Conservation Implications of Genetic Differentiation in Southern African Populations of Black Rhinoceros (*Diceros bicornis*)

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Abstract: *We analyzed 30 protein-coding loci of four southern African black rhinoceros populations in order to calculate fixation indices and genetic distances for the different populations. We concluded that one of these populations is of the subspecies* Diceros bicornis bicornis *and the other three of* Diceros bicornis minor. *No evidence of inbreeding within populations was found.* F- *statistics revealed significant differentiation between populations. Small genetic distances found among the four populations reveal that they are conspecific, and no evidence was found to support the claim that the populations belong to discrete subspecies. Rather, an east-west cline in genetic characteristics appears to exist with G6pd and HB-2 alleles peculiar to western populations and Es-2 and GP-3 alleles peculiar to eastern populations.*

Implicaciones de la Diferenciacion Genética en la Conservación de Poblaciones del Rinoceronte Negro *Diceros bicornis* en el Sur de Africa

Resumen: *Un análisis de 30 genes proteínicos se cuadro poblaciones de rinocerontes negros en Africa austral, posibilitó calcular estadísticas F y distancias genéticas entre las diferentes poblaciones. Una de estas poblaciones de rinocerontes negros es considerada como perteneciente a la sub-specie* Diceros bicornis bicornis *y las otras tres a* Diceros bicornis minor. *No se presento evodencia de procreatión consanguínea dento de las poblaciones. Las poblaciones. Las estidísticas F presentaron una diferenciación significativa entre las poblaciones. Pequeñas distancias genéticas entre las quatro poblaciones manifiestan que partenecen a la misma especie, y no se encontró evidencia para apoyar la teoría que estos grupos pertenencen a subespecies distintas. Mas bien, una transición gradual de este a oeste fue una de las características que parecen existir con genes G6pd y HB-2 con características a poblaciones del occidente y con genes Es-2 y GP-3 con características a poblaciones orientales.*

Introduction

The genetic management of small populations of endangered species has been the focus of many publications over the last 15 years. One of the issues that has often been considered is the steady and inevitable attrition of genetic variation due to genetic drift in small populations. One of the ways of reducing the effects of genetic drift in such populations is by maximizing their effective population size (Franklin 1980). This could be achieved partly by translocation of individuals among the few surviving populations of an endangered species (Lande & Barrowclough 1987). On the other hand, it may not always be advisable to translocate individuals between distinct populations because this could lead to the break-up of genetic combinations that reflect local adaptations of each remaining population (Allendorf & Leary 1986). The inadvertent mixing of genetic material from locally adapted populations may lead to outbreeding depression (Templeton 1986). The judicious management of endangered species therefore requires intimate knowledge of the genetic structure of the component popula-

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tions. This is even more important when the populations under consideration have been categorized as different subspecies. In this case wildlife managers have even stronger responsibilities toward the maintenance of extant biodiversity.

The black rhinoceros (*Diceros bicornis*) comprises a quintessential example of these problems. The number of black rhinoceroses has declined rapidly over the last two decades, from some 65,000 in 1970 to fewer than 2500 in 1993 (du Toit & Cummings 1986; du Toit, personal communication.) This decline occurred mostly because of poaching. The situation is made even more complex by the fact that the species has been divided into six subspecies, of which none comprises more than 600 individuals (Cumming et al. 1990). The subspecific designation of these populations (Groves 1987) has been the subject of debate, and du Toit et al. (1987) suggested four conservation units in Africa. From a genetic perspective, black rhinoceros managers are in a dilemma. On the one hand many of the extant populations are small, and loss of genetic variation is expected to take place relatively rapidly in these populations unless genetic management is implemented. On the other hand, this species occurs in diverse habitats; for example, *D. b. bicornis* is found in arid southwestern Africa, whereas *D. b. minor* roams the moister, eastern parts of the continent. This leads to a fear that the integration of all these populations in a single genetic management plan may lead to outbreeding depression which, in turn, would actually counter the aims of any conservation plan. Investigations of the genetic variation in extant black rhinoceros populations conflict. Results from Osterhoff and Keep (1970), Merenlender et al. (1989), and Ashley et al. (1990) suggest that the species is depauperate in genetic variation. Ashley et al. (1990) attempted the only existing analysis of geographic variation in *D. bicornis* and could not detect significant regional differentiation in mtDNA. The view emerging from these studies is that *D. bicornis* is genetically depauperate and uniform. In contrast Swart et al. (1994) showed that black rhinoceroses in southern Africa have a degree of genetic variation that approximates that of large, outbred populations. But, they did not perform a geographic analysis of genetic variation. Since then, we have had the unique opportunity of obtaining more material, especially for *D. b. bicornis*, from Namibia; therefore, an analysis of geographic differentiation of southern African black rhinoceros populations is now possible. Our study includes 169 individuals, the largest collection of genetic material for wild-living black rhinoceroses analyzed to date. We attempt to answer two questions: (1) How much genetic differentiation exists among southern African populations of black rhinoceros? and (2) Is this variation consistent with the current delineation of subspecies in the area? We hope that our analysis will aid the genetic management of these populations.

