Animal Conservation

Animal Conservation. Print ISSN 1367-9430

The history and management of black rhino in KwaZulu-Natal: a population genetic approach to assess the past and guide the future

M. Karsten[1], B. Jansen van Vuuren[1,2], P. Goodman[3] & A. Barnaud[1,2]

1 Evolutionary Genomics Group, Department of Botany and Zoology, University of Stellenbosch, Stellenbosch, South Africa
2 Centre for Invasion Biology, University of Stellenbosch, Stellenbosch, South Africa
3 Ezemvelo KZN Wildlife, Cascades, South Africa

Keywords
rhinoceros; microsatellite; translocation; management; inbreeding; *Diceros bicornis*.

Correspondence
Bettine Jansen van Vuuren, Department of Botany and Zoology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa. Tel: +27 21 808 4862; Fax: +27 21 808 2405 Email: bjvv@sun.ac.za

Received 30 June 2010; accepted 3 January 2011

doi:10.1111/j.1469-1795.2011.00443.x

Abstract

The numbers of black rhino *Diceros bicornis* in Africa declined dramatically during the last century due primarily to poaching and latterly habitat transformation and fragmentation and as such, significant concerns exist with regard to the long-term population viability and the management of these fragmented populations. A considerable proportion of the remaining black rhino (ssp. *minor*) are found within South Africa where they largely fall under the protection and management of Ezemvelo KwaZulu-Natal (KZN) Wildlife. Here we provide information on the genetic diversity, population differentiation and level of inbreeding among 77 *Diceros bicornis minor* individuals sampled in seven protected areas within the KZN Province of South Africa and a single population from Zimbabwe founded from the KZN population. For reference purposes with the cluster analyses, we included four individuals from ssp. *bicornis* and four individuals from ssp. *michaeli*. We found low levels of differentiation among ssp. *minor* populations across the KZN Province; this result is not unexpected given the history of establishments and translocations between reserves. In fact, we argue that the translocations conducted by Ezemvelo KZN Wildlife have contributed to the acceptable levels of heterozygosity and minimal inbreeding which characterize the majority of protected areas in the province. Although the overall genetic diversity in *D. b. minor* is lower than that present in both *Diceros bicornis bicornis* and *Diceros bicornis michaeli*, we do not feel that it is any cause for concern at this stage as it still falls within the range reported for other large mammals across Africa. The information presented here forms the basis of an ongoing monitoring programme aimed at providing vital information which, when taken with ecological and other data, will direct the future management decisions regarding translocations between reserves in South Africa and the exchange of individuals with other countries.

Introduction

Biodiversity and the conservation of species and natural processes are at a critical juncture. Unprecedented levels of extinction face many animal and plant groups mostly as a result of anthropogenic actions, climate change and/or invasive alien species (Dukes & Mooney, 1999; De Salle & Amato, 2004; Heller & Zavaleta, 2009; see also http://www.maweb.org/). As such, the long-term survival of many species is closely tied to areas of protected natural habitat (Shaffer, 1981; Heller & Zavaleta, 2009). However, increased competition for available land means that very few of the world's protected natural areas are of adequate size to sustain long-term viable populations for many of the species occurring within them (Ceballos *et al*., 2005; Venter *et al*., 2008). In terms of reserve design, the 'single large or several small' debate has therefore become somewhat obsolete, and a more critical question is how to optimally manage populations spread across several smaller protected areas to ensure long-term survival, minimize inbreeding and maximize the retention of genetic diversity.

A case in hand concerns the black rhino *Diceros bicornis*, where population numbers have declined dramatically (Gakahu, 1993; http://www.iucn.org; http://www.cites.org) mostly as a result of anthropogenic factors including habitat

destruction, fragmentation and poaching (Cunningham, Harley & O'Ryan, 1999). In addition, factors associated with K-selected species such as a relatively low reproductive rate and high mortality of calves (Skinner & Chimimba, 2005) have contributed to the slow recovery of the species. It is perhaps not surprising that several of the recognized subspecies (Groves, 1967) are thought to be extinct or close to extinction. Following an African Rhino Workshop in Cincinnati in 1986 (Du Toit, Foose & Cumming, 1987), it was agreed that conservation efforts should focus on four ecological groups which broadly corresponds to the remaining subspecies namely south-central (*Diceros bicornis minor*), south-western (*Diceros bicornis bicornis*), eastern (*Diceros bicornis michaeli*) and north-western (*Diceros bicornis longipes*) groups. Subsequent to this meeting, *D. b. longipes* was declared tentatively extinct in July 2006 (IUCN, 2009). Approximately 2000 of the remaining 4000 black rhino individuals in Africa belong to *D. b. minor*, the majority of which are found in South Africa. Most of the current South African *D. b. minor* individuals, which are distributed across 16 state- and 25 private-protected areas in five of South Africa's nine provinces, are descendants from only two populations that survived to the middle of the previous century namely those of the Hluhluwe-iMfolozi Park and Mkhuze Game Reserve in KwaZulu-Natal (KZN) (a few individuals from Zimbabwe were translocated to the Kruger National Park). It has been estimated that the combined size of these two founder populations comprised no more than 110 individuals at their lowest point thought to have been during the 1930s (see Emslie & Brooks, 1999).

