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RESEARCH ARTICLE

Genetic restoration of black rhinoceroses in South Africa: conservation implications

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Abstract

Globally, wildlife populations are becoming increasingly small and isolated. Both processes contribute to an elevated risk of extinction, notably due to genetic factors related to inbreeding depression and a loss of adaptive potential. Wildlife translocation is a valuable conservation tool to reintroduce species to previously occupied areas, or augment existing populations with genetically divergent animals, thereby improving the viability of endangered populations. However, understanding the genetic implications of mixing gene pools is key to avoid the risk of outbreeding depression, and to maximise translocation effectiveness. In this study we used mitochondrial and microsatellite DNA collected from 110 black rhinoceroses (*Diceros bicornis minor)* in Kruger National Park, South Africa, to determine levels of genetic diversity, inbreeding and relatedness. We compared this diversity with the two source populations (KwaZulu-Natal, South Africa and Zambezi River, Zimbabwe) using data from previously published studies, and assessed changes in the relative contribution of source lineages since their reintroduction in the 1970s. Our results show that Kruger's black rhinoceroses are genetically more diverse than those from KwaZulu-Natal, with levels closer to those from the Zambezi Valley. Furthermore, our findings indicate a relative increase in the Zimbabwean lineage since reintroduction, suggesting a possible selective advantage. From a conservation perspective, our results demonstrate the benefits of mixing multiple source populations to restore gene flow, improve genetic diversity and thereby help protect small, isolated populations from extinction.

Keywords *Diceros bicornis* · Kruger National Park · Reintroduction · Genetic diversity · Gene flow · South-central black rhinoceros · Population genetics

Introduction

Compared to their historical counterparts, many wildlife populations remaining today are small and isolated with limited gene flow. These populations may exhibit an increased population differentiation and reduced genetic diversity as a function of genetic drift and/or inbreeding (Lacy 1987; Primack 2002). Negative genetic impacts are intensified in fenced populations, as mechanisms that evolved to reduce inbreeding, such as dispersal, are inhibited. Thus, a key challenge in wildlife conservation is to prevent or minimise the loss of genetic diversity to enable the long-term persistence of threatened species (Weeks et al. 2011; Keller et al. 2012).

Reintroductions and translocations are important conservation tools used to increase the effective population size, maintain genetic diversity (Hedrick and Fredrickson 2010; Heber et al. 2013), reduce inter-population differentiation (Thavornkanlapachai et al. 2019) and improve the geographic range of endangered species (Armstrong and Seddon 2008). Translocations that simulate the immigration of genetically divergent individuals (Tallmon et al. 2004) have successfully improved the demographic performance of populations of wildlife species such as adders (*Vipera berus*, Madsen et al. 1999), Florida panthers (*Puma concolor coryi*, Johnson et al. 2010), and bighorn sheep (*Ovis Canadensis*, Poirier et al. 2019). However, despite its potential benefits, mixing highly differentiated populations that lack local adaptations may render translocations counterproductive, as

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it may lead to reduced survival or fitness in the offspring (outbreeding depression; Templeton 1986; Edmands 2007; Frankham 2015; Ralls et al. 2018).

The black rhinoceros (*Diceros bicornis*) epitomises the threats facing many endangered species. The south-central black rhinoceros *(D. b. minor)* historically occurred from Tanzania, through Zambia, Zimbabwe and Mozambique to the north-eastern parts of South Africa (Emslie and Adcock 2016). However, wide-scale poaching and unregulated trophy hunting reduced the south-central black rhinoceros in southern Africa to two remnant populations, one in the KwaZulu-Natal region of South Africa, and the other in the Zambezi Valley and Sebungwe regions along the Zambezi River in Zimbabwe (Cumming et al. 1990; Emslie and Brooks 1999; Emslie 2012). The drastic reduction in population size and geographic isolation resulted in extremely low genetic diversity within the KwaZulu-Natal black rhinoceroses, with only a single mitochondrial DNA haplotype remaining (Anderson-Lederer et al. 2012; Kotzé et al. 2014); historically, South Africa harboured at least six haplotypes (Moodley et al. 2017). The Zambezi River black rhinoceros population retained substantially more genetic variation - possibly because of a milder bottleneck event – with six mitochondrial DNA haplotypes identified thus far (Kotzé et al. 2014). Black rhinoceroses remain vulnerable due to relentless poaching for their horns (Amin et al. 2006; Ferreira et al. 2015, 2017; Knight 2017) and range expansion continues to be a conservation priority in South Africa (SANParks 2002; WWF-South Africa 2020).

Black rhinoceroses were declared extinct in the Kruger National Park, South Africa (24°0'41"S, 31°29'7"E; Kruger), in the 1930s after decades of hunting eradicated the population (SANParks 2002). A new population was founded during the 1970 and 1980 s with reintroductions from both the KwaZulu-Natal and Zambezi River source populations (Hall-Martin and Knight 1994; Ferreira et al. 2011). This founder population provides a unique opportunity to explore the outcome of mixing two source population gene pools. In this study, we used genetic data from 110 black rhinoceroses to (i) quantify the genetic variation and relatedness and (ii) evaluate any changes in the proportion of source population ancestry present in the founded Kruger population. Finally, we compared the genetic diversity between the Kruger black rhinoceroses with published estimates of both source populations. Describing the diversity and lineage composition of the Kruger black rhinoceroses allowed us to evaluate the impact of this population admixture and provide information necessary for optimising the recovery and growth of this subspecies throughout its range.

