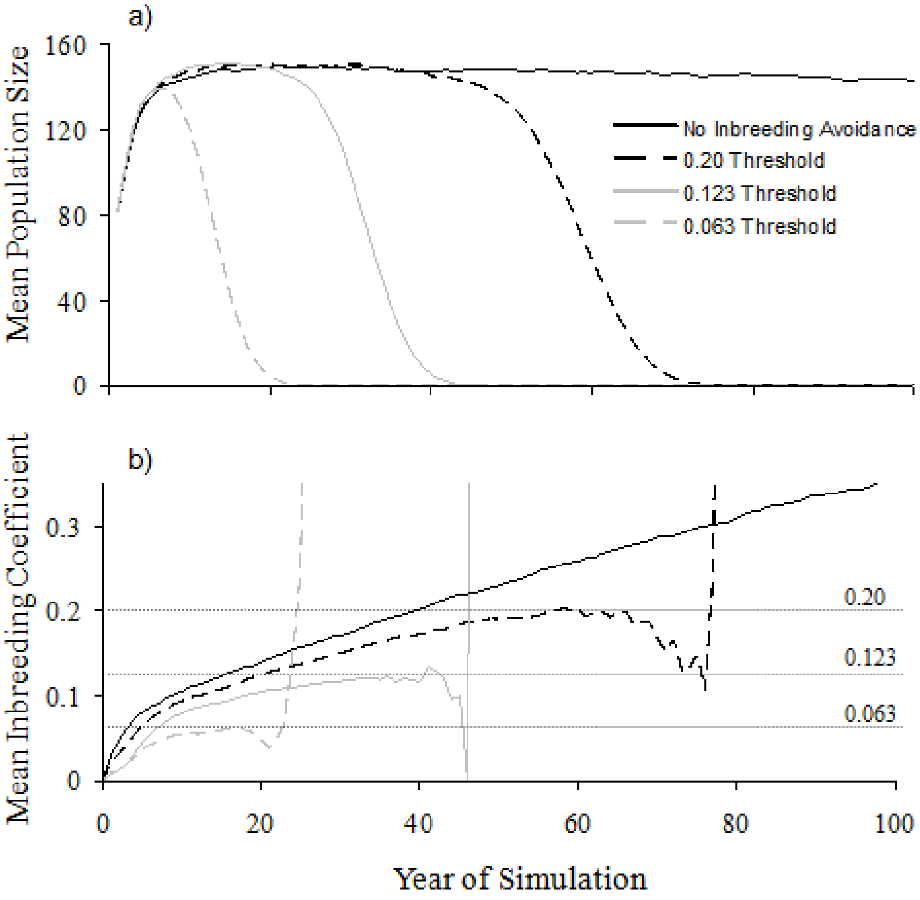

Figure 5.3 Mean projected population size (a) and mean inbreeding coefficients (b) of simulated African wild dog populations over 100 years without inbreeding avoidance behaviours, with prevention of parent-offspring and full-sibling matings (F = 0.20), with prevention of aunt-nephew/uncle-niece and half-sibling matings and higher (F = 0.123), and with prevention of first cousin matings and higher (F = 0.063). Dotted horizontal lines in (b) indicate inbreeding thresholds. The erratic behaviour of mean inbreeding coefficients just before extinction is the result of very small population sizes that lead to unusual mean values near F = 1.0.

The model not preventing inbreeding had only a 1.6 ± 0.4% chance of extinction before 100 years, and the small percentage of simulations declining to $N = 0$ lasted 50.6 ± 7.4 years. All population models grew rapidly in the first 6 years of the simulation with mean stochastic growth of 13.8 ± 0.01% (no threshold), 15.3 ± 0.02% (F = 0.20), 15.0 ± 0.02% (F = 0.123), and 13.9 ± 0.02% (F = 0.063) until reaching a carrying capacity set at twice the size of the initial population. During years of population expansion, genetic diversity was maintained more effectively in cases that presented the strongest inbreeding avoidance behaviours (Fig. 5.3b and Table 5.3). Populations with inbreeding thresholds preventing first-order relative matings (t-test: $t_{10} = -2.75$, P = 0.02), second-order matings ($t_{10} = -5.58$, P < 0.001) and third-order matings ($t_{10} = -7.18$, P < 0.0001) retained gene diversity better than those without inbreeding avoidance. Despite improved retention of gene diversity in the absence of inbreeding, the limited availability of suitable mates eventually led to demographic failure in these populations. While populations without an inbreeding threshold remained near carrying capacity for the 100 year simulation, in cases where inbreeding was prevented, reproduction slowed, and then pup production stopped due to the presence of only related individuals remaining as potential mates (Fig. 5.4). Reproduction ceased completely when, for example, first-order relatives were prevented from mating, even when 8 females and 12 males remained in the population (Fig. 5.4).

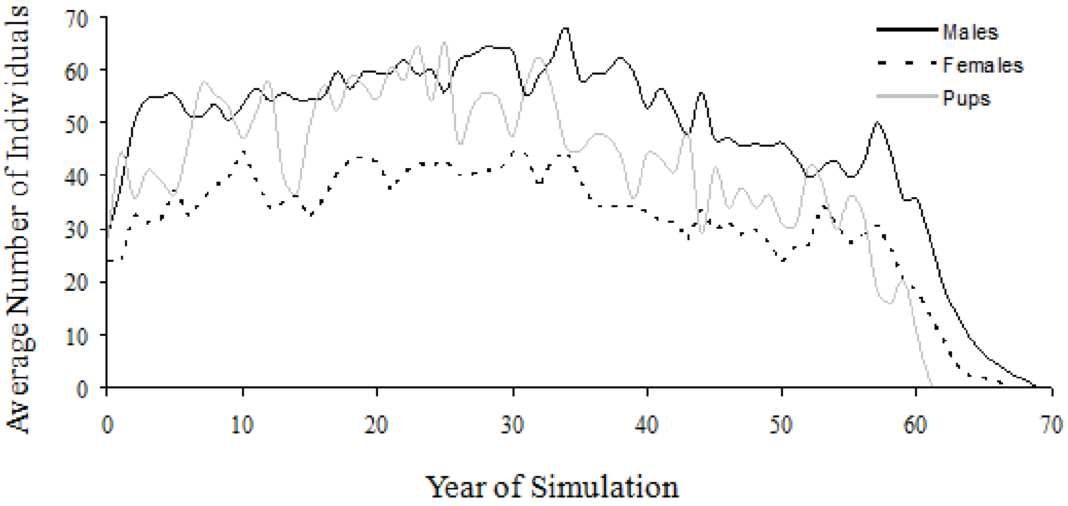

Figure 5.4 Average number of adult male, adult female and juvenile wild dogs in simulated populations maintaining an inbreeding threshold of F = 0.20.

Once inbreeding thresholds began to influence the numbers of individuals that were able to breed, there were dramatic population declines that superseded previous benefits from retained genetic diversity (Fig. 5.3b and Table 5.3).

Table 5.3 Average percent gene diversity lost annually before inbreeding thresholds were reached and population growth rates after thresholds began influencing mate availability in simulated African wild dog populations experiencing a range of levels of inbreeding avoidance.

	Loss of gene diversity before inbreeding threshold (%)	Population growth rate after inbreeding threshold (%)
No Inbreeding Avoidance	-0.43	11.57
F = 0.200	-0.39	-5.73
F = 0.123	-0.32	-13.62
F = 0.063	-0.27	-28.65

Varying the carrying capacity also influenced growth potential and long-term viability of populations (Fig. 5.5). Simulations with carrying capacities one, two, and three times the initial population size (81, 162, and 243 individuals, respectively) had a 100% chance of extinction before 100 years and survived an average of 40.9 ± 0.2 years, 62.9 ± 0.3 years, and 80.9 ± 0.4 years, respectively. In contrast, models set to carrying capacities of four and five times the initial population (324 and 405 individuals, respectively) were considerably more likely to persist than smaller areas with only 54.1 ± 1.6% and 17.3 ± 1.2% probabilities of extinction, respectively (Fig. 5.5).

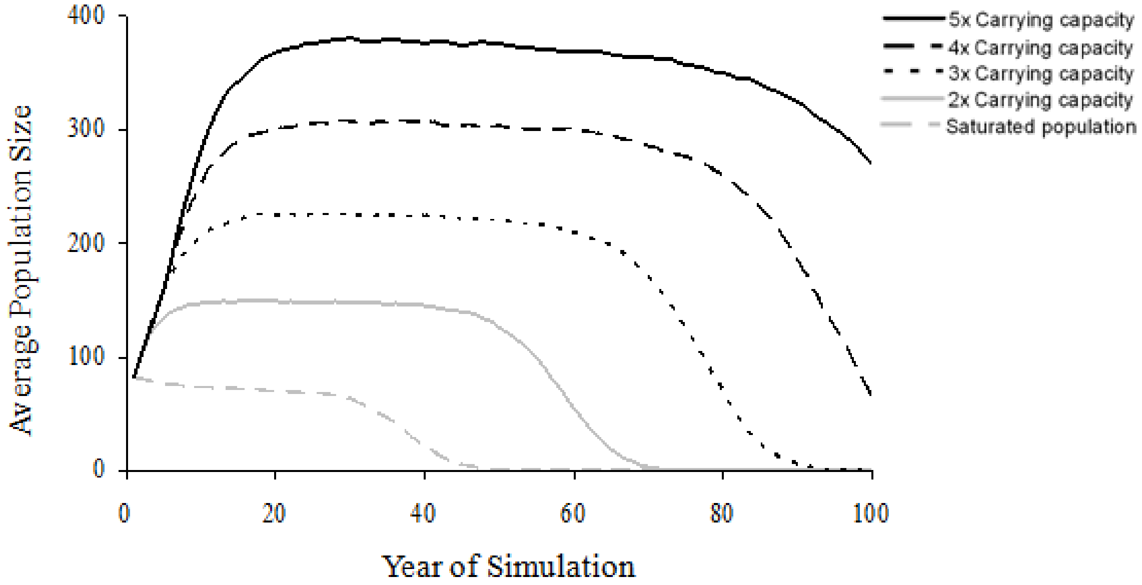

Figure 5.5 Average projected size of simulated wild dog populations over 100 years with the carrying capacity parameter set at varying levels in relation to initial population size. Model assumes an inbreeding avoidance threshold of F = 0.20.

5.5 Discussion

Results from our integrated behavioural, genetic, and demographic evaluation support our hypothesis that strong inbreeding avoidance is present in the African wild dog, and indicate that individuals within this species have the capacity to discriminate between kin and non-kin through ‘recognition by association’. It is likely that these animals learned during rearing to recognize familiar individuals (Blaustein *et al*. 1987). In the KZN study population, we observed only one individual breeding with a parent in the natal pack. We also observed a case of sibling cohorts forming a breeding pack and just one reproductive vacancy being filled by offspring of the breeding pair. Our molecular genetics assessment illustrated that a majority of wild dog mating pairs (~74%) were unrelated or distantly related individuals. Familiarity appeared key to avoiding incestuous matings as only one of the six inbreeding cases was associated with individuals that knew each other well. The other five instances involved relatives that were previously unfamiliar as they had not resided together earlier within the same pack.