To minimize the loss of genetic heterozygosity in the remaining populations, the recovery strategy for the South African black rhino aimed to increase the population size(s) as rapidly as possible (Emslie & Brooks, 1999). To achieve this, the founder populations in the Hluhluwe-iMfolozi Park and Mkhuze Game Reserve were live harvested and these live removals translocated to establish additional populations in suitable habitat across South Africa (Emslie & Brooks, 1999; see Fig. 1). Animals have been routinely translocated among these populations, with no population acting as a sink *per se*, but rather feeding into a meta-population framework. Although several studies using allozymes, mitochondrial sequence data and/or microsatellite markers have documented variable levels of genetic diversity among ecological groups and selected populations within these groups (Ashley, Melnick & Western, 1990; O'Ryan, Flamand & Harley, 1994; Swart & Ferguson, 1997; Brown & Houlden, 1999, 2000; Harley *et al.*, 2005), no study to date has directly assessed the genetic impacts of management actions on South African black rhino populations. Here we use microsatellite markers to survey genetic diversity and the pattern of genetic differentiation for different populations of *D. b. minor* in KZN and place our findings within the context of a comprehensive management plan for this ecological type. We provide data regarding levels of inbreeding within populations, and use our findings to direct translocation efforts.

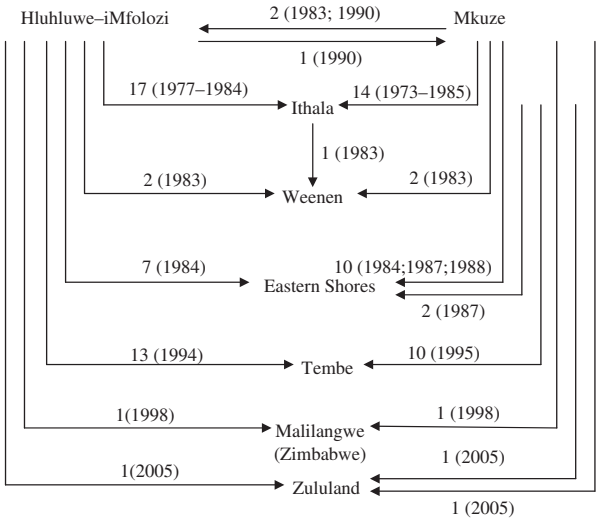

Figure 1 Translocation history of *Diceros bicornis minor* in KwaZulu-Natal from 1973 onwards, including individuals translocated to Malilangwe, Zimbabwe. The number of translocation events as well as dates for translocations (in parentheses) is indicated. (Adapted from Emslie & Brooks, 1999; Skinner & Chimimba, 2005; J. Craigie, pers. comm.).

Methods

Samples

Samples (skin biopsies stored in a saturated salt solution supplemented with 15% dimethyl sulfoxide) were collected by Ezemvelo KZN Wildlife from 60 black rhino individuals from seven state- and one private-protected area in KZN (South Africa) and three individuals from a privately protected area in Zimbabwe. Sampling locations from KZN include the Weenen Nature Reserve, Hluhluwe-iMfolozi Park, Cape Vidal State Forest, Zululand Rhino Reserve, Ithala Game Reserve, Mkhuze Game Reserve and Tembe Elephant Park (see Table 1 and Fig. 2). A Biological Resource Bank, sponsored by the Department of Science and Technology, National Government of South Africa and housed at the Pretoria Zoological Gardens, was established with the aim to acquire, process, bank and ultimately provide biomaterials for scientific and conservation research (Bartels & Kotze, 2006). Seventeen black rhino fibroblast cultures, available to us as part of this national facility, were included in the present study. These samples were from the Hluhluwe-iMfolozi Park ($n = 7$) and the Mtubatuba holding station ($n = 10$). The latter is not a protected area but rather a holding station, and samples that had Mtubatuba as their recorded location represent samples taken from a number of protected areas containing black rhino in the province. In the light of our results (no significant structure among protected areas, see below), these samples were included only in the combined analyses and were not considered as a separate population. A total of 77 *D. b. minor* individuals

Table 1 The populations, sample size (N), expected (H_E) and observed (H_O) heterozygosity, inbreeding coefficient (F_{IS}) and allelic richness (A_r) of each ecological type (subspecies) of *Diceros bicornis* included in the present study