Methods

Starch and polyacrylamide gel-electrophoresis were used to analyse 30 protein-coding loci. The products of 13 of these were in blood plasma and the rest in erythrocytes (rbc). Details of the buffers and of the methods and conditions of electrophoresis are given in Swart et al. (1994). Six of these 30 loci were polymorphic and form the basis of our geographic analysis: Esterase-2 (EC 3.1.1.1), General Protein-3, General Protein-5, Glucose-6-phosphate dehydrogenase (EC 1.1.1.49), Hemoglobin-2 (EC 4.4.1.5), and Phosphoglucomutase (EC 2.7.5.1.).

Material from four black rhinoceros populations were analyzed: *D. b. bicornis* from Etosha National Park, Namibia (19°0′S 15°30′E; plasma $n = 15$; rbc $n = 6$) and *D. b. minor* from the Zambezi Valley, Zimbabwe (16°45′S 28°30′E; plasma $n = 95$; rbc $n = 40$), Hluhluwe-Umfolozi Park, Natal (28°15′S 31°45′E; plasma and rbc $n = 25$), and Mkuzi, Natal: (27°35′S 32°15′E; plasma and rbc $n = 34$).

Gene diversity (i.e., expected heterozygosity) within each population and its standard error (SE) were calculated from the observed allele frequencies at each locus (Nei 1987). To measure genetic differentiation between black rhinoceros populations, Wright's fixation indices (F_{IS}, F_{IT}, F_{ST}) were calculated following the method of Weir and Cockerham (1984), who use analysis of variance to determine the different sources of genetic variation when several populations are sampled. Standard errors of the fixation indices obtained were calculated by jackknifing (Weir 1991*a,* 1991*b*). Standard genetic distances (D) between different black rhinoceros populations were calculated by the method of Nei (1972), modified by Hillis (1984). Sampling variances of the genetic distances were calculated as suggested by Nei and Rouychoudoury (1973). Genetic similarities between populations were depicted by multidimensional scaling (Kruskal & Wish 1978).

Results

Genotypic frequencies for polymorphic loci within each of the populations did not differ from Hardy-Weinberg expectations (Table 1), the only exception being the GP-5 locus in the Zambezi population (excess homozygotes, $G = 9.2$, $df = 1$, $p < 0.05$). Gene-diversity values ranged from 0.057 (G6pd at Mkuzi) to 0.5 (GP-5 at Zambezi; Table 1). Mean gene diversity in the four rhino populations ranged from 0.037 (Mkuzi) to 0.062 (Zambezi; Table1). These measures of overall genetic variation are consistent with those of Swart et al. (1994).

Several differences in the geographical occurrence of alleles were observed: polymorphism for GP-3 was observed only in the Zambezi population and for HB-2 only in the Etosha population. The Hluhluwe-Umfolozi popu-

Table 1. Allele frequencies for six polymorphic loci for the four black rhinoceros populations surveyed.*

	Es-2	*GP-3*	*GP-5*	*Pgm-2*	*G6pd*	*HB-2*	*Total ± SE*
Etosha							
p	1.000	1.000	0.633	0.084	0.250	0.75	
H	0	0	0.464	0.153	0.487	0.391	0.053 ± 0.027
n	15	15	15	15	6	15	
Zambezi							
p	0.859	0.445	0.541	0.400	0.880	1.00	
H	0.247	0.484	0.500	0.368	0.234	0	0.062 ± 0.022
n	95	95	95	15	15	15	
Hluhluwe							
p	0.840	1.000	0.760	0.400	1.000	1.000	
H	0.296	0	0.365	0.480	0	0	0.038 ± 0.022
n	25	25	25	25	25	25	
Mkuzi							
p	0.955	1.000	0.617	0.382	0.980	1.000	
H	0.084	0	0.472	0.472	0.057	0	0.037 ± 0.022
n	34	34	34	34	34	34	

**In all cases two alleles were observed. The allele frequency of the first allele (*p*) at a locus, gene diversity (*H*; i.e., expected heterozygosity), and the number of individuals successfully assayed (*n*) for that locus are indicated. Total represents the weighted mean gene diversity based on all six loci, with its associated standard error.*

lation was the only one with no variation at the G6pd locus, and the Etosha population was the only one with no variation at the ES-2 locus. This suggests measurable genetic differentiation between the four populations sampled, which was confirmed by the values of fixation indices.

