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A POPULATION VULNERABILITY ANALYSIS OF SOUTH AFRICAN BLACK RHINOCEROS *DICEROS BICORNIS*

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A population vulnerability analysis of South African black rhinoceros *Diceros bicornis*

by

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To my mother

ABSTRACT

A population vulnerability analysis of South African black rhinoceros *Diceros bicornis*

by

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To determine the vulnerability of black rhinoceros populations in South Africa it was necessary to study all aspects of population biology. Since most of the remaining black rhinoceros populations are small the emphasis was on the genetics and demography of these populations. The aim was to identify those factors that have a negative effect on the effective population size. Thirty protein and enzyme loci of four black rhinoceros populations were studied using starch and polyacrylamide gel electrophoresis. All four populations still carry high levels of genetic variation which is reassuring for the long-term survival of the species. A genetic polymorphism of the erythrocyte enzyme glucose-6-phosphate dehydrogenase was observed. Evidence suggest that a deficiency of this enzyme is associated with intravascular haemolysis similar to the situation in humans. Since haemolytic anaemia is the major cause of rhinoceros mortality in captivity, stress and the administration of some drugs, may induce haemolytic anaemia in G-6-PD deficient rhinoceroses. A demographic extinction model (DEMM) was developed to project the persistence time of South African black rhinoceros populations. The model uses a Leslie matrix

with age-specific fecundity and mortality data and was written in Pascal. Other extinction models (Vortex and Goodman) based on the small population paradigm were also used to project the persistence time of different black rhinoceros populations and the results compared to that of DEMM. A population persistence analysis model (PPA) which is based on the declining population paradigm was used to predict persistence time for black rhinoceros populations. PPA is useful in identifying the first stages of a population decline and because the model uses census data (which is easy obtainable), the model is accessible to managers. Poaching, haemolytic anaemia and captive breeding are a few of the problematic issues that emerged from this project and valuable management reccommendations are made reagarding these.

To my mother

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CHAPTER 1

INTRODUCTION:

CONSERVATION BIOLOGY

The world is on the threshold of a catastrophic loss of biological diversity. This loss of diversity is caused by : 1) destruction and fragmentation of some habitats and pollution and degradation of others, 2) overkilling of plants and animals by humans, 3) introduction of alien animals and plants and 4) secondary effects of extinctions - the extinction of one species caused by the extinction of another (Western *et al.* 1989). The number of species (as estimated in 1982) ranges from three to five million species and conservationists have to find convincing arguments to conserve and protect the multitude of wild taxa (Huntley 1989). As humans we have duties to protect biodiversity, especially threatened species, not for the sake of the species themselves as such but rather for future human beings (Rolston 1985).

Conservationists strive to maintain diversity within natural systems. This has become increasingly difficult (Soulé 1987), since the human population explosion has established political and economic behaviour which conflict with conservation policies and goals (Soulé 1985). Conservation in the past focused on proclaiming parks and reserves and protecting the animals and plants inside these proclaimed areas but, since 1982, conservation entered the arena of conventional science when conservation biology came to life (Huntley 1989).

There has been much controversy surrounding the justification of protecting natural diversity (Huntley 1989), and prejudice against conservation biology because it is an applied as well as a pure science (Soulé & Wilcox 1980). Conservation biology focuses the knowledge and tools of all biological disciplines, from molecular biology to population biology, on one issue - nature conservation (Soulé & Wilcox 1980). The aims of conservation biology are to provide scientific conservation principles, identifying conservation problems, establishing corrective procedures and to bridge the gap between theory and management (Western 1989). Conservation biology is mission-oriented and therefore ethics cannot be excluded from this multidisciplinary science (Soulé 1985). Conservation biology has two threads: 1) the small population paradigm which deals with the effects of smallness on the persistence of a population (Soulé & Wilcox 1980) and 2) the declining population paradigm which deals with the cause of this smallness and with the prevention of this state (Caughley 1994).

In short, the aims of conservation biology entail not only to preserve nature, but also to avoid extinctions and to restore ecological damage (Western 1989). Vrijenhoek (1989) concludes that human disturbance artificially reduces gene flow by fragmenting habitats and preventing migration and for these reasons, humans should intervene by re-establishing gene flow. On the other hand, ill-considered hybridisation between genetically discrete subpopulations may lead to outbreeding depression because of differentially adapted genotypes (Vrijenhoek 1989). Distinct subpopulations are the product of naturally divergent processes which may contribute to local adaptation, and which thus need to be maintained (Vrijenhoek 1989). Nevertheless, care should be taken when identifying different groups and subpopulations.

Evidence suggests that the large herbivores and large carnivores are the most vulnerable components of an ecosystem due to their vast requirements (Eisenberg & Harris 1989). To preserve larger organisms, large areas must be set aside and the conservation of smaller organisms will often occur simultaneously. It is economically more viable to conserve a species in nature than in captivity. For instance, the cost of maintaining viable populations of five primate species in North American zoos is the same as that of maintaining the entire Serengeti ecosystem (Woodruff 1989).

It is necessary to study all disciplines of population biology to shed light on conservation issues to prevent extinction (Soulé & Wilcox 1980). Population genetics and population ecology are the two main themes of population biology (Elseth & Baumgardner 1981) and to find proper solutions for conservation problems, both the genetics and ecology of populations must be understood. Population genetics deals with the genetic changes that occur within and between populations (Hartl 1988). These changes result in genetic variation, which is a necessary condition for evolution (Ayala 1982). Population ecology deals with the relationships between a population and the environment, which determines its size and internal organization with the focus on the number of individuals as determined by demographic parameters (Elseth & Baumgardner 1981).

Extinction occurs when the genetics and/or the ecology of a population are disturbed. Factors contributing to the extinction of a local population are: 1) island formation, 2) isolation, 3) predation, 4) competition, 5) habitat destruction and 6) poaching (Soulé 1983). Soulé (1983) describes the intrinsic factors that become operative when the population size has been reduced as follows: 1) demographic stochasticity, 2) behavioral dysfunction and 3) genetic deterioration. The ultimate test of conservation biology is the application of its theories in management situations (Soulé 1986). Numerous authors have set directives in conservation biology on

how to approach a conservation problem, how to analyse it and how to take appropriate management action for the conservation of a species (Western *et al.* 1989). When a project is undertaken to conserve a species, emphasis should be placed on the practical implications of management recommendations that result from the scientific investigations.

Although controversial, six subspecies of the black rhinoceros, *Diceros bicornis* are recognized: *D. b. brucii* - Ethiopia and Somalia, *D. b. longipes* - Cameroon, *D. b. chobiensis* - Angola, *D. b. michaelii* - Kenya and Tanzania, *D. b. bicornis* - Namibia and *D. b. minor* - Kenya to South Africa (Cumming *et al* 1990). The latest estimates of black rhinoceros numbers in Africa are as follows: *D. b. bicornis* = 519, *D. b. longipes* = 35, *D. b. michaeli* = 489 and *D. b. minor* = 1413 (SSC-ARSG\RHNO-STA 1992). Black rhinoceros numbers have declined by 95% over the past 20 years, from approximately 65000 in 1970 to fewer than 3500 in 1990 (Balfour & Balfour 1991). The black rhinoceros is now threatened with extinction, mostly due to man and his actions, especially poaching. The severity of poaching is clearly illustrated by the decrease in rhinoceros numbers in Zimbabwe. In 1990, the black rhinoceros estimate was 1600 animals, with only 425 surviving in 1992 (SSC-ARSG\RHNO-STA 1992). During 1985 alone, a minimum of 71 rhinoceroses were killed in the Zambezi Valley by well-armed poachers that crossed the Zambezi river. Despite a new clause (November 1985) in the the Parks and Wild Life Amendment Bill, specifying mandatory sentences of imprisonment and fines for killing rhino, the poaching continued. In 1985, the Zimbabwean Ministry of Natural Resources and Tourism announced their intention to recruit armed game scouts, of which the first 100 were ex-combatants, to actively protect the rhinos. They also launched a fund-raising campaign for purchasing equipment to assist the anti-poaching campaign, but still poaching did not stop (Du Toit & Cumming 1986).

At present, 53% of all *D. b. minor* individuals are located in South Africa. This emphasises the importance of conservation of the black rhinoceros in this country. Kenya and Zimbabwe are no longer the strongholds of the black rhinoceros. To protect this species from extinction, urgent conservation action must be taken that addresses: 1) the decreasing population sizes, 2) fragmentation and 3) genetic similarity.

AIMS and OBJECTIVES:

The aim of this project is to determine the vulnerability status of the South African black rhinoceros (*Diceros bicornis*) populations and captive populations worldwide. Informed management recommendations can only be made by studying genetic variation within and between populations and by investigating the demography and ecology of each population. This will be achieved by:

1) Analysis of plasma and red blood cell enzymes of the different black rhinoceros populations in Southern Africa by using protein electrophoresis. This will give an indication of the amount of genetic variation present within each population and between the different populations. The distribution of genetic variation can give an indication of how much genetic variation has been lost due to population bottlenecks, genetic drift and poaching. The amount of genetic variation can also yield information on the restriction of gene flow between different populations that has occurred as a result of population subdivision. The potential of a population to adapt to changing environmental conditions can be influenced negatively by low levels of genetic variation. To compensate for very low levels of genetic variation, it might be necessary to introduce genetically variable animals into some of the extant populations.

2) By reviewing literature on the life-histories of different wild black rhinoceros

populations, it will be possible to understand their demography and to identify demographic factors that may cause a decline in the population size.

3) Since it is essential to establish a viable captive population of this endangered species, the genetics and demography of captive bred black rhinoceroses need to be studied and compared with the situation in the wild. This will enable one to identify problems that may exist in the captive breeding of black rhinoceroses.

4) The construction of a demographic computer model that will enable an estimate of the vulnerability of a population given certain parameters and then to project the survival time of each population.

CHAPTER 2

GENETIC VARIATION

INTRODUCTION:

The large human population and associated industrialization are causing deteriorating environmental conditions (Smith *et al.* 1976) including habitat destruction, fragmentation and poaching. Consequently the remaining populations of many species, including the black rhinoceros, are small and this raises management and conservation questions.

Small populations are challenged by intrinsic factors (random variation of genetic and demographic events within the population occurring without reference to environmental events), as well as extrinsic factors (environmental events acting on the genetics and demography of a population) (Ballou 1991). The loss of genetic variation is the primary genetic consideration. Measurement of genetic variation can be accomplished by studying single-locus variation as well as quantitative variation. With single-locus variation it is possible to monitor the levels of genic variation for several different classes of loci; blood group antigens as well as restriction sites in mitochondrial and nuclear DNA. However, the most easily measured genetic polymorphisms are the structural gene polymorphisms revealed by protein electrophoresis (Lande & Barrowclough 1987). The level of heterozygosity in a managed population, may actually be a reflection of demographic events that the source population experienced in the very distant past. A trend of loss of this variability would be easily observed and could serve as a warning to managers that selection or

random genetic drift is taking place in their stock (Lande & Barrowclough 1987). The level of genetic variation for quantitative traits can be monitored through heritability studies. Some of the phenotypic variation in a population has an additive genetic component. Narrow-sense heritability is the ratio of the additive genetic variance to the total phenotypic variance and can therefore be used to monitor the level of quantitative genetic variation in a population. In zoological gardens it is relatively easy to monitor the heritability of representative quantitative traits, such as size, because pedigrees are known for many animals (Lande & Barrowclough 1987).

Genetic variation in a population can be reduced by: 1) genetic drift and 2) inbreeding.

1) **Genetic drift**: In every generation the alleles of the parents are passed to their offspring and these alleles are a random sample of the alleles of the parents. In a small population, each random sample of alleles is a small sample and represents only a fraction of the genes of the parental generation. Some of the genetic variation present in the parents may by chance not get passed on to the offspring. Genetic drift therefore results in temporal fluctuations of the genetic characteristics of a population (Ballou 1991).

2) **Inbreeding**: Inbreeding occurs as a consequence of the geographic division of a population into a number of isolated subpopulations or through the choice of mates according to phenotype or genetic relationship (Selander 1983). Since relatives share many of the same alleles, they have lower levels of genetic diversity. The rate of loss of genetic variation is a function of the effective population size (N_e).

Numerous studies have shown that inbreeding can significantly reduce reproduction and survival in a wide variety of species and inbreeding depression results from two effects: 1) the increase in homozygosity allows deleterious recessive alleles in the

genome to be expressed and 2) reduced heterozygosity, caused by inbreeding, can reduce the fitness of the inbred individuals in cases where heterozygous individuals are more fit than homozygous individuals (Ballou 1991). The loss of genetic diversity can result in decreased long-term survival because future natural selection depends on genetic variation (Clegg & Brown 1981, Hartl 1988). Since small populations are more susceptible to loss of genetic variation (Franklin 1980, Allendorf 1986), they are therefore also more susceptible to extinction (Shaffer 1981, Soulé 1986, 1987).

The disadvantageous effects of inbreeding in nature have been illustrated in a number of species. The uniform homozygosity in the northern elephant seal (*Mirounga angustirostris*) apparently makes them less able to adapt to changing environmental conditions (Bonnell & Selander 1974). The cheetah (*Acinonyx jubatus*) is an example of a species which is highly monomorphic at the protein level. O'Brien *et al.* (1985) examined 50 allozyme loci, none of which were polymorphic. In evolutionary terms this means that they lack genetic variation that is a pre-requisite for selection. Any environmental change or disease can result in a serious decline in population numbers as illustrated by the disease the cheetahs contracted. During 1983, 90 % of the cheetahs in Oregon contracted feline infectious peritonitis, possibly because they lacked genetic variants which could confer disease resistance to these animals (O'Brien *et al* 1985). Nevo (1984) found that the fitness of individuals in ecologically uncertain environments appears to depend on their level of heterozygosity.

A population may experience demographic stochasticity, environmental stochasticity, natural catastrophes and genetic stochasticity. Under these conditions, a population is vulnerable and can become extinct (Shaffer 1981). Demographic factors such as population size and reproduction are important for short-term conservation

(Lande 1988), while the maintenance of genetic variation is vital for the long-term survival of the population (Lande & Barrowclough 1987).

Aims:

Population bottlenecks and inbreeding have negative effects on many species, the aim of this chapter therefore is to monitor the level of genetic variation to determine whether black rhinoceros populations have reduced genetic variation.

The level of genetic variation in black rhinoceros populations determines whether the emphasis should be on demographic or genetic management to conserve this species. Levels of genetic variation in a population can be increased by translocations which replace natural migrations to re-establish gene flow between different populations that existed in the past. If high levels of genetic variation exist, there is no need for intensive management. If genetic variability is non-existent, then the emphasis should rather be placed on ecological management to ensure that the population does not experience any demographic risk which may result in extinction.

2.1 GENETIC VARIATION

Genetic variation is a prerequisite for the evolutionary process (Ayala 1982, Hartl 1988, Hedrick 1984, Lande & Barrowclough 1987) and the chances for an organism to adapt to environmental changes are greater if genetic variation is high (Clegg & Brown 1981; Lande & Barrowclough 1987). It is important to define genetic variation since it comprises several facets. Lande & Barrowclough (1987) consider quantitative genetic variation as measured by narrow-sense heritability. Additionally, these authors also explain the maintenance of genetic variation by equilibrium and non-equilibrium approaches. The equilibrium approach centres on how much

genetic variation of a given type is maintained in a population with a certain spatial and temporal structure. The non-equilibrium approach focuses on how long it will take to lose genetic variation by genetic drift, how much loss can be tolerated and whether variation can be regained by mutation in a large population (Lande & Barrowclough 1987). All genetic information distributed among an interbreeding group of individuals collectively forms a gene pool (Mettler & Gregg 1969). The genotypes that develop into individuals of one generation result from the union of gametes produced by the preceding generation. The advantage of subdivision of a population into small, semi-isolated demes gives the best chance for the populations to explore the full range of their adaptive topography (surface of mean fitness plotted as a function of allele frequencies) (Hartl & Clark 1989).

The maintenance of genetic variation within a managed population depends on the duration of the conservation programme and the generation time (Soulé *et al.* 1986). Genetic management is also affected by the need for intra- or interpopulation diversity (Melnick & Western 1987). Ralls & Ballou (1986) state that small populations face genetic problems related to inbreeding which will result in increased juvenile mortality. The maintenance of genetic diversity is thus important and has been emphasized by many authors (Frankel 1974; Greig 1979; Soulé 1980, 1985, 1987; Lande 1988).

There is an urgent need for the genetic management of the remaining rhinoceros populations (Merenlender *et al.* 1989) in order to understand the genetic composition of each and the difference between populations. The first step in genetic management is to assess levels of genetic variability (Smith *et al.* 1976) to determine intra- and interpopulation variation, and to devise a sound conservation strategy based on these findings.

2.2 MEASURES OF GENETIC VARIATION:

Heterozygosity

The most widespread measure of genetic variation within a population is average **observed** heterozygosity in that population (Hedrick 1984) and this measure is convenient for identifying levels of genetic variability in captive and natural populations of endangered species. In self-fertilizing populations and populations where matings between relatives are common, observed heterozygosity does not reflect the amount of genetic variation accurately. A major objective in genetic conservation should be to maintain high levels of heterozygosity. Only additional introductions of conspecific individuals can increase the amount of genetic variation within a population (Smith *et al.* 1976).

Gene diversity

By calculating the **expected** heterozygosity, the amount of genetic variation can be calculated more accurately (Ayala 1982). Nei (1987) defines gene diversity/**expected** heterozygosity (H) as the expected proportion of heterozygotes per locus in a random mating population. Gene diversity is therefore the expected proportion of heterozygous loci in a randomly chosen individual (Nei 1987). Gene diversity is the preferred measure of genetic variation because of its statistical tractability (Nei 1978), enabling calculation of confidence limits to the estimates of gene diversity.

Number of alleles per locus

Leberg (1992) found that the numbers of polymorphic loci and number of alleles per

locus are more sensitive indicators of differences in genetic diversity between pre-bottleneck and post-bottleneck populations than multilocus heterozygosity.

2.3 PROTEIN POLYMORPHISM

To get an indication of genetic variation a large number of loci need to be studied because 60 - 70% of protein loci show no genetic variability (Nei 1987). Two major opposing theories exist regarding the determinants of protein polymorphism. They are: 1) the Neutral theory of molecular evolution and 2) Selectionism.

Neutral theory of molecular evolution:

Kimura proposed the neutral theory of molecular evolution in 1968 and according to this theory the great majority of evolutionary mutant substitutions are not caused by positive Darwinian selection but by random fixation of selectively neutral or nearly neutral mutants (Kimura 1968). Kimura (1983) claims that intraspecific genetic variability at the molecular level, such as manifested in the form of protein polymorphisms, is selectively neutral or naerly so and maintained in the species by the balance between mutational input and random extinction or fixation of alleles. The effective population size (N_e) and mutation rate per locus (v) are the key parameters determining the level of protein polymorphism (heterozygosity or gene diversity) in a population (Kimura & Ohta 1971).

The interaction of expected single-locus gene diversity with effective population size is expressed by: $H = 4N_e v / (1 + 4N_e)$ (Infinite alleles model).
where H = the expected heterozygosity and v = the mutation rate for a locus each generation (Nei 1987). Nei & Graur (1984) used data from 77 different species and

concluded that available data on protein polymorphisms are most easily explained by the neutral theory of molecular evolution where the effects of bottlenecks are taken into account.

Selectionism:

In contrast, supporters of selectionism argue that the various forms of selection, such as balancing selection, maintain molecular variation (Hedrick 1984). Nevo (1984) conducted several allozyme studies on invertebrates and found some cases in which heterozygosity is not a function of the effective population size, and in which genetic diversity is partly correlated to and predicted by ecological factors. In the genetic patterns observed by Nevo (1984), natural selection appears to be a differentiating and orienting force of evolutionary change in protein and DNA polymorphisms. Nevo suggested that ecological factors explain 90 % of the genetic variance, while demography and life history account for the remaining 10 %. Selectionists regard protein polymorpisms as adaptive and that it is maintained by balancing selection (Hedrick 1984).

When studying the amount of genetic variation (heterozygosity in particular), it is evident from these two theories that the following factors should be taken into account: N_e, mutation rate, ecological factors, genetic variation to give a complete picture in the biological conservation of a species. The results of Ward *et al.* (1992) indicate that a substantial proportion of the variation in mean heterozygosity between populations can be accounted for statistically by enzyme- or taxon-specific effects. This finding is consistent with both neutral and selection theories by assuming that the degree of constraint varies in an enzyme- or taxon-specific way.

2.4 HETEROZYGOSITY AND FITNESS

The relationship between observed heterozygosity and fitness is controversial and has far reaching applications, particularly in the field of conservation genetics. Mitton & Grant (1984) suggest a positive correlation between heterozygosity and fitness while others like Hedrick (1984) oppose this view. Fitter individuals are predicted to increase in frequency with time and will eventually replace the less fit ones (Krimbas 1984). Fitness can thus be viewed as a relative or absolute measure of reproductive efficiency or reproductive success. Koehn *et al.* (1983) use the term "genotypic fitness" to describe differential survival and reproduction among genotypes. If there are no differences in fitness among the genotypes at a locus, the evolution of that locus is determined by mutation and genetic drift.

In some species, vigour and fecundity decline at a rate proportional to the level of homozygosity and that, in turn, is inversely related to population size (Turelli & Ginzburg 1983). Correlations between protein heterozygosity and growth rate were reported in the American oyster (*Crassostrea virginica*), blue mussel (*Mytilus edulis*) and tiger salamander (*Ambystoma tigrinum*). In the white-tailed deer (*Odocoileus virginianus*), correlations involving individual heterozygosity, weight, fecundity and fetal growth exist (Mitton & Grant 1984). In domesticated animals, for example sheep, individuals heterozygous for isocitrate dehydrogenase grow 10 % faster than homozygotes. Pigs, heterozygous at most loci, were also examined for growth and food consumption and it was found that they gained more weight while consuming less food than pigs with homozygous loci. A correlation also exist between the level of polymorphic loci and fetal growth in humans where babies that weigh less, have less polymorphic loci (Mitton & Grant 1984).

The associations between heterozygosity and fitness measures, such as developmen-

tal stability and growth rate, must reflect the underlying influences of enzyme polymorphisms upon metabolism (Mitton & Grant 1984). These authors suggest several explanations of why variation in the number of heterozygous proteins is related to developmental stability, growth rate and oxygen consumption, i.e.:
1) The enzymes are accidentally linked to genes that affect growth and development.
2) Isozymes typically exhibit different kinetic characteristics - these differences affect the flow of energy through metabolic pathways and thereby influence growth, development and oxygen consumption.

A small number of loci cannot accurately be used to determine the level of heterozygosity within a population (Chakraborty 1981). Metabolic pathways chosen in electrophoretic surveys are likely to affect estimates of heterozygosity. Different groups of proteins (structural, storage, nonmetabolic and regulatory) have different levels of genetic variation and proteins with different qauternary structures and subunit sizes vary regularly in their levels of genetic varition. Heterozygosity at a dozen enzyme polymorphisms is much more likely to estimate enzyme heterozygosity within one or two metabolic pathways than at several thousand variable loci. This suggest that metabolic pathways sampled in electrophoretic analyses are likely to influence variability in growth rates (Mitton & Grant 1984). Enzymes are protein catalysts that control the flow through metabolic pathways. Many enzymes in natural populations are polymorphic and for those enzymes whose kinetics have been investigated, there are typical differences in performance among genotypes (Mitton & Grant 1984).

Mitton & Grant (1984) admit that there are a few exceptions, for example in young oysters and pine seedlings, heterozygosity and mean growth rates are associated, but not in adults of these species. The surplus energy of larval oysters and pine seedlings is put into growth whereas the surplus energy in adults is partitioned between repro-

duction and growth. However, the authors still agree that the relationships between protein heterozygosity and both growth rate and development seem to be the rule.

In contrast, Hedrick *et al.* (1986) argue that ecological measures such as rate of increase, productivity and mortality give an indication of the potential of that population to adapt to environmental changes. The authors conclude that homozygotes at allozyme loci may be less fit than allozyme heterozygotes when inbreeding is present, because they are more likely to be homozygous for linked detrimental alleles. Hedrick (1984) and Hedrick *et al.* (1986) state that there may be a relationship between fitness and heterozygosity, but it is difficult to substantiate the cause-effect relationship.

2.5 SUBDIVIDED POPULATIONS

Natural populations are often divided into subpopulations and display differences in allelic and genotypic frequencies depending. Population subdivision leads to an increase in homozygotes and a decrease in the proportion of heterozygous genotypes, an inbreeding-like effect (Hartl 1988). The extent of genetic differentiation needs to be studied in subdivided populations to determine the amount of genetic variation and whether a population is experiencing a local depletion of genetic variation, because of fragmentation, that can lead to inbreeding depression (Nei 1987).