Methods

Study area and population history

The Kruger National Park (Kruger) is situated on the eastern side of Limpopo and Mpumalanga provinces of South Africa and covers an area of 19,485km^2 (Ferreira et al. 2011), extending 360 km from North to South and 90 km from East to West at its widest part (Fig. 1; Foxcroft et al. 2008). Between 1971 and 1989 a total of 81 black rhinoceroses were reintroduced into southern Kruger (SANParks 2002). These black rhinoceroses originated from the Zambezi River, Zimbabwe (n = 14) and KwaZulu-Natal, South Africa (n = 67). In 2009, the population was estimated at 627 black rhinoceroses living in southern Kruger (Ferreira et al. 2011), making it the largest south-central black rhinoceros population in Africa. However, by 2019 the population had declined to 268 (95% CI: 191–342) (Ferreira et al. 2020) predominantly due to poaching (Ferreira et al. 2018). With the possible exception of a few individuals, the remaining black rhinoceroses in Kruger are found south of the Olifants River.

Samples and genetic data

Blood samples (n = 110; female = 60, male = 50) were collected from black rhinoceroses in southern Kruger (Fig. 1) from 2014–2019 during various management interventions, performed in accordance with South African National Parks (SANParks) Wildlife Capture Standard Operating Procedures. Sex, date and location were recorded at the time of capture. DNA was extracted using DNeasy Blood & Tissue Kits (Qiagen, Valencia, CA, USA), following the manufacturer's instructions. A fragment of the mitochondrial DNA control region was sequenced by ZooOmics™ (Inqaba Biotec, Pretoria) using primers mt15996L (5'-TCCACCATCAGCACCCAAAGC-3'; Campbell et al. 1995) and mt16502H (5'-TTTGATGGCCCTGAAGTAAGAACCA-3'; Moro et al. 1998). Individuals were genotyped by ZooOmics™ using the standard rhinoceros forensic panel in South Africa (Harper et al. 2013) comprised of 23 microsatellite markers (Supplementary Table S1).

Genetic analysis

i. Quality control

Control region sequences were edited in Mega v 10.0.5 (Kumar et al. 2018) after visually inspecting individual chromatograms using Chromas v 2.6.6 (Technelysium Pty Ltd, Australia). The sequences were aligned using Clustal