Subspecies	Population	N	H_E	H_O	F_{IS}	A_r[a]
South-western (*Diceros bicornis bicornis*)	Etosha National Park, Augrabies Falls National Park, Karoo National Park	4	0.43 ± 0.20	0.46 ± 0.35	0.09	
Eastern (*Diceros bicornis michaeli*)	Hluhluwe-iMfolozi Park, Ngorongoro Crater, Addo Elephant Park	4	0.54 ± 0.25	0.54 ± 0.31	0.16	
South-central (*Diceros bicornis minor*)	All populations	77	0.44 ± 0.19	0.38 ± 0.19	0.14	
	Cape Vidal State Forest	2	0.31 ± 0.28	0.40 ± 0.46	0.06	2
	Hluhluwe-iMfolozi Park	32	0.43 ± 0.17	0.36 ± 0.19	0.19*	1.87
	Ithala Game Reserve	6	0.37 ± 0.20	0.41 ± 0.24	0	1.78
	Malilangwe (Zimbabwe)	3	0.29 ± 0.27	0.32 ± 0.37	0.17	1.8
	Mkhuze Game Reserve	15	0.43 ± 0.19	0.40 ± 0.21	0.11	1.9
	Mtubatuba (holding station)	10	–	–	–	–
	Tembe Elephant Park	2	0.23 ± 0.19	0.30 ± 0.26	0	1.6
	Weenen Nature Reserve	4	0.31 ± 0.26	0.40 ± 0.36	−0.13	1.74
	Zululand Rhino Reserve	3	0.33 ± 0.21	0.47 ± 0.32	−0.22*	1.79

[a]Calculation based on a minimum sample size of two diploid individuals.
*Significant ($P \leq 0.05$).

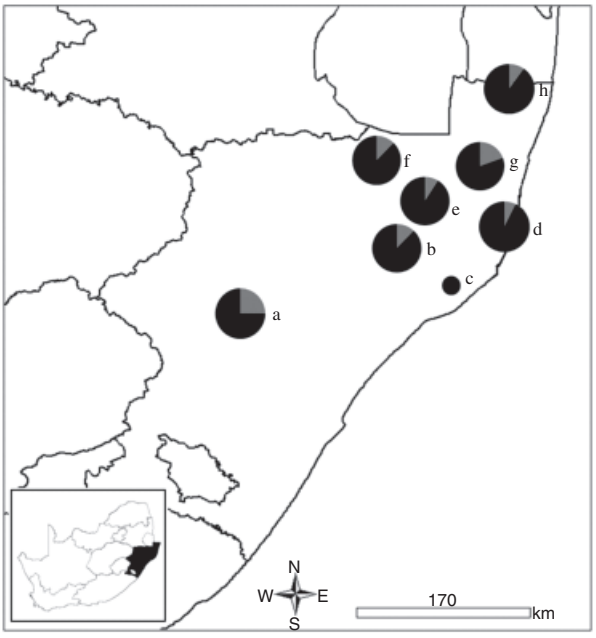

Figure 2 Geographic locations of protected areas in KwaZulu-Natal, South Africa where *Diceros bicornis minor* were sampled: (a) Weenen Nature Reserve, (b) Hluhluwe-iMfolozi Park, (c) Mtubatuba (information on these individuals are unavailable), (d) Cape Vidal State Forest, (e) Zululand Rhino Reserve, (f) Mkhuze Game Reserve, (g) Ithala Game Reserve, (h) Tembe Elephant Park. The size of the pie reflects the relative size of the rhino population maintained in each protected area. The proportion of black rhino individuals sampled (gray) are indicated in relation to the total number of individuals within each reserve (black).

were analyzed, and for reference purposes we also included four *D. b. bicornis* individuals and four *D. b. michaeli* individuals (Table 1).

DNA extraction and microsatellite loci amplification

Cell cultures were placed in 25 cm^3 tissue culture flasks containing 4 mL of tissue culture medium (15% fetal calf serum in Dulbecco's modified Eagle medium). Cultures were grown in an incubator set at 37 °C and 5% carbon dioxide (ppm). After flasks reached confluence, cells were harvested for DNA extraction. DNA was extracted from tissue biopsies and harvested fibroblast cultures using a Wizard® SV Genomic DNA purification system (Promega, Madison, WI, USA) and the DNA was subsequently purified using a Wizard® SV-Gel and PCR clean-up system (Promega). The black rhino samples were analyzed using 10 polymorphic microsatellite markers (SSR loci, Table 2). The forward primer of each pair of microsatellites was 5′-labelled with one of four fluorophores (6-FAM, HEX, VIC and NED). Microsatellite loci with the same fluorophore and with no signal inhibition of the polymerase chain reaction (PCR) products were pooled for amplification. A multiplex PCR kit (Qiagen Inc., Hilden, Germany) was used with a final reaction volume of 10 μL containing 6 μL of 2 × Qiagen multiplex master mix, 1 μL of primer mix (2 mM), 1 μL water and 2 μL of template DNA (~30 ng). PCR amplification included an initial denaturation of 15 min at 95 °C followed by 35 cycles of denaturation (30 s at 94 °C), annealing (90 s at primer-specific temperatures; see Table 2) and extension (50 s at 72 °C). A final extension step of 10 min at 60 °C completed reactions. PCR products [1 μL diluted (1/80)] were combined with 15 μL of deionized formamide and 0.2 μL of the GS500LIZ size standard (Applied Biosystems, Foster City, CA, USA). Samples were genotyped on an ABI 3130 Prism (Applied Biosystems) using ABI Prism GENEMAPPER software 3.7 (Applied Biosystems). A negative control was used to check the quality of the PCR products and ultimately the genotypes. Loci were tested for genotypic linkage disequilibrium (GENEPOP'007, Raymond & Rousset, 1995; Rousset, 2008).