The following theoretical models were developed to investigate the effects of geographical stucture of populations: 1) isolation-by-distance model (Wright 1943), 2) stepping-stone model (Kimura & Weiss 1964), and 3) the island model (Wright 1943, Maruyama (1970) and Nei *et al.* (1977).

In the isolation-by-distance-model, individuals are continuously distributed throughout an area. A finite distance exists between birth- and breeding site, therefore it will take a number of generations for genes to flow across the range of the species. The population is subdivided into "neighbourhoods" and random mating takes place (Wright 1943). A large amount of local differentiation takes place if the effective number in the neighbourhoods is of the order of 20, and a negligible amount if neighbourhood is larger than 1000. According to the stepping-stone model there is no continuous distribution of the species. The distribution of individuals is colonial and gene flow exist between different colonies. However, a significant reduction in genetic variation is inevitable (Kimura & Weiss 1964). The island model was developed by Wright (1943). Large number of colonies exist and each exchanges a fraction, m, of its individuals each generation randomly with an immigrant gene pool made up from contributions from all colonies. This model could be appropriate for zoo populations and reserves where gene flow is mediated by managers.

F-statistics were developed in terms of one locus with two alleles (Wright 1931). The F-statistics can be thought of as correlations between uniting gametes and are related in the following manner:

$$(1 - F_{IS})(1 - F_{ST}) = (1 - F_{IT})$$ (Wright 1969).

F_{IS} is a measure of inbreeding that takes into account the effects of nonrandom mating within subpopulations (the correlation of genes within individuals within a subpopulation) and (F_{IT}) measures the inbreeding within individulas relative to the total population. The F_{ST}-value is concerned with inbreeding of subpopulations relative to the total population (Wright 1931). The fixation index (F_{st}) was calculated by using the formula:

$$F_{ST} = (H_T - H_S) / H_T$$

where H_t is the expected heterozygosity of the total population and H_s is the mean

expected heterozygosity of the subpopulations (Hartl & Clark 1989).

Analysis of G_{ST} (the multiple allelic equivalent of F_{ST}), enables one to study gene diversity of the total population and the subpopulations. G_{ST} is not related to genotypic frequencies except in randomly mating populations (Nei 1987). Expected G_{st} is estimated by using the following formula:

$$G_{ST} = 1 / (4Nmt + 1)$$

where N = the number of individuals in a subpopulation, m = the fraction of genes that are from migrants, n = the number of subpopulations and $t = [n/(n - 1)]^2$ (Hartl & Clark 1989). Gene diversity values can be calculated by the formula:

$$G_{ST} = D_{ST}/H_T \text{ (eq. 8.27 in Nei 1987),}$$

where H_t = the gene diversity in the total population, D_{ST} = mean pairwise genetic differences between populations.

A large number of loci need to be studied to get a general picture of gene differentiation among subpopulations. It is important to note that if the number of individuals sampled in each subpopulation is small (less than 50 individuals), certain biases may arise in the measurement of gene diversities but unbiased estimates exist (Nei 1987).

The black rhinoceros (*Diceros bicornis*) is no exception to population subdivision and this is an important factor for the conservation of the species. Black rhinoceroses are scattered in parks and reserves in Africa in very small populations mostly due to human interference. The populations are fragmented and isolated since migration is impossible. This can lead to the depletion of genetic variation if no corrective management action is taken. Intra- and interpopulation genetic variation are both important for the genetic conservation of the black rhinoceros. An example of two Asian primate species, the rhesus monkey and the long-tailed macaque illustrates

intra- and interpopulation variation (Melnick & Western 1987). Only 5 % of the genetic diversity found among rhesus monkeys across Asia can be attributed to differences between animals from different regions (interpopulation diversity). The remaining 95 % of species diversity is represented by intrapopulation diversity that can be found in any single region. In contrast, 41 % of the genetic diversity found among long-tailed macaques can be attributed to differences between regional populations. A strategy devised to conserve the greatest amount of genetic diversity in these primate species would thus entail the conservation of many more regional populations of the long-tailed macaque than the rhesus monkey (Melnick & Western 1987).

2.6 MEASUREMENT OF LARGER GENETIC DIFFERENCES BETWEEN POPULATIONS

Conservation of biodiversity requires taxonomic knowledge and therefore it is important to determine the genetic similarity of different populations within a species. Electrophoretic studies provide data in the form of genotypic frequencies which can be used to calculate allele frequencies (Ayala 1982). In order to estimate the genetic differentiation between two populations, a random sample of proteins must be assayed. The results obtained from a survey of a large number of gene loci can then be extrapolated to the whole genome. The statistic that is used in estimating genetic differentiation between populations is genetic distance (Ayala 1982).

Genetic distances (D) were calculated using the formulae of Nei (1972, 1987). Genetic identity (I) of genes from different populations (X and Y), with respect to one locus, is defined as:

$I_j = j_{XY} / (j_X j_Y)^{1/2}$ and identity between different populations respect to all loci, = $J_{XY} / (J_X J_Y)^{1/2}$, where J_{XY}, J_X and J_Y are the arithmetic means of j_{XY}, j_X and j_Y over

all loci respectively, j_{XY} is the probability of identity of a gene from X and a gene from Y. $j_{XY} = \Sigma x_i y_i$ where x_i and y_i are the frequencies of the *i*th alleles in X and Y respectively. Genetic distance between population X and Y is then defined as D = -ln I (Nei 1987).

Avise and Aquadro (1982) reviewed literature of genetic distances between vertebrate species based on conventional electrophoretic analyses of proteins. To calculate genetic distances (D), the majority of studies these authors reviewed, utilized Nei's (1972) coefficients. Grant *et al.* (1988) examined the use of genotypic data derived from protein electrophoresis in taxonomic and phylogenetic research. They studied the phylogenetic relationships among the taxa of two gastropod genera, *Bulia* and *Burnupena* and came to the conclusion that an increasing the number of loci in a study increases the phylogenetic resolution. However, the accuracy of protein electrophoresis when inferring phylogenies becomes weaker when the evolutionary timespan exceeds that of 20 million years. In addition, it is very difficult to resolve recent speciation events if the sample sizes are small.

The discovery that genes code for proteins and the development of the techniques of gel electrophoresis have made it possible to estimate the amount of genetic change during the speciation process (Ayala 1982). Therefore, analysis of genetic variation of black rhinoceros populations by protein electrophoresis can also give an indication of the relationships between different populations.

MATERIALS AND METHODS

Blood samples were collected from black rhinoceros populations from (1) the Zambezi Valley, Zimbabwe (N = 90); (2) Mkuzi Game Reserve, Natal (N = 34); (3) the Hluhluwe - Umfolozi Park, Natal (N =25); and (4) Etosha National Park, Namibia (N =15) (Fig.2. 1). In the field, the blood samples were stored on ice and centrifuged as soon as possible to separate the plasma, buffy coat and red blood cells. The plasma and red blood cells were subsequently frozen in liquid nitrogen, transferred to the laboratories and the blood were kept at - 20°C. Red blood cells preferably need to be preserved with a glycerol-containing preserving fluid to prevent lysis of the red blood cells. However, some of the blood samples collected in 1986 were not preserved in this way. One-dimensional vertical polyacrylamide and horizontal starch gel-electrophoresis were performed to determine the variation in protein mobilities. The staining methods of Harris & Hopkinson (1976) were adapted (using histidine in some buffers) for use on rhino material. Red blood cells were lysed with a 5% mercaptoethanol and 10% Triton X-100 solution prior to electrophoresis. For some of the enzymes tested a reduction in sample size of the Zambezi and Etosha population was inevitable because of the low activity of some of the red blood cell enzymes. This decrease in enzyme activity can be attributed to the age of some of these samples and the preservatives used. In order to analyse a random sample of enzymes, enzymes representing different enzyme groups were analysed, such as the oxidoreductases, transferases, hydrolases and lyases, as well as nonenzymatic proteins. Table 2.1 presents the electrophoretic conditions for each of the 30 enzyme and protein loci analysed.

Three measures of genetic variation were used: heterozygosity (proportion of heterozygous individuals), gene diversity (Nei 1973) and proportion of polymorphic loci. Standard errors of gene diversity (Nei & Roychoudhoury 1974) were calculated.

Deviations of genotypic frequencies from Hardy-Weinberg equillibrium were tested using a G-test (Sokal & Rohlf 1981). F-statistics were calculated using Wright's formula (Wright 1931, Hartl & Clark 1989). Weir & Cockerham's (1984) methods to estimate F-statistics that do not make assumptions concerning numbers of populations, sample sizes and heterozygote frequencies, were used to calculate corrected F-stastistics. A jackknife procedure was used in calculating variances associated with estimates of F-statistics (Weir & Cockerham 1984). The null hypothesis, $F_{ST} = 0$, was tested for each allele with:

$X^2 = 2N\ F_{ST}$; d.f. = N - 1 (Workman & Niswander 1970).

The null hypothesis, $F_{IS} = 0$ was tested with:

$X^2 = N\ F_{IS}^2$, d.f. = 1 (Li & Horvitz 1953);

and the null hypothesis, $F_{IT} = 0$, was tested with:

$t = \left| F_{IT}\sqrt{N} \right|$; d.f = ∞ (Brown 1970).

Genetic distances were calculated using the formulae of Nei (1972, 1987) and by using genetic distance values, a phylogenetic tree was constructed to illustate the relationships between the different black rhinoceros populations. A phenogram was constructed using the average distance method or unweighted pair-group method with arithmetic mean (UPGMA). UPGMA is based on the assumption of a constant rate of evolution and reduces the effect of large stochastic error which genetic distance measures are subjected to (Nei 1987). Sampling variances of the genetic distances were calculated according to the procedures of Nei & Roychoudhury (1973).

RESULTS AND DISCUSSION

Genetic variation:

Six of the 30 loci studied were polymorphic: esterase-2, general protein-3 (possibly transferrin) and general protein-5, glucose-6-phosphate dehydrogenase, haemoglobin and phosphoglucomutase-2 (Table 2.2). Gene diversity of individual polymorphic loci varied between 0.153 and 0.500 in the various populations (Table 2.2). The Zambezi samples yielded the largest number of polymorphic loci, with some loci being monomorphic in the other populations. The haemoglobin locus was the only polymorphic locus which did not show variation in the Zambezi population (Table 2.2). Appendix IV shows the allele frequencies for the four black rhinoceros populations.

Analysis of the four black rhinoceros populations resulted in mean gene diversities ranging from 0.037 to 0.062 (Table 2.3). The Zambezi Valley population had the highest gene diversity, while the Mkuzi population had the lowest value. Mean sample heterozygosity ranged between 0.018 and 0.046 (Table 2.3).

Estimates of gene diversity among southern African black rhinoceroses were similar to those found in outbreeding mammal species where gene diversity values of about 0.05 appear to be common (Nei & Graur 1984; Nevo 1984; Selander & Johnson 1973). The results of three other studies suggested low levels of genetic variation in black rhinoceroses.

Merenlender *et al.* (1989) found a heterozygosity of 0.013 over 31 loci for 9 East African black rhinoceroses *D. b. michaeli* in zoological gardens. These authors found only two polymorphic loci: a general protein locus (stained with Amido

Black) and Phosphoglucomutase-2. In adition Osterhoff & Keep (1970) observed no genetic variation in the haemoglobin, transferrin and albumin of 10 individuals of *D. b. minor* from Hluhluwe-Umfolozi. Ramey (unpublished) analysed serum of 16 individuals of *D. b. minor* from Zimbabwe and three individuals of *D. b. bicornis* from Namibia and found no variation for 12 loci. Ashley *et al.* (1990) also analysed mitochondrial DNA of three populations from Kenya (*D. b. michaeli*), Zimbabwe and South Africa (*D. b. minor*) and found little mitochondrial DNA differentiation: only 4 out of 18 restriction enzymes revealed mtDNA polymorphisms, and the sequence divergence between *D. b. minor* and *D. b. michaeli* was only 0.29% (Ashley *et al.* 1990).

In contrast, Stratil *et al.* (1990) found variation among serum proteins by using one-dimensional polyacrylamide gel-electrophoresis of white rhinoceros (*Ceratotherium simum*). These authors found intraspecific polymorphism at several loci and questioned the conclusion of Merenlender *et al.* (1989) that white rhinoceroses, like black rhinoceroses are depauperate in genetic variation. In addition, Dinerstein & McCracken (1990) found that the erosion of genetic variability after a genetic bottleneck, may be overemphasized. The authors came to the conclusion that the high levels of genetic variation in the endangered greater one-horned rhinoceros (*Rhinoceros unicornis*) can be explained by the long generation time of rhinoceroses. The authors found high levels of genetic variation in *R. unicornis* after it experienced a bottleneck. Repeated bottlenecks, however can result in depletion of genetic variation (Dinerstein & McCracken 1990).

The observed gene diversities for black rhinoceros were higher than the equilibrium values expected from theory, when taking into account the population sizes of each population (Table 2.3). The relatively long generation time for the animals - about ten years (Conway & Goodman 1989, Hitchins & Anderson 1983) would contribute

to this phenomenon, causing the bottleneck effect on genetic variation to be small at this point in time. This applies in particular to the Zambezi populations where any reduction in gene diversity is not noticeable at present.

Gene diversity values for the two Natal populations were lower (but not statistically significantly so) than that of the Zambezi population. The values of around 0.035 (Table 2.3) suggested that the Natal populations are not strongly depauperate in genetic variation, since some outbreeding mammal species have similar amounts of gene diversity (Nei & Graur 1984). The reduced gene diversity of these animals (Fig. 2.2) can be explained by demographic changes in the Natal black rhinoceros population during the last century. 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 (Du Toit & Cumming 1986).

Black rhinoceroses were common in Natal during the first part of the 19th century, but their numbers decreased rapidly during the last half of the century, (Findlay 1903; FitZimmons 1920 and Lacey 1899) as in the interior of southern Africa (Selous 1908). After the establishment of the Natal Game Parks (including Hluhluwe-Umfolozi and Mkuzi) during 1947, small populations of black rhinoceros were protected. Records of the Natal Parks Board indicated that approximately 150 animals inhabited the Hluhluwe-Umfolozi area and 6-20 were to be found in Mkuzi between 1930 and 1940 (Hitchins & Brooks 1986). Gene flow between the Hluhluwe-Umfolozi and Mkuzi ceased before about 1940 (Brooks PM, pers. comm.*). Numbers in these parks have increased to 290 in Hluhluwe-Umfolozi, and 70 in Mkuzi (Flamand J, pers. comm.**).

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*Dr. P.M. Brooks. 1992. Natal Parks Board.

**Dr. J. Flamand. 1992. Natal Parks Board.

Expected gene diversities were simulated given population bottlenecks of the magnitudes outlined above (Fig. 2.2) and a non-overlapping generation time of 10 years was assumed.

Effective population sizes (N_e) are always smaller than census polulation sizes (Lande & Barrowclough 1987). Available data (Hitchins & Brooks 1986 and Schenkel & Schenkel- Hulliger 1969) suggest that sex ratio and social behaviour are relatively unimportant in affecting the N_e of black rhinoceros populations, and that the variance in number of offspring in a population may have the largest effect on N_e. If the sex ratio at birth is close to 1:1, the mean number of progeny per individual must be nearly equal for the two sexes because they contribute the same total number of gametes to the next generation. The variances in progeny number are usually larger in males than in females. Even the actual number of males and females are equal, a larger variance in progeny number in males implies that the efftive number of males is less than that of females (Lande & Barrowclough 1987).

Expected single-locus gene diversity, given N_e and the mutation rate, were calculated using the formula:

$$H = 4N_e v /(1 + 4N_e v)$$

where H = the expected heterozygosity and v = the rate at which a locusmutates per generation (Nei 1987). The N_e was varied between 0.6 and 1.0 of the census population size and only small changes on expected gene diversity were found. The simulation results were also not significantly affected by the initial population size. The results showed: 1) that curent gene diversities in the existing populations largely reflected that of populations during the previous century and, 2) that the observed levels of gene diversity in the Natal populations can be explained by the recent decline in these populations. The calculations suggested mean gene diversities at 1990 of 0.033 for the Mkuzi population and 0.046 for the Hluhluwe-Umfolozi

population (Fig. 2.2). These figures do not differ statistically from the observed values. The simulation results were not considered as a quantitative, predictive tool but merely as a heuristic aid to understand why the Natal populations still have a moderate 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. The results of this study do not support this view since no genetic data could be found suggesting a bottleneck in the distant past and the recent bottleneck in the Natal populations did not reduce gene diversity to anything close to the low levels suggested by these authors. The heterozygosity estimate for the population with the least genetic variation (Mkuzi, Table 3) was higher than that observed by these authors. These data indicate that free-living black rhinoceros populations in southern Africa are not depauperate in genetic variation and that the extant variation largely reflects that of populations prior to the recent population decline.

As a result of isolation, gene flow between the different populations are prevented. The high F_{ST}- values obtained (Table 2.4) suggest that there are genetic differences between subpopulations. However, the large standard error associated with F_{ST} (Table 2.4) suggests that this estimate does not differ significantly from 0. The F_{IS}, values differed significantly from 0 at 3 loci, GP-3, GP-5 and G-6-PD. This could suggest local inbreeding. In the Zambezi Valley population, statistically non-significant excesses of homozygotes were observed at 3 of the 6 loci; GP-3, PGM-2 and G-6-PD. In addition, the GP-5 locus had a statistically significant deviation from Hardy-Weinberg expectations (Table 2.2).

The subdivision in the Zambezi Valley population rather than the occurrence of inbreeding can explain the high F_{IS} value (Table 2.4). The material analysed from the Zambezi Valley included samples from different regions within the greater Zambezi Valley and explain why the total population still carries high levels of genetic variation even though an excess of homozygotes was observed at 4 of the 6 polymorphic loci. The effect of population subdivision in the Zambezi Valley population illustrates Wahlund's principle where pooling the data of subpopulations results in an increase in homozygotes with respect to the total population (Li 1978). On the other hand, the small effective population size of the Mkuzi black rhinoceros population can explain the excess of homozygotes. However, the high observed value of gene diversity for this population does not suggest that the Mkuzi black rhinoceros population is inbred.

Analysis of the F_{ST} and F_{IT} values suggest that the excess of homozygotes as seen in the whole southern African population is rather the result of a high F_{IS} (SE = 0.147) than as a result of the F_{ST} value (SE = 0.128). To summarize, no consistent and firm evidence exists that inbreeding has taken place in southern African black rhinoceros populations and the high F_{IT} value results from the Wahlund effect, mostly because of population subdivision in the Zambezi population.

The high levels of heterozygosity present in all four populations (Table 2.3) are reassuring for the genetic conservation of this species. However, it is important to keep in mind that the remaining black rhinoceros populations are small and translocation of individuals between different subpopulations are necessary to

maintain genetic variation within subpopulations.

Table 2.5 indicates the genetic distances calculated for the four different black rhinoceros populations. The two Natal black rhinoceros populations are very closely related (Fig. 2.3) while the biggest difference exists between the Etosha and Zambezi populations. According to Thorpe's interpretation of Nei's genetic distances among pairs of taxa, the Zambezi and Etosha populations are conspecific (Thorpe 1982).

Diceros bicornis is divided into several subpopulations in Africa, ranging from western to eastern Africa and south to Zimbabwe and South Africa. Population subdivision in black rhinoceroses is a very important factor to consider for the conservation of this species. There is much controversy surrounding black rhinoceros classification (Ashley *et al.* 1990). These authors analysed mitochondrial DNA of *D. b. michaelii* from Kenya and *D. b. minor* from Zimbabwe and South Africa and found the sequence divergence between the subspecies is 0.29% and values that range between 0 - 4% have been observed among members of the same local population. This means that the different subspecies shared a common ancestor approximately 145 000 years ago (Ashley *et al.* 1990). The authors conclude that there is no evidence from mitochondrial DNA of *D. b. bicornis* they sampled, are "evolutionary distinct units" and that these populations may be considered as a single population for breeding purposes, which might increase the genetic variation of the species and be favourable for the conservation of this species.

Although no individuals of the subspecies *D. b. michaeli* were analysed during this study, it seems if *D. b. minor* and *D. b. bicornis* are closely related, and this is in concordance with the results of Ashley *et al.* (1990). The Etosha population is classified as belonging to the subspecies *Diceros bicornis bicornis* (the so-called desert

black rhino). Calculation of genetic distances between this subspecies and *D.b. minor* revealed only a slight difference. The controversy regarding the classification of the black rhinoceros subspecies remain unresolved. Harley (pers. comm.*) analysed material of the different black rhinoceros subspecies by DNA fingerprinting and found no evidence to support the classification into different subspecies. Even if *D. b. minor* and *D. b. bicornis* shows little divergence at the protein level (Table 2.5) and at the DNA level, it would still be a risk to allow the mixing of the two subspecies since they occupy two totally different habitats and it might be that the two groups constitute discrete collections of genetic traits which have resulted from adaptation to the diverse environments. Keeping the different subspecies seperated and isolated could lead to loss of distinct genetic material or even to extinction. However, thorough investigation of the environmental and habitat differences is necessary before pooling the different "subspecies".

The Zambezi Valley black rhinoceros population was, until recently, the largest remaining natural population with the highest level of heterozygosity which supports the belief that larger populations usually have more genetic variation. As a result of poaching, this population declined rapidly from ±700 in 1990 to only 400 in 1992. Hereafter, the remaining black rhinoceroses in the Zambezi Valley were translocated to other parks where better precautions can be taken against poachers. The decline in population size implies that the population will experience a genetic bottleneck that will reduce the genetic variability of this population. This can be overcome by exchanging animals between the different smaller reserves. If extant black rhinoceros populations in other parts of the geographic range of this subspecies were to be supplemented to increase its genetic diversity, the Zambezi Valley population would be a suitable source population because of its substantial genetic variation.

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*Dr. E. Harley. 1993. University of Cape Town.

However, the real threat to the survival of the black rhinoceros in Zimbabwe and many other parts in the world, is poaching. Preventative action results in many small reserves with small effective population sizes which may guarantee short-term conservation but the genetic aspects on long-term conservation should not be ignored. Translocations between different populations are neccessary to maintain the genetic variation of such small populations. Hearne & Swart (1991) developed a non-linear differential equation model for optimal translocations for the black rhinoceroses. This involves translocating animals from areas where the population is approaching carrying capacity and establishing new viable populations in other suitable reserves. However, the authors do not consider genetic factors for translocations.

In conclusion, the high levels of genetic variation present in the four black rhinoceros populations suggest that they do not experience genetic depletion and that inbreeding does not pose an immediate threat to the survival of the species. The high levels of genetic variation indicate that these rhinoceros populations theoretically possess the potential to adapt to different environmental conditions. This is very positive for long-term conservation of this species. However, the remaining black rhinoceros populations are very small and therefore it is essential not to ignore genetic management. Small populations can lead to genetic drift and inbreeding, both of which result in the depletion of genetic variation.

Since the Zambezi Valley, Mkuzi, Hluhluwe-Umfolozi and Etosha black rhinoceros populations carry high levels of genetic variation it is inappropriate to spend large amounts of funds towards the genetic management of southern African black rhinoceros populations at present. However, genetic management should be kept in mind when planning a long-term conservation strategy for the species.

TABLE 2.1 : PROTEIN AND ENZYME LOCI EXAMINED AND ELECTROPHORETIC CONDITIONS

PROTEIN	EC NO.	LOCUS	TISSUE	BUFFER	pH	MEDIUM
Acid phosphatase	3.1.3.2	Acp-1	Rbc	6-PGD	7.5	Starch
Adenylate kinase	2.7.4.3	Ak-1	Rbc	6-PGD	7.5	Starch
Diaphorase	1.6.2.2	Dia-1	Rbc	6-PGD	7.5	Starch
Esterase	3.1.1.1	ESA-1	Plasma	Tris-glycine	8.3	Page
		ESA-2	Plasma	Tris-glycine	8.3	Page
		ESD	Rbc	Glyoxalase	7.6	Starch
General Proteins		Gp-1	Plasma	Tris-glycine	8.3	Page
		Gp-2	Plasma	Tris-glycine	8.3	Page
		Gp-3	Plasma	Tris-glycine	8.3	Page
		Gp-4	Plasma	Tris-glycine	8.3	Page
		Gp-5	Plasma	Tris-glycine	8.3	Page
		Gp-6	Plasma	Tris-glycine	8.3	Page
Glucose-6-phospha-te dehydrogenase	1.1.1.49	G6pd	Rbc	PGM-3	7.4	Starch
Glutamate-pyruvate transaminase	2.6.1.2	Gpt	Rbc	6-PGD	7.5	Starch
Glyoxalase I	4.4.1.5	Glo	Rbc	PGM-3	7.4	Starch
II		Glo	Rbc	PGM-3	7.4	Starch
Haemoglobin		Hb	Rbc	PGM-3	7.4	Starch
Lactate dehydrogenase	1.1.1.27	Ldh-1	Plasma	Tris-glycine	8.3	Page
		Ldh-2	Plasma	Tris-glycine	8.3	Page
		Ldh-3	Rbc	Tris-glycine	8.3	Page
Malate dehydrogenase	1.1.1.37	Mdh-1	Rbc	Tris-glycine	8.3	Page
		Mdh-2	Plasma	Tris-glycine	8.3	Page
Malic enzyme	1.1.1.40	Me-1	Rbc	6-PGD	7.6	Starch
Phosphoglucomutase	2.7.5.1	Pgm-1	Rbc	Glyoxalase	7.6	Starch
		Pgm-2	Rbc	PGM-3	7.4	Starch
Phosphogluconate dehydrogenase	1.1.1.44	Pgd-1	Plasma	Tris-glycine	8.3	Page
Purine nucleoside phosphorylase	2.4.2.1	Np-1	Rbc	6-PGD	7.6	Starch
Pyruvate kinase	2.7.1.40	Pk-1	Rbc	6-PGD	7.5	Starch
Superoxide dismutase	1.15.1.1	Sod-1	Plasma	Tris-glycine	8.3	Page
		Sod-2	Rbc	Tris-glycine	8.3	Page

Rbc = red blood cells. 6-PGD, Glyoxalase and PGM-3 buffers (Dr. S. Bissbort. Chemical Pathology,

TABLE 2.2. Values of gene diversity (H; Nei 1972: estimates ± standard error of estimates) for polymorphic protein loci in southern African black rhinoceros. Allele fequencies for GP-5 in the Zambezi population (asterisk) did not follow Hardy-Weinberg expectations (G=9.2, df=1, p<0.05).