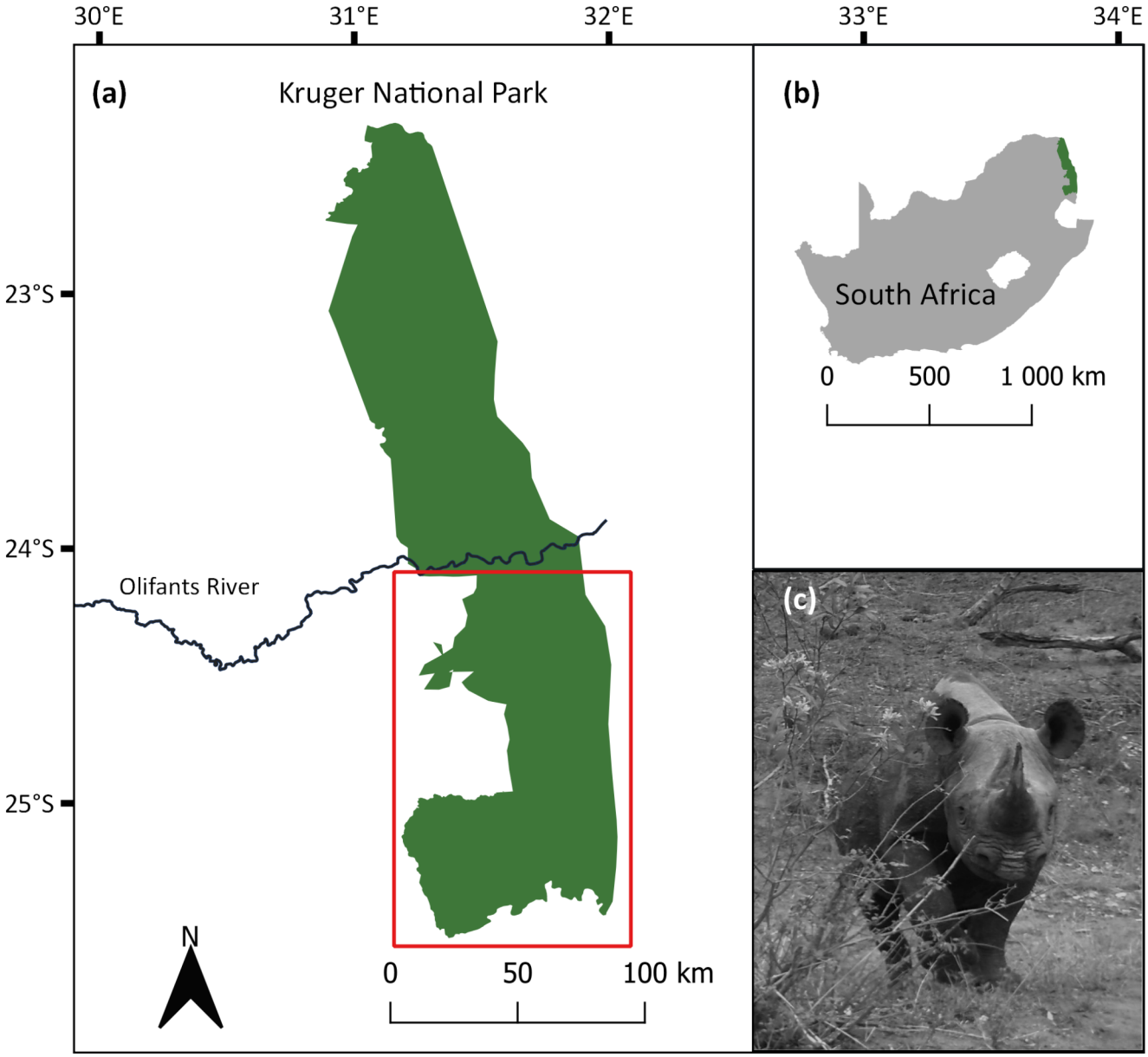

Fig. 1 (a) Map showing the sampling area (red box) within the Kruger National Park (green polygon); (b) Kruger National Park's location and total extent within South Africa; (c) photograph of a black rhinoceros taken during the 2019 census (photo credit: SANParks, C. Dreyer)

W (Larkin et al. 2007) as implemented in Mega. Published control region mtDNA sequences of south-central black rhinoceros from the Zambezi River (Zambezi Valley and Sebungwe region), Zimbabwe and KwaZulu-Natal (South Africa) source populations were included in a combined dataset (GenBank accession numbers AF187825-AF187831, Brown & Houlden 2000; JN593089 (n = 64); Anderson-Lederer et al. 2012; KM095529-KM095627, Kotzé et al. 2014; KY472322-KY472346, Moodley et al. 2017).

Genotype profiles were checked for genotyping errors, allele dropout and null alleles using MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004). Null allele frequencies were estimated using FREENA (Chapuis and Estoup 2007). Frequencies of null alleles may cause moderate (> 0.08; Chapuis and Estoup 2007) to significant (> 0.20; Dakin and Avise 2004) bias in F-statistics, and thus loci with null alleles with a frequency greater than 0.08 were removed from further analysis. Genepop v 4.7.2 (Raymond and Rousset 1995) was used to test for deviations from Hardy–Weinberg equilibrium (HWE) for each locus and genotypic linkage disequilibrium (LD) between loci. All probability tests were based on Markov Chain Monte Carlo (MCMC) default parameters. Sequential Bonferroni correction was

applied to determine significance thresholds for HWE and LD to account for multiple comparisons.

ii. Diversity

Genetic diversity within the mtDNA control region sequences was assessed by calculating nucleotide diversity (π), haplotype diversity (h) and the number of unique haplotypes present. These diversity metrics were calculated in DnaSP v 6 (Rozas et al. 2017). Microsatellite diversity was inferred from the range and number of alleles per locus (Na), observed heterozygosity (H_o) and expected heterozygosity (H_e; Nei 1978). The effective number of alleles per locus (A_e), i.e., the number of alleles one would expect in a population with the same heterozygosity but with an equal distribution of allele frequencies (Crow and Maruyama 1971), was also calculated. All calculations were performed in GenAlEx 6.5 (Peakall and Smouse 2012).

iii. Relatedness and inbreeding

Average pairwise relatedness (r) for the population was calculated using the package 'related' (Pew et al. 2015) in R version 3.6.1 (R Core Team 2019). The estimator of relatedness chosen for this analysis was based on a simulation analysis comparing different estimators using the 'compareestimators' function. Given similar performance, the Wang (2002) estimator (having the highest correlation between observed and expected values) was chosen for further analysis. Pairwise relatedness was calculated using the 'coancestry' function. Wright's inbreeding coefficient (F_{IS}) was calculated using GENETIX 4.05.2 (Belkhir et al. 2004). Confidence intervals for inbreeding coefficient values for each locus and over all loci in each population were obtained by bootstrapping 1000 times.

Source and founder population comparison

The combined mtDNA sequence dataset was used to compare the nucleotide (π) and haplotype (h) diversity between source (Zambezi River and KwaZulu-Natal) and founder (Kruger) populations. The genetic structure among source and founder haplotypes in the combined dataset was visualised by constructing a median-joining network (Bandelt et al. 1999) in PopART (Leigh and Bryant 2015). Finally, the relative maternal lineage contributions of the source populations were determined and compared to the initial ratio of Zambezi River and KwaZulu-Natal founder females obtained from historic records (SANParks, unpublished data).

Although a direct comparison of microsatellite diversity between population is not possible due to the different number and type of microsatellite loci used between studies, a comparison of relative nuclear diversities was conducted between source and founder populations from published studies.

Results

Quality control

No evidence of genotyping errors or allelic dropout was found. Signatures of null alleles were detected at seven loci, namely SR74, IR12, SRS262, 7 C, BlRh1B, DB44 and DB66; loci with null allele frequencies greater than 0.08 (SR74, IR12, SRS262, 7 C and BlRh1B) were removed from subsequent analysis. ZF1 (Zinc Finger locus; Peppin et al. 2010) was used to confirm individual sex. Seven of the total of 254 combinations of paired loci were in linkage disequilibrium after applying a Bonferroni correction ($P < 0.0001$). Loci IR12 and SR74 were removed as they appeared to be sex-linked. Locus 12 F (originally isolated from white rhinoceros) and locus BlRh37D were removed from further analysis. Loci 7B and 32 A, both originally isolated from white rhinoceros, were monomorphic in this study and were also removed. Finally, individuals with more than 30% missing data (n = 1) were removed from further analysis.