Table 2 Summary of the microsatellite markers used in the analyses of *Diceros bicornis minor*

Locus name	Repeat motif	Multiplex	Label	Temperature (°C)	Size range (bp)	N_A	H_O	H_E
DB1[a]	(CA)14	1	6FAM	58	121–130	5	0.50	0.52
DB14[a]	(CA)13	2	VIC	58	272–288	4	0.03	0.08
DB49[a]	(CA)14	1	6FAM	58	152–162	3	0.55	0.53
DB66[a]	(CA)7TA(CA)16	2	6FAM	58	187–222	6	0.65	0.66
BR4F[b]	(CA)19	3	VIC	52	100–146	5	0.50	0.65
BR6F[b]	(CA)15	3	PET	52	126–158	4	0.20	0.28
BR17F[b]	(AT)6(GT)18	1	NED	58	115–135	3	0.20	0.28
AY606078c[c]	(GT)13GCA(TG)3	3	6FAM	52	235–257	4	0.30	0.37
AY606080c[c]	(CA)14GA(CA)4	3	VIC	52	234–253	3	0.33	0.55
AY606083[c]	(TG)6(AG)11GA(AG)5	3	NED	52	190–253	2	0.23	0.23

The locus name, repeat motif, multiplex composition, annealing temperature as well as allele size range is given. We also present the number of alleles (N_A), expected (H_E) and observed (H_O) heterozygosity per locus.
[a]Brown & Houlden (1999).
[b]Cunningham *et al.* (1999).
[c]Nielsen *et al.* (2008).

Genetic analyses

To assess levels of genetic diversity, basic statistics were computed for the 10 SSR loci. Number of alleles (GENALEX 6, Peakall & Smouse, 2006) as well as observed and expected heterozygosities (GENETIX, Belkhir *et al.*, 1996–2004) were calculated for each SSR locus and for each individual as well as each reserve. Allelic richness was calculated using FSTAT 2.9.3.2 (Goudet, 2001). F_{IS}, the coefficient of inbreeding, measured as the proportion of the variance in a population that is contained in an individual, was calculated using GENETIX (Belkhir *et al.*, 1996–2004). F_{IS} values that approach 0 indicate random mating in the population, whereas positive F_{IS} values indicate an excess of homozygotes (potentially as a result of the mating of related individuals in the absence of genetic structure) and negative F_{IS} values indicate an excess of heterozygotes (potentially indicating outbreeding) (see Braude & Templeton, 2009 for further discussion on inbreeding). Permutation procedures (10 000 permutations) were performed to test for deviations from the null hypothesis of no inbreeding/outbreeding ($F_{IS} = 0$).

To assess population structure we used two complementary approaches namely a neighbor-joining analysis and a Bayesian model-based clustering method. A dissimilarity matrix (Simple Matching) was first calculated in DARWIN 5.0.158 (Perrier & Jacquemoud-Collet, 2006). We then constructed a weighted neighbor-joining tree using 10 000 bootstraps. As suggested for inferring population substructure at low levels of population differentiation (Latch *et al.*, 2006), we also used a Bayesian clustering method implemented in STRUCTURE (Pritchard, Stephens & Donnelly, 2000; Falush, Stephens & Pritchard, 2003). For this, we selected uncorrelated allele frequencies for the combined dataset including the three ecological groups as we do not expect gene flow between these, and correlated allele frequencies when considering only *D. b. minor* as gene flow occurs among populations. We assumed that the number of populations (K) varied between one and 10 with 10 independent runs per K-value. We used a burn-in period length of 2×10^6 followed by 2×10^6 MCMC steps, which allowed stability to be reached for statistical parameters and gave consistent results across runs. First, we performed our analysis using the entire sample including all ecological types. Secondly, we performed the analysis considering only ssp. *michaeli* and ssp. *bicornis*. Thirdly, we performed our analysis only within ssp. *minor*, considering the two founding populations namely Hluhluwe-iMfolozi Park and Mkhuze Game Reserve. Lastly, we considered all the populations within ssp. *minor*. We used the method described in Evanno, Regnaut & Goudet (2005) to determine the number of true clusters (K) in our sample which uses the second-order rate of change in the log probability to calculate the number of clusters.