The values for F_{ST} for each of the six loci each ranged from 0.03 to 0.5 (Table 2). The F_{ST} estimates larger than 0.25 all resulted from loci that were fixed in at least one of the populations. The F_{ST} of 0.34 based on the combination of these six loci differed significantly from zero, with a 95% confidence interval of [0.14 – 0.42]. If the Zambezi population (*Diceros bicornis minor*) is omitted from the analysis, F_{ST} for the remaining populations drops to 0.08. Even though this last value differs significantly from zero (bootstrap $p < 0.05$), it does not differ in a statistically significant way from the estimate for all four of the populations combined. Likewise, exclusion of the Etosha population (*D. b. bicornis*) from the analysis resulted in a F_{ST} of 0.195, a value significantly different from zero (bootstrap $p < 0.05$), but not statistically different from the figure of 0.34 for all four the populations.

When genetic distance is used as a measure of genetic differentiation between black rhinoceros populations, the two Natal black rhinoceros populations are similar, where as the Etosha population, Zambezi population, and Natal populations form three discrete groups (Table 3). The Natal populations were about equally dissimilar to the Zambezi population and the Etosha population. (Fig. 1).

We have already indicated that, apart from the GP-5 locus in the Zambezi population, genotypic frequencies followed Hardy-Weinberg expectations. In addition, bootstrapping indicated that F_{IS} for the overall data set did not differ significantly from zero (bootstrap $p > 0.05$). But the F_{IT} estimate based on the combined data differed significantly from zero (Table 2).

Table 2. Wright's *F* statistics for the four black rhinoceros populations reflected by the six polymorphic loci studied and the overall means and their associated standard errors (SE).

Locus	F_{IS}	F_{IT}	F_{ST}
Es-2	−0.150	−0.109	0.036
GP-3	0.323*	0.661*	0.500*
GP-5	0.568*	0.582*	0.033
Pgm-2	0.104	0.133	0.032
G6pd	0.497*	0.628*	0.261
HB-2	−0.348	0.048	0.294
Mean	0.270	0.413*	0.195*
SE	0.147	0.141	0.128

**Differ in a statistically significant way from zero (bootstrapping $p < 0.05$).*

Discussion

The significant $83F_{ST}$ for the combined data indicates that a marked degree of genetic differentiation has taken

Table 3. Genetic distances (above diagonal)* and associated standard errors (below diagonal) among the four black rhinoceros populations studied.

	Etosha	*Hlubluwe*	*Mkuzi*	*Zambezi*
Etosha	——	0.0175	0.0146	0.0225
Hluhluwe	0.0119	——	0.0005	0.0153
Mkuzi	0.0107	0.0006	——	0.0135
Zambezi	0.0142	0.0125	0.0123	——

**Nei (1973) and Hillis (1984).*

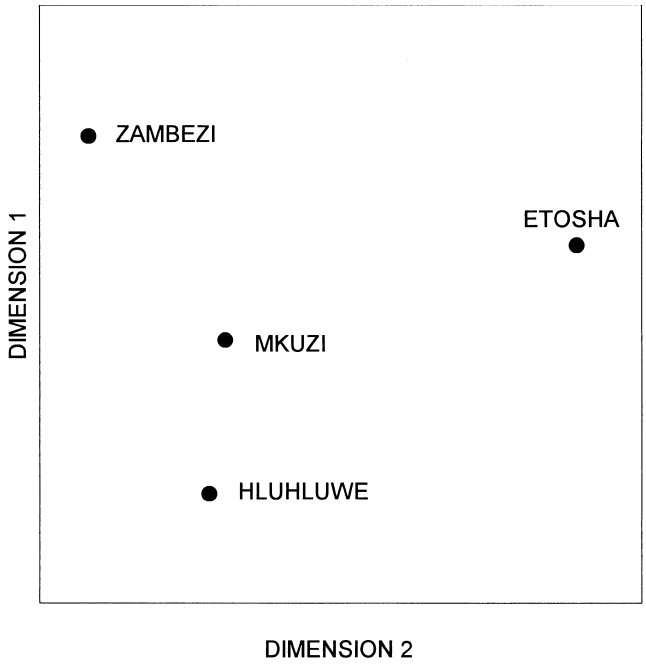

*Figure 1. Two-dimensional multidimensional scaling representing the genetic characteristics of the four black rhinoceros populations (*Diceros bicornis*) (Stress = 0.00).*

place among the four populations studied. This answers our first question. The four populations could be considered isolated remnants of a much larger ancestral population. Gene flow between the Natal, Zambezi, and Etosha populations ceased during the last half of the nineteenth century, and the two Natal populations became isolated soon after 1900 (P. M. Brooks, personal communication). We do not attach any particular importance to the statistically significant large F_{IT} values obtained in our analysis. Because F_{IT} is determined by F_{ST} and F_{IS}, we assume that the large F_{IT} value is mostly the result of the large F_{ST} observed.