Diversity, relatedness and inbreeding

The final mtDNA dataset contained 103 sequences of 469 bp in length. Four haplotypes were identified. These haplotypes were characterised by five polymorphic sites, all containing transition nucleotide substitutions (G↔A and/or C↔T). Haplotype diversity (h) and nucleotide diversity (π) were 0.48 (±0.05 SD) and 0.29 (±0.20 SD), respectively. The final microsatellite data set comprised 109 animals. All 13 microsatellite loci retained for analyses were polymorphic, with two to 14 alleles each (Table 1). The effective number of alleles (A_e) ranged from 1.3 to 5.03 (mean = 2.26 ± 0.25 SE) alleles per locus. Expected heterozygosity (H_e) varied greatly among loci, ranging from 0.23 (SR281) to 0.80 (DB66) and averaged 0.51 across all loci. Population level F_{IS} was 0.04 (95% CI -0.01- 0.07) (Table 1). Mean pairwise relatedness (r) was − 0.03.

Source and founder population comparison

The combined Kruger, Zambezi River and KwaZulu-Natal mtDNA control region dataset comprised 296 sequences of 363 bp after alignment. A total of seven mtDNA haplotypes were found across the three populations, containing seven

Table 1 Genetic diversity of the Kruger black rhinoceros population across 13 microsatellite loci (n = 109)

Locus	Na	A_e	H_o	H_e	uH_e	F_{IS} (95% CI)
DB23	3	2.01	0.54	0.50	0.50	-0.07 (-0.26-0.13)
DB1	2	1.99	0.44	0.50	0.50	0.11 (-0.09-0.29)
DB52	5	2.08	0.51	0.52	0.52	0.02 (-0.15- 0.17)
32 A	5	1.88	0.41	0.47	0.47	0.12 (-0.02- 0.24)
SR281	2	1.30	0.25	0.23	0.23	-0.07 (-0.18-0.09)
IR22	2	1.98	0.47	0.50	0.50	0.05 (-0.15- 0.22)
BIRh1C	5	2.16	0.56	0.54	0.54	-0.04 (-0.14- 0.08)
SR63	4	2.53	0.59	0.60	0.61	0.04 (-0.11- 0.17)
DB44	6	1.71	0.35	0.41	0.42	0.16 (-0.03- 0.33)
BR6	8	2.66	0.68	0.62	0.63	-0.09 (-0.18-0.00)
DB66	14	5.04	0.73	0.80	0.81	0.10 (-0.01- 0.19)
SR268	5	2.30	0.53	0.57	0.57	0.06 (-0.09- 0.19)
IR10	3	1.71	0.41	0.41	0.42	0.02 (-0.12- 0.17)
Mean	4.92	2.26	0.50	0.51	0.52	0.04 (-0.01- 0.07)
SE	0.90	0.25	0.04	0.04	0.04	

N_a number of alleles, A_e number of effective alleles, H_o observed heterozygosity, H_e expected heterozygosity, uH_e unbiased expected heterozygosity, F_{IS} inbreeding coefficient

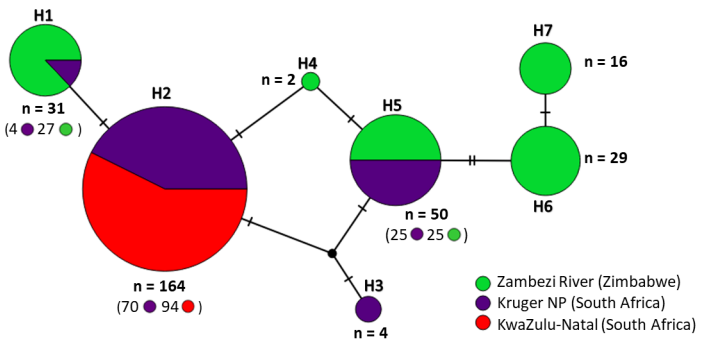

Fig. 2 Median-joining haplotype network among 296 mtDNA sequences from three *D. b. minor* populations. The circle sizes are proportionate to the numbers of individuals representing each haplotype. Colours represent black rhinoceros populations. Hatch marks represent the number of mutation steps between haplotypes. The label above or below the circles represent the haplotype number (H1 – H7). n = number of samples. Numbers in brackets indicate numbers of individuals from the colour-coded population within that haplotype

polymorphic sites (Table 2). The nucleotide and haplotype diversities calculated in the Kruger population was approximately mid-way between those of the two source populations (Table 2). The relationship between haplotypes can be seen in Fig. 2. The Kruger population shared two haplotypes (H1 and H5) with the Zambezi River population; these two haplotypes together represented 28.15% of the Kruger black rhinoceros in this study. The extant KwaZulu-Natal population is represented by only a single haplotype (H2), and this haplotype was shared with 67.96% of the Kruger population. Haplotype 3 (H3) was unique to Kruger, two mutational steps from both H2 and H5 (Fig. 2). Haplotypes 4, 6 & 7 were unique to the Zambezi River population. The current haplotype distribution among the three populations is illustrated in Fig. 3.

In this study, the level of nuclear variation revealed in Kruger black rhinoceroses (H_o = 0.50; H_e = 0.51) was similar to that reported for the Zambezi population (H_o = 0.54; H_e = 0.52; Kotzé et al. 2014) and high relative to prior reports of nuclear diversity of *D. b. minor* in Kwa-Zulu Natal (H_o = 0.38; H_e = 0.44; Karsten et al. 2011; Table 2).

