Conservation Genetics of the Black Rhinoceros (*Diceros bicornis*), I: Evidence from the Mitochondrial DNA of Three Populations

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Abstract: *A drastic decline in the number of black rhinoceroses (*Diceros bicornis*), primarily as a result of poaching, places this species in imminent danger of extinction. The remaining black rhinos are divided into small, isolated populations that are vulnerable to demographic extinction, disease epidemics, genetic drift, and inbreeding. Some conservationists have suggested minimizing these threats by moving as many animals as possible from different isolated populations to a few safe "rhino sanctuaries." To examine the possible long-term genetic consequences of such a strategy, we focused our efforts on determining the level of genetic differences among the remaining black rhino populations by examining restriction fragment length polymorphisms of the rapidly evolving mitochondrial DNA molecule. The 23 black rhinos in our survey, including animals from*

Resumen: *Una disminución drástica en el número de rinocerontes negros (*Diceros bicornis*), principalmente debido a la caza ilegal, pone a dicha especie en peligro de extinción inminente. Los rinocerontes negros restantes estan divididos en poblaciones pequeñas y aisladas, que son vulnerables a la extinción demográfica, las epidemias, la deriva genética y la endogamia. Algunos conservacionistas han sugerido disminuir estas amenazas, trasladando tantos animales como sea posible, de diferentes poblaciones aisladas, a un par de "santuarios seguros" para rinocerentes. Para examinar las posibles consecuencias genéticas a largo plazo de dicha estratégia, enfocamos nuestros esfuerzos en determinar el nivel de diferencia genética entre las poblaciones restantes de rinocerontes negros. Examinamos polimorfismos de fragmentos de longitud restringida de la molécula ADN, de evolución mitocondrial rápida. Los 23 ejemplares de rinocerontes negros de nuestro estudio, que incluyen a animales provenientes de tres regiones geográficas y de dos subespecies descritas, denotaron muy poca diferenciación del ADN mitocondrial. Unicamente 4 de 18 enzimas re-*

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three geographic regions and two named subspecies, showed very little mitochondrial DNA differentiation. Only 4 out of 18 restriction enzymes revealed any mtDNA polymorphisms, and the average estimated percent sequence divergence between the four mtDNA genotypes observed as 0.17%. Mitochondrial DNA divergence between the two named subspecies, D. b. minor *and* D. b. michaeli, *was estimated to be only 0.29%. These results indicate a very close genetic relationship among the black rhinos in our survey. Thus, the mitochondrial DNA data suggest that within national boundaries, the black rhino populations we sampled may be considered single populations for breeding purposes, which might increase the species' probability of survival.*

strictivas, denotaron poliformismo mitocondrial del ADN (ADNmt) y, el porcentaje promedio estimado de divergencia secuencial entre los cuatro genotipos ADNmt observados, fue de 0.17%. La divergencia de ADN mitocondrial entre las dos subespecies descritas, D. b. minor *y* D. b. michaeli, *se estima que fue solo de 0.29%. Estos resultados indican una relación genética muy cercana entre los rinocerontes negros de nuestro estudio. Por lo tanto, los datos de ADN mitocondrial sugieren que, dentro de las fronteras nacionales, las poblaciones de rinocerontes negros que estudiamos pueden considerarse una sola población para finalidades de reproducción, lo cual puede incrementar la probabilidad de supervivencia de dicha especie.*

Introduction

Despite its reputation as a powerful and invincible beast, the rhinoceros has suffered precipitous declines in number and is threatened with extinction. Fewer than 11,000 individuals of all five species survive in small scattered populations throughout Africa, India, and Southeast Asia. The black rhino (*Diceros bicornis*), the focus of this study, has suffered the most dramatic decline, disappearing faster than any other large mammal. The species once occupied most of sub-Saharan Africa and numbered in the hundreds of thousands (Fig. 1). Even by the turn of the century, large, nearly contiguous populations of black rhino were spread across much of central, eastern, and southern Africa. However, by 1970 their numbers had declined to 65,000 and over the past 18 years poaching has reduced this number by 95%. The remaining 3,800 animals are split into some 75 populations, only ten of which have more than 50 animals (Western & Vigne 1985; Du Toit et al. 1987; Wildlife Conservation International News 1988).