Results

Genetic diversity

All loci included here were polymorphic, with the number of alleles ranging from two (AY606083) to six (DB66). No linkage disequilibrium was detected among the 10 loci. In total, 39 alleles were detected in *D. b. minor* across the 10 loci (Table 2), 36 alleles in *D. b. michaeli* and 25 alleles in *D. b. bicornis*. Observed heterozygosity (H_O) ranged from 0.03 (DB14) to 0.65 (DB66) and expected heterozygosity (H_E) ranged between 0.08 (DB14) and 0.66 (DB66).

Genetic diversity as indicated by mean H_E was 0.54 for *D. b. michaeli*, 0.43 for *D. b. bicornis* and 0.44 for *D. b. minor* (see Table 1 and supporting information Table S1). For *D. b. minor*, genetic diversity (H_E) within each reserve ranged from 0.23 (Tembe Elephant Park) to 0.43 (Hluhluwe-iMfolozi and Mkhuze) (Table 1). Allelic richness ranged from 1.60 (Tembe Elephant Park) to 2.00 (Cape Vidal). F_{IS} ranged from −0.22 (Zululand Rhino Reserve) to 0.19 (Hluhluwe-iMfolozi). These values should be taken with caution as most of the reserves are represented by less than 10 samples. H_E were also calculated for individuals, and are

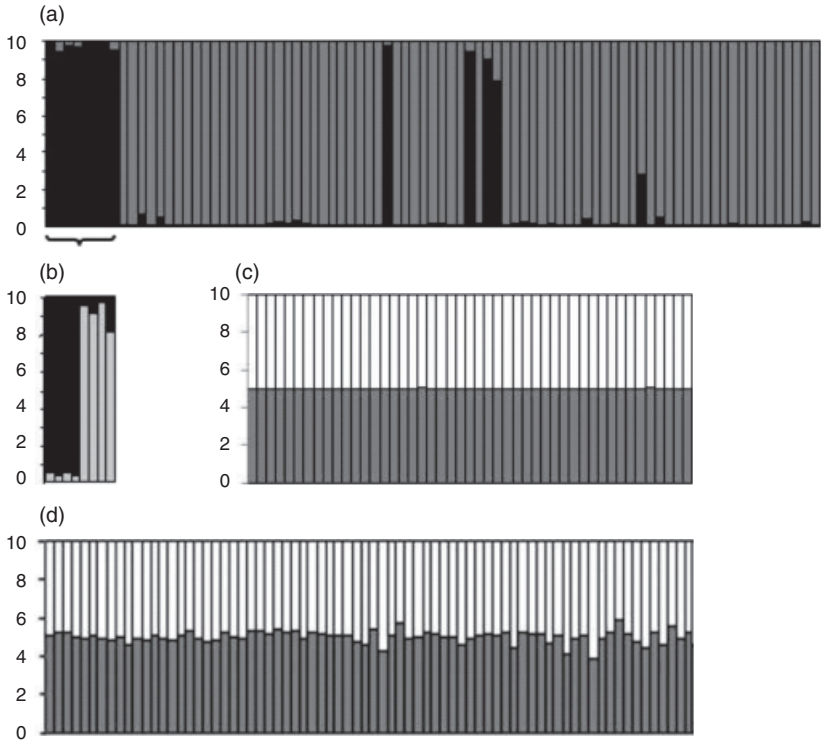

Figure 3 Hierarchical cluster analysis of 85 black rhino individuals using the Bayesian model-based algorithm implemented in the program STRUCTURE (Pritchard *et al.*, 2000). Each individual is represented by a vertical line, which is partitioned into segments that represent the individual's estimated membership fractions in the *K* clusters. (a) For $K=2$, the clusters identified correspond well to subspecies (*Diceros bicornis bicornis* and *Diceros bicornis michaeli* group in the black cluster and *Diceros bicornis minor* in the gray cluster); (b) the *D. b. bicornis* and *D. b. michaeli* cluster can be further subdivided in two clusters distinguishing the two ecological types; (c) no genetic structure can be found among the two founding populations (Hluhluwe-iMfolozi and Mkhuze); (d) no genetic structure can be found among all reserves when only *D. b. minor* individuals are included.

presented in supporting information Table S2. As a comparison between reserves, these values were plotted and are presented in supporting information Fig. S2.