Our finding that wild dogs had an apparent ability to recognize related kin by association was compatible with our recent discovery of a higher than expected rate of reproductive sharing in this same population (Spiering *et al*. 2010). This previous investigation determined that in packs containing siblings and half siblings of the alpha individuals, subordinate males sired up to 45% of pups and subordinate females whelped litters in half of all years. Although facilitating the maintenance of genetic diversity in this small, reintroduced population (Miller *et al*. 2009), this strategy of shared parentage could, in theory, make it challenging for offspring to distinguish parents from aunts, uncles, and non-relatives. In such circumstances, kin recognition by association would be strongly favoured. Our observations of no inbreeding between siblings, aunts, uncles, nieces, and nephews that shared packs, along with limited cases of interbreeding between unfamiliar relatives, suggested that kin recognition based on familiarity exists in this species to avoid inbreeding. Our finding also is consistent with observations for other species where individuals identify relatives based on prior association, including the long-tailed tit (*Aegithalos caudatus*) that learns contact calls of close relatives (Sharp *et al*. 2005) and naked mole rat (*Heterocephalus glaber*) that differentiates between odours of kin and non-kin (Clark & Faulkes 1999). In canids, both young wild dog and coyote (*Canis latrans*) pups have been observed to have been successfully fostered by unrelated adults, suggesting that genetic relatedness is not necessary for parents to recognize offspring as their own (Kitchen & Knowlton 2006; McNutt *et al*. 2008). Similarly, familiar olfactory signals could logically act as important recognition cues in African wild dogs because scent marking is important in this species, and pack members are often in close physical contact with each another (Mills 1993).

Because of the relative stability and long tenure of African wild dog packs (deVilliers *et al*. 2003), avoidance behaviours based on familiarity would almost always prevent matings of first-order relatives while likely decreasing the chances of mating with second-order relatives. However, some second-order relatives and most cousins would be unfamiliar to each other if originating from different packs. In this study, we observed that pairings between unfamiliar kin are avoided less often, therefore, we suspect that avoidance mechanisms in wild dogs have maintained an inbreeding threshold in KZN in the range of $F = 0.123$ to $F = 0.063$, which takes into account some matings by third-order relatives (and a few others of second- and third-order relatives). This threshold may increase with time when the population becomes more interrelated and cases of inbreeding associated with third-order relatives increase as individuals becoming increasingly temporally separated or are born into different, albeit related, packs.

Although we determined that wild dogs sustain significantly more genetic variation annually by avoiding incestuous matings, our simulations illustrated the potential adverse consequences on future demographics, especially while populations are declining and becoming more isolated. Specifically, without the supplementation of unrelated individuals through natural immigration or management, modelling demonstrated that the number of suitable, unrelated mates continued to dwindle and inbreeding thresholds eventually were reached. This, in turn, led to marked, negative population growth, rapid population decline, and (even in the presence of only mild avoidance to prevent breeding of first-order relatives) nearly certain population extinction within 100 years. Although population growth was as high as 15% annually in models before reaching carrying capacity, a similar real population within KZN (presenting strong avoidance behaviours as demonstrated in our study) would approach extinction within 20 to 40 years without the supplementation of unrelated individuals. There is some evidence that inbreeding avoidance may have led to population decline previously in KZN since the African wild dog population stopped breeding completely in the 1980s and 1990s when it was suspected that all remaining individuals were related (Maddock 1996). Reproduction recommenced after translocations of new individuals to rebuild the population (Somers *et al*. 2008). A similar event occurred with Scandinavian wolves (*Canis lupus*), where the population remained fewer than 10 individuals within one breeding pack for several years and then grew exponentially after the arrival of a single immigrant in 1991 (Vilà *et al*. 2003).

Our simulations also were useful for generating new insight into the significance of long-distance dispersal, which already has been recognized as a significant means for avoiding inbreeding and maintaining gene diversity in the African wild dog (Girman *et al*. 1997). It is well established that opportunities for offspring to reproduce are only created by leaving close relatives in the natal pack to find mates and form new breeding packs (McNutt 1996; Girman *et al*. 1997). But, emigration also poses significant risks in this species, predominantly mortality that is 1.5 and 1.4 times higher annually for dispersing males and females, respectively, compared to non-dispersing counterparts (Creel & Creel 2002). This risk also tends to be 1.5-fold greater for females than males due to a longer duration of 'floating' between packs (Creel & Creel 2002). When the corresponding high mortality rates in KZN that include dispersal costs were incorporated into our simulations, the population was particularly sensitive to the loss of adult females, thus creating demographic vulnerability. Others have suggested that emigration exceeding immigration within isolated populations of cooperative breeders can lead to dispersal becoming detrimental, especially as groups drop below a critical threshold size needed for hunting (Creel & Creel 1995; Courchamp *et al*. 1999) and caring for young (Malcolm & Marten 1982). Consequently, inbreeding avoidance in small, fragmented groups of African wild dogs could drive both dispersal and a mate-finding Allee effect (Courchamp *et al*. 1999; Somers *et al*. 2008) that, in turn, pushes the overall population into a steady decline.

The advantage for males or females to avoid or accept incestuous matings has been reported to depend on the degree of reproductive investment for each sex and the strength of inbreeding depression (Kokko & Ots 2006). Specifically, mutual mate choice for avoiding interbreeding with relatives should evolve when reproductive investment is symmetrical between the sexes and when inbreeding costs are high, thus favouring outbreeding (Parker 2006). Supporting this hypothesis, both male and female African wild dogs display high reproductive investment in young within their cooperative breeding system (Malcolm & Marten 1982) and here we found evidence of inbreeding avoidance likely through kin recognition. This also suggests that the deleterious effects of inbreeding in this species have the potential to be severe. Therefore, wild dogs may have evolved inbreeding avoidance behaviours because the cost of investing in energetically expensive gestation and/or parent care of pups is larger than the fitness payoff of producing more homozygous offspring. Additionally, because higher dispersal risks broaden the conditions favouring inbreeding tolerance (Waser *et al*. 1986), the detrimental effects of wild dog relatives interbreeding must be severe to warrant costly dispersal behaviours.

Our modelling indicated that, paradoxically, the same mechanism that evolved to prevent incestuous matings and to maintain genetic diversity could promote population extinction in KZN within 2 to 4 decades simply because too few potential mates are available for dispersing individuals. Other species also have experienced the negative effects of inbreeding avoidance on population demographics, including contributing to extinction prior to the onset of serious inbreeding depression (Koenig & Haydock 2004). For example, the red-cockaded woodpecker (*Picoides borealis*), which avoids mating with first-order relatives while having a short dispersal distance, has been found to be highly susceptible to population decline and extinction in the absence of translocations of new individuals (Daniels *et al*. 2000). Additionally, it has been discovered that offspring of the acorn woodpecker (*Melanerpes formicivorus*) do not fill reproductive vacancies to breed with parents, which has resulted in population declines of 1.8 to 2.3% annually (Koenig *et al*. 1999). Because populations in the southwestern United States are demographically fragile and dependent on immigration, this species has become particularly vulnerable to extinction (Koenig & Haydock 2004). Similarly, the sensitive African wild dog faces demographic failure because historically important dispersal corridors have divided populations that were previously connected. Strong selection pressures are possibly currently acting against the very same kin recognition and dispersal behaviours that long ago evolved to prevent the negative demographic effects of inbreeding. However, it is unlikely that sufficient time has passed for natural selection to take effect and cause wild dogs to alter these behaviours because the most dramatic increases in disperser mortality and decreases in population size has occurred over a very short period of time.

5.5.1 Conservation implications

Our findings demonstrated the presence of inbreeding avoidance in the African wild dog, which suggested that, given adequate resources (habitat, prey) and low persecution, populations of this species would naturally evade incestuous matings and, thereby, sustain robust genetic diversity. However, given the current dire status of wild dog habitat coupled with high levels of persecution (Woodroffe *et al*. 2004) and adult mortality (Woodroffe & Ginsberg 1997; Creel & Creel 2002), our results indicate that inbreeding avoidance could further compromise the conservation status of this endangered species. Naturally low population densities and high numbers of human induced deaths may compound Allee effects created by inbreeding avoidance, leading to continuous population declines and extinction of small populations. Although carrying capacity was not the most sensitive variable in our models, results showed that larger carrying capacities afforded by protected areas may have a marked influence on growth potential and long-term viability of African wild dog populations. Securing even larger expanses of protected area to satisfy the habitat and prey requirements of wild dogs will be a challenge future conservation strategists must consider to ensure adequate population sizes for long-term viability Specifically, mitigating strategies should include maintaining and linking as much prey-filled, protected areas as possible. However, it also appears useful to continue translocating individuals between isolated populations to mimic natural immigration and to ensure that more dispersers locate unrelated mates. Finally, this study supports the value of integrating longitudinal and detailed monitoring of species demography, behaviour, and genetic status for making informed management decisions.

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CHAPTER 6: INTEGRATING GENETICS INTO POPULATION MODELS IMPROVES VIABILITY ASSESSMENTS FOR ENDANGERED SPECIES MANAGEMENT

6.1 Abstract

Population viability analysis (PVA) is a powerful tool for conservation, yet it is not often used to evaluate the success of endangered species reintroductions, in part, due to inadequate information on the genetic status of free-ranging species. We integrated extensive demographic and genetic data from an African wild dog population in the KwaZulu-Natal Province, South Africa into PVA models to: 1) determine those factors most influential to population persistence; 2) assess the viability of the current population; and 3) recommend a reintroduction strategy that we predicted would produce a demographically *and* genetically viable population for at least 50 yr. Modelling simulations revealed that wild dog populations were influenced most by the proportion of adult females that had the capacity to begin breeding, the mortality of females 3 yr and older, and the severity of disease outbreaks. Under current conditions, simulated populations persisted for an average of only 34.7 yr, thus failing to meet demographic or genetic viability criteria. Within the limited habitat currently available, the model predicted that demographic viability could be maintained by translocating a dispersal group into the area every 4 yr. In contrast, the ability to retain at least 90% of genetic diversity for 50 yr involved more intensive management, requiring supplementation of two dispersal groups every 2 yr. Our findings illustrate the value of including genetic information for wild populations and how such data can be incorporated into PVA modelling to produce more realistic expectations for guiding reintroduction strategies.

6.2 Introduction

Population viability analysis (PVA) has become a popular means of evaluating management strategies for small populations. Specifically, PVAs are used to identify the probability of population success, the financial or logistical costs of different management actions, and the time needed to accomplish conservation goals (Beissenger & Westphal 1998; Brook *et al*. 2000). Interestingly, this process has been used sparingly for forward planning of endangered species' reintroductions (Morris *et al*. 2002), in part, due to incomplete knowledge of basic population demographics and/or the most appropriate model structure to use (Frankham *et al*. 2002; Reed *et al*. 2002). Furthermore, genetic considerations have not been integrated well into most models (Beissenger 2002; Frankham *et al*. 2002), which has been a significant criticism about the usefulness of PVAs for making informed conservation decisions (Oostermeijer 2000; Kirchner *et al*. 2006). Although there is some evidence that genetic factors may lead to the extinction of reintroduced populations (Frankham 2009), genetic goals or inputs have been rarely incorporated into PVA predictive planning for, or testing of the effectiveness of, endangered species restorations.