Although the loss of any species is tragic, the plight of the rhinoceros is particularly apalling because the overwhelming cause of their demise is not destruction of their preferred habitat but continued slaughter by poachers to supply two major markets. Horns are fashioned into ornamental dagger handles costing up to $30,000 in the Near East, particularly in North Yemen. In many parts of East Asia, rhino horn is valued at $8,000 to $15,000 per kilogram for various medicinal purposes (Martin 1983; Penny 1988). Increasing affluence in the Fat East and oil wealth in the Near East, coupled with declining availability, have drastically increased the value of rhino horn in recent years, creating devastating repercussions for rhinos in Africa. The market value of rhino horn is currently so high that all age and size classes are susceptible to poachers (Leader-Williams 1988).

Assuming for the moment that the governments of countries still harboring sizeable numbers of black rhino (e.g., Kenya, Tanzania, Zimbabwe, and South Africa) are successful in controlling poachers, another major problem exists for the species. The small, isolated populations of black rhino that remain are vulnerable to the effects of demographic fluctuations, local ecological perturbations and disease epidemics, and loss of genetic variability due to drift and inbreeding (e.g., Crow & Kimura 1970; Gilpin & Soulé 1986; Wilcove et al. 1986). Additionally, low population density reduces the probability that a male will find a female during the 1–2-day period in her estrous cycle when she is sexually

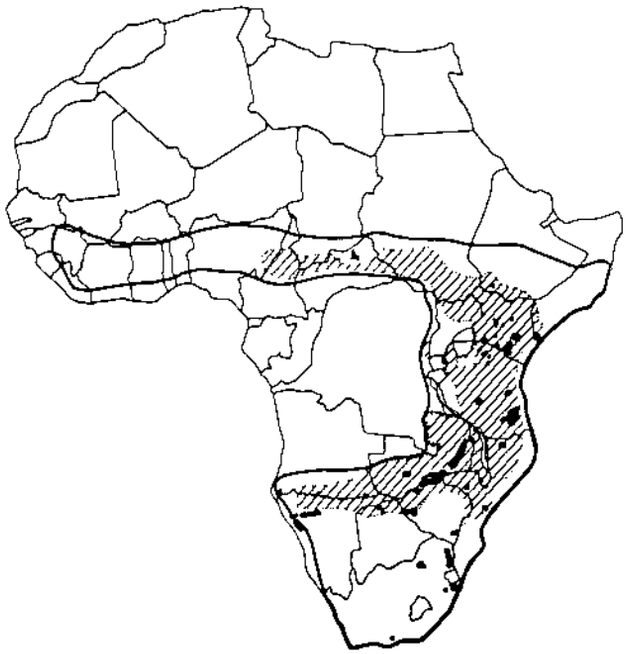

Figure 1. Map of Africa showing black rhino distributions during historical times (heavy black line), 1900 (hatched area), and 1987 (black area). Redrawn from Western & Vigne (1985), incorporating data from WCI for 1987.

receptive (Hitchins & Anderson 1983). Hence, what were once thought to be minimum viable populations on the basis of genetic parameters (i.e., inbreeding depression and loss of genetic heterogeneity) are now in many instances considered underestimates (Soulé 1987).

The shortage of manpower and resources within national conservation departments across Africa is a primary factor in the decline of the rhino (Leader-Williams & Albon 1988). Consolidating groups is an easier and less expensive way to reestablish former densities. The idea of creating more sanctuaries, which are effectively "species parks," has received widespread interest following the increase in Kenya's rhino populations in such sanctuaries. However, management strategies such as this are complicated by the fact that each remaining population has been assigned to one of several subspecies, based upon aspects of external morphology such as horn shape and body size. The genetic and evolutionary relationships of these morphologically defined subspecies are unknown. The most widely accepted classification (Groves 1967) recognizes seven subspecies of *Diceros bicornis,* one of which *D. b. ladoensis,* is probably extinct. Three other subspecies — *D. b. brucii,* found in Ethiopia and Somalia; *D. b. longipes,* which remains only in Cameroon and perhaps Chad; and *D. b. chobiensis,* found in Angola — are down to a few dozen animals, if they are not already extinct (Western & Vigne 1985). *D. b. bicornis,* if it can be considered a distinct taxon at all, is found in Namibia and probably numbers less than 100 (Hall-Martin 1985; Du Toit et al. 1987). The remaining two subspecies, *D. b. michaeli* and *D. b. minor,* or populations designed as such, will figure most importantly in the return of the black rhino, should this be accomplished. *D. b. michaeli,* found in Kenya and Tanzania, has declined drastically as well, but still numbers between 500 and 1,000 and has increasingly received more protection in Kenya. The vast majority of the approximately 150 black rhinos in North American and European zoos are of this subspecies (Du Toit et al. 1987) and so will be important for future captive breeding efforts. *D. b. minor* is the most common remaining race, ranging from Kenya to South Africa, and with numbers at about 2,500, it is relatively the most secure.