Pattern of genetic structure

The Bayesian clustering approach revealed strong structure at the species level when all samples were included (see Fig. 3a). According to Evanno's method, $K = 2$ is the most likely number of clusters. For $K = 2$, 94% of *D. b. minor* individuals are grouped in cluster 1 with an average membership of 99%. Hundred per cent of *D. b. michaeli* and *D. b. bicornis* individuals grouped in cluster 2 with an average membership of 98%. When the analysis is repeated within the cluster formed by the subspecies *D. b. michaeli* and *D. b. bicornis*, $K = 2$ is the most likely number of clusters separating the *D. b. michaeli* individuals (average membership of 96%) from the *D. b. bicornis* individuals (average membership of 91%) (see Fig. 3b). When only *D. b. minor* individuals were considered, no structure was detected among reserves including when only the two founding populations were included (see Fig. 3c and d). Evanno's method cannot calculate a ΔK value for $K = 1$, as ΔK is a measure of the rate of change. We therefore examined plots of the log posterior probability of the data [ln $P(D)$] for each K. Ln $P(D)$ was maximized for $K = 1$ as is expected when there is no detectable structure. Furthermore, α plots remained unstable across all values of K and the cluster membership for different individuals was equally admixed (Pritchard *et al.*, 2000). The weighted neighbor-joining tree confirmed the weak genetic structure within *D. b. minor* (see supporting information Fig. S1).

Discussion

After the drastic decline in the number of black rhinos, intensive conservation efforts were employed to conserve the species. Arguably the most significant area for the conservation of black rhino (ssp. *minor*) is the KZN Province in South Africa, and in this respect the current role of Ezemvelo KZN Wildlife is crucial. To elucidate the patterns of genetic diversity among state- (and private-) protected areas, and to provide an indication of genetic fitness (including heterozygosity and inbreeding) on these protected areas (see Reed & Frankham, 2003 for a discussion on genetic parameters and conservation), we embarked on a genetic survey of *D. b. minor* rhinos housed on reserves and game farms in the province. Our aim was to provide

information that would feed into the management of this rare species and that would aid decisions regarding translocations among reserves.

Our results indicated clear albeit small differences among the three ecological types included in the present study (see Fig. 3a and b). When only the *D. b. minor* individuals were included, no genetic structure was found among populations (see Fig. 3d) (see also e.g. Ashley *et al.*, 1990; O'Ryan *et al.*, 1994; Swart & Ferguson, 1997 who similarly reported no genetic structure among South African *D. b. minor* populations). Although not unexpected given the history and management strategy for the subspecies in South Africa (all *D. b. minor* individuals in South Africa are descendants from two founder populations which are genetically very similar and experienced a drastic bottleneck in population sizes; see 'Introduction' for more detail and Fig. 3c for STRUCTURE comparison between founding populations), these results hold important implications for their management. The lack of significant structure across populations means that individuals can freely be moved among reserves and protected areas, and that new populations can be established with excess individuals from a mixture of protected areas (i.e. no sink populations *sensu stricto* exists for black rhino in South Africa). This situation is in sharp contrast to a pattern of significant structure among protected areas (possibly as a result of genetic differences among founding populations) resulting in a lower effective population size where the mixing of individuals from different genetic groups could potentially lead to problems with outbreeding depression and the disruption of locally adapted gene complexes (see Braude & Templeton, 2009).

Notwithstanding that *D. b. minor* today has the highest population size of the remaining black rhino subspecies, it is characterized by the lowest levels of heterozygosity when compared with the other black rhino subspecies. Within our study, the average H_E for *D. b. minor* was estimated at 0.44 compared with 0.51 reported for *D. b. bicornis* (Harley *et al.*, 2005) and 0.68 for *D. b. michaeli* (see also Brown & Houlden, 1999) (in our study, H_E was estimated at 0.43 for *D. b. bicornis* and 0.54 for *D. b. michaeli* based on a sample size of four individuals per subspecies). Although the lower heterozygosity for *D. b. minor* may reflect a sampling bias in our study, we do not believe this to be the case as our finding are in line with previous studies which similarly documented lower diversity within *D. b. minor* ($H_E = 0.46$, Harley *et al.*, 2005; $H_E = 0.37$, Nielsen *et al.*, 2008). Rather, these lower diversity values may reflect past demography and a small founding population (both in terms of number of individuals as well as the geographic area from which samples were taken) where all extant South African *D. b. minor* individuals are descendants from populations on only two reserves namely Hluhluwe-iMfolozi Park and Mkhuze Game Reserve. In addition, the other two subspecies (*D. b. bicornis* and *D. b. michaeli*) historically both occupied larger ranges and the higher levels of diversity within them may simply reflect historical genetic patterns. We do not feel that the genetic diversity with *D. b. minor* is any cause for concern as it is in line (and even higher) than values reported for other large mammal species across their distributions (see supporting information Table S1).

Conservation strategies in black rhino are aimed to increase population sizes to prevent the potential catastrophic effects of stochastic events. Black rhino (ssp. *minor*) were distributed across several protected areas in KZN and indeed South Africa to further minimize the risks associated with local populations going extinct because of external factors. However, these populations are managed in a meta-population framework with individuals exchanged between reserves when needed. New populations are established with excess animals when carrying capacity is reached on established protected areas. Our findings indicate that the use of translocations has helped the ssp. *minor* to retain acceptable amounts of genetic diversity overall as well as on each of the protected areas.