Population: Locus	Zambezi	Etosha	H-U	Mkuzi
ES-2	0.247±0.026	0	0.269±0.052	0.084±0.025
GP-3	0.484±0.037	0	0	0
GP-5	0.500±0.037 *	0.464±0.088	0.365±0.060	0.472±0.059
PGM-2	0.368±0.050	0.153±0.080	0.480±0.069	0.472±0.059
G6PD	0.234±0.040	0.486±0.142	0	0.057±0.021
HB-2	0	0.391±0.081	0	0

TABLE 2.3. Genetic variation in four southern African black rhino populations, based on electrophoretic analysis of 30 serum and red blood cell protein-encoding loci. P=proportion of polymorphic loci, H=gene diversity (Nei 1973), H_m =proportion of heterozygous individuals, H_e =expected gene diversity according to the neutrality hypothesis, assuming mutation rate=10^{-5}.locus^{-1}.generation^{-1} (Nei 1985), N_t = population size (1990) and N = sample size.

Population	N	N_t	H_e	H±S.E.M.	H_m	P
Zambezi	90	750	0.029	0.062±0.022	0.046	0.167
Etosha	15	350	0.013	0.053±0.027	0.036	0.133
H-U	25	300	0.012	0.038±0.022	0.032	0.100
Mkuzi	34	70	0.003	0.037±0.022	0.018	0.133

FIGURE 2.1. Map of southern Africa indicating the geographic origin of material analysed: Zambezi(1), Mkuzi(2), Hluhluwe/Umfolozi(3) and Etosha(4).

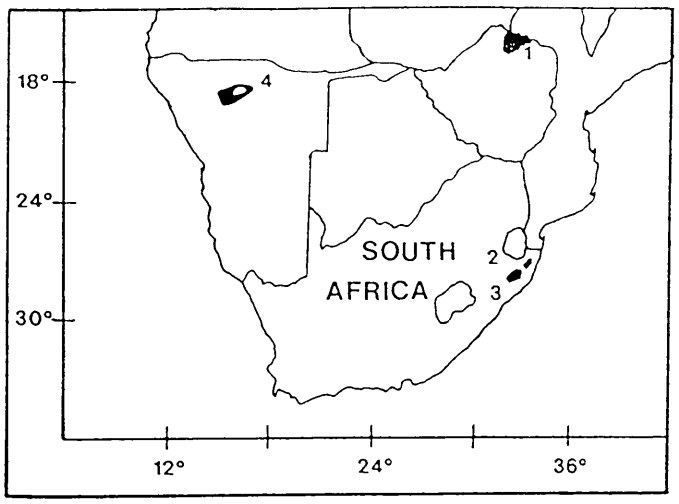


FIGURE 2.2. Estimates of effective population sizes of Natal black rhino populations over the last 150 years (sources quoted in text). Numbers on graph indicate expected gene diversity at 1850, 1910 and 1990 for the two indicated populations. Even though the gene diversity for Mkuzi is lower because of a severe population bottleneck around 1940, substantial genetic variation remains in that population because of the relatively long generation length for black rhinoceros.

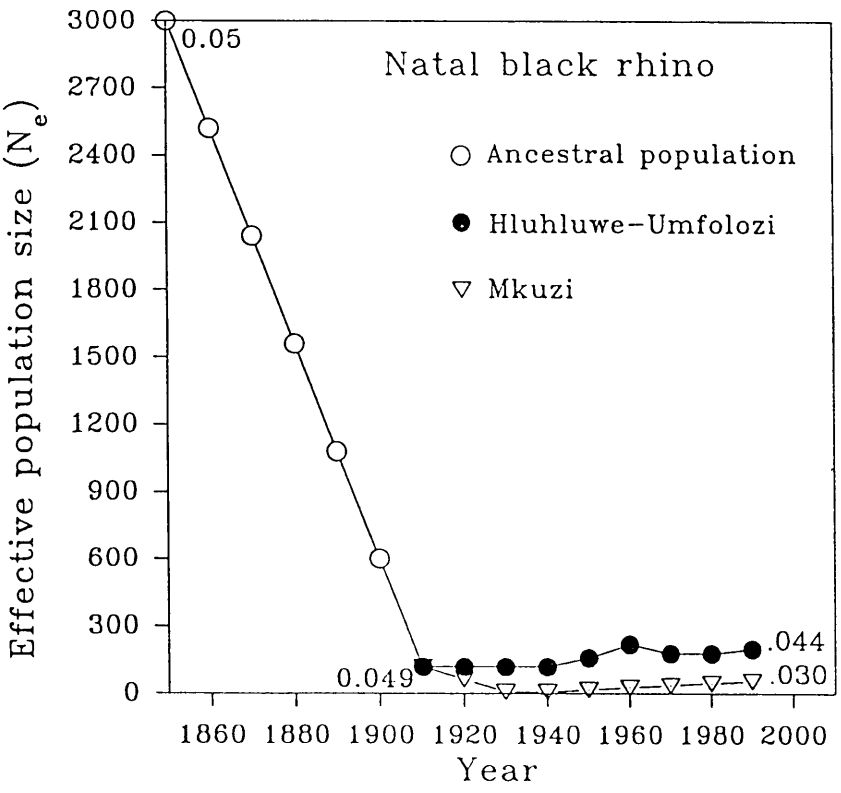


TABLE 2.4: Wright's F-statistics for the four black rhinoceros populations. Asterisks indicate statistically significant differences from zero.

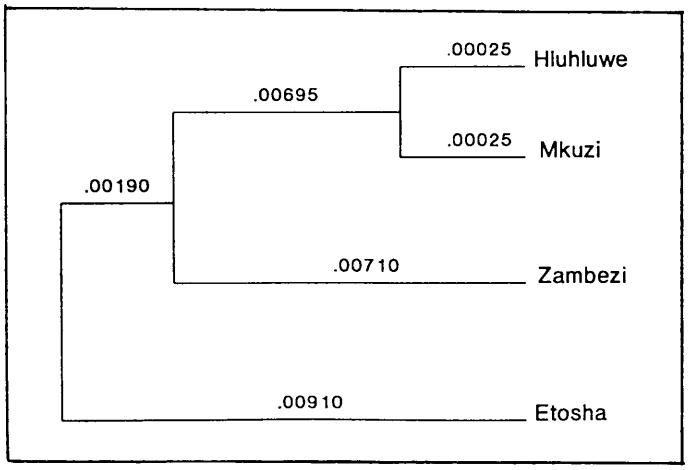
	Uncorrected			Corrected		
Locus	F_{is}	F_{it}	F_{st}	F_{is}	F_{it}	F_{st}
Es-2	-0.161	-0.095	0.057	-0.150	-0.109	0.036
GP-3	0.312	0.668	0.518	0.323*	0.661*	0.500*
GP-5	0.600	0.615	0.035	0.568*	0.582*	0.033
Pgm-2	0.079	0.154	0.081	0.104	0.133	0.032
G6pd	0.571	0.726	0.361	0.497*	0.628*	0.261
HB-2	-0.364	-0.071	0.214	-0.348	0.048	0.294
*MEAN	0.260	0.360	0.211	0.271	0.413	0.195
SE				0.147	0.141	0.128

* Includes analysis of 24 monomorphic loci.
SE = standard error derived from jackknife variances.

TABLE 2.5: Genetic distances (above the diagonal) - (Nei 1973) and associated standard errors (below the diagonal) between the four black rhinoceros populations studied.

	Etosha	H-U-C	Mkuzi	Zambezi
Etosha	-	0.0175	0.0146	0.0225
H-U-C	0.0119	-	0.0005	0.0153
Mkuzi	0.0107	0.0006	-	0.0135
Zambezi	0.0142	0.0125	0.0123	-

FIGURE 2.3: Phenogram (UPGMA) for the four black rhinoceros populations (*Diceros bicornis*) to show their genetic relatedness.



CHAPTER 3

HAEMOLYTIC ANAEMIA IN BLACK RHINOCEROS

INTRODUCTION:

Since 1982 it has been well documented that black rhinoceroses (*Diceros bicornis*) suffer from acute intravascular haemolytic anaemia. Rhinoceroses, when stressed, can develop intravascular haemolysis, often accompanied by fatal kidney failure. Sudden death due to spontaneous haemolysis occurs frequently after capture or immobilisation (Chaplin *et al.* 1986). Paul *et al.* (1988) obtained baseline haematological data by analysing blood samples of 31 wild black rhinoceroses from Zimbabwe and suggested that an unstable haemoglobin is involved in haemolytic anaemia. However, Fairbanks & Miller (1990) described a polymorphism of the rhinoceros' haemoglobins and concluded that this polymorphism appears to be unrelated to acute haemolytic anaemia. The red cell enzymes of rhinoceroses were studied (Paglia *et al.* 1986) but no evidence for red cell enzyme abnormalities was found.

Van Vliet (1991) examined blood samples of 22 white and 31 black rhinoceroses and found that 30% of the black and 50% of the white wild-captured rhinoceroses suffered from intravascular haemolytic anaemia as described for captive rhinoceroses. Kock *et al.* (1990) found that, in Zimbabwe, no animal died at capture, but there was a mortality rate of 14 %, one week to two months after capture. Paglia (1993) performed enzyme assays on black rhinoceros cell isolates and observed that 10 - 15% of circulating erythrocytes in apparently normal rhinoceroses, contained Heinz

bodies which are characteristic of haemolytic anaemia. The author also observed marked deficiencies of intracellular adenosine triphosphate (ATP), catalase, adenosine deaminase and in some enzymes involved in glycolysis, glutathione cycling and nucleotide metabolism. Paglia (1993) proposed that erythrocyte ATP deficiency in rhinoceroses may be an evolutionary adaptation conferring selective advantage against common blood parasites, comparable to the effect of human glucose-6-phosphate dehydrogenase (G-6-PD) deficiency on falciparum malaria. Human G-6-PD deficiency served as the paradigm because of its strong clinical resemblance to the rhinoceros haemolytic syndrome (Paglia & Miller 1993). Still, all the above-mentioned authors could not identify a basic biochemical or metabolic defect that would account for a number of factors or agents initiating haemolysis (Paglia & Miller 1993).

Evidence found by Paglia (1993), however, support the hypothesis that the product-deficiency disorder in rhinoceros erythrocytes is analogous to human G-6-PD deficiency.

3.1 Haemolytic anaemia (HA) in humans

In the case of humans, G-6-PD deficiency is well described and has been studied extensively. G-6-PD deficiency is inherited and can lead to haemolytic anaemia when triggered by some drugs, the ingestion of fava beans or infections (Luzzatto and Mehta 1989). Inheritance of human G-6-PD further shows a characteristic X-linked pattern. A selective advantage of G-6-PD deficiency in humans is that it can confer relative resistance against malaria and clinical data indicate that this is confined to heterozygous females (Luzzatto and Mehta 1989). It has been postulated that significantly diminished red cell ATP concentrations contribute to the relative resistance of certain erythrocytes to malarial parasitization (Paglia 1993). An indi-

vidual may be asymptomatic but clinical expression of haemolytic anaemia results from an interaction of the molecular properties of each individual G-6-PD variant with exogenous factors (Table 3.1).

Clinical haemolysis starts 2 to 3 days after the drugs are administered to the individual. The anaemia worsens until the seventh to eighth day. Drug-induced HA is characterized by intravascular haemolysis and haemoglobinuria. Heinz bodies are found in the peripheral blood. A reticulocyte response then sets in and the haemoglobin level begins to recover on the eighth to tenth day. However, increased drug dosage or the presence of a severely deficient G-6-PD variant will cause more protracted haemolysis. Certain drugs are reported to cause oxidative haemolysis (but not necessarily anaemia) in some human population groups (Table 3.2) (Luzzatto & Mehta 1989). Even in humans, it is difficult to link a specific drug to haemolytic anaemia since clinical haemolysis is not always reproducible after administration of a particular drug. Infection can also cause additional oxidant stress, especially in neonates. Infection is probably the most common cause of haemolysis in individuals with G-6-PD deficiency.

3.2 The biochemical pathway of the enzyme: Glucose-6-phosphate dehydrogenase

Glucose-6-phosphate dehydrogenase catalyses the first step in the hexose monophosphate pathway (the oxidation of glucose-6-phosphate to 6-phosphogluconate). Nicotinamide adenine dinucleotide phosphate (NADPH)is generated from NADP. NADPH is responsible for the regeneration of reduced glutathione and the stability of catalase. Glutathione and catalase are essential for the detoxification of hydrogen peroxide and of oxygen radicals to ensure functional red blood cells (Giblett 1969). Some drugs may stimulate the hexose monophosphate shunt pathway and then more

NADPH is needed for the detoxification of hydrogen peroxide. The cells are unable to produce enough NADPH, and the result is red cell destruction. (It is, however, not yet fully understood why inadequate production of NADPH leads to haemolysis). In the case of G-6-PD deficiency, the red blood cells are unable to withstand the oxidative damage. Red cell destruction is intravascular and may result in haemoglobinuria and kidney failure (Luzzatto & Mehta 1989).

In order to diagnose G-6-PD deficiency, the specific enzymatic activity of G-6-PD must be measured and not the amount of G-6-PD protein. Heterozygotes have a mixture of normal and deficient erythrocytes and the proportion between the two cell types can vary enormously between the two extremes of completely normal activity and complete deficiency. A cell lysate may therefore not reveal heterozygosity if the proportion of enzyme-deficient cells is small. Therefore microscopic examination of individual cells on a blood film slide is preferable.

3.3 Haemolytic anaemia in animals

Haemolysis has been observed in domestic animals, particularly horses after they have consumed wild onions, oak and red maple leaves (Paglia & Miller 1993). Du Toit & Paul (1987) and Paglia (1993), emphasized that the die-off of about 300 black rhinoceroses during 1960 - 1961 may well have been due to haemolytic episodes, initiated by nutritional factors (such as postdrought blooms of toxic plants or unusual variations in vegetation available for browse) and not as previously thought the result of nutritional anaemia. Paglia & Miller (1993) warned that all rhinoceroses should be regarded as clinically equivalent to G-6-PD deficient humans and should therefore be protected from agents and conditions that can initiate haemolytic episodes.

3.4 Aims:

In humans the most common cause of haemolytic anaemia is a deficiency of the red blood cell enzyme, Glucose-6-phosphate dehydrogenase. The aim of this chapter was to examine glucose-6-phosphate dehydrogenase in the black rhinoceros to see if any correlation exists between haemolytic anaemia and an enzyme deficiency.

MATERIALS AND METHODS

Starch gel electrophoresis: Blood samples of 102 black rhinoceroses from four different populations (see Chapter 2: materials and methods) were analysed. Prior to electrophoresis, 200 µl of erythrocyte lysate was pipetted into a reaction tube, 20 µl of an aqueous solution containing 5%/vol. mercaptoethanol and 10%/vol. Triton X-100 was added. Another 200 µl of a water-based gel consisting of 20% sucrose and 4.5% Sephadex G-200 was added and mixed. The samples were applied into preformed slots in the starch gel about 8 cm from the cathodal end.

Starch gel electrophoresis was performed using a water cooled electrophoretic apparatus. The bridge buffer consisted of 0.2 M Tris and 0.23 M Histidine - HCl, (pH 7.4). The gel buffer was a 1:7.7 dilution of the bridge buffer. The conditions for electrophoresis were as follows: 18 h at 4°C at 300 V/300 mA at 5 V/cm. After electrophoresis, the first 10 cm of the anodal part of the gel was sliced into slabs of 2 mm thickness and stained. The staining solution consisted of 10 mg NADP, 15 mg Glucose-6-phosphate, 20 mg MTT, 1 mg PMS dissolved in Buffer (pH 7.4): 0.1 M Tris-HCl + 0.2 M $MgCl_2$. Filter paper (Schleicher and Schüll, no. 2316) (10 x 19 cm) was soaked with the staining solution, laid over the cut starch gel and covered with a transparent household plastic wrap. The gel was incubated in the

dark for ± 30 min at 37°C until the banding pattern could easily be detected.

Scanning: In order to measure the relative intensities of the isoenzyme bands, a Cliniscan 2, (Helena Labarotories, Beaumont, Texas) was used with wavelength of 540 nm.

Specific Activity tests: The glucose-6-phosphate dehydrogenase activity was determined spectrophotometrically using a Lambda II spectrophotometer (Perkin-Elmer). Glucose-6-phosphate dehydrogenase and NADP were used as substrates. The rate of increase of NADPH was measured at 340 nm. A blank sample, without glucose-6-phosphate, was used. The reaction solution consisted of the following: 2 ml Buffer (pH 7.2): 0.1 M Tris - HCl + $MgCl_2$, NADP 100 µl (10 mg/ml), Glucose-6-phosphate 100 µl (20 mg/ml), erythrocyte lysate (500 µl buffer + 100 µl lysate from compacted erythrocyte sediment).

Haemoglobin determination: A haemoglobin was observed by Paul *et al.* (1986) and since haemoglobin plays an important role in the transport of metabolites, gases and hormones, the concentration of haemoglobin can give an indication of any abnormalities in the blood (Schmidt-Nielsen 1983). The haemoglobin concentration was determined by the ferricyanide method (Drabkin and Austin 1932).

A longer period elapsed between the collection of samples from Etosha and laboratory analysis compared with the corresponding period for the Zimbabwe samples. Direct comparison between the values for Etosha and Zimbabwe was complicated because of a higher degree of denaturation of G-6-PD in the samples form Etosha. Separate analyses were therefore performed on the two sets of samples.

RESULTS

A polymorphism for G-6-PD was found in the black rhinoceros material examined. Three different banding patterns (Fig. 3.1) enabled the formal genetic interpretation of two alleles at one locus. The homo- and possible hemizygous animals showed a single band with a minor pre-band, whereas the heterozygous animals showed a double-banding pattern with minor pre-bands.

Table 3.3 shows the distribution of the three phenotypes and the gene frequencies of the two alleles within different black rhinoceroses populations. The allele frequencies at the G-6-PD locus were in Hardy-Weinberg equillibrium for all four black rhinoceros populations. The frequency of the F allele is much higher in the Etosha population than in the other populations ($X^2 = 40.69$, d.f. = 6, $P < 0.001$). The frequency of the faster variants exceeded that of the slower allele in the Etosha population, while in the Zambezi population, the slow allele was more common. Hluhluwe-Umfolozi specimens were monomorphic for this locus and revealed only the slow migrating allele (S). A single F allele was detected in the material from Mkuzi.

By scanning the enzyme pattern of heterozygous animals, two gene products with different activities were identified. The results indicated the staining intensity of the faster moving (F) isoenzyme to be up to 20% of the slow (S) isoenzyme. Similar results were obtained with spectrophotometric activity tests when the red cell G-6-PD activity of five black rhinoceroses (two from Zambezi and three from Etosha) were measured. Table 3.5 shows that the F phenotype had a lower activity than S, and the heterozygote (FS) had an intermediate activity. The activity of the F phenotypes ranged between 35% and 57% of the activity of the slow phenotypes.
All the heterozygous individuals were females. Only the Etosha black rhinoceros

population revealed a haemoglobin polymorphism and this polymorphism was not correlated with the G-6-PD polymorphism (Table 3.4).

DISCUSSION

It is evident that animals with the normal allele (S), are found far more frequently in the two Natal populations and those with the faster variant (F) in Etosha and Zimbabwe. The F phenotypes are characterized by a glucose-6-phosphate dehydrogenase deficiency and if this hypothesis holds true, animals with the fast variant are more likely to develop haemolytic anaemia, especially the Etosha and Zambezi populations because of the higher frequency of the deficient faster migrating allele.

The allele frequencies correspond with reported cases of haemolytic anaemia in black rhinoceroses. The haemolytic attacks in Zambezi Valley black rhinoceroses are alarming (Du Toit R, pers. comm.*) and the high mortality rate of the Zambezi rhinoceroses after capture and immobilisation (Kock *et al.* 1990) might be explained by anaemia inducing agents e.g. drugs. Measurable mortalities were experienced in Etosha after capture and dehorning of black rhinoceroses due to a range of causes (Fox B, pers. comm.**). In Natal however, rhinoceros deaths related to haemolytic anaemia are non-existent (Brooks P, pers. comm.***). It might be that capture procedures and the administration of drugs to the rhinoceroses induce haemolytic anaemia in G-6-PD deficient individuals. This might explain the high mortality rate of wild black rhinoceroses just after capture, since haemolytic anaemia accounts for

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*R. Du Toit. 1991. Zambezi Rhino Project, Harare, Zimbabwe.

**Dr. B. Fox. 1992. Dept. Tourism and Nature Conservation, Namibia.

***Dr. P. M. Brooks. 1991. Natal Parks Board.

40% of adult mortality of black rhinoceros in captivity (Paglia 1993).

In all mammals studied so far, the X-chromosomal location of the G-6-PD locus has been confirmed. All the heterozygous rhino individuals were females. This is consistent with the hypothesis that the gene for this enzyme is also X-linked in the rhinoceros. Rhinoceroses are exposed to endemic blood parasite diseases such as trypanosomiasis, piroplasmosis and rickettsiosis. It may be that a G-6-PD polymorphism evolved as a protection mechanism in the black rhinoceros which confers relative resistance against these parasites.

Du Toit & Paul (1987) also proposed a hypothesis that the low levels of ATP would similarly provide a less hospitable host for intra-erythrocytic and other hemic parasites that commonly infect rhinoceros in the wild. A haemoglobin polymorphism was observed in the Etosha population, but this polymorphism appears unrelated to the G-6-PD polymorphism.

Paglia (1993) and Paglia & Miller (1993) also came to the conclusion that haemolytic disease in rhinoceros is analogous to G-6-PD deficiency in humans. Paglia (1993) formulated the hypothesis that "rhinoceros red cells in affected animals (animals with HA) were deficient in G-6-PD or some other enzyme essentail to antioxidant metabolism". Paglia (1993) found that rhinoceros erythrocytes appear highly susceptible to oxidant stresses *in vitro* as well as *in vivo* and like G-6-PD deficient cells, exhibit positive ascorbate-cyanide and Heinz body tests and marked glutathione instability with increased Heinz bodies when challenged by oxidants such as acetylphenylhydrazine or ascorbate-generated hydrogen peroxide. Paglia (1993) concludes that the results of his experiments, support the hypothesis that multiple metabolic deviations from other mammals produce a product-deficiency disorder in rhinoceros erythrocytes that is functionally analogous to human G-6-PD

deficiency.

The results of **this** study, are the first to identify a G-6-PD variant with reduced enzyme activity in a wild animal. The results and findings of Paglia (1993) and Paglia & Miller (1993) further substantiate the hypothesis formulated in this chapter. This will then be the first analysis to actually pin-point that haemolytic anaemia in black rhinoceroses has a genetic origin, that of a deficiency of the enzyme: glucose-6-phosphate dehydrogenase.

The black rhinoceros is an endangered species and in order to survive, they have to undergo intensive management which includes immobilisation, handling, treatment and translocations. However, in the effort to conserve the rhinoceros, it is inevitable that they will experience severe stress. Stress in animals with the enzyme deficiency or even the drugs used during capture, immobilisation and for treating the rhinoceroses, may trigger haemolytic disease. Managers will therefore have to minimize stress and should take care when administering drugs to rhinos (the drugs listed in Table 3.2 should be avoided). Managers have to avoid unnecessary translocations and handling of rhinos to reduce stress in a population. Translocation of rhinoceroses can only be considered if the population is threatened by poaching and needs to be moved to a safer reserve or if genetic depletion poses a threat for the long-term survival of the population. Positive identification of drugs (that can trigger haemolytic anaemia in rhinos), will enable managers to perform translocations where mortalities, as a result of HA, can be eliminated.