It appears imperative to consider genetic aspects in reintroduction planning and evaluation since these are critical for assuring the long-term sustainability of populations (Moritz 1999; Frankham 2009). Exclusion of genetic information from PVA models may produce overly optimistic predictions of population persistence and lead to flawed management recommendations, especially in cases where populations are small, reproductively isolated and/or have short generation intervals (Frankham *et al*. 2002; Keller & Waller 2002). The VORTEX software package for population viability analysis (Version 9.98; Lacy *et al*. 2005; Miller & Lacy 2005) is especially valuable as a tool for incorporating a variety of genetic variables into demographic models that examine alternative management options (Frankham *et al*. 2002). However, producing reliable simulations and projections of population persistence requires both detailed demographic and genetic information (Lindenmayer *et al.* 1995). Such thorough data are rare, and we are aware of only two species that have permitted integrated *in situ* investigations (Ballou *et al*. 2008: *Equus caballus*, feral horse; Vonholdt *et al*. 2008: *Canis lupus,* grey wolf).

In this paper, we illustrate that the African wild dog (*Lycaon pictus*) is a species that demonstrates the predictive powers of the PVA process for guiding future reintroductions. Our reasoning is that there are substantial amounts of population-specific data available (Girman *et al*. 1997; Creel & Creel 2002; Creel *et al*. 2004; Moueix 2006; McNutt & Silk 2008), especially for our study region of the KwaZulu-Natal (KZN) Province of South Africa (Graf *et al*. 2006; Gusset *et al*. 2006; Somers *et al*. 2008; Spiering *et al*. 2010). There are approximately 3,000 to 5,500 free-ranging wild dogs in all of Africa (Woodroffe *et al*. 2004), with only 300 to 400 individuals remaining in South Africa (Lindsey 2009). Of the latter, there is a viable population in Kruger National Park (n = ~150 animals) with the second largest assemblage existing in our study area. The KwaZulu-Natal population was established from multiple reintroductions into Hluhluwe-iMfolozi Park (HiP) during the 1980s followed by additional translocations into HiP in 1997 (Somers & Maddock 1999), 2001, and 2003 (Graf *et al*. 2006; Gusset *et al*. 2006) and into additional protected areas in the province in 2005 and 2006 (Davies-Mostert *et al*. 2009).

The overall goal of the KZN Wild Dog Management Group (comprised of conservation authorities, private land owners, researchers, and other stakeholders) is to establish and assist in the management of a viable population of this endangered species within the province through reintroductions. The KZN wild dog population has increased steadily in size through translocations, reproduction, dispersal, and natural group formations and as of November 2009 consisted of 10 breeding packs comprised of 114 individuals. The habitat for these animals is protected areas that are enclosed with electrified, game-proof fencing which is sufficiently ‘porous’ to allow single-sex groups to disperse from natal packs and travel throughout the province in search of mates. Wild dogs in KZN are known to travel more than 200 km to multiple game reserves to find opposite-sex groups (unpublished data). Therefore, although current and potential wild dog habitat in KZN is not contiguous, a level of connectivity is achieved through successful dispersal.

One of our highest conservation priorities is to determine how to better guide reintroduction strategies. As the population of KZN wild dogs is one of the world’s most thoroughly monitored assemblages of wildlife, demographically and genetically, we were keen to determine if our collective data could provide new insights for guiding future species restoration planning. We were especially interested in addressing four critical questions. First, what demographic and/or genetic variables most influence population growth and success of the KZN population? Second, what is the expected viability of the current KZN wild dog population without further reintroductions? Third, how many releases are needed to maintain a demographically viable wild dog population in the area for at least 50 yr? And fourth, how many additional reintroductions or supplementations are needed to maintain a demographically *and* genetically viable population for at least 50 yr? We anticipate that our results will provide new scholarly information on the value of both long-term assessments of demographic and especially genetic diversity for improving the usefulness of the PVA process. This exercise will also be of practical use for wild dog specialists tasked with ensuring effective long-term management of this endangered species.

6.3 Methods

6.3.1 Demographic and genetic sampling

Intensive demographic and behavioural monitoring was conducted for the KZN wild dog population from August 1997 through November 2009. Data on pack composition (number of animals, age, and gender), life history information (births, dispersals, pack formations, deaths), dominance (hierarchy of individuals of each sex per pack), and reproductive status (mating, denning) were collected at least once and as often as 10 times per month. Detailed methods for these procedures can be found in Spiering *et al*. (2009, 2010). In brief, over the 12 yr study period, 327 individual wild dogs were identified by unique coat patterns and were known from birth or the date of translocation to KZN from other sites. At least one and as many as four individuals per pack were fitted with VHF radio-collars to facilitate group monitoring. Genetic sampling and analyses were conducted from January 2003 through January 2008 using a combination of hands-on and non-invasive approaches. Sample collection, DNA extraction, microsatellite genotyping and characteristics of the 19 loci used are described elsewhere (Spiering *et al*. 2009, 2010). Briefly, wild dog tissue, blood or faeces were collected to obtain a representative genetic sample across the population (n = 113 individuals from 10 packs).

6.3.2 PVA model and population inputs

We used the VORTEX software package (Version 9.98; Lacy *et al*. 2005; Miller & Lacy 2005) to model the impact of deterministic forces, stochastic events, genetics, and reintroduction strategies on the KZN wild dog population. Simulations were repeated 1,000 times and results predicted over 50 yr. For the purposes of this study, a population was considered demographically viable when it had a ≤10% probability of extinction during a 50 yr period (Hilton-Taylor 2000) and genetically viable when populations had a ≤10% decrease in expected heterozygosity during a 50 yr period (Foose *et al*. 1986; Foose 1993).

The extensive database on the KZN wild dog population was used for model development with input from established publications (Creel & Creel 2002; Creel *et al*. 2004; McNutt & Silk 2008). A description of the model structure and population parameters is presented in Appendices A and B, and a summary of the PVA input data provided in Table 6.1. To simulate the social and reproductive characteristics of African wild dogs within VORTEX, we used a combination of settings in the model to reflect accurate reproductive rates, including proportions of animals within packs and across the population that actually mated. To mimic a realistic population demographic structure and pedigrees, a studbook file including all individuals alive in the KZN population in November 2009 was used as the initial population for the model.

Table 6.1 Inputs used to model the reintroduced African wild dog population in KwaZulu-Natal, South Africa.

Model input	Value[a]
Breeding system	long-term monogamy
Maximum breeding age	10
Minimum female breeding age	2
Minimum male breeding age	2
Pup sex ratio (% males)	51
Females producing litters (%)	
2 yr olds	7.7 (3.0)
3 yr olds	29.7 (10.0)
3 yr olds	95.6 (10.0)
Mean litter size	7.6 (3.2)
Largest litter size	14
Males in breeding pool (%)	48
Female mortality	
Pups	24.4 (8.0)
Yearlings	23.0 (7.0)
2 yr olds	22.6 (8.0)
3 yr olds	42.9 (8.0)
4 yr and older	50.0 (8.0)
Male mortality	
Pups	22.5 (7.3)
Yearlings	8.2 (7.5)
2 yr olds	23.8 (4.8)
3 yr olds	27.3 (4.8)
4 yr and older	32.0 (4.8)
Lethal equivalents	1.7
Due to recessive lethals (%)	50
Inbreeding threshold (F)	0.123
Probability of catastrophe (disease)	8.0
Severity on reproduction	0.8
Severity on survival	0.8
Environmental variation, survival, and reproduction	concordant

[a] Standard deviations due to environmental variation (Miller & Lacy 2005) are shown in brackets.

6.3.3 Genetic considerations

Tests for parentage completed with CERVUS software (Marshall *et al*, 1998; Spiering *et al.* 2009, 2010) were used to verify pedigrees and construct the initial population studbook file. Including all genotypes for genetically sampled individuals from the population, we calculated pairwise relatedness estimates (r) with the program KINSHIP (version 1.3.1; Goodnight & Queller 1999) and used these to determine Wright's inbreeding coefficients (F; Wright 1922). Relying on the genetic management options within VORTEX, we applied rules that limited the genetic suitability of potential mates by preventing breeding between individuals that would produce offspring with inbreeding coefficients greater than F = 0.123. This quantitative threshold prohibits matings between second-order kin and more closely related individuals (i.e., parents-offspring, full-siblings, half-siblings, aunts-nephews, or uncles-nieces), which is consistent with our earlier observations (Spiering *et al*. submitted).

6.3.4 Sensitivity analyses

To determine the factors that drive growth dynamics of our simulated wild dog population, we conducted a sensitivity analysis by varying a suite of parameters for our wild dog population (initial population size, percentage of females breeding, percentage of males in the breeding pool, carrying capacity, disease severity, and mortality rates) by ± 25% while keeping other parameters constant. The standard sensitivity index for each variable was calculated as $S = [(\lambda_{\text{Base - 25\%}} - \lambda_{\text{Base + 25\%}}) / (0.5 * \lambda_{\text{Base}})]$, where λ was the annual rate of population growth calculated from the simulation, and subscripts *Base, Base - 25%, and Base + 25%,* referred to growth rates from models using the baseline parameter value and those increasing or decreasing that parameter value by 25%, respectively (Heppell *et al*. 2000).

6.3.5 Carrying capacities and reintroductions

The ecological carrying capacity of each protected area in the current range and the proposed reintroduction sites were calculated based on the abundance ratio of the most important prey species in KZN (i.e., impala, *Aepyceros melampus* and nyala, *Tragelaphus angasii*) and using prey-specific requirements of wild dogs (Lindsey *et al*. 2004; Table 6.2). These estimates assumed an average of 12 dogs per pack, including seven adults and yearlings and five pups. The protected areas incorporated into reintroduction strategies 1, 2 and 3 (hereafter R1, R2 and R3; Fig. 6.1) were estimated based on planning and correspondence with provincial wildlife authorities and private land owners within KZN (Whittington-Jones, pers. comm). In the model, new breeding packs formed by artificial or natural means were reintroduced into the protected areas included in R1, R2, and R3 (currently devoid of wild dogs), with the number of packs translocated to each area determined by carrying capacity estimates. Due to the long distance dispersal capacity of wild dogs and a history of movement among the current and proposed protected areas (unpublished data), the KZN wild dogs were modelled as a single, connected population. Natural immigration from areas outside KZN was not included in the simulations because there have been no reports of immigrant wild dogs entering the area from outside the province.

Table 6.2 Summary of protected areas (PA), habitat size, carrying capacity, and number of packs within the current range (CR) and proposed reintroduction stages (R1, R2, R3) for African wild dogs of KwaZulu-Natal Province.