The ratio of female black rhinoceros founders introduced into Kruger from 1971 to 1989 from the two source populations was 5: 1 (KZN: ZIM). The lineage ratio found in the current Kruger population was 2.41: 1 (KZN: ZIM), indicating approximately a two-fold increase in the proportion of Zimbabwean ancestry since reintroduction (Fig. 4).

Discussion

The Kruger black rhinoceros population offered a unique opportunity to evaluate the outcome of mixing the two remnant south-central black rhinoceros source populations. Using mtDNA and microsatellite markers, we found that mixing gene pools substantially enhanced both the mitochondrial and nuclear diversity of the founded Kruger population relative to the source *D. b. minor* population in South

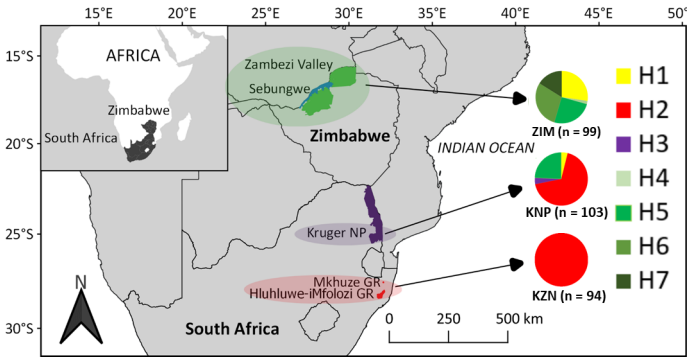

Fig. 3 Current mtDNA control region haplotype distribution in the Zambezi River, Zimbabwe (ZIM), KwaZulu-Natal (KZN) and Kruger National Park (KNP) source populations. n = sample size. H1–H7 = haplotype number

Table 2 Mitochondrial and Nuclear genetic diversity among black rhinoceros populations in Zimbabwe, Kwa-Zulu Natal and Kruger National Park

	Mitochondrial DNA Diversity						Nuclear DNA Diversity			
Population	**N**	**#H**	**#P**	**HD (SD)**	**π (%)**	**Published**	**N**	**Ho**	**He**	**Published**
Zambezi River, Zimbabwe	104	6	7	0.77	0.70	Kotzé et al. 2014	236	0.54	0.52	Kotzé et al. 2014
KwaZulu-Natal, South Africa	65	1	1	0*	0*	Anderson-Lederer et al. 2012	77	0.38	0.44	Karsten et al. 2011
Kruger National Park, South Africa	103	4	5	0.48 (±0.05)	0.29 (±0.20)	This study	109	0.50	0.51	This study

N = number of individuals sampled; #H = number of haplotypes; #P = number of polymorphic; h = haplotype diversity; π = nucleotide diversity; * no variation; Ho = observed heterozygosity; He = expected heterozygosity

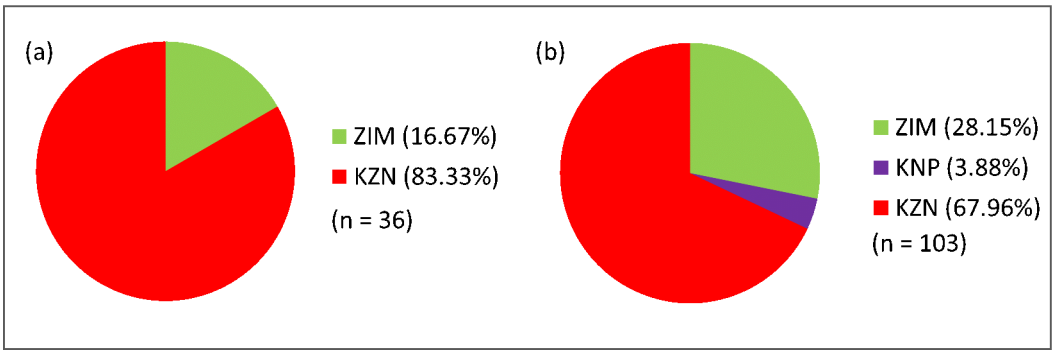

Fig. 4 The proportion of Zambezi Valley (ZIM) and KwaZulu-Natal (KZN) animals in the (a) founder, and (b) current Kruger populations

Africa. Together with the low levels of relatedness and no evidence of non-random mating, our results confirm that the Kruger black rhinoceros population is a diverse, outbred, panmictic population. This study provides a baseline for informing black rhinoceros metapopulation management strategies and indicates that Kruger black rhinoceroses would be ideal candidates for translocation and reintroduction efforts aimed at improving diversity in other *D. b. minor* black rhinoceros populations.

Maintaining adequate levels of genetic diversity is essential for ensuring both the short-term health and long-term survival of isolated populations of endangered species. Small population numbers, genetic drift and/or inbreeding may all contribute to a substantial loss in genetic diversity of such populations, and consequently may negatively impact their viability. For example, the small, reintroduced population of black rhinoceroses in Addo Elephant National Park, South Africa has comparatively low genetic diversity and high relatedness relative to their source populations, resulting in low fitness, manifesting as low population growth rate and reduced male survival (le Roex et al. 2018).