FIGURE 3.1. Electrophoretic isoenzyme separation indicating the different G-6-PD phenotypes (F, FS and S) among black rhinoceros from Zimbabwe.

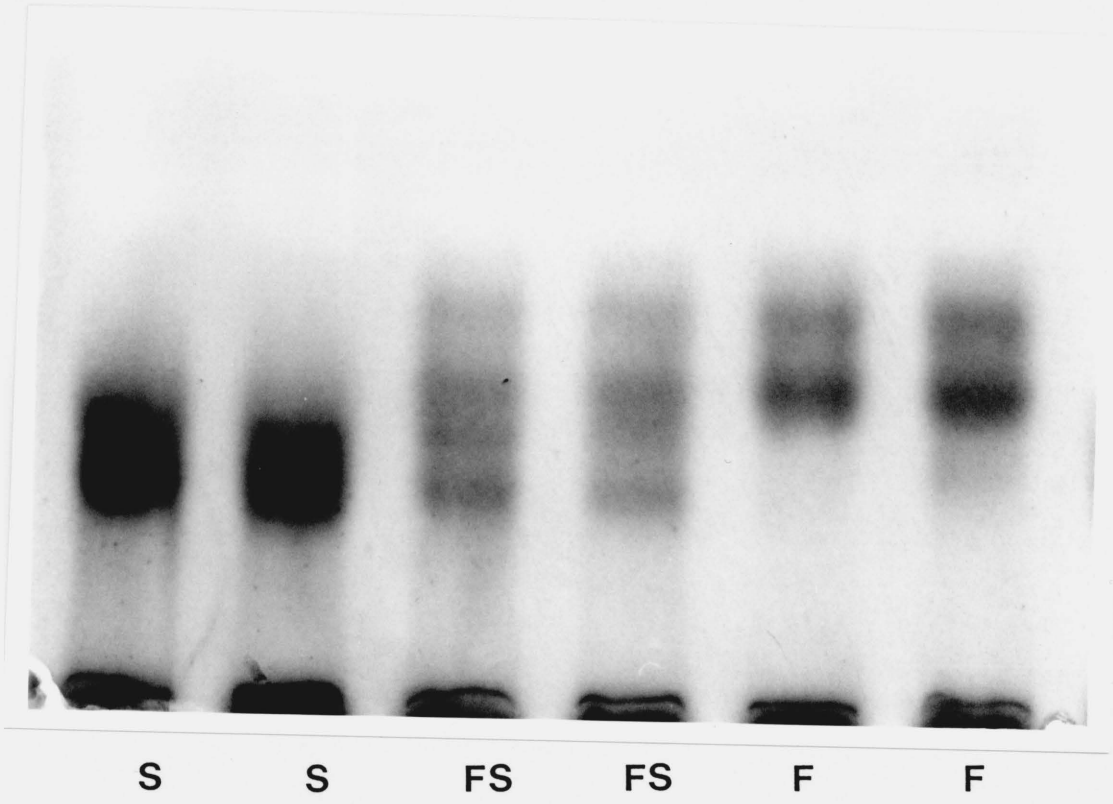

TABLE 3.1: Factors which influence individual susceptibility and severity of drug-induced oxidative hemolysis in humans.

Inherited:
Metabolic integrity of the erythrocyte, Precise nature of enzyme defect, Genetic differences in pharmacokinetics
Aquired:
Age, Dose, absorption, metabolism and excretion of drug Presence of additional oxidative stress; eg. infection, Effect of drug on enzyme activity Preexisting haemoglobin level, Age distribution of red blood cell population

TABLE 3.2: Drugs and chemicals associated with significant hemolysis in humans with G6PD deficiency

Drugs	Definite association	Possible association
Antimalarials	Primaquine, pamaquine	Chloroquine, quinacrine pentaquine
Sulfonamides	Sulfanilamide Sulfacetamide Sulfapyridine Sulfamethoxazole	Sulfamethoxypyridazine, Sulfoxone, sulfadimidine, Sulfadiazine, sulfamerizine, Sulfisoxazole
Sulfones	Thiazolesulfone Diaminodiaphenylsulphone (DDS, Dapsone)	
Nitrofurans	Nitrofurantoin	
Antipyretic-analgesic	Acetanilid	Aminopyrine, Acetominophen, phenacetin, aspirin
Others	Nalidixic acid Naphtalone Niridazole Phenylhydrazine Toluidine blue Trinitrotoluene (TNT) Methylene blue Phenazopyridine	Chloramphenicol, PAS, Vitamin K analogues, L-Dopa, Vitamin C, Dermicaprol, Doxorubicin, Probenecid

Table 3.1 & 3.2 from: Luzzatto & Mehta (1989)

TABLE 3.3: Phenotypes and allele frequencies for the glucose-6 -phosphate dehydrogenase locus in the four studied populations. S=slow allele, F=fast allele.

	POPULATION			
	Mkuzi	**H-U**	**Zambezi**	**Etosha**
Sample size:	31	25	37	6
Phenotype:				
F	0	0	3	4
FS	1	0	4	1
S	30	25	30	1
Allele frequencies: (S)	0.98	1.0	0.88	0.25

TABLE 3.4: Densitometric measurements on the staining intensities of electrophoretic isoenzyme bands of the G-6PD phenotypes. Values in the "Density" collumn represent the proportion of areas under the densitometric peaks associated with the relevant isoenzyme bands.

Rhino #	**Sex**	**G-6PD phenotype**	**Density**	**Haemoglobin genotype**
Etosha 1	M	S	97.25	A
Mkuzi 20	F	S	100.00	A
Mkuzi 23	F	S	93.95	A
Etosha 2	F	FS	95.32	B
Etosha 3	F	F	76.11	B
Etosha 4	F	F	80.67	B
Etosha 5	F	F	83.06	A

TABLE 3.5: Glucose-6-phosphate dehydrogenase activity of 3 black rhinoceros phenotypes determined spectrophotometrically. Five individuals were assayed and separate analyses were conducted for samples from Etosha and from Zimbabwe (see methods). Specific activity is expressed as M substrate converted per minute per gram of haemoglobin.

Rhino #	Sex	Phenotype	Activity micro M. $min^{-1}.g^{-1}$	Relative activity %
Etosha 1	M	S	1.47	100.0
Etosha 2	F	FS	1.13	76.2
Etosha 3	F	F	0.83	56.6
Zambezi 6	F	S	2.23	100.0
Zambezi 10	M	F	0.71	34.8

CHAPTER 4

DEMOGRAPHIC CHARACTERISTICS OF BLACK RHINOS IN CAPTIVITY

INTRODUCTION:

In the past, breeding of animals in captivity was not a major goal of most zoological gardens but this has changed drastically since the potential of zoos to maintain a variety of self-sustaining captive populations of rare and endangered species has been recognized (Ralls & Ballou 1983). As areas of suitable habitats for wild animals decrease and become fragmented, an ever-increasing number of species will exist in relatively small populations and a certain degree of inbreeding will inevitably occur (Ralls & Ballou 1983).

Breeding systems can be manipulated to increase effective population size and the following are important demographic factors to keep in mind to achieve this goal (Senner 1980): 1) high fecundity (high reproduction rates result in an excess of offspring which permits selection for vigour or other traits); 2) large number of viable offspring (offspring that survive to adulthood and which are fit for breeding); and 3) prevention of skewed sex ratios. Senner (1980) argued that the stochastic extinction of small populations is inevitable. The probability of such extinctions depends on fecundity, viability and sex ratio. An unbalanced sex ratio increases both the rate of inbreeding and the probability of stochastic extinction in small populations. Inbreeding, in turn, should be avoided since it often results in viability-, fecundity- and sex ratio depression (Senner 1980). Managers of captive black

rhinoceros populations should therefore strive to achieve demographic stability to ensure the survival of this species in captivity.

4.1 Inbreeding

The incidence of inbreeding depression is much higher in zoo populations than in wild populations (Ralls & Ballou 1983). The frequency of close inbreeding in natural populations of birds and mammals appears to be relatively low and analysis of the literature indicates that incestuous matings, between close relatives, in nature are between 0 - 6% of those observed in zoos (Ralls *et al.* 1986). The evolution of behavioural patterns that prevent inbreeding of close relatives suggest that inbreeding in the wild is disadvantageous. Ralls *et al.* (1979) found that in 15 out of 16 captive ungulate species, juvenile mortalities were higher in inbred than in non-inbred young. Genetic management in captive breeding is essential to develop a captive population where as much as possible of the original genetic variability is preserved (Frankel & Soulé 1981). It is inevitable that the size of a population in captivity is much smaller than in the wild and that is therefore more susceptible to inbreeding.

Franklin (1980) and Allendorf (1986) concluded that genetic variance for complex traits is lost when the effective population size is small. Ryder *et al.* (1981) analysed genetic variation in the endangered Przewalski's horse (*Equus przewalskii*) and Père David's deer (*Elaphurus davidianus*) and came to the conclusion that the preservation of genetic variability, in small populations, depends on maximizing the effective population size and minimizing inbreeding. Conserving genetic variability in managed populations requires the monitoring of allozymic and quantitative variation. These monitoring procedures, however, are rarely carried out in captive populations (Lande & Barrowclough 1987).

4.2 Effective population size of rhinoceroses in captivity.

One of the major problems of populations in captivity, is that the population sizes are small and the effective population sizes are even smaller since all individuals in the population are not reproductively active. The concept of effective population size was introduced by Wright (1931) to calibrate the amount of genetic drift in actual populations with a non-Poisson distribution of progeny numbers, separate sexes, or fluctuations in population size. The effective size of a population, N_e, is the size of the ideal population that would undergo the same amount of genetic drift as does the actual population (Lande & Barrowclough 1987). The calculation of N_e values, enables one to determine the amount of genetic variation (heterozygosity) expected for selectively neutral alleles at a locus. According to the infinite allele model, every locus mutates at a rate μ per generation, with each new mutant of a type not currently existing in the population (Lande & Barrowclough 1987). At equilibrium between mutation and random genetic drift, the predicted gene diversity is:

$$H = 4 N_e \mu / (1 + 4 N_e \mu)$$

where μ = mutation rate (taken as 10^{-5} per locus per generation for single loci (Dobszhansky 1970).

Aims:

The aim of this chapter is to determine whether zoological gardens are succeeding in establishing a viable black rhinoceros population in captivity.

In order to answer this question, thorough investigation of the following is needed: detailed information on life-history parameters such as birth rate, mortality rate, calving interval and age at sexual maturity.

Analysis of birth and death rates will give an indication if the captive population is experiencing a negative or positive growth rate. Other factors that can reduce growth rate (such as poor nutrition, climatic conditions and the occurrence of disease and parasites) will also be studied.

Inbreeding can have a negative effect on reproductive rate and therefore it was important to determine whether black rhinoceroses in captivity are subject to in-breeding depression.

The size of the effective population in captivity enables one to predict the gene diversity of the population.

METHODS

Data on captive breeding of black rhinoceros were obtained by reviewing literature of the International Studbook of African Rhinoceros (Klös & Frese 1989, 1991 and Smith & Read 1992). Data on age-specific fecundity and mortality of black rhinoceroses were analysed. The relationship between mortality and age was investigated. The reproductive success of cows belonging to different age classes was analysed. The effective population sizes (N_e) were calculated for each black rhinoceros population in captivity. There are two approaches in calculating the N_e of the black rhinoceros population in captivity:

1) calculating N_e for each zoo population, followed by the summation of these N_e's to arrive at a total N_e for the black rhinoceros population in captivity. The effective population size (N_e) was calculated by using the following formula:

$$N_e = (4\ N_m\ N_f)/N_m + N_f$$

where N_m = the number of breeding males in the population and N_f = the number of

breeding females in the population.
2) All black rhinoceroses in different zoos are regarded as one metapopulation and the N_e is calculated accordingly.

Inbreeding was analysed by studying black rhinoceroses pedigrees. By calculating the N_e of black rhinoceroses in captivity, it was also possible to predict the gene diversity in the population.

RESULTS AND DISCUSSION

Demography of captive black rhinoceroses.

Birth rate of captive black rhinoceroses: The number of black rhinoceroses (*D. b. michaeli* and *D. b. minor*) captured in the wild and held in captivity totals 72 animals, of which 40 are *D. b. minor*. Since 1956, thirteen black rhinoceroses (*D. b. minor*) were born in captivity of which 4 died. For *D. b. michaeli*, 187 were born in captivity and 88 of these animals died (Klös & Frese 1991).

At the end of 1991, 204 black rhinoceroses (*D. b. michaeli* and *D. b. minor*) existed in captivity (91 males : 113 females) at 72 locations (Klös & Frese 1991). From 1987 to 1990, 24 births (10:14) of *D. b. michaeli* and six (2:4) of *D. b. minor* were recorded. The long gestation period and long calving intervals are some of the demographic factors causing this low population growth rate. The gestation period of animals in zoos can be monitored accurately and for black rhinos it ranges from 438 days to 476 days. The average calving intervals for *D. b. michaeli* in captivity is about 37 months (intervals of 18 to 108 months have been recorded in zoos) and 46 months for *D. b. minor* (Klös & Frese 1991). Calving intervals of captive black

rhinos are much longer than for black rhinos in nature (Table 4.1). This suggests that the breeding of black rhinos in captivity has not yet reached its full potential.

Rhinoceros cows can start reproducing from age class three (6 - 9 years). A rhinoceros cow which reaches age class 10 (28 - 30 years old) can produce seven offspring per lifetime (Table 4.2). The age group, 15 - 24 years, is still the most successful in breeding, but this is still alarmingly low considering that each cow can produce three calves in nine years. Smith & Read (1992) consider the production of a calf every 2 years from the time the cow is six years old, as optimal reproduction.

Two factors, calving interval and high calf mortality, need urgent attention in order to assure the viability of breeding this endangered species in captivity. To solve these problems it will be necessary to monitor the condition of the cows very closely and to ensure that, when they reach sexual maturity (from six to nine years), a sexually mature bull is available. Violent encounters between cows and bulls is another problem that zoo-keepers are confronted with. Research regarding artificial insemination and embryo transplants in black rhinoceros is being conducted by the Department of Veterinary Science, University of Pretoria. This may eliminate the problem of long calving intervals and slow reproductivity of captive rhinoceros.

Smith & Read (1992) made two suggestions to achieve a desirable rate of reproduction: 1) female calves approaching three years of age should be moved to situations where they can be bred and 2) birth intervals should be kept as close to two years as possible. It will also be important to exchange rhino bulls between different zoos in order to maintain genetic variation and avoid inbreeding. Zoos do not appear to have a lot of success with the rearing of rhinoceros calves and the high mortality of rhinoceros calves under two years of age is of great concern to rhinoceros breeders.

Mortality of black rhinoceroses in captivity: From 1987 to 1990, 26 black rhinos died, sex ratio (14:12), at an average age of 18.5 years. Seven of these animals were younger than one year. From 1956 to 1990, a high death rate was observed in black rhinoceroses under four years of age, but the highest death rate was found for animals under two years of age (Fig. 4.1). Sixty percent of all dead rhinoceroses are less than 20 years old. A slight increase in deaths of rhinoceroses in captivity occurred world-wide between December and April of every year. During the winter months in the northern hemisphere fatal disorders may occur due to vitamin A, C and E deficiencies because of the unavailability of lucerne for zoos (Klös & Frese 1991).

The cause of high juvenile mortality in rhinoceroses cannot be attributed to inbreeding as zoos take special care not to use inbred animals for further breeding and since they keep breeding records for every animal. The first two years of a captive bred calf is the most critical stage of its life since many cows abandon their calves which then die from malnutrition and vitamin deficiencies. Abandoned calves have to be reared with a special milk formula. A milk formula was successfully developed by Nestlé for a black rhino calf at ARC (Animal Rehabilization Centre) near Pretoria (Trendler pers. comm.*).

Climatic conditions, especially in the northern hemisphere, also play a major role in the breeding failure of rhinoceroses. In North-America, rhino deaths are seasonal with death rates being high during the winter months (December - May) due to poor nutrition. Growth of the captive black rhinoceros population has been limited by four diseases; haemolytic anaemia, oral and skin ulcers, encephalomalacia and fungal pneumonia. Haemolytic anaemia is the leading cause of death among captive

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*Me. K. Trendler. 1992. ARC, Pretoria, South Africa.

black rhinoceros (Paglia 1993).

The mortality rate of rhinoceroses in captivity is alarmingly high. For the period 1987 to 1990, a low growth rate was observed for rhinoceroses in zoos.

Effective population size of black rhinoceroses in captivity

N_e = **116.3** (when zoo populations are considered separately), while the N_e = **150.36** when all individuals of different zoos are regarded as one metapopulation. In both cases the N_e for captive black rhinoceroses is small and it is evident that in case 1) the N_e makes up only 57% of the total captive black rhinoceros population whereas in 2) the N_e = 74% of the captive population. Twenty-six zoos (40% of the total zoos), have N_e's of zero, because of unbalanced sex ratios (some zoos only have animals of one sex). This is alarming and shows that these zoos do not contribute in increasing the number of black rhinoceros.

Since rhinoceroses only reach sexual maturity at age class three, 30% of the animals (individuals of the first two age classes) are not reproducing (Fig. 4.2). The age-structure of the captive population reflects that of a growing population but the high mortality rate of the calves, reduces the number of offspring that survives to reach sexual maturity, resulting in a decrease in N_e. Although the mortality rate of calves is high, one would expect the growth rate to be much higher since 57% of the total number of females and 55% of males are in the reproductive age class (Fig. 4.2). The sex ratio for the total number of reproductive individuals in zoos is 1:1.3, however, this does not reflect the variation of N_e's and sex ratios of individual zoos.

The results of this study indicate that the black rhinoceros populations in nature carry high levels of genetic variation (Table 2.3), therefore the captive population

should also consist of black rhinoceroses with high levels of genetic variation. Predicted gene diversity was calculated for the black rhinoceros population in captivity. The gene diversity value for the captive population where N_e = 116.3, is 0.004 and for a N_e of 150.36 = 0.006. The heterozygosity values predicted for the captive population in both these cases are much lower compared to the observed heterozygosity values for wild populations. The poor breeding success of black rhinoceroses in captivity might be explained by low levels of genetic variation of the breeding animals. Analysis of the genetic varition of captive black rhinoceroses is necessary in order to determine whether captive black rhinoceroses have adequate levels of genetic variation to ensure a positive growth rate of black rhinoceroses in captivity.

Inbreeding of rhinoceroses in captivity.

Inbreeding in black rhinoceroses (*Diceros bicornis michaeli*) in zoos has been recorded (Fig. 4.3) and the inbreeding coefficients calculated. Four father-daughter matings and one brother-sister mating took place. In one case the offspring of these inbred animals died on the day of birth. The records indicate that none of the off-spring of the inbred matings have reproduced (Klös & Frese 1991).

Of these five rhinoceroses, three animals are still alive and according to zoo records, no further breeding is planned with these animals (Taronga Zoo, Australia and Buenos Aires Zoo, Argentinia). It is therefore difficult to study the consequences of inbreeding in the black rhinoceroses and whether rhinoceroses are susceptible to inbreeding depression and to what degree. Knowledge of the genetic variation of the world's captive black rhinoceroses would be very valuable in assisting zoo-keepers to make informed decisions about breeding this endangered species.

It is very difficult to come to a conclusion regarding the effects of inbreeding in small black rhinoceros populations. Where inbreeding has taken place, the zoo authorities did not allow further breeding of the inbred animals, hence the lack of information to determine the effects of inbreeding on rhinoceroses. Since rhinoceroses carry high levels of genetic variation, it might be that they are relatively tolerant of inbreeding but evidence is needed to substantiate this argument.

In conclusion:

Individual zoos have to contribute more in order to achieve the goals of captive breeding and it would be more viable to treat all rhinoceroses in captivity as one metapopulation. To keep one metapopulation in captivity is idealistic but impractical. Every effort should be made by zoo-keepers to balance sex ratios and to maximize N_e.

The high mortality rate of calves under 4 years of age needs urgent attention. Elimination of the high calf mortalities will result in an increase in growth rate.
Each zoo-keeper will have to participate in breeding black rhinoceroses in order to increase the effective population size and improve the growth rate. This can be achieved by: 1) balancing sex ratios, 2) limiting calving intervals, 3) assuring adequate and nutritionally balanced food is available and 4) monitoring the levels of genetic variation in the rhinoceroses to determine whether tranlocations are needed.

It seems that many zoos are only keeping black rhinoceroses for display purposes and are not actively involved in breeding black rhinoceroses. One of the *goals* of rhinoceros conservation is to establish a viable captive population which can serve as a reservoir to stock natural populations since poaching is destroying the remaining populations in the wild.

Curators of zoological gardens have not yet managed to implement a captive breeding programme which achieves this goal and which is essential for the survival of the species.

TABLE 4.1: Comparison of life-history parameters of captive and wild black rhinoceroses.

Sex ratio (m:f)	Calving interval (months)	Gestation period (months)	Rate of increase (%)	Age at sexual maturity (years)
WILD:				
East Africa:				
(1:0.8)	27 - 28	15 - 18	6.8 - 10.9	4.3 - 5 [a]
South Africa:				
(1:0.8)	33 - 38	15 - 18	9.6	5 - 10 [b]
	22 - 55		5.3 - 11	6 - 10 [c]
CAPTIVITY:				
(1:0.8)	37 - 46 (18 - 108)	14 - 16	1.15	6 - 8 [d]

a Goddard (1970)
b Hall-Martin (1986)
c Hitchins & Anderson (1983)
d Klös & Frese (1991)

TABLE 4.2: Demographic data of black rhinoceroses in captivity.

Age class	# cows	# calves	Mean # calves/ cow	calving interval (months)	# dead calves	% dead* calves
3	2	2	1	-	1	50
4	5	5	1	-	0	0
5	4	6	1.5	56.5	2	33.3
6	2	3	1.5	38	2	66.6
7	4	14	3.5	62.6	4	28.8
8	1	4	4	27.6	0	0
9	-	-	-	-	-	-

Age class : 3 = 7 - 9 years, 4 = 10 - 12 years, 5 = 13 - 15 years, 6 = 16 - 18 years, 7 = 19 - 21 years, 8 = 22 - 24 years, 9 = 25 - 27 years, 10 = 28 - 30 years, 11 = 31 - 33, 12 = 34 - 36 years.

* Dead-born and neonatal deaths

From: Klös & Frese (1991).

FIGURE 4.1: Age distribution of black rhinoceroses that died in captivity since 1956.