	Protected areas (PAs)*	Number of PAs	Size of PAs	Carrying capacity	Number of packs
CR	HiP, Thanda and Hlambanyathi	3	960 km² + 85 km² + 60 km² = 1,105 km²	150	11-13
R1	CR + Mkhuze and Tembe	5	1,105 km² + 400 km² + 300 km² = 1,805 km²	224	14-18
R2	CR + R1 + Somkhanda and Phongolo	7	1,805 km² +108 km² + 140 km² = 2,053 km²	262	17-21
R3	CR + R1+ R2 + Opathe and Phinda	9	2,053 km² + 210 km² + 90 km² = 2,353 km²	295	19-24

*Complete names of reserves included are: Hluhluwe-iMfolozi Park, Thanda Private Game Reserve, Hlambanyathi Private Game Reserve, uMkhuze section of iSimangaliso Wetland Park, Tembe Elephant Park, Somkhanda Game Reserve, Phongolo Nature Reserve, Emakhosini Opathe Heritage Park, Phinda Private Game Reserve.

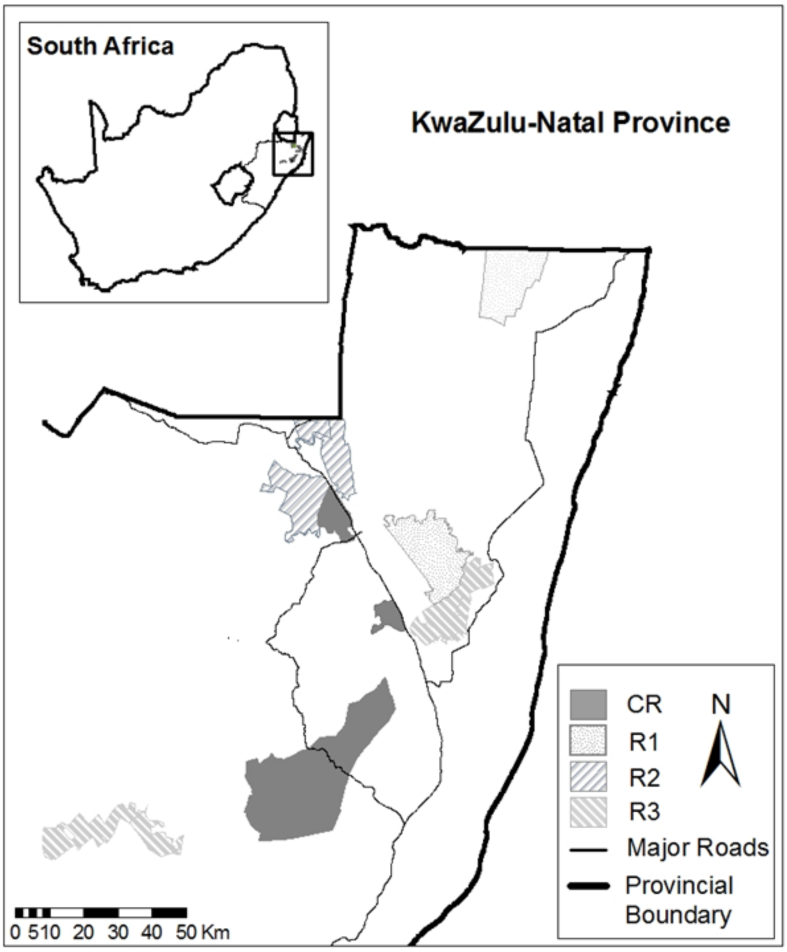

Figure 6.1 Map of the current range (CR) and potential African wild dog habitat (within reintroduction strategies R1, R2 and R3) in northern KwaZulu-Natal Province (larger map), South Africa (inset).

6.3.6 Supplementations

Supplementation via translocation from other sources was assessed as a means of maintaining demographic and/or genetic viability within the current and future wild dog ranges. In all cases, these additive events were modelled to consist of single-sex groups of at least two individuals ≥

2 yr old to mimic natural dispersals from natal packs to new areas. For the purposes of this model, the sex of dispersal groups was alternated with each supplementation.

6.3.7 Statistical analyses

We used a Student's t-test to compare the simulated and real population growth rates in order to validate our model structure. All statistical analyses were performed using JMP software version 3.2.2 (SAS Institute Incorporated) with means given ± standard error.

6.4 Results

To ensure that simulation findings were displaying characteristics similar to those of the KZN wild dog population, we compared growth rates produced by the model to real world results. From 2002 to 2009, the actual wild dog population in HiP (within KZN province) expanded 21.3% in size and had not yet reached the theoretical carrying capacity of 125 individuals based on prey and area (Lindsey *et al*. 2004). In comparison, the average growth rate for the simulated populations was 15.7% during the first 7 yr of the model (Fig. 6.2). The model yielded a slightly more conservative estimate of the expansion possible within real wild dog populations, although the two growth rates did not differ significantly ($t_{14} = 0.75$, $P = 0.47$).

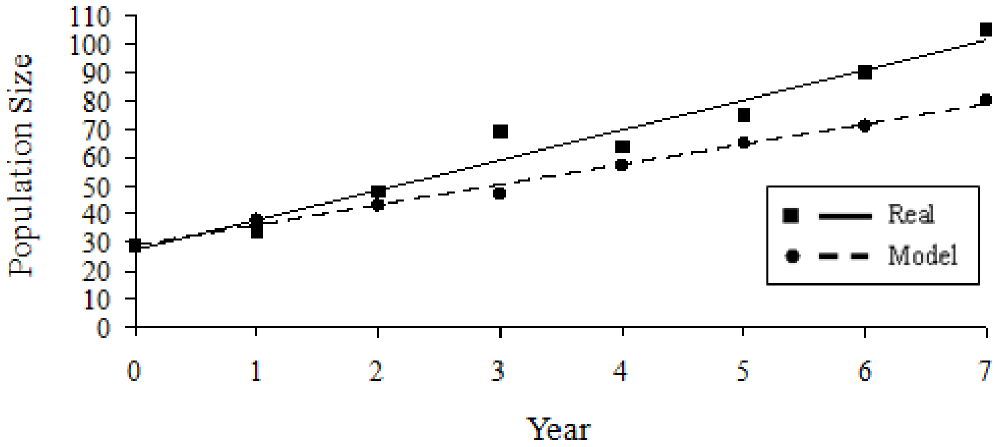

Figure 6.2 Annual population sizes of the real KZN (squares, solid line) and the modelled (circles, dashed line) African wild dog population in seven years before reaching carrying capacity of the habitat.

The sensitivity analysis indicated that simulated wild dog populations were influenced significantly by (1) the proportion of adult females (>2 yr) that are able to begin breeding (i.e., they were in established breeding packs and gained reproductive privileges in the hierarchy), (2) mortality of females 3 yr and older, and (3) severity of disease outbreaks (Table 6.3). Other tested factors were less sensitive to variation in the model (Table 6.3).

Table 6.3 Sensitivity analyses for selected model inputs parameters with a ± 25% variation range in values for a simulated African wild dog population.

Model Parameter	Minimum	Baseline	Maximum	S
*Females >2 yr first breeding (%)	22.3	29.7	37.1	-0.151
*3 yr old female mortality (%)	32.2	42.9	53.9	0.090
*>3 yr old female mortality (%)	37.2	50.0	62.5	0.089
*Disease severity (%)	57.0	76.0	95.0	-0.076
2 yr old female mortality (%)	17.0	22.6	28.3	0.063
Yearling mortality (%)	F = 17.3 M = 6.2	F = 23.0 M = 8.2	F = 28.8 M = 10.3	0.059
Pup mortality (%)	F = 18.3 M = 16.9	F = 24.4 M = 22.5	F = 30.5 M = 28.1	0.057
Carrying capacity (individuals)	113	150	188	-0.029
Males in breeding pool (%)	36	48	60	-0.010
Initial population size	78	104	130	-0.008

*Indicates the highest sensitivity (S) factors.

VORTEX modelling indicated that the current range of African wild dogs in KZN consisting of three protected areas and an expected carrying capacity of 150 individuals (Table 6.2) was inadequate to meet demographic or genetic viability criteria for the next 50 yr. Without further supplementation with dispersal groups into the existing habitats, simulated populations persisted for only 34.7 yr (Fig. 6.3a) and had a 100% probability of extinction within 50 yr (Fig. 6.3b).

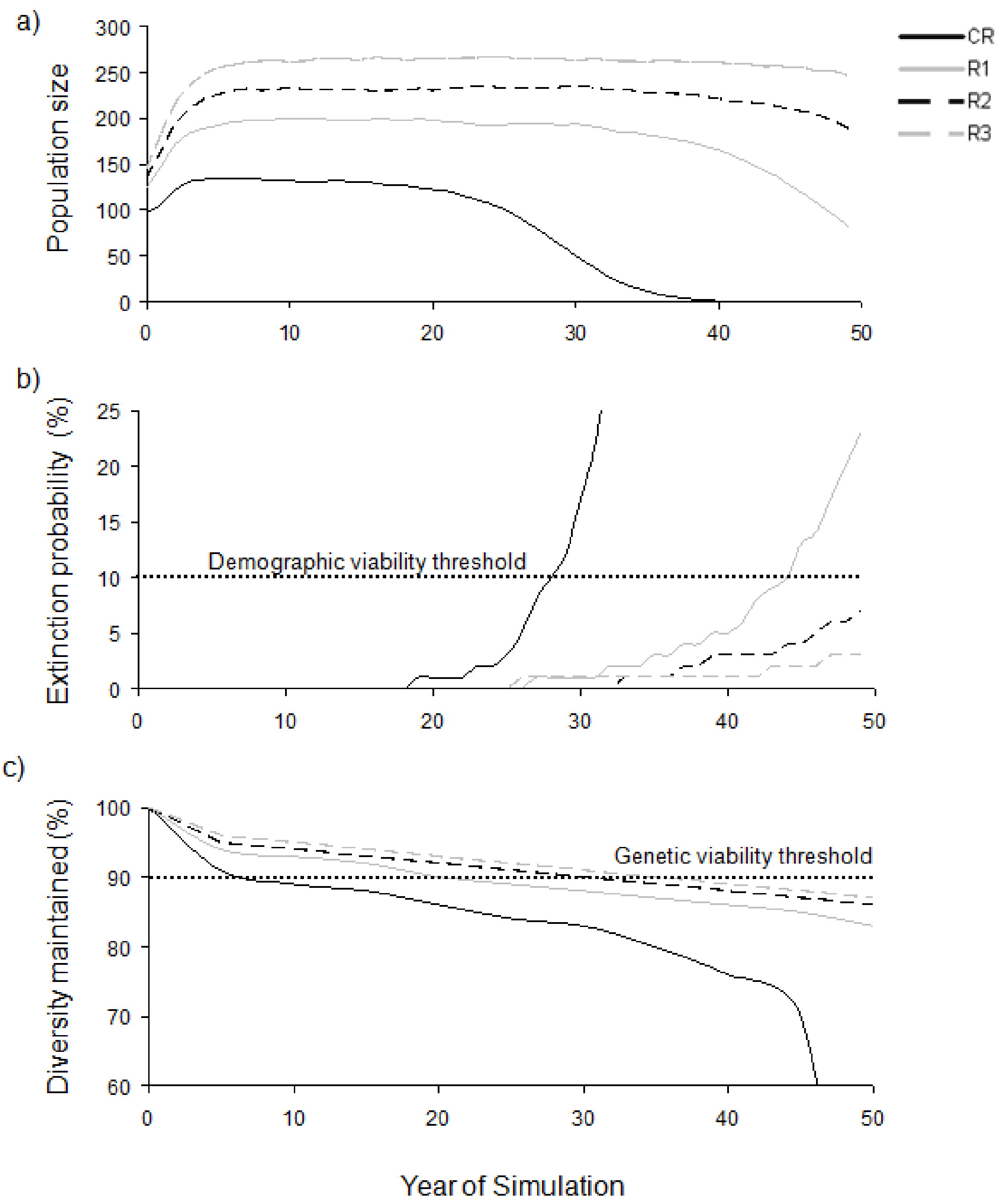

Figure 6.3 Average projected population size (a), probability of extinction (b), and genetic diversity maintained for the African wild dog population in the current range (CR; solid, black line) and in reintroduction stages R1 (solid, grey line), R2 (dashed, black line) and R3 (dashed, grey line) over 50 yr.