Should all the remaining black rhinos be considered as a single population for breeding purposes? This tactic might increase their chances of survival by increasing effective population sizes and thus forestalling stochastic demographic extinctions, inbreeding depression, and loss of the species' existing genetic variability (Soulé 1983; Gilpin & Soulé 1986; Ralls et al. 1986; Goodman 1987). Alternatively, do different populations (which may or may not coincide with subspecies designations) merit separate conservation as genetically and possibly ecologically distinct units? The latter strategy might prevent outbreeding depression or the production of animals with genetic makeups inappropriate for a given environment (Templeton 1986). These questions should, ideally, be tackled from both an ecological and genetic standpoint. Ecologically, it might be possible to distinguish locally adapted traits. Statistically significant differences in serum vitamin E levels, for example, have been found between Kenyan and southern African samples, which may reflect substantial differences in diet (Dierenfield, personal communication). Ecological differences also distinguish the desert rhinos of Namibia from the highland forest rhinos of Kenya (Du Toit 1987).

In the absence of any clear morphometric differences, IUCN's African Elephant and Rhino Specialist Group has placed a high priority on genetic studies of black rhinos to resolve whether discrete populations could be identified (Du Toit et al. 1987). As a first step in applying molecular genetic techniques to questions of black rhino conservation, we have examined the mitochondrial DNA (mtDNA) of 23 black rhinos representing two morphologically defined subspecies and three geographic populations. We chose mtDNA because its rapid evolutionary rate has shown it to be a useful molecule for determining intraspecific relationships of many animals (e.g., Wilson et al. 1985; Avise & Lansman 1983). If the rhino populations surveyed here have had separate evolutionary histories for a considerable length of time, it should be reflected in the divergence of mtDNA's from animals in different populations.

The mitochondrial genome consists of a closed circular DNA molecule which codes for 13 proteins and a complete set of transfer RNAs. It is extremely conserved in size (about 16,000 base pairs in all mammals that have been examined) and gene arrangement (Brown 1983). It lacks the complicating features of repetitive DNA or introns; therefore, a relatively simple restriction enzyme analysis of the molecule can be undertaken to yield good estimates of genetic relationships among fairly large numbers of individuals. It is maternally inherited without recombination and thus represents an unambiguous marker of maternal phylogeny. Because it evolves 5–10 times more rapidly than single-copy nuclear DNA (Brown et al. 1979) and intraspecific mtDNA variability has been widely demonstrated, this approach seemed the most likely to uncover genetic differentiation among black rhinos, should it exist.

Materials and Methods

With the cooperation of field biologists, wildlife managers, and zoo personnel in both Africa and the United States, we were able to obtain whole blood from both

captive and wild-caught black rhinos (Table 1). Our sample included 11 *D. b. michaeli* of Kenyan origin now kept in U.S. zoos, 11 *D. b. minor* taken from wild populations in Zimbabwe, and one captive (U.S.) *D. b. minor* of South African origin. While blood was separated into plasma, red blood cells, platelets, and white blood cells or buffy coats, the latter two components being our primary source of DNA. Total DNA was extracted from white blood cells or buffy coats by standard procedures. We also obtained frozen organ tissue from three animals that died during the period of our study (Table 1). This frozen tissue served as a source of purified mtDNA, which was isolated by the method of differential centrifugation (Lansman et al. 1981).