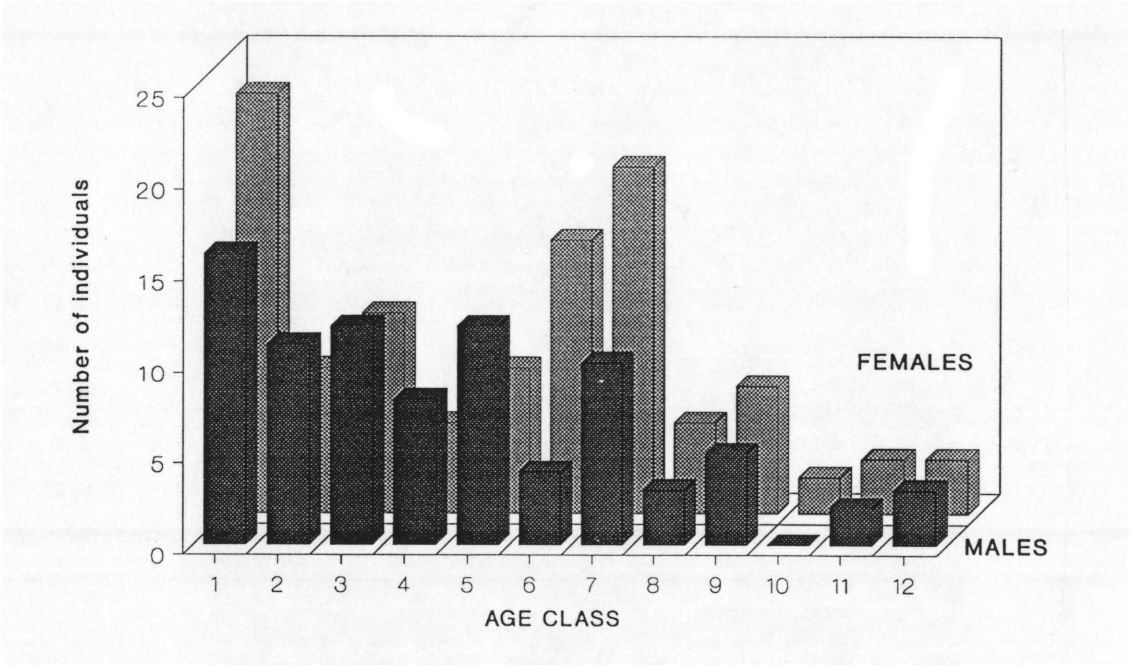
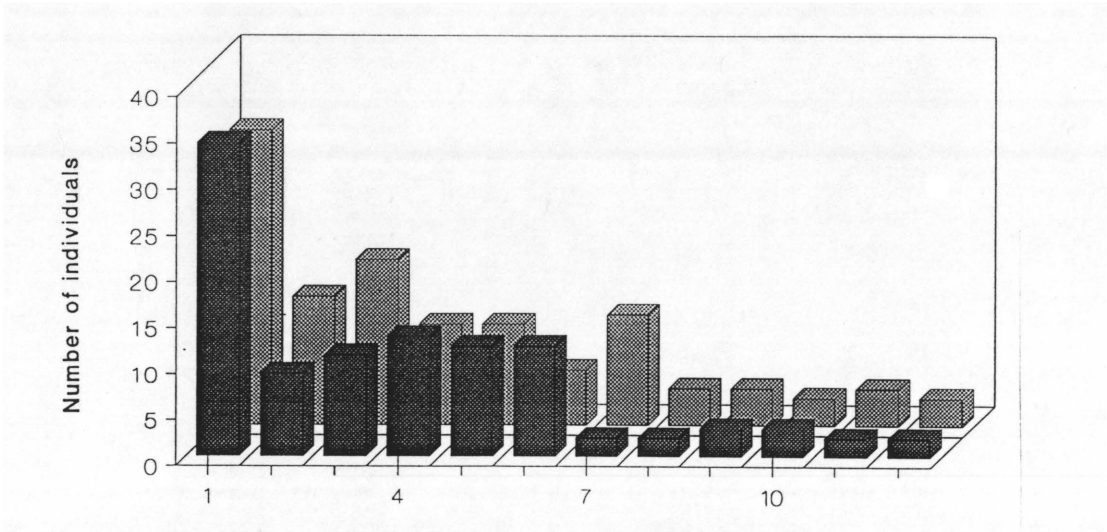
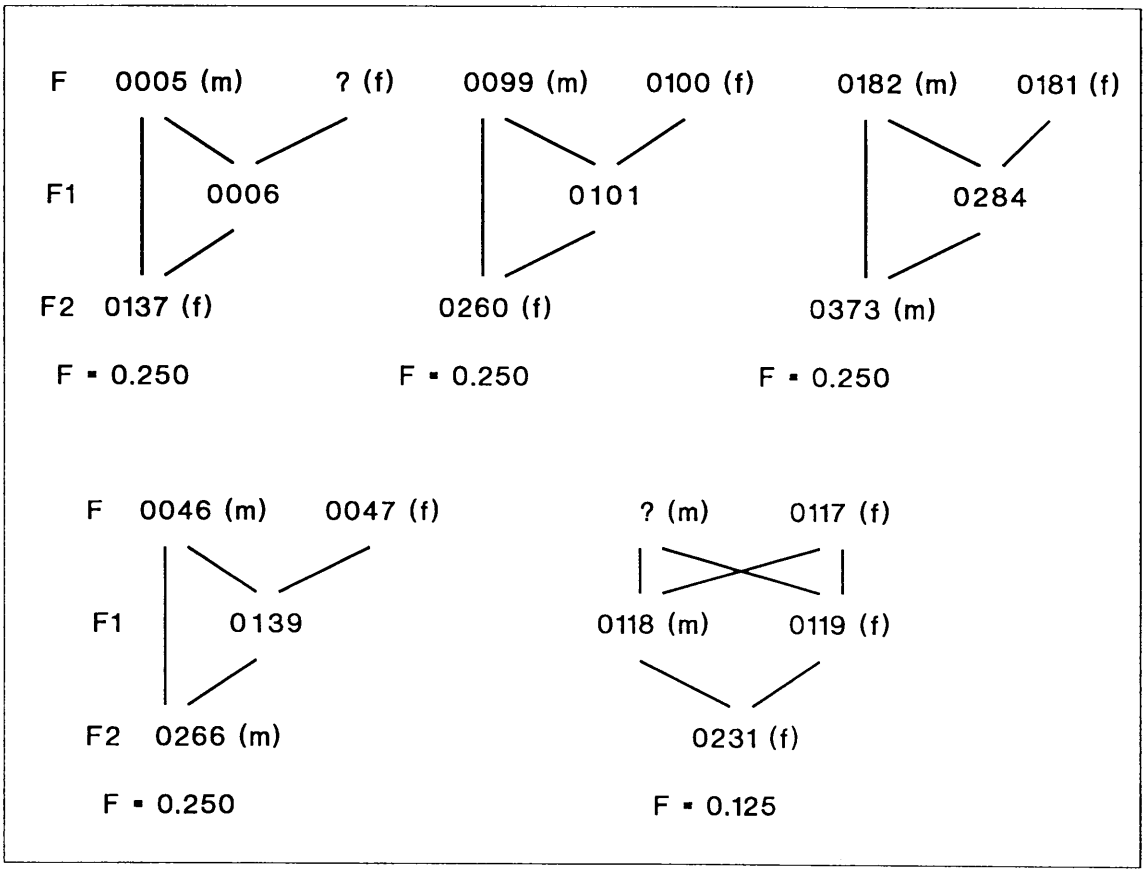


FIGURE 4.2: The age distribution of living captive black rhinoceroses.



Age class: (on the X-axes) 1 ▪ 1-3 years, 2 ▪ 4-6, 3 ▪ 7-9, 4 ▪ 10-12,
5 ▪ 13-15, 6 ▪ 16-18, 7 ▪ 19-21, 8 ▪ 22-24, 9 ▪ 25-27

FIGURE 4.3: Genealogies of inbred black rhinoceroses in zoos. Rhinoceroses are identified according to their studbook numbers (0005), the sex of the animal is in parentheses and F at the bottom of each genealogy indicate the inbreeding coefficient.



CHAPTER 5

POPULATION ECOLOGY

INTRODUCTION:

Population ecology deals with the relationships between a population and its environment. This relationship ultimately determines a population's size, internal organization, dispersal, reproductive rate, age-structure as well as the relevant regulatory mechanisms and interspecies relationships (Elseth & Baumgardner 1981; Hedrick 1984). Ecological factors that affect the distribution and abundance of a population can be divided into abiotic (physical) and biotic factors. Biotic factors include the effects of other species (competitors and predators) and intrinsic aspects such as dispersal, birth- and death rates (Hedrick 1984). Therefore it is vital to understand the factors that affect the distribution, density, dispersion, population growth, interspecific competition, predator-prey interactions and demography of a population.

Three ecological factors influence the demogrpahy of a population: 1) static demographic factors, 2) demographic stochasticity and 3) environmental stochasticity. Age-specific survival and fecundity reflect the ecological conditions under which the animals live and give insight in the dynamics of a population (Caughley 1977, Pimm 1991). Age-dependent birth and death rates can be used to estimate a number of demographic measures: population growth rate, generation length, life expectancy and reproductive value. The ecological evaluation of the actual status of a population includes the determination of the following: age at sexual maturity (male and female), interval between parturitions and the reproductive life-span (female) (Schenkel & Schenkel-Hulliger 1969). Any temporal variations or changes in the birth and

death rates lead to demographic stochasticity which can best be explained as the random fluctuation in the observed birth- and death rate and sex ratio of a population even if the probabilities of birth and death remain constant (Lacy 1991). Burgman *et al.* (1988) define demographic stochasticity as the result of chance events in the survival and reproduction of a finite number of individuals. Environmental stochasticity is the fluctuations in the probabilities of birth and death rate that results from fluctuation in the environment (weather, disease and predation). Stochastic events are unpredictable and the outcome of these depend on a number of probabilities. By using computer models, it is possible to simulate these stochastic events of a population and to project the results in terms of the persistence time of the population.

Aims:

Because demographic factors can be very important for the short-term survival of populations (Goodman 1987), it is essential to study the demographic characteristics of black rhinoceros populations to determine whether they are experiencing demographic risks that may lead to local extinctions.

The aim of this chapter is also to construct a demographic extinction model that takes into consideration important demographic factors and that can allow one to make statements about the probability of survival of each black rhinoceros population.

5.1 Demography

Demographic population factors can lead to a decrease in the reproductive rate, resulting in a decline in the population size which can, in turn, lead to extinction (Berry 1979). Small populations would be affected more severely than larger populations (Soulé 1980, Goodman 1987). Woodruff (1989) states that in a crisis,

the ecological interactions of a population are more important than other factors, eg. genetics.

The impact of birth and death rates on population growth rates and age-structures determines the demography of that population. Four factors affect the size of a population: births, deaths, immigrations and emigrations. These are subject to genetic variation, especially variation within populations. However, it is difficult to disentangle cause and effect when studying the relationship between genetics and demography (Berry 1979). In fact, the demography and genetics of a population are inseparable and should be considered jointly in order to resolve many of the problems that face small populations.

An understanding of the interactions between genetics and demography (Fig. 5.1) is important to provide a reliable basis for determining the vulnerability of the black rhinoceros (*Diceros bicornis*) in southern Africa. The impact of genetics is best explained by the rate at which genetic variation is lost by random genetic drift resulting in changes in individual properties such as fitness. A decrease in individual fitness affects demographic population characteristics, for example a reduction in fecundity which, in turn, results in an altered age-structure which could result in a further decline of the effective population size. On the other hand, changes in environmental factors cause changes in demographic characteristics which may in turn influence the effective population size. Positive feedback loops of biological and environmental interactions have further negative impacts on the population, possibly leading to its extinction (Soulé 1986). These events have been referred to as extinction vortices. The demographic aspect of population vulnerability analysis focuses on extinction through random changes in demographic parameters (Ewens *et al.* 1987).

The Life cycle

Classical demographic information is acquired through analysis of the life cycle of a population by tabulating age-specific survival and reproduction. The life cycle is the fundamental unit of description of the organism. Ecology, genetics, evolution, development and physiology are important to study the life cycle (Caswell 1989). The vital rates (birth, growth, development and mortality rates) on which demography depends, describe the development of individuals throughout the life cycle. The response of these rates to the environment, determines population dynamics in ecological time and the evolution of life histories in evolutionary time. By focusing on the vital rates in the context of an organism's life cycle, demography addresses both the dynamics and structure of a population (Caswell 1989).

By reviewing the literature on the ecology and demography of black rhinoceros (*Diceros bicornis minor*) populations, it is possible to establish a database containing valuable information regarding the ecology and demography of this species. Knowledge of the demographic characteristics of black rhinoceros populations will enable one to make management recommendations and take appropriate conservation action.

5.2 Implications of ecology and demography of the black rhinoceros for Population Vulnerability Analysis (PVA)

Social organisation: The only stable social group in the black rhinoceros is the cow - calf unit. It persists when the cow mates again and even during most of the new pregnancy (Schenkel & Schenkel-Hulliger 1969). Goddard (1967) suggested that later intolerance of the cow for her old offspring may serve an evolutionary function assuring population dispersal in a species which is very sedentary and therefore

susceptible to the effects of inbreeding. The adult male rhinoceros is essentially a solitary animal. However, it may associate with other animals of both sexes and all ages (Goddard 1967). Males and females associate only temporarily and no strong bond exists between them. Goddard (1967) found both sexes sharing the same home range in areas with low population densities.

The black rhinoceros is one of the species living in the transitional habitat between grassland and forest (Schenkel & Schenkel-Hulliger 1969). It is well-adapted as a browser with its prehensile upper lip (Penny 1987) and feeds on a variety of bushes and shrubs in *Acacia* woodlands (Schenkel & Schenkel-Hulliger 1969). However, it can also pick up fallen fruits from the ground and from the trees where it can reach. Black rhinoceroses are dependent on water and keep within 5 km of water during dry seasons (Penny 1987). The home ranges of the animals are therefore centered on waterholes and for rhinoceros bulls a home range with a radius of 5 km appears typical. The home range size of female black rhinoceroses varies depending on the availability of food. In thickets, which are the most suitable habitats for these animals, home ranges can be as small as 3 km^2 while in more arid areas they can be up to 90 km^2 (Penny 1987). Studies in the Hluhluwe Game Reserve showed that the black rhinoceros is a sedentary species with no records of movement out of their home ranges (Hitchins & Brooks 1986). Since female and male rhinoceroses keep close to waterholes, this increases the potential of rhinoceroses finding mates when they reach sexual maturity. Joubert (1969) also found that both sexes of *Diceros bicornis bicornis* in Namibia shared the same territory. The results indicate that because of the social organisation of rhinoceroses, an individual would not experience difficulty in finding a mate (as long as the sex ratio does not become skew). Most animals are likely to be mated since a sex ratio of 1:1 exists in the wild (Goddard 1969) and this will therefore have a positive effect on the effective population size (N_e). This is reassuring, because to achieve maximum growth rate,

any factors that can cause variations in growth rate should be prevented.

Reproduction: Although it has been found that rhinoceroses can start reproducing at the age of four to five years (Goddard 1970), rhinoceroses in some populations only start reproducing at eight years of age, e.g. in Hluhluwe (Hitchins & Brooks 1986). Generally, rhinoceroses reach sexual maturity at about six or seven years (Goddard 1966, Gowda 1967, Hallstrom 1967, Hays 1967, Yamamoto 1967 and Penny 1987) and the gestation period of black rhinoceroses vary from 450 and 480 days. Only one calf is born. Black rhinoceroses can attain ages of 45 - 60 years (Balfour & Balfour 1991), easily producing 10 offspring per lifetime. The growth rate is potentially high in terms of rhinoceros generation time, but very low per annum. To ensure stable populations, the population size have to be maximised since it is only the reproducing individuals that will make postive contributions to population growth. The size (N) of extant black rhinoceros populations are small, resulting in a slow absolute growth rate. Poaching results in a decrease in population size, since poachers select mostly adult rhinoceroses, thus lowering the breeding rate even further.

Food competition: Competition between rhinoceros and other verebrates is limited. Where elephant and rhinoceros occur concurrently, the impact of the elephant on its habitat is not entirely favourable to the rhinoceros since there is some overlap of the food preferences of the two species (Schenkel & Schenkel-Hulliger 1969). Food competition between elephant and rhinoceros should not be overrated, however, as browsing elephants eat mainly leaves or chew the bark of branches and they hardly ever tear off the thin twigs which are the main diet of the rhinoceros (Schenkel & Schenkel-Hulliger 1969). Evidence suggests that interspecific competition for food, is not an important factor in affecting the demography of rhinoceroses and is unlikely to have a negative impact on their population sizes.

Predation: As rhinoceroses are short-sighted, they have difficulty in detecting enemies at a distance. However, they have a good sense of smell and hearing (Schenkel & Schenckel-Hulliger 1969 and Balfour 1991). Their sensory organs function primarily in intraspecific communication and contact, in the selection of food and in orientation in the environment (Schenkel & Schenkel-Hulliger 1969). Predators do not pose a serious threat to black rhinoceroses. Lions take calves if they can separate them from their mothers, but the cows defend their calves so that this is actually a rare event (Penny 1987). Spotted hyaenas, on the other hand, can be a more serious threat because they can take calves so successfully that it can actually limit population growth (Penny 1987). In any event, predation is not considered an important limiting factor for the survival of the black rhinoceros (Schenkel & Schenkel-Hulliger 1969), and it therefore does not strongly affect the size of a population. Guggisberg (1966), Schenkel & Schenkel-Hulliger (1969) and Penny (1987) agree that poaching by man is the greatest limiting factor of rhinoceroses.

Effects of natural catastrophes: Goddard (1968) reported that damage to the vegetation in Ngorongoro caused by fire did not appear to have any marked unfavourable effect on the habitat of rhinos. Rhinoceroses continued to browse on charred shrubs without adverse effects in an area that had been completely burnt. Even after the first rains had stimulated green growth, the animals continued to feed on charred *Indigofera arrecta* (Goddard 1968). Goddard (1968) also observed that they utilize herbs in heavily overgrazed areas that are unsuitable for other ungulates such as the wildebeest, *Connochaetes taurinus*. The tree-bush complex of Tsavo National Park, Kenya, was reduced and destroyed by elephants and fire in many areas and replaced by bush-grassland and grassland. Goddard (1970) concluded that the black rhinoceros population was stable in spite of the destruction of habitat since the elephants did not destroy plant species which are important food for the black rhinoceros. However, during a drought period (August 1960 - September 1961) in

East Tsavo, at least 282 rhinoceroses died of nutritional anaemia (Goddard 1970). Foster (1965) also recorded this drought period in East Tsavo Park and suggested that the ultimate cause of death was probably lack of sufficient quantity and/or quality of food due to massive destruction of vegetation by elephants which concentrated along the rivers during the drought. Schenkel & Schenkel-Hulliger (1969) also attributed the deaths of these animals to malnutrition. However, Du Toit & Paul (1987), suggested that haemolytic anaemia may have caused this massive die-off. (See Chapter 3). Rhinoceroses in Tsavo can survive by utilizing the abundant ground legumes or shrubs which exist in abundance in the present grassland areas. Goddard (1970) made the observation that a black rhinoceros population may experience severe environmental pressures but it has a minimal effect on the population structure (age-structure and sex ratio). Schenkel & Schenkel-Hulliger (1969) found the sex ratio to be 1:1 even after a decline in population numbers.

Joubert (1969) conducted an extensive study on *Diceros bicornis bicornis* in Namibia. These rhinoceroses are exposed to severe weather and habitat conditions and yet (Joubert 1969) concluded that habitat conditions were favourable and that the animals tend to breed from December to June. From the literature it is evident that periods of drought do not frequently have severe impacts on rhinoceros population size as they compensate for the lack of browse by utilizing other plants where possible.

The only well-documented case of a rapid decline in population size due to natural causes, occurred in the Hluhluwe Game Reserve, Natal, from 1961 to 1985. During 1961, 55 black rhinoceroses died in Hluhluwe, the cause being unknown (possibly disease or environment related causes and not as a result of poaching) (Hitchins 1969). Thomson (1986) attributed the decline in rhinoceros numbers in Hluhluwe (from 300 in 1961 to less than 100 in 1985) to a change in habitat. Due to the

removal of predominantly grazing animals such as wildebeest (*Connochaetes taurinus*), zebra (*Equus burchelli*), warthog (*Phacochoerus aethiopicus*), impala (*Aepyceros melampus*) and nyala (*Tragelaphus angasii*) the *Acacia* spp. thickets changed to wooded grasslands. Fires further reduced the quality of the black rhinoceros thicket habitats: the animals were observed to be in poor condition, they reached sexual maturity much later and the calving interval was increased (Thomson 1986). Hitchins & Anderson (1983) recorded the earliest age of sexual maturity as 10 years for the Hluhluwe population, which also had a slow recruitment rate. Thomson (1986) concluded that the Hluhluwe population had experienced environmental stress and that a population crash was inevitable. This is clearly a case of environmental stochasticity which influenced the demography of a population. The change of habitat and fires resulted in an increase in mortality resulting from insufficient nutrition and/or diseases. This lead to a decline in the population size, longer calving intervals and older ages at sexual maturity.

Effects of poaching: The decline in black rhinoceros numbers is alarming, and even the last strongholds of this endangered species are threatened. Several examples exist where the decline in rhinoceros numbers are due to human agencies. A decline in black rhinoceros, *Diceros bicornis michaeli* population numbers in the Amboseli National Reserve, Kenya, can be attributed to poaching (Western 1982): from 1971 to 1977 human agencies accounted for 94% of rhinoceros mortalities whereas only 2% died from natural causes. Western (1982) concluded that the animals face extermination more through their susceptibility to poaching than through loss of habitat. In Amboseli the most important consideration in the conservation of the rhinoceros is to prevent population numbers from declining further. In 1987 only 10 animals were recorded in Amboseli, which is far below the potential carrying capacity of this region (±100 animals) (Cumming *et al.* 1990). It is difficult for a black rhinoceros population to recover from such low numbers because of their slow reproduction

rate. Western (1982) suggested translocations in order to maintain genetic viability. In the Ngorongoro Crater, Tanzania, the population number fell from 108 to 25 during a period of 16 years, a decline of 77%, and poaching was also the main reason for this decline (Kiwia 1989). However, the population structure did not change significantly. Kiwia (1989) expressed doubts regarding the genetic viability of a population consisting only of 25 individuals.

The black rhinoceros population of the Luangwa Valley National Park, Zambia has a high biological and conservation status (Cumming & Jackson 1981). Leader-Williams (1988) reported on the devastating decline in rhinoceros numbers in the Luangwa Valley as a result of poaching. This population decreased from about 3000 animals to only 75 within a period of only 10 years.

Poaching of rhinoceroses in Zimbabwe has taken on serious proportions. A large number (556) of these rhinoceroses were killed by legal hunting or by Tsetse Control Staff between 1919 and 1964 and this figure does not include illegal hunting and poaching prior to 1952 (Roth 1967). In 1971 the number of animals in the Zambezi Valley was estimated at 590 - 750 (Kerr & Fothergill 1971). The estimate of rhinoceros numbers in 1987/1988 was 500 - 1000 in the Zambezi Valley (Du Toit 1989). The total number of black rhinoceroses in Zimbabwe, including the Zambezi Valley, was estimated at 1550 - 2200 in 1987/1988 (Du Toit 1989) but has fallen to about 400 animals in 1992 (Du Toit pers. comm.*). These examples clearly illustrate the influence and impact of human activities on black rhinoceros populations.

Poaching results in artificial selection and the population size declines drastically since the individuals which are reproductively active are removed from the

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*R. du Toit. 1992. Zambezi Rhino Project, Harare, Zimbabwe.

population. Poaching is responsible for big variances in growth rate (unbalanced sex ratio and a change in age-structure since mostly adults are removed). From the above-mentioned it is evident that the decline in rhinoceros numbers is appalling. The distribution of eastern Africa black rhinoceros subspecies and numbers in 1992 are illustrated in Fig. 5.2 (a) and those of southern Africa in Fig. 5. 2 (b) (SSC-ARSG\RHINO-STA 1992).

What makes the decline in rhinoceros numbers even worse is that the remaining black rhinoceroses populations are small (See Fig. 5.3). Only **five** populations survive with a population size exceeding 100 individuals and only the Etosha population exceeds 300 individuals. Analysis of the genetic-, environmental- and demographic factors that affect black rhinoceros populations, illustrate that even when conditions do not favour the rhinoceroses, the impact of these factors on the effective population size is minimal compared to that of poaching.

5.3 The Small population approach *versus* the Declining population approach:

Small populations, as is the case for the black rhinoceros populations mentioned above, are challenged by a number of factors that increase the likelihood of the population becoming extinct simply because the population is small (Ballou 1991). Wilcox (1980) views extinction as a process which is now most frequently initiated by human-induced environmental change. It starts with a deterministic pressure (habitat loss, poaching, competition or predation) causing a gradual reduction in size, number and proximity of populations. As populations become smaller, fewer and more isolated, demographic stochasticity and genetic erosion start to predominate. Inbreeding leads to inbreeding depression causing a reduction in viability and fecundity that directly affects the demographic parameters and leads to further decline in population size. The stochastic processes with feedback loops results in a

downward spiral to extinction (Wilcox 1980). The aim of the declining population approach is to discover the cause of the decline and to prescribe an antidote (Caughley 1994). The research effort is aimed at determining **why** the population is declining and what might be done about it. In contrast, the small population approach deals largely with the genetic and dynamic problems faced by a population at risk of extinction (Caughley 1994).

These two approaches are not necessarily opposing, and can prove valuable in the conservation of a species. Firstly, the declining population approach enables early identification of a decline in a population. Secondly, the ecological factors that cause the decline should be identified and, thirdly, every effort should then be made to avoid the small population problem. Small populations are treated according to the small population approach. I feel that these two approaches are complimentary and that such a perspective is likely to add a new dimension to conservation issues.

For minimising the probability of extinction, a minimum viable population size (MVP) is needed and to calculate a MVP, information on the birth-and-death process is required for the species being studied (Lande & Barrowclough 1987).

5.4 Minimum viable population size (MVP)

Franklin (1980) employed an equilibrium model for the maintenance of additive genetic variance in quantitative characters by mutation in the absence of selection. He proposed a minimum effective population size of 500 individuals for long-term conservation and 50 individuals for short-term conservation. Franklin observed that purely additive genetic variance is lost by random genetic drift at the same rate as heterozygosity: 1/2 N_e per generation (Lande & Barrowclough 1987). The input of additive genetic variance per generation from mutation was taken to be 10^{-3} of the

environmental variance that would be expressed in a highly inbred line. To maintain a heritability of 0.5, it is necessary that the genetic and environmental variances be about equal (Lande & Barrowclough 1987). Accordingly, an approximate equilibrium between gain and loss is expected when $1/2N_e = 10^{-3}$, that is $N_e = 500$. The amount of genetic variation maintained will depend on the effective population size. However, the above-mentioned minimum viable population concept remains controversial. Woodruff (1989) concludes that the effective population size indeed plays an important role in the conservation of a species but it does not lead to magic numbers of 50 or 500.