In the absence of additional space for wild dogs in the province, ensuring demographic viability required that single-sex dispersal groups (≥ 2 individuals) be translocated into the current available habitat every 4 yr at minimum (Table 6.4). Although it is the goal of the KZN Wild Dog Management Group to reintroduce wild dogs in as many provincial and private protected areas as possible to establish a viable population, the number of sites available for the species is limited by lack of funding, supportive land owners and local politics. Therefore, we modelled alternative scenarios, including the impact of reintroducing wild dogs via different reintroduction strategies (R1, R2, and R3; Table 6.2). The R1 plan provided 63% more wild dog habitat than the current range (Table 6.2), but the simulated population had a 26.0% probability of extinction within 50 yr (Fig. 6.3b) and those populations that went extinct survived only 45.4 yr on average (Fig. 6.3a). Further simulations revealed that demographic viability was achievable in reintroduction strategy R1 by introducing dispersal groups at least every 17 yr to breed with the existing dogs (Table 6.4). If the KZN reintroduction program reached stages R2 (86% more habitat than current range) or R3 (113% greater than current range), the model estimated that the resulting populations became demographically viable, with probabilities of extinction of 6.0% and 3.2%, respectively (Fig. 6.3b).

Table 6.4 Estimated number of dispersal group supplementations required for demographic and genetic viability, and resulting African wild dog population sizes after 50 yr based on population viability analysis simulations. Ranges include current range (CR) and three reintroduction strategies (R1, R2, R3).

Range	Demographic viability supplementation	Population size	Genetic viability supplementation	Population size
CR	1 group, 4 yr	107	2 groups, 2 yr	135
R1	1 group, 17 yr	133	2 groups, 2 yr	203
R2	no supplementation	189	2 groups, 3 yr	240
R3	no supplementation	245	2 groups, 3 yr	270

Within the current range of KZN wild dogs, simulations revealed that 90.5% of the initial gene diversity in the population was retained with the release of one male and one female dispersal group into the available habitat every 2 yr. Although modelling findings demonstrated that demographic viability was attained with few supplementations (one dispersal group every 17 yr) in the R1 scenario and none in the R2 and R3 plans, none of these reintroduction strategies achieved the goal of genetic viability without additional translocations of dispersers (Fig. 6.3c). Model evidence for R1 similarly suggested that supplementation of at least one male and one female dispersal group every 2 yr into the province allowed maintaining 90.8% of gene diversity for 50 yr (Table 6.4). In the scenario including expanded habitat for wild dogs (R2 and R3), genetic viability was retained as a result of importing one male and one female dispersal group to the province every 3 yr (90.0% and 91.0% gene diversity, respectively).

6.5 Discussion

We used this well-studied population of endangered African wild dogs to demonstrate the importance of integrating genetic considerations with demographic inputs into PVA models designed to test alternative population management scenarios. A majority of earlier efforts with rare wildlife species (e.g., Przewalski's horse, *Equus caballus przewalskii*, Slotta-Bachmayr *et al*. 2004; pygmy rabbit, *Brachylagus idahoensis:* Zeoli, Sayler & Wielgus 2008) have not had the benefit of genetic information combined with thorough, longitudinal demographic data (as in Table 6.1). Our study population of wild dogs is particularly valuable because it exists in an area where the species had been extirpated and then re-established through translocations followed by extensive monitoring for more than 12 yr (Somers & Maddock 1999; Somers *et al*. 2008; Spiering *et al*. 2010). Besides conventional demographic data collections, this population has undergone recent genetic assessments to evaluate the extent of reproductive sharing (Spiering *et al*. 2010), relatedness and inbreeding avoidance (Spiering *et al*. submitted). Although extremely useful for understanding the status of the growing, contemporary population, such data also have value in guiding management protocols to ensure that wild dogs remain in this native region of southern Africa. As is the case with most reintroduced animals, genetic considerations are critical for long-term persistence, and thus the inclusion of genetic parameters into PVA models is essential to properly determine probabilities of persistence and to generate effective management recommendations (Allendorf & Ryman 2002; Keller & Waller 2002). The African wild dog is highly vulnerable to extinction because of short generation times (4.5 yr; Quick 2003), the tendency to exist in small, reproductively isolated groups (Davies-Mostert *et al*. 2009) and hyper-sensitivity to a host of threats, including disease and persecution by humans (Woodroffe *et al*. 2007). Our findings demonstrated that, despite the overall apparent robustness of the population, it will be challenging to sustain a demographically and genetically viable population without continued intensive management, including the recruitment of more reintroduction sites and supplements of unrelated individuals.

Results strongly supported the notion that incorporating genetic parameters into the VORTEX model facilitated the creation of a realistic depiction of population dynamics and a flexible tool for considering alternative population management scenarios. Specifically, VORTEX allowed including three important genetic inputs and settings: 1) a studbook of the incipient population with known relationships among the founders; 2) restrictions on mates based on relatedness; and 3) quantitative expression of the degree of inbreeding depression (genetic load) as defined by number of lethal equivalents in the starting population and the distribution between lethal and detrimental alleles. Because many individuals in the actual KZN wild dog population were related within (and even between) packs (Spiering *et al*. submitted), the ability to include a starting population pedigree markedly decreased the amount of initial genetic variation within the model compared to simulations without those critical structural data. Imposing mating restrictions in the model that reflected our first hand observation of real inbreeding avoidance in this species (Spiering *et al*. submitted) created yet another constraint that needed to be considered in making important management decisions pertaining to additional reintroductions and supplementations.

VORTEX has been criticized for its inability to accurately portray the social complexities of some species, including the African wild dog (Vucetich & Creel 1999; Gusset *et al*. 2009). Although we observed definite improvements by having the capacity to integrate genetic information, significant refinement would be accomplished by adding more social system-specific settings to the software. For this species, it would be especially helpful if the user were able to manipulate the relationships within dispersal groups and newly formed packs and also include reproductive sharing within breeding packs in a more direct way. Regardless, we are confident that the settings currently available allowed us to capture the most important demographic and genetic aspects of wild dog populations in our PVA model structure. This was confirmed in two ways, including discovering that the simulated population growth rate was comparable to our long-term, real-life data. Additionally, we found that the proportion of adult females breeding, adult female mortality, and disease were the most sensitive and important factors in the model, which was congruent with other wild dog PVA models that did not have the advantage of genetic data (Ginsberg & Woodroffe 1997; Vucetich & Creel 1999; Hofmeyr *et al*. 2000; Gusset *et al*. 2009). We were not surprised to find that the proportion of females breeding so strongly influenced the model because we have field observations that many packs contain only one female potential breeder (Spiering *et al*. 2010), hierarchy within packs prevents some adults from mating (Spiering *et al*. 2010) and, adult female offspring residing in natal packs do not reproduce (Spiering *et al*. submitted).

Expanding simulations for the next half century was especially interesting in predicting the level of intensive management required to sustain a demographically and genetically robust population within different amounts of habitat. Results of our simulations confirmed that the probability of persistence of the KZN wild dog population was dramatically influenced by the number of breeding pack reintroductions into new areas. Without further reintroductions into unoccupied space as well as supplementations into areas already containing wild dogs, the current range was found to be inadequate to maintain a population of wild dogs, even for the relative short time span of 50 yr. However, realistically, this will be possible only if the connectivity between areas is maintained through dispersal, corridor creation and improved protection. Achieving the goal of retaining 90% gene diversity was more challenging and required considerably more management than simply establishing demographic viability. The translocation recommendations derived from our modelling to achieve the genetic target were similar to suggestions that immigrants should be added to carnivore populations each generation (Frankham 2009) and slightly more intensive than the translocations suggested every 6 yr in a recent wild dog modelling study (Gusset *et al*. 2009). Regardless, there remains no established consensus of what constitutes a viable population in terms of acceptable risk of extinction (Beissinger 2002). Although our scenarios focused on a target of retaining 90% gene diversity for 50 yr, plans that would hit more stringent viability criteria (e.g., 95% for 200 yr) would help ensure long-term population persistence (Allendorf & Ryman 2002), but require even more intensive restoration and management actions.

The number of reintroductions and supplementations calculated to accomplish demographic and/or genetic viability in our population of African wild dogs should not be considered a ‘goal’, but rather a lower threshold to the amount of management required (Allendorf & Ryman 2002). In general, it is prudent to avoid relying on absolute value results from PVAs (Beissinger & Westphal 1998) because the fate of populations largely depends on stochastic elements that are difficult to predict from these analyses (Frankham *et al*. 2002). ‘Living’ environments are constantly changing, meaning that longitudinal monitoring, data recovery and analysis are required consistently over time. A once-off PVA may be useful in one point of time, but decisions for future management actions must operate in an iterative fashion, benefiting from the most contemporary data and periodically updated PVA projections (Frankham *et al*. 2002). For example, we assumed that male and female dispersal groups would be supplemented alternately after each translocation. However, in reality, the most advantageous approach will be to determine through continued demographic monitoring which gender-specific group most benefits the overall population. Bolstering numbers of the most needed sex could mitigate mate-finding Allee effects (Somers *et al*. 2008) by ensuring that natural dispersal groups find opposite-sexed counterparts, form new packs and breed. Furthermore, research into high-priority topics such as the impacts of inbreeding on fitness traits will improve scholarly knowledge about this endangered species, but will also add more quantitative data to strengthen PVA analyses, thereby enhancing the ability to make the best management decisions for ensuring species sustainability.