The Zambezi population (*Diceros bicornis minor*) contributes in a larger way to the calculated F_{ST} than does the Etosha population (*D. b. bicornis*). This is because many more animals from the Zambezi population were sampled than from Etosha, causing estimates involving the Zambezi animals to have high statistical significance. Conversely, the sample size of 15 *D. b. bicornis* results in relatively large standard error of the F_{ST} estimate involving rhinoceroses from Etosha, which therefore have less effect in the calculation of fixation indices.

There is no clear evidence, however, that genetic differences between the Etosha population (*D. b. bicornis*) and the other three populations (*D. b. minor*) is the major contributing factor in causing a large F_{ST}. This is evident from the fact that exclusion of the Zambezi population from the analysis reduced the magnitude of F_{ST} more than did exclusion of the Etosha population. This interpretation is supported by the genetic distance calculations in which the Zambezi population does not clearly cluster with the two Natal populations. In fact, a multidimensional scaling representation of the data from the four populations (Fig. 1) suggests that the genetic differences within the three populations of *D. b. minor* is as large as the differences between *D. b. bicornis* (Zambezi) and *D. b. bicornis* (Etosha). Genetic distance data therefore also suggest that *D. b. bicornis* is not a subspecies, genetically distinct from *D. b. minor*. Rather, the patterns of genetic variation in the four populations studied suggest a west-to-east genetic continuum of which the Etosha and Natal population are the extremes, being subsets of the genetic variation in the relatively large Zambezi population. This is reflected by, on the one hand, genetic variation at the HB-2 locus peculiar to the Etosha population and at the G6pd locus, which was common in the Etosha and Zambezi populations and, on the other hand, variation at the Es-2 locus, which was shared by the Natal and Zambezi populations. The two Natal populations and the Etosha population each represented a subset of the genetic variation extant in the Zambezi population, the only anomalous locus being HB-2. This answers the second question we posed.

Importance of the Zambezi Valley Population

This study did not reveal any clear evidence of inbreeding or of an excess in homozygosity in any of the studied populations. We suggest that inbreeding is not an important conservation problem in the rhinoceros populations that we studied.

Even though each of the four populations are not genetically distinct, it is obvious that some genetic differentiation has occurred among them because they became isolated some 100 years ago (i.e, 10–15 rhinoceros generations). This indicates that mixing of these populations will affect their genetic constitution. If translocations were deemed a necessary management tool for the conservation of southern African black rhino populations, the genetic constitution of the populations could thus be best preserved if translocations were performed among populations that are closely situated geographically or among the descendants of such populations.

We regard the Zambezi population as a particularly important population because of the large degree of genetic variation within that population. Merenlender et al. (1989) performed genetic analyses on captive black rhinoceroses, mostly of the east African subspecies *D. b. michaeli*, and found very little genetic variation. Our results, seen in the context of earlier studies, might indicate that the Zambezi Valley population is the only remaining population containing the full complement of genetic variation that existed before the turn of the cen-

tury, at least in southern African populations and possibly in eastern African populations. The Zambezi Valley population, however, has been translocated to a number of smaller reserves in Zimbabwe. This conservation action could, if the small resulting populations in Zimbabwe are not well managed, result in rapid loss of genetic variation both from inbreeding and genetic drift. Because of this, the Zambezi Valley population needs special care in genetic management.

Conclusion

Genetic variation in four southern African black rhinoceros populations clearly indicates that genetic management of the species as a whole is not a priority for the short-term conservation of this species because (1) a large degree of genetic variation remains in these populations and (2) The Etosha population, which belongs to the subspecies *D. b. bicoris*, does not differ in a discrete way from the other populations of the subspecies *D. b. minor*. But there appears to be an east-west cline of genetic variation, suggesting that, if the genetic structure of the species in southern Africa is to be maintained, individuals from populations on the western side of the subcontinent should not be translocated to the eastern side of the continent, or vice versa. In addition, the Zambezi Valley population appears to be the only remaining population containing approximately the full set of genetic variation that existed before rhinoceros populations were severely reduced by hunting and poaching. This population needs genetic management to maintain the extant genetic variation. The situation for these populations is therefore intermediate because there is no immediate crisis that requires the genetic management of the species, but the genetic effects of translocation and population fragmentation should be managed, especially in Zimbabwe, to ensure that the long-term existence of this species is not affected by a rapid loss of genetic variation.

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