Shaffer (1981) defined a MVP as the smallest isolated population having a 99% chance of remaining extant for 1000 years despite the effects of demographic, environmental and genetic stochasticity and natural catastrophes (Shaffer 1987). It is important to know the minimum population size which is likely to yield a required level of genetic variation to ensure the long-term survival of a species (Frankel 1974). The minimum viable population (MVP) concept has both genetic and demographic aspects. The effective population size is affected by variance in progeny production, fluctuation in adult sex ratio and overlapping generations (Hill 1972). Grumbine (1990) argues that a MVP needs a minimum area within which to survive and that this has profound implications for a society that has yet to grasp that national parks and reserves do not automatically protect the species that depend on them. Soulé (1987) defined a MVP as a set of population estimates that are the product of a systematic process for estimating species-, location- and time specific criteria for persistence. He refers to the process as a population vulnerability (or viability) analysis (PVA). PVA confronts some of the most difficult problems of ecology and evolutionary biology and determines whether current theory leads to long-term predictions as to the probable persistence of populations or species (Woodruff 1989).

5.5 The use of computer models in Population Vulnerability Analysis

A computer model was constructed to make statements about the vulnerability of black rhinoceros populations and to estimate the persistence time of each population. Starfield & Bleloch (1986) describe a model as an intellectual tool that enables one to define problems, organise thoughts, understand the data and to make predictions. In the non-physical sciences it is inevitable that management decisions have to be made despite the lack of data and of understanding. Several modeling approaches exist. Deterministic models are mathematical models in which all relationships are fixed and the concept of probability does not feature. A given input results in one exact prediction as output (Starfield & Bleloch 1991). A deterministic model therefore can not account for any environmental stochasticity or variation because it does not take into account the variation in parameters of mathematical functions which describe rhinoceros demography.

5.6 STOCHASTIC POPULATION MODELS

The aim of stochastic population models is to estimate the probability of extinction of a population within a finite time, taking into account environmental and/or demographic stochasticity (Burgman *et al* 1988). Stochastic models are mathematical models based on probabilities. The prediction of the model is a range of possible numbers. These models are of particular importance to small populations. Biological systems are probabilistic or stochastic and not deterministic (Krebs 1985) and it is therefore more accurate to use probabilities when predicting, for example, the expected persistence time of a population.

1) **Goodman's demographic extinction model:**

Goodman (1987) developed an extinction model which enables one to estimate the viabilities of small populations and to estimate the minimum extent of a reserve that can support a population for a period before that population goes extinct. Goodman's model examines the relationship between the expected survival time of a population and its associated demographic variables (life history and environmental variation). The model is a generalized model of population dynamics that requires as input, the minimal description of the population's essential demographic statistics. It then calculates the expected time to extinction.

Goodmans's model can be formulated as:

$$T = \sum_{x=1}^{N_{max}} \sum_{y=x}^{N_{max}} [2/\ y(yV - r)] \prod_{z=x}^{y-1} [zV + r/\ zV - r]$$

where T is the expected persistence time, r = average growth rate of the population, V = the variance of r attributed to environmental fluctuations and N_{max}= the maximum population size (Belovsky 1987). Long-term demographic data are required to solve an extinction model. The model does not use an age-structured approach. Growth rate is calculated as follows: r = the difference between birth and death rates. Simple body mass dependent relationships exist for the maximum r values (r_m) for unicellar animals to mammals and r_m can be used as a first approximation for r for different mammals in the extinction model. One basic assumption is necessary to estimate N_{max} and V. The rate of increase in animal populations are limited by energy. Therefore, the upper limit of population size (N_{max}) is set by available energy levels. The best way to approximate V, given some environmental fluctuation is to scale the inherent variability in r by the environmental fluctuations and call the increase in variance, V. Environmental variances were calculated by the Department

of Environmental Health, USA, 1960, and can be used when data is unavailable to determine V for different types of climatic conditions such as temperate forest rainfall, dry temperate forest, grasslands (temperate and tropical). The lowest environmental varaince ($V = 1.43r$) was found for temperate forest rainfall and the highest values ($V = 7.32r$) were found for grassland rainfall (Belovsky 1987). However, this method of estimation has limitations and direct estimates of r, $N_{max}$and V, are much more useful.

Goodman used the birth-and-death process model because this model describes a population in terms of the number of individuals, the mean birth rate and mean death rate and variances, and it leads to a direct algebraic solution for the expected time to extinction. The extinction model provides an expected or average persistence time; however, this value is distributed as a negative exponential which means that, although a population of a give size may have a large expected persistence time, a majority of populations that size will persist for a far shorter period and only a few will persist for far longer.

Goodman (1987) concluded that environmental stochasticity poses a more severe problem than demographic stochasticity and his model confirmed suspicions that earlier estimates of population survival times based only on demographic stochasticity were conservative. Belovsky (1987) addressed the accuracy of Goodman's extinction model by comparing the predicted persistence times for different boreal mammal distributions on mountain tops in the southwestern USA (where observed values of r and V were used). The comparison indicated a close agreement between the predictions from the model and the observed persistence. Belovsky concluded that the model appears to capture the dynamics of the extinction process and may be a useful first approximation for the probability of extinction. Given carnivore and herbivore populations of equal size, the model's predictions are supported more

closely for herbivores; extinctions are much greater in carnivores than expected. This may be because carnivores have very specialized habitat requirements. The minimum population size (N) required to permit a 95% probability of persistence for a given time perios can be examined for the impact of the relative environmental variance on *r*; (*V/r*). This minimum N increases dramatically over a small range of *V/r* values and then increases at a much slower rate. In short, the simple presence of environmentally-induced variance has a dramatic impact on ectinction. Goodman's extinction model considers the important aspects of the extinction process and provides a first approximation of the necessary population sizes and habitat area for averting extinction over some time period (Belovsky 1987). The model also provides a useful methodology for estimating the sizes of viable populations (Belovsky 1987).

The concept of MVP (minimum viable population size), does not inspire optimism since comparison of the areas required for mammal persistence with that of the sizes of parks leads to the conclusion that no park is large enough to guarantee persistence for 1000 years (Belovsky 1987).

2) **VORTEX:**

A stochastic simulation of the extinction process (better known as Vortex) was developed by Lacy (1991) and is a Monte Carlo simulation of the effects of deterministic forces as well as demographic, environmental, and genetic stochastic events on wildlife populations (Kreeger 1991). Vortex models population dynamics as sequential events that occur according to defined probabilities. The simulation is based on the events that describe the typical life cycle of sexually reproducing, diploid organisms and is most applicable to species with low fecundity and long lifespans (Lacy 1991). By sampling birth rates, death rates and the carrying capacity from binomial or normal distributions, Vortex can model environmental variation, linear

trends in carrying capacity, catastrophes and the transmission of genes through generations (Lacy 1991). Demographic stochasticity in Vortex is modelled by determining the occurrence of probabilistic events such as reproduction, litter size, sex determination and death with a pseudo-random number generator. Fecundity is assumed to be independent of age after an animal reaches reproductive age. Mortality rates are specified for each pre-reproductive age-sex class and for reproductive age animals. Since no analytical model exists to describe the combined effect of demographic stochasticity and loss of genetic variation on the probability of population persistence time, Lacy (1991) wrote Vortex with the objective to incorporate genetics as well. Genetic drift is modelled in Vortex by simulation of the transmission of alleles at a hypothetical locus. Each animal is assigned two unique alleles at the beginning of the simulation. Each offspring is randomly assigned one of the alleles from each parent. Inbreeding depression is modelled as a loss of viability during the first year amongst inbred animals. The impact of inbreeding is determined by using one of two models: 1) the recessive lethals model or 2) the heterosis model.

In summary, Vortex simulates many of the processes which influence the size, behaviour and viability of populations. The output is as follows: 1) the probability of extinction at specified intervals, 2) the median time to extinction, 3) the mean time to extinction and 4) the mean size and genetic variation of extant populations (Lacy 1991). Lacy (1993) admits that some aspects of population dynamics are not modelled by Vortex. In particular Vortex is a single-species dynamics model and is therefore inappropriate for use where the fate of a species is strongly determined by interspecific interaction. Lacy (1993) also makes the distinction that PVA does not predict in general what will happen to a population but it forecasts the likely effects only of those factors incorporated into the model. However, the methods and formulas used in the program are not described in detail and it constitutes somewhat of a black box approach.

3) **Population Persistence Analysis (PPA):**

Dennis (*et al.* 1991) developed statistical methods for estimating quantities related to growth rates and extinction probabilities from time series data on the abundance of a single population. Nicholls *et al.* (in prep.) realised the application of this model as an objective method of introducing "risk assessment" into the conservation management and planning of large populations. PPA requires only census data of a population to determine the persistence time. Therefore, the application of this model to wild populations is unlimited since census data is available for many populations. Nicholls *et al.* (in prep.) named this approach, population persistence analysis. This represents a first step towards integrating the strengths of risk management into the declining population approach (Nicholls *et al.* in prep.). Extinction models such as Vortex, Goodman and DEMM (see 5.7) require more sophisticated data that are difficult to collect.

The statistical methods of PPA are based on a stochastic diffusion model of exponential growth which is based on the Wiener-drift process as a general approximation for age- or stage-structured populations. The model incorporates environmental stochastic fluctuations and yields a lognormal probability distribution of population abundance. Maximum likelihood estimates of two parameters: the diffusion process drift parameter and the process variance parameter are determined by statistical procedures (Table 5.3). Various growth and extinction related quantities are functions of these two parameters, including the continuous rate of increase, the finite rate of increase, the geometric finite rate of increase, the probability of reaching a lower threshold population size, the mean, median and most likely time to reach the threshold and the projected population size (Dennis *et al.* 1991). Dennis *et al.* (1991) concluded that the model and associated statistical methods can be useful for investigating various scientific and management questions concerning species

preservation.

Nicholls *et al.* (in prep.) used PPA on 12 herbivore populations from the Kruger National Park and concluded that risk assessment models (assuming that past performance can be used to predict future demographic trends) can be valuable for conservation managers. Managers should focus on those populations that have a significant probability of declining drastically within the immediate future.

5.7 DETERMINISTIC and PSEUDO-DETERMINISTIC MODELS

1) **Matrix models:**

An important factor in the dynamics of small populations is age-structure. Age-structured stochastic models are often based on a Leslie matrix with stochasticity introduced as randomly varying cells of the matrix (age-specific survival and/or fecundity) (Burgman *et al.* 1988). The inclusion of a simulation process in the model not only allows the exploration of the variance in all life-history parameters, but also covariances and density dependent relations (Burgman *et al.* 1988). However, most of these models include environmental variation which is manifested in certain assumed distributions of population parameters.

It is possible to construct models that can simulate environmental- as well as demographic stochasticity. This is achieved by including variances of population parameters (to obtain environmental stochasticity) and through the explicit simulation of individuals (to obtain demographic stochasticity) (Burgman *et al.* 1988). The model constructed for the purpose of the present study (Demographic extinction matrix model - DEMM) is more correctly called a pseudo-deterministic model because of

its dualistic approach. It can give a straightforward deterministic outcome (called DEMM 1) as well as the stochastic outcome (called DEMM 2) by including age-specific variation in birth and death rates. A population vulnerability analysis can become very complicated and the implementation of a computer model can facilitate the solution of this problem.

METHODS

A pseudo-deterministic population model that projects the probability of extinction of a population within a finite time, accounting for demographic stochasticity (Burgman *et al.* 1988) was developed. The demographic extinction matrix model (DEMM) is an age-classified matrix model that requires life table data regarding the survivorship (l_x) and reproduction or maternity function (m_x) of a population.

Age-specific birth- and death rates are necessary to calculate the l_x and m_x values. Although it was very difficult to get age-specific fecundity data for wild black rhinoceros populations, records exist for some black rhinoceroses in captivity, where data of 22 known age black rhinoceros cows were used to construct a life table following the methods of Caughley (1977). Information on age-specific birth- and death rates of wild black rhinoceros populations is incomplete and impedes the analysis. Limited fecundity and mortality data exist for a few populations (See Table 5. 2).

The model was written using Pascal (Appendix 1) and only requires age-specific l_x and m_x data and the effective population size of a particular population. It calculates

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*Dr. P. Goodman. 1993. Mkuzi Game Reserve, Natal.

the survival and fecundity probabilities according to Caswell's (1989) approach, using the l_x and m_x data. Formulation of the matrix projection model can be divided into several steps:

1) division of a continuous age variable x into age-classes (all of the same duration), 2) defining a projection interval of the same duration as the age class width, 3) projecting the abundance of each age class from one time to the next, in other words the calculation of birth-flow survival probabilities by
using the following formula;

$$P_i = l(i) + l(i + 1) / l(i - 1) + l(i)$$

where P_i= the probability that an individual in age class i will survive from time t to time $t + 1$ (Caswell 1989). The black rhinoceros does not seem to be a seasonal breeder and calving takes place throughout the year (Goddard 1967) and throughout every age class from the time they reach sexual maturity. Therefore it was decided to use birth-flow survival and fecundity probabilities rather than birth-pulse probabilities.

4) calculation of birth-flow fertilities;

$$F_i = \{ l (0.5)\} \quad m_i + P_i m_i + 1 / 2$$

where $l(0.5)$ can be estimated by: $\{l(0) + l(1)\} / 2$ (Caswell 1989).

The survival and fecundity values are then entered in the Leslie matrix. A projection of the population at time $t + 1$, can be made and is written in matrix form as follows: $\mathbf{n}(t + 1) = \mathbf{A}\mathbf{n}(t)$. The matrix **A** is a population projection matrix and **n** is the population vector. Analysis of the matrix starts with an initial age distribution vector $\mathbf{n}(0)$ and the subsequent state of the population is projected by repeated matrix multiplica-

tion (Caswell 1989).

The first assumption of this type of model is that age is the only relevant factor in determining the animal's demographic fate. Other factors will only be relevant if they are highly correlated with age. The demography of many organisms depends much more on their size or developmental stage than absolute age, however, this does not make the model less reliable since these variables are mostly correlated with age. The second assumption is that the model discards all information on the age dependency of vital rates within age classes. The model assumes that fertilities and survival probabilities remain constant over time and this requires that the vital rates be independent of density. This is an inconsistency since the vital rates of most organisms vary conspicuously in time and space and the effects of density on population dynamics are well documented. To justify the above-mentioned analyses, careful interpretation of the demographic analyses is necessary and a distinction should be made between forecasting and projection: a forecast is an attempt to predict what will happen and a projection is an attempt to describe what would happen given certain hypotheses (Caswell 1989).

The model can be used solely as a deterministic model or by including variances in fecundity and mortality it can give a more stochastic outcome. Standard deviations were calculated from the fertility coefficients of several females in each age class. The standard errors calculated from the standard deviations, were used as a vector-type. The standard deviation, mean and standard error vectors (Table 5.2) were used to calculate normally-distributed deviations (Matloff 1988) of fecundity for each age class. Age-specific fecundity data were obtained from the black rhinoceros population in captivity and the Addo black rhinoceros population. The product of each deviation and the mean fertility coefficient gives a new fertility coefficient that is used in the matrix. Several series of matrix multiplications were performed, each

time resulting in different times to extinction. The program is terminated when the population size reaches a value of less than one animal.

The extinction process was studied in three different black rhinoceros populations by using three extinction models: 1) Goodman's deterministic demographic extinction model; 2) Vortex - a stochastic simulation of the extinction process that includes genetic factors (heterosis model); and 3) DEMM - Demographic extinction matrix model - using age-specific life-history patterns in a Leslie matrix to compute extinction. In addition, PPA (Population persistence analysis) was used to identify black rhinoceros populations that are vulnerable to a decline in population numbers. As DEMM requires age-specific data, only three black rhinoceros populations could be analysed and compared to the other two extinction models.

RESULTS AND DISCUSSION

Simulation of the captive black rhinoceros population (Fig. 5.4 a), using DEMM 1 (no stochasticity), predicted extinction after 12 generations. Depending on the age at which they reach sexual maturity (6 or 8 years), the population will go extinct in 72 - 96 years time. Analysis of this population using Goodman's extinction model predicts extinction of this population after 91 years (assuming a growth rate of 2% per year and the the mean environmental variance, V = 4.37). The black rhinoceros population in captivity is experiencing a low growth rate compared to high growth rates of wild populations (Table 5.1). A growth rate of 1 - 2% was observed for black rhinoceros in captivity (Klös & Frese (1991) and used in Goodman's simulation. Simulation using Vortex, forecasts extinction of the captive population between 70 and 162 years with a mean of 90 years. From Fig. 5.4 (a) it is evident that the predictions of the three extinction models do not differ strongly, which provides

some confidence in the time of extinction predicted for the rhinos in captivity.

In the case of the Mkuzi black rhinoceros population (Fig. 5.4 b), Vortex predicts that the population will go extinct earlier than predicted by Goodman's model and DEMM. Fecundity values of the Addo black rhinoceros population were used. These latter values reflect the maximum growth rate observed in a wild black rhinoceros population while minimum growth rate is reflected by the captive black rhinoceros population. To achieve reliable projections, it may be more realistic to use the average of these two extreme values. DEMM (Fig. 5.4 b), also results in an early extinction for the Mkuzi population and illustrates the effect a decline in birth-rate will have on this population. The reason why Vortex predicts that this population will go extinct in about 80 years might be explained by the fact that Vortex includes the genetic factors, with the emphasis on inbreeding, and because Mkuzi is a small population (N = 70), the effects of genetic drift and inbreeding in a small population can have adverse effects on survival. However, the predictions of Goodman's model and the projections of DEMM are once again similar, strengthening the forecasting of the demographic time to extinction. Vortex and DEMM predict an extinction time for the Tsavo population (Fig. 5.4 c), between 150 and 250 years while Goodman's model is more conservative and predicts extinction only at 283 years. Since there is no estimate of the growth rate for the Tsavo population, a growth rate of 8%, which was measured in the Addo population, was used. These values are relatively high compared to growth rates of the wild black rhinoceros populations and this might explain the outcome of Goodman's model. Except for the Mkuzi black rhinoceros population, the results of the different models overlap and this suggests relative consistency in forecasts regarding the extinction probability of a population.

Population Persistence Analysis (PPA):

Census data for five black rhinoceros populations (Appendix III) were used to determine their persistence time by using the population persistence analysis model (Dennis *et al.* 1991). The growth parameters are provided in Table 5.3 and the extinction parameters in Table 5.4. The probabilities of attaining the survival threshold (one individual remaining), (Table 5.4), shows that the Etosha and Hluhluwe-Umfolozi black rhinoceros populations are at risk of extinction. These are the only two populations that experienced a decline in numbers in the past seven years. The black rhinoceros populations of Addo and Kruger National Park, are however, experiencing high growth rates (Table 5.3). Although the populations of Hluhluwe-Umfolozi and Etosha are at risk of going extinct, the persistence time for Etosha is over 1000 years whereas for Hluhluwe-Umfolozi it is only 159 years (Table 5.4). The Etosha population is larger than the Hluhluwe-Umfolozi population. However, the difference in population size can not be the only measure responsible for such a big difference in persistence time. The process variance parameters (σ^2) of the two populations (Table 5.3), as well as the population size, might explain this difference. Population numbers of Hluhluwe-Umfolozi varied dramatically and in one stage the population numbers fell from 300 to 200. This resulted in a higher variance in the Hluhluwe-Umfolozi population than in the Etosha population, causing a shorter estimated persistence time for the Natal population.

PPA is important for the conservation of populations since it enables one to identify a population at risk of losing its *LARGE population* status. This eventually results in the small population syndrome where intensive management of environmental, demographic and genetic factors is essential to prevent the population from entering the extinction vortex. The declining population approach sees the smallness of a

population as a pathological condition that must be treated to get the population out of that danger zone as fast as possible (Caughley 1994).

Demographic extinction models and predictions

Simulation models give some estimates on how vulnerable a population is so that management can make recommendations regarding the population size or other problems that are facing that population. Depending on the demographic, environmental and genetic information available, different models can be used to predict or project the survival time of a population.

The demographic model (DEMM) (Appendix 1), requires age-specific data and the more complete the data, the better results will be achieved as illustrated by the projection of DEMM for the captive black rhinoceros population where sufficient fecundity and mortality data exists for this population. The results of DEMM correspond to that of other extinction models (Goodman and Vortex), and more accurate predictions on the extinction time of each population can be made especially when considering that each model is unique and uses different parameters. Goodman's model and DEMM (1) are deterministic but the big difference between these two models is that Goodman's model only uses growth rate and variance in growth rate, whereas DEMM (1) uses age-specific fecundity and mortality data in a Leslie matrix.

On the other hand, stochastic simulations such as Vortex and DEMM (2), are different because Vortex is a Monte Carlo simulation of deterministic, demographic, environmental and genetic stochastic events and DEMM (2) is stochastic since it uses the variance in fecundity. The predictions from the different extinction models overlapped and were very similar (Fig. 5.4). This leads to the conclusion that each

model can be used separately (depending much on the data available for a particular population) or in conjunction with each other to yield more reliable projections.

Projections of the extinction models for black rhinoceros populations

It is clear from the genetic analysis of the black rhinoceros populations (Chapter 2), that they are in no immediate danger regarding genetic impoverishment. It was also necessary to see if any demographic risk exists. By implementing demographic extinction models, one can make predictions on the time of extinction of a population so that preventative action can be taken to improve it's management.

The three extinction models project that in 300 years, the black rhinoceros populations of Mkuzi, Tsavo and in captivity will be extinct. These models only include environmental, demographic and genetic factors and not human-induced factors such as poaching. Poaching affects demographic variables such as sex ratio and age-structure that further decreases the population size. Intensive management of the small remaining and scattered black rhinoceros populations is absolutely necessary to ensure the survival of this species. It is clear that the time to extinction increases when the population size is bigger. This was also the case for the other black rhinoceros populations including the Zambezi Valley population. However, this population no longer comprises such large numbers of rhinoceroses. If all the black rhinoceros populations in South Africa are pooled, the population comprises 770 individuals. The effect of population size is clearly illustrated in Fig. 5.5 where simulations involving the three models are compared. Assuming a rate of increase of 6% per year (which has been recorded in the wild for populations of this size), the combined population will persist for 430 years according to Goodman's extinction model. Vortex and DEMM project that a population of this magnitude will go extinct after 200 years. The simulations of Vortex and DEMM for a population of this size differ

from Goodman's predictions. This can be explained by the fact that Goodman's model only takes into account variations in growth rate and not other limiting factors such as carrying capacity or decrease in fecundity. The projections of DEMM and Vortex tend to flatten as the population size increases (Fig. 5.5). The population numbers asymptotically approach the carrying capacity forming a flat logistically shaped curve (Hedrick 1984). However, Goodman's model exhibits an exponential population growth curve (Fig. 5.5). As long as V, due to environmental stochasticity and catastrophes remains bigger than r (intrinsic rate of increase), persistence time exhibits a logistic pattern (Caughley 1994).

However, when a population starts to recover from the small population syndrome, age-specific fecundity increases and mortality decreases. A population of 500 individuals will therefore persist far longer than predicted in Fig. 5.5, since the fecundity values will be higher and mortality lower. The V in Goodman's model will become smaller and r will increase and therefore result in a more exponentially shaped curve.

Conservation strategies for black rhinoceros populations based on the declining population- and small population approaches:

The black rhinoceros is vulnerable to environmental, demographic and genetic risks. Extinction models were used which implement the declining population as well as the small population approach. By implementing PPA, one can identify a black rhinoceros population that is experiencing unusually large fluctuations in population numbers - the first warning sign of a population at risk of extinction. Of the five black rhinoceros populations analysed by PPA, the Hluhluwe-Umfolozi population was identified as having a high probability of reaching extinction within the foreseeable future (Table 5.4).

Some of the fluctuation in population numbers in Hluhluwe-Umfolozi is due to the translocation of animals. This needs to be taken into account in order to arrive at an accurate estimate that reflects environmental stochasticity. The Etosha black rhinoceros population consists of 346 individuals, with high levels of genetic variation, suitable habitat with no environmental risks or catastrophes, and no skewed sex ratio. PPA, however indicates that this population is experiencing a decline and if this decline should continue, the probability is high that the population will eventually become extinct. The next step should then be to identify the agents responsible for this decline and to eliminate these before the population experience further declines. Poaching is the most important agent responsible for the decline in population numbers in Etosha.

The Zambezi Valley population is an example of a large population that experienced fluctuations due to poaching. However, it was impossible to eliminate poaching and the once large population declined so rapidly that the remaining black rhinoceroses from the Zambezi Valley are scattered in several small populations that need intensive management. The black rhinoceros populations of the Kruger National Park and Addo, are the only two populations which are experiencing a positve growth rate. The Kruger National Park and the Etosha black rhinoceros populations, are however, the only remaining large populations. Maintaining large populations is more advantageous for the conservation of a species (Lande & Barrowclough 1987).