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CHAPTER 7: GENERAL DISCUSSION

7.1 Summary

African wild dog numbers have declined throughout their range, although reintroduction efforts in South Africa have been moderately successful at reversing this decline, at least on a regional level. This dissertation aimed to elucidate the behaviour and genetic structure of the wild dog population of the province of KwaZulu-Natal in South Africa in order to better understand this species and to inform managers on how to adequately sustain this population into the future.

Working with an endangered species required successful non-invasive methods for genetic monitoring. This study used faecal samples to obtain DNA to characterize heterozygosity and relatedness within and among packs in the population. Extracting DNA from faecal samples has been highly successful in many species (e.g., brown bear, *Ursus arctos*: Murphy *et al*. 2002; Eurasian badgers, *Meles meles*: Frantz *et al*. 2003), and methods for conducting this type of analysis are described in Chapter 2. Results from DNA extraction and analysis revealed that sampling errors may be prevalent when collecting faecal samples in the field for genetic analyses of social carnivores such as the African wild dog, but that these errors may be detected and eliminated using the proper methodologies. Without this non-invasive technology, sample sizes for genetic analyses of wild dogs and other sensitive endangered species would be dramatically smaller and much less representative of overall populations. The comprehensive sampling and genotyping accomplished early in this study (Chapter 2) was vital to the completion of the social and genetic analyses conducted later in this study (Chapters 3 through 6).

By genotyping as many individuals as possible over a five year period (2003-2008) and combining that information with the long-term demographic and behavioural database available for the KZN wild dog population, Chapter 3 explored the effects of decreased genetic diversity on the fitness traits of individuals. Despite previous suggestions that molecular metrics of heterozygosity are likely to be indicative of inbreeding levels in endangered species (Grueber *et al*. 2008), all three metrics I tested were relatively poor indicators of pedigree inbreeding coefficients. Results suggest that inbred wild dogs had shorter lifespans, but these results were confounded by pack effects since they were confined to a small number of packs in KZN. Nevertheless, these findings may point to significant disadvantages for inbred and/or less heterozygous individuals, even if these effects are not yet observed in a large number of individuals in the population.

Although inbreeding that reduced heterozygosity was detected in some packs, the significant discovery in Chapter 4 of a high level of reproductive sharing between dominants and subordinates suggests that African wild dogs may have developed a mechanism to maintain genetic diversity within their populations. This sharing behaviour means that more individuals are able to pass on their genes than in a system where reproduction is skewed solely in the direction of a dominant pair. In addition to incomplete reproductive suppression, multiple adults succeeded in attaining dominance status in their lifetime to ensure breeding opportunities. However, males and females employed different tactics to attain dominance. Males frequently used aggression to overthrow dominants while females generally waited for opportunities to reproduce when alpha counterparts died.

While Chapter 4 showed that opportunities to breed were clearly sought after in many ways, Chapter 5 demonstrated that African wild dogs avoided mating with kin in an overwhelming majority of situations. Wild dogs likely use familiarity to differentiate between potential mates and relatives since most cases of inbreeding occurred between related individuals that had no prior association in natal packs. Along with reproductive sharing, these behaviours serve to maintain the genetic diversity of the population. However, using computer modelling, it was revealed that reducing the number of potential mates by avoiding inbreeding within wild dog populations that are mostly small and isolated may cause demographic instability and greatly decrease the future viability of these populations.

I determined in Chapter 6 that the current KZN wild dog population is not demographically or genetically viable and probably will not persist as long as 50 years. This is likely due to small populations, limited habitat, as well as the inbreeding avoidance behaviours highlighted in Chapter 5. Additional reintroductions and habitat are essential for the population to persist in the future, and results suggest that supplementations of unrelated dispersal groups will always be needed to achieve viability. More specifically, attaining and maintaining a genetically viable population of African wild dogs in KZN is much more management intensive than simply aiming for predetermined demographic goals for this and other reintroduction programs.

7.2 Conclusions and Recommendations

To adequately conserve populations of African wild dogs in KZN and worldwide into the future, continual multidisciplinary monitoring, including non-invasive sampling is essential. Specifically, the collection of faeces or urine will be important for genetic and hormone analyses without the physiological stress of anaesthesia on these sensitive and socially complex animals (Kohn & Wayne 1997; Monfort 2003; Hausknecht *et al*. 2007). As shown in Chapter 2 and previously noted in the endocrine literature (Keay *et al*. 2006), it is critical that the individual source of each sample is precisely known since even a single incorrect identification can severely perturb results. A multi-faceted approach like the one demonstrated in Chapter 2 for detecting sampling errors and laboratory errors such as allelic dropout (Navidi *et al*. 1992) and false alleles (Taberlet *et al*. 1996) must be employed in each analysis. Only when these steps are followed can proper longitudinal monitoring be accomplished successfully to extend current population databases and further the understanding of wild dog biology.

A topic in wild dog biology in particular that requires further attention is to confirm whether inbreeding depression is present in this species and to determine additional fitness metrics that might be affected. This is especially important to examine quickly since severe inbreeding depression has been recorded in many canids (e.g., Liberg *et al*. 2005; Raikkonen *et al*. 2006; Asa *et al*. 2007). Because a majority of the remaining wild dog populations throughout Africa are small (<50 individuals: Woodroffe *et al*. 1997) and it is generally difficult to attain genetic samples of free-ranging individuals, the very large sample size necessary to study this question could only be facilitated by using non-invasive sampling of several wild dog populations. However, testing for inbreeding depression may still prove to be difficult since few populations have the extensive demographic and life history data that have been collected for KZN wild dogs for the past 13 years. In the future, I strongly encourage wild dog population managers to record life histories and social pedigrees within their populations, as well as collect genetic material as often as possible. Given that results in Chapter 3 illustrate that molecular metrics of heterozygosity were not good predictors of inbreeding levels, it is best to document pedigrees as accurately as possible and then verify parentage assignments through genetic analyses.

The confirmation of pup parentage is especially important considering the high levels of reproductive sharing observed within packs in KZN (Chapter 4). My findings indicate that reproductive suppression is likely mediated by a combination of behavioural and physiological mechanisms and that these mechanisms are different for males and females. Although results roughly compare to conclusions made by Creel *et al.* (1997) based on hormone profiles, subordinate females did not ovulate in Selous, Tanzania, while KZN females certainly ovulated as many of them became pregnant and gave birth. It would be beneficial to ascertain whether hormone profiles in the small reintroduced population in KZN mimic those of the larger and extant Selous population.

The sharing of breeding opportunities between dominants and subordinates and the fact that many wild dogs were able to attain the alpha position to reproduce are important positive findings for wild dog conservation. These observations suggest that, in contrast to a highly skewed and more stable breeding structure often assumed for wild dogs, many more individuals in the KZN population (and likely other populations as well) are contributing genes to offspring. In addition, wild dogs are maintaining the genetic diversity of the population through active inbreeding avoidance by recognizing relatives with which they have had prior associations. African wild dogs appear to have developed multiple methods of ensuring the genetic health of their populations, which suggests that these behaviours evolved in order to circumvent severe consequences of inbreeding (Koenig *et al.* 1999), making it even more critical to accurately quantify inbreeding depression in this species.

Paradoxically, the mechanism of inbreeding avoidance that evolved to benefit the persistence of African wild dog populations may now be promoting increased extinction risk for this endangered species by limiting the number of unrelated potential mates in increasingly small and isolated populations. In today's current conditions, small populations like the one in KZN that lack supplementation of unrelated individuals (through natural immigration or management intervention) could go extinct in as few as two to four decades. To decrease the demographic vulnerability of these smaller populations, it is essential that corridors linking wild dog populations be maintained or created whenever possible and that artificial supplementation be conducted in fragmented landscapes. In addition, because my results suggest that population growth and persistence is dramatically affected by high adult female mortality and severe disease outbreaks, mitigating these threats should be of the highest priority. Specifically, efforts need to focus on reducing human induced mortality such as snaring and road accidents (Woodroffe *et al*. 2007) to lower adult wild dog mortalities. Females are likely most vulnerable to high mortality during dispersal and especially when outside of protected areas since they spend longer time 'floating' between packs than males (Creel & Creel 2002). Since disease transmission is also most likely to occur while wild dogs are in contact with domestic dogs during dispersal outside of protected areas (Flacke 2007), intensive vaccination campaigns to eradicate pathogens such as the rabies virus should be conducted in adjacent villages.

Overall, concentrated management planning and intervention are needed to make the KZN African wild dog population viable into the future. Although the original goal for provincial wild dog conservation was to create a self-sustaining population, meeting demographic viability and challenging genetic maintenance goals means that continual translocations of unrelated individuals are needed. The frequency of supplementations can be reduced if additional habitat is made available to establish breeding packs throughout the province, but realistically, it appears that achieving wild dog conservation in KZN will carry logistical and financial costs into the intermediate future.

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APPENDICES

Appendix A: Model Inputs for Population Viability Analysis in VORTEX

Demographic inputs

Rather than attempting to model individual packs within our population, we chose to simulate the demographic and genetic consequences of a pack structure within the larger population. To reflect that African wild dog packs generally are relatively stable with the same groups of individuals mating over several years and that the dominant individuals often breeding annually (Girman *et al*. 1997; Creel & Creel 2002), 'long-term monogamy' was selected as the reproductive system. Our reproduction input values differed slightly from those in models constructed previously in order to portray recent findings of extensive reproductive sharing within those packs including adult siblings of the alpha pair (Spiering *et al*. 2010). Despite high levels of reproductive sharing in breeding packs that contained adult siblings of the alpha pair, most pups in the KwaZulu-Natal (KZN) population were still produced by dominants (93% whelped by alpha females and 72% sired by alpha males) because many packs were comprised of only the alpha pair and their offspring (Spiering *et al*. 2010). Therefore, long-term monogamy captured the most important highlights of classical wild dog reproduction traits and was the closest approximation of the breeding system available. We then calculated the percentages of males and females in the breeding pool, thereby dealing with the normative that only adult members of the pack had the opportunity to reproduce (i.e., excluding dispersing individuals or offspring remaining in natal packs). Thus, it was determined that an average of 48% of all adult males in the KZN population comprised the breeding pool, a value used throughout the analysis. For females, we added a function to allow the expression of the percentage of individuals breeding based on specific age classes and to include whether or not a female had produced offspring in previous years. Only 7.7 ± 3.0% (SD) of 2 yr old females normally reproduce because younger wild dogs are less likely to have already dispersed and joined a breeding pack (Spiering *et al*. submitted). While only 29.7 ± 10.0% of females >2 yr old that have not yet whelped pups in their lifetimes produce young annually, 95.6 ± 10.0% of females that had already reproduced previously as the alpha or beta individual continued to breed, most often until death (Spiering *et al*. 2010). In KZN, African wild dog females whelp pups at 1.3 to 10 yr old, and males sire offspring from 1.1 to 10 yr old (Spiering *et al*. 2010). As most individuals did not breed before 2 yr of age, this metric was set as the age at first offspring production with maximum breeding age fixed at 10 yr. Mean litter size in this population is 7.6 ± 0.6 pups, with the largest litter recorded as 14 pups and near gender parity at this age class (0.51 ± 0.04; Spiering et al. 2010).