Our results are consistent with several other studies across taxa that have reported improved genetic diversity, and ultimately genetic rescue, in bottlenecked populations through translocation, including Australia's mountain pygmy possum (*Burramys parvus*; Weeks et al. 2017), adders (*Vipera berus*; Madsen et al. 1999), Scandinavian wolves (*Canis lupis*; Åkesson et al. 2016) and New Zealand's South Island

robins (*Petrioica australis*; Heber et al. 2013). Similarly, Poirier et al. (2019) reported that outbreeding with a few translocated individuals significantly increased the low genetic diversity observed in a post-bottleneck population of bighorn sheep (*Ovis canadensis*). Following this recovery in genetic diversity, first-generation (F_1) admixed lamb survival rates improved and population size consequently increased, i.e. genetic restoration resulted in genetic and evolutionary rescue.

The reconstructed mtDNA haplotype network in this study demonstrates that the Zimbabwean mitochondrial lineages are well established in the Kruger black rhinoceros population. Genetic analyses of museum specimens identified at least four mtDNA haplotypes historically found in South African black rhinoceroses (Moodley et al. 2017). Thus our results suggest that mixing the two source populations has restored a comparable level of mtDNA diversity to South African black rhinoceroses. The single haplotype (H2) found in all KwaZulu-Natal black rhinoceros (Anderson-Lederer et al. 2012; Kotzé et al. 2014) was the most common haplotype present in the Kruger population (67.96%). We also found two Zambezi River haplotypes (H1 and H5) within the Kruger population; the remaining Kruger haplotype (H3) was reported in a single captive Zimbabwean black rhinoceros (Fernando et al. 2006), confirming its origin within the Zimbabwean population, as well as its presence within the translocated individuals. Thus at least two (or three, if including H3) of the six known Zimbabwean haplotypes (33–50%) have been restored in the Kruger black rhinoceros population. It is also possible that with more extensive sampling, additional Zimbabwean haplotypes would be detected. Future studies that directly compare the nuclear contributions of the two source populations within the current Kruger population would also provide further insight into this genetic admixture.

While mixing individuals from different source populations may increase genetic diversity and reduce the likelihood of inbreeding depression, it may increase the risk of outbreeding depression (Edmands 2007). For example, if source populations are under unique environmental pressures (e.g., different climates or habitats), local adaptations may arise, especially in long-isolated populations. Thus, outbreeding with genetically diverse individuals is counterproductive if the hybrid offspring face lowered fitness due to the loss of locally adapted genetic variants (Edmands 1999). A classic case of outbreeding depression occurred when two subspecies populations of Alpine ibex (Capra ibex) were translocated from the Sinai Peninsula and Turkey into the European Alps. Unfortunately, the introduced Ibex bred earlier in the season than their European counterparts, resulting in hybrid offspring born in midwinter, reducing survival and ultimately leading to the hybridised herd's extinction (Templeton 1986). Although this is an exceptional case and outbreeding depression is relatively uncommon, (Frankham et al. 2011; Ralls et al. 2018), it remains a significant concern among conservation managers of critically endangered species.

Outbreeding depression from mixing KwaZulu-Natal and Zambezi River black rhinoceroses is unlikely when considering primary risk factors, such as chromosomal differences, lack of gene flow for more than 500 years, and substantial environmental differences between populations (Frankham et al. 2011). The Zambezi River and KwaZulu-Natal black rhinoceros populations were historically connected (Kotzé et al. 2014) and a healthy population of translocated KwaZulu-Natal black rhinoceros in Malilangwe, Zimbabwe suggests that the different environment between populations is unlikely to contribute to outbreeding depression. Furthermore, the increase in Zimbabwean lineage proportion seen in the extant Kruger population (relative to the ratio of founder females) contradicts any potential loss of local adaptation; if anything, selection over the generations may have favoured the more diverse Zambezi River black rhinoceros. Further research, however, is required to test whether a selective advantage or stochastic events are responsible for the lineage proportion increase seen in this study.

In conclusion, this study indicates that the admixture of black rhinoceroses from different gene pools substantially enhanced both the nuclear and mitochondrial diversity of the founded Kruger population relative to the source *D. b. minor* population in South Africa. In the absence of threat alleviation, metapopulation management strategies (such as population supplementation through translocation) aimed at increasing the range and securing the genetic health of black rhinoceros are critical. The improved genetic diversity found in the Kruger population is encouraging for the long-term survival of this subspecies as a managed metapopulation within South Africa, possibly improving its adaptive potential to respond to environmental change. Given the encouraging levels of diversity observed, this also makes the Kruger black rhinoceros population an ideal source candidate for founding new populations or improving the genetic variation (and thus reducing extinction risk) for genetically depauperate *D. b. minor* populations in South Africa.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10592-022-01486-y.

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Conflict of interest The authors declare that they have no conflicts of interest.

Data availability GenBank accession numbers OK376773-OK376875. Microsatellite data to be submitted to Dryad upon acceptance of the manuscript.

Code availability Not applicable.

Declarations

Consent to participate Samples collection was performed by South African National Parks (SANParks) veterinary staff in accordance with national Wildlife Capture Standard Operating Procedures.

Consent to participate Not applicable.

Consent for publication Not applicable.