The extinction models, Vortex, Goodman and DEMM, are constructed to deal with problems of small populations and therefore information on the genetics and demography is very important. The Mkuzi and captive black rhinoceros populations are small, with short persistence times and therefore they need intensive demographic, genetic and environmental management. The conservation aim of managers of small

populations should be to achieve maximum growth rate in order to lose small population status. PPA, Vortex, Goodman and DEMM projected a short persistence time for the Hluhluwe-Umfolozi population. The declining population approach as well as the small population approach yielded corroborating results for the Hluhluwe-Umfolozi population. This population should be treated as a small population with the emphasis on genetic and demographic management.

For managers it is important to determine whether their black rhinoceros population can be classified as a small population or a declining population. Management actions can then be taken accordingly:

Managing a declining population involves the following: 1) use scientific method to determine why population is declining and to identify the agents that cause the decline, 2) remove or neutralize agent of decline, 3) release a probe group to confirm that the cause of the decline has deduced correctly, 4) restock areas by translocation or breed up a protected stock and 5) monitor the subsequent re-establishment (Caughley 1994).

Managing a small population.
Management actions that can be taken in order to maintain small populations and improve population size include:
Wild populations: Translocations, raising carrying capacity, restricting dispersal, fostering young, reducing mortality, preserving habitat, restoring habitat.
Captive populations: Maintaining captive breeding populations for re-introductions, genetic and demographic management. Re-introduction of captive individuals to occupied or unoccupied habitat and as a last resort capture of wild animals for captive propagation.

This chapter highlights the urgency of taking action against poachers but also provides managers of black rhinoceros populations with two approaches (declining or small populations), to tackle problems in rhinoceros conservation.

1) From the projections of the extinction models it is evident that the captive black rhinoceros population has a short persistence time and is not viable.

2) Most remaining black rhinoceros populations in South Africa are small (eg. Addo, Mkuzi) and intensive demographic management is necessary.

3) The Etosha black rhinoceros population is large but is experiencing a survival threatening decline as a result of poaching.

4) Since South Africa is now the last stronghold for black rhinoceros in the world, poaching has to be eliminated.

5) The Hluhluwe-Umfolozi black rhinoceros population is a large population but remains vulnerable due to fluctuation in the population size. However, demographic factors rather than poaching, are threatening this population.

6) The persistence time for one large black rhinoceros meta-population (all the South African reserves combined), does not guarantee a very long survival period. This suggests that black rhinoceroses are vulnerable over and above the effects of poaching.

FIGURE 5.1: The functioning of a population system - the interaction between population dynamics, the environment and population genetics.

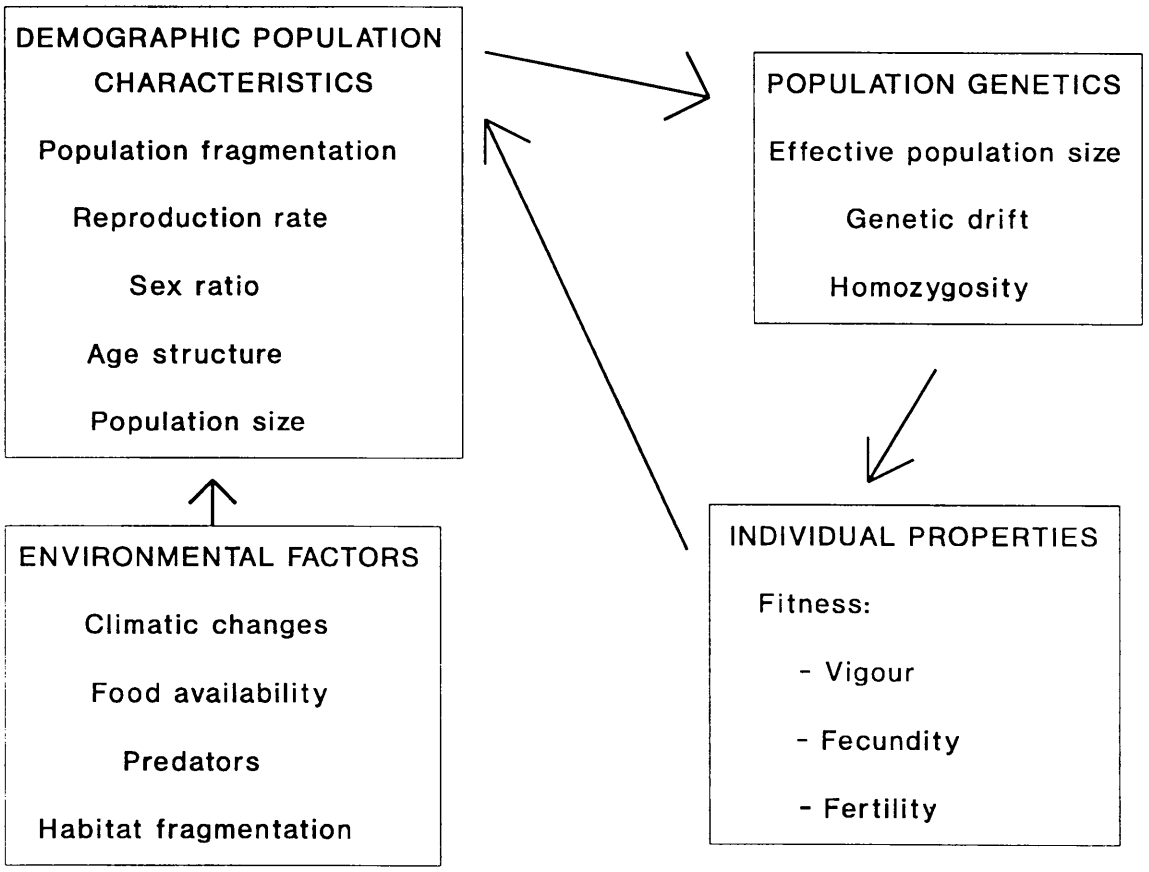

FIGURE 5.2(a): Distribution of black rhinoceros (*D.b. minor* and *D.b. michaelli*) numbers (1992) in East African countries.

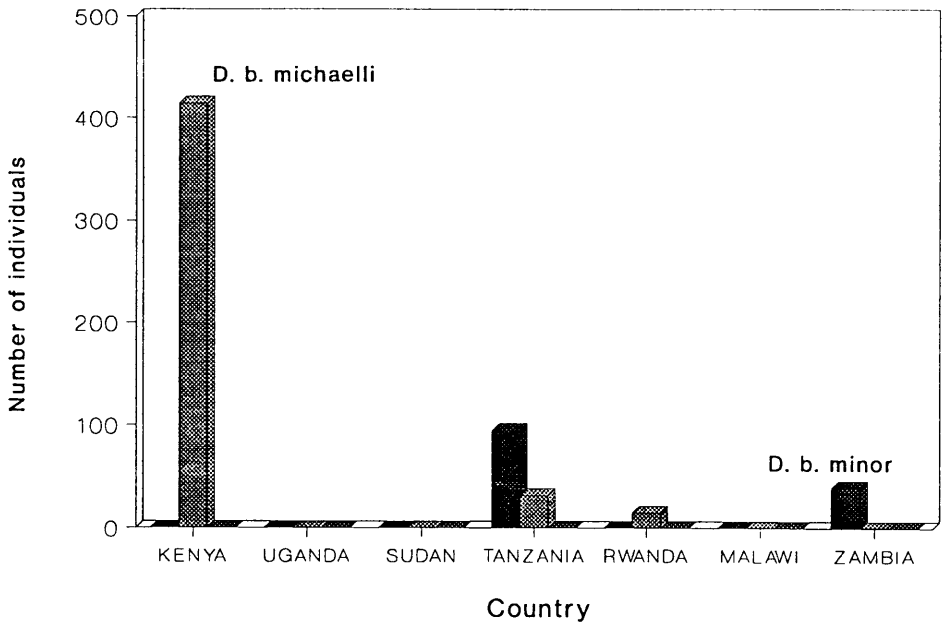

FIGURE 5.2(b): Distribution of black rhinoceros numbers (1992) in southern Africa.

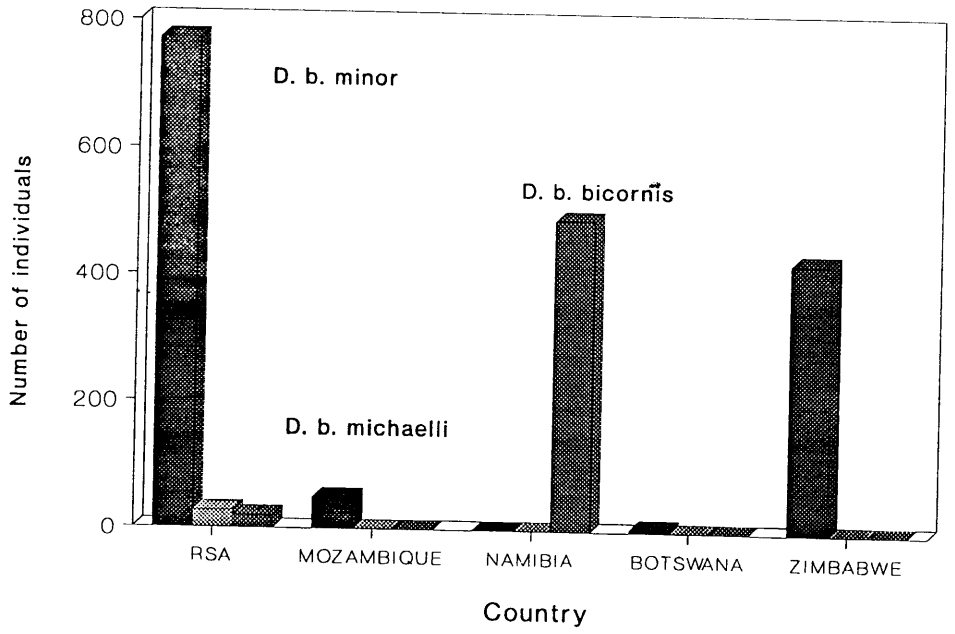

FIGURE 5.3: Black rhinoceros populations (> 50 individuals) in southern African parks and reserves (1992).

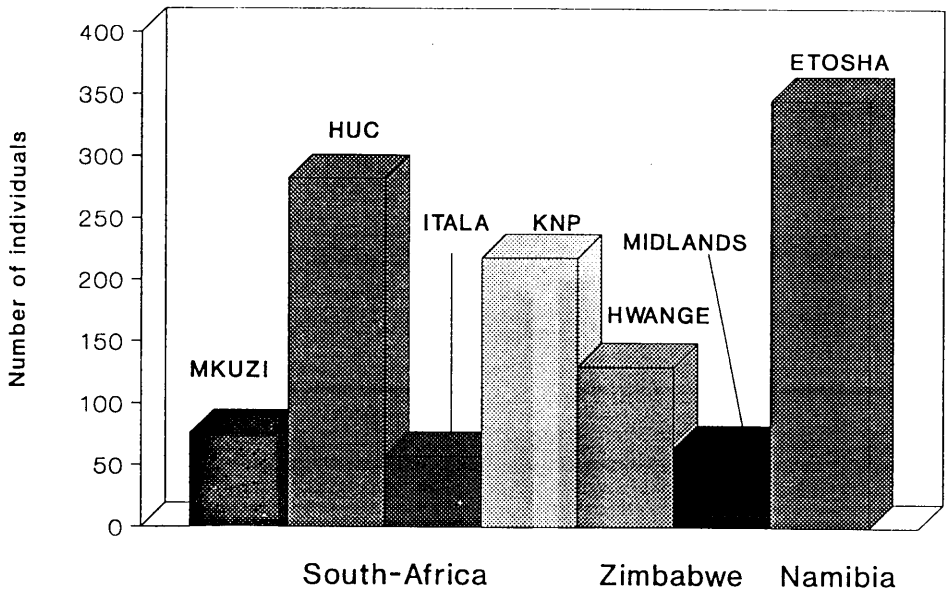

FIGURE 5.4(a-c): Time to extinction simulated for each of 3 black rhinoceros populations using 3 extinction models: Vortex, Goodman and DEMM.

Fig. 5.4(a): Black rhinoceros population in captivity (100 simulations).

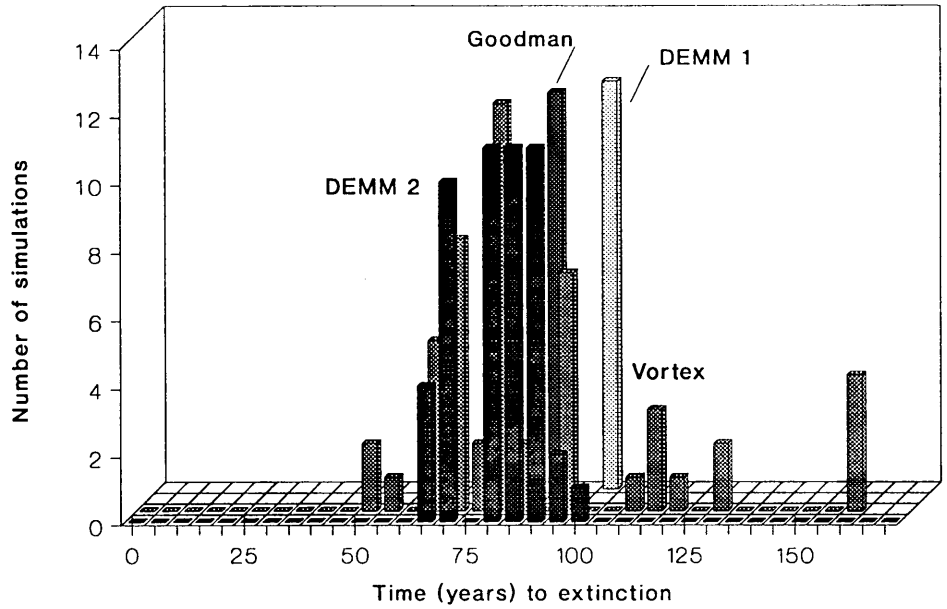

Fig.5.4(b): Simulations for the Mkuzi black rhinoceros population (100 simulations)

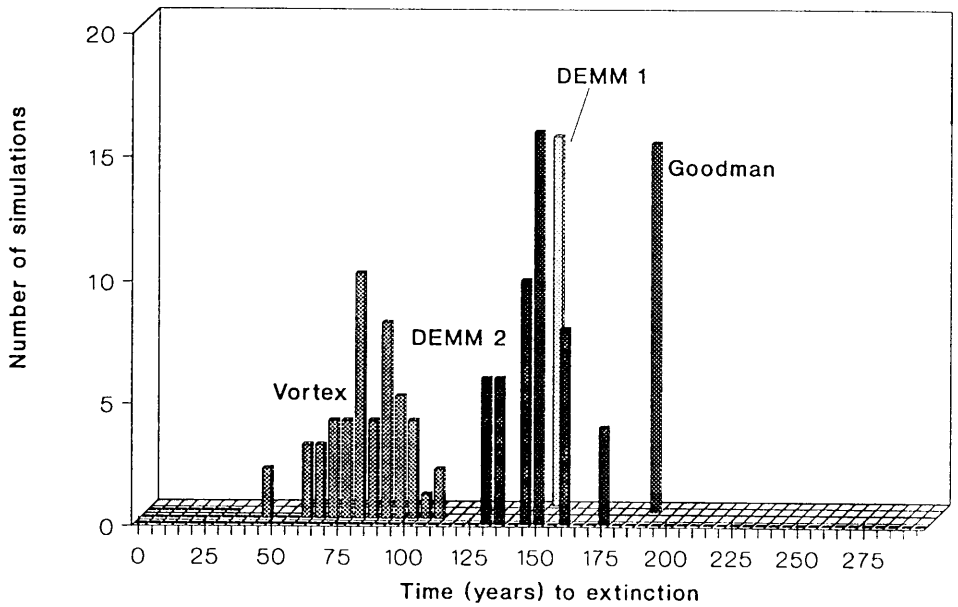

Fig.5.4(c): Simulations for the Tsavo black rhinoceros population (100 simulations)

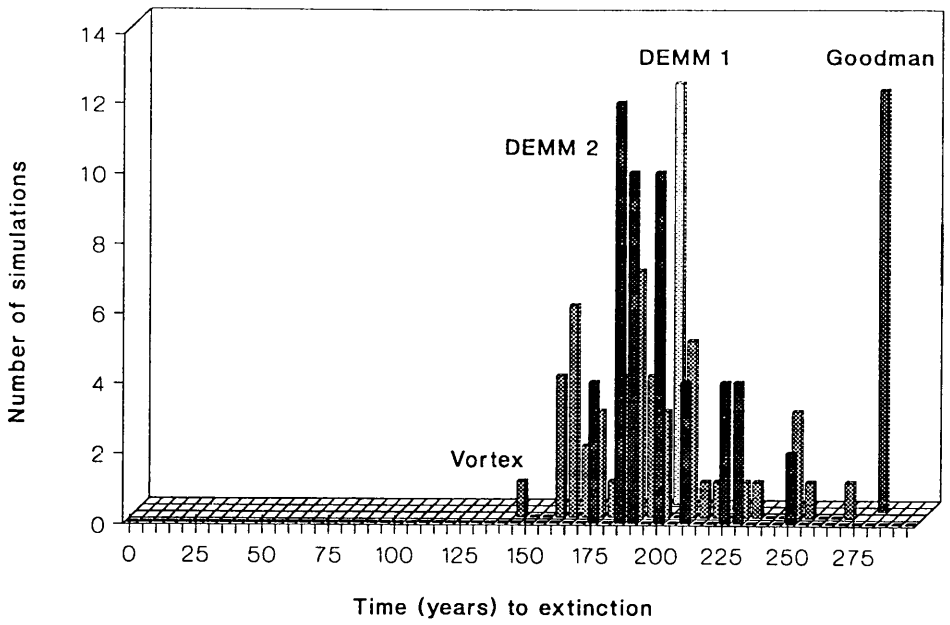

FIGURE 5.5: A comparison of simulations of the 3 extinction models for different sized black rhinoceros populations.

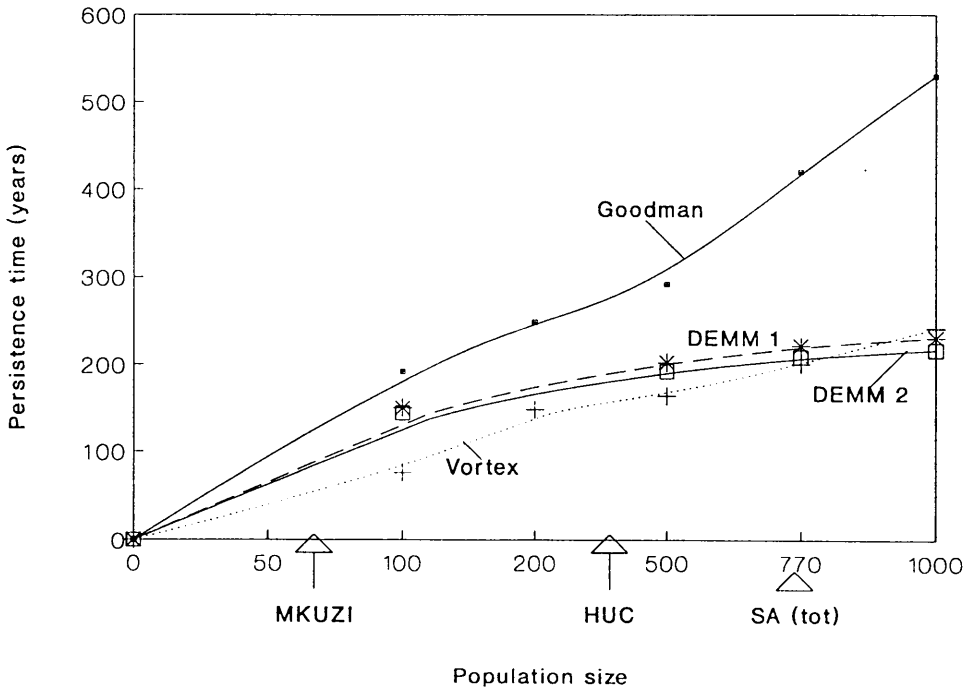

TABLE 5.1: Aspects on the demography and ecology of East- and Southern African black rhinoceroses populations

Park	Area [a] km^2	N	Pop. density /km^2	Calving interval (months)	Rate of increase % /year	Home range km^2
Amboseli	400	10			6.8 - 10.9[b,e]	
Luangwa	16600	75				
Ngorongoro	8300	25	0.08[f]	27 - 28[f]	6[f]	2 - 15[d]
Tsavo	20200	150		30[c]		
Addo	80	17		33 - 38[h]	9.6[h]	
H-U-C	900	270	0.4[g]	22 - 55[g]	5 - 11[g]	
Kruger	19485	140				
Mkuzi	251	82				
Ndumu	100	42	0.38[i]			4.3 - 13.8[i]
Etosha	22270	350		19 - 80[j]		

N = Total population size
Sex ratio = 1:1 - 1:1.2

a Cumming, du Toit & Stuart (1990)
b Goddard (1970)
c Schenkel & Schenkel-Hulliger (1969)
d Goddard (1967)
e Western (1982)
f Kiwia (1989)
g Hitchins & Anderson (1983)
h Hall-Martin (1986)
i Conway and Goodman (1989)
j Joubert (1969)

TABLE 5.2: Life-history data of three black rhinoceros populations. Standard deviations (std.dev) and standard errors (std.err) of age-specific fecundity data to calculate normally-distributed deviations (Matloff 1988) used in DEMM 2 to arrive at more stochastic projections.

	CAPTIVE [1]				EAST TSAVO [2]				MKUZI [3]	
Age class	l_x	m_x	std. dev	std. dev	l_x	m_x*	std. dev	std. dev	l_x	m_x**
0	1.0	0	0	0	1.0	0	0	0	1.0	
1	0.73	0.5	0.269	0.037	0.455	0.25	0.245	0.021	0.468	
2	0.318	0.35	0.34	0.06	0.286	0.5	0.235	0.023	0.281	
3	0.045	0.125	0.25	0.072	0.148	0.75	0.23	0.04	0.25	
4	0.045	0.25	0.25	0.05	0.074	0.5	0.23	0.05	0.218	
5	0.03	0.5	0.2	0.05	0.03	0.45	0.2	0.045	0.156	

[1] Klös & Frese (1991), [2] Goddard (1966), [3] Goodman (pers. comm. 1993).

* m_x data of Addo black rhinoceros population were used since lack of age specific fecundity data.
** m_x data of captive and of Addo used in model.

Table 5.3: Estimated growth parameters for wild black rhinoceros populations using PPA.

Pop.	μ	σ^2	r	δ	α
Addo*	0.866×10^{-1}	0.245×10^{-1}	0.987×10^{-1}	1.104	1.091
Etosha	-0.164×10^{-2}	0.961×10^{-2}	0.316×10^{-2}	1.003	0.998
H-U	-0.884×10^{-2}	0.628×10^{-1}	0.225×10^{-1}	1.023	0.991
KNP	0.853×10^{-1}	0.487×10^{-2}	0.877×10^{-1}	1.092	1.089
Mkuzi	0.117×10^{-1}	0.242×10^{-3}	0.119×10^{-1}	1.012	1.012

μ = diffusion process drift parameter
σ^2 = process variance parameter
r = continuous rate of increase
δ = finite rate of increase
α = geometric finite rate of increase

Transitions used = 4, length of time populations have been observed = 7
* Transitions = 11, length of time population has been observed = 15.

Table 5.4: Estimated extinction parameters for South African black rhinoceros populations, calculated by PPA using growth parameter estimates from Table 5.3.

Population	N	Nt	π	ϕ	$E_{0.5}$	T
Addo	33	1	0.181×10^{-10}	40	38	36
Etosha	346	1	1.0	3560	2613	1078
H-U	282	1	1.0	638	439	159
KNP	218	1	0.0	63	62	62
Mkuzi	76	1	0.0	369	367	366

N = Population size at present
Nt = threshold population size
π = probability of attaining threshold
ϕ = mean time to reach threshold
$E_{0.5}$ = median time to reach threshold
T = most likely time to reach threshold (years)

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

From the preceding chapters it is clear that demographic factors as well as population genetic factors can lead to a decrease in the effective population size (N_e), ultimately leading to extinction. Analysis of those factors that result in a decrease in N_e is essential and should result in control over some of the demographic factors to which the population is most sensitive. A balanced sex ratio and age-structure, high fecundity, low mortality and the prevention of population fragmentation and inbreeding are important for the medium to long-term conservation of the black rhinoceros.

Genetic analysis:

Black rhinoceroses still carry high levels of genetic variation which indicates that they have the potential to survive environmental changes and are not at risk to the deleterious effects of inbreeding. However, if the population sizes should become smaller, genetic drift and inbreeding can have negative results on the remaining black rhinoceros populations. The interaction of demographic factors and genetic factors (Fig. 5.1) emphasises the importance of analysing the demography and genetic composition of a population before management decisions can be made. A decline in effective population size (caused by demographic factors) leads to an increase in genetic drift and homozygosity that in turn affects the individual fitness of an animal.