Using our extensive demographic database, we calculated that age-specific mortality in the KZN population was similar to those reported by Creel and Creel (2002) for wild dogs in the Selous Game Reserve, Tanzania. Pup mortality (through 1 year) in our region was 24.4 ± 8.0% for females and 22.5 ± 7.3% for males. Yearling mortality was 23.0 ± 7.0% for females and 8.2 ± 7.5% for males, with this rate remaining similar for 2 yr old females and increasing for males (females, 22.6 ± 8.0%; males, 23.8 ± 4.8%). However, the incidence of mortality in females aged 3 yr (42.9 ± 8.0%) and older (50.0 ± 8.0%) was higher than for counterpart males (27.3 ± 4.8% and 32.0 ± 4.8%, respectively). The latter finding is known to lead to increasing male bias in this species in older age classes (Frame *et al*. 1979; Reich 1981; Creel & Creel 2002; Spiering *et al*. 2010).

Genetic inputs

The percentage of gene diversity reported in the present paper was the amount retained relative to the starting point at Year 0 of the model. Inbreeding depression was simulated as a reduction in juvenile (i.e., first year) survival of inbred individuals according to the general model of Morton *et al*. (1956):

$$S_f = S_0 e^{-Bf}$$

where S_f and S_0 are survival rates for individuals with inbreeding coefficients equal to f and 0, respectively, and B is a constant describing the rate of decline in survival with increasing coefficient of inbreeding. The severity of inbreeding depression is typically expressed in terms of the number of lethal equivalents per diploid genome in the population of interest, which is conveniently expressed as $2B$. We used the nonlinear maximum likelihood approach of Kalinowski & Hedrick (1998) to estimate the number of lethal equivalents, using data on inbreeding coefficients derived from studbook records and observed survival rates (Fig. S1). The maximum likelihood method produces results that are more statistically robust than more traditional linear regression methods (Kalinowski & Hedrick 1998). From this analysis, we estimated a total of $2B = 1.7$ lethal equivalents per diploid genome for the KZN wild dog population. We assumed that 50% of this genetic load was attributable to lethal alleles, with the remainder composed of detrimental alleles, or those alleles that conferred only a probability of dying for an individual that was homozygous for the allele in question. The distribution of the genetic load of a mammal population (i.e., lethal vs. detrimental) is impossible to determine through field observation. The choice of 50% of the genetic load due to lethal alleles is derived from extensive research in *Drosophila* that is routinely applied to analyses across mammals, birds, and other animals of conservation concern (Simmons & Crow 1977). Therefore, given this information, VORTEX reduces the survival probability of an inbred individual during their first year of life by the factor:

$$e^{-B(1-\Pr[Lethals])f}$$

where Pr[Lethals] is the proportion of the total inbreeding effect (number of lethal equivalents) attributable to lethal alleles.

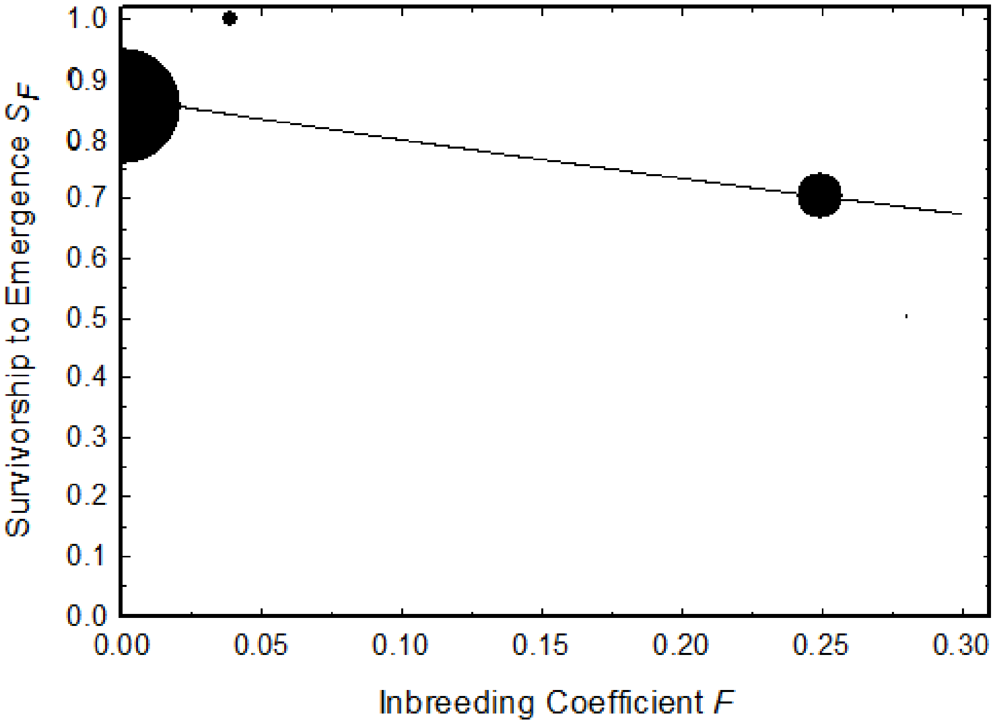

Figure S1 Observed survival rates for African wild dogs of KwaZulu-Natal Province, South Africa, and line representing best fit to the data using the maximum likelihood model of Kalinowski & Hedrick (1998). The size of the circle at each data point is proportional to the number of individuals born with the associated inbreeding coefficient. Data points at $F = 0.0625$ and $F = 0.281$ may not be visible due to the small number of individuals within those inbreeding classes ($N = 1$ and $N = 4$, respectively).

Catastrophes and competition

For the purposes of this evaluation, a ‘catastrophe’ was analogous to a disease outbreak and was (1) set to occur with an average frequency of 8.0% and (2) lead to a 24% average decline in reproduction and survival (compared to normative times in the absence of an epidemic). These values were consistent with recorded events in KZN during the study period as well as the influence of 'mild' catastrophes modeled by Ginsberg and Woodroffe (1997). The effect of interspecific competition with lions was not integrated into our model because: 1) demographic rates used as inputs were based on data from HiP, where lion densities are considered moderate to high and would have inherently included effects of mortality by lions, and 2) recent evidence suggests that this factor may not be as critical a threat as was once considered (Hayward & Kerley 2008; Rasmussen *et al*. 2008; Gusset *et al*. 2009).

Appendix B: Studbook File Used as Initial Population in VORTEX

Current KZN wild dog population as of November 2009

#ID	Dam	Sire	Sex	Selected	Living	Age
Jane	WILD	WILD	1	1	0	-1
DJ	WILD	WILD	0	1	0	-1
Karong	WILD	WILD	1	1	0	-1
UAM	WILD	WILD	0	1	0	-1
UBM	WILD	WILD	0	1	0	-1
DB	Karong	UAM	0	1	0	-1
UBF	WILD	WILD	1	1	0	-1
CAF	WILD	WILD	1	1	0	-1
CAM	WILD	WILD	0	1	0	-1
Kele	WILD	WILD	1	1	0	-1
Musina	WILD	WILD	1	1	0	-1
Shane	WILD	WILD	1	1	0	-1
Step	Jane	DJ	0	1	0	-1
KC	Kele	WILD	1	1	0	-1
Musho	Kele	WILD	1	1	0	-1
Dube	Kele	WILD	1	1	0	-1
Lia	WILD	WILD	1	1	0	-1
Luna	Kele	WILD	1	1	0	-1
Mamba	Kele	WILD	1	1	0	-1
Amstel	WILD	WILD	0	1	0	-1
Carlin	WILD	WILD	0	1	0	-1
Castle	WILD	WILD	0	1	0	-1
Light	Karong	DB	0	1	0	-1
Guines	WILD	WILD	0	1	0	-1
Isi	WILD	WILD	0	1	0	-1
RG	Karong	UAM	1	1	0	-1
Toro	Kele	WILD	0	1	0	-1
Nero	Jane	DJ	0	1	0	-1
LP	Musina	WILD	0	1	1	7
Cobra	Kele	WILD	0	1	0	-1
RP	Musina	WILD	0	1	0	-1
DP	Musina	WILD	0	1	1	7
Azim	Karong	DB	0	1	1	5
Bointj	Jane	DJ	1	1	0	-1
Brindl	Jane	DJ	1	1	1	5
Furaha	Jane	DJ	1	1	1	4
Willy	Jane	DJ	0	1	1	4

Chevro	Karong	UAM	0	1	1	7
Frodo	Karong	UAM	0	1	1	7
Orion	RG	Chevro	0	1	1	3
Jupit	Jane	DJ	0	1	0	-1
Wasabi	CAF	LP	1	1	1	5
Pinstr	Jane	DJ	0	1	0	-1
Skye	WILD	WILD	0	0	1	4
Stitch	Lia	Step	1	0	1	3
Saffro	Karong	DB	1	1	1	6
Frida	Jane	DJ	1	1	0	-1
TK	Jane	DJ	0	1	0	-1
Mlenze	Jane	DJ	0	1	0	-1
Tutu	CAF	CAM	0	1	0	-1
Ananku	CAF	DP	0	1	0	-1
Baleka	Musho	Carlin	0	1	0	-1
Bastar	Jane	DJ	0	1	0	-1
Bhobho	Musho	Carlin	0	1	0	-1
Biko	CAF	LP	0	1	0	-1
Boggom	Jane	DJ	0	1	0	-1
Cairo	CAF	DP	0	1	0	-1
Carame	Karong	DB	0	1	0	-1
Chilli	Karong	DB	0	1	0	-1
Chino	Karong	DB	0	1	0	-1
Diza	Luna	Amstel	0	1	0	-1
Duma	Luna	Amstel	0	1	0	-1
DZ	Karong	DB	0	1	0	-1
Gobi	Jane	DJ	0	1	0	-1
Goliat	Musho	Carlin	0	1	0	-1
H05p8	Jane	DJ	0	1	0	-1
LB	Karong	UAM	0	1	0	-1
Matrix	Karong	UAM	0	1	0	-1
Mickey	Karong	DB	0	1	0	-1
Milo	Karong	DB	0	1	0	-1
M06p3	Musho	Carlin	0	1	0	-1
M06p4	Musho	Carlin	0	1	0	-1
Nemo	Karong	DB	0	1	0	-1
Night	Karong	Pinstr	0	1	0	-1
Omega	Karong	DB	0	1	0	-1
Pamp	Jane	DJ	0	1	0	-1
Phiva	Karong	DB	0	1	0	-1
Phoeni	RG	Chevro	0	1	0	-1
Prince	Karong	DB	0	1	0	-1
Regal	Wasabi	Orion	0	1	0	-1