References

Åkesson M, Liberg O, Sand H et al (2016) Genetic rescue in a severely inbred wolf population. Mol Ecol 25:4745–4756. https://doi.org/10.1111/mec.13797

Amin R, Emslie KT et al (2006) An overview of the conservation status of and threats to rhinoceros species in the wild. Int Zoo Yearb 40:96–117. https://doi.org/10.1111/j.1748-1090.2006.00096.x

Anderson-Lederer RM, Linklater WL, Ritchie PA (2012) Limited mitochondrial DNA variation within South Africa's black rhino (*Diceros bicornis minor*) population and implications for management. Afr J Ecol 50:404–413. https://doi.org/10.1111/j.1365-2028.2012.01333.x

Armstrong D, Seddon P (2008) Directions in reintroduction biology. Trends Ecol Evol 23:20–25. https://doi.org/10.1016/j.tree.2007.10.003

Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48

Belkhir KP, Borsa P, Chikhi L et al (2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire du Génome et populations, interactions, CNRS UMR 5171. Université de Montpellier II, Montpellie

Brown SM, Houlden BA (2000) Conservation genetics of the black rhinoceros (*Diceros bicornis*). Conserv Genet 1:365–370. https://doi.org/10.1023/A:1011579807460

Campbell NJH, Harriss FC, Elphinstone MS, Baverstock PR (1995) Outgroup heteroduplex analysis using temperature gradient gel electrophoresis: high resolution, large scale, screening of DNA variation in the mitochondrial control region. Mol Ecol 4:407–418. https://doi.org/10.1111/j.1365-294X.1995.tb00234.x

Chapuis M-P, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621–631. https://doi.org/10.1093/molbev/msl191

Crow JF, Maruyama T (1971) The number of neutral alleles maintained in a finite, geographically structured population. Theor Popul Biol 2:437–453. https://doi.org/10.1016/0040-5809(71)90033-5

Cumming DHM, du Toit R, Stuart SN (1990) African elephants and Rhinos: Status Survey and Conservation Action Plan. Gland, Switzerland, IUCN

Dakin EE, Avise JC (2004) Microsatellite null alleles in parentage analysis. Heredity (Edinb) 93:504–509. https://doi.org/10.1038/sj.hdy.6800545

Edmands S (2007) Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Mol Ecol 16:463–475. https://doi.org/10.1111/j.1365-294X.2006.03148.x

Edmands S (1999) Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. Evol (N Y) 53:1757–1768. https://doi.org/10.2307/2640438

Emslie R, Brooks M (1999) Donors to the SSC Conservation Communications Programme. and the African Rhino Action Plan

Emslie RH (2012) *Diceros bicornis*. IUCN 2013. IUCN Red List of Threatened Species. Version 2013-1

Emslie RH, Adcock K(2016) The Red List of Mammals of South Africa, Swaziland and Lesotho. In: Child MF, Roxburgh L, Do Linh San E, Raimondo DD-MH (eds).South African National Biodiversity Institute and Endangered Wildlife Trust, South Africa

Fernando P, Polet G, Foead N et al (2006) Genetic diversity, phylogeny and conservation of the Javan rhinoceros (*Rhinoceros sondaicus*). Conserv Genet 7:439–448. https://doi.org/10.1007/s10592-006-9139-4

Ferreira SM, Bissett C, Cowell CR et al (2017) The status of rhinoceroses in south african National Parks. Koedoe 59:1–11. https://doi.org/10.4102/koedoe.v59i1.1392

Ferreira SM, Greaver CC, Knight G et al (2015) Disruption of rhino demography by poachers may lead to declines in Kruger National Park, South Africa. PLoS ONE 10:e0127783. https://doi.org/10.1371/journal.pone.0127783

Ferreira SM, Greaver CC, Knight MH (2011) Assessing the population performance of the black rhinoceros in Kruger National Park. 41:192–204. https://doi.org/10.3957/056.041.0206

Ferreira SM, le Roex N, Greaver CC et al (2020) Comparison of mark-recapture and block-count-based estimation of black rhinoceros populations. Afr J Wildl Res 50:167–175. https://doi.org/10.3957/056.050.0167

Foxcroft LC, Richardson DM, Wilson JRU (2008) Ornamental plants as invasive aliens: problems and solutions in Kruger National Park, South Africa. Environ Manage 41:32–51. https://doi.org/10.1007/s00267-007-9027-9

Frankham R (2015) Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Mol Ecol 24:2610–2618. https://doi.org/10.1111/mec.13139

Frankham R, Ballou JD, Eldridge MDB et al (2011) Predicting the probability of outbreeding depression. Conserv Biol 25:465–475. https://doi.org/10.1111/j.1523-1739.2011.01662.x

Hall-Martin AJ, Knight MH (1994) Conservation and management of black rhinoceros in South African national parks. In: Penzhorn BL, Kriek NPJ (eds) Proceedings of a symposium on rhinos as game ranch animals. Onderstepoort, South Africa., pp 11–19

Harper CK, Vermeulen GJ, Clarke AB et al (2013) Extraction of nuclear DNA from rhinoceros horn and characterization of DNA profiling systems for white (*Ceratotherium simum*) and black (*Diceros bicornis*) rhinoceros. Forensic Sci Int Genet 7:428–433. https://doi.org/10.1016/j.fsigen.2013.04.003

Heber S, Varsani A, Kuhn S et al (2013) The genetic rescue of two bottlenecked south island robin populations using translocations of inbred donors. Proc R Soc B Biol Sci 280:20122228. https://doi.org/10.1098/rspb.2012.2228

Hedrick P, Fredrickson R (2010) Genetic rescue guidelines with examples from mexican wolves and Florida panthers. Conserv Genet 11:615–626. https://doi.org/10.1007/s10592-009-9999-5