We have used restriction enzymes to survey the black rhinos for mtDNA polymorphisms. Restriction enzymes recognize specific oligonucleotide sequences, usually 4 to 6 base pairs in length, and cleave double-stranded DNA wherever these sequences occur. By surveying mtDNAs with a set of restriction enzymes, we can obtain an accurate estimation of similarity by determining the proportion of restriction fragments and/or restriction sites they share.

Samples of total DNA were digested with 14 restriction enzymes (Bethesda Research Laboratories) having 5 or 6 base pair (b.p.) recognition sites, according to manufacturer's instructions. These enzymes typically cleave mtDNA into 1–7 fragments. The DNA fragments once obtained were separated electrophoretically in 1% agarose gels along with a radioactively labeled (α-32-P) one-kilobase ladder (Bethesda Research Laboratories), then transferred to GeneScreen-plus membranes (New England Nuclear) by an alkaline blotting procedure (Southern 1975; Reed & Mann 1985). Purified mtDNA obtained from tissue was then nick-translated with α-32-P labeled nucleotides and was used to "probe" the southern blots. Membranes were then washed under high-stringency conditions and exposed to Kodak XAR film.

Additionally, to increase our resolution, we digested the three purified mtDNA samples (one from each population) with four enzymes having 4 b.p. recognition sites. These enzymes cleave the mtDNA into 20–30 fragments and thus have a greater likelihood of revealing differences between individuals. Because each sample contained only purified mtDNA, the resulting restriction fragments could be directly labeled with α-32-P (Brown 1980) before being separated electrophoretically on 3.5% polyacrylamide gels. Again, an appropriate radioactively labeled molecular weight/size standard was included in the gel. Gels were subsequently dried under vacuum and exposed to Kodak XAR film.

The proportion of shared restriction fragments was calculated between the observed mtDNA genotypes. The percent sequence divergence between the mitochondrial genotypes was estimated using equation 6b of Upholt (1977). Calculations for restriction enzymes having 6, 5, and 4 b.p. restriction sites were calculated separately, then weighted according to the total number of base pairs recognized by each type of restriction enzyme. This procedure allowed an overall estimate or weighted average of nucleotide sequence divergence, based on the differences revealed by all the restriction enzymes used, between the mtDNA of different individuals.

Results

Each restriction fragment pattern produced by a given enzyme was arbitrarily assigned a letter. The results of the enzymes having 5 or 6 base-pair recognition sites for all 23 animals are listed in Table 2. The results of enzymes for a smaller set of three animals, including en-

Table 1. Black rhino samples.

Sample	*Origin**	*Subspecies***	*Tissue*	*Source*
1–8	Zimbabwe	*D. b. minor*	Buffy coat	Department of Parks and Wildlife Management Zimbabwe
9	Zimbabwe	*D. b. minor*	Frozen liver	Department of Parks and Wildlife Management Zimbabwe
10, 11	Zimbabwe	*D. b. minor*	W.B.C.***	Los Angeles Zoo
12	South Africa	*D. b. minor*	Frozen brain	Calvin Bentsen Ranch Brownsville, Texas
13–16	Kenya	*D. b. michaeli*	W.B.C.	Denver Zoo
17–19	Kenya	*D. b. michaeli*	W.B.C.	St. Louis Zoo
20	Kenya	*D. b. michaeli*	W.B.C.	Zoo Atlanta
21	Kenya	*D. b. michaeli*	W.B.C.	Busch Gardens
22	Kenya	*D. b. michaeli*	Frozen liver	Kansas City Zoo
23	Kenya	*D. b. michaeli*	W.B.C.	Detroit Zoo

** For animals born in captivity, the origin of the individual's mother is given.*
*** Subspecific designation according to range distributions given by Groves, 1967.*
**** White blood cells.*

Table 2. mtDNA Patterns for enzymes with 5 and 6 b.p. sites.

		D. b. minor		*D. b. michaeli*
Enzyme	*# Sites**	*South Africa n = 1*	*Zimbabwe n = 11*	*Kenya n = 11*
AvaI	2	A	A	A
BamHI	2	A	A	A
BglII	1	A	A	A
ClaI	1	A	A	A
DraI	5	A	A	A
EcoRI	2(1)	A	A(B)	A
EcoRV	2	A	A	A
HaeII	4	A	A	A
HindIII	4	A	A	A
ScaI	7	A	A	A
XbaI	4	A	A	A

** The number in parentheses represents the number of restriction sites for haplotype B.*

Table 3. mtDNA Patterns for additional enzymes.