For long-term conservation the maintenance of high levels of genetic variation can be achieved by translocations. Since the Zambezi black rhinoceros population has

the highest level of genetic variation (Table 2.3), animals originating from this population are possibly the most suitable to restock other populations. The Etosha black rhinoceros population revealed different polymorphism from the two Natal and the Zambezi black rhinoceros populations, however, the difference between Etosha and the other three populations according to allozyme electrophoresis suggests that the genetic differences are too small (Table 2.5) to support the different subspecies theory.

The maintenance of genetic variation is not an important factor for the short-term survival of the black rhinoceros. Managers should at this time rather concentrate on the demographic factors which affect the population size. Poaching poses a more severe threat to N_e than any other ecological, demographic or genetic factor.

Haemolytic anaemia:

Haemolytic disease is an important factor that can lead to depletion of black rhinoceros numbers in captivity and in the wild. The hypothesis of this study, that haemolytic anaemia in rhinos results from a Glucose-6-phosphate dehydrogenase deficiency similar to the situation in humans, finds support in the results of this thesis and is complemented by the work of Paglia (1993).

It is important to identify captive animals that carry the G-6-PD deficient allele. This can be accomplished by allozyme electrophoresis. Spectrophotometric techniques can also be used to determine the activity of the enzyme but this technique requires fresh blood, whereas with allozyme electrophoresis blood can be frozen and analysed later. Translocations and immobilisations of animals can have disastrous consequences if some of these animlas are G-6-PD deficient.

All wild rhinoceroses being handled (except animals from Natal) should be regarded as G-6-PD deficient and treated accordingly. In captivity and in the wild, treatment and the administration of drugs to rhinoceroses should be performed with utmost care and those drugs listed in Table 3.2 should be avoided.

Demographic management of black rhinoceroses

The actual population size is the primary critical factor for the conservation of the black rhinoceros. The future of the black rhinoceros indeed looks bleak since most of the remaining populations are very small, consisting of five to 35 individuals per population. Poaching makes it impossible to keep populations of 500 or more individuals. Needless to say it is impossible under these conditions, to maintain a viable population of black rhinoceroses that is consistent with the criteria for a MVP.
This is regrettable since the persistence time of a population of even as many as 70 animals is only between 76 and 192 years. To be viable such small populations need intensive management which includes protection against poaching.

Even with habitat changes, sex ratio (an important demographic factor) and age-structure do not change drastically (Chapter 5). Demographic management should focus on maintaining short birth intervals to ensure an increase in the effective population size. The projections of the demographic extinction models again emphasized the importance of a large population size for persistence of a population. Simulations of the captive and Tsavo black rhinoceros populations using DEMM (Demographic extinction matrix model) revealed that a population with high fecundity and a large effective population size (Tsavo) will persist for far longer but then a big area is needed to maintain a population of this size. The disadvantage of a big area is that it attracts poachers because it is more difficult to catch them. Managers should focus on maintaining a demographic viable population - one with high

fecundity, a large effective population size and low mortality. A decrease in probability of extinction can be achieved by decreasing *V*. Environmental and demographic stochasticity can result in high *V* values. However, poaching is mostly responsible for the large variation in growth rate. Demographic management is very important since in the absence of poaching as in the Hluhluwe-Umfolozi black rhinoceros population, demographic factors threaten this population.

Demographic management of the remaining small black rhinoceros populations should include the grouping of clusters of these small populations as effective demographic management units. Demographic management should aim at the following:

1) Minimising fluctuations in the numbers of black rhinoceros in each demographic unit. Managers of especially the Kruger National Park and Etosha should use the PPA model regularly to detect any decline since these are the only two remaining large populations;

2) Ensuring favourable sex ratios within each small population inside a management unit;

3) Ensuring maximum calf survival.

Rhinoceroses in captivity:

The importance of establishing a viable black rhinoceros population in captivity can not be overemphasized. The estimated world carrying capacity for rhinoceroses in captivity is 200 - 250 individuals and the N_e is only 116 individuals. To establish a captive black rhinoceros population in captivity, selection of breeding pairs should

be made by considering the following conditions: 1) the genetic composition of the individual and 2) the reproductive success of the individual. The captive black rhinoceros population will play an important role to supplement populations in the wild. Smith & Read (1986) found that the captive black rhinoceros population has not yet been able to sustain itself without recruitment from the wild and that birth rates approximately equal death rates. A low birth rate can lead to loss of genetic diversity. Smith & Read (1986) concluded that although the rhinoceroses experience medical and nutritional problems, the real problem may be breeding management.

The black rhinoceros is endemic to Africa and the climatic conditions and the availability of food favour the breeding of black rhinoceroses in Africa rather than in Europe or in the USA. However, no serious attempt has been made in Africa to breed black rhinoceroses in captivity.

It is therefore important that zoological gardens in temperate areas participate in a scientifically planned captive breeding program. This will eliminate the stress that winter conditions induce in captive populations outside of temperate areas. Moreover, the high mortality of captive rhinoceros younger than five years of age, needs to be eliminated.

Many authors attribute the high mortality of black rhinoceroses in captivity to haemolytic anaemia. The importance of this syndrome in causing deaths of young captive rhinoceroses needs to be ascertained.

Demographic management of captive black rhinoceroses should be performed. Forty percent of zoos with black rhinoceroses do not contribute to the breeding of these animals. For the moment the keeping of captive black rhinoceros purely for purposes of display can not be justified. Agencies like the IUCN Captive Breeding Specialist

Group should do their utmost to encourage zoological gardens to keep black rhinoceros of both sexes and to actively conduct captive breeding.

To summarize:

It is important to prevent extant **large** black rhinoceros populations from declining, because this will result in small populations that are more at risk of extinction. Extinction models have been developed to assist managers in making decisions on the scientific management of the remaining black rhinoceros populations and whether a population should be treated as a declining population or as a small population. This enables managers to follow two approaches in the conservation of a population: the small population approach (intensive management); and the declining population approach (identifying a decline in a population as soon as possible and try to avoid the small population scenario). PPA will determine whether a population is experiencing a decline and if this decline is drastic, extinction models such as DEMM, Goodman and Vortex can be very useful in identifying the small population problem.

The Human Factor:

During this study the results of the genetic analysis of the black rhinoceros are reassuring for their future survival. However, during a period of three years (from 1990 - 1992), more than 1000 rhinos died, mostly as a result of poaching. The survival of the rhino, as for so many other species, is in the hands of humans. Poaching inevitably leads to a loss in genetic variation because it has a direct influence on the effective population size as well as on the demographic factors such as sex ratio and age-structure that leads to a further decline in population size.

From this study, quite a few fields emerged where the conservation of the rhinoceros needs attention. A captive population that reproduces successfully is needed to supplement the black rhinoceros populations in the wild. The subject on haemolytic anaemia was only touched during this study and since it is responsible for rhinoceros mortalities, further investigations are essential. For managers of natural black rhinoceros populations the monitoring of sex ratios, **effective** population size, age-specific fecundity and mortality and age-structure are essential to maintain black rhinoceros populations. Managers of wild black rhinoceros populations can use extinction models as aids to assist them in decision making. Vortex, DEMM and Goodman can be used in the case of small populations and PPA (Nicholls *et al.* in prep.) within the declining population context.

SUMMARY

It was necessary to study a diverse number of aspects concerning black rhinoceros population biology to determine the vulnerability of these populations. The focus of this project was on population genetics and population demography which are the two main themes of population biology. The effective population size is the critical factor for the conservation of the rhinoceros since most of the remaining black rhinoceros populations are small. The aim therefore was to identify those factors that influence the effective population size.

Thirty protein and enzyme loci of four black rhinoceros populations were analysed to determine the levels of genetic variation. Protein electrophoresis revealed that all four black rhinoceros populations still carry high levels of genetic variation. This is reassuring for the long-term survival of the species.

A polymorphism of glucose-6-phosphate dehydrogenase was observed and my results suggested that haemolytic anaemia in the black rhinoceros is probably caused by a deficiency of this enzyme. Stress associated with capture and the administration of some drugs to rhinoceroses may induce haemolytic anaemia in animals that carry an allele that is G-6-PD deficient. Haemolytic anaemia is responsible for 40% of rhinoceros mortalities in zoos therefore each individual should be analysed to determine whether it carries the deficient allele.

The total effective population size of black rhinoceroses in captivity is alarmingly low. It is important to establish a viable black rhinoceros population in captivity and each zoo should participate in a captive breeding programme based on scientific knowledge. From the literature reviewed, it is evident that captive breeding of rhinoceroses has not yet reached its full potential.

Analysis of the demographic factors indicate that wild rhinoceros populations remain stable (e.g. sex ratio and age-structure do not change dramatically) even if they experience ecological threats such as competition, predation, droughts and fire. Poaching however, has a negative effect on the sex ratio and age-structure and will result in a serious decline in effective population size.

A demographic extinction model (DEMM) was written to determine the persistence time of black rhinoceros populations. The model uses a Leslie matrix with age-specific fecundity and mortality data. The results of DEMM compare well with other extinction models e.g. Vortex and Goodman's models. All three models (based on the small population approach) suggest that demographic and environmental stochasticity will cause the three black rhinoceros populations (captive, Mkuzi and Tsavo) to become extinct within 300 years. This highlights the urgent need for intensive management of the remaining black rhinoceros populations. The population persistence analysis model (PPA) was also used to predict the persistence time of larger black rhinoceros populations. PPA is based on the declining population approach and only uses census data which makes it more accessible to managers of rhinoceros populations. Ideally, PPA can be used to prevent the small population situation, by identifying a decline in a population at an early stage. If a population is already classified as "small", extinction models such as DEMM, Vortex and Goodman, which address the problems faced by small populations, are invaluable. Managers of black rhinoceros can use extinction models as valuable tools to assist them in making scientific decisions for the conservation of the species.

OPSOMMING

Ten einde die kwesbaarheid van swartrenosterbevolkings te bepaal, was dit nodig om verskeie aspekte van hul bevolkingsbiologie te bestudeer. Die fokus van die projek het geval op die twee hooftemas van bevolkingsbiologie naamlik bevolkingsgenetika en bevolkingsdemografie. Die effektiewe bevolkingsgrootte is die kritiese faktor vir die bewaring van die renoster omdat die oorblywende swartrenosterbevolkings baie klein is. Die doel van die projek was om die faktore te identifiseer wat die effektiewe bevolkingsgrootte beïnvloed.

Dertig proteïen- en ensiemlokusse van vier swartrenosterbevolkings is bestudeer om die genetiese variasie te bepaal. Proteïenelektroforese dui daarop dat al vier swartrenosterbevolkings steeds hoë vlakke van genetiese variasie besit. Dit is gerusstellend vir die langtermyn oorlewing van die spesie.

'n Polimorfisme van glukose-6-fosfaat dehidrogenase is waargeneem en my resultate dui daarop dat 'n tekort aan die ensiem waarskynlik verantwoordelik is vir hemolitiese anemie. Stres wat geassosieer word met die vang van renosters en die toediening van sekere geneesmiddels mag lei tot hemolitiese anemie in individue wat die alleel besit met 'n G-6-FD tekort. Hemolitiese anemie is verantwoordelik vir 40% van renoster sterftes in dieretuine en daarom moet elke individu getoets word om vas te stel of die individu 'n draer is van die alleel met die ensiemtekort.

Die totale effektiewe bevolkingsgrootte van swartrenosters in gevangenskap is onrusbarend laag. Dit is belangrik om 'n lewensvatbare swartrenosterbevolking in gevangenskap te vestig en alle dieretuine moet deelneem aan 'n teelprogram wat gebaseer is op wetenskaplike kennis om sodoende die effektiewe bevolkingsgrootte

te laat styg. Uit die literatuuroorsig is dit duidelik dat die teling van swartrenosters in gevangenskap nog nie volle potensiaal bereik het nie.

Bestudering van die demografiese faktore dui daarop dat wilde swartrenosterbevolkings stabiel bly (bv. geslagsverhouding en ouderdomstruktuur verander nie drasties) al word hulle ekologies bedreig deur kompetise, predasie, droogtes en vuur. Wildstropery het egter 'n negatiewe uitwerking op die geslagsverhouding an ouderdomstruktuur en kan lei tot 'n ernstige afname in die effektiewe bevolkingsgrootte.

'n Demografiese uitsterwingsmodel (DEMM) is geskryf om vas te stel wat die tyd tot uitsterwing van swartrenosterbevolkings sal wees. Die model maak gebruik van 'n Leslie matriks en ouderdomspesifieke data oor fekunditeit en mortaliteit. Die resultate van DEMM vergelyk goed met die resultate van ander uitsterwingsmodelle soos Vortex en Goodman. Al drie modelle (klein bevolking benadering) voorspel dat die drie swartrenosterbevolkings (gevangenskap, Mkuzi en Tsavo) binne 300 jaar sal uitsterf as gevolg van demografiese- en omgewingsstogastisiteit. Dit beklemtoon dat die oorblywende swartrenosterbevolkings dringend intensiewe bestuur benodig. 'n Ander uitsterwingsmodel, PPA, is ook gebruik om die tyd tot uitsterwing van swartrenosterbevolkings te bepaal. PPA is gebaseer op die afnemende bevolking benadering en maak gebruik van sensus data wat maklik verkrygbaar is en die model dus toeganklik maak vir bestuurders van renosterbevolkings. Die ideaal is om PPA te gebruik om die "klein bevolking" toestand te voorkom deur vroegtydig 'n afname in die getalle van die bevolking te identifiseer. Indien die bevolking reeds "klein" status bereik het, is modelle wat "klein bevolking" probleme hanteer soos DEMM, Vortex en Goodman van onskatbare waarde. Uitsterwingsmodelle is waardevolle hulpmiddels wat bestuurders van swartrenosterbevolkings kan ondersteun in wetenskaplike besluitneming oor die bewaring van die spesie.

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APPENDIX I: Demographic Extinction Martix model (DEMM 1 & 2)

```
program rhino;
uses crt, dos;

   {============================================================
              DEMOGRAPHIC EXTINCTION MATRIX MODEL

============================================================}

CONST no_ageclasses = 5;

TYPE matrixtype = array[0..no_ageclasses, 1..no_ageclasses] of
     real;
     vectortype = array[0.. no_ageclasses] of real;
     texttype = string[10];

VAR  f, f1:text;
     stochasticity: BOOLEAN;
     inmatrix:matrixtype;
     invector, outvector :vectortype;
     mean,stddev,sem,normalval,ndev,saveF: vectortype;
     row,collumn,i,j,age:integer;
     count,seed,range : word;
     x,y,std_dev,newf,NTotal : real;
     filename : texttype;
     ans : char;

{=========================================================
 Life History Data, Calculation of Fi and P values,
 Writing Fi and P values into LESLIE MATRIX
=========================================================}

PROCEDURE LeslieMatrix(VAR matrix: matrixType);

VAR
    ageclass: ARRAY[0..no_ageclasses] of INTEGER;
    f : text;
    lx, mx, p, r :vectortype;
    surv1 :real;
    i,j,row,collumn:integer;
BEGIN
   assign(f,'lifedata.zoo');
   reset(f);

 {writeln('=======Datafile=======');}

   for row:= 0 to no_ageclasses do begin
       readln(f,ageclass[row],lx[row],mx[row]);
       writeln(ageclass[row]:6, lx[row]:6:2, mx[row]:6:2);
   end;

{====Calculation of survival from Ageclass 1 to 2====}

      surv1 := (lx[0] + lx[1]) / 2;
      writeln(surv1:2:3);

{===Calculation of Probability of survival ====}
   p[0] := 0;
   p[no_ageclasses] := 0;
```

```
   for row := 1 to no_ageclasses-1 do begin
       p[row]:= (lx[row] + lx[row+1]) / (lx[row] + lx[row-1]);
       writeln('prob. of survival(',row:2,')=',p[row]:2:3);
   end;

{===Calculation of the Fertility coefficients====}

    for row:= 1 to no_ageclasses do begin
          if row < no_ageclasses then
              r[row]:= survl * (mx[row]+(mx[row+1]*p[row])) /2
          else
              r[row]:= survl * mx[row] / 2;
      writeln(' Fi:',row:3,r[row]:9:4);
    end;

{====Construction of LESLIE MATRIX =======}

{====Assigning Fi & P values to MATRIX====}

   for row := 1 to no_ageclasses DO
       for collumn := 1 to no_ageclasses do
           inmatrix[row,collumn] := 0;
   for collumn:= 1 to no_ageclasses do
       inmatrix[1,collumn]:=r[collumn];
   for row:= 0 to no_ageclasses do
       inmatrix[row,row-1]:= p[row-1];
END;


{====Print LESLIE MATRIX======}

PROCEDURE Printmatrix(Inmatrix:MatrixType);
begin
 for row := 1 to no_ageclasses do begin
     for collumn := 1 to no_ageclasses do
        write(Inmatrix[row,collumn]:9:4);
     writeln;
 end;
end;

{====VECTOR=====}

PROCEDURE Readvector(VAR InVector:vectortype; FileName:TextType);
begin
  assign(f, FileName);
  reset(f);
  for row:= 1 to no_ageclasses do begin
     read(f,invector[row]);
  end;
end;


PROCEDURE Printvector(InVector:vectortype);
begin
  for row:= 1 to no_ageclasses do begin
      write(invector[row]:14);
  end;
end;
```

```
{====LESLIE MATRIX * VECTOR====}


{Matloff, 1988}

FUNCTION normal(mean,Std_dev: real): REAL;
var T1,T2: real;
     a,b:REAL;
     X,Y:REAL;
begin
  T1 := 6.28 * random;
  T2 := sqrt(-2.0 * ln(random));
  X := cos(T1) * T2; X := Std_dev * X + Mean;
  Y := sin(T1) * T2; Y := Std_dev * Y + Mean;
  normal := X;
end;


PROCEDURE   Matrixmultiply(var   outvector:vectortype;
Inmatrix:matrixtype; Invector:vectortype);
var
i,j:longint;
age: real;
begin
   if stochasticity = TRUE then begin
      for i := 1 to no_ageclasses do begin
         normalval[i] := normal(0,1);
         if invector[i] = 0 then sem[i] := 0
             else sem[i] := stddev[i]/sqrt(invector[i]);
         ndev[i] := normalval[i] * sem[i];
         { writeln(normalval[i],saveF[i],newf); }
         newf := saveF[i] + ndev[i];
         if newf < 0 then newf := 0;
         inmatrix[1,i] := newf;
         end;
      end; { if stochasticity }
   for i:= 1 to no_ageclasses do begin
      outvector[i]:= 0;
      for j:= 1 to no_ageclasses do begin
         outvector[i]:=   outvector[i]   +   (invector[j]   *
inmatrix[i,j]);
         end;
   end;
end;

{====CHANGE OUTVECTOR TO INVECTOR====}

PROCEDURE   invector_outvector(var   invector:vectortype;var
outvector:vectortype);
var
i:longint;
begin
  for i:= 1 to no_ageclasses do
  invector[i]:= outvector[i];
  writeln;
end;
```

```
PROCEDURE Invectorprint(invector:vectortype);
begin
   NTotal := 0;
   for row:=1 to no_ageclasses do begin
       NTotal := NTotal + invector[row];
       write(invector[row]:9:3);
   end;
   write('| ',NTotal:9:3);
end;


{=========================================================
          M A I N     P R O G R A M
=========================================================}

BEGIN
  ClrScr;
  writeln('Karen se super-duper simmulasie');
  stochasticity := FALSE;
  Randomize;
  lesliematrix(inmatrix);
  for age := 1 to no_ageclasses do saveF[age] := inmatrix[1,age];
  printmatrix(inmatrix);
  writeln;
  readvector(invector,'vector.zoo');
  readvector(mean,'mean.vec');
  readvector(stddev,'stddev.vec');
  printvector(mean);
  writeln('===============');
  writeln('Do you prefer stochasticity in the model? (Y/N)  ');
  readln(ans);
  if UpCase(ans) = 'Y' then stochasticity := TRUE;
  {matrixmultiply(outvector,inmatrix,invector);
  printmatrix(inmatrix);}
  i := 1;
  repeat
      matrixmultiply(outvector, inmatrix, invector);
      invector_outvector(invector, outvector);
      write(i:3,' '); invectorprint(invector);
      i := i+1;
      until NTotal < 1;
  writeln('DONE');
  readln;
end.
```

APPENDIX II: Published papers.

1) Polymorphism of glucose-6-phosphate dehydrogenase in black rhinoceros: a possible link with haemolytic anaemia. *South African Journal of Science* 90: 14-16. 1994

2) Substantial genetic variation in Southern African black rhinoceros (*Diceros bicornis*). *Journal of Heredity* 85(4). 1994.

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

M. K. J. Swart, J. W. H. Ferguson, R. du Toit, and J. R. B. Flamand

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 1981) 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).

From the Department of Zoology, University of Pretoria, Pretoria, 0001, South Africa (Swart and Ferguson), the WWF Multispecies Project, Causeway, Harare, Zimbabwe (du Toit), and the Natal Parks Board, Mtubatuba, South Africa (Flamand). We sincerely thank Dr. S. Bissbort, Department of Chemical Pathology, Pretoria University, for invaluable assistance in some of the electrophoretic runs and Dr. P. M. Brooks, Natal Parks Board, for valuable discussion of the rhino position in Natal. This work was supported by grants to J.W.H.F. from the FRD and from Pretoria University. Address correspondence and reprint requests to J. W. H. Ferguson at the address above.

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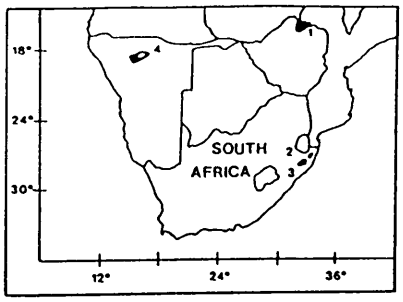

Figure 1. Map of southern Africa indicating the geographic origin of material analyzed: (1) Zambezi, (2) Mkuzi, (3) Hluhluwe-Umfolozi, and (4) Etosha.

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 jth 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. 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. minor*) and found

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*	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)

* *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 conform to Hardy-Weinberg expectations.

Table 3. Genetic variation* 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	Hetero-zygosity	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

* 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).

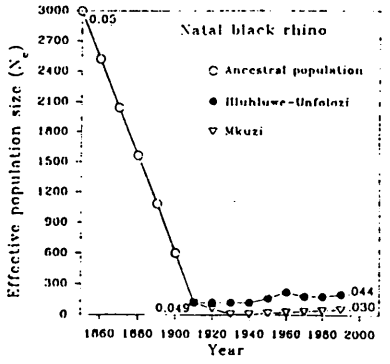

Figure 3. Estimates of effective population sizes of Natal black rhinoceros populations over the last 150 years (sources quoted in text). Numbers on graph indicate expected gene diversity at 1850, 1910, and 1990 for the two indicated populations, assuming $H_{t+1} = H_t(1 - 1/2N_e)$[39] and N_e = 0.6 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.

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.

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APPENDIX III: Census data of black rhinoceros populations used in the population persistence analysis model.

HLUHLUWE *

Year	N
1985	300
1986	200
1987	220
1988	220
1992	282

MKUZI *

Year	N
1985	70
1986	70
1987	70
1988	70
1992	76

ETOSHA **

Year	N
1985	350
1986	350
1987	300
1988	300
1992	346

ADDO ***

Year	N
1978	9
1979	11
1981	16
1983	19
1985	16
1986	17
1992	29
1993	33

* Brooks (pers. comm).

** Fox (pers. comm).

*** Hall-Martin (pers. comm).

APPENDIX IV: Allele frequencies of the 6 polymorphic loci for the four black rhinoceros populations (*Diceros bicornis*).

	ETOSHA			ZAMBEZI		
Locus	**A**	**B**	**N**	**A**	**B**	**N**
Es-2	1.00	0.00	15	0.859	0.141	90
GP-3	1.00	0.00	15	0.445	0.555	90
GP-5	0.633	0.367	15	0.541	0.459	90
Pgm-2	0.084	0.916	15	0.4	0.6	15
G6pd	0.25	0.75	6	0.88	0.12	15
HB-2	0.75	0.25	15	1.00	0.00	15
	HLUHLUWE			**MKUZI**		
Es-2	0.84	0.16	25	0.955	0.045	25
GP-3	1.0	0.0	25	1.0	0.0	25
GP-5	0.76	0.24	25	0.617	0.383	25
Pgm-2	0.4	0.6	25	0.382	0.618	25
G6pd	1.0	0.0	25	0.98	0.02	25
HB-2	1.0	0.0	25	1.0	0.0	25

N = sample size

A = frequency of the A allele

B = frequency of the B allele