Although the majority of populations show no signs of inbreeding (notably Cape Vidal State Forest, Ithala Game Reserve and Tembe Elephant Park) or even inbreeding avoidance as result of the translocation policy (as measured by excess heterozygotes; Braude & Templeton, 2009) (Weenen Nature Reserve and Zululand Rhino Reserve), few display F_{IS} values that should be viewed with concern (such as Hluhluwe-iMfolozi Park; $F_{IS} = 0.19$, $P \leq 0.05$). It should be noted that our sample sizes for many of the protected areas are low and inbreeding coefficient values should therefore be viewed with caution. However, if the precautionary principle is applied, management on reserves with moderate to high F_{IS} values could consider bringing in stock that are unrelated which will result in inbreeding values being returned to 0 (random) in a single generation.

Population genetic data such as those presented in this study have conservation relevance and are helpful in management decision-making. It has been well established that reduced genetic diversity and inbreeding has significant negative effects on the continued survival of species and in fact, may even decrease the time to extinction under variable environmental conditions (Frankham, 1995; Frankham *et al.*, 1999; Frankham, Ballou & Briscoe, 2003). Black rhino populations under the management of Ezemvelo KZN Wildlife are doing well as measured by genetic diversity (H_E) and inbreeding (F_{IS}), and the health of these populations results from proper management. Future management recommendations would involve the continuation of a translocation policy which is advised by genetic data, where sample sizes for some of the protected areas are increased to obtain more robust results.

Acknowledgments

This work was carried out under export permit numbers 164/2009, 252/2010, 005460 and 020905. Tissue culture samples were kindly provided by BioBankSA. We thank John Craigie from Ezemvelo KZN Wildlife for assistance with the *D. b. minor* samples, information and field assistance, and Adriaan Engelbrecht for assistance with cell cultures. Genotyping was performed by the Central

Analytical Facility at Stellenbosch University. We would also like to thank Dr Trent Garner and two anonymous reviewers that provided constructive criticisms. This study was funded by WWF (Black Rhino Range Expansion Programme), a National Research Foundation grant to B.J.v.V. (NRF UID 65735) and the South African National Biodiversity Institute's Threatened Species Programme.

References

Ashley, M.V., Melnick, D.J. & Western, D. (1990). Conservation genetics of the black rhinoceros (*Diceros bicornis)*, I: evidence from the mitochondrial DNA from three populations. *Conserv. Biol.* **4**, 71–77.

Bartels, P. & Kotze, A. (2006). Wildlife biomaterial banking in Africa for now and the future. *J. Environ. Monit.* **8**, 779–781.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N. & Bonhomme, F. (1996–2004). *GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations*. Montpellier: Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II.

Braude, S. & Templeton, A.R. (2009). Understanding the multiple meanings of 'inbreeding' and 'effective size' for genetic management of African rhinoceros populations. *Afr. J. Ecol.* **47**, 546–555.

Brown, S.M. & Houlden, B.A. (1999). Isolation and characterization of microsatellite markers in the black rhinoceros (*Diceros bicornis*). *Mol. Ecol.* **8**, 1559–1561.

Brown, S.M. & Houlden, B.A. (2000). Conservation genetics of the black rhinoceros (*Diceros bicornis*). *Conserv. Genet.* **1**, 365–370.

Ceballos, G., Ehrlich, P.R., Soberón, J., Salazar, I. & Fay, J.P. (2005). Global mammal conservation: what must we manage? *Science* **309**, 603–606.

Cunningham, J., Harley, E.H. & O'Ryan, C. (1999). Isolation and characterization of microsatellite loci in black rhinoceros (*Diceros bicornis*). *Electrophoresis* **20**, 1778–1780.

DeSalle, R. & Amato, G. (2004). The expansion of conservation genetics. *Nature* **5**, 702–712.

Dukes, J.S. & Mooney, H.A. (1999). Does global change increase the success of biological invaders? *TREE* **14**, 135–139.

Du Toit, R.F., Foose, T.J. & Cumming, D.H.M. (1987). Proceedings of African rhino workshop, Cincinnati, October 1986. *Pachyderm* **9**, 1–33.

Emslie, R. & Brooks, M. (1999). *African rhino. Status Survey and Conservation Action Plan*. Gland/Cambridge: IUCN/SSC African Rhino Specialist Group.

Evanno, G., Regnaut, S. & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**, 2611–2620.

Falush, D., Stephens, M. & Pritchard, K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* **164**, 1567–1587.

Frankham, R. (1995). Inbreeding and extinction: a threshold effect. *Conserv. Biol.* **9**, 792–799.