Johnson WE, Onorato DP, Roelke ME et al (2010) Genetic restoration of the Florida panther. Sci (80-) 329:1641–1645. https://doi.org/10.1126/science.1192891

Karsten M, Van Vuuren BJ, Goodman P, Barnaud A (2011) The history and management of black rhino in KwaZulu-Natal: a population genetic approach to assess the past and guide the future. Anim Conserv 14:363–370. https://doi.org/10.1111/j.1469-1795.2011.00443.x

Keller LF, Biebach I, Ewing SR, Hoeck PEA (2012) The Genetics of Reintroductions: Inbreeding and Genetic Drift. In: Ewen JG, Armstrong DP, Parker KA, Seddon PJ (eds) Reintroduction Biology. pp 360–394

Knight M (2017) African Rhino specialist Group report. Pachyderm 58:17–35

Kotzé A, Dalton DL, du Toit R et al (2014) Genetic structure of the black rhinoceros (*Diceros bicornis*) in south-eastern Africa. Conserv Genet 15:1479–1489. https://doi.org/10.1007/s10592-014-0632-x

Kumar S, Stecher G, Li M et al (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Lacy RC (1987) Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. Conserv Biol 1:143–158. https://doi.org/10.1111/j.1523-1739.1987.tb00023.x

Larkin MA, Blackshields G, Brown NP et al (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948. https://doi.org/10.1093/bioinformatics/btm404

le Roex N, Paxton M, Adendorff J et al (2018) Starting small: long-term consequences in a managed large-mammal population. J Zool 306:95–100. https://doi.org/10.1111/jzo.12572

Leigh JW, Bryant D (2015) popart: full-feature software for haplotype network construction. Methods Ecol Evol 6:1110–1116. https://doi.org/10.1111/2041-210X.12410

Madsen T, Shine R, Olsson M, Wittzell H (1999) Restoration of an inbred adder population. Nature 402:34–35. https://doi.org/10.1038/46941

Moodley Y, Russo IRM, Dalton DL et al (2017) Extinctions, genetic erosion and conservation options for the black rhinoceros (*Diceros bicornis*). Sci Rep 7:1–16. https://doi.org/10.1038/srep41417

Moro D, Campbell JH, Elphinstone NS, Baverstock MR P (1998) The Thevenard Island mouse: historic and conservation implications from mitochondrial DNA sequence-variation. Pac Conserv Biol 4:282–288

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update. Bioinformatics 28:2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Peppin L, McEwing R, Ogden R et al (2010) Molecular sexing of african rhinoceros. Conserv Genet 11:1181–1184. https://doi.org/10.1007/s10592-009-9912-2

Pew J, Muir PH, Wang J, Frasier TR (2015) Related: an R package for analysing pairwise relatedness from codominant molecular markers. Mol Ecol Resour 15:557–561. https://doi.org/10.1111/1755-0998.12323

Poirier MA, Coltman DW, Pelletier F et al (2019) Genetic decline, restoration and rescue of an isolated ungulate population. Evol Appl 12:1318–1328. https://doi.org/10.1111/eva.12706

Primack RB (2002) Conservation Biology, 3rd edn. Sinauer Associates, Inc, Sunderland, Massachusetts U.S.A.

R Core Team (2019) R: a language and environment for statistical computing. R Foundation for Statistical Computing

Ralls K, Ballou JD, Dudash MR et al (2018) Call for a paradigm shift in the genetic management of fragmented populations. Conserv Lett 11:1–6. https://doi.org/10.1111/conl.12412

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. J Hered 86:248–249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Rozas J, Ferrer-Mata A, Sanchez-DelBarrio JC et al (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol 34:3299–3302. https://doi.org/10.1093/molbev/msx248

SANParks (2002) Strategic plan for the conservation and management of rhinoceros (*Diceros bicornis* and *Ceratotherium simum*) within South. African National Parks, pp 2003–2013

Tallmon DA, Luikart G, Waples RS (2004) The alluring simplicity and complex reality of genetic rescue. Trends Ecol Evol 19:489–496. https://doi.org/10.1016/j.tree.2004.07.003

Templeton AR (1986) Coadaptation and outbreeding depression. In: Soulé ME (ed) Conservation biology: the science of scarcity and diversity. Sinauer Associates Inc., Sunderland, Massachusetts, pp 105–116

Thavornkanlapachai R, Mills HR, Ottewell K et al (2019) Mixing genetically and morphologically distinct populations in translocations: asymmetrical introgression in a newly established population of the boodie (*Bettongia lesueur*). Genes (Basel) 10:1–18. https://doi.org/10.3390/genes10090729

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Wang J (2002) An estimator for pairwise relatedness using molecular markers. Genetics 160:1203–1215

Weeks AR, Heinze D, Perrin L et al (2017) Genetic rescue increases fitness and aids rapid recovery of an endangered marsupial population. Nat Commun 8:1071. https://doi.org/10.1038/s41467-017-01182-3

Weeks AR, Sgro CM, Young AG et al (2011) Assessing the benefits and risks of translocations in changing environments: a genetic perspective. 4:709–725. https://doi.org/10.1111/j.1752-4571.2011.00192.x

World Wildlife Fund (WWF-South Africa) (2020) Black Rhino Range Expansion. https://www.wwf.org.za/our_work/initiatives/black_rhino_expansion/

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