		D. b. minor		*D. b. michaeli*
Enzyme	*# Sites**	*South Africa n = 1*	*Zimbabwe n = 1*	*Kenya n = 1*
AvaII	4	A	A	A
BclI	6(5)	A	A	B
HincII	7	A	A	A
HinfI	30(29)	A	A	B
HpaII	16	A	A	A
MboI	23	A	A	A
TaqI	24(25)	A	A	B

** The number in parentheses represents the number of restriction sites for haplotype B.*

zymes having 4 b.p. recognition sites (i.e., HinfI, HpaII, MboI, TaqI), are presented in Table 3. The restriction enzymes used in our survey yielded an average of 140 restriction sites per mitochondrial genome. This corresponds to a recognized total of over 630 b.p., or 3.9% of the mitochondrial genome. For 14 out of a total of 18 restriction enzymes, absolutely no mtDNA variability was observed. That is, all rhinos surveyed had the identical restriction fragment pattern (designated as "A" in Tables 2 and 3) for any one of these 14 restriction enzymes. One enzyme, EcoRI, was found to be polymorphic among the Zimbabwe rhino, with 3 of 11 animals possessing only one EcoRI restriction site instead of the two sites found in the other 8 members of this population. Three enzymes, BclI, HinfI, and TaqI, revealed a difference between the Kenyan population and the Zimbabwe and South African populations. In each case, the result could be interpreted as a single loss or gain of a restriction site.

In total, then, for our sample of 23 animals, only three mtDNA haplotypes could be distinguished: (1) the Kenyan haplotype with fragment pattern "B" for BclI, HinfI, and TaqI; (2) the Zimbabwe haplotype with fragment pattern "B" for EcoRI; and (3) the Zimbabwe and South African haplotype with fragment pattern "A" for all 18 restriction enzymes. These three mtDNA haplotypes are extremely similar to one another (Table 4), with an average estimated percent sequence difference between any pair of haplotypes and/or populations of 0.17%. The average difference between subspecies was only slightly higher, 0.29%.

Discussion

The results of the mtDNA analysis strongly suggest a very close genetic relationship among all the black rhinos in our survey. Because of the generally rapid rate of mtDNA evolution in mammals, differences observed among rhino populations appear to indicate a very recent common ancestry. If mtDNA evolves at a rate of 2% per million years as suggested (Brown et al. 1979; Wilson et al. 1985), this common ancestry probably dates back no farther than 100,000 years. Indeed, the level of differentiation between the so-called subspecies is well within the range (0–4%) observed among members of other mammalian species (e.g., Avise & Lansman 1983), and even within the range (0–2%) that has been observed among members of the same local population (Ashley & Wills 1987). Thus, there is no evidence from these data that the black rhinos we sampled represent "evolutionarily distinct units."

These findings for the black rhino stand in sharp contrast to the level and distribution of mtDNA differences reported for the white rhino. The southern white rhino, *Ceratotherium simum simum,* has recovered quite well from a population bottleneck that occurred at the turn of the century, and now more than 3,000 members of this population can be found in South Africa and other African countries (Penny 1988). The status of the northern white rhino, *Ceratotherium simum cottoni,* is much bleaker, with only 22 individuals known to exist in the wild (Western 1987). Management plans originally proposed supplementing the northern race with members from the southern population. Based on a survey of one individual from each of the two races, however, George et al. (1983) reported a very high level of mtDNA divergence (approximately 4.0%). Partially as a result of this study, managers decided against interbreeding the two races. Unlike the black rhino, the white rhino subspecies have existed in nonoverlapping

Table 4. Estimated percent sequence divergence between mtDNA types, based on the proportion of shared restriction fragments (Upholt, 1977).

	Zimbabwe 1	*Zimbabwe 2*	*S. Africa*
Kenya	0.24	0.39	0.24
Zimbabwe 1	—	0.08	0.00
Zimbabwe 2		—	0.08
S. Africa			—

ranges, at least during historical times. George et al. suggest, on the basis of their molecular data, that the two white rhino subspecies have been isolated from each other for at least two million years. However, recent research on variability of nuclear-coded allozymes found little differentiation between the northern and southern subspecies (Merenlender et al. 1989), suggesting a more recent isolation.

We chose mtDNA analysis because we thought it would be most likely to uncover genetic differences between the black rhino populations, should they exist. It seems unlikely from our results that significant barriers to successful interbreeding would exist, given what we estimate to be a brief history of separation between the populations in question. However, more information should be obtained before final management decisions are made. The mitochondrial genome represents only a tiny fraction of an organism's genetic makeup, and problems that might arise from interbreeding might not necessarily be reflected in mtDNA differentiation. For this reason, we are conducting an allozyme survey in our laboratory to determine if the findings regarding the mtDNA hold for nuclear-coded genes as well. The allozyme survey will also be more informative for determining if the black rhinos suffer from reduced levels of genetic variability, as has been reported for some species that have passed through recent population bottlenecks (Bonnell & Selander 1974; O'Brien et al. 1983). Thus far, we have found no allozyme polymorphisms within or between populations, despite the fact that (1) we have included in our analysis animals from Kenya, Zimbabwe, and three different populations in South Africa (Etosha, Ado, and Zululand) and (2) our initial surveys have included all three allozyme loci identified by Merenlender et al. (1989) as polymorphic in African rhinos (Amato & Melnick, unpublished data).

Karyotype analysis is also recommended, because chromosomal differences reducing the fertility of hybrids could conceivably exist in the absence of either allozyme or mtDNA differentiation. There have been no known crosses of black rhinos from different subspecies in captivity, which might indicate reduced viability or fertility.

The application of genetics to conservation issues is a practical endeavor and should yield concrete recommendations for management strategies. Black rhinos from the populations included in our study will probably be the ancestors of all future black rhinos, as their successful breeding is the only hope for the survival of the species. Our results provide strong evidence for a very close genetic relationship among these populations. At the national level, the level at which management decisions are currently made, the pooling of black rhinos carries with it little risk of mixing distinct genetic adaptations worthy of separate conservation efforts. This finding should allow managers to aggregate individuals to create larger local populations or demes. Preserving the black rhino in relatively large local populations would have several beneficial effects. These include retarding the rate of loss of genetic variability, buffering each aggregate against the possibility of demographic extinction, restoring previous population densities, and allowing the wildlife managers with limited resources to provide better production against poachers. Taken together, these effects should, in the long run, increase the probability of survival of this critically endangered species.

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Literature Cited

Ashley, M., and C. Wills. 1987. Analysis of mitochondrial DNA polymorphisms among Channel Island deer mice. Evolution 41:854–863.

Avise, J. C., and R. A. Lansman. 1983. Polymorphism of mitochondrial DNA in populations of higher animals. Pages 147–164 in M. Nei and R. K. Koehn, editors. Evolution of genes and proteins. Sinauer, Sunderland, Massachusetts.

Bonnell, M. L., and R. K. Selander. 1974. Elephant seals: genetic variation and near extinction. Science **184**:908–909.

Brown, W. M. 1980. Polymorphism in mitochondrial DNA of humans as revealed by restriction endonuclease analysis. Proceedings of the National Academy of Sciences of the United States of America **77**:3605–3609.

Brown, W. M. 1983. Evolution of animal mitochondrial DNA. Pages 62–88 in M. Nei and R. K. Koehn, editors. Evolution of genes and proteins. Sinauer, Sunderland, Massachusetts.

Brown, W. M., M. George, Jr., and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proceedings of the National Academy of Sciences of the United States of America **76**:1967–1971.

Crow, J. F., and M. Kimura. 1970. An introduction to population genetics theory. Harper & Row, New York.

Du Toit, R. 1987. Conservation biology of the black rhino. Zimbabwe Science News **21**:63–67.

Du Toit, R., T. J. Foose, and D. H. M. Cumming. 1987. Proceedings of the African Rhino Workshop, Cincinnati, October, 1986. Pachyderm Special Issue, No. 9.

George, M., Jr., L. A. Puentes, and O. A. Ryder. 1983. Genetic differences between subspecies of white rhinoceroses. International studbook of African rhinos. Berlin Zoological Garden. Berlin, East Germany.

Gilpin, M. E., and M. E. Soulé. 1986. Minimum viable populations: the process of species extinction. Pages 13–34 in M. E. Soulé, editor. Conservation: the science of scarcity and diversity. Sinauer, Sunderland, Massachusetts.

Goodman, D., 1987. The demography of chance extinction. Pages 11–34 in M. E. Soulé, editor. Viable populations for conservation. Cambridge University Press, Cambridge, England.

Groves, C. P. 1967. Geographic variation in the black rhinoceros *Diceros bicornis* (L., 1758). Z Saugetierkunde **32**:267–276.

Hall-Martin, A. 1985. The nabab of Aukoerebis. African Wildlife **39**:245–247.

Hitchins, P. M., and J. L. Anderson. 1983. Reproduction, population characteristics and management of the black rhinoceros *Diceros bicornis minor* in the Hhuhluwe/Corridor/Umfolozi game reserve complex. South African Journal of Wildlife Research **13**:78–85.

Lansman, R. A., R. O. Shade, J. F. Shapira, and J. C. Avise. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. Journal of Molecular Evolution **17**:214–226.

Leader-Williams, N. 1988. Patterns of depletion in a black rhinoceros population in Luangwa, Zambia. African Journal of Ecology **26**:181–187.

Leader-Williams, N., and S. D. Albon. 1988. Allocation of resources for conservation. Nature **336**:533–535.

Martin, E. B. 1983. Rhino exploitation. World Wildlife Fund, Hong Kong.

Merenlender, A. M., D. S. Woodruff, O. A. Ryder, R. Kock, and J. Váhala. 1989. Allozyme variation in African and Indian rhinoceroses. Journal of Heredity **80**:377–382.

O'Brien, S. J., D. E. Wildt, D. Goldman, D. R. Merril, and M. Bush. 1983. The cheetah is depauperate in genetic variation. Science **221**:459–462.

Penny, M. 1988. Rhinos: endangered species. Facts on File, New York.

Ralls, K., P. H. Harvey, and A. M. Lyles. 1986. Inbreeding in natural populations of birds and mammals. Pages 35–56 in M. E. Soulé, editor. Conservation biology: the science of scarcity and diversity. Sinauer, Sunderland, Massachusetts.

Reed, K. C., and D. A. Mann. 1985. Rapid transfer of DNA from agarose gels to nylon membranes. Nucleic Acid Research **13**:7207–7221.

Soulé, M. E. 1983. What do we really know about extinction? Pages 111–124 in C. M. Schonewald-Cox, S. M. Chambers, B. MacBryde, and L. Thomas, editors. Genetics and conservation. Benjamin/Cummings, London, England.

Soulé, M. E. 1987. Introduction. Pages 1–10 in M. E. Soulé, editor. Viable populations for conservation. Cambridge University Press, Cambridge, England.

Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. Journal of Molecular Biology **98**:503–517.

Templeton, A. R. 1986. Coadaptation and outbreeding depression. Pages 105–116 in M. E. Soulé, editor. Conservation genetics: the science of scarcity and diversity. Sinauer, Sunderland, Massachusetts.

Upholt, W. B. 1977. Estimation of DNA sequence divergence from comparison of restriction endonuclease digests. Nucleic Acids Research **4**:1257–1265.

Western, D. 1987. Africa's elephants and rhinos: flagships in crisis. Trends in Ecology and Evolution **2**:343–345.

Western, D., and L. Vigne. 1985. The deteriorating status of African rhinos. International Union for Conservation of Nature/Species Survival Commission Report **1985**:1–15.

Wilcove, D. S., C. H. McLellan, and A. P. Dobson. 1986. Habitat fragmentation in the temperate zone. Pages 237–256 in M. E. Soulé, editor. Conservation biology: the science of scarcity and diversity. Sinauer, Sunderland, Massachusetts.

Wildlife Conservation International News. 1988. Rhino rescue fund to implement emergency action plan. Summer issue.

Wilson, A. C., R. L. Cann, S. M. Carr, M. George, U. B. Gyllensten, K. M. Helm-Bychowski, R. G. Higuchi, S. R. Palumbi, E. M. Prager, R. D. Sage, and M. Stoneking. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biological Journal of the Linnear Society **26**:375–400.