Frankham, R., Ballou, J.D. & Briscoe, D.A. (2003). *Introduction to conservation genetics*. Cambridge: Cambridge University Press.

Frankham, R., Lees, K., Montgomery, M.E., England, P.R., Lowe, E.H. & Briscoe, D.A. (1999). Do population size bottlenecks reduce evolutionary potential? *Anim. Conserv.* **2**, 255–260.

Gakahu, C.G. (1993). African rhinos: current numbers and distribution. In *Proceedings of an international conference: rhinoceros biology and conservation*: 160–165. Ryder, O.A. (Ed.). San Diego: Zoological Society.

Goudet, J. (2001). FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). Available at http://www.unil.ch/popgen (accessed November 2009).

Groves, C.P. (1967). Geographic variation in the black rhinoceros. *Z. Saugetierkd.* **32**, 267–276.

Harley, E.H., Baumgarten, I., Cunningham, J. & O'Ryan, C. (2005). Genetic variation and population structure in remnant populations of black rhinoceros, *Diceros bicornis*, in Africa. *Mol. Ecol.* **14**, 2981–2990.

Heller, N.E. & Zavaleta, E.S. (2009). Biodiversity management in the face of climate change: a review of 22 years of recommendations. *Biol. Conserv.* **142**, 14–32.

IUCN (World Conservation Union). (2009). Red List of Threatened Species. Version 2009.1. Available at http://www.iucnredlist.org (accessed November 2009).

Latch, E.K., Dharmarajan, G., Glaubitz, J.C. & Rhodes, O.E. (2006). Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conserv. Genet.* **7**, 295–302.

Nielsen, L., Meehan-Meola, D., Kilbourn, A. & Alcivar-Warren, A. (2008). Characterization of microsatellite loci in the black rhinoceros (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*): their use for cross-species amplification and differentiation between the two species. *Conserv. Genet.* **9**, 239–242.

O'Ryan, C., Flamand, J.R.B. & Harley, E.H. (1994). Mitochondrial DNA variation in black rhinoceros (*Diceros bicornis*): conservation management implications. *Conserv. Biol.* **8**, 495–500.

Peakall, R. & Smouse, P.E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**, 288–295.

Perrier, X. & Jacquemoud-Collet, J.P. (2006). DARwin software. Available at http://darwin.cirad.fr/darwin (accessed November 2009).

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Raymond, M. & Rousset, F. (1995). An exact test for population differentiation. *Evolution* **49**, 1280–1283.

Reed, D.H. & Frankham, R. (2003). Correlation between fitness and genetic diversity. *Conserv. Biol.* **17**, 230–237.
Rousset, F. (2008). Genepop'007: a complete re-implementation of the Genepop software for Windows and Linux. *Mol. Ecol. Res.* **8**, 103–106.
Shaffer, M.L. (1981). Minimum population sizes for species conservation. *BioScience* **31**, 131–134.
Skinner, J.D. & Chimimba, C.T. (2005). *The mammals of the Southern African subregion*. 3rd edn. New York: Cambridge University Press.
Swart, M.K.J. & Ferguson, J.W.H. (1997). Conservation implications of genetic differentiation in southern Africa populations of black rhinoceros (*Diceros bicornis*). *Conserv. Biol.* **11**, 79–83.
Venter, F.J., Naiman, R.J., Biggs, H.C. & Pienaar, D.J. (2008). The evolution of conservation management philosophy: science, environmental change and social adjustments in Kruger National Park. *Ecosystems* **11**, 173–192.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Weighted neighbor-joining tree based on 10 SSRs, using the simple matching index. Ten thousand bootstraps were performed. Rhino individuals are identified by the name of their reserve except for ssp. *bicornis* and ssp. *michaeli*.

Figure S2. Graphic representation of expected heterozygosities (H_E) per individual per protected area. The scale on the X and Y axis are similar for comparisons. Weenen Nature Reserve (WNR), Ithala Game Reserve (IGR), Hluhluwe-iMfolozi Park (HMP), Zululand Rhino Reserve (ZRR), Mkhuze Game Reserve (MGR), Tembe Elephant Park (TEP), Cape Vidal State Forest (CVSF), Malilangwe (Zimbabwe) (MAL), Mtubatuba (M).

Table S1. Table listing the observed (H_O) and expected (H_E) heterozygosities for a number of larger African mammal species. Sample sizes as well as references are included.

Table S2. Table listing the expected heterozygosities (H_E) and standard error for each individual included in the present study. Weenen Nature Reserve (WNR), Ithala Game Reserve (IGR), Hluhluwe-iMfolozi Park (HMP), Zululand Rhino Reserve (ZRR), Mkhuze Game Reserve (MGR), Tembe Elephant Park (TEP), Cape Vidal State Forest (CVSF), Mkhuze Game Reserve (MGR), Mtubatuba (M).