Rholi	CAF	CAM	0	1	0	-1
Sebast	Karong	UAM	0	1	0	-1
Silver	Jane	DJ	0	1	0	-1
Sirius	RG	Chevro	0	1	0	-1
Spress	Karong	DB	0	1	0	-1
T06p7	Lia	Step	0	1	0	-1
T08p5	Stitch	Skye	0	1	0	-1
T08p6	Stitch	Skye	0	1	0	-1
T08p7	Stitch	Skye	0	1	0	-1
T08p8	Stitch	Skye	0	1	0	-1
Tuck	Karong	DB	0	1	0	-1
Two	Jane	DJ	0	1	0	-1
Yankee	Musina	Frodo	0	1	0	-1
Remb	Brindl	Light	0	1	1	2
Rico	Brindl	Light	0	1	1	2
Bambis	Musho	Carlin	0	0	1	3
Bassai	CAF	DP	0	1	1	3
Caden	CAF	DP	0	1	0	-1
Calder	CAF	DP	0	1	1	2
Calvin	CAF	DP	0	1	1	2
Chance	CAF	DP	0	1	1	2
Cosmo	CAF	DP	0	1	1	2
DO7	Saffro	Willy	0	1	1	2
J08p1	Wasabi	Orion	0	1	1	1
J08p2	Wasabi	Orion	0	1	1	1
J08p3	Wasabi	Orion	1	1	1	1
Kamai	CAF	DP	0	1	1	3
Kanshu	CAF	LP	0	1	1	3
Klippy	Musina	Frodo	0	1	1	4
Mduna	Shane	Nero	0	1	1	3
Nash	RG	Chevro	0	1	1	3
N08p3	Saffro	Willy	0	1	1	1
N08p4	Saffro	Willy	1	1	1	1
N08p5	Saffro	Willy	1	1	1	1
Polloc	Bointj	Tutu	0	1	0	-1
Reno	Frida	Light	0	1	1	2
Saint	Saffro	Willy	0	1	1	2
Sepai	CAF	DP	0	1	1	3
U08p8	Brindl	Azim	1	1	1	1
U08p9	Brindl	Azim	1	1	1	1
U08p10	Brindl	Azim	0	1	0	-1
U08p11	Brindl	Azim	0	1	0	-1
U08p12	Brindl	Light	0	1	0	-1

U08p13	Brindl	Light	0	1	0	-1
Yoko	CAF	DP	0	1	1	3
Angel	Jane	DJ	1	1	0	-1
Aum	Jane	DJ	1	1	0	-1
Butter	Jane	DJ	1	1	0	-1
Cici	Karong	DB	1	1	0	-1
Cinn	Karong	DB	1	1	0	-1
C04p4	CAF	DP	1	1	0	-1
Cygnus	Wasabi	Orion	1	1	1	2
Dipst	Shane	Step	1	1	0	-1
Dolly	Jane	DJ	1	1	0	-1
Flent	Karong	Pinstr	1	1	0	-1
Gigi	Karong	UAM	1	1	0	-1
I03p2	Karong	UAM	1	1	0	-1
I04p7	Karong	DB	1	1	0	-1
Khanda	Jane	DJ	1	1	0	-1
Lilly	CAF	Tutu	1	1	0	-1
Marion	Karong	DB	1	1	0	-1
Marula	Musina	Frodo	1	1	0	-1
Mocha	Karong	DB	1	1	0	-1
Molly	Karong	DB	1	1	0	-1
Nala	Karong	DB	1	1	0	-1
Nandi	Luna	Amstel	1	1	0	-1
Naomi	Karong	DB	1	1	0	-1
Ntombi	Luna	Amstel	1	1	0	-1
Okuma	Jane	DJ	1	1	0	-1
Perfec	Jane	DJ	1	1	0	-1
Pippi	Karong	DB	1	1	0	-1
Rita	Karong	UAM	1	1	0	-1
Sahara	Jane	DJ	1	1	0	-1
SM	Jane	DJ	1	1	0	-1
Sentio	CAF	LP	1	1	0	-1
Shadow	Karong	DB	1	1	0	-1
Snow	Jane	DJ	1	1	0	-1
Socks	Jane	DJ	1	1	0	-1
Spica	Wasabi	Orion	1	1	0	-1
Sushi	CAF	DP	1	1	0	-1
T08p1	Stitch	Skye	1	1	0	-1
T08p2	Stitch	Skye	1	1	0	-1
T08p3	Stitch	Skye	1	1	0	-1
T08p4	Stitch	Skye	1	1	0	-1
Thandi	Luna	Amstel	1	1	0	-1
Toffee	Karong	DB	1	1	0	-1

Vanill	Karong	DB	1	1	0	-1
Ghiz	Shane	Nero	1	0	1	3
Kanga	Lia	Step	1	0	1	3
Ursa	Wasabi	Klippy	1	1	0	-1
Caia	CAF	DP	1	1	1	2
Calico	CAF	DP	1	1	1	2
Calyps	CAF	DP	1	1	0	-1
Cameo	CAF	DP	1	1	0	-1
Cloud	CAF	DP	1	0	1	2
Barlow	Bointj	Tutu	1	1	1	2
Bekeze	Musho	Carlin	1	0	0	-1
Grace	Bointj	Tutu	1	1	1	2
Hadlee	Bointj	Tutu	1	1	1	2
J08p4	Wasabi	Orion	1	0	0	-1
Lara	Bointj	Biko	1	1	1	2
Mata	Saffro	Willy	1	1	1	2
Nidan	CAF	LP	1	1	1	3
N08p1	Saffro	Willy	1	1	0	-1
N08p2	Saffro	Willy	1	1	0	-1
Petra	CAF	CAM	1	1	0	-1
Raqui	Brindl	Azim	1	1	1	2
Riley	Frida	Light	1	1	1	2
Roxie	Frida	Light	1	1	1	2
Ruby	Brindl	Azim	1	1	1	2
Sachin	Bointj	Biko	1	1	1	2
Uchi	CAF	DP	1	1	1	3
U08p1	Furaha	Azim	1	1	1	1
U08p2	Furaha	Azim	1	1	1	1
U08p3	Furaha	Azim	1	1	1	1
U08p4	Furaha	Azim	1	1	1	1
U08p5	Furaha	Light	1	1	1	1
U08p6	Furaha	Light	1	1	1	1
U08p7	Furaha	Light	1	1	1	1
V08p4	Bointj	Tutu	1	1	0	-1
V08p7	Bointj	Biko	1	1	0	-1
Zuki	CAF	DP	1	1	1	3
C04p5	CAF	LP	1	1	0	-1
C04p6	CAF	CAM	1	1	0	-1
C04p7	CAF	DP	1	1	0	-1
C04p8	CAF	LP	1	1	0	-1
C04p9	CAF	CAM	1	1	0	-1
C04p11	CAF	DP	1	1	0	-1
H02p10	Jane	DJ	1	1	0	-1

H02p11	Jane	DJ	1	1	0	-1
I02p6	Karong	UAM	1	1	0	-1
I02p7	Karong	UAM	1	1	0	-1
I03p1	Karong	UAM	1	1	0	-1
I04p10	Karong	DB	1	1	0	-1
I04p11	Karong	DB	1	1	0	-1
I04p12	Karong	DB	1	1	0	-1
I04p13	Karong	DB	1	1	0	-1
I04p8	Karong	DB	1	1	0	-1
I04p9	Karong	DB	1	1	0	-1
I05p12	Karong	DB	1	1	0	-1
I05p13	Karong	DB	1	1	0	-1
I05p14	Karong	DB	1	1	0	-1
I06p1	Karong	DB	1	1	0	-1
I06p2	Karong	DB	1	1	0	-1
I06p3	Karong	DB	0	1	0	-1
I06p4	Karong	DB	0	1	0	-1
I06p5	Karong	DB	0	1	0	-1
I06p6	Karong	DB	0	1	0	-1
I06p7	Karong	DB	0	1	0	-1
I06p8	Karong	DB	0	1	0	-1
I06p9	Karong	DB	0	1	0	-1
J05p4	Musina	Frodo	0	1	0	-1
J05p5	Musina	Frodo	0	1	0	-1
J05p6	Musina	Frodo	0	1	0	-1
J05p7	Musina	Frodo	0	1	0	-1
M05p5	Luna	Amstel	0	1	0	-1
M05p6	Luna	Amstel	0	1	0	-1
M05p7	Luna	Amstel	0	1	0	-1
N07p10	Saffro	Willy	0	1	0	-1
N07p4	Saffro	Willy	0	1	0	-1
N07p5	Saffro	Willy	0	1	0	-1
N07p6	Saffro	Willy	0	1	0	-1
N07p7	Saffro	Willy	0	1	0	-1
N07p8	Saffro	Willy	0	1	0	-1
N07p9	Saffro	Willy	0	1	0	-1
V08p8	Bointj	Biko	0	1	0	-1
N08p6	Saffro	Willy	1	1	1	1
U09p1	Furaha	Azim	0	1	1	1
U09p2	Furaha	Azim	0	1	1	1
U09p3	Furaha	Azim	0	1	1	1
U09p4	Furaha	Azim	0	1	1	1
U09p5	Furaha	Azim	1	1	1	1

U09p6	Furaha	Azim	1	1	1	1
U09p7	Furaha	Azim	1	1	1	1
U09p8	Furaha	Azim	1	1	1	1
D09p1	Riley	Bassai	0	1	1	1
D09p2	Riley	Bassai	0	1	1	1
D09p3	Riley	Bassai	0	1	1	1
D09p4	Riley	Bassai	1	1	1	1
D09p5	Riley	Bassai	1	1	1	1
D09p6	Riley	Bassai	1	1	1	1
S09p1	Ruby	DP	0	1	1	1
S09p2	Ruby	DP	0	1	1	1
S09p3	Ruby	DP	0	1	1	1
S09p4	Ruby	DP	1	1	1	1
S09p5	Ruby	LP	0	1	1	1
S09p6	Ruby	LP	0	1	1	1
S09p7	Ruby	LP	1	1	1	1
N09p1	Saffro	Willy	0	1	1	1
N09p2	Saffro	Willy	0	1	1	1
N09p3	Saffro	Willy	0	1	1	1
N09p4	Saffro	Willy	0	1	1	1
N09p5	Saffro	Willy	1	1	1	1
N09p6	Saffro	Willy	1	1	1	1
N09p7	Saffro	Willy	1	1	1	1
N09p8	Saffro	Willy	1	1	1	1
J09p1	Wasabi	Orion	0	1	1	1
J09p2	Wasabi	Orion	0	1	1	1
J09p3	Wasabi	Orion	1	1	1	1
J09p4	Wasabi	Orion	1	1	1	1
T09p1	Skye	Stitch	0	1	1	1
T09p2	Skye	Stitch	0	1	1	1
T09p3	Skye	Stitch	1	1	1	1
T09p4	Skye	Stitch	1	1	1	1

“WILD” as a dam or sire indicates a founder or individual from the wild.
Sex is coded as 0 male and 1 for female.
The “Selected” column indicates whether or not an individual was included in the starting population, with 1 for included and 0 excluded.
The “Living” column indicates if the animal in the pedigree is currently alive (1) or dead (0).
The age of each individual rounded to the nearest year is included with -1 indicating dead animals.