# Substantial Genetic Variation in Southern African Black Rhinoceros (*Diceros bicornis*)

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**Thirty protein-coding loci of southern African black rhinoceros (*Diceros bicornis*) from four isolated populations were studied using starch gel electrophoresis and polyacrylamide gel electrophoresis. Gene diversity estimates varied between 0.036 and 0.058, with the Zambezi Valley population having the largest amount of protein variation. These levels are higher than those in other studies of genetic variation in black rhinoceros and are similar to the amount of genetic variation observed for outbred natural populations that are not genetically depauperate. Because the observed levels of genetic variation vastly exceed the expectations for current effective population sizes, the current levels apparently reflect large black rhinoceros populations which have existed until recently. Observed levels of genetic variation within populations are consistent with the expectations when recent demographic events are taken into account.**

The maintenance of genetic variation is a primary goal of conservation genetics (Soulé 1980). Genetic variation is thought to be essential to ensure the evolutionary adaptability of species in the long term (Clegg and Brown 1983) and to maintain individual fitness and vigor in the short term (Franklin 1980; Lande and Barrowclough 1987). The mean proportion of heterozygous individuals per locus (heterozygosity) is a common measure of genetic variation (Hedrick 1984) and is convenient for identifying levels of genetic variability in captive or natural populations of endangered species. The level of heterozygosity in a population can also give an indication of inbreeding (Hartl 1988). This information can be used to make informed management decisions on the conservation of that population.

In the case of the black rhinoceros, *Diceros bicornis*, estimates of the amount of genetic variation are essential for the conservation of this species since these affect, among others, management policies about translocations and breeding schedules. Declining black rhinoceros numbers pose a serious threat for the survival of this species. Their numbers declined from an estimated 65,000 in 1970 to about 3,500 in 1990, mostly due to poaching (Leader-Williams 1988). Small population sizes will inevitably lead to the loss of genetic variation (Ryder et al. 1981), as was apparently the case of the Northern elephant seal (*Mirounga angustirostris*), which experienced a severe bottleneck as a result of decimation by sealers (Bonnell and Selander 1974). Another example of the presumptive consequences of bottlenecks is high levels of homozygosity in cheetah (*Acinonyx jubatus*), which is reflected in a high incidence of sperm abnormalities and is also thought to result in isogenicity at its major histocompatibility complex (no rejection of skin grafts) and the occurrence of diseases that affect animals in the population (O'Brien et al. 1983).

Several studies have been performed on genetic variation in rhinoceroses. Osterhoff and Keep (1970), Merenlender et al. (1989), and Ashley et al. (1990) all found very low levels of genetic variation. Merenlender et al. (1989) examined electrophoretic variation for 31 protein-coding loci and found a mean heterozygosity of 0.013 in nine captive individuals of black rhinoceros originating from east Africa (*D. b. michaeli*). They hypothesized that recent and ancient population bottlenecks account for this relatively low level of genetic variation. However, Stratil et al. (1990) found variation among serum proteins of white rhinoceros (*Ceratotherium simum*) and suggested that genetic variation for that species may be larger than that observed by the first-named authors. Similar high levels of genetic variation were also observed in the Indian one-horned rhino (Dinerstein and McCracken 1990).

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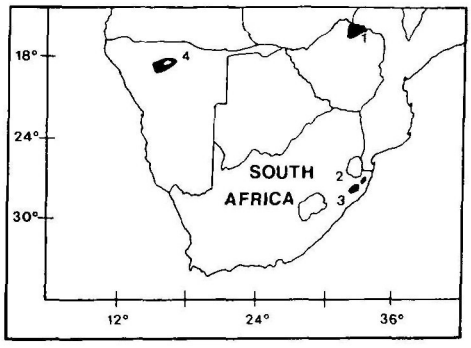


**Figure 1.** Map of southern Africa indicating the geographic origin of material analyzed: (1) Zambezi Valley, (2) Mkuzi Game Reserve, (3) Hluhluwe-Umfolozi Game Park, and (4) Etosha National Park.

The results from these studies indicate that intensive surveys of genetic variation are needed for the respective rhinoceros species so that a clear impression of their genetic constitution can be obtained throughout the geographical range of each species. This knowledge would place planned genetic management as an integral part of rhinoceros conservation plans. This contribution investigates the genetic variation in four southern African populations of the black rhinoceros. An extensive protein-electrophoretic survey of these populations has not been done before.

## Materials and Methods

We analyzed material collected from free-ranging individuals of two of the seven subspecies (Groves 1967): *D. b. bicornis* from Etosha National Park, Namibia, and *D. b. minor* from Natal, South Africa, and from Zimbabwe. Blood samples were collected from black rhinoceros populations from (1) the Zambezi Valley, Zimbabwe ($n$ = 90 for plasma; $n$ = 15 for red blood cells allozymes), (2) the Mkuzi Game Reserve, Natal ($n$ = 34), (3) the Hluhluwe-Umfolozi Park, Natal ($n$ = 25), and (4) the Etosha National Park, Namibia ($n$ = 6) (Figure 1). Samples were treated with an anticoagulant (heparin or acid citrate dextrose), centrifuged as soon as possible to guarantee good separation of plasma, red blood cells (RBC), and buffy coat. Where possible, the plasma and buffy coat were frozen in liquid nitrogen and kept at −70°C while the red blood cells were kept at −20°C. Red blood cells preferably need to be preserved with a glycerol-containing preserving fluid to prevent lysis; however, some of the blood samples dating from 1986 were not preserved in this way, resulting in smaller sample sizes for the Zimbabwe blood cell allozymes. We carried

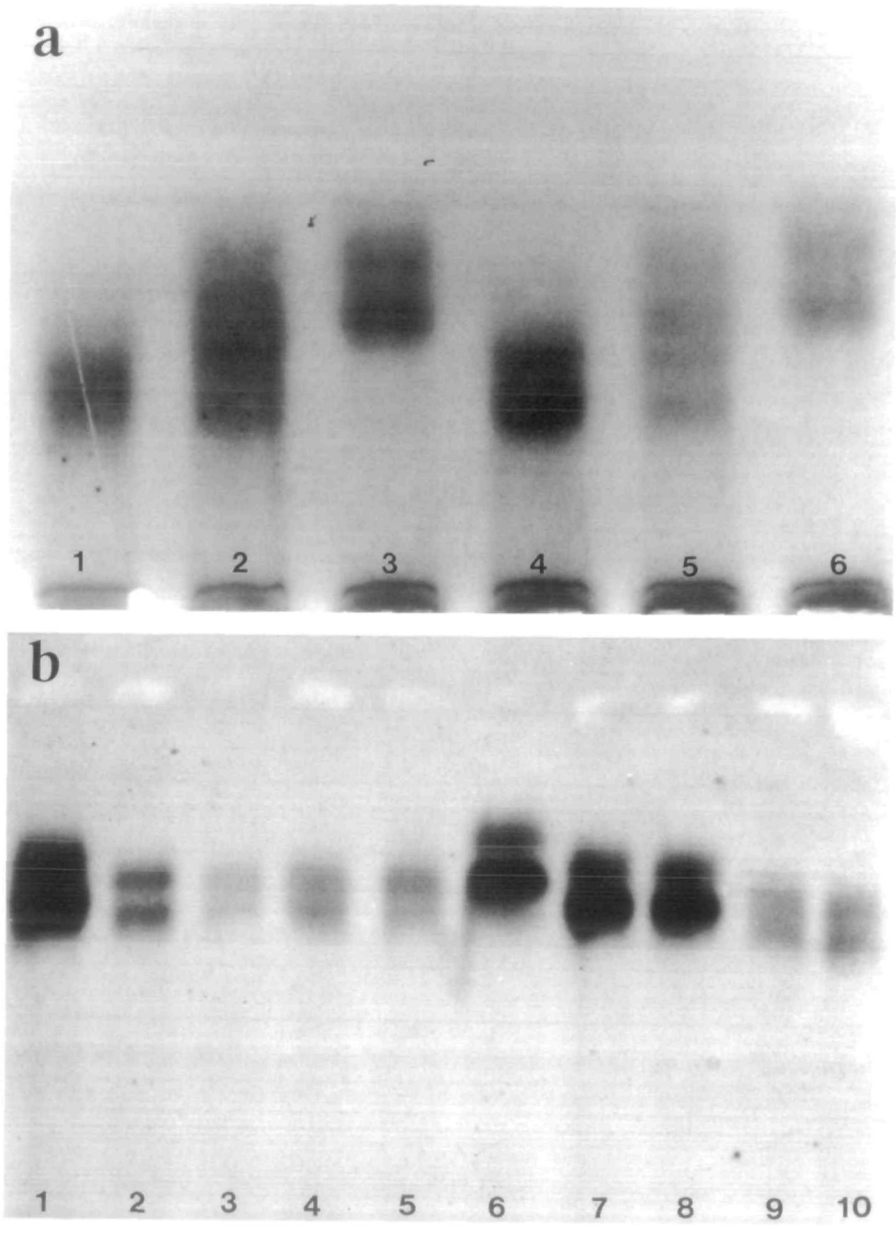


**Figure 2.** Electrophoretic variation observed in G-6PD and *Est-2*: (**a**) A starch gel electrophoretic separation of red blood cells indicating the different G-6PD phenotypes (F, FS, and S). Each isoenzyme is represented by two bands on the gel due to posttranslational modification of the enzymes. 1 = S male; 2 = FS female; 3 = F male; 4 = SS female; 5 = FS female; 6 = F male. (**b**) Variability at the *Est-2* locus analyzed via vertical polyacrylamide electrophoresis of plasma. Lane 6 = FF; lanes 1, 7, 8 = SS; lanes 2, 3, 4, 5, 9, 10 = FS. Posttranslational effects are also evident in this separation.

out one-dimensional vertical polyacrylamide and horizontal starch gel electrophoresis to determine the variation in protein mobilities. The staining methods (Harris and Hopkinson 1976) were adapted for use on rhinoceros material, as indicated below. Red blood cells were treated with a 5% mercaptoethanol and 10% Triton X-100 solution before electrophoresis. To achieve a random sample, as many enzymes as possible representing different enzyme groups were analyzed, such as the oxidoreductases, transferases, hydrolases, and lyases, as well as nonenzymatic proteins (Figure 2). Table 1 presents the electrophoretic conditions for each of the 30 enzyme and protein loci analyzed. Modified bridge buffers used were PGM-3 (starch): 0.97 M Tris, 0.87 M histidine (diluted 1:7.8 for gel buffer); 6-PGD (starch): 0.5 M Tris, 0.16 M citric acid (diluted 1:14 for gel buffer); glyoxalase (starch): 1 M Tris, 0.76 M histidine (diluted 1:13 for gel buffer); and Tris-glycine (PAGE): 0.005 M Tris, 0.038 M glycine.

We used three measures of genetic variation: heterozygosity (proportion of heterozygous individuals), gene diversity

(expected proportion of heterozygous individuals, taking into account extant allele frequencies; Nei 1978), and proportion of polymorphic loci (Nei 1987). If, among $n$ loci sampled, the proportion of individuals heterozygous at locus $i$ is indicated by $h_i$ and the $j$th allele frequency at the same locus is signified by $x_{ij}$ then the mean heterozygosity for a population is signified by $\Sigma\, h_i/n$ and the gene diversity at the locus by $1 - \Sigma\, x_j^2$. Mean gene diversity over all loci is $\Sigma\, (1 - \Sigma\, x_{ij}^2)/n$. We prefer gene diversity as a measure of genetic variation because of its statistical tractability. Standard errors (SE) of gene diversity (Nei and Roychoudoury 1974) were calculated, and conformation of allele frequencies to Hardy-Weinberg expectations were tested using a Fisher's exact $G$ test (Sokal and Rohlf 1981).

## Results and Discussion

Allele frequencies for the serum and RBC loci in the four populations conform to Hardy-Weinberg expectations, suggesting that the polymorphism is genotypic in origin and not due to biochemical effects. The only exception was the *Gp-5* locus for the subdivided Zambezi population (Table 2). For the *G6pd* locus, all the heterozygotes were females, supporting the supposition that the locus is sex linked. Six of the 30 loci studied were polymorphic. Gene diversity of individual polymorphic loci varied between 0.167 and 0.530 in the various populations (Table 2). The Zambezi samples yielded the largest number of polymorphic loci, with some loci being monomorphic in the other populations. *Hb-2* was the only polymorphic locus that did not show variation in the Zambezi population (Table 2). Analysis of the 30 loci of plasma and red blood cells combined produced mean gene diversity levels ranging from 0.036 to 0.059 (Table 3). The Zambezi Valley population had the highest gene diversity, while the Mkuzi population yielded the lowest value. Mean heterozygosity ranged between 0.018 and 0.046 (Table 3).

Our estimates of gene diversity among southern African black rhinoceros are similar to those found in outbreeding mammal species in which gene diversity values of about 0.05 appear to be common (Nei and Graur 1984; Nevo 1984; Selander and Johnson 1973). However, our estimates contrast with those of a number of authors who found little genetic variation among black rhinoceroses. Merenlender et al. (1989) found a mean heterozygosity of 0.013 over 31 loci for nine eastern African black rhinoceroses, *D. b. michaeli*, in zoological gardens with only two polymorphic loci: a general protein locus (Amido Black) and phosphoglucomutase-2. Osterhoff and Keep (1970) observed no genetic variation after analysis of hemoglobin, transferrin, and albumin of 10 individuals of *D. b. minor* from Hluhluwe-Umfolozi. R. R. Ramey (unpublished) analyzed serum of 16 *D. b. minor* from Zimbabwe and three *D. b. bicornis* from Namibia and found no variation for 12 loci and no allele frequency differences between the two populations. Ashley et al. (1990) analyzed mitochondrial DNA of three populations from Kenya (*D. b. michaeli*), Zimbabwe, and South Africa (*D. b.*

**Table 1. Protein loci examined, electrophoretic conditions and number of loci observed at each locus**

| Protein | EC no. | Locus | Tissue | Buffer | pH | Medium | No. of alleles |
|---|---|---|---|---|---|---|---|
| Acid phosphatase | 3.1.3.2 | *Acp-1* | RBC | 6-PGD | 7.5 | Starch | 1 |
| Adenylate kinase | 2.7.4.3 | *Ak-1* | RBC | 6-PGD | 7.5 | Starch | 1 |
| Diaphorase | 1.6.2.2 | *Dia-1* | RBC | 6-PGD | 7.5 | Starch | 1 |
| Esterase | 3.1.1.1 | *Est-1* | Plasma | Tris-glycine | 8.3 | Page | 1 |
| | | *Est-2* | Plasma | Tris-glycine | 8.3 | Page | 2 |
| | | *Est-3* | RBC | Glyoxalase | 7.6 | Starch | 1 |
| General Proteins | | *Gp-1* | Plasma | Tris-glycine | 8.3 | Page | 1 |
| | | *Gp-2* | Plasma | Tris-glycine | 8.3 | Page | 1 |
| | | *Gp-3* | Plasma | Tris-glycine | 8.3 | Page | 2 |
| | | *Gp-4* | Plasma | Tris-glycine | 8.3 | Page | 1 |
| | | *Gp-5* | Plasma | Tris-glycine | 8.3 | Page | 2 |
| | | *Gp-6* | Plasma | Tris-glycine | 8.3 | Page | 1 |
| Glucose-6-phosphate dehydrogenase | 1.1 1.49 | *G6pd* | RBC | PGM-3 | 7.4 | Starch | 2 |
| Glutamate-pyruvate transaminase | 2.6.1.2 | *Gpt* | RBC | 6-PGD | 7.5 | Starch | 1 |
| Glyoxalase I | 4.4.1 5 | *Glo* | RBC | PGM-3 | 7.4 | Starch | 1 |
| Hemoglobin | | *Hb-1* | RBC | PGM-3 | 7.4 | Starch | 1 |
| | | *Hb-2* | RBC | PGM-3 | 7.4 | Starch | 2 |
| Lactate dehydrogenase | 1.1.1.27 | *Ldh-1* | Plasma | Tris-glycine | 8 3 | Page | 1 |
| | | *Ldh-2* | Plasma | Tris-glycine | 8.3 | Page | 1 |
| | | *Ldh-3* | RBC | Tris-glycine | 8.3 | Page | 1 |
| Malate dehydrogenase | 1.1.1.37 | *Mdh-1* | RBC | Tris-glycine | 8.3 | Page | 1 |
| | | *Mdh-2* | Plasma | Tris-glycine | 8.3 | Page | 1 |
| Malic enzyme | 1.1.1.40 | *Me-1* | RBC | 6-PGD | 7.6 | Starch | 1 |
| Phosphoglucomutase | 2.7.5.1 | *Pgm-1* | RBC | Glyoxalase | 7.6 | Starch | 1 |
| | | *Pgm-2* | RBC | PGM-3 | 7.4 | Starch | 2 |
| Phosphogluconate dehydrogenase | 1.1.1.44 | *Pgd-1* | Plasma | Tris-glycine | 8.3 | Page | 1 |
| Purine nucleoside phosphorylase | 2 4 2.1 | *Np-1* | RBC | 6-PGD | 7. 6 | Starch | 1 |
| Pyruvate kinase | 2.7.1.40 | *Pk-1* | RBC | 6-PGD | 7.5 | Starch | 1 |
| Superoxide dismutase | 1.15.1.1 | *Sod-1* | Plasma | Tris-glycine | 8.3 | Page | 1 |
| | | *Sod-2* | RBC | Tris-glycine | 8.3 | Page | 1 |

**Table 2. Values of gene diversity for polymorphic protein loci in southern African black rhinoceros**

| Locus | Test[a] | Zambezi *D b minor* $N$ = 90/15 | Mkuzi *D. b minor* $N$ = 34 | Hluhluwe-Umfolozi *D. b. minor* $N$ = 25 | Etosha *D. b. bicornis* $N$ = 6 |
|---|---|---|---|---|---|
| *Est-3* | *H* | 0.249 ± 0.004 | 0.086 ± 0.008 | 0.274 ± 0.014 | 0 |
| | *G* | 0.053 (0.97) | 0 008 (0.97) | 0 002 (0.96) | n/a |
| *Gp-3* | *H* | 0.487 ± 0.001 | 0 | 0 | 0 |
| | *G* | 4 39 (0.81) | n/a | n/a | n/a |
| *Gp-5* | *H* | 0.503 ± 0.001 | 0 479 ± 0.005 | 0.372 ± 0.013 | 0.409 ± 0.054 |
| | *G* | 9.204 (0.02) | 3.400 (0.07) | 0.091 (0.76) | 0.005 (0.94) |
| *Pgm-2* | *H* | 0.331 ± 0.023 | 0 479 ± 0.005 | 0.490 ± 0.006 | 0.167 ± 0.055 |
| | *G* | 0.001 (0.97) | 0.277 (0.87) | 0.346 (0.84) | 0.001 (0.97) |
| *G6pd* | *H* | 0 186 ± 0 023 | 0.029 ± 0.005 | 0 | 0.530 ± 0.031 |
| | *G* | 0.170 (0.68) | 0.000 (0 99) | n/a | 0.075 (0.79) |
| *Hb-2* | *H* | 0 | 0 | 0 | 0.485 ± 0.0430 |
| | *G* | n/a | n/a | n/a | 0.037 (0 84) |

[a] $H$ = Hardy-Weinberg estimates ± SE (Nei 1978); $G$ = result of Fisher's exact $G$ test, testing whether genotypic proportions were related to allele frequencies in accordance with Hardy-Weinberg expectations. Values in parentheses following $G$ values are probabilities that particular loci depart from Hardy-Weinberg expectations.

**Table 3. Genetic variation[a] in four southern African black rhinoceros populations, based on electrophoretic analysis of 30 serum and red blood cell protein-encoding loci**

| Population | Sample size | Population size (1990)[35] | Mean $H \pm$ S.E. | Expected $H$ | Heterozygosity | $P$ |
|---|---|---|---|---|---|---|
| Zambezi Valley | 90 | 750 | 0.059 ± 0.026 | 0.02 | 0 046 | 0.167 |
| Etosha | 6 | 35 | 0.053 ± 0 027 | 0 013 | 0.028 | 0.133 |
| Mkuzi | 34 | 300 | 0.036 ± 0.022 | 0.012 | 0.018 | 0.133 |
| Hluhluwe-Umfolozi | 25 | 70 | 0.038 ± 0 021 | 0.003 | 0.032 | 0.100 |

[a] Mean $H$ = measured gene diversity ± SE of estimates, expected $H$ = expected gene diversity (Nei 1978) of a similarly-sized population in mutation-drift equilibrium (Nei 1987) and assuming mutation rate = $10^{-5}$·locus$^{-1}$·generation$^{-1}$; heterozygosity = proportion of heterozygous individuals; $P$ = proportion of polymorphic loci. See methods section for definitions of gene diversity ($H$), heterozygosity, and proportion of polymorphic loci ($P$).

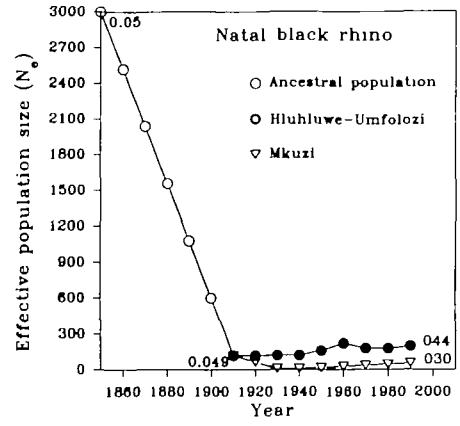


**Figure 3.** Estimates of effective population sizes of Natal black rhinoceros populations over the last 150 years (sources quoted in text). Numbers on graph indicate expected gene diversity at 1850, 1910, and 1990 for the two indicated populations, assuming $H_{t+1} = H_t(1 - 1/2N_e)$[36] and $N_e = 0.6$ of census population size. Open circles indicate the decline in the ancestral black rhinoceros population before the Mkuzi and Hluhluwe populations became separated by about 1910. Even though the gene diversity for Mkuzi is lower because of a severe population bottleneck around 1940, substantial genetic variation remains in that population because of the relatively long-generation length for black rhinoceros

*minor*) and found little variation in mitochondrial DNA. Our study is the first one, assaying a relatively large number of blood-based proteins, which indicates substantial genetic variation in black rhinoceroses. Our results are similar to those of Stratil et al. (1990), who studied the serum proteins of white rhinoceroses, *Ceratotherium simum*, by using an array of electrophoretic and immunoelectrophoretic techniques and who found intraspecific polymorphism at several loci. In addition Dinerstein and McCracken (1990) found high levels of heterozygosity in the Indian one-horned rhinoceros, *Rhinoceros unicornis*.

If one considers the present effective population sizes, the observed gene diversities are higher than the equilibrium values expected in similarly-sized populations in mutation-drift equilibrium (Nei and Graur 1984; Table 3). However, we believe that we are measuring the genetic diversity of the large black rhinoceros populations immediately before the precipitous decline in rhinoceros numbers during the last century. The relatively long generation time for black rhinoceroses—about 10 years (Conway and Goodman 1989; Hitchins and Anderson 1983; Hitchins and Brooks 1986)—would contribute to this phenomenon, causing the bottleneck effect on genetic variation to be small. This applies in particular to the Zambezi populations where this effect is minimal because of the recent date of the population decline (Cumming and du Toit 1986). A similar situation exists in the case of the Indian one-horned rhinoceros (Dinerstein and McCracken 1990).

Gene diversity values for the two Natal black rhinoceros populations appear to be lower (but not statistically different) from that of the Zambezi population. However, the values of around 0.035 (Table 3) suggest that the Natal populations are not strongly depauperate in genetic variation, since some outbreeding mammal species have similar amounts of gene diversity (Nei and Graur 1984). We suspect that this difference, although relatively small, is biologically real. Demographic changes in the Natal black rhinoceros population during the last century appear to explain the reduced gene diversity of these animals. There are some estimates of population sizes in Natal during the last century, but most of these are based on qualitative observations rather than on accurate counts. Black rhinoceroses were common in Natal during the first part of the 19th century, but their numbers decreased rapidly during the last half of that century (Findlay 1903; Fitzsimmons 1920; Lacey 1899), as happened in the interior of southern Africa (Selous 1908). After proclamation of the Hluhluwe and Mkuzi game reserves during 1895, small populations of black rhinoceros were protected. Records of the Natal Parks Board indicate that, during the period 1930–1940, between 100 and 130 individuals inhabited the Hluhluwe-Umfolozi area and six to 20 individuals were to be found in Mkuzi (Hitchins and Brooks 1986). Gene flow between the Hluhluwe-Umfolozi and the Mkuzi black rhinoceros populations effectively ceased about 1900 or very soon thereafter (Brooks PM, personal communication). Their numbers in these parks have increased to 220 in Hluhluwe-Umfolozi Park and 70 in Mkuzi (Cumming et al. 1990).

We simulated expected gene diversities given population bottlenecks of the magnitudes outlined above (Figure 3) and assumed a nonoverlapping generation time of 10 years. The results showed, first, that gene diversity in the extant populations (excluding Mkuzi in Natal) largely reflects that of black rhinoceros populations during the previous century and, second, that the lower observed levels of gene diversity in the Mkuzi population can be explained by the recent decline in this population followed by a prolonged bottleneck that lasted roughly 10 generations. Effective population sizes are always smaller than census population sizes (Li 1976). Available data suggest that a skewed sex ratio does not play an important part in causing a low effective population number. The population sex ratio appears to be close to unity, as 52% of the population in the Hluhluwe-Umfolozi Park were males (Hitchins and Anderson 1983; Hitchins and Brooks 1986) and 55% of East African animals sighted were males (Goddard 1970; Schenkel and Schenkel-Hulliger 1969): these figures did not differ significantly from an equal sex ratio. The operational sex ratio also does not appear to be skewed since black rhinoceroses are mostly solitary animals with the only significant grouping being cow/calf combinations, and male home ranges do not determine those of females (Goddard 1967): a harem effect therefore does not appear to play a role in reducing the effective population number. Present evidence (Goddard 1967, 1970; Hitchins and Anderson 1983; Hitchins and Brooks 1986; Schenkel and Schenkel-Hulliger 1969) indicates that both sexes mature at ages varying between 5 and 10 years and that about 20% of the census population is immature. We therefore assume that variance in reproductive rate and fluctuations in population size are the most important factors influencing effective population sizes of black rhinoceros populations. Mean calving interval for the Hluhluwe-Umfolozi Park is some 42 months (Hitchins and Anderson 1983), which is

similar to the figure of 40.4 months for animals in captivity (Smith and Read 1992).

Very little is known on the variance of reproductive success in wild black rhinoceroses. Variance in reproductive success of only three cows in the Addo Elephant National Park was 2.7 (Hall-Martin and Penzhorn 1977). Variance of reproductive success of 19 captive cows (Klös and Frese 1991) was 3.6 after extrapolation for cows that have not lived through their whole reproductive life. If pre-reproductive animals constitute 0.2 of the population and the variance in reproductive output is 3.5, $N_e$ is 0.58 of the census population size. This figure is consistent with estimates of reduction in effective population size due to variance in reproductive rate of a number of free-living species (Crow and Morton 1955). The simulated gene diversity was also not significantly affected by the initial population size. The calculations suggested mean gene diversities at 1990 of 0.030 for the Mkuzi population and 0.044 for the Hluhluwe-Umfolozi population (Figure 3). These figures do not differ statistically from the observed values. We do not consider the simulation as a quantitative, predictive tool but merely as a heuristic aid to understand why the Natal black rhinoceros populations still have a significant amount of genetic variation, even after a marked population bottleneck.

Merenlender et al. (1989) invoked "recent demographic bottlenecks coupled with possible prehistoric bottlenecks" to explain the lack of genetic variation among the rhinoceroses they assayed for genetic variation. Our results suggest that this does not apply to all the African populations of black rhinoceros and that their results apply to *D. b. michaelli*, the east African black rhino race. Our results also indicate that further genetic investigation of the east African populations is required for a fuller understanding of the genetic variation among extant black rhinoceroses. Our results, combined with those of Dinerstein and McCracken (1990), are real-life examples of the fact that population bottlenecks by themselves do not necessarily reduce genetic variation dramatically: this effect is only obtained if the bottleneck extends over at least several generations.

Genetic variation in several southern African populations resembles that of several outbred mammal species and largely represents heterozygosity levels before the precipitous decline in black rhinoceros numbers in Africa which took place during the last century. Furthermore, knowledge of the population sizes in each area allows us to predict heterozygosity levels resembling that were measured during the present study. This knowledge affects captive breeding attempts. If black rhinoceroses had the same low levels of genetic variation as cheetahs, one approach to captive breeding would be to merely select for the best breeders, regardless of origin or genotype. However, the existence of significant genetic variation adds an additional perspective to the captive breeding of black rhinoceroses since the preservation of as much genetic variability as possible now needs to be included in these programs. The preservation of genetic variation in wild rhinoceroses also needs to be considered in the management of this species. However, these considerations can only be secondary to the importance of free-living rhinoceroses surviving the present onslaught of poaching.

**References**


Ashley MV, Melnick DJ, and Western D, 1990. Conservation genetics of the black rhinoceros (*Diceros bicornis*): 1. Evidence from the mitochondrial DNA of three populations. Conserv Biol 4(1):71–77.

Bonnell ML and Selander RK, 1974. Elephant seals: genetic variation and near extinction. Science 184:908–909.

Clegg MT and Brown AHD, 1983. The founding of plant populations. In: Genetics and conservation (Schoenewald-Cox CM, ed). Menlo Park, California: Benjamin Cummings; 216–228.

Conway AJ and Goodman PS, 1989. Population characteristics and management of black rhinoceros *Diceros bicornis minor* and white rhinoceros *Ceratotherium simum simum* in Ndumu Game Reserve, South-Africa. Biol Conserv 47:109–122.

Crow JF and Morton NE, 1955. Measurement of gene frequency drift in small populations Evolution 9:202–214.

Cumming DHM and du Toit R, 1986. Rhino poaching in Zimbabwe. Pachyderm 6:18.

Cumming DHM, du Toit RF, and Stuart SN, 1990. African elephants and rhinos· status survey and conservation action plan. Harare: IUCN/SSC African Elephant and Rhino Specialist Group

Dinerstein E and McCracken GF, 1990. Endangered greater one-horned rhinoceros carry high levels of genetic variation. Conserv Biol 4:417–422.

Findlay FRN, 1903. Big game shooting and travel in South-East Africa. London: T Fisher Unwin.

Fitzsimmons FW, 1920. Natural history of South Africa. London: Longmans Green.

Franklin IR, 1980. Evolutionary changes in small populations. In: Conservation biology: an evolutionary-ecological perspective (Soulé ME and Wilcox BA, eds) Sunderland, Massachusetts: Sinauer.

Goddard J, 1967. Home range, behaviour and recruitment rates of two black rhinoceros populations East Afr Wildl J 5:133–150.

Goddard J, 1970. Age criteria and vital statistics of a black rhinoceros population. E Afr Wildl J 8:105–121

Groves CP, 1967. Geographic variation in the black rhinoceros *Diceros bicornis* (L., 1756). Z Saugetierkunde 32:267–276.

Hall-Martin AJ and Penzhorn BJ, 1977. Behaviour and recruitment of translocated black rhinoceros *Diceros bicornis*. Koedoe 20:147–162.

Harris H and Hopkinson DA, 1976. Handbook of enzyme electrophoresis in human genetics. Amsterdam: North/Holland.

Hartl DL, 1988. A primer of population genetics. Sunderland, Massachusetts: Sinauer.

Hedrick PW, 1984. Population biology: the evolution and ecology of populations. Boston: Jones and Bartlett.

Hitchins PM and Anderson JL, 1983. Reproduction, population characteristics and management of the black rhinoceros *Diceros bicornis minor* in the Hluhluwe/Corridor/Umfolozi Game Reserve Complex. S Afr J Wildl Res 13:78–85.

Hitchins PM and Brooks PM, 1986. Preliminary report on the population size and trend (1973–1985) of the black rhinoceros in the Hluhluwe and Umfolozi Game Reserves, 1985. Mimeographed report to Natal Parks, Game and Fish Preservation Board, P.O Box 662, Pietermaritzburg, 3200, South Africa.

Klös HG and Frese R, 1991. International studbook of African rhinoceroses. Berlin: Zoologischer Garten Berlin

Lacey G, 1899. Pictures of travel, sport and adventure. London: C. Arthur Pearson

Lande R and Barrowclough GF, 1987. Effective population size, genetic variation, and their use in population management. In: Viable populations for conservation (Soulé ME, ed). Cambridge: Cambridge University Press; 87–124

Leader-Williams N, 1988. Patterns of depletion in a black rhinoceros population in Luangwa Valley, Zambia. Afr J Ecol 26:181–187

Li CC, 1976. First course in population genetics. Sunderland, Massachusetts: Boxwood.

Merenlender AM, Woodruff DS, Ryder OA, Kock R, and Vahala J, 1989 Allozyme variation and differentiation in African and Indian rhinoceros. J Hered 80(5):377–382

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

Nei M, 1987. Molecular evolutionary genetics. New York. Columbia University Press.

Nei M and Graur D, 1984. Extent of protein polymorphism and the neutral mutation theory. Evol Biol 17: 73–118.

Nei M and Roychoudoury AK, 1974. Sampling variances of heterozygosity and genetic distance. Genetics 76·379–390.

Nevo E, 1984. Genetic diversity in nature Evol Biol 17: 217–246.

O'Brien SJ, Wildt DE, Goldman D, Merril CR, and Bush M, 1983. The cheetah is depauperate in genetic variation. Science 221:459–462.

Osterhoff DR and Keep ME, 1970. Natural variation in the blood proteins of white and black rhinos. Lammergeyer 11:50–53.

Ryder O, Brisbin P, Bowling A, and Wedemeyer E, 1981. Monitoring genetic variation in endangered species. In: Proceedings 2nd International Congress Systematic Evolutionary Biology (Scudder G and Reveal J, eds). Pittsburgh: Hunt Institute for Botanical Documentation; 417–424.

Schenkel R and Schenkel-Hulliger L, 1969 Ecology and behaviour of the black rhinoceros (*Diceros bicornis*): a field study. Hamburg: Paul Parey

Selander RK and Johnson WE, 1973. Genetic variation among vertebrate species. Annu Rev Ecol Syst 4·75–91.

Selous FC, 1908. African nature notes and reminiscences. London: Macmillan.

Smith RL and Read B, 1992. Management parameters affecting the reproductive potential of captive, female black rhinoceros, *Diceros bicornis*. Zoo Biol 11:375–383.

Sokal RR and Rohlf FJ, 1981. Biometry. San Francisco W. H. Freeman.

Soulé ME, 1980. Thresholds for survival. maintaining fitness and evolutionary potential. In: Conservation biology. an evolutionary-ecological perspective (Soulé ME and Wilcox BA, eds). Sunderland, Massachusetts: Sinauer

Stratil A, Bobak P, Kalab P, Cizova D, and Pokorny R, 1990. Serum proteins of rhinoceros. inter- and intraspecific variation. Comp Biochem Physiol 95B(4):803–810.

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