African wild dogs: Genetic viability of translocated populations across South Africa

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Highlights

- The metapopulation plan for African wild dogs successfully achieved population growth.
- Without intervention, the metapopulation would lose 48% of its genetic diversity.
- Genetic differentiation is apparent, although population admixture occurs.
- Translocations between reserves remain essential for future population viability.
- Genetic data form a critical part of conservation management.

Abstract:

South Africa holds a viable population of the endangered African wild dog (*Lycaon pictus*), with almost 500 individuals divided into (1) an unmanaged population in the Kruger National Park (KNP), (2) a free-roaming population, and (3) a managed metapopulation (MTP) that originated from reintroductions. Because metapopulation reserves are geographically isolated, translocations are ongoing to mimic natural dispersal. During this study, we questioned whether the metapopulation management plan for wild dogs has been successful at maintaining healthy levels of genetic diversity and avoiding inbreeding in packs. We evaluated whether the current approach is effective for long-term population viability and assessed whether population admixture occurs between the three populations. To achieve this, we amplified 20 microsatellite loci for genetic analysis. We found high levels of genetic variation, likely resulting from translocations and artificial pack formation. Results showed that in the absence of any management intervention, the MTP would lose 48% of its heterozygosity over a 100-year trajectory, and KNP 12% heterozygosity. Under the current management scenario, the MTP will maintain 95% of its heterozygosity. We found genetic evidence that limited recent dispersal occurs between the MTP and KNP (F_{ST}=0.06). In conclusion, the metapopulation management plan can be considered successful based on the achieved population growth and preservation of genetic diversity. Our study highlights that genetic data form a critical part of conservation management, and that translocations can be a vital tool to restore genetic variability of species.

Keywords: Conservation management, genetic diversity, *Lycaon pictus*, metapopulation, population viability, reintroduction.

1. Introduction

The African wild dog (*Lycaon pictus*) is listed as Endangered by the International Union for Conservation of Nature (IUCN, 2012), with only approximately 6,600 individuals left in the wild (Woodroffe and Sillero-Zubiri, 2012; Kuiper et al., 2018). The vast majority of the species' former range, which spanned from Algeria to South Africa, has been lost and only two strongholds remain in eastern and southern Africa (Woodroffe and Sillero-Zubiri, 2012; Kuiper et al., 2018). Habitat destruction and fragmentation have been the main drivers of their population decline (Davies and du Toit, 2004; Woodroffe et al., 2004). Wild dogs have also been intentionally eliminated from many areas, mainly due to the negative attitude of land owners and local communities towards this predator (Fanshawe et al., 1997; Woodroffe et al., 1997; Lindsey et al., 2005). Additionally, illegal poaching, road accidents, poisoning, and accidental snaring still contribute major anthropogenic threats (Ginsberg et al., 1995; Woodroffe and Ginsberg, 1999; Davies and du Toit, 2004) and as a consequence, populations struggle to persist outside protected areas (Woodroffe et al., 1997).

South Africa holds a viable wild dog population of almost 500 individuals (Page et al., 2015), which are divided into three main populations: (1) an unmanaged population in the Kruger National Park, (2) a free-roaming population in the northern part of South Africa, and (3) a managed metapopulation that originated from reintroductions (Lindsey et al., 2004). Kruger National Park is the only protected area in South Africa that has had a constant population of wild dogs (Mills et al., 1998), which includes the majority of the national population (Page et al., 2015). Here, population numbers have naturally been fluctuating between 140 and 360 individuals (Maddock and Mills, 1994; Davies, 2000). The second population includes free-roaming wild dogs in and around the Waterberg area, in the northern part of South Africa. These animals primarily inhabit private land which makes them more vulnerable to human persecution (Thorn et al., 2012). It is estimated that only 20 wild dogs currently occur in this area (WAG-SA minutes 2010 – 2018).

The only wild dog population that has been increasing in South Africa is the managed metapopulation, due to intensive conservation strategies (Davies-Mostert et al., 2015). Before the development of the metapopulation management plan, wild dogs were completely eradicated from private reserves and national parks outside Kruger National Park. The metapopulation approach aimed to reintroduce wild dogs into other reserves to reduce the impact of stochastic events, by establishing a minimum of nine packs within 10 years of initiating the project (Mills et al., 1998). Wild dogs were first reintroduced into Hluhluwe-iMfolozi Park in 1980, and coordinated movements started in 1998 (Mills et al., 1998). The network subsequently grew with an additional 13 reserves that currently hold wild dogs (Figure 1). In 2018, the South African metapopulation supported 227 individuals (adults, yearlings and pups) spread across 20 packs (WAG-SA minutes, 2018). Because the metapopulation reserves are fenced and geographically isolated, wild dog translocations are ongoing to mimic natural dispersal (Davies-Mostert et al., 2009). To this end, sixty-nine individuals were moved between reserves from 1998 to 2007 (Davies-Mostert et al., 2015).

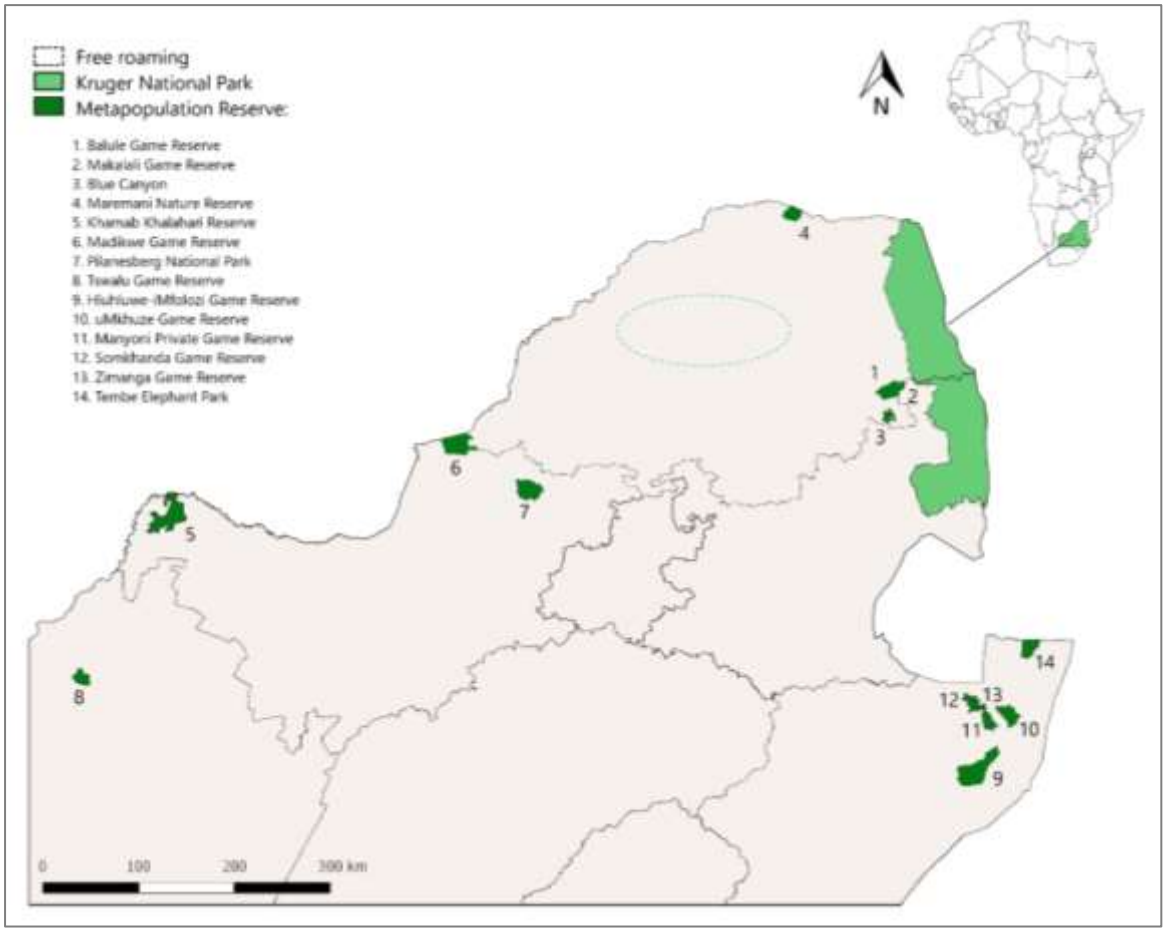

Figure 1: Distribution map of African wild dogs (*Lycaon pictus*) in South Africa

It is widely accepted that translocation is a viable conservation tool, particularly for threatened species, to enhance metapopulation viability and increase reproductive fitness (Griffith et al., 1989; Lubow, 1996; Weeks et al., 2011). From a genetic perspective, translocations between small populations can assist in enhancing genetic diversity, conserving evolutionary processes, and combatting inbreeding depression (Moritz, 1999; Storfer, 1999; Goossens et al., 2002). Consequently, genetic assessments have been proposed as one of the most efficient tools to measure the success of reintroduction and translocation programmes (Weeks et al., 2011; Dresser et al., 2017). In wild dogs, small population sizes due to habitat fragmentation and restricted gene flow are two main drivers of the strong genetic subdivision seen among populations in southern Africa (Marsden et al., 2012; Tensen et al., 2016). The lack of gene flow and low population numbers have already resulted in the loss of genetic variability in the Kruger National Park population (Girman et al., 1993; Tensen et al., 2016). Marsden et al. (2009) also found a reduction in adaptive variation in genes of the major histocompatibility complex (MHC), which affect disease susceptibility (Marsden et al., 2009; Flacke et al., 2010). Spiering et al. (2011) found evidence of inbreeding in wild dogs in South Africa, but only in a few, segregated packs. Although no reduced fitness or impacts on reproductive success was observed, the authors found that inbred wild dogs ($f \geq 0.25$) had reduced lifespans. In wolves (*Canis lupus*), the deleterious effects of inbreeding have been illustrated more profoundly, such as reduced litter size and pup survival (Liberg et al., 2005), physiological malformations (Robinson et al., 2018), and reduced fitness and longevity (Vilà et al., 2003). This further highlights the importance of maintaining acceptable levels of genetic diversity, and the value added by well-considered translocations.

Here, we report on the population demographics (i.e. population size, translocations and dispersal events) of wild dogs in South Africa, primarily focussing on the metapopulation and Kruger National Park. In essence, we questioned whether the metapopulation management plan for wild dogs has been successful at maintaining healthy levels of genetic diversity and avoiding inbreeding in packs during the recent population expansion. We further evaluated whether the current approach is effective for long-term population viability by simulating changes in heterozygosity into the future under different management scenarios. This allows a unique opportunity to understand translocations as a management tool to preserve genetic diversity (Goossens et al., 2002). Finally, we assessed whether there is evidence for population admixture between the metapopulation, free-roaming wild dogs and those in Kruger National Park. To our knowledge, our study is the first to provide genetic data revealing the impacts of reintroductions and translocations on metapopulation viability of wild dogs. Our findings can further serve as a proxy for other metapopulation programmes for endangered species that became restricted by habitat fragmentation and local extinctions.

2. Methods

2.1 Sample collection

Blood samples were obtained between 2008 and 2017 when wild dogs were immobilized for translocation, radio-collaring or vaccination against rabies. No animal was immobilized specifically for the purpose of this study. A total of 120 samples, from nine different reserves and 27 packs, were collected from animals that belong to the metapopulation (MTP). Currently, the metapopulation reserves cover 5 710 km^2 combined. Six samples were retrieved from the free-roaming population (FRM) around the Waterberg region, which is approximately 14 500 km^2 in size. In addition, 58 samples were collected from Kruger National Park (KNP), which sizes 19 485 km^2, from 25 different packs. All samples were stored at -20°C after collection.

2.2 Microsatellite genotyping

DNA was extracted using the protocol for blood samples with the NucleoSpin Tissue kit (Macherey Nagel, Germany). We used 200 µL blood, 25 µL Proteinase K and 200 µL lysis buffer to isolate the DNA during an incubation of 15 minutes at 70°C. The DNA was eluted in a final volume of 100 µL buffer. We amplified 20 microsatellite loci that were originally developed for the domestic dog *Canis lupus familiaris*: FH2001, FH2010, FH2611, FH2658, FH2848, FH3399, FH3965, CPH1, CPH2, PEZ01, PEZ08, PEZ12, PEZ15, AHTh137, AHTh171, AHTh253, REN162, REN169, CXX279, INU30 (Francisco et al., 1996; Thomas et al., 1997; Neff et al., 1999). These loci were selected because they are highly polymorphic and widely distributed throughout the reference genome. Polymerase chain reactions (PCR) were performed in 50 µL volumes containing 2 µL (~ 20 ng) genomic DNA, 23 µL (1x) Platinum Multiplex PCR Master Mix (Applied Biosystems), 5 µL (1x) GC Enhancer, and 1 µL (100 nM) of each primer. Forward primers were 5'-labelled with 6-FAM, VIC, NED or PET fluorescent dyes based on their allelic size range. Amplifications were carried out in a Multigene Optimax Thermal Cycler (Labnet, USA) with the following PCR cycling protocol: 2 min of initial activation at 95°C, 40 annealing cycles consisting of 95°C for 30 s, 60°C for 90 s, and 72°C for 60 s, and a final denaturation step of 72°C for 10 min. Two microliters of the amplified products were processed for fragment analysis on a 3500xL Genetic Analyzer (Applied Biosystems).

2.3 Statistical analysis

2.3.1 Population demographics

Data on wild dog demographics were available from the Wild Dog Advisory Group of South Africa (WAG-SA), who have been documenting the population dynamics of wild dogs in South Africa since the start of the metapopulation management plan. Each year's final WAG-SA report was used to evaluate population numbers of wild dogs in the MTP, FRM and KNP between 2008 and 2017. The reports were also used to give an overview of the number of translocations. The decision to translocate animals was based on various criteria. Principally, individuals were chosen based on their genealogy, to avoid inbreeding in closed reserves where possible. However, management decisions were also often in response to conflicts with land owners or local communities, for instance when individuals skipped park fences or when population numbers became too high for reserves. Furthermore, reserves could only receive animals when land owners agreed and finances allowed. As a result, not all reserves have been supplemented during our study period.

2.3.2 Genetic diversity

Microsatellite fragment scoring was done in Geneious 6.1.5 (Biomatters Ltd., 2013). MICRO-CHECKER v2.2.3 (Van Oosterhout et al., 2004) was used to test for genotypic errors. This program was also used to test whether alleles were in Hardy-Weinberg equilibrium, based on 1 000 bootstraps and 95% confidence intervals (CIs). Linkage disequilibrium (LD) between all pairs of loci was assessed in Arlequin v3.5 (Excoffier and Lischer, 2010), with 10 000 permutations and a significance level fixed at 0.05. Arlequin was also used to calculate observed (H_O) and expected heterozygosity (H_E) across loci, following bootstrap and 95% CIs. FSTAT v2.9.3.2 (Goudet, 2002) was used to calculate inbreeding fixation index (F_{IS}) and allelic richness (A_R). We also calculated allelic richness using the rarefraction method (R_S) in HP-Rare v1.0 (Kalinowski, 2005) to compensate for differences in sample size between populations and allow for comparisons. We also measured F_{IS} and LD with members from the same pack excluded, to detect potential bias caused by comparing closely related individuals.

The effective population size (N_e), which is the ideal population size under which allele frequencies and heterozygosity values change at the same rate as the observed population, was estimated using NeEstimator v2 (Do et al., 2014). For this, we used the LD method for monogamous mating (Waples and Do, 2010) and parametric confidence intervals with critical value P=0.05 (Waples, 2006). LD is considered the most common and accurate method for small populations (Saura et al., 2015). Monogamous mating was chosen because wild dogs live in social, cooperatively breeding packs, in which normally only the alpha pair reproduces (Courchamp et al., 2002). Even though subordinate individuals do occasionally breed when unrelated packs members are available (Spiering et al., 2010), monogamous mating was found to be most appropriate for genetic analysis (Fuller et al., 1992a; Davies-Mostert, 2012). We also calculated the ratio between the effective and census population size (N_e/N_c) for the MTP and KNP, to assess the populations' vulnerability to genetic stochasticity (we could not calculate this statistic for the FRM due to the small sample size). Pairwise relatedness was calculated using the Triadic Likelihood estimator (Wang, 2007) in COANCESTRY (Wang, 2011) for the whole group (MTP and KNP), within reserves, within packs, between packs, for females and males separately, and between alpha pairs (MTP only). We also calculated overall relatedness with only one representative per pack to avoid a potential overestimation.

2.3.3 Population Viability Analysis

We performed a Population Viability Analysis using the simulation program VORTEX (Lacy et al., 2005) to predict future changes in genetic diversity (i.e. heterozygosity) under different scenarios for the MTP. We simulated population dynamics for 100 years and ran the model for 1 000 iterations. Allele frequencies calculated with FSTAT v2.9.3.2 (Goudet, 2002) were uploaded in the program. Population sizes were based on the WAG-SA report of 2017. The minimum carrying capacity of reserves was set at 10 individuals, which is the minimum viable pack size and considered reasonable for small reserves (Lindsey et al., 2004). Because the carrying capacity in larger reserves is often artificially managed, we applied a value of N+1 (Davies-Mostert et al., 2009). We used life history data (e.g. reproductive and mortality rates) of the MTP provided by Davies-Mostert (2010) and Spiering et al. (2010), see Table A1 for complete overview of all the input parameters.

We included all 14 reserves in the metapopulation tally, and run the program for five scenarios: (1) no population growth and no translocations, (2) population growth of 10% over 10 years and no translocations, (3) no population growth and translocations [set at two individuals (one female and one male) at two year intervals for each reserve], (4) population growth of 10% over 10 years and translocations, (5) population growth of 10% over 10 years and translocations [set at four individuals (two females and two males) at two year intervals]. Translocations were entered in VORTEX by the supplementation option, and the frequency (two individuals per two years) was based on the total number of translocation events during our study period (*N*=217), divided by the number of years (*N*=10) and populations (*N*=14). Even though translocation events for single reserves realistically occur less often and involve more individuals at a time, this frequency was considered most suitable for the program, which selects the order of recipient populations at random for translocations. We added an additional translocation frequency (four individuals per two years) for comparison.

For KNP, we calculated the change in heterozygosity in the absence of translocations under two scenarios: (1) no change in population size (*N*=304), (2) an increase in population size according to the observed/ maximum density ratio of 0.61 (Lindsey et al., 2004). This ratio was measured for southern KNP and predicts that the wild dog population has the potential to grow by 39% based on area requirements and prey densities (Lindsey et al., 2004).

2.4.4 Population admixture

To test whether genetic differentiation has occurred, we used AMOVA applications to calculate genetic distance (F_{ST}) with Arlequin v3.5 (Excoffier and Lischer, 2010). To test whether the MTP, FRM, and KNP form genetically distinct populations, we used STRUCTURE v2.3 (Pritchard et al., 2000), which uses a Bayesian framework for inferring population structure. The parameter Delta K was used to determine the most likely number of genetic clusters (Earl and vonHoldt, 2012). We applied admixture for the ancestry of individuals, assumed correlated allele frequencies, and tested both the simple and logistic models. The program was run from K value 1 to 5 with 1 000 000 MCMC generations (discarding 10% as burn-in) and 20 iterations. To estimate rates of recent movement between the MTP and KNP, we used BIMR v1.0 (Faubet and Gaggiotti, 2008) which estimates the probability that an individual migrated during the last generation. We applied the same parameter set as for the population structure analysis.

3. Results

3.1 Population demographics

Between 2008 and 2017, the MTP has increased from 84 to 148 adults, which indicates an annual growth rate of 7% (Table 1). Numbers of wild dogs in KNP have been fluctuating between 127 and 255 individuals. Sightings of free-roaming wild dog have been increasingly rare in the northern parts of South Africa, with a potential population decrease of approximately 90%. A total of 217 wild dogs were moved between reserves in the metapopulation network (Table 1); of these, 120 were females, 95 were males, and two individuals were of unknown sex (see Table A2 for a complete overview). Most of these translocation events (80%) involved a single sex. Eleven reserves, three holding facilities, captive animals from the De Wildt Cheetah & Wildlife Trust and free-roaming individuals from the KwaZulu-Natal Province (KZN) served as donors. Ten reserves within the metapopulation network, as well as Kruger National Park and Northern Tuli Game Reserve in Botswana, received animals (Figure 2).

Table 1. Population sizes of African wild dogs of adults and yearlings (pups) in South Africa, and the number of individuals (*N*) that were translocated between 2008 and 2017 in the metapopulation.

Year	**Population size**			**Translocations**
	Metapopulation	*Kruger NP*	*Free-roaming*	*N*
2008	84 (37)	144 (unk)	104 (unk)	0
2009	93 (49)	151 (unk)	96 (unk)	9
2010	134 (74)	163 (unk)	81 (unk)	16
2011	155 (31)	180 (unk)	30 (unk)	11
2012	142 (52)	127 (55)	24 (unk)	9
2013	140 (32)	171 (30)	20 (unk)	14
2014	165 (72)	197 (83)	37 (unk)	55
2015	173 (84)	199 (98)	20 (unk)	22
2016	148 (87)	200 (95)	8 (unk)	50
2017	148 (94)	255 (49)	4 (unk)	31

unk = unknown

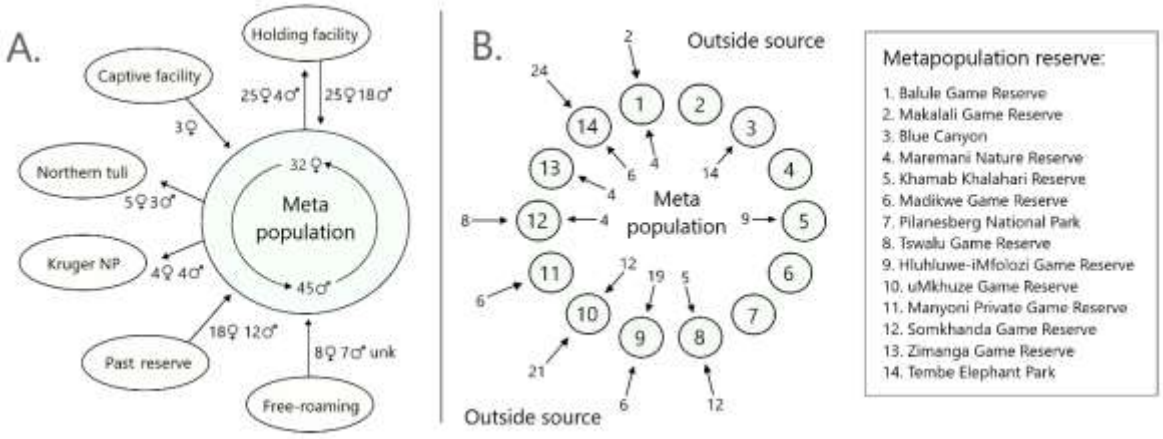

Figure 2A. Visualisation of wild dog translocations from and into the metapopulation between 2008 and 2017. Past reserves no longer partake in the metapopulation, free-roaming wild dogs originate from KwaZulu-Natal in South Africa, Northern Tuli is a game reserve in Botswana, and 'unk' refers to unknown sex. 2B. An overview of reserves (presented as circles) that received individuals from either the metapopulation or outside sources (i.e. holding facilities, captive facilities, free-roamers and past reserves). Numbers at the end of each arrow represent the number of translocated individuals.

3.2 Genetic diversity

We found no evidence for stuttering, large allele dropout or null alleles in our microsatellite data except for loci REN162, which was subsequently removed from further analyses. All microsatellite loci were polymorphic, with an average of 7.6 alleles (SD 3.4) per locus across all populations (see Table A3 for microsatellite data). The loci all appeared to be in Hardy-Weinberg equilibrium, but some LD was observed across pairs of loci (24 out of 171 pairwise comparisons). This is likely caused by the population structure of African wild dogs and because some packs, which consist of close relatives, were sampled multiple times (Hedrick, 2000; Girman et al., 2001). When excluding multiple members of the same pack, LD was absent.

Levels of genetic diversity were high and varied little between the study populations (Table 2). Allelic richness, measured as the average number of alleles per locus, ranged from 4 to 12 in the MTP (A_R=6.8), 2 to 6 in FRM (A_R=4.2), and 3 to 14 in KNP (A_R=6.1). The lower allelic richness observed in the free-roaming population stems from the small sample size, as the rarefraction method indicated little difference between populations (MTP R_S=4.9, FRM R_S=4.1, KNP R_S=4.8). The expected heterozygosity varied from 70 to 75 percent in all the populations. Fixation indexes were negative for all populations, ranging between -0.02 and -0.10. Within pack FIS averaged -0.13, and when members from the same pack were excluded the F_{IS} for the MTP was 0.024 and for KNP -0.08. The estimated effective population size (N_e) of wild dogs was 64.9 for the MTP and 62.7 for KNP. The census size (N_c) of the MTP in 2017 was 148 adults and yearlings, which translates into a ratio of 0.44 between the effective and census population sizes (N_e/N_c). This ratio was lower for KNP (N_e/N_c=0.25). The overall relatedness in the metapopulation and KNP was identical and low (r=0.06), typical for fourth-order relatives (Figure 3). We found no difference between male and female relatedness values. We similarly found low levels of relatedness within reserves, within packs, and in two breeding pairs (Table 2). As expected, between pack relatedness was lower (r=0.04).

Table 2. Genetic diversity estimates (SD) for African wild dogs in the managed metapopulation, free-roaming population, and Kruger National Park (NP) in South Africa. Effective and census population sizes are based on population demographics from 2017 (adults and yearlings).

Genetic diversity	**Metapopulation**	**Kruger NP**	**Free-roaming**
Sample size (N)	120	59	6
Allelic richness (A_R)	6.8 (2.4)	6.1 (2.8)	4.2 (0.8)
Rarefraction A_R	4.9 (1.3)	4.8 (1.6)	4.1 (1.1)
Observed heterozygosity (H_O)	0.71 (0.10)	0.75 (0.11)	0.81 (0.18)
Expected heterozygosity (H_E)	0.70 (0.11)	0.71 (0.10)	0.75 (0.11)
Fixation index (F_{IS})	-0.016 (0.1)	-0.10 (0.2)	-0.07 (0.2)
Effective population size (N_e)	64.9 (6.5)	62.7 (5.8)	n.c.
Population census size (N_c)	148	255	n.c.
N_e/N_c ratio	0.44	0.25	n.c.
Overall relatedness (r)	0.06 (0.1)	0.06 (0.1)	n.c.
Within reserve r	0.06 (0.1)	n.c.	n.c.
Within pack r	0.06 (0.1)	n.c.	n.c.
Between pack r	0.04 (0.1)	n.c.	n.c.
Alpha pair r	0.07 (0.03)	n.c.	n.c.

n.c. = not calculated

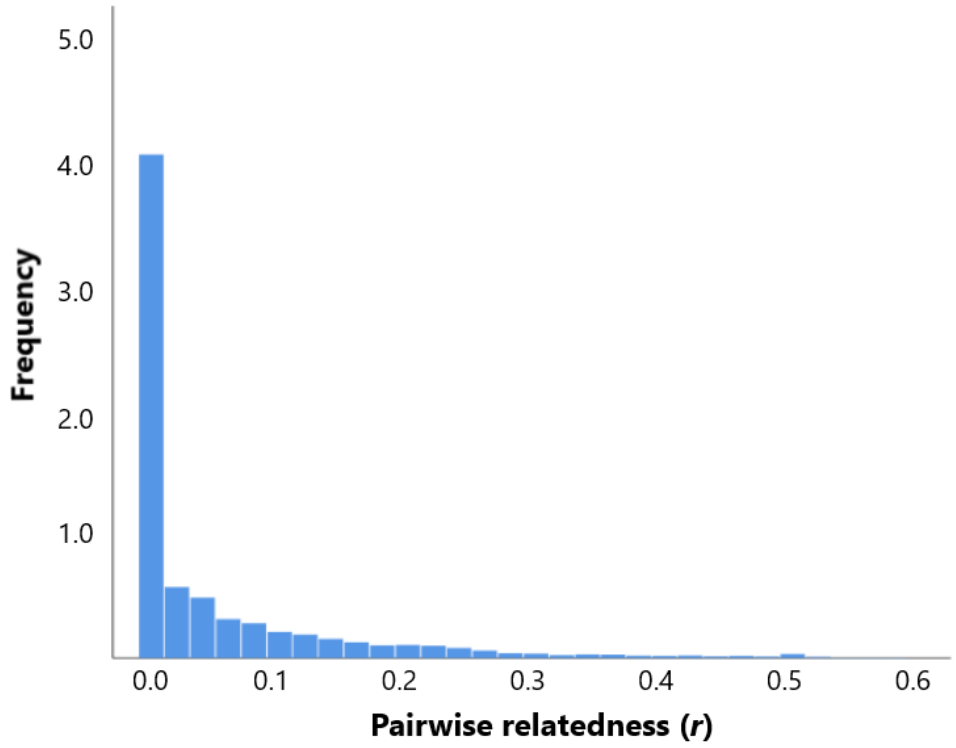

Figure 3. Frequency distribution of pairwise relatedness in the African wild dog metapopulation

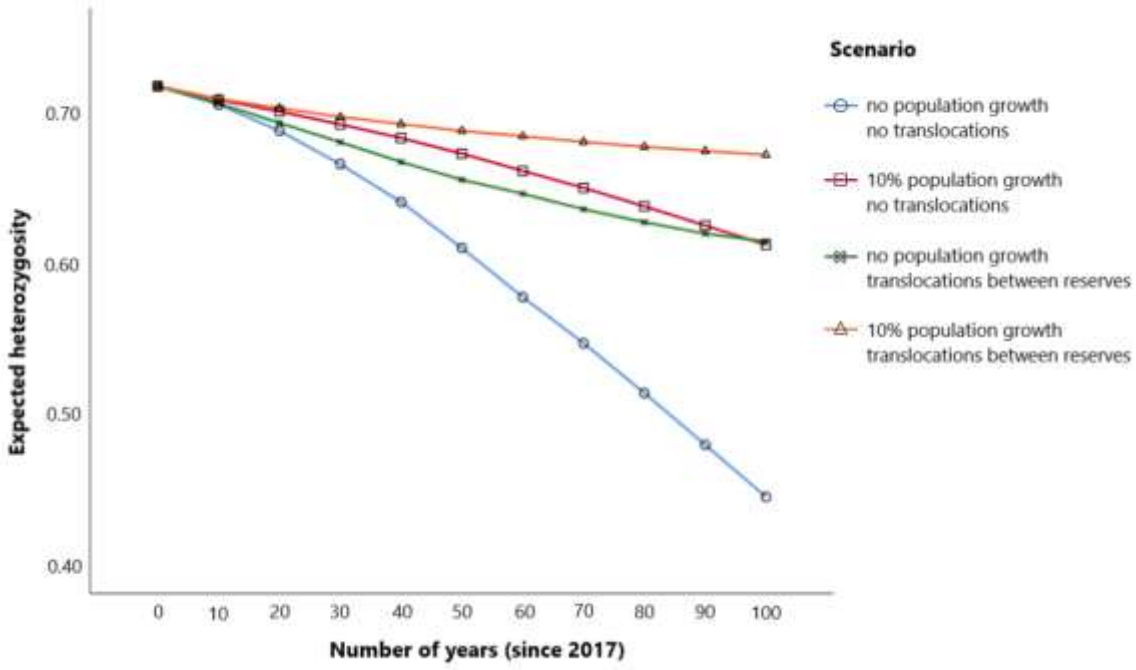

Figure 4. Population viability simulations of the wild dog metapopulation under five scenarios, and the resulting loss in heterozygosity over a time period of 100 years.

3.3 Population Viability Analysis

Our simulations predict that in the absence of supplementation and population growth, the metapopulation would lose 48% of its heterozygosity (Figure 4) in the future. Only scenario 4 and 5 (which allow for a population growth of 10% over 10 years and supplements each population with two and four individuals respectively) was successful at maintaining 95% stable heterozygosity values over a trajectory of 100 years. Under both scenarios, the mean growth rate across all years was 0.11 (0.17 SD). Translocating four instead of two individuals at two-year intervals had only a minor impact on the heterozygosity. For KNP, the wild dog population would lose 12% of its heterozygosity over 100 years if the population was not supplemented and if no population growth were to occur. If the KNP reached its potential carrying capacity (*N*=423 individuals), the population would lose 8% of its heterozygosity over 100 years.

3.4 Population admixture

An analysis of molecular variation showed that 6% of the overall variation was accounted for by the between population (MTP, FRM and KNP) component (F_{ST}=0.06; P<0.05), with the remainder of the variation (94%) accounted for by variation between individuals within populations. The highest delta K value determined by STRUCTURE was 220, for two genetic clusters, which further indicates that there is a level of genetic differentiation between wild dogs in South Africa. The genetic clusters are not representative for either of the populations (Figure 5). This is in line with the estimated migration rates, which indicated some movement between the MTP and KNP. Our analyses showed that 0.16 (SD 0.03) individuals have moved into MTP from KNP in the last generation, and that 0.05 (SD 0.02) individuals moved into KNP from MTP. The generation time of wild dogs is approximately four years (O'Grady et al., 2008) which means that at the measured rates, one individual disperses from KNP to the MTP every 24 years, and 1 individual disperses from the MTP to KNP every 80 years.

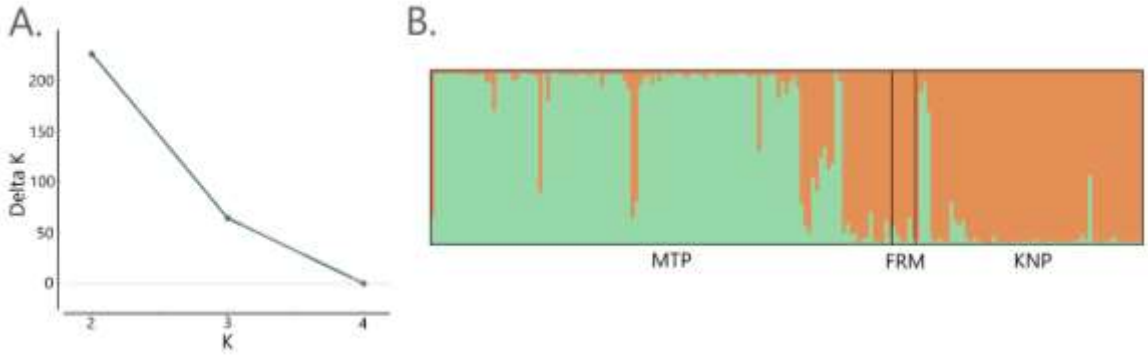

Figure 5A. Plot showing the number of genetic clusters based on Delta K for African wild dogs in South Africa. 5B. Histogram showing two genetic clusters (K) among three African wild dog populations: the managed metapopulation (MTP), a free-roaming population (FRM) and Kruger National Park (KNP).

4. Discussion

4.1 Patterns of genetic diversity

The metapopulation plan for African wild dogs in South Africa has been one of the most extensive efforts to recover population numbers for an endangered carnivore (Gusset et al., 2008; Davies-Mostert et al., 2009). Population monitoring has been ongoing to evaluate the success of the

programme (Davies-Mostert et al., 2015), and genetic data form an essential part of these efforts (Spiering et al., 2011). Maintaining acceptable levels of genetic diversity is considered one of the best strategies to improve reproductive fitness and maximize the success of carnivore translocations (Miller et al., 1999; Spiering et al., 2011; Weeks et al., 2011). Ongoing gene flow at the rate of one-migrant-per-generation was originally recommended to counter the expression of deleterious genes in populations and maintain adaptive potential (Mills and Allendorf, 1996). However, other studies have shown that three to ten migrants per generation are required to avoid extensive loss of genetic diversity, dependent on the population size and temporal fluctuations (Storfer, 1999; Vucetich and Waite, 2000; Couvet, 2002). Based on a population size of 242 individuals, the metapopulation management plan for wild dogs in South Africa meets the minimum required number of migrants, as an average of 22 individuals per year (approximately 5 per generation) have been moved between reserves during our study period (from 2008 to 2017).

In coherence, the metapopulation showed high levels of genetic variation (H_O=0.71, A_R=6.8). Similar indicates of genetic diversity were found for wild dogs in KNP and FRM, although for the latter only a small sample size was included and we are cautious of any inferences related to the FRM population. The current genetic variability within the metapopulation is higher than was previously estimated (2003-2008) for wild dogs in the Hluhluwe-iMfolozi Park (H_O=0.59, A_R=4.9, F_{IS}=0.07; Spiering et al., 2011), which indicates that genetic diversity appears to have increased, in part driven by extensive translocation efforts. Genetic variation in the MTP was also found to be higher than a recovering wild dog population in Zimbabwe (Tensen et al., 2016), and instead is more similar to KNP and other large, free-roaming populations in Africa (Marsden et al., 2012).

Besides translocation efforts, the high level of genetic variation seen in the metapopulation is likely to have been influenced by other factors. First, the metapopulation was founded by a large number of individuals (66 wild dogs; Davies-Mostert et al., 2009), which is one of the most important determinants of reintroduction success (Wolf et al., 1996; Miller et al., 2009). Secondly, the rapid population growth of wild dogs in South Africa (7% mean annual increase) is likely to have prevented loss of diversity (Sugg et al., 1996; VonHoldt et al., 2008). Thirdly, efforts have continuously been made to match unrelated individuals during pack establishment (Mills et al., 1998; Spiering et al., 2011) as pedigrees were known from intensive monitoring (Graf et al., 2006). Lastly, strong inbreeding avoidance in social carnivores, such as wild dogs, naturally sustains genetic variability and enhances the efficiency of translocations (Saccheri and Brakefield, 2002; Vilà et al., 2003).

Fixation indexes were negative in all populations, which normally corresponds to a heterozygote excess. However, this approach may be limited for isolated monogamous populations, as is the case for wild dogs (Balloux, 2004). Negative F_{IS} values can occur when multiple members from the same pack are sampled, as this has a negative bias on the expected heterozygosity (Van Hooft et al., 2018). For instance, in the MTP we found that within pack F_{IS} was higher (-0.13), and when we only incorporated unrelated individuals a significant increase is F_{IS} was observed (i.e. from -0.016 to 0.024), due to which we cannot dismiss the possibility of inbreeding. In KNP we found a negative F_{IS} (-0.08) even when pack members were excluded, which suggests possible outbreeding in our study population.

Relatedness was found to be typical for fourth-order relatives (r=0.06). We predict that cooperative breeding in wild dogs could partly explain the low relatedness, as subordinate individuals often lack access to unrelated mates in packs. Inbreeding avoidance thereby limits their reproduction and

subordinates primarily assist in rearing offspring other than their own (Jennions and Macdonalds, 1994; Cooney and Bennet, 2000). Nonetheless, this should normally mean that relatedness within packs is expected to be higher. For instance, it was previously found that within pack relatedness in wild dogs averaged 0.27 (Girman et al., 1997). It is possible that our values were lower because not all individuals in packs were sampled at once (i.e. an average of 7 individuals per pack at various time intervals).

We found an effective population size of 65 for the MTP and 63 for KNP, which illustrates that a larger proportion of the MTP animals reproduce, possibly also resulting from translocations and artificial pack formation (Lande and Barrowclough, 1987). It has been suggested that the N_e/N_c ratio in wildlife populations is normally close to 0.50, but this is strongly dependent on mating systems (Nunney, 1993). A smaller median N_e/N_c ratio of 0.14 was reported by Palstra and Ruzzante (2008), who combined data from 83 studies. In cooperative breeders, N_e is expected to be much smaller than N_c due to a limited number of breeders (Frankham, 1995; Creel et al., 2004). For instance, previous studies that applied a similar methodology reported a N_e/N_c ratio of 0.02-0.25 in wild dogs (Marsden et al., 2012), 0.20 in Ethiopian wolves *Canis simensis* (Randall et al., 2010) and 0.26-0.42 in wolves (Aspi et al., 2006; VonHoldt et al., 2008). Therefore, our estimates of KNP appear to be average (N_e/N_c ratio of 0.25), whereas the ratio of the MTP (0.44) is relatively high. This could, partially, have accounted for the high population growth seen in African wild dogs that are part of the MTP (Frankham, 1995).

4.2 Population viability predictions

At the onset of the metapopulation plan, it was suggested that the supplementation of new wild dogs into the population at 5-year intervals would be sufficient to maintain genetic diversity (Mills et al., 1998). It was further predicted that in the absence of supplementation, the metapopulation would lose 16.4% of its heterozygosity within 25 years (Davies-Mostert, 2010). Our PVA simulation showed that in the absence of any management intervention, the metapopulation would lose 48% of its heterozygosity over a 100-year trajectory, and that KNP would lose 12%. Under the current management scenario (population growth and translocations), the metapopulation will maintain 95% of its heterozygosity over the next 100 years. Hence, predictions are that it is adequate to supplement each reserve with two individuals (one female, one male) at two-year intervals for preserving genetic variation (WAG-SA, 2017). However, the required supplementation frequency differs per reserve, as long-term persistence is practically impossible when a population contains only a single pack or less than thirty individuals (Gusset et al., 2008; Davies-Mostert, 2010). The PVA also showed that allowance for population growth is essential to maintain levels of heterozygosity. These results are comparable to a study done on the management of a reintroduced wolf population in Yellowstone National Park, where it was similarly concluded that if no increase in population size can be realized, inbreeding depression is likely to occur without ongoing translocations or dispersal from outside sources (VonHoldt et al., 2008).

This means that in the current scenario, KNP is the only self-sustainable wild dog population in South Africa. Ideally, the wild dog population outside KNP will become self-sustainable in the future without the requirement of ongoing translocations (Hanski and Simberloff, 1997). In the revised management strategy, one of the main aims is to establish populations in larger areas outside of KNP to lower the need for interventions. However, with limited space, such areas are few and managers are often forced to intervene to ensure safe habitat and keep good relationships with managers. Hence, intensive management will remain crucial to avoid local extinctions and maintain genetic diversity in wild dogs (Mills et al., 1998). Similarly, metapopulation programmes are essential for the population

viability of locally endangered species such as white rhino (*Ceratotherium simum*; Emslie et al., 2009), black rhino (*Diceros bicornis*; Foose et al., 1993) and cheetah (*Acinonyx jubatus*; Buk et al., 2018).

4.3 Population admixture and dispersal

Natural dispersal from outside populations will substantially increase the chance of unrelated individuals adding to the metapopulation's gene pool (McNutt, 1996; Mills et al., 1998). We found genetic evidence that dispersal occurs between the MTP and KNP, although only an estimated 0.05 to 0.16 individuals per generation (i.e. every four years; O'Grady et al., 2008). Despite the fact that wild dogs from KNP spent 6.3% of their time outside park boundaries (Louis van Schalkwyk, unpublished data) and that some metapopulation reserves are in close proximity to KNP, it is possible that effective dispersal (i.e. migration with successful reproduction) remains low due to high mortality rates of wild dogs outside protected areas (Woodroffe et al., 2004; Page et al., 2015). Our results show that wild dogs in South Africa are divided into two genetic clusters, with a significant degree of differentiation (F_{ST}=0.06). These results are comparable to Marsden et al. (2012) and Tensen et al. (2016), who found that KNP has been isolated from other regions, which caused them to become genetically distinct over many generations through the effect of genetic drift. However, the clusters are not restricted to either the MTP, FRM or KNP, which suggests some level of population admixture. That admixture seems to occur is promising with regards to the long-term viability of African wild dogs in South Africa (Fuller et al., 1992b; Mills et al., 1998). It also suggests that translocations between the three populations will pose acceptably low levels of risk with regards to the loss of local adaption or unique lineages (Storfer, 1999; Weeks et al., 2011).

4.4 Conservation implications

In conclusion, the metapopulation management plan for African wild dogs in South Africa can be considered successful based on the achieved population growth and subsequent preservation of genetic diversity. Our study highlights that genetic data form a critical part of conservation management, and that translocations can be a vital tool to restore genetic variability and conserve reproductive and adaptive potential of threatened species. We recommend other reintroduction and translocation programmes to include population genealogies to assess the effectiveness of their management practices for the long-term viability. We further recommend that serious consideration be given to translocations of wild dogs between populations within the same cluster that can alleviate loss of genetic diversity over multi-generations; this might not only be pertinent to South Africa but applicable across the region as safe space becomes reduced due to increase human populations and pressures.

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Supplementary Material

Table A1. Population demographic data of African wild dogs used as input parameters for the VORTEX Population Viability Analysis

Table A2. Translocation history of African wild dogs as part of the metapopulation approach in South Africa

Table A3. Microsatellite data retrieved for African wild dogs